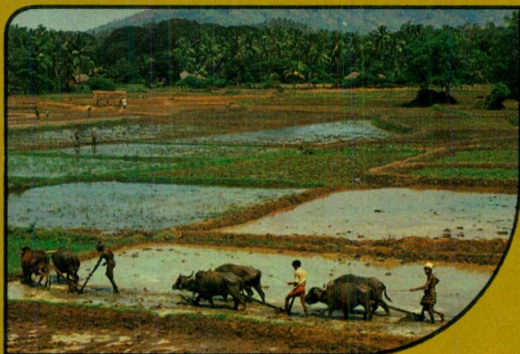


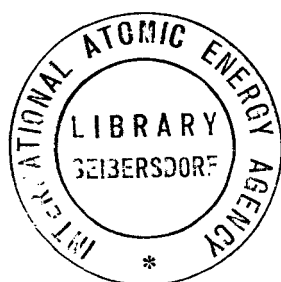


NUCLEAR AND RELATED TECHNIQUES IN ANIMAL PRODUCTION AND HEALTH

PROCEEDINGS OF A SYMPOSIUM, VIENNA, 17–21 MARCH 1986
JOINTLY ORGANIZED BY IAEA AND FAO



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1986



**NUCLEAR AND RELATED TECHNIQUES
IN ANIMAL PRODUCTION AND HEALTH**

PROCEEDINGS SERIES

NUCLEAR AND RELATED TECHNIQUES IN ANIMAL PRODUCTION AND HEALTH

PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM
ON THE USE OF NUCLEAR TECHNIQUES
IN STUDIES OF ANIMAL PRODUCTION AND HEALTH
IN DIFFERENT ENVIRONMENTS
JOINTLY ORGANIZED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
AND HELD IN VIENNA, 17-21 MARCH 1986

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1986

EDITORIAL NOTE

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FOREWORD

Developing countries have undergone a rapid population growth in the last two decades, and as a consequence the demand for high quality food and fibre of animal origin has increased substantially. Reflecting this demand have been increases in the production of animal products, but these have been achieved largely through increased livestock numbers rather than through improved individual animal productivity. Indeed, in some regions animal productivity has actually decreased with the result that per caput consumption of meat and milk is currently the same as it was 20 years ago, despite the fact that many countries now also import substantial quantities of animal feeds and products. In developed countries by contrast, there have been impressive increases in livestock production over the same period, increases which have been achieved not by increasing numbers but through enhancing productivity. This, coupled with population stabilization, has meant that the demand within these countries can now largely be satisfied, and that in some instances there is substantial over-production.

The reasons for the disparities in livestock productivity which currently exist between developing and developed countries and even between developing countries themselves are variable and complex, and therefore efforts to improve productivity must involve a variety of strategies taking account of the diversity of traditional patterns of farming and social practices. These strategies should, where possible, be based on local animal as well as feed resources, and encourage appropriate low-cost modifications to the animal and/or its environment. All this may seem obvious, but the goal of improving livestock productivity in developing countries is made all the more difficult by the paucity of basic and applied research conducted within the countries themselves on animal feeding, breeding and disease-control strategies, and on the interactions between these. Within and across these disciplines nuclear and related techniques, employed in association with standard methods, help veterinary and animal scientists to assess such parameters as the nutritional value of feeds, and the reproductive and disease status of livestock. This approach not only enables identification of constraints, but also investigation of the methods to minimize their impact.

The primary function of the FAO/IAEA Programme on Animal Production and Health is to assist in the establishment and/or strengthening of the capability of veterinary and animal science institutes in developing countries to conduct research into the causes of and solutions to low animal productivity. This programme, which is carried out in close collaboration with the animal production and health programme of FAO, operates through a variety of mechanisms including Technical Co-operation Projects and Research Contracts. Training, not only in techniques per se but also in experimental design and data interpretation, is of central importance to the

programme. When new techniques need to be developed or existing techniques need to be modified to suit conditions in developing Member States, this task is conducted at the FAO/IAEA Animal Production Unit of the IAEA Laboratory at Seibersdorf.

The FAO/IAEA International Symposium on the Use of Nuclear Techniques in Studies of Animal Production and Health in Different Environments was organized jointly by the IAEA and FAO and held in Vienna from 17–21 March 1986. The Symposium was attended by about 130 participants from 45 countries and included 83 scientific presentations of which 42 were posters. Many of the participants were from developing countries and the majority of presentations described work conducted within developing countries, often with assistance from the Joint FAO/IAEA programme. However, without the generous support provided by other international and bilateral funding organizations, the effectiveness and extent of this programme and of the Symposium itself would have been substantially reduced. It is therefore with pleasure that the IAEA and FAO acknowledge the contributions made to these activities by: the Australian Council for International Agricultural Research; the British Council; the Italian Department of Co-operation for Development; the International Foundation of Science; the Department of Technical Assistance, Ministry of Foreign Affairs, Netherlands; the Overseas Development Administration of the United Kingdom; the Swedish Agency for Research Co-operation with Developing Countries; the Swedish International Development Authority and the United States Agency for International Development.

This volume covers four principal and interrelated topics: adaptation of animals to the environment, and animal reproduction, health and nutrition. Within each topic, consideration is given to those nuclear and related techniques currently employed in investigative research and their usefulness in studying animal production systems. Progress towards new areas of application and new techniques is also covered, particularly the development and practicability of immunoassay and related biotechnological methods for the diagnosis of livestock diseases. It is hoped that by bringing together information from a variety of disciplines and countries, and by focusing heavily on the problems of developing countries, this volume will assist scientists within these countries in their investigations towards improving livestock productivity.

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OPENING SESSION

OPENING ADDRESS

H. Blix

Director General of the International Atomic Energy Agency,
Vienna

On behalf of the Director General of the Food and Agriculture Organization of the United Nations and on behalf of the IAEA, I would like to welcome you to this FAO/IAEA International Symposium on the Use of Nuclear Techniques in Studies of Animal Production and Health in Different Environments. I should also like to welcome you to the Headquarters of the IAEA here in Vienna.

There are a number of points that I would like to mention in connection with this symposium.

First of all, when I looked back over the statistics of previous symposia on this subject, I noticed that the first symposium in 1972 attracted 19 Member States and that the latest one in 1976 was attended by 90 participants from 28 countries. Today, I am pleased to note that not only do we have many more participants, but we also have 45 Member States represented. These figures show clearly that this area of activity has become increasingly important and the subject of growing interest over the past decade. Equally gratifying to me is that when I look at the programme for the week's activities, I see that more than half of the paper and poster presentations come from developing Member States in Africa, Asia and Latin America, where the need to improve performance in animal production and health is most acute. This is an encouraging sign.

This symposium is being co-sponsored by the Food and Agriculture Organization of the United Nations. The word 'co-sponsorship' can have many different meanings, but in this particular case it means that the symposium is truly a joint endeavour of both organizations. For this excellent spirit of co-operation I would like to thank H.A. Jasiorowski, the Director of FAO's Animal Production and Health Division in Rome. Apart from the fact that he has agreed to give the keynote address, which will consider some of the major problems facing livestock producers in developing countries and thereby set the scene for later discussions, he has been the driving force in ensuring that this word 'co-sponsorship' really means something.

A vote of thanks is also due to those other international and national funding organizations whose support has contributed in no small way, not only to the fact that there are so many participants at this symposium but also to the overall programme of the Joint FAO/IAEA's Animal Production and Health Section. In these days of emphasis on bilateral funding and zero-growth budgets, it is a pleasure for me to note that organizations such as the Department of Co-operation for Development in Italy, the Department of Technical Assistance, Ministry of Foreign Affairs, Netherlands, and the Swedish International Development Authority (SIDA), consider the Joint FAO/IAEA programme in animal science sufficiently relevant to the needs of developing Member States that they are willing to provide substantial support for this

programme. As far as the symposium itself is concerned, and apart from the support provided by FAO which I mentioned earlier, I would like to acknowledge the contributions made by the Australian Council for International Agricultural Research (ACIAR), the British Council, the Overseas Development Administration of the United Kingdom, the International Foundation of Science, the Swedish Agency for Research Co-operation with Developing Countries (SAREC) and the United States Agency for International Development (USAID). Without these contributions, the wide exchange of information which will take place over the next five days would not have been possible.

Turning now to the topic of the symposium, there is hardly a need to stress the global economic and social importance of livestock, or the constraints currently hindering increased livestock productivity. Nor do I need to dwell on the ways of overcoming these constraints because these are going to be covered during this week. What I would like to mention, however, is what is known as the 'productivity gap' between North and South, or between temperate and tropical or subtropical countries in terms of livestock production. This productivity gap has two components, although they overlap to some extent. The first is the 'application gap': many experts say that livestock productivity in the developing world could be doubled at least using available technologies. What are these technologies? This question leads to the second component, namely the 'research gap' — that is, research aimed at understanding livestock systems, overcoming weaknesses in the present range of technologies and establishing the technologies needed for sustained livestock development in the next century.

Developing Member States recognize all too well the existence of the gaps to which I have referred, and they know that bridging them will not be a simple task. I hope that this symposium — so well attended by experts from these countries — will make a useful contribution to this task. Some might perhaps feel pessimistic about the prospects for real breakthroughs when they observe the present critical food situation for both man and his livestock in much of Africa and realize that these events have taken place against a background of substantially increased foreign aid from international and bilateral organizations. However, in the IAEA — and I am sure Professor Jasiorowski will agree that the same applies in FAO — we believe that even if many of those involved in livestock production in developing countries have not yet come up with the right answers, then at least they have begun to ask the right questions. More importantly, they have also begun to realize that solutions can only come from within the countries and regions themselves.

The IAEA and FAO have a duty to help Member States to define and solve their own problems, but not to attempt to solve them for the Members. The organizations also have a duty to serve as a channel for the transfer of the most appropriate technology. In this context, I would like to mention the use of radio- and enzymeimmunoassay techniques, since in the field of animal reproduction and disease diagnostics these have found ever-increasing application, and indeed are now carried out more or less routinely in virtually every country in the northern hemisphere.

But what about the situation in developing countries? Ten years ago at our last symposium on animal production and health, there were three presentations on the use of hormone radioimmunoassays for defining the constraints on livestock reproductive efficiency in developing countries. Today, we have over 40, and interestingly many of these are reporting work on indigenous breeds and species of livestock — animals such as the buffalo in South-East Asia, and the llama and alpaca in Latin America. This increasing awareness of the value of immunoassay methods is encouraging; so are the serious efforts now being made to support work on local livestock resources, and especially on animals kept on small farms.

Support for the development of local livestock resources requires a different infrastructure from that used in studies dealing with the problems associated with large-scale animal production systems. It also requires great care with regard to technology transfer, because if this is inappropriate then nothing will be achieved by way of narrowing either the 'application' or 'research' gaps I referred to earlier. Indeed, inappropriate technology only serves to widen these gaps, since scientists and planners alike quickly become disillusioned.

To transfer nuclear and related technology such as radio- and enzyme-immunoassay successfully requires research — it is not enough simply to take a piece of equipment, a technique, or an approach which has proved successful in a developed country and put them somewhere in a tropical developing country and expect them to work. The technologies have to be adapted to the environment in which they are going to be used.

Two years ago we established a laboratory unit at the Agency's Seibersdorf Laboratory in support of the Joint FAO/IAEA programme for the specific purpose of developing cheap, simple, and above all, reliable methods which would enable scientists in developing Member States to conduct radio- and enzymeimmunoassays. Now, any Agency and FAO field project which requires one can have a fully operational radioimmunoassay laboratory for under US \$2000 and a kit for measuring hormones which has in-built quality control and which works even if it lies around a customs shed for three weeks at 35°C! To my mind, this is appropriate equipment and hopefully it will not be long before we can develop similar kits for disease diagnostics.

To conclude, ladies and gentlemen, I sincerely hope that you enjoy this symposium. There are a great many papers and posters to get through over the next five days, so clearly you will be kept busy. However, not too busy, I hope, to have time for informal exchanges of experience and ideas, or to see something of Vienna and what it has to offer.

KEYNOTE ADDRESS

LIVESTOCK PRODUCTION IN DEVELOPING COUNTRIES *Problems and policies*

H.A. Jasiorowski

Animal Production and Health Division,
Food and Agriculture Organization of the United Nations,
Rome

1. INTRODUCTION

The purpose of this keynote address, as I understand it, is to present some of the basic problems of livestock production in developing countries and some policy alternatives which in our opinion offer possible solutions.

Livestock are kept in both developing and developed countries for the same reasons: to provide high quality food and fibre of animal origin, to create income for rural producers and to contribute foreign exchange through exports or import substitution.

While the development of livestock production in developed countries is never questioned and trends to consume more and more animal protein seem natural, efforts to develop animal production in poor countries are sometimes questioned.

The arguments behind this are that animal production is expensive. First, three to ten times the amount of protein or energy of plant origin is needed to produce protein or energy of animal origin. Second, it is argued by some that animal protein is not essential for human growth and survival and that it can be replaced by a cheaper combination of plant proteins. Why, therefore, it is asked, do we not encourage the people in poor countries to satisfy themselves with grain, roots, pulses and fruits?

I am sure that to us such statements sound heretical and absurd, but they are nevertheless sometimes raised during discussions on priorities for aid and donations, and they are theoretically correct — but only theoretically. The reality of the situation is quite different. In the first place, the wish to consume more food of animal origin is common to all of humanity and no power in the world can change this tendency for improved quality of life by the majority of the human population. Second, a great proportion of the land and vegetation of the developing world cannot be used except by ruminants.

These two facts alone should be sufficient to convince those who oppose the development of animal production in poor countries to change their minds. In fact, the importance of livestock to many developing countries cannot be overemphasized, since for many nations it is an essential pivot to human existence. In vast areas of

Africa and parts of Asia with nomadic and transhumant systems there would be no human existence based on natural vegetation without the use of livestock. For semi-sedentary and sedentary systems livestock provide the necessary power to cultivate sufficient areas of land for subsistence-type farming. However, I believe that we, the representatives of the livestock science and production profession, would make a great mistake if we rejected completely the arguments that food of animal origin may be too expensive for poor people. It may be too expensive if we are not careful in advising them to use the appropriate, correct systems of animal production. In fact, promotion of capital- and grain-intensive systems of animal production has already proved in many cases to be too great an economic burden for developing countries.

I offer these views at the beginning of my presentation because I consider them fundamental to the policy for livestock development in developing countries. We should, and even must, encourage livestock production in these areas, but the methods chosen should be based on local resources, on using mainly feeds unsuitable for human nutrition, and they should be labour intensive, with low capital inputs, and should not be dependent on imports.

In other words, the cheapest methods of production should be selected and introduced at the outset. This would seem obvious, but perhaps it is not so to all. Too many mistakes have already been made which justify, I think, my raising this problem here.

2. TRENDS IN CONSUMPTION OF ANIMAL PRODUCTS

Before I come to current problems and policy issues in animal production in developing countries, let me first consider here world trends in the consumption of food of animal origin over the last 20 years.

The total per caput meat consumption in different parts of the world differs greatly. During the last 15 years, meat consumption in developed countries increased rapidly and by 1985 was five times higher than the average of developing countries. What is more important is that the gap between meat consumption in developed and developing countries has increased. In fact, in Africa meat consumption per caput even decreased. If we look deeper into meat consumption patterns in the developing countries we may observe that the real increases in consumption were only for poultry and pork. The consumption of beef and small ruminant meat decreased in Africa, or remained unchanged, as in Latin America (Tables I-IV).

In addition, the increase in poultry and pork consumption in developing countries was due mainly to importations and the introduction of grain- and capital-intensive, so-called industrial methods of production which are often based on imported inputs.

In considering milk consumption trends in developing countries, which is the cheapest source of animal protein, the picture does not change (Table V). The per capita consumption of milk, which is already very low in developing countries,

TABLE I. PER CAPUT CONSUMPTION OF BEEF/VEAL (kg)

	1970	1985	1990
Total developed	26.2	27.1	26.8
Africa	5.9	5.2	5.0
Latin America	20.8	19.9	19.0
Near East	5.2	7.3	7.4
Asia and Far East	0.9	0.9	1.0
Other developing	8.9	10.4	10.7
Total developing	3.8	4.0	4.0

Source: FAO Commodities Statistics.

TABLE II. PER CAPUT CONSUMPTION OF MUTTON AND GOAT MEAT (kg)

	1970	1985	1990
Total developed	3.5	2.8	2.8
Africa	2.7	2.3	2.2
Latin America	1.6	1.0	0.9
Near East	6.5	7.4	7.7
Asia and Far East	0.5	0.7	0.7
Other developing	1.1	3.0	3.1
Total developing	1.2	1.3	1.4

Source: FAO Commodities Statistics.

TABLE III. PER CAPUT CONSUMPTION OF PIG MEAT (kg)

	1970	1985	1990
Total developed	24.1	29.6	29.8
Africa	0.7	0.8	0.8
Latin America	6.1	7.0	7.0
Near East	0.1	0.1	0.1
Asia and Far East	4.0	7.2	7.7
Other developing	7.2	7.8	8.2
Total developing	5.5	5.6	5.8

Source: FAO Commodities Statistics.

TABLE IV. PER CAPUT CONSUMPTION OF POULTRY MEAT (kg)

	1970	1985	1990
Total developed	10.4	16.6	17.3
Africa	1.4	2.2	2.2
Latin America	4.4	8.6	9.0
Near East	2.2	6.7	7.0
Asia and Far East	0.7	1.3	1.4
Other developing	1.9	3.8	3.9
Total developing	1.3	2.5	2.7

Source: FAO Commodities Statistics.

TABLE V. PER CAPUT CONSUMPTION OF MILK (kg)

	1970	1985	1990
Total developed	193	193	196
Africa	24	26	26
Latin America	82	88	90
Near East	59	80	78
Asia and Far East	17	23	23
Other developing	26	28	29
Total developing	28	36	36

Source: FAO Commodities Statistics.

dropped in Africa and remained practically unchanged elsewhere, with the exception of the Near East. I am sure you will agree with me that the outlook does not appear to be promising.

3. BASIC ANIMAL PRODUCTION PATTERNS IN DEVELOPING COUNTRIES

It is interesting to examine the main factors which have influenced the consumption of animal products in developing countries. Because imports can only play a minor role here, the main factor is production. (For simplicity I will omit the problem of demand and purchasing power which varies greatly between different groups of society in developing countries.)

Since average population growth in developing countries during the last two decades has been rapid, it is obvious that even maintaining the previous level of consumption can only be possible if the total production of animal products continues to increase. This has actually happened (Table VI). However, the important fact is that the increase of animal production during the last 20 years has been mainly due to the increase in the number of livestock (Table VII), while productivity per head remained low and in some regions even decreased (Table VIII).

This is, in my opinion, an alarming fact, especially when we consider that it will probably be very difficult to reduce population growth to any extent in the near future. Can we continue in the future to increase livestock numbers in developing

TABLE VI. ANIMAL PRODUCTS PRODUCTION IN DEVELOPING COUNTRIES (million tonnes)

	1970	1985	1990
Cow's milk	51.7	85.4	98.5
Beef and veal	11.2	14.5	15.4
Pig meat	9.3	19.5	22.8
Mutton and goat meat	3.2	4.4	5.0
Poultry meat	3.3	8.7	10.3
Eggs	6.5	10.5	11.0

Source: FAO Commodities Statistics.

TABLE VII. NUMBER OF ANIMALS IN DEVELOPING COUNTRIES (million head)

	1970	1985	1990
Cattle	808	977	1014
Cows	85	122	135
Sheep and goats	896	1114	1242
Pigs	285	494	542
Poultry	2423	4260	4960

Source: FAO Commodities Statistics.

countries parallel to the human population growth? The answer is certainly no. The only solution is to increase individual animal productivity in developing countries. It is easy to reach this conclusion, but much more difficult to achieve such a goal.

This is an urgent matter. In practice, it is found that in developing countries lack of progress in production per caput causes them to turn to imports of animal products (Table IX). Once a new consumption habit is started it is difficult to turn it back. However, the large external debts of the developing countries are likely to stop this trend.

TABLE VIII. PRODUCTIVITY OF ANIMALS IN DEVELOPING COUNTRIES
(kg/head)

	1970	1985	1990
Cow's milk	608	700	730
Beef and veal	14	15	15
Pig meat	32.6	39.5	42.1
Mutton and goat meat	3.6	4.0	4.0

Source: FAO Commodities Statistics.

TABLE IX. NET IMPORTS BY DEVELOPING COUNTRIES
(thousand tonnes)

	1970	1985	1990
Milk equivalent	7539	21 352	21 201
Beef and veal	(1166)	225	722
Pig meat	(62)	548	93
Mutton and goat meat	(4)	402	604
Poultry meat	81	419	511

() = net export.

Source: FAO Commodities Statistics.

4. MAIN CONSTRAINTS TO IMPROVING ANIMAL PRODUCTION IN DEVELOPING COUNTRIES

The major role that livestock play in human nutrition is the reason for all the efforts in the last two decades to improve their productivity in developed countries.

The results are impressive and well known. They were achieved through improved methods of selection, wide exchange of genetic material (importation of frozen semen) and intensification of feeding, especially with large amounts of grain and concentrates.

The great progress made in industrialized countries is fascinating to breeders and producers as well as to economists. Many less developed countries are attempting to follow the developed countries' methods of intensifying livestock production. For example, semen of the same best bulls can now be found in the United States of America and Europe as well as in Africa and Asia. It often happens that similar patterns of selection methods and technologies are introduced on a large scale all around the world, often without taking into account the stage of development, resources and climate of a given country.

The major constraints, grouped into three main categories — ecological, biological and socio-economic — which influence, or even determine, livestock production in different parts of the world, are too often forgotten.

Ecological constraints are mainly land and climate. It is obvious that such factors as land topography, soil fertility, rainfall, temperature and plant growing season have a direct effect on livestock production, its management and utilization.

Biological constraints such as feed and water supply are of primary importance for livestock production. Animal diseases are another constraint (of a biological nature) which may strongly influence livestock production.

Livestock genotype used to be a severe constraint on production systems but now, with the possibility of semen shipments and cross-breeding, it plays, perhaps, a more limited role, provided other biological factors allow improved animals to survive and produce.

Socio-economic constraints are rather complex. We may include here such factors as the supply-demand relationship, input-output ratios, prices, as well as cultural, religious and political aspects.

5. LIVESTOCK PRODUCTION SYSTEMS

The above-mentioned constraints influence to a great extent livestock production systems in the world. Taking into consideration all the ecological, biological and socio-economic constraints, countless different livestock production systems can be distinguished. For practical reasons let us group them into two main categories: migratory and sedentary systems.

5.1. Migratory systems

5.1.1. *Nomadic system*

This is probably the oldest style of animal utilization. It consists of constant migration of whole tribes and families together with their herds, composed usually of cattle, goats, sheep and camels. The direction of the migrations is dictated by seasonal changes of grass growth and accessibility to water.

Nomads use semi-arid areas, which in principle are not suited for settlement, often uninhabited for most of the year, and which would otherwise be left unused.

The prospects of increasing nomadic herd output are rather poor because of lack of possibilities in grazing improvement through ways other than gradual destocking. No genetic improvement of productivity in such a situation is possible.

5.1.2. Seasonal herd migration system

This method of livestock utilization makes use of sometimes distant pastures during rainy seasons and of fodder and post-harvest residues in dry periods. It can be found not only in Africa and Asia but also in some regions of southern Europe, the Arctic and the Asian republics of the USSR and Mongolia.

In some regions this system of pastoral migration of livestock plays a very important role. In Africa, nomadism and transhumance use 35% of grazing land [1]. In both systems, practically no controlled cross-breeding with temperate breeds is practised.

5.1.3. Shifting agriculture

This system falls between the nomadic and settled forms of livestock production. It is practised in regions with enough rain, a low population density and with plenty of 'no man's land'. Regions which were once cultivated and then abandoned offer good opportunities for livestock grazing. In some regions of West Africa this system is used for the breeding of N'Dama and the North African Shorthorn cattle, which are resistant to trypanosomiasis.

5.2. Sedentary systems

Increasing population density, the shrinking of available land resources and the emergence of markets are the main reasons for settlement.

The existing sedentary systems of livestock production in the world differ widely from one another, depending on soil and climatic conditions and the level of development of individual countries.

5.2.1. Subsistence production

This system is quite frequent in the tropics in regions provided with enough rainfall or with irrigation. Under this system livestock are fed chiefly waste fodder and what remains in the fields after harvesting. Pigs and poultry run around taking care of themselves.

Subsistence farmers use cattle and buffaloes extensively for draught purposes. Some cross-breeding is also practised.

5.2.2. Livestock production under permanent plantations

This system plays a certain role in tropical regions, mainly for beef production. Waste products have considerable importance. Sugar-cane plantations, for instance, provide cattle feed in the form of sugar-cane tops, molasses and fibrous residues after the sugar extraction process. Pineapple plantations, cleared of fruit, also provide feed for cattle, as do plantations of agave. Recently, in the tropics, more attention has been paid to the utilization for feed of such by-products and wastes. Large feedlots are organized in the vicinity of fruit-canning factories using pineapple, citrus and other by-products.

Integration of cattle and buffalo production with the possibility of grazing under coconut, oil palm and rubber trees is gaining popularity.

5.2.3. Ranching

This is the commercial form of pastoralism adapted to urbanized conditions. Popular in regions with poor soil and little rain, this extensive system is used chiefly for beef production. This system of production can be found in classical farms in North and Latin America and in Australia. Attempts to organize ranches in Africa have mostly failed because of the relatively high investment needed.

5.2.4. Livestock production based on crop rotation

In this system cattle are fed on fodder grown in the fields or they are allowed to graze on pastures in the summer. This system is predominant in temperate climates, but also appears sporadically in the tropics.

Owing to high labour costs and the scarcity of land, livestock production in the developed world is becoming more and more capital intensive.

5.2.5. Large-scale industrial livestock units

This very capital-intensive system can be considered the latest stage in the evolution of livestock production systems in the world. In their most developed form such as in large piggeries, chicken and dairy units, the operations are independent of local land for fodder and grain farming. These systems are usually capital- and grain-feeding intensive. They predominate in highly developed countries but they can also be seen sometimes — I would say too frequently — in developing countries.

The purpose of this short presentation of livestock production systems is to illustrate the great variation in situations with which we have to deal. It is obvious that our policy for livestock development in developing countries should be based on the different production systems that have existed for centuries.

6. FEED RESOURCES AND FEEDING

There is not the slightest doubt that in addition to endemic disease, inadequate feeding (and even occasional starvation) of livestock in developing countries is the main reason for their low productivity under practically all production systems, and it is a most difficult problem to resolve.

A very large part of the ruminants in developing countries live on rangeland in arid and semi-arid areas which are characterized by abundant, palatable vegetation in the rainy season and very poor feed resources during the dry season. In addition, occasional prolonged droughts cause starvation and even the death of animals. This situation predominates under nomadic and transhumant systems as well as under the shifting agriculture and ranching systems.

Improvements in the quality and management of waste rangelands represent a great potential and are the subject of a large part of FAO's efforts; however, they call for large investment, which in practice is very difficult to achieve. We know from experience that high investments and the creation of certain types of ranches on rangelands, for example in Africa, have largely failed due to the great increase in the costs of production.

However, there are several other options which can lead to the improvement of rangelands.

Under nomadic and transhumant systems one method is to avoid overgrazing by destocking. This may not appear to be an easy solution because there may be no alternative support for the people living there. A better solution would be to remove the threat of the tsetse fly in large areas in Africa which are under-utilized by livestock because of trypanosomiasis. This would allow the herds to be moved from overgrazed areas. Seven to nine million square kilometres of the best land in Africa are not available to livestock and are thus under-utilized because of the presence of tsetse, justifying a large FAO programme in this field. It calls, however, for large investment, which until now has been largely lacking.

Another possibility would be to provide watering points in areas that are undergrazed. Long-lasting effects would result in improvements in vegetation; introduction of leguminous plants can be in the form of annuals and small bushes. Introduction of hardy, drought-resistant, nitrogen-capturing plants offers possibilities both in rangeland areas and as a substitute for fallow land in areas of shifting agriculture.

One of the most important problems of rangeland utilization is the dry season. Theoretically, part of the rangeland could be cut during the good quality stage and be preserved as hay or silage. This, however, is not practised and has little chance of being introduced on a larger scale. Feed supplementation for livestock during this season with non-protein nitrogen, possibly in the form of urea-molasses blocks, is a more realistic approach. This, however, can be introduced primarily in emergency situations. To fight drought starvation of livestock in the Sahelian region, FAO has launched a programme of feed security in which we work with the governments to improve feed resources and to adjust livestock numbers accordingly.

However, we must be prepared to accept continuation in the future of the present common practice of leaving ungrazed or partly grazed rangeland to mature and be consumed in the dry season as very poor quality, low nitrogen containing materials which, even if available *ad libitum*, cause animals to lose weight.

Therefore, we should look for natural possibilities for the better utilization of rangeland resources. We know, for example, that excess rangeland production is preserved as fat accumulated in the tail or hump of certain species for utilization during the dry season. Ørskov has suggested that we should help to select animals with a large capacity to eat and a high propensity for accumulation of fat outside the body cavity. Accumulation of fat in the abdominal cavity is likely to restrict roughage intake.

Assuming a 20% loss of material in hay and silage making, this strategy seems to be biologically the most efficient. If pursued, it would need long-term breeding strategies for animals which have the best ability to perform under such conditions just to strengthen the process, which has in any case taken place through natural selection. No doubt we will devote a great deal of thought to this theory and if justifiable, we will try to implement it in practice.

I will not elaborate here on the obvious necessity to increase fodder production whenever possible under subsistence farming and in crop rotation systems, since this is the concern of the Crop and Grasslands Service in the Plant Production and Protection Division. Under tropical and subtropical conditions with sufficient rain, the possibilities are great. However, on the other hand, it will only pay if the productivity of the animals is high enough.

In the Animal Production and Health Division we are now concentrating our efforts on the utilization of a wide range of crop by-products for feeding animals.

Straw and stovers, the by-products from cereals, are by far the greatest and most under-utilized resource. The extent and the reasons for this vary according to a range of factors. In some areas the nomadic herds cannot reach the straw left in cultivated areas because of lack of watering points. In other areas farmers are simply led to believe that it is not good for cattle and should be used only as a last resort. In other countries most of the maize stovers are left to be grazed *in situ* and much is wasted.

The FAO effort is directed towards increasing straw and stover utilization as they comprise the largest amount of under-utilized resources. We have now launched a broad programme on straw treatment using different methods for increasing its feeding value.

Sugar-cane and sugar-beet tops, bagasse, molasses, citrus and pineapple industrial by-products — even poultry manure — are all examples with a great potential for feed resources. It is useful to mention in this context FAO work on the use of sugar-cane as a feed and improvement of utilization of molasses (urea-molasses blocks).

Many developing countries produce large amounts of high protein by-products from the plant processing industry. These highly nutritious products are usually

exported to developed countries and are seldom used for feeding local animals. This cannot be criticized as long as it is more economic to export than to feed local low-producing livestock.

In general, I wish to stress that the improvement of rangeland utilization, use of high fibre, low quality roughages and utilization of by-products are our highest priorities in work on feed resources in developing countries.

We hope that new horizons will be opened in this field by research involving rumen microflora manipulation, such as, for example, the elimination of protozoa, by increasing the proportion of cellulolytic bacteria therein and even, in the future, by introducing genetically engineered microorganisms which would be able to degrade lignin.

7. GENETIC IMPROVEMENT

The livestock population in developing countries is usually characterized by low productivity, but at the same time it is highly adapted to local, frequently harsh, conditions. To improve the productivity of these animals it is necessary, in addition to improving nutrition, to increase their genetic potential. This is obvious, but in our day to day work we are faced with the question of how to do it.

I am sure we all realize that this is an old and difficult question to which there is no simple answer. In fact, different answers have been given over the years and have varied from making maximum use of the indigenous breeds through selection and improvement to replacing them totally by temperate breeds — or synthesizing a new breed by different methods of cross-breeding of local and exotic breeds.

In fact, the answer will depend on several important conditions, mainly of an environmental and managerial nature.

In general, we may say that the most radical method of replacing local breeds with an exotic one usually takes place through intensification of poultry and pig production rather than improvement of dairy production, which is usually based on cross-breeding with exotic breeds. Beef producing cattle, sheep, goats and buffalo are less frequently the subject of cross-breeding.

Unfortunately, selection of the best method for genetic improvement of local breeds cannot only be based on economic grounds. Biological factors such as adaptability to local conditions must also play an important role.

The contrast in potential production between high producing temperate breeds and low producing tropical breeds of livestock appears most attractive at first sight and the first reaction of the authorities responsible for livestock development is often to import. The matter is, however, not a simple one, as the following points seek to illustrate.

Total replacement of indigenous by temperate breeds can be, and has been, accomplished successfully with a complete change and control of environment and management. This can best be illustrated with poultry and pigs. However, for dairy

production such a system is viable only in large-scale commercial units where the increased capital costs can be spread over many cows. It is clearly not possible to apply this solution to the majority of family or village situations with the scarce feeding resources that exist in most developing countries.

The exceptional situation prevailing in the Gulf States in recent years, where oil income permitted the import of turnkey packages of dairy cows (pure-bred Black and White) with controlled environment and skilled management of temperate systems, allowed total substitution of local breeds. Clearly this demands either the improved local production of grain or alternatively the import of grain in addition to the improved production of succulent fodder crops by irrigation and adequate fertilizer application. This production of feed at appropriate levels is also in itself a capital-intensive enterprise. The improved environment requires not only the careful control of ambient temperatures and humidity but also that of endemic diseases.

7.1. How should the main options be implemented?

In advising developing countries on which method of livestock improvement to choose our main tool is the special scheme organized to help countries establish sound breeding plans. Examples related to dairy cattle are given.

Sizeable, economically sound, large-scale, commercial dairy farms based on exotic breeds have been encouraged by FAO, but only when they are compatible with national economic conditions and possess an organized processing system, as well as a market in addition to proper environmental and disease control and management. These enterprises may have a particular impact when placed near the growing urban areas of developing countries. Under these specific conditions the 100% temperate dairy cow can be successful as it has been not only in the tropical highlands of Kenya, Sri Lanka and Indonesia but also in India and close to large cities in China.

However, in FAO we distinguish very carefully between this rather specific situation and the quite different options open to small farmers using the shifting agriculture or subsistence systems that exist in most developing countries. Here, it is essential to help breed a cow with the percentage of temperate genes appropriate to their socio-economic possibilities. This is often achieved by use of an exotic or cross-bred bull, either through artificial insemination or in natural service.

In FAO's experience it has been found desirable to modify appropriately the genotype of animals by introducing as high a proportion of temperate genes as possible under the actual circumstances so that the ability of the cross-bred cow to withstand the unfavourable environment still remains. Generally, under harsh conditions — with endemic diseases still uncontrolled — it is prudent to keep the temperate genes at a level no higher than 50 to 60%. Higher levels bring the risks of increased calf mortality, later age for first calving, poorer conception rate, increased calving interval, limited milk production and curtailed life expectancy.

With increasing levels of environmental modification and management improvement the percentage of temperate genes can be increased up to the 100% tem-

perate animal. However, marked skill is required to introduce temperate genes successfully. There are many examples of disappointed family and village cattle owners who were unable to introduce sufficient changes in feeding management or to control disease. The result was that the expected increases in production were lost, since genotype and environment in successive generations were not properly matched.

Therefore, we should avoid introduction of exotic genes to an environment which cannot be improved and where management levels will remain low. However, on the other hand, we must accept that in a breeding policy some flexibility is necessary. The best example for this can be seen in some of the Caribbean countries. The Dominican Republic, for example, established a number of years ago a relatively high producing dairy population through the importation of Holstein-Friesians from the USA and Canada. At the time, the economy of the country and the ratio of the peso to the dollar allowed grain and feed importation. Now the situation has changed drastically and the response of the dairy farmers has been to put Criollo and Zebu bulls to pure dairy Holstein-Friesian herds to obtain lower producing but stronger cows which can be satisfied with the local pastures. We cannot condemn such actions and should not even consider them as a failure. Our policy should be to help farmers to adjust the type of livestock and system of production to changing conditions.

In contrast, beef production in the tropics differs in so many aspects from those dealing with dairy cattle and we usually recommend improvement within the indigenous breeds. This approach is particularly necessary in Africa within nomadic transhumant and self-subsistence production. However, when more intensive beef production systems are possible as on ranches, for example, in some parts of Latin America or the Far East, it is appropriate to consider a beef industry based on temperate beef breeds or synthetic breeds adapted to the tropical environment as, for example, the Santa Gertrudis.

The buffalo has so far been neglected in large cross-breeding programmes but (in co-operation with FAO) in some developing countries consideration has already been given to its genetic improvement. A large-scale project involving FAO, the United Nations Development Programme and the Government of the Philippines is now showing substantial benefits from crossing the indigenous buffalo of the Philippines with the Nili-Ravi of Pakistan and the Murrah of India. Both these imported breeds are higher milk producers and larger animals. The crosses between these and the indigenous breeds have not only shown higher yields but have also been found better for work. So far partial infertility, which could arise as a result of the different chromosome complements, has not occurred. Artificial insemination is being used in the cross-breeding programme and the problems of oestrus identification are being overcome by the use of controlled oestrus.

I have already said much about cross-breeding and breed improvement, but I cannot omit at this point our programme on genetic material conservation. The purpose of this programme is to save endangered breeds and species and it is carried out in close co-operation with the United Nations Environment Programme. We plan to organize special databanks on native breeds in each region. For native breeds

representing a genetic potential for improvement, the breeding plans will be elaborated, including individual performance recording and progeny testing.

7.2. Role of new methods involving biotechnology

The possibilities offered by the new techniques of molecular engineering (molecular biology/genetic engineering) for animal production in developing countries need to be reviewed briefly in concluding this section on animal breeding and genetics.

We are all aware of the rapid strides and the great potential in this area in developed countries and we are faced with many requests from developing countries to help them make use of these new technologies. We reject the theory that developing countries should wait to introduce them until they reach the necessary stage of development. We feel that many of the new technologies, especially those which do not need expensive equipment, can and should be introduced in developing countries if only for teaching and research purposes. This is the reason for calling, later this year, an FAO expert consultation on biotechnology applied to animal production and health which could be introduced to developing countries.

One of the most interesting technologies for animal breeding and genetics is the possible use of restriction fragment length polymorphisms (RFLP) for the improvement of quantitative trait loci (QTL). The pioneering work of Soller and Beckmann [2] in this area carried out with domestic animals offers the promise of mapping, for the first time, part of the genome for economically important traits. Such work has already been successful with *Drosophila* and wheat. No doubt it will be applied shortly to dairy cattle in developed countries as a means of identifying genetic markers for the quantitative traits of milk production. Theoretically, it even offers the possibility of replacing traditional methods of progeny testing if the QTL for milk are found to be more easily manipulated and selected directly than in the traditional and indirect progeny testing method.

At FAO we feel that efforts should be made to apply these techniques in the case of important traits in the developing world. We are therefore examining the possibilities of using RFLP for mapping the genome of trypanotolerant cattle in Africa, together with the newly established International Trypanotolerant Centre in The Gambia. This centre has access to a large number of animals for the several generations of cross-breeding and back-crossing that are needed to quantify this trait in the genome. This may appear to be a very bold proposal, but trypanosomiasis is a problem of tragic proportions in animal production in so many parts of Africa that resistance to it should surely be one of the first topics to be explored using new methods in animal breeding.

8. ROLE OF ARTIFICIAL INSEMINATION IN DEVELOPING COUNTRIES

If we take the well known and impressive increase in livestock production in industrialized countries over the last three or four decades, we see clearly that artifi-

cial insemination (AI) has made a considerable contribution to these achievements. It is only natural that developing countries put much hope in using this tool for improving their livestock.

Some of the first attempts at AI on a more regular basis in developing countries can be traced back to the early 1950s or even earlier. At that time, in most cases these attempts frequently copied procedures in developed countries, regardless of the requirements of the local situation. Almost all international and bilateral agencies supporting livestock development programmes in developing countries have implemented AI breeding programmes and built special stations for this purpose. However, the available data indicate that in only a few developing countries did the number of cows served by AI exceed 1 % of the cattle population. Our own observations and the information we have received from the countries concerned support these data. Thus, we have no alternative but to agree that in spite of great efforts and a great deal of investment AI, and its impact on livestock development in these countries, is far from satisfactory.

There are probably five major reasons for the disproportionately high input compared with the results.

First, it should be recognized that the AI technique as applied and developed in industrialized countries should not necessarily be that applied to developing countries.

Second, AI services will only develop and find increased demand from farmers if there is an incentive for them to use it. AI cannot be implemented successfully without providing additional services. An AI programme should thus always be an essential part of an integrated livestock development programme and should never be implemented on its own.

Third, overcapitalization. When visiting developing countries, I noticed that there were several AI centres housed in very large new buildings with extremely well equipped laboratories, bull pens to accommodate large numbers of bulls and an impressive number of staff. Looking at the available statistics on performance it was clear that only a relatively small number of semen doses were being produced and, as a result, only a few cattle were being inseminated. We therefore need to ask whether this assistance would not have been more effective if several small centres had been planned and effectively organized for siting in the middle of the areas with milksheds — but with less sophisticated equipment.

Fourth, instead of establishing semen production units would it not have been better, in many cases, to rely only on imported semen either purchased or donated? Large quantities of bull semen from sires with a high genetic value are available and are often not utilized in developed countries employing advanced AI breeding programmes. This semen is now available to developing countries through the FAO semen donation scheme, and a large FAO semen bank will be established close to Rome for this purpose. Should we not advise most of the developing countries to use such semen for the best local cows to produce F_1 bulls which could then be used on

a large scale through natural service? Such a scheme could eventually then be developed to provide '¾ blood' bulls for use.

We should learn our lesson from past failures, which should by no means imply the abandoning of AI systems in developing countries. We should adapt our plans to the existing realities. Even more, dairy cattle should by no means be the only species for which AI is used. Much can be done by using AI for the improvement of goats, sheep, pigs, buffaloes — and even poultry and bees. Our policy and our action in the field should be both bold and clear in character, as well as more realistic.

8.1. New techniques in livestock reproduction manipulation

As you are aware, the embryo transfer technique is gaining considerable popularity in modern cattle breeding programmes in industrialized countries. It is expected that in 1986 there will be 100 000 pregnancies in the USA and some 30 000 in Europe based on embryo transfer. Problems related to non-surgical embryo recovery and transfer as well as the deep-freezing of zygotes have now been overcome and the technique has become a routine one. Even embryo splitting and the production of identical twins are now becoming practically a routine procedure in commercial embryo transfer programmes. Other very interesting areas under current development are those of micromanipulation of the ova and the embryo; sexing, in vitro fertilization, gene infusion and the production of chimeras are being actively studied by various research groups and we may expect interesting results with a potential for application in the very near future.

We must clearly examine the potential of the embryo transfer technique for the production and improvement of livestock in developing countries. The drive to implement embryo transfer programmes is not confined to industrialized countries; a number of developing countries are seeking support in their venture to initiate these programmes with the use of deep-freezing and micromanipulation of zygotes.

The pressure for new technology from developing countries exists; FAO cannot adopt a negative attitude and advise a blanket delay.

In the present circumstances we would tend to advise use of embryo transfer and embryo manipulation technology in developing countries in the following cases for:

- (1) Education and demonstration purposes
- (2) Research and development; one good example could be embryo transfer from the N'Dama in The Gambia to Zebu in Kenya for studying the influence of trypanosomiasis challenge on the level of resistance
- (3) Transfer of genetic material in the form of embryos, instead of live animals
- (4) Conservation of endangered breeds in the form of deep-frozen embryos.

9. ROLE OF LIVESTOCK IN RURAL DEVELOPMENT

Since helping the poorest farmers and contributing to rural development are among FAO's first priorities, our strategy in the Animal Production and Health Division must be directed towards this same goal.

9.1. Dairy cow

We think that one of the ways of achieving this is by support given to milk production and dairy development. There are many reasons for such a policy. The production of milk from ruminants is far more efficient biologically than the production of meat.

The dairy cow has been and remains a powerful instrument of social change in raising a rural economy from a low level.

Milk production can increase by a factor of ten under excellent conditions (400 to 4000 L of milk per lactation), which is much higher than production increases in any other domestic animal. Added to this is the possibility of improving the reproduction rate from the commonly experienced calf every 2 to 3 years in unimproved conditions to a calf (and lactation) every 15 months under top management.

I have mentioned dairy cows, but milking buffaloes and goats should also be included.

FAO is traditionally very much involved in dairy development in developing countries. For this purpose the International Scheme for the Co-ordination of Dairy Development has been established. Many dairy development projects, including the famous one in Anand, India, profited from these activities. The scheme deals with all dairy aspects from production to consumption, including marketing and economics.

When advising countries on dairy development we encourage them to take all the necessary steps not to allow imported or donated milk products to compete unfairly with local production.

9.2. Small ruminants

Development of small ruminant production is another area of activity related closely to rural development.

There are very good reasons for this interest in sheep and goat production. Most notable is their comparative biological efficiency in meat, milk and fibre production, their inherent suitability to small-scale production — a characteristic of the vast majority of farms in developing countries — their adaptive capacity to produce well on low cost feeds and finally their comparatively low capital demand.

Much of the technical information necessary for the development of sheep and goat production is already known and established. However, there is a real need to

select and adapt these technologies in developing countries and to ensure they have an impact; there is also a need for further research.

An example we can mention here is the problem of the economic impact of flock replacement rate, selective grazing behaviour and ability to ingest and utilize highly fibrous feeds, especially by goats. We need to study and better understand the biochemical and physiological basis of appetite, digestion and rumen metabolism.

This is why FAO is currently initiating a worldwide structure of linkage networks throughout the developing countries. Networks are being established in Latin America and the Caribbean, in Asia and the Far East and in Africa. In addition to the networks already established by FAO, namely the European Network and the Near East and North African Network, these will complete a global network structure on small ruminants.

9.3. Poultry

The attraction of poultry as farm livestock is clear. As we all know, the domestic fowl is one of the most widespread forms of livestock on a worldwide basis. Its ability to adapt to a wide range of different climates (from cold to hot, humid and arid) enables them to maintain suitably high growth rates and egg production levels even under relatively difficult conditions. The general acceptance on the market of poultry meat and eggs, their nutritive value and the relative ease of production frequently leads them to play a useful role in nutrition at the family and village level.

Unfortunately, much controversy surrounds the problem of poultry production in developing countries.

Certain reservations have been shown by donors and international organizations when it comes to including poultry in their livestock development policies. This is based on the common assumption that poultry competes with man for food grains. The earlier failures of large capital-intensive, industrial-style poultry production in some developing countries, where virtually everything from day-old chicks to feed, equipment and spare parts had to be imported, left a slightly negative image.

The alternative is, of course, backyard poultry keeping which, however, has low productivity but hardly needs increased feed inputs. It is true that in this case marketing is a serious problem, but this can be overcome by special efforts such as, for example, co-operative organizations.

In helping to organize the supply of genetic material, supplement feeds and the infrastructure needed for backyard poultry production, we foresee a major role for FAO.

Ladies and gentlemen, it was not my intention to expose all the problems and policy issues related to livestock production in developing countries. Even less so was it my intention to list here all our activities in this field, but I think I cannot omit the serious problems of animal diseases which hamper so much livestock development in developing countries. Let me mention only the large FAO programmes on tsetse and trypanosomiasis control, the rinderpest campaign in Africa, Asia and the Near

East, our activities in fighting ticks and tick-borne diseases, and our efforts to control foot and mouth disease.

I have given you our views on some of the problems and policy issues which we face in our work in the field of livestock development. I hope that your meeting will help our Animal Production and Health Division in Rome and our sister division here in Vienna to cope better with the task we are facing.

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**ADAPTION OF ANIMALS
TO THE ENVIRONMENT**
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Chairmen

H.A. JASIOROWSKI
FAO

J.V. WILKINS
United Kingdom

PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF CATTLE IN THE TROPICS*

J.V. WILKINS

Overseas Development Administration/
British Tropical Agricultural Mission,
Santa Cruz, Bolivia

Abstract

PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF CATTLE IN THE TROPICS.

The interactions of management system, environment and genotype are discussed and the importance of the selection of the optimum system and the appropriate genotype for the economic and environmental climate is emphasized. Productivity is indicated as a function of yield, fertility and survival rate; realistic estimates of all three parameters must be considered when planning livestock development. Intensive, semi-intensive and extensive milk producing systems are briefly discussed in this context. It is suggested that research workers should have a firm understanding of the most valid systems in their areas so that the solution of problems limiting production can be given research priority. It is finally emphasized that the estimates of yield, fertility and survival rate are not constants and will change with increases in the knowledge of animal health, nutrition and forage plant breeding, while the most appropriate system will also change with this new knowledge and with changes in costs of production and market price.

Cattle performance in the tropics is influenced by the climate, the production system and the genotype of the animals. In arid areas of the tropics the only alternative to nomadism with small ruminants and camels is irrigation and the necessarily intensive management system needed for such high investment. However, in the more humid areas a great number of viable management systems exist and it is emphasized that a good system in the tropics may be very different from those currently practised in temperate zones. A good cattle management system is here defined as any sustainable system that produces a livestock product at a cost that is sufficiently below the market price to justify the investment necessary for the establishment of the system and its subsequent maintenance. It may be noted that many traditional systems using indigenous cattle meet the criteria of this definition, while many so-called improved systems that are introduced into the same areas do not.

The management system may modify the effect of the climate on the herd by the provision of shade or by cooling, while management directly influences the nutrition and health of the herd. Thus, the product of the climate and management system is the environment of the herd and the interaction of the herd environment and herd genotype determines the productive and reproductive performance of the herd.

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The management system and genotype of animal appropriate to that system should be chosen after determining the probable market prices of milk or meat, calculating the costs of production and estimating cattle productivity within that system. This may appear to be obvious, but the existence of very many economically disastrous livestock development projects in the tropics would suggest that these preliminary calculations contained some grave error. The market price of milk and meat can certainly fluctuate, but those failed projects that this writer has studied erred most greatly in the estimation of productivity. Production parameters such as lactation milk yield were often overestimated, but greater differences were seen for the estimated economic productivity where production parameters were adjusted for the fertility and survival rate. At first this may appear to be surprising, but it is in fact very rare for one published paper to contain all the required information for the calculation of herd productivity in a specific production system, and indeed many papers, while being truthful in what they say, may be erroneous in their implication. Authors will not be cited to demonstrate this, but a study of growth of ranch-bred cattle in feedlot may well show a breed or cross to be very superior when yarded with no suggestion that on the range it has low fertility and high calf mortality. All three parameters are important and must be included in the calculation. Another example was seen in a three-year study of Friesian cattle that concluded "when appropriate steps are taken to mitigate the effects of climate and a high level of management practice is maintained, there appears to be no reason why high ambient temperatures and humidities should be inimical to successful dairy development with European breeds in the Near East". This conclusion may well be correct, but the only evidence presented to support it was the mean first lactation yield of 19 heifers, although it was mentioned that 94 calvings took place during the study.

This paper discusses the productive and reproductive parameters of different genotypes in several milk producing systems in the tropics. Production in temperate areas within the tropics is not considered.

The lowland rain forest is one extreme climate of the tropics and Brazilian work in the lower Amazon suggests that buffaloes' grazing behaviour is more appropriate [1], their efficiency of digestion of rations of high fibre and low protein is more efficient [2], their fertility and growth rate higher and calf mortality lower than those of Zebu cattle in the same environment [3].

The arid environment lies at the other extreme and in some of these areas a very high milk price and the availability of suitable soil and water have permitted the establishment of intensive dairy units of Holstein/Friesian cattle maintained in shaded yards and fed on irrigated forages. Reliable production data are scarce, but the rapid multiplication of such projects suggests that profitable production levels are being attained. It must be noted that in this system high quality forage is available, disease and parasites can be readily controlled and a milk price of approximately US \$1/L does not require extremely high yields in order to cover costs that include very considerable investment in infrastructure. The amelioration of stress by management of British Friesians in the Persian Gulf has been described by Ansell [4]. Appetite

decreases with increasing temperature and a ration of high nutrient density must be fed to meet the cow's nutritional needs within the limits of its daily intake. McDowell [5] suggests that very high feeding levels can be counterproductive, even in the environment of New York State. There, the genetic potential for lactation yield is probably over 11 000 kg, but most farmers only feed for 6000 to 8000 kg as they are aware that very high feeding levels lead to reduced breeding efficiency and increased health problems. Such effects of feeding stress will occur at much lower milk production levels, where the temperature greatly exceeds that of New York State. With increasing temperature and higher food intake, efficiency of food conversion will decline as the cow expends considerable energy in combating heat stress. The study of Epstein and Herz [6] on the production of Holstein cattle at three different altitudes in Israel (which is, of course, outside the tropics) clearly showed the depressing effect of heat on fertility and milk production. Maximum yield and fertility were obtained when calving took place at the end of the hot season and mating took place in the cool season.

Intensive milk production has been successfully carried out in the tropics where rainfall is sufficient for forage production, but here many problems exist that are not encountered, or are much reduced, in intensive production systems in the arid zone. High humidity exacerbates the effect of high temperature, there is a greater risk of parasites and disease and the available pastures have low digestibility [7]. Minson [8] has indicated that pasture digestibility is negatively correlated with temperature so that tropical pastures, although high yielding, have low digestibility. Alfalfa cannot usually be grown in this environment because of pests and disease. Puerto Rico is the most advanced dairying country within the tropics, but it should be remembered that it is an island where sea breezes and altitude modify the tropical environment and make it far from severe. Despite this, McDowell [5] stated that the mean lactation milk yield of recorded herds of 4380 kg was close to the level for 'best biological efficiency' for the environment.

TABLE I. LACTATION PERFORMANCE OF HOLSTEIN COWS
IN VENEZUELA [9]

	Imported cows	Venezuelan born cows
305-day milk yield (kg)	4564	4041
Calving interval (d)	434	440
Cow live weight (kg)	606	517
Services/conception	2.32	3.1

Venezuela has a large, well established dairy industry. A study of the performance of imported and Venezuelan born Holsteins kept in shaded yards at an altitude, mean annual temperature and mean annual rainfall of 452 m, 25°C and 1000 mm, respectively, was made by Martinez et al. [9]. The mean performance data are shown in Table I.

The authors concluded that "acceptable reproductive efficiency and production levels can be obtained from yarded Holsteins in the tropics". Their following statement "this, however, does not imply that this system should be adopted in practice" was given considerable validity by the findings of Pearson-Vaccaro et al. [10] who studied survival rates in the same herd and one other at an altitude of 1200 m with a mean temperature and annual rainfall of 19°C and 1130 mm, respectively. A total of 252 Holstein and 150 Dutch Friesian cattle were imported at 5 to 18 months of age and these, together with 896 female descendants born in Venezuela, were studied over a 10-year period. No differences in survival rates between cattle at the two altitudes could be detected. It was found that 68.1% of the imported cattle did not survive until third calving and only 9% were sold because of low yield; 16% died, 50% were sold because of breeding problems and 12% because of a poor physical condition. There were only 1.8 calvings per imported female and 2.2 calvings per imported female that calved at least once. Of the locally born animals 8.5% died at birth or were aborted and 14.6% died or were culled before one year of age. Of the heifers that lived to one year, 63.6% left the herd before the third calving; only 13% of these were sold because of low milk yield. Survival rate is a very important parameter of productivity and the mean calving interval, as given in Table I, is shown to have little value when so many cows were culled for infertility. The authors note that "realistic estimates of herd life are essential to proper planning" and conclude that "the fact that losses of the magnitude reported were obtained in herds supervised by professionals suggests the urgent need to revise carefully the economic aspects of dairy enterprises using similar animals in tropical regions". This admirable study emphasizes the inadequacy of many published results for use in project planning and indeed the risks that may be run if the study of lactation performance is used for such a purpose without the information contained in the second study.

It is very unfortunate that organizations that initiate the importation of dairy cattle to the tropics very rarely publish the subsequent performance of the cattle, although it is necessary to attempt to distinguish between losses that could have been easily reduced by common sense and good animal husbandry and those which are practically unavoidable. The pregnant Friesian cows sent from western Europe in the winter by sea around the Cape of Good Hope to East Africa were unlikely to benefit the Kenyan dairy industry. Vaccaro et al. [11] describe the losses that occurred when 98 heavily pregnant Holstein heifers were sent from Virginia, USA, in January 1981 to Acarigua, Venezuela, at latitude 9°35'N at 220 m above sea level with a mean temperature of 27.2°C. Eleven heifers aborted and 12 calves were born dead. Of the 75 calves born alive, 61 died before January 1982. Eight of the cows died in the first year. The mean first lactation milk yield of the survivors was estimated to be

2605 kg, which was inferior to the mean for cross-bred Holstein/Zebu heifers in the area. The study, which contains a good review of the literature of calf mortality in the tropics, suggests that the results provide further evidence concerning the impracticability of basing dairy development plans in the tropics on the importation of pregnant females.

Wilkins et al. [12] studied cattle performance on milk producing farms in the Bolivian lowlands at latitude 17°S with a mean altitude, annual temperature and annual rainfall of 400 m, 24°C and 1100 mm, respectively. They reported on losses incurred on five farms that imported European dairy cattle. They stated that they neither wished to criticize the owners or to suggest that all the losses were unavoidable. They pointed out that professional consultants actively encouraged these importations in the belief that the properties were sufficiently developed to receive them.

On Farm A: 35 Holstein cows were imported, of which 18 cows and 8 calves died within 15 months. The surviving cows had an average lactation yield of 507 kg of milk in 191 days. The farm and herd were sold.

On Farm B: 190 Holstein cows were imported and 40 cows and 18 calves died within the first 12 months. The average daily milk yield was 3.5 kg per cow before the farm and herd were sold.

On Research Station C: 82 Brown Swiss cows were imported, of which six died in the first year. No calf born during the first two years survived to six months of age.

On Farm D: 120 Holstein cows were imported and in the first 18 months six cows died, nine aborted and calf mortality was 26%. The mean lactation yield, mean lactation length and mean calving interval were 2245 kg, 316.1 d and 663.6 d, respectively. The herd and farm have been sold.

On Parastatal Farm E: This farm was under the constant supervision of a veterinarian and a livestock specialist. The cows were kept in shaded yards and the calves were housed individually. A group of 72 Brown Swiss were imported and in the first year three cows died, four were sold for slaughter and three aborted. The mean first lactation yield, mean lactation length and mean calving interval were 1722.2 kg, 259.4 d and 490.0 d, respectively. A group of 50 Holstein heifers were then imported, of which 47 were pregnant. Fourteen cows aborted and the mean first lactation yield was 3585.5 kg; the mean calving interval was 545.3 d. Two further importations were made of Holsteins and Brown Swiss and at the end of the first two years of operation, of 301 calves born alive 100 had so far died despite skilled and energetic veterinary attention.

On two other farms in the area, 84 Holsteins that had passed through a period of adaptation and were kept in shaded yards had a mean annual yield of 3040.7 kg and a calving interval of 416.7 d; calf mortality was 5.3%. These animals were kept free of ticks and had no immunity to babesiosis or anaplasmosis, as was demonstrated when the sale of 30 cows to a farm where they had to graze resulted in 50% mortality within three months.

Seventy-nine Holsteins on the same feeding regime as those maintained in shaded yards but grazing day and night had a mean annual yield of 2402.9 kg and a calving interval of 470 d. Calf mortality was 25% and it was presumed that the lower performance of the grazing animals was caused by the stress of solar radiation and walking and the effect of poorly controlled parasites, particularly ticks. It is highly probable that increased calf mortality was caused by babesiosis and anaplasmosis. Several of the farms included in the study contained both pure Brown Swiss or Holstein cows and the crosses between these breeds and the Zebu/Criollo animals of the zone. These crosses produced more milk per year, were more fertile and had a lower calf mortality than the pure-breds on the same farm.

The productivity of the Holsteins when yarded and when on pasture was too low to cover the costs of the operation, while the cross-breds were profitable when grazing improved pastures supplemented by concentrates. The cross-breds in the study were half or three-quarter Holsteins or Brown Swiss whose sires were always of the European dairy breed. Wilkins et al. [13] demonstrated that the reverse cross was also superior when they compared the performance of pure Brown Swiss heifers and half-bred Criollo/Brown Swiss heifers produced by crossing Criollo bulls on Brown Swiss cows. The cross-breds had a lower calf mortality, faster live weight gain, lower first calving age and higher fertility than the pure-breds and first lactation milk yields that were insignificantly superior.

The study of commercial herds demonstrated that Holstein cattle could be maintained in a state of good health when kept in shaded yards in lowland Bolivia, but their mean annual production of 3041 kg was nearly 2000 L lower than that required to cover the costs of production in that system with the existing milk price. Costs were reduced when the cows grazed, but production yields fell and the cows remained unprofitable. In this environment at the existing milk price level, cross-bred cattle grazing improved tropical pastures supplemented by concentrates were profitable and superior in productivity to pure-bred Holsteins. This general result is in agreement with most other studies in a similar environment [14, 15] and is entirely logical. Tropical forages cannot form the basis of a feeding regime for high milk production in a hot environment, so a Holstein cow will be restricted in its production to the level limited by the nutritive value of its feed intake to a level that is well below its genetic potential. At the same time its production is adversely affected by the environment, resulting in lower fertility, further yield depression and higher mortality. The cross-bred on the same nutritional regime may be able to realize virtually its maximum genetic potential, resulting in a lactation yield that may be superior to that of the Holsteins under the same management, but it is more fertile, healthier and grows faster because of heat tolerance, parasite resistance and, perhaps, greater appetite.

While the cross-bred can be demonstrated to be a suitable genotype for milk production systems based on grazing improved tropical pastures supplemented with concentrates, there is no clear consensus of opinion on what is the most suitable cross and how it may be maintained [16]. The relative merits of a number of mating

systems for cross-bred dairy cattle production are being investigated with large numbers in Brazil [17]. Reciprocal crossing between two breeds would maximize the benefits of heterosis and would permit the use of progeny-tested bulls from large pure-bred populations. This would necessitate the use of the semen of two breeds on a farm or two breeds of bull would have to be maintained; this is not feasible for many farmers. The other alternative is to form a new breed that incorporates the high potential of the European dairy breed and the adaptability of the tropical breed. This facilitates management for the farmer but presents formidable problems in its realization. Among these are the need for a very large initial population in order to permit the heavy selection of females needed in the F_2 and subsequent generations and for the progeny testing of bulls of the new breed. It may be noted that the Jamaica Hope and the Australian Milking Zebu have not been outstanding commercial successes in their home countries [18, 19] and the Brazilian Pitangueiras breed that was formed from the inter se mating of 5/8 Red Poll and 3/8 Zebu cattle was found to have a lower mean lactation yield and longer calving interval than 5/8 Red Poll and 3/8 Zebu cows produced by mating 3/4 Zebu and 1/4 Red Poll cows with pure Red Poll bulls [20], presumably because of the benefit of heterosis in the latter genotype. However, there are now some promising results from the Australian Friesian Sahiwal that show this new breed to have a superior lactation performance to the Friesian in the environment of latitude 12°28'S in northern Australia [21].

Cross-breds between European dairy breeds and Zebras have been reported to have an undesirable dairy temperament in some situations and this usually implies that the cow required the stimulus of the calf in order to let down her milk. This is a problem in many Zebu cattle and it is possible to select for good dairy temperament in this population. This was responsible for the rapid increase in the mean lactation yield in Sahiwal cattle in Kenya [22]. However, this necessitates a progeny testing programme, which is not possible in the very small number of Zebu dairy cattle available in Australia. The bad dairy temperament of cross-breds in this country under the prevailing management system is responsible for the inferior performance of the cross-breds compared with Jersey cattle [18]. The inferior performance of cross-breds in the USA was also largely due to the same reason [23]. It is certain that cross-breds can be most easily managed when handled frequently and hand milked, but the problem is not insurmountable and the author has seen large herds of Holstein/Brahman cows milked by machine in the absence of their calves in the lowlands of Venezuela without apparent problem. It should also be remembered that the milking of the cow with the calf at foot can be an appropriate system. Calf milk consumption is high, but this may be of little importance in a dual purpose system where the price of milk is low compared with the value of weaners and where milk substitutes are expensive or unobtainable. The handling of calves is also laborious, but calf mortality and mastitis are reduced [24–26].

While the European dairy breed cross Zebu cow can be demonstrated to be the appropriate genotype for the system already described where the cows graze improved tropical forages supplemented by concentrates, there are many situations

and large areas of the tropics where it is not. If concentrates are not available for any reason, the productivity of the cross-bred will be limited to that yield permitted by the nutritional value of the pasture, and this may not exceed the production potential of the local tropical breed that has a lower calf mortality than the cross-bred and greater resistance to parasites [27]. McDowell [5] reported that many farmers in India were dissatisfied with their cross-breds' low performance and complained that 'they tended to die'. Dairy ranching is a common commercial-scale production system in Latin America on cheese producing farms that are far from the market and can be more lucrative than pure beef production. The cows graze 12 h/d with their calves and 12 h without, and are milked at the end of the period of separation. The cows do not normally receive concentrates and often graze unimproved pasture or browse. The criticism that the system produces little milk and emaciated weaners is not valid if the cows are only milked at those times of the year and those stages of lactation when the cow has milk surplus to the requirements of the calf. This system is, of course, carried out in large areas of Africa and Asia and although productivity per cow is low, productivity per unit of capital invested will be found on many occasions to exceed that of introduced capital-intensive systems. This system, under good management, will be the most appropriate where scarcity of capital is the limiting factor to increased productivity. It is suggested that the most suitable cattle for this system are those selected from within the indigenous population and that the chief criteria for selection be high fertility, low calf mortality and milk production to the nutritive limit of the pasture. Low calf mortality is very important under this system as apart from the value of the weaner, the death of the calf usually leads to the immediate termination of lactation and consequent loss of milk. The weaning rate of Zebus can be very low due to low fertility [28, 29] and high calf mortality [30, 31]. Such cases may be caused by inadequate nutrition, including mineral deficiencies, and by poor calf management, but Amble and Jain's study [14] of the performance of Zebu and cross-bred cattle on military farms in India showed that cross-breds had a lower calf mortality than Zebus; other studies in India had a similar result [32]. Zebu calves can be described as physiologically less well developed at birth than cross-breds and European cattle because their birth weight is a lower percentage of their mature weight and they may be more susceptible to enteritis and pneumonia. However, the author has observed that Zebu calves are more tick resistant than cross-breds and manifest much less severe symptoms of babesiosis and anaplasmosis. A study of the pedigrees of one well known Zebu breeding farm in India indicated a high degree of inbreeding, which may also be partly responsible for the Indian results. Wilkins et al. [12] presented some slight evidence to suggest that consanguinity in Criollo cattle, which must exist in all herds where bulls are bred and never bought into the farm, is responsible for elevated calf mortality and reduced fertility, growth rate and milk yield. If this is demonstrated to have widespread validity in consanguineous herds in the tropics, an exchange of bulls between populations would result in an increase in productivity at little cost. Whatever the cause of high mortality and low fertility in some populations of indigenous cattle, a solution to the

problem is more urgently required than an increase in lactation milk yield or speed of growth.

This discussion may appear to be of interest only to those involved in livestock development. However, it is suggested that workers in applied research should also thoroughly understand the importance of identification of the most appropriate system as well as identification of the problems limiting production within that system. Investigation should then concentrate on determining the methods to resolve these key problems. Finally, it must be added that a production system and genotype will only remain appropriate if there are no great relative changes in costs of production and market prices of livestock products and no increase in the knowledge of nutrition, animal health and plant breeding. Those who said in the 1930s that pure Friesian cattle should not be kept in the Jordan Valley were almost certainly correct at that time. However, what was true then was evidently no longer valid twenty years later.

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APPLICATIONS OF HORMONE RADIOIMMUNOASSAYS ON STUDIES OF ENVIRONMENT AND REPRODUCTION INTERACTIONS IN LARGE RUMINANTS*

W.W. THATCHER**, R.J. COLLIER**, M. DROST***,
J. PUTNEY**, D.K. BEEDE**, C.J. WILCOX**

**Department of Dairy Science,
Institute of Food and Agricultural Sciences

***Department of Reproduction,
College of Veterinary Medicine

University of Florida,
Gainesville, Florida,
United States of America

Abstract

APPLICATIONS OF HORMONE RADIOIMMUNOASSAYS ON STUDIES OF ENVIRONMENT AND REPRODUCTION INTERACTIONS IN LARGE RUMINANTS.

The effects of environment on the reproductive processes of female cattle are described. Variation in fertility responses is partitioned into environmental and genetic effects. Environmental effects are defined as all effects that are non-genetic, and emphasis is placed on quantitation of the climatic measurements (e.g. maximum environmental temperature) associated with reproductive performance. Sensitivities of various reproductive events to thermal stress are defined and include such responses as reproductive behaviour, hormonal balance, alterations in uterine blood flow, embryonic death, conceptus development, placental function and restoration of the postpartum reproductive function. Several management strategies are discussed to improve animal productivity, including environmental modification, reproductive management and evidence of genetic adaptation to local environments through selection.

1. ENVIRONMENTAL EFFECTS ON REPRODUCTIVE PERFORMANCE

When addressing the area of environmental effects on reproductive performance, it is important to note that environmental effects are those effects which are not genetic and encompass such factors as temperature, humidity, solar radiation, nutrition, age, physiological state of the animal (e.g. lactating, suckled, non-lactating), parasites, disease organisms, photoperiod, season, management (extensive, intensive), regions and years. These various environmental components are not

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necessarily independent. Fertility is controlled by a multiplicity of physiological, endocrinal and biochemical events. The per cent of total variation (genetic effects (V_G)) and non-genetic environmental effects (V_E)) for measurement of fertility that is controlled by additive gene effects (V_A) is defined as heritability ($V_A/(V_G + V_E)$). Heritability estimates for various reproductive measurements are low.

In subtropical and tropical areas, a predominant environmental effect on fertility is the suppression of conception rates during stressful months of the year. A considerable number of studies have quantified various climatic measurements and evaluated their associations with the conception rate [1]. However, it is important to characterize the particular location and management system of each study, since in certain localities associated climatic effects (e.g. temperature during wet and dry seasons) may be confounded with nutrient and energy availability under extensive management systems with beef cattle. Such effects are somewhat minimized in modern intensive management systems with dairy cows. For example, we evaluated [2, 3] the various climatic effects (air temperature, solar radiation, minimum relative humidity) on the fertility of 6555 inseminations over a 3-year period (1975–1977) with three breeds (Jersey, Brown Swiss and Holstein) within a commercial dairy herd of Florida (subtropical environment). Heifers had higher conception rates than lactating cows (47% > 32%); the conception rates of cows decreased much more in the summer months of June to August; the conception rates did not recover until November, suggesting carry-over effects during the months of September and October. Furthermore, differences in conception rates among breeds (Jersey > Holstein, Brown Swiss: 45% > 39% and 41%, respectively) probably also reflected the differences associated with milk production and thermoregulatory responses during summer thermal stress periods.

Of practical significance was whether specific climatic measurements could be related quantitatively to fertility on an insemination to insemination basis. We reported previously [4] that the maximum environmental temperature on the day after insemination was associated negatively with the conception rate. This was also the case for the present experiment [2, 3]. A clear difference in the conception rates was detected between lactating dairy cows and heifers as the maximum environmental temperature increased the day after insemination. The conception rates decreased in lactating cows, and the decrease was accelerated when maximum environmental temperatures exceeded 30°C. In contrast, the conception rates increased slightly for heifers. The decrease in conception rates of lactating cows is probably due to an inability to maintain a normal body temperature under heat stress conditions because of the high rate of internal heat production associated with production of milk. In contrast, non-lactating heifers are more likely to have a higher upper critical environmental temperature beyond which body temperature is elevated, but not to the point where fertility is diminished. In relating various climatological measurements (e.g. maximum, minimum and average daily temperature; average daily rainfall; average wind movement; average daily solar radiation; average maximum and minimum daily atmospheric pressure) with fertility, the maximum environmental temperature on the

day after insemination was the most potent environmental measurement associated with fertility in the subtropical environment of Florida [3, 4]. Obviously many of the environmental measurements are correlated, and the most consequential environmental measurements may vary, depending on the geographical location. For example, during the summer in northern Israel, minimum ambient temperature was associated negatively with conception rates [5]. Likewise, it has been reported that the conception rates for nullipara heifers were unchanged during the summer months, whereas for cows fertility was suppressed.

Changes in climatic measurements represent alterations in the animals' microenvironment that are related to conception rates. A logical question is whether similar associations can be detected within the potential embryo's microenvironment, e.g. the uterus. Uterine temperatures recorded on the day of insemination (UT_0) and the day after insemination (UT_1) were related negatively to the conception rates [6]. An increase of 0.5°C above the mean UT_0 (38.6°C) and UT_1 (38.3°C) decreased the conception rates by 12.8 and 6.9%, respectively.

Several excellent studies suggest that seasonal differences in reproductive performance can be ascribed to factors (e.g. photoperiod) other than elevated environmental temperatures and humidity during summer periods (see review in Ref. [7]).

2. EFFECTS OF THERMAL STRESS ON THE REPRODUCTIVE PROCESSES OF FEMALES

2.1. Oestrus detection

Herd reproductive inefficiency results from two main problems: (1) an absence of expressed oestrus or inefficient detection of oestrus; and (2) infertile services, of which embryonic mortality in dairy cattle is estimated to be approximately 15% [8]. The magnitude of these problems is amplified during hot seasonal periods. One of the most consistent gross observations during thermal stress exposure is a reduction in the length of oestrus to approximately 10 hours and a lower intensity of oestrous behaviour [9]. Such behavioural alterations would contribute to a reduction in success of heat detection for artificial insemination programmes. This is documented in a study with a large commercial dairy herd of Jersey cows [3]. Approximately 66% of potential heats were not detected for the calendar year 1982. The striking observation was that the hypothetical percentage of potential heats not detected increased to a plateau of approximately 80% during the hot summer months of June to September. This, coupled with lower conception rates to detected heats, accounts for a tremendous amount of herd infertility during the summer period of June to October.

2.2. Hormonal alterations during the oestrous cycle

When examining the effects of thermal stress on the array of hormones controlling reproductive processes, it is important to realize that both acute and chronic thermal stress require metabolic adaptations (e.g. altered secretion rates of metabolic hormones) to accommodate altered processes associated with dissipation of heat, water and electrolyte turnover, and altered metabolism. These changes, both directly and indirectly, may alter control and secretion of the reproductive hormones (see review in Ref. [7]). With regard to the reproductive hormones controlling the oestrous cycle, daily changes in plasma hormonal concentrations (oestradiol, LH, progesterone and corticoids) were monitored throughout the oestrous cycle of five cows maintained in a shade management system versus four cows managed under a stressful no-shade management system [10]. The transitory hormonal concentrations among the two groups indicated that thermal stress, of the magnitude observed in this experiment, did not inhibit recurrence of oestrous cycles. In both groups, a fall in progesterone concentrations associated with corpus luteum regression and a pro-oestrous rise in oestradiol were sufficient to induce a pre-ovulatory surge of LH and oestrus followed by ovulation. However, in cows managed with no shade, slight elevations were detected in progesterone and corticoids during the luteal phase, whereas the pro-oestrous rise in oestradiol concentrations was slightly lower. A smaller pro-oestrous rise of oestradiol in heat-stressed heifers (32 versus 21 °C) was also detected when samples were collected at 6-hour intervals in an environmental chamber [11]. The biological significance of these subtle but statistically significant differences in both luteal progesterone concentrations and pro-oestrous oestradiol/progesterone ratios may be related to the quality of the developing pre-ovulatory follicle, the intensity of oestrous behaviour, the quality of the new corpus luteum and the subsequent microenvironment of the uterus and oviduct which control such processes as capacitation, sperm and ova transport, fertilization and development of the embryo. The ovarian function was influenced by these controlled environments, and it is interesting that McNatty et al. [12] also detected seasonal differences in the ovarian activity of cows between autumn and winter versus spring in New Zealand. The mean diameters of large healthy follicles (> 8 mm) and the dominant oestrogen secretory follicles were larger and contained more granulosa cells in autumn and winter than during spring. Likewise, corpora lutea were heavier and secreted more progesterone in autumn and winter than in spring. The mechanism controlling the specific environmental and seasonal effects on ovarian activity are not known. Such factors as ovarian sensitivity and gonadotrophin secretion warrant additional research.

Since elevated environmental and uterine temperatures near the time of fertilization are related to conception rates, the factors controlling uterine temperature are important. The greatest rate of uterine blood flow during the oestrous cycle occurs during the peri-oestrous period in association with high ratios of oestradiol/progesterone concentrations [13]. In cows exposed to the sun with no shade during the sum-

mer, the increase in uterine blood flow induced by oestradiol (200 μg) was less than that of the same cows treated with oestradiol (200 μg) but exposed to an effective shade structure [14]. Thus, during the animal's thermoregulatory response to thermal stress, there is a decreased rate of blood perfusion to the uterus after injection of oestradiol. Such a thermal stress-induced reduction in uterine blood flow would preferentially elevate uterine temperature and probably affect the availability of water, electrolytes, nutrients and hormones to the uterus. Such induced inhibitory responses, as a consequence of thermal stress, would have a high probability of increasing rates of embryo death during early pregnancy.

2.3. Early embryo development

Specific periods during which the early bovine embryo is sensitive to heat stress have not been documented extensively. Dunlap and Vincent [15] exposed 23 Hereford heifers to a temperature of 32.2°C for 72 hours following breeding, whereas 25 control heifers were maintained at a temperature of 21.1°C. None of the heifers exposed to 32.2°C maintained pregnancy compared with 12 of 25 heifers at 21.1°C. The period of embryo sensitivity to heat stress has been studied much more extensively in sheep. Ewes subjected to high ambient temperature (32.2°C) at about the time of mating had lower fertilization and increased early embryonic death rates [16]. When ewes were not exposed to the treatment room until 8 days post-breeding, embryo losses were not significantly different from the control ewes. The specific time during which the detrimental effects of high temperatures were exerted on the sheep embryo was evaluated by embryo transfer [17]. Transfers were performed approximately 72 hours after onset of oestrus. When both donor and recipient were maintained at 21.1°C, 55.6% of the transfers were successful compared with 9.5% when donors were maintained at 32.2°C and recipients at 21.1°C. It was concluded that some detrimental action had occurred by 3 days after onset of oestrus in ewes at 32.2°C. It was also observed that embryonic death occurred at a later stage of development in utero. When ewes were exposed to 32°C at the time of breeding and at 1, 3 and 5 days after breeding, no differences in fertility were detected in the different groups [18]. Exposure to heat resulted in an increase in morphologically abnormal ova; embryo loss, estimated as the percentage of fertilized ova that failed to survive, was significantly higher in all the treated groups. Embryo loss for the combined 0- and 1-day groups was significantly higher than in the 3- and 5-day ewes. The sheep zygote is most sensitive to the harmful effects of high ambient temperatures during the initial stages of cleavage while in the oviduct. Eighty-five per cent of the control ewes lambed compared with 10% for ewes in the 0- and 1-day treatment groups, 35% in the 3-day group and 40% in the 5-day group. Fertilized ova grown in vitro through the first cell division at 40°C had a lower rate of embryo survival than those grown at 38°C. As the period of culture at 40°C was delayed to either the second, third or fourth cell division, differences in post-implantation death losses disappeared. Collectively, these studies in the female indicate that heat stress

of the ovum immediately after fertilization causes the resulting conceptus to perish some time later in its development.

We recently conducted a series of experiments in cattle that were acclimatized to environmental chambers at 20°C for 9 days, superovulated with FSH-P (40 mg total for 10 injections at 12-hour intervals), inseminated (3 times at 10-hour intervals), and then maintained either at thermal neutrality (20°C) or under hyperthermic conditions (16 hours at ~30°C and 8 hours at 42°C) for an additional 7-day period beginning at ~30 hours after onset of oestrus [19]. On day 7 post-oestrus, embryos were recovered non-surgically and evaluated by light microscopy for their stage of development, quality score and number of cells. Differences in rectal temperatures, respiration rates and embryo responses of heifers between environmental treatment groups were evaluated by least squares analysis of variance and chi-square analyses. Heifers maintained under hyperthermic conditions had higher ($P < 0.01$) rectal temperatures (41.3 > 38.8°C) and respiration rates (155 > 39 per minute). The distribution of embryos classified as normal (N), retarded (R) and unfertilized ova (UFO) differed ($P < 0.02$) between heat stress (number = 49: 42.9% N, 40.8% R and 16.3% UFO) and control treatments (number = 44: 65.9% N, 15.9% R and 18.2% UFO). The mean cell number for embryos collected from heifers in the heat stress group was less ($P < 0.01$) than those from the control group ($38 \pm 6 < 61 \pm 7$). These responses indicated that thermal stress (from 30 hours post-onset of oestrus) increased the incidence of abnormal or retarded embryos in superovulated heifers. It is of extreme interest to determine if embryos continue to be sensitive to heat stress beyond the period of day 7 until the period of pregnancy recognition (see review in Ref. [20]) at day 17.

Biggers et al. [21] evaluated whether elevated ambient temperature between days 8 and 16 post-insemination altered the hormonal profiles and conceptus development in beef cattle. Thirty-two multiparous cows were acclimatized to metabolism stalls in a temperature-controlled building (20°C for 7 days). Oestrus was synchronized for group matings with fertile bulls. On day 7, cows were assigned randomly to the following three groups: control (C: 22°C, 25% RH), mild heat stress (MHS: 37°C, 27% RH) and severe heat stress (SHS: 37°C, 40% RH), until day 16 when they were slaughtered. Compared with the control cows, cows in the MHS and SHS groups had increased ($P < 0.01$) respiration rates (C: 56; MHS: 99; SHS: 103 per minute) and rectal temperatures (C: 38.9°C; MHS: 39.3°C; SHS: 39.8°C). Although heat stress did not significantly alter pregnancy rates, there was a trend towards greater embryonic mortality in heat-stressed cows (C: 83%; MHS: 64%; SHS: 50%). Conceptus weights were decreased ($P < 0.01$) in MHS (0.1106 g) and SHS (0.0729 g) compared with C (0.1579 g). Results indicated that heat stress during the period of blastocyst development, elongation and pregnancy recognition reduces conceptus weights, and this may lead to increases in embryonic mortality. However, thermal sensitivity after day 7 does not appear to be as great as during the earlier period of development before day 7.

It is clear from the results of Biggers et al. [21] that conceptuses exposed to heat stress, between 8 and 16 days post-insemination, are reduced in their development. Maintenance of the corpus luteum (CL) in cattle is required if pregnancy is to persist. Uterine production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes CL regression in cyclic cattle. Maintenance of the CL and hence survival of the conceptus or embryo depends on the ability of the conceptus-produced products to 'signal' the uterine endometrium and/or ovary to maintain the CL. The signal is initiated or produced by the conceptus between 15 and 17 days after oestrus. A protein produced by the conceptus is involved; we have demonstrated that conceptus secretory proteins (produced by the conceptus during a 24-hour incubation in a culture dish at 37°C) will extend the life span of the CL when injected twice daily into the uterine lumen from days 15.5 to 21 [20]. Cows given conceptus secretory proteins returned to oestrus at a mean of 33.4 days ($n = 3$ cows) versus 23.5 days ($n = 3$ cows) for the control group. These results combined with plasma progesterone analyses indicated that the conceptus is able to prevent regression of the CL, which is essential for maintenance of pregnancy. Research is being continued to characterize and purify the specific protein so that we may be able to improve conceptus survival in the future [22, 23]. At present, we know that conceptus secretory proteins reduce the ability of the uterus to secrete $PGF_{2\alpha}$ when oestradiol is injected at day 18 post-oestrus [20].

Any treatment which delays regression of the CL may allow conceptuses additional time to develop and have a higher probability of exerting an anti-luteolytic effect. This may indeed be beneficial in situations, such as heat stress, where embryos have survived but are slightly behind in their development [21].

A single injection of HCG (10 000 i.u.) on day 10 of the oestrous cycle prolonged the luteal life span and delayed oestrus by 6 to 7 days [24]. In an additional study, HCG (10 000 i.u.) extended the oestrous cycle when injected on day 10 or 15 but not on day 17 [25]. Thus, extended CL function could provide additional time for embryos to develop (e.g. elongate) so that they may be in a better state to block the endogenous luteolytic secretion of $PGF_{2\alpha}$ that normally occurs in the absence of a conceptus. Furthermore, the timing of the HCG injection at day 15 would be important, since it is the window just preceding the time of pregnancy recognition. A single injection of HCG (10 000 i.u.) on day 10, 11 or 12 after first or second insemination did not improve pregnancy rates (control 63% versus HCG 59%). However, the injection was not given at day 15 [26]. Additional support for a beneficial effect of HCG on pregnancy rate is its efficacy in bovine embryo transfer recipients [27].

We conducted a series of six experiments with HCG in order to evaluate its potential role of improving fertility [28]. In all our experiments, HCG (3300 i.u.) was injected intravenously at day 15 of the oestrous cycle; day 15 just precedes the critical time when the conceptus 'signals' or initiates the pregnancy recognition process or extends the life span of the CL. In Experiment I, injection of HCG caused animals ($n = 15$) to have an oestrous cycle length of 24.0 days versus 20.0 days for control animals ($n = 15$). Analyses of plasma progesterone indicated that CL regression was delayed by approximately 3 days for animals injected with HCG. HCG may delay

CL regression via several possible mechanisms, such as blocking the luteolytic action of $\text{PGF}_{2\alpha}$, reducing uterine secretion of $\text{PGF}_{2\alpha}$, altering ovarian activity that delays ovarian-induced secretion of uterine $\text{PGF}_{2\alpha}$, or induce a new ovulation and CL formation.

Experiment II indicated that injection of HCG on day 15 did not block the luteolytic effect of a $\text{PGF}_{2\alpha}$ injection (25 mg) given 24 hours later on day 16. Both groups of cows (HCG or saline treatments) had an abrupt decrease in plasma progesterone associated with CL regression that occurred on days 17 and 18 following injection of $\text{PGF}_{2\alpha}$. All control cows ($n = 5$) had an induced oestrus on day 18.5. In contrast, none of the HCG-treated cows ($n = 5$) had a detected oestrus by day 25. Although HCG-treated cows had an abrupt decrease in plasma progesterone concentrations, values did not fall below 1 ng/mL, and these values were sustained until at least day 28. When additional cows ($n = 3$) were treated (HCG on day 15 plus $\text{PGF}_{2\alpha}$ on day 16) and ovariectomized on day 18, the ovarian responses were clear. The original CL was regressed, several follicles were luteinized and a new CL ($\cong 2.5$ days old) was present. Consequently, HCG did not block the luteolytic effects of exogenous $\text{PGF}_{2\alpha}$ but ovulated a follicle that was present on day 15. Formation of a new CL and luteinization of follicles were probably responsible for the sustained basal levels of P_4 in the range of 1 to 2.5 ng/mL. An important finding of the experiment was that HCG did not block the luteolytic effect of the $\text{PGF}_{2\alpha}$ (25 mg) that was injected.

Experiment III was conducted to determine if HCG reduced the uterine secretion of $\text{PGF}_{2\alpha}$ when ovariectomized cows were injected intravenously with oestradiol (3 mg). The experimental model was that 10 cows were ovariectomized on day 14 of the oestrous cycle, injected with either HCG ($n = 5$ cows) or saline ($n = 5$ cows) on day 15, and all were injected with oestradiol on day 16. Progesterone concentrations of 2 ng/mL of plasma were maintained by installation of a 'progesterone-releasing intravaginal device' (PRID) on day 13 or 24 hours before ovariectomy (day 14). The uterine secretion of $\text{PGF}_{2\alpha}$ induced by oestradiol injected on day 16 was monitored over a 10-hour period (30 min sample) in which plasma concentrations of 15-keto-13,14-dihydro- $\text{PGF}_{2\alpha}$ were measured. Results indicated that oestradiol induced $\text{PGF}_{2\alpha}$ secretion, but the response was the same for cows treated with either HCG or saline. Implications are that HCG does not directly inhibit uterine secretion of $\text{PGF}_{2\alpha}$ in ovariectomized cows treated with PRIDs, and that a delay of CL regression in intact cows (Experiment I) is probably due to some ovarian effect (e.g. follicle luteinization) which delays the normal follicle-induced secretion of $\text{PGF}_{2\alpha}$ from the uterus that would cause CL regression. It is important to recognize that mechanisms of luteal maintenance brought about by HCG and conceptus secretory proteins are probably different. The reason for this assumption is that uterine $\text{PGF}_{2\alpha}$ secretion in response to an injection of oestradiol is reduced in cows treated with conceptus secretory proteins [20] but not when treated with HCG (Experiment III).

At this point, a series of field experiments (IV, V and VI) was conducted at Larson's Dairy, Inc., Okeechobee, Florida, to determine if injection of HCG on day 15

TABLE I. PREGNANCY PER CENT FOR THREE EXPERIMENTS IN WHICH DAIRY HEIFERS WERE TREATED WITH HCG^a OR SALINE AT DAY 15 POST-INSEMINATION

Experiment	Overall	HCG	Saline	Difference
IV: October 1983	46.6 (204) ^b	51.9 (104)	41.0 (100)	+10.9 ^c
V: February 1984	61.2 (206)	54.8 (104)	69.6 (102)	-12.8 ^c
VI: July 1984	64.6 (266)	63.2 (133)	66.2 (133)	- 3.0

^a 3300 i.u. given intravenously.

^b No. of heifers in parentheses.

^c $P < 0.10$.

post-insemination affected pregnancy rates in dairy heifers. The rationale for the treatment was that an induced delay in CL regression may permit slower developing conceptuses an additional 24 to 48 hours to expand. This may provide a greater probability of blocking the normal cyclic release of PGF_{2α} that causes CL regression. A total of 676 heifers were randomly assigned to receive intravenous injections of either HCG (3300 i.u.) or saline on day 15 post-insemination (Table I). The protocol was replicated three times during October 1983 (Experiment IV), February 1984 (Experiment V) and July 1984 (Experiment VI). The fertility rate was considerably lower in Experiment IV versus Experiments V and VI. At a time of poor overall fertility (46.6%; Experiment IV), HCG treatment caused a 10.9% increase in fertility ($P < 0.10$). When the experiment was replicated in the cooler time of the year and overall fertility (61.2%) was higher (Experiment V), HCG had a significant ($P < 0.10$) detrimental effect of -12.8% on fertility. A third replicate was completed (Experiment VI) during early summer and overall fertility was also high (64.6%); HCG treatment was associated with a slight non-significant decrease (-3%) in the pregnancy rate.

The contrasting results between experiments were interesting. A pooled statistical analysis with 676 heifers indicated that there was a significant 'experiment by treatment' interaction. Biologically this means that HCG had effects on fertility in the three experiments, but that the effects differed (positive in Experiment IV and negative in Experiments V and VI) between experiments. It also is important to recognize that all three experiments represented sensitive systems for testing the treatment effect on pregnancy rate. The experiments were conducted on one farm and were completed in a short period of time (7 to 20 days). Inseminations were made by essentially one inseminator (for all three experiments) and heifers within each experiment were quite uniform in quality and assigned randomly to treatments. Fertility was

indeed lower in Experiment IV. If this was due to heat stress or some other environmental-management factor, then conceptuses that are affected and survive may benefit from a delay in luteal regression which would permit an additional period for elongation of extra-embryonic membranes before exerting an anti-luteolytic signal. Such a benefit may account for the 10.9% increase in fertility found in Experiment IV. In contrast, heifers in Experiments V and VI did not have the overall lower level of fertility, and at this higher level of fertility HCG exerted a detrimental effect on pregnancy rates. Perhaps HCG effects on ovarian function are detrimental to pregnancy rates when overall fertility is elevated. Although the reasons for the differential response of pregnancy rate to HCG are not as yet apparent, the potential benefit in periods of lower fertility warrants further investigation. These experiments were conducted in dairy heifers because of the availability of animals and better experimental control of the experiments. A field study with lactating dairy cows during the hot summer months would be a more stringent test of HCG treatment, since these animals are more sensitive to heat stress.

2.4. Periods of late pregnancy and peripartum

Several reports indicate that thermal stress during late pregnancy in sheep causes reductions in uterine blood flow, decreased placental weights and retardation of foetal growth [29–31]. Approximately 60% of the bovine foetal growth occurs during the last 90 days of pregnancy [32]. Coupled with foetal growth is simultaneous development of the dam's mammary gland [33]. Conceptus growth and development clearly control maternal function to some degree. The birth weight of the calf is positively associated with the subsequent milk yield of the dam [34, 35]. Analysis of a large herd dataset for a 23-year period indicated that the birth weight of Holstein calves was lower during the hot summer months than in the cool winter months (range of 6 kg) in our subtropical environment of Florida [9, 35]. These observations led Head et al. [36] to characterize placentas ($n = 187$), collected within 24 hours of parturition, from Holstein and Jersey cows during a 1.5 year period. Cows undergoing parturitions in July and August had reduced weights of total placental dry tissue, whereas cotyledonary dry weights appeared to be lower during May to September. The month effects on calf birth weights could not be detected for the 187 parturitions in this study. Collectively, these results indicated that the late pregnant cow and conceptus are sensitive to seasonal changes in environment. The ultimate size of placenta and calf are influenced by seasonal periods of heat stress, and these reductions may contribute partially to the seasonal depression in milk production.

Collier et al. [37] designed an experiment to test whether a stressful thermal environment would alter conceptus and maternal functions during the periparturient period. Twenty-one cows and 10 heifers were assigned randomly in June 1978 to shade (S: $n = 16$) or no shade (NS: $n = 15$) treatments during the last trimester of pregnancy. Animals in S were provided full access to the shade management system

described in Ref. [38], whereas animals in NS had no natural or artificial shade. At parturition, all animals were removed from treatments and managed uniformly as one group in the milking herd. Calf birth weight was lower in the NS group (NS: 36.6 kg; S: 39.7 kg) and cows in the NS group produced less milk after calving. The decrease in the calf birth weight of NS cows was associated with a lower mean prepartum concentration of E_1SO_4 in maternal plasma (NS = 2505 pg/mL < S = 4433 pg/mL). Since E_1SO_4 is produced by the cotyledon (foetal placenta) and was lower, it is an indication of reduced conceptus function during thermal stress and is consistent with a lower calf birth weight in the NS group. Prepartum heat stress increased concentrations of 15-keto-13,14-dihydro-PGF $_{2\alpha}$ (PGFM) postpartum and increased the rate of uterine involution [39]. This may reflect a functional difference in the maternal placenta (caruncle) between groups that was established prepartum and expressed postpartum as a difference in PGFM. Lindell et al. [40] found that the rate of uterine involution with no clinical signs of genital tract disturbances was increased in cows with higher concentrations of PGFM. Others have detected both seasonal and photoperiod effects on postpartum uterine involution in cattle [41, 42]. Thus, restoration of normal postpartum uterine function, a prelude to normal cyclicity, is influenced by prepartum environmental factors.

The results of these studies indicate that environmental heat stress prepartum has pronounced effects on the conceptus and dam that influence the well-being and subsequent performance of each. Pregnant cows should not be ignored during the dry period since their physical environment and nutritional management programmes affect postpartum performance.

3. MANAGEMENT STRATEGIES AND PERSPECTIVES TO IMPROVE REPRODUCTIVE EFFICIENCY DURING HEAT STRESS

Various practical and economical systems to modify the microclimate of the cow are needed to improve fertility. Workers in Arizona [43] reduced the air temperature by 10 to 12°C by using evaporative cooling under practical farm conditions in large herds. Both milk production and fertility were improved.

Environmental conditions in tropical and subtropical areas are not ideally suited to evaporative cooling systems because of the high relative humidity. Evaporative cooling requires an addition of moisture to the air, and its effectiveness is limited by the ability of air to absorb additional water. Consequently, evaporative cooling systems cool more effectively in dry arid climates than in areas with higher relative humidities. Often the relative humidity is 90% or higher during the early morning hours in the subtropical environment of Gainesville, Florida. However, when the temperature is 32°C the relative humidity is seldom higher than 60%. Under such climatic conditions, the potential exists for operating an evaporative cooling system, for at least several hours per day. Whether this has potential benefit on production responses in cattle needs to be tested.

Perhaps the simplest environmental modification is use of shade structures. The principal function of shade is to reduce the heat load of animals by reducing incident solar radiation. If this reduction is sufficient to maintain a near normal body temperature, then increases in production should be detected or production should be maintained near normal. However, the type of shade and associated management of animals will be critical in obtaining a production benefit. Exposure of cows, managed as a group, to an insulated shade structure in which cows also had access to feed, water and an adjacent sod area for loafing resulted in increased milk yield and reproductive performance. Cows exposed to the shade management system produced 10.7% more milk and their per cent conception was 44.4 versus 25.3% for controls without shade [38].

Environmental modifications such as air-conditioning, evaporative cooling, zone cooling, various shade structures, and intermittent upper body sprinkling with forced ventilation have been used with different degrees of success to reduce the detrimental effects of heat stress. However, use of various systems to alter the environment should be coupled with management that provides adequate feed (quantity and quality) and availability of drinking water. Use of a particular system to alter the environment depends on the severity of that environment, characteristics of the climate and economic considerations.

Additional physiological strategies should be combined with environmental modification. In dairy systems, utilization of frozen semen from proven bulls is essential for improving genetic merit. Furthermore, heat stress effects on the bull are avoided by use of artificial insemination in which semen can be collected and frozen during cooler times of the year. Prostaglandin $F_{2\alpha}$ works effectively to regress the CL in heat-stressed cattle [44], and effective synchronization systems should be implemented with environmental management systems. Parts of the herd could be synchronized and bred to avoid the heat stress season. Alternatively, groups of cows could be synchronized during hot weather to intensify the accuracy of heat detection; these synchronized groups could be managed under shade management systems for a limited time (e.g. 20 to 40 days) to maximize conception rates. It is anticipated that with continued technological advancements, frozen embryos will be utilized successfully (with a normal rate of fertility) for embryo transfer in cattle. Furthermore, on-farm non-surgical embryo transfers may develop into as routine a reproductive manipulation as artificial insemination is today.

Hypothetically, these techniques may offer a means of bypassing the early post-ovulatory period of thermal sensitivity. For example, excellent quality embryos that are frozen could be transferred into recipients at day 7, when the embryo appears to be less sensitive to heat stress. Alternatively, embryos could be collected from heat-stressed donors, screened for quality and development, and only good quality embryos transferred to recipients that are not as sensitive to heat stress (e.g. non-lactating recipients). At present, management systems have not been developed that integrate reproductive management programmes with the environmental management system.

An additional alternative to attenuate the effects of thermal stress on reproduction and milk production is consideration of the genetic development of less heat-sensitive animals.

Practically all physiological and nutritional modifications to increase animal productivity also increase body heat production. Higher milk yield is apparently always associated with higher heat increment (heat of fermentation plus heat of nutrient metabolism). The metabolic heat load accounts for 40 to 50% of the total heat load requiring dissipation [45]. This must inevitably put higher yielding animals at a disadvantage in warm climate areas where thermal stress is a problem. Numerous reports indicate that environmental temperatures exceeding 27 to 29°C depressed milk yield. In general, higher yielding breeds (i.e. European dairy breeds) are more sensitive to thermal stress than lower producing *Bos indicus* cattle; one may assume that this is primarily due to increased body heat load associated with increased heat increments of high milk production. Selection for higher yielding cattle, which are also less sensitive to thermal stress under existing natural conditions, would not seem plausible physiologically.

Most, if not all, indices for measuring the efficiency of heat loss (rectal temperatures, respiration rates and sweating rates) do not have precise estimates of heritabilities. Turner [46] reported heritabilities of rectal temperatures of 0.25 ($n = 800$ to 900 ; SE 0.12), and its genetic correlation with fertility was -0.76 (SE 0.35). It was of interest that in Hereford $\times$ Shorthorn crosses the lower fertility of lactating cows versus dry cows was accounted for completely by their higher rectal temperature. However, in Zebu crosses, with either Hereford or Shorthorns, lactation had a large detrimental effect, independent of the negative effect of higher rectal temperature. In spite of these differences in the physiological state on mean fertility rates, all breed groups had parallel negative response curves between rectal temperature and fertility. Heritability estimates for other traits associated with heat loss and their genetic correlation with fertility and milk yield are not to be found in the literature. Heritability estimates obtained from small data sets have large sampling errors. Equations for estimating the standard errors for heritabilities are well known [47]. For example, to obtain heritability estimates with narrow confidence limits, e.g. 0.02 to 0.04, requires 500 families of 5 to 10 individuals each.

In beef cattle production systems it is of paramount importance to know whether genetic adaptation to the local environment is important in production. The classical studies of Koger et al. [48] indicated that there were clear genotype by environment interactions in Hereford cattle for pregnancy and weaning rates. In two unrelated lines of Hereford cattle that were developed in Florida (F_6) and Montana (M_1), compared at each location over 10 years, the advantages of local over introduced lines were 6.7 and 6.1% for pregnancy and weaning percentages, respectively. A second phase of the study was also to compare a foundation line of Montana Hereford cows that were subsequently selected in Florida (F_4) and tested in both locations. Time trends for pregnancy and weaning rates indicated that selection in Florida among Montana origin cattle (F_4 in Florida) resulted in positive effects on reproduction in

Florida. During the same period of time there was a negative trend in the pregnancy rate of F_4 cattle maintained in Montana. Simultaneously, there was a decline in pregnancy rate among M_1 cattle maintained in Florida. Although these environments were extremely different, responses indicated that genetic adaptability to local environmental conditions is important in beef cattle production. Furthermore, measurable improvement in genetic adaptation can be achieved through appropriate selection and culling procedures.

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UTILIZING GENOTYPE $\times$ ENVIRONMENT INTERACTIONS FOR IMPROVING THE PRODUCTIVITY OF CATTLE IN THE TROPICS*

J.E. VERCOE, J.E. FRISCH
Tropical Cattle Research Centre,
Division of Tropical Animal Science,
Commonwealth Scientific and Industrial
Research Organization,
Rockhampton, Queensland,
Australia

Abstract

UTILIZING GENOTYPE $\times$ ENVIRONMENT INTERACTIONS FOR IMPROVING THE PRODUCTIVITY OF CATTLE IN THE TROPICS.

Genotype $\times$ environment interactions occur because genotypes differ both in their inherent levels of productivity, which determine the maximum levels of productivity that are possible, and in their resistance to environmental stresses, which governs the proportion of that maximum which is actually expressed. The major environmental stresses considered are climatic factors, external parasites, internal parasites, diseases and variations in the quantity and quality of feed available. Improvements in productivity depend either on increasing the inherent levels of resistance while enhancing or maintaining the existing levels of inherent productivity, or on modifying the environment to control the stresses to enable a higher proportion of the inherent levels of productivity to be manifested. In extensive grazing systems the depressing effects of high air temperatures, humidity and solar radiation can best be reduced through the use of genotypes of high heat resistance. Controlling parasites and parasite-borne diseases by chemotherapy is limited by a number of factors. Prospects for the success of an immunological approach to the control of disease organisms through the use of improved vaccination methods and by the use of resistant genotypes are analysed. Ways of developing genotypes that have high resistance are discussed. The most intractable problem is the quantity and quality of the available feed. The possible role of supplementary feeding with non-protein N, rumen-soluble S and other minerals in increasing productivity is considered by comparing the responsiveness of different genotypes to these supplements under controlled conditions. Manipulating growth rates through use of growth promotants and metabolic depressants is compared with the potential advantages associated with use of genotypes that have an inherent ability to achieve the same result. The role of various strategies to improve fertility is discussed in relation to the genotype $\times$ environment interactions that are known to occur. An understanding of genotype $\times$ environment interactions in quantitative terms should enable decisions to be made regarding whether modification of the genotype or the environment is the appropriate course of action.

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1. INTRODUCTION

The components of cattle productivity in the tropics are calving rate, mortality, growth rate and the quantity and quality of the output of milk, meat and draught power. These components are determined by two sets of factors: one related to the inherent level, which is expressed in the absence of any environmental constraint, and the other to the resistance to environmental stresses. The latter governs the proportion of the inherent level that is expressed in a particular environment.

Different genotypes vary in both the inherent level of productivity and the level of resistance to environmental stresses. In the tropics the major environmental factors that mask and depress the expression of the inherent levels of the components of productivity are:

- (1) Climatic factors (e.g. high temperatures, humidity and solar radiation)
- (2) External parasites (e.g. ticks and other biting arthropods)
- (3) Internal parasites (e.g. gastrointestinal helminths)
- (4) Diseases (e.g. protozoal, bacterial, fungal, viral)
- (5) Variations in the quantity and quality of the available feed.

Genotype $\times$ environment interactions occur because in different environments genotypes express different proportions of their inherent productivity, depending on their level of resistance to the environmental stresses that are present in those environments. If the inherent productivity and the degree of resistance to particular environmental stresses are known for various genotypes, the most productive genotype or breed for an environment can be identified. In addition, if the responsiveness of various breeds to environmental modifications is known, rational and economically sound decisions can be made regarding the potential benefits resulting from changes to the environment and the genotype.

This paper deals with the nature and causes of genotype $\times$ environmental interactions with particular reference to growth rate and fertility, and how they may be exploited to improve cattle production in the tropics through either genetic or environmental modifications.

2. GENOTYPE $\times$ ENVIRONMENTAL INTERACTIONS

2.1. Heat stress

An increase in rectal temperature caused by climatic factors will depress growth rate and fertility in all breeds [1, 2]. However, the environmental conditions that cause the same increase in rectal temperature differ between breeds. Thus, although a rise in rectal temperature of 1°C has been shown to depress the growth rate of both heat-resistant Brahman and heat-susceptible British breeds by 0.04 kg/d when growth rates are ca 0.4 kg/d [1], the environmental conditions necessary to produce

such an increase are markedly different. For example, the rectal temperature of British cattle increased 0.2°C for every 10°C increase in mean radiant temperature compared with only a 0.04°C increase in Brahman [3].

There are similar effects on fertility. Marked differences have been reported between breeds in calving rate at a rectal temperature of 38.5°C , i.e. when the breeds were not heat stressed, but as the rectal temperature increased from 39 to 40°C the calving rate was depressed by 14% in a number of breeds and crosses [2].

Provision of shade, water and feed will only partly alleviate the deleterious effects of heat stress on the productivity of grazing animals. Shade reduces the environmental heat load and the rate at which rectal temperature rises. However, animals and breeds that seek shade have reduced grazing times and lower growth rates than those that do not [4]. Thus, in situations of high environmental heat load such as occur in most of the tropics and subtropics the only practical way of overcoming the reduction in productivity of grazing animals associated with an increase in rectal temperature is to utilize heat-resistant breeds. While in Australia and probably elsewhere the breeds of high heat resistance, e.g. Brahman and Africander, have lower inherent feed intake capacities than British and European breeds, they are able to exhibit a higher proportion of their lower potential in hot environments. Genetic improvement of the inherent level of production depends on increasing the feed intake capacity without simultaneously or significantly reducing the level of heat resistance that already exists. In situations where cattle are housed intensively for a considerable part of the day the need for breeds of high heat resistance is reduced. Shading to eliminate solar heat load perhaps combined with a cooling sprinkling system may make use of breeds with lower heat resistance and higher inherent productivity more economical than indigenous breeds that do not require any environmental modifications but have a low inherent productivity. Economic success depends on the relative rather than the absolute returns from each system.

2.2. Parasites

Breeds differ considerably in both their resistance to internal and external parasites and their response to the elimination or control of the parasites. Ticks (*Boophilus microplus*) and worms (gastrointestinal helminths) reduce productivity, primarily by depressing food intake [5, 6] and also by affecting digestion and metabolism [7]. The effect of these parasites on growth is related to the numbers carried, and generally breeds and individuals that have high resistance grow faster in the presence of the parasite than those that have low resistance. This is illustrated in Table I [8].

The Brahman was relatively resistant to both ticks and worms and in consequence grew faster than the susceptible Hereford $\times$ Shorthorn (HS) cross-bred when ticks and worms were uncontrolled and responded less to dipping and drenching. The BX, which is a stabilized F_n population of cross-breds derived from the Brahman $\times$ HS, responded more than the Brahman to treatment but not as much as the least resistant HS.

TABLE I. EFFECTS OF TICKS AND WORMS ON GROWTH RATE FROM BIRTH TO 15 MONTHS OF AGE FOR DIFFERENT CATTLE BREEDS [8]

Breed	Growth (kg/d)		Response to treatment (kg/d)	Ticks (No./side)	Worms (eggs/g)
	C ^a	T ^b		C ^a	
Brahman (B)	0.56	0.63	0.07	4	340
BX ^c	0.55	0.70	0.15	13	460
Hereford × Shorthorn (HS)	0.38	0.63	0.25	26	660

^a C = no treatment.

^b T = dipped and drenched every three weeks.

^c BX = stabilized B × HS.

Thus, given that breeds differ in their resistance to parasites and respond to different extents to treatment, decisions can be made about the most economical methods for improving productivity in parasite-infested environments. In the present example, the cost of obtaining the increased gain through chemical control of parasites must be weighed against the extra returns. Likewise, the economics of using a breed that has a higher resistance than the Brahman can be assessed. In extensive pastoral systems, use of breeds of high resistance is likely to remain an economically attractive option for the long-term control of parasites. If resistance has to be increased, the relative merits of cross-breeding to breeds of higher resistance or selection within existing breeds for higher resistance can be evaluated.

There appear to be no measurements of the direct loss to production due to tick infestation on indigenous African breeds, so estimates of the costs and benefits of acaricide treatment are difficult to ascertain. Among the exotic breeds and crosses in Africa regular use of chemicals has been the sole method of control. In Australia resistant strains of ticks are evolving with increasing frequency and the cost of acaricide protection is continuing to rise. Complete reliance on chemotherapy as the major method of control is therefore inadvisable, particularly in countries where production systems are based on low-input, low-output methods.

More information is needed on the comparative productivity and resistance to parasitic diseases of breeds of cattle in the developing countries of Africa and Asia. Without this information rational decisions cannot be made as to which breeds are likely to enhance resistance to parasites if used in cross-breeding programmes. Such assessments of resistance must be made on a strictly comparative basis under either

natural or artificial infestations. Similarly, if resistance is to be increased by within-breed selection for higher resistance it is essential that accurate and practical techniques for identifying superior individuals are available. This has been discussed in more detail elsewhere [9, 10]. However, there is no doubt that within-breed selection will increase resistance, both within breeds that are already relatively resistant [11] and within very susceptible breeds [12]. In herds of Hereford $\times$ Shorthorn cattle that have been selected mainly for growth rate in the presence of parasitic burdens, animals have been identified that rapidly acquire (first six weeks of life) complete resistance to *Boophilus microplus* [13].

There are some promising early indications that vaccination against ticks and worms may offer protection and that it may be technically and economically feasible to produce these vaccines through the application of biotechnology [14]. If vaccination does become an economically attractive option, and provided the target parasite cannot readily develop resistance to this immunological attack, there would be no need to seek or develop breeds of very high resistance to the major parasites. Increased attention could then be directed towards directly improving other components of productivity.

2.3. Vector-borne and other diseases

Genetic resistance exists to a variety of vector-borne and other diseases, e.g. babesiosis, trypanosomiasis, eye cancer, bovine infectious keratoconjunctivitis (BIK) and dermatophylosis. While there is no point in seeking the nature or existence of genetic resistance to diseases that can be controlled readily by immunization, the case for greater utilization of trypanotolerant breeds in the tsetse belt of Africa is a strong one and has been made forcefully elsewhere [15].

2.4. Variations in nutrition

In most of the tropics and particularly in developing countries, the most difficult environmental constraint to productivity that needs to be overcome in both the short and long term is that of shortage of feed.

In the traditional African grazing systems of animal production, expansion of cropping into marginal areas and onto traditional grazing lands, associated with the rapid increase in human population and below average rainfall, has led to increases in stocking pressure and degradation of rangelands. In more intensive agricultural systems such as those in parts of South-East Asia reduction in farm size has also contributed to reduced availability of livestock feed. These deficiencies in feed quantity are more difficult to overcome than any deficiencies in quality of standing dry feed.

One way in which a chronic shortage of feed could be combated is to use animals with a low maintenance requirement. This may be associated with a small body size or with a high efficiency of maintenance. Where animals of small body size are undesirable, larger animals of high efficiency of maintenance might be sought.

TABLE II. BREED $\times$ NUTRITION INTERACTION [8]

Breed ^a	Ad libitum		Restricted
	Intake (g·kg ⁻¹ ·d ⁻¹)	Gain/d (kg)	Relative weight maintained (%)
B	27.8	0.84	100
BX	28.5	0.94	93
HS	33.1	1.04	86

^a See Table I.

There are genetic differences between breeds in maintenance although, in general, breeds that have a high efficiency also have a low inherent feed intake capacity and consequently do not grow as well when feed is plentiful as breeds with a higher maintenance requirement. This latter feature is seldom a disadvantage in low-input, low-output systems. The interaction is illustrated in Table II [8]. The Brahman breed which has the lowest feed intake capacity and gain when fed ad libitum maintains a higher weight when fed a fixed amount of the same feed.

The extent of genetic variation in the efficiency of maintenance between indigenous breeds in developing countries is not known. However, it is almost certainly present and should be particularly pronounced between breeds that have evolved in arid areas and are used predominantly for meat and draught and those that are kept predominantly for milk in more benign areas. The prospects for genetically manipulating maintenance requirement while maintaining or increasing both the size and milk yield have not been investigated. Perhaps the best prospects for increasing milk production without concomitant increases in maintenance requirement could be achieved by reducing body size.

In environments where there is a marked seasonal variation in the quantity and quality of feed, as in most tropical grazing areas, the type of animal required is one that has a high feed intake capacity and grows rapidly when feed is plentiful, but has a low maintenance requirement and a fast rate of reduction of metabolic rate when the quantity and quality of feed deteriorate in the dry season. Such breeds and individuals do exist. For example, the breeds of Southern Africa, as represented by the Africander and its derived breed the Belmont Red in Australia, appear to have a similar feed intake capacity to that of the Hereford $\times$ Shorthorn (HS) on good quality pasture hay but they can reduce the fasting metabolic rate, after being fed low quality roughages, to that of the BX [16]. An alternative approach to achieve these

desirable responses to variations in the feed supply is through use of growth promotants and metabolic depressants, e.g. anabolic steroids and other hormonal manipulations as yet undefined.

Efficient utilization of crop residues, by-products and other low quality roughage feeds will depend on cheap sources of supplements to stimulate rumen function and increase intake of these materials and on breeds that respond to supplementation. The addition of rumen soluble nitrogen (N) and sulphur (S), for example, has an effect on feed intake that depends on both the basal diet and the genotype of the animal. This is illustrated in Table III [17, 18]. The intake response of the breed with the relatively low inherent feed intake capacity (Brahman) to N and S supplementation was less than the breed with the higher inherent feed intake capacity (Hereford), and the response of each breed was greater on the pangola grass hay than on the spear grass hay. Presumably these sorts of differences also exist where the basal diet is crop residues.

Despite occasional reports to the contrary, most evidence suggests that there are no consistent differences between cattle breeds in their ability to digest organic matter or nitrogen in roughage diets. However, there is good circumstantial evidence that the chemical composition of the rumen microorganism synthesized in the rumen of Brahman and Hereford cattle may differ [19]. It has been suggested that the differences in lipid metabolism between *Bos indicus* and *Bos taurus* [20] have their origins

TABLE III. EFFECTS OF RUMEN SOLUBLE N AND S ON FEED INTAKE (g/kg LIVE WEIGHT) AND RATE OF RUMEN DIGESTION OF LOW QUALITY ROUGHAGE BY DIFFERENT BREEDS [17, 18]

Breed	Spear grass		Pangola grass	
	US ^a	S ^b	US ^a	S ^b
<i>Intake (g/kg)</i>				
Brahman	11.8	10.6	16.1	18.5
Hereford	11.3	14.0	17.8	25.2
<i>Rate of digestion (k)^c</i>				
Brahman	0.031	0.032	0.045	0.066
Hereford	0.020	0.032	0.023	0.066

^a US = unsupplemented.

^b S = supplemented.

^c k = rate constant (change in rate of digestion per unit time).

in the rumen and result from a higher lipid content of microorganisms in the rumen of *Bos indicus* breeds. Lipid is utilized with a higher energetic efficiency than other substrates and a higher proportion of nutrients absorbed from the gut as lipid could be a contributory factor to the lower maintenance requirement of Brahman cattle. In addition to these likely differences in the composition of rumen microorganisms between different breeds, there may also be differences in the proportion of microorganisms as protozoa, bacteria and fungi that may be functionally important and capable of manipulation to improve feed utilization and productivity [21].

No differences have been reported in the relative susceptibility of different breeds to plant toxins such as dihydroxy pyridine (DHP), a metabolite of mimosine. However, the incidence of lantana poisoning has been observed to be greater in Hereford $\times$ Shorthorn cattle that carried large tick burdens than in similar cattle in which ticks had been controlled or in tick-resistant cattle [22]. In the sense that the effect of one environmental stress increases the effect of another environmental stress, there may be an interaction between genotype and plant toxins.

Although no published data are available on whether different breeds of cattle respond differently to growth promotants, particularly anabolic steroids, some preliminary results from the Tropical Cattle Research Centre, Rockhampton, suggest that this might be the case. In the presence of environmental stresses the response to anabolic steroids has been greater in Brahman and Brahman $\times$ HS than in HS animals. The response of the HS to growth promotants increased when they were treated to control ticks and worms, but this did not occur in more parasite-resistant Brahmans.

The genotype $\times$ nutrition interaction should be assessed and utilized to maximize returns from particular production systems.

3. GENOTYPE $\times$ ENVIRONMENT INTERACTIONS IN FERTILITY

The fertility of different breeds responds differently to environmental changes, as illustrated in Table IV [23]. Except when environmental conditions are particularly severe, they have only a minor influence on the fertility of non-lactating cows of both adapted and unadapted breeds. There is, however, a marked effect on lactating cows that varies with breed. The environmental factors that contribute to the 'above' and 'below' average years, undefined in this study, caused a marked change in the fertility of the unadapted HS but had a relatively small effect on the well adapted Brahman breed. Brahmans are apparently inherently more susceptible to lactation anoestrus than breeds of British or European origin, but the underlying neuro-endocrinological factors responsible for this have not been identified.

Table IV suggests that while improvements to the environment are likely to be successful in increasing fertility in breeds with a high reproductive potential, in breeds such as the Brahman a more fundamental approach will be necessary to either

TABLE IV. GENOTYPE $\times$ ENVIRONMENT INTERACTION FOR FERTILITY [23]

Breed ^a	Calving (%)			
	Above average years ^b		Below average years ^b	
	Lactating	Non-lactating	Lactating	Non-lactating
B	40	77	34	76
BX	55	76	45	71
HS	78	78	47	75

^a See Table I.^b Mean of 10 years of records; overall mean 61%.

modify the genotype or compensate for a hormonal insufficiency by direct intervention. Past experience has shown that genetic improvement through mass selection is likely to be ineffective. However, application of new statistical procedures such as BLUP, which utilize information from performance pedigrees and increase the accuracy of selection, may increase the chances of success. Another avenue worth exploring is very intense selection of females for the absence of lactation anoestrus. To enable intense selection, multiple ovulation and embryo transplant technology need to be applied. Alternatively, if well adapted breeds that have a high potential fertility can be identified they could be used in cross-breeding or upgrading programmes.

Where the reduced fertility of a breed can be linked to a hormonal insufficiency or imbalance the possibility of using hormonal therapy to redress the imbalance exists, either by slow release implants or hormone immunoneutralization to disrupt feedback mechanisms, e.g. the principle of use of Fecundin in sheep to increase the ovulation rate [24] may be applied to lactating cattle to initiate fertile cycles. Current research on releasing hormones holds promise for improvement in the control of breeding cycles in cattle that are susceptible to lactation anoestrus.

4. CONCLUSIONS

This paper has presented evidence for a number of genotype $\times$ environment interactions that result in the relative performance of different genotypes, measured as the output of milk, meat and draught, differing in different environments.

It is important to evaluate the performance of different breeds on a strictly comparative basis, not only in terms of the direct components of productivity such as growth, reproductive rate, milk production and draught power output, but also for the factors that determine these components such as heat, parasite and disease resistance, and feed utilization including the efficiency of maintenance (see Ref. [25]). It is also important that evaluation of these factors be carried out in contrasting environments in order that the response of a breed to a change in environmental conditions can be assessed as well as the relative merit of different breeds to a fixed set of conditions. Environment in this sense includes not only the broad physical environment but also the microenvironment in a physiological sense. In other words, contrasting environments may be established by geographical separation but, more importantly, by such experimental procedures as dipping and drenching, feed supplements, and hormonal treatments to modify growth or reproduction in order to measure how different breeds respond to the sorts of options that may be implemented to improve productivity in particular production systems.

It is only when these characteristics have been assessed for a variety of genotypes that practical decisions can be made regarding the desirability and likely effectiveness of particular strategies to improve livestock productivity.

There are only two avenues by which productivity can be increased: by changing the genotype or modifying the environment. Changing the genotype will only be effective if the new inherent potential can be expressed and can be realized in the presence of the existing environmental stresses. Changing the environment to improve productivity assumes that the genotype is able to respond by manifesting more of its inherent potential. If the genotype is changed its level of resistance to the environmental stresses that are operating must not be decreased. If the environment is changed some other genotype may respond more to that change than the existing one. If both the genotype and the environment are to be changed simultaneously the most appropriate combinations can be selected to ensure the maximum returns for the minimum inputs.

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USE OF ISOTOPES IN THE ASSESSMENT OF ANIMAL RESPONSES TO THE ENVIRONMENT*

D. ROBERTSHAW

Department of Physiology and Biophysics,
College of Veterinary Medicine and
Biomedical Sciences,
Colorado State University,
Fort Collins, Colorado,
United States of America

Abstract

USE OF ISOTOPES IN THE ASSESSMENT OF ANIMAL RESPONSES TO THE ENVIRONMENT.

A great source of economic loss of domestic ruminants and other food-producing species occurs as a result of maladaptation to the thermal environment. Furthermore, the introduction of high producing species into harsh environments has usually resulted in severe depression of their genetic potential. Therefore, it is important to understand the factors that enable an animal to thrive under harsh environmental conditions, particularly those associated with heat stress. The water loss of any animal must be matched by the water intake and therefore it is important, particularly under range conditions, to know the water requirements of an animal. Tritiated water can be used for this purpose. This isotope is relatively safe to use and can be applied under field conditions. Of the main avenues of water loss, i.e. faeces, urine and evaporation, the animal can make only slight adjustments to its urinary and faecal loss; the evaporative component, therefore, is the most variable. Changes in evaporation are largely associated with the need to use evaporative heat loss to maintain a constant body temperature. Therefore, tritiated water, which can be used to measure the water turnover rate and give an index of water requirements, can also be used to provide an indication of the degree of heat stress and the need to use water for evaporative heat loss. Such measurements can be made under free range conditions. Certain assumptions have to be made in order to ascertain these calculations, but tritiated water has proved to be a very useful tool in identifying those individuals and species which are best adapted to their thermal environment, and which are therefore the most productive.

1. INTRODUCTION

Domestic animals that are adapted to their natural environment, i.e. one that allows them to survive and reproduce, cannot be said to be environmentally stressed. However, man, in the course of his close association with domestic animals, has altered not only their natural environment but also the expression of their normal behavioural pattern. For example, in hot environments it may be normal for an animal to graze in either the morning or the evening and seek shade during the heat

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of the day. However, because man may only allow an animal to graze during the daylight hours and confine it during the night-time hours, the animal is forced to be exposed to the harshness of the thermal environment. The animal, therefore, is unable to respond to its environment in an appropriate manner and is, to some extent, stressed by this management practice. In many parts of the world, these types of management practices have been in existence for many hundreds of years. It is possible, and indeed likely, that the animals have become physiologically adapted to this management practice but they are still unable to express their full genetic potential for production. In addition, in this century there has been a practice of relocating animals to less productive parts of the world. Such animals have been developed for high production, whether it be meat, milk or fibre, or a high reproductive performance in terms of either the frequency of their reproductive cycles or their fecundity. Invariably the less productive parts of the world have been the developing countries located in the tropical and subtropical regions of the world, and animals have been moved from temperate regions without a clear understanding of the physiological consequences of such a move. The results have been disappointing in that such animals fall far short of achieving their full genetic potential. The reasons for this are many. Invariably the quality of the food is less than optimal, it may be high in fibre and low in nitrogen; animals have no innate resistance to the diseases to which they become exposed; animals are maladapted to the high temperature and humidity. Radioisotopes can be used to study the physiological responses of domestic animals to an environment to which they are not adapted, whether it be the adverse nutritional environment or the impact of the thermal environment on the productive process. One of the most useful isotopes for such studies has been the use of tritiated water for studying aspects of production and adaptation in ruminants. Its use was the topic of a publication of the International Atomic Energy Agency in 1982 [1]. This review summarizes the use of tritiated water in interactions between animals and their thermal environment.

2. NATURE OF THE THERMAL ENVIRONMENT

It is a feature of homoeothermic animals that they maintain a constant body temperature, either by physiological or behavioural means, over a wide range of environmental temperatures.

The heat that has to be dissipated is derived from two sources: (1) the heat produced by metabolic energy transformations, and (2) the heat absorbed from the radiant environment. Most experimental work involves exposing animals to environments in which the air temperature is controlled. As the air temperature rises, the ability to lose heat by non-evaporative means declines, so that when air temperature is equal to body temperature the transfer of heat is zero (Fig. 1 [2]). Environments in which air temperature exceeds body temperature are not widely distributed throughout the world, and in most situations such air temperatures exist for a limited

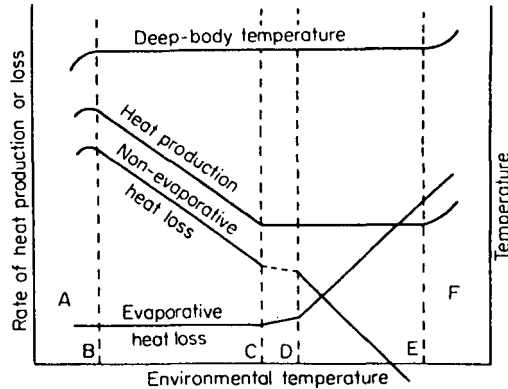


FIG. 1. This diagram defines the various avenues of heat loss and heat production over a range of environmental temperatures. The temperature of the environment is not simply air temperature but is a non-dimensional index of the total thermal environment which includes the solar heat load. If there is no solar heat load, then air temperature is a reasonable measure of environmental temperature. The range of temperatures between C and D is the zone of minimal thermoregulatory effort being defined by the lower critical temperature (C) below which heat production must be increased and the upper critical temperature (D) above which evaporative heat loss must be increased in order to maintain a constant body temperature. The zone will vary with the degree of adaptation or acclimatization. Animals which are neither genetically adapted nor non-acclimatized to a hot environment may spend a considerable amount of time at environmental temperatures above D, that is, they will be 'heat stressed'. Temperatures above E or below B define the extremes of the system and unless some relief is provided will result in death [2].

season and for a limited time each day. The natural environment is much more complex, since significant radiant heat exchanges take place. In the case of an animal standing in the shade, the net flow of heat by radiation is away from the animal [3]. However, an animal standing in the sun is subject to solar radiation and the amount of heat absorbed from the sun depends in part on the structure of the fur and in part on the colour of the fur. For example, animals with fur in which the hair extends in many directions will present a relatively rough surface to the incident solar beam which is then dispersed within the coat; the degree of dispersion is a function of the coat colour in that in white fur there will be a greater reflection into the coat and the heat will be absorbed closer to the skin than it would be in a black coat where most of the heat would be absorbed at the fur surface. Most animals that are genetically adapted to the tropics have a relatively short and dense fur with the hair lying in one direction, giving it a sleek appearance. Under these circumstances, animals with a white coat will reflect back more of the incident solar beam than animals with a dark coat. Thus, coat structure is critical in assessing the significance of coat colour. Table I shows some measurements made by Finch and Western [4] in which it can be seen that the heat absorbed at the fur surface is greatest in a black animal and least in a white, with brown animals being intermediate. Heat absorbed at the fur surface

TABLE I. MEAN VALUES ($\pm$ SE) OF THE HEAT LOAD ON CATTLE OF DIFFERENT COLOURS BETWEEN 09:00 AND 15:00 h STANDING AT A POSITION APPROXIMATELY 160 km SOUTH OF THE EQUATOR. MEASUREMENTS ARE EXPRESSED IN W/m^2

	Radiation absorbed	Sensible heat loss	Inward sensible heat flow
Black	691 $\pm$ 14	573 $\pm$ 7	118 $\pm$ 24
Brown	630 $\pm$ 6	531 $\pm$ 7	99 $\pm$ 17
White	549 $\pm$ 14	499 $\pm$ 8	50 $\pm$ 15

TABLE II. HEAT BALANCE OF A STEER (*Bos indicus*) MEASURED AT NOON IN A LOCATION APPROXIMATELY 160 km SOUTH OF THE EQUATOR. MEASUREMENTS ARE IN W/m^2 AND ARE DIRECT MEASUREMENTS. ERRORS IN THE MEASUREMENTS LEAD TO THE HEAT LOAD NOT EXACTLY EQUALLING THE HEAT LOST. (Figures in parentheses are percentages of the total heat dissipated by the various avenues of heat loss [5])

Metabolic heat gain	Radiant heat gain	Reradiated heat loss	Convective loss	Evaporative heat loss	Heat storage
50.7	638	397.3 (57.7)	63.6 (9.2)	182.3 (26.4)	7.6 (1.1)

can be either reradiated back to the atmosphere or dissipated by convection. Both routes of heat loss are termed sensible heat loss and the difference between the radiation absorbed and the sensible heat loss is the inward sensible heat flow. A white animal receives only 10% of the radiation absorbed, whereas it is approximately twice as much for a black animal. The inward sensible heat flow together with the heat of metabolism must be dissipated by evaporative means. Another feature of animals adapted to hot environments is that they have a relatively low metabolic rate and therefore a low internal heat load.

Table II shows the heat balance of a Zebu steer (*Bos indicus*) broken down into the various components of heat load and heat loss. In this instance, approximately 25% of the total heat load is dissipated by evaporation [5]. Thus, it can be seen that an animal not adapted or acclimatized to hot environments and subjected to a high heat load will require more water to dissipate that heat load than one which is naturally adapted to a hot environment. Thus, the physical and physiological features of animals adapted to hot environments are: (1) a relatively low metabolic rate and therefore a low internal heat load; (2) a short, sleek and lightly coloured coat which reduces the inward sensible heat flow.

3. WATER BALANCE

Animals have physiological homoeostatic mechanisms which balance water intake with water loss and ensure a constant body water volume. In addition to water taken via the thirst mechanism, water originates from water in the food as well as water derived from metabolic reactions. Should water in the food and metabolic water be sufficient for water loss, then the animal will show no thirst and will not drink water. Water is lost from an animal in the urine and faeces and by evaporation. In the non-heat-stressed animal water loss by evaporation from the skin and respiratory tract greatly exceeds that from the urine and faeces [6]. However, as heat stress increases and an animal is required to use water for evaporative cooling, then evaporative water loss becomes an even more significant component of water loss. Water turnover rate is a term used for measuring either the total water intake or the total water loss and can be determined using tritiated water (TOH). Since evaporation is the main mode of water loss, and since evaporation is increased in heat stress, determinations of the water turnover rate will provide an index of heat stress.

4. MEASUREMENT OF WATER TURNOVER USING TOH

Although tritium is radioactive and has a relatively long half-life, its emission of beta particles has a relatively low energy value; it is therefore safer to use than some other forms of radioactive isotopes when used in tracer amounts. Where there are adequate sewage disposal systems, the amount of tritium used in animal experiments is small enough to represent only a very minor public health hazard. However, since tritium is radioactive, certain precautions must be taken and the isotope must be handled by acceptable procedures. TOH has the advantage that its specific activity is fairly readily determined from samples of body fluids, whether they be blood or urine. Indeed, Springell and Wright [7] have validated a method for measurement of TOH in plasma by applying plasma directly to the liquid scintillation fluid.

If the assumption is made that TOH is biologically indistinguishable from unlabelled water (HOH), then the dilution of a known amount of TOH administered to an animal gives a measure of the total body water. Once this has been determined, addition of any further HOH will dilute the total body water pool at the rate determined by the water intake, and if the body water volume remains constant, the water intake will equal the water loss. Thus, the rate of dilution of TOH will be a measure of water turnover. TOH can theoretically be used to measure not only the total body water but also the water turnover rate. From a knowledge of the water turnover rate several derivatives of either water intake or water loss can be determined. Since water intake is considered to be the sum of water drunk, water in food and metabolic water, then a measure of water turnover combined with a measure of two of the intake parameters will lead, by difference, to a measure of the third. The technique of water turnover using TOH has been used to determine the food intake of grazing

animals. Thus, if the water content of food is known, the amount of free drinking water measured and the metabolic water predicted from metabolic body weight, then the total water intake provided by the food can be calculated, and the dry matter intake determined. Dry matter intake measured from TOH turnover will only be precise when food water is a significant part of water intake, i.e. animals grazing lush pastures. Likewise, if the urine and faecal water losses are known, the evaporative water loss can be calculated.

5. ESTIMATION OF TOTAL BODY WATER

TOH can be administered either by mouth or by injection. Adequate time for mixing of the TOH within the body HOH pool is necessary before a sample is withdrawn for total body water determination. The change in blood concentration following intravenous administration of TOH is shown in Fig. 2. Equilibration occurs when TOH concentration remains relatively constant. Since mixing of the TOH is dependent on blood flow for distribution throughout the body, it is obvious that intravenous administration would lead to the fastest rate of equilibration. In ruminants, however, the rate of equilibration with the water in the rumen is relatively slow and probably takes place via the saliva rather than across the rumen wall. After intravenous injection in sheep, the specific activity of rumen contents may not reach equilibration until approximately 8 hours after administration [8]. However, equilibration can be achieved some 5 hours after administration if the TOH is administered both intrarumenally as well as intravenously [8]. Equilibration following either intramuscular or subcutaneous administration of TOH will take even longer. Theoretically, intravenous administration of TOH could lead to an initial high blood concentration of the isotope that could be filtered in the kidney before equilibration is complete and lead to loss of the isotope. However, experimentally,

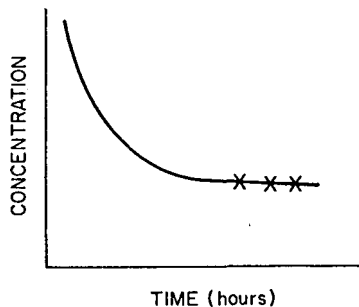


FIG. 2. Changes in concentration of TOH in blood after intravenous administration of TOH. The crosses indicate times of sampling.

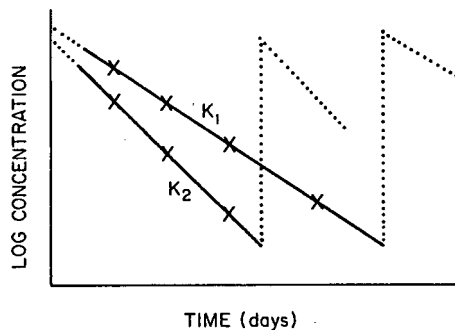


FIG. 3. Changes in concentration of TOH with time for two animals with differing turnover rates (K_1 and K_2). A second injection of TOH (dotted lines) is necessary if a change in total body water is suspected between the beginning and end of the experiment. The crosses indicate times of sampling.

Smith and Sykes [8] were unable to substantiate the fact that intravenous administration introduced any significant error in the technique. During the equilibration period it is useful to reduce the water intake to a minimum, since the time for equilibration will be prolonged by the need for the ingested HOH molecules to become evenly distributed throughout the body. Therefore, access to water and food should be denied during the equilibration period as well as for a short period before administering TOH. In the case of a ruminant, the easiest way to overcome these difficulties is to deprive the animal of food and water for approximately 2 hours before administration of the TOH, which can then be given by intramuscular injection. The animal should be confined in a relatively cool environment without food or water overnight for 12 to 16 hours. A urine or blood sample is then taken first thing in the morning at which time equilibration should be complete. From calculation of the dilution, the total body water can be determined. However, since the animal has not had access to water overnight, and since the animal is constantly losing water during the equilibration period, the specific activity of TOH will give a measure of the total body water at the time of sampling and not at the time of administration. If the animal now drinks and replaces the fluid lost during the period of equilibration, a new value of total body water close to that at the time of injection would be obtained, but a further 5 to 8 hours for equilibration would be required. To overcome this difficulty, an alternative method of total body water measurement is applied using extrapolation to zero time. In this method, the concentration of TOH is measured by serial sampling over a period of days and the theoretical concentration at the time of administration is calculated by extrapolating these values to zero time (Fig. 3). In studies on grazing animals, the extrapolation technique has the advantage that the animal can be allowed to return to its natural or normal lifestyle immediately after administration of the isotope. It does, however, require several samples to ensure a sufficient number of points for accurate extrapolation. Thus, if only a few samples are taken small differences in the slope of the regression, relating specific activity of TOH with time, could cause major errors in estimation of the total body water.

6. MEASUREMENT OF WATER TURNOVER RATE

As molecules of HOH are added to a mixture of TOH and HOH the concentration of TOH in HOH will decline exponentially (Fig. 3). Under a set of environmental conditions which do not vary to any major extent from day to day, the rate of water loss, and therefore the rate of water intake, will be constant. However, for the rate of decline to be a measure of body water turnover it is essential that the total body water does not change between the time of administration of the isotope and the time of the final sample. If a change has taken place, then a correction for the change in total body water needs to be made. It is therefore advisable to measure total body water at the beginning and at the end of the experimental period, as shown in Fig. 3.

7. BASIC ASSUMPTIONS AND POSSIBLE ERRORS

One of the basic assumptions associated with use of TOH to study water metabolism is that the tritium in TOH is not incorporated into other compounds. However, this is not so and Lewis and Phillips [9] have calculated that approximately 3% of the body weight may contain bound tritium. The magnitude of the error resulting from tritium incorporation is less likely to be of significance when the equilibration method of total body water measurement is used since there is little time for TOH incorporation to occur, but it has been reported to overestimate total body water by 1.2% [10].

Another assumption is that water lost from the body has the same concentration, i.e. specific activity, of TOH in HOH as that of body fluids. However, many authors have noted that evaporative water may have a lower specific activity than that of body water [11], and that there is, therefore, over a period of time a net retention of TOH. The actual error due to fractionation of the isotope will depend on the degree of fractionation and on the proportion of total body water loss due to evaporation. A partial explanation of the phenomenon of fractionation is that the vapour pressure of TOH is approximately 93% of HOH at body temperature [12]. If fractionation is greater than 93%, then some other source of HOH must be contributing to the low proportion of TOH in evaporative water. In some recent experiments, Robertshaw and Zine-Filali [13] have examined the cutaneous water loss and measured the specific activity of evaporated water from the skin before and after stimulation of the sweat glands. They noted that the percentage of TOH in HOH before sweating was approximately 70% before and 93% after initiation of sweating. These results suggest that the extra HOH molecules, which give rise to a low specific activity of evaporated water, come from water absorbed from the atmosphere in the outer layers of the skin. The fractionation, therefore, is in part an artifact of the technique, although it is still a reality due to the differing physical properties of TOH and HOH.

The possible interchange of HOH in the ambient air with that of the body water pool is a much more serious error in the technique. Animals inspiring air of high humidity could potentially be provided with an alternative source of HOH which is not part of the true water intake. Nagy and Costa [14] have initially investigated this problem and their results using the kangaroo rat (*Dipodomys merriani*) show that the error in water turnover is proportional to the ambient humidity. At zero humidity, TOH turnover underestimated water turnover, probably due to fractionation of evaporated water.

8. USE OF THE TECHNIQUE

It is obvious that application of TOH in studies of animal production is a useful technique for understanding the reactions of domestic animals to their environment under natural conditions. Measurements of the total body water can be used for determination of the body composition and water turnover gives a measure of both the water requirements of free ranging animals as well as their water losses. In addition, measurements of certain components of water gain or water loss lead to the determination of individual components of water exchange.

9. WATER TURNOVER AND THE ENVIRONMENT

As indicated, evaporation is the major mode of heat loss and is influenced by the level of heat stress. Figure 4 represents some data from King et al. [15] where

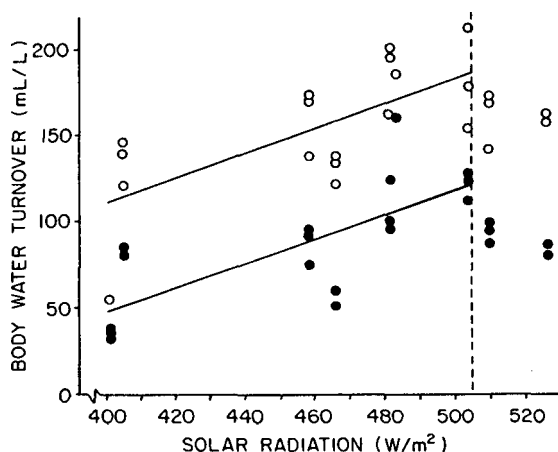


FIG. 4. Relation between water turnover rate and intensity of solar radiation in the eland (o) and the oryx (●) (from King et al. [15]).

they related body water turnover to solar radiation in two species of East African antelope, the eland and the oryx. It will be seen that up to a solar heat load of 510 W/m^2 good correlation exists. At heat loads in excess of this there appears to be a decline in body water turnover, which may reflect maximal evaporation and use of heat storage as a strategy to combat heat stress. It is worth noting that the intercept is different for the two species; the oryx lives under xeric conditions very often in areas where there is no free drinking water and probably has better urinary and faecal water conservation mechanisms than the eland which inhabits a more semi-arid type of habitat.

10. CONCLUSIONS

Use of TOH for studies of the response of animals to hot environments has great potential, but the amount of data that can be obtained from this relatively simple technique goes beyond animal-environment interactions and extends to studies involving many other parameters which are significant in studies of animal production.

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FLUID BALANCE AND UREA RECYCLING DURING PREGNANCY AND LACTATION IN SMALL RUMINANTS*

S. BENLAMLIH, M. OUKESSOU

Département de physiologie et thérapeutique,
Institut agronomique et vétérinaire Hassan II,
Rabat, Morocco

Abstract

FLUID BALANCE AND UREA RECYCLING DURING PREGNANCY AND LACTATION IN SMALL RUMINANTS.

Studies on the physiological adjustments of water balance and urea kinetics during pregnancy and lactation in small ruminants are described and discussed in relation to adaptation to arid zones. The water intake increases markedly during the course of pregnancy and, in parallel, the urine volume increases and the urine osmolality decreases. These changes persist during lactation, but to a lesser extent. The increased water turnover appears to be caused by a decrease in the water storage capacity of the rumen during pregnancy and by a lower ability of the kidney to concentrate the urine both during pregnancy and lactation. Recycling of urea into the digestive tract increases during pregnancy and lactation and, in parallel, the rumen ammonia concentration is elevated. The increased renal conservation of urea during pregnancy and lactation is achieved by a rise in the renal tubular reabsorption of urea. Thus, pregnant and lactating small ruminants are more susceptible to a shortage of water but can make better use of low protein vegetation than non-pregnant, non-lactating animals.

1. INTRODUCTION

Small ruminants are important components in the agricultural systems of arid regions. Most of the sheep and goats graze extensive areas and are therefore subjected to the severe effects of climate. During the long dry season the environmental constraints on productivity are sparse vegetation which is high in fibre and low in nitrogen, low availability of water and extreme variations in air temperature.

Pregnancy and lactation are critical phases of the production cycle and impose considerable demands on water, energy and nitrogen. In arid zones this situation is aggravated by the additional stresses of low water availability and the low nitrogen content of food. To fully utilize and exploit this situation in the most efficient manner, it is essential to know and understand the physiological adjustments of the water and nitrogen balances which occur during pregnancy and lactation.

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2. WATER BALANCE STUDIES

It is well known that water intake increases during pregnancy and lactation in sheep [1-3] and recent studies carried out on pregnant and lactating goats have shown that augmented water intake is accompanied by excretion of large amounts of diluted urine [4]. The studies presented in Table I show that similar changes in water balance occur in sheep. The water intake increased progressively and reached a maximum during the last month of pregnancy. During lactation, water intake was related to milk production. In parallel to the increased water intake, the urine volume increased and the urine osmolality decreased during pregnancy (Table I). Urine osmolality remained low during lactation.

Determination of the total body water using tritiated water (TOH) showed that it increased during the course of pregnancy by 4 to 8 litres and then returned to the initial level during lactation. These results show that part of the additional water consumed during pregnancy is retained within the body fluids but that most is excreted in the form of diluted urine.

The increased water turnover during pregnancy may be due to primary polydipsia or to enhanced loss of water via the urine, leading to secondary polydipsia. Because animals in arid zones are faced with the problem of water availability, it is important to know if the physiological mechanism controlling the water economy capacity changes during pregnancy and lactation.

TABLE I. WATER INTAKE, URINE VOLUME AND URINE OSMOLALITY OF THE SAME EWES DURING THE NON-PREGNANT, NON-LACTATING PERIOD (CONTROL), THE LAST 3 MONTHS OF PREGNANCY AND LACTATION

	Control period	Pregnancy			Lactation
		Month 3	Month 4	Month 5	
Water intake (L/d)	1.8 ± 0.1	2.4 ± 0.1	2.8 ± 0.2	3.0 ± 0.3	2.4 ± 0.2
Urine volume (L/d)	0.6 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.6 ± 0.2	0.8 ± 0.1
Urine osmolality (mosm/kg)	1270 ± 100	1030 ± 90	990 ± 100	820 ± 100	900 ± 110

Mean ± SE; N = 11 ewes; mean body weight: 31 kg.

3. WATER ECONOMY CAPACITY DURING PREGNANCY AND LACTATION

The surface grazed by the animals around water sources is determined by their capacity to store large amounts of water and to limit body water loss. This ability appears to decrease during pregnancy and lactation. As shown in Fig. 1, in all experiments intraruminal administration of 3 litres of water in sheep induced water diuresis. This response was strong and precocious during the last month of pregnancy compared with non-pregnancy states. Similar reactions have been observed in goats by Olsson et al. [5]. Fast excretion of the water load during pregnancy may be caused by a lower capacity of the rumen to store water and/or by a lower ability of the kidney to concentrate urine.

3.1. Rumen water storage capacity

The rumen constitutes a reservoir of water in which large volumes of drinking water can be stored. An increase in rumen water outflow into the larger absorptive surface of the intestine may increase the digestive absorption of water [6]. During pregnancy the rumen volume is reduced, probably due to compression of the gravidic uterus [7, 8]. It is thought that the rates of rumen water outflow and digestive absorption of water increase; therefore, the water storage capacity of the rumen may be reduced in pregnant small ruminants. In an experiment carried out to measure the rumen volume using polyethylene glycol as a non-absorbable marker (Fig. 2), 3 litres of water were administered into the rumen and the rumen volume was measured during the subsequent 3 hours. It was shown that in pregnant ewes an equivalent of 3 litres of water load were emptied out of the rumen within 1 hour. In contrast, during lactation and the non-pregnant, non-lactating (control) period, the rumen volume remained above the prehyperhydration level 3 hours after the water load.

In a similar experiment, the water load administered into the rumen was labelled with TOH. The kinetics of plasma TOH expressed as a per cent of the steady-state value is shown in Fig. 3. Clearly, the rate of appearance of TOH in the plasma was higher during the last month of pregnancy than during the control period. The steady state was approached at 4 and 6 hours after intraruminal administration of labelled water during pregnancy and the control period, respectively. In lactating sheep, the rate of absorption of TOH was not significantly different from the control period (Fig. 3).

These experiments indicate that during pregnancy the intake of water is followed by higher rumen fluid outflow and fast water absorption, leading to enhanced renal loss. This may explain the high water turnover that took place during pregnancy.

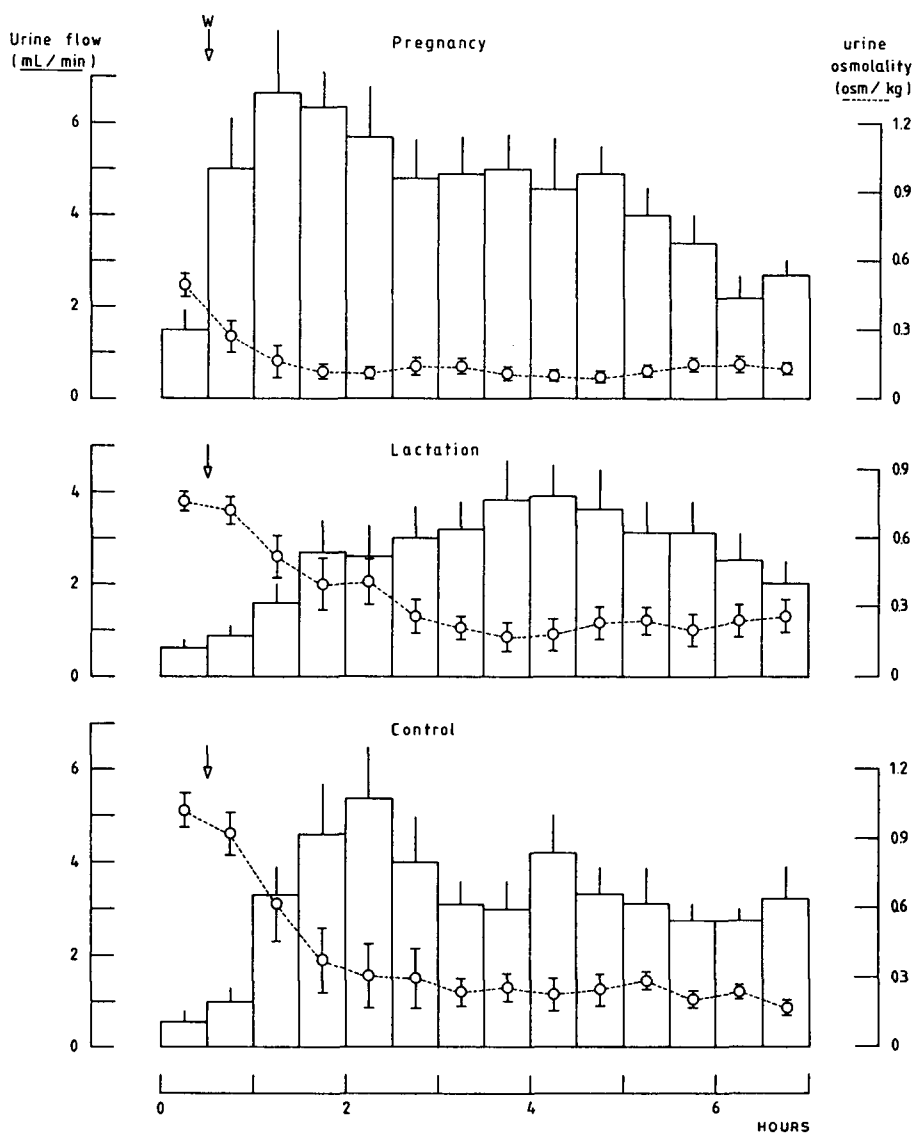


FIG. 1. Effects of hyperhydration (W indicates administration of 3 L of warm water into the rumen) on urine flow and urine osmolality during pregnancy, lactation and the non-pregnancy, non-lactation (control) period. (N = 4 ewes; mean $\pm$ SE).

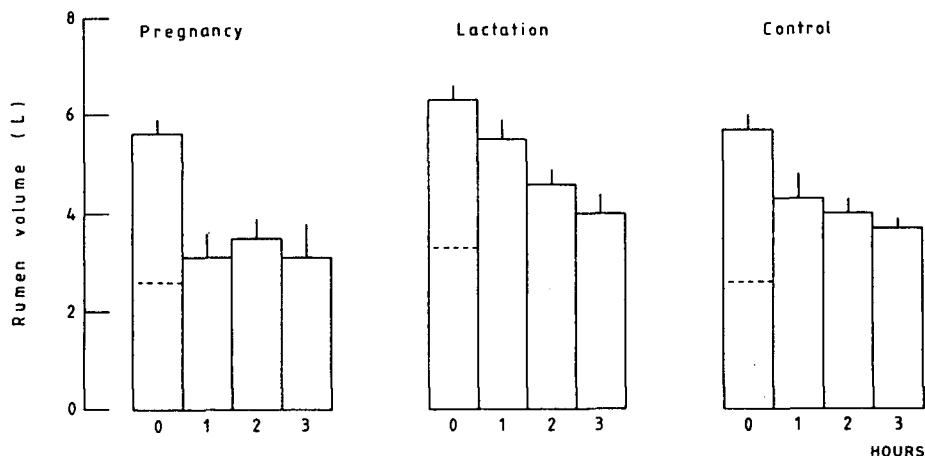


FIG. 2. Changes in rumen volume following hyperhydration with 3 L of warm water. The volume of the rumen immediately before the water load is indicated by broken lines ($N = 3$ ewes; mean $\pm$ SE).

3.2. Renal capacity to concentrate the urine

An alternative explanation for the increased water turnover during pregnancy and, to some extent, during lactation might be that increased renal water excretion is caused by a lower renal ability to concentrate the urine. The maximum urine concentration found in sheep during water deprivation was lower during pregnancy and lactation than during the control period [9]. In normal-hydrated goats, Olsson et al. [5] observed that plasma arginine-vasopressin (AVP) was similar in pregnant, lactating and anoestrous animals. However, during water deprivation plasma AVP increased to a greater extent in pregnant and lactating goats, but these animals concentrate their urine less than anoestrous goats. The efficiency of AVP in concentrating the urine was therefore tested during pregnancy, lactation and the control period. As shown in Fig. 4, hyperhydrated sheep in a steady state of water diuresis responded to an injection of AVP, with immediate inhibition of the water diuresis. However, duration of the antidiuresis was shorter during pregnancy and (to a lesser extent) also during lactation than during the control period. This indicates that the lower ability of the kidney to economize water during pregnancy and lactation is caused by a decrease in the renal efficiency of vasopressin.

4. UREA METABOLISM DURING PREGNANCY AND LACTATION

It is well known that rumen ammonia arises from food nitrogen as well as from endogenous urea which enters the rumen through saliva and the epithelium wall.

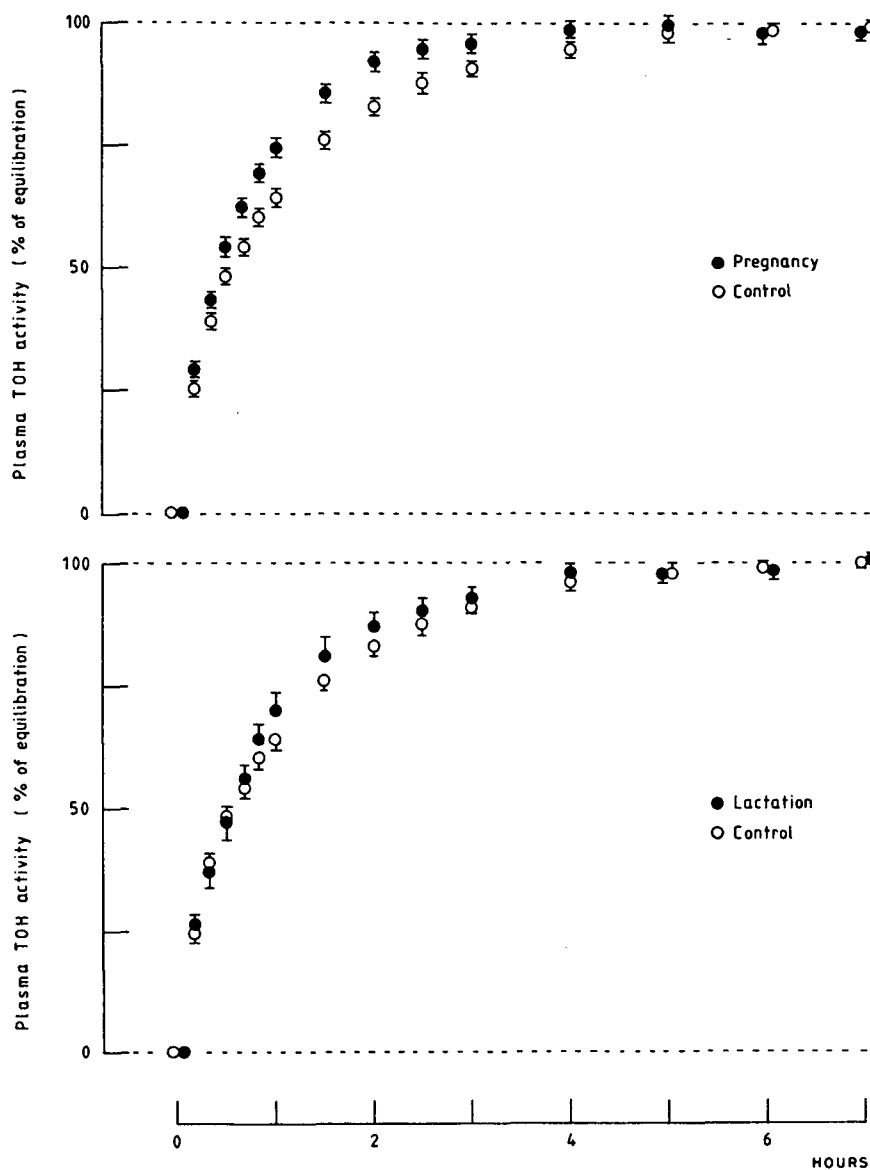


FIG. 3. Increase of plasma TOH activity (as per cent of equilibration value) after intraruminal administration of 3 L of TOH in the same ewes during pregnancy, lactation and the non-pregnant, non-lactating (control) period ($N = 4$ ewes; mean $\pm$ SE).

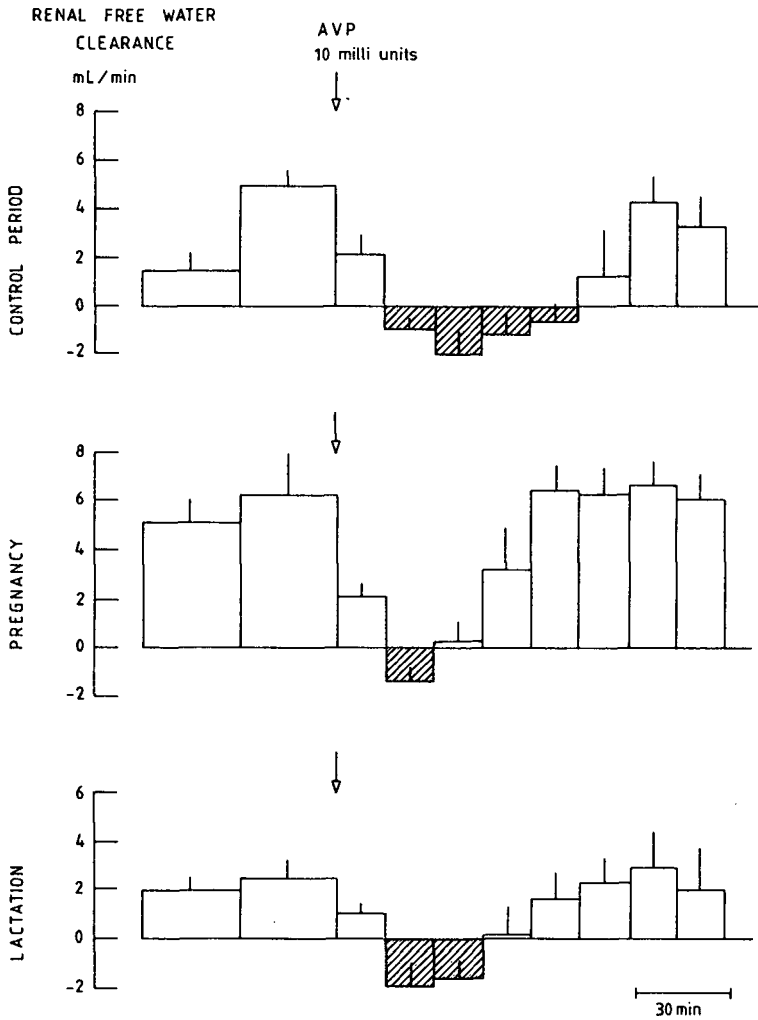


FIG. 4. Effect of intravenous injections of 10 milli units of arginine-vasopressin (AVP) on renal free water clearance. All ewes had 3 L of water administered into the rumen 2 h before the AVP injection (from Ref. [9]).

TABLE II. UREA KINETICS IN THE SAME EWES DURING THE CONTROL PERIOD, PREGNANCY AND LACTATION

	Control period	Pregnancy	Lactation
Urea pool (mmol/animal)	160 $\pm$ 20	220 $\pm$ 20	160 $\pm$ 10
Plasma urea (mmol/L)	7.4 $\pm$ 0.5	7.2 $\pm$ 0.5	6.7 $\pm$ 0.4
Urea half-life (h)	3.8 $\pm$ 0.5	3.8 $\pm$ 0.3	3.4 $\pm$ 0.2
Urea entry rate (mmol/h)	30 $\pm$ 2	40 $\pm$ 3	33 $\pm$ 3
Renal urea excretion rate (mmol/h)	24 $\pm$ 2	15 $\pm$ 1	17 $\pm$ 1
Recycling rate/entry rate (%)	20 $\pm$ 2	63 $\pm$ 2	48 $\pm$ 5

Mean $\pm$ SE; N = 6 ewes; mean body weight: 45 kg.

Recycling of endogenous urea to the rumen is an important means of supplying ammonia for microbial cell growth and microbial protein synthesis. This provides the ruminant with an additional source of available protein. Because pregnant and lactating animals are faced with an additional protein demand, it is important to know if the proportion of endogenous urea which is recycled into the digestive tract changes during these two phases of the production cycle.

4.1. Endogenous urea recycling

Nolan and Leng [10] observed that the amount of urea recycled into the digestive tract is higher in pregnant than in non-pregnant animals. Using the same technique [11] we compared urea kinetics during the last month of pregnancy, lactation and the control period in the same animals on the same diet. The results in Table II show that the urea entry rate increased during pregnancy, while renal urea excretion decreased. As a result, recycling of urea increased markedly. During lactation the amount of recycled urea was also elevated, but was lower than during pregnancy.

The proportion of urea which is recycled depends on the rate of urea transfer into the digestive tract and the rate of renal urea excretion. Under similar experimental conditions we found that the rumen ammoniac concentration was 26 ± 2 , 24 ± 2 and 15 ± 1 mg/100 mL during pregnancy, lactation and the control period, respectively. In addition, the daily duration of rumination (when saliva secretion is maximal) was increased by about 1 h/d during pregnancy. These results suggest that a significant part of the additional urea recycled into the digestive tract is effectively transferred into the rumen.

4.2. Renal urea handling

Renal conservation of urea is essential for the maintenance of a high recycling rate. Renal urea excretion depends on the plasma urea concentration, the glomerular filtration rate and renal tubular reabsorption. These parameters were measured in order to elucidate the mechanism whereby pregnant and lactating animals reduce the irreversible loss of urea. As in humans [12] and goats [4], the glomerular filtration rate in sheep increased markedly during the last month of pregnancy (Table III). The filtered urea load was slightly elevated during pregnancy and lactation. Therefore,

TABLE III. GLOMERULAR FILTRATION RATE (GFR) AND AMOUNTS OF UREA FILTERED, REABSORBED AND EXCRETED DURING THE CONTROL PERIOD, PREGNANCY AND LACTATION

	Control period	Pregnancy	Lactation
GFR (mL/min)	144 ± 13	214 ± 23	160 ± 11
Urea filtered (mmol/min)	1.07 ± 0.10	1.26 ± 0.13	1.16 ± 0.08
Urea excreted (mmol/min)	0.46 ± 0.04	0.34 ± 0.03	0.36 ± 0.03
Urea reabsorbed (mmol/min)	0.61 ± 0.06	0.92 ± 0.12	0.79 ± 0.06
Reabsorbed/filtered (%)	56 ± 3	73 ± 3	68 ± 4

Mean $\pm$ SE; N = 7 ewes; mean body weight: 47 kg.

the reduction in renal urea excretion was mainly caused by a higher renal tubular reabsorption of urea. Whether renal tubular reabsorption of urea is controlled by an active process is still a controversial question, but the possibility cannot be excluded that some hormonal and/or metabolic factor related to pregnancy and lactation may favour the renal reabsorption of urea.

5. CONCLUSIONS

Pregnancy and lactation induce a rise in water turnover caused by a reduction in the water storage capacity of the rumen and a decrease in the renal ability to concentrate the urine. The animals therefore become more susceptible to a shortage of water during these two critical phases of the production cycle.

The recycling of endogenous urea increases during pregnancy and lactation. In arid zones this may have beneficial effects of supplying additional ammonia for microbial protein synthesis in the rumen, stimulating microbial cell growth and therefore cellulose breakdown, and saving some water which ought to be excreted with urea in the urine.

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COPING WITH SHORTAGE OF ADEQUATE FOOD BY LIVESTOCK IN THE EXTREME DESERT*

I. CHOSHNIAK

Department of Zoology,
George S. Wise Faculty of Life Sciences,
Tel Aviv University,
Ramat Aviv, Israel

Abstract

COPING WITH SHORTAGE OF ADEQUATE FOOD BY LIVESTOCK IN THE EXTREME DESERT.

Goats, camels and donkeys are the traditional livestock of man in the extreme deserts of the Middle East. In the harsh environment where these animals are raised precipitation is sparse. Following a short rainy season, pasture in this area dries out and its quality deteriorates. To meet their energy requirements, livestock graze unselectively and consume mostly dry plant material that is high in fibre and low in protein. This behaviour allows for high stocking rates. In the present study the energy and nitrogen metabolism of livestock characteristic of the extreme desert were studied in the laboratory under mid-summer conditions (maximum daily temperature: 35°C; RH: 60 to 80%; maximum solar radiation: 0.959 W/cm²). Experiments were conducted on eight Bedouin goats, four camels and five donkeys. Throughout the study the animals were maintained in unshaded metabolism cages and were tested in sequence under two different diets: high quality roughage (alfalfa hay, 25% crude fibre, 18% crude protein) and low quality roughage (wheat straw, 35% crude fibre, 5% crude protein). The gross energy intake, dry matter digestibility, resting oxygen consumption and urea nitrogen kinetics were assessed. When on alfalfa hay, all three species gained virtually the same amount of digestible energy on a mass specific basis. When on wheat straw, the gross energy intake in the goat, camel and donkey was lower by 64, 76 and 25%, respectively, than on alfalfa hay. Oxygen consumption on alfalfa hay was lowest in the Bedouin goat and highest in the donkey. When tested on wheat straw, the oxygen consumption amounted to 66, 52 and 43% of the values recorded when on alfalfa hay in the goat, donkey and camel, respectively. Urea recycling, as a percentage of urea entry rate in animals fed alfalfa hay, amounted to 40.3, 16.8 and 59.0% in goats, donkeys and camels, respectively, and when on wheat straw 69.3, 85.3 and 90.6%, respectively. It is concluded that livestock indigenous to the desert are all capable of balancing their metabolism under traditional Bedouin husbandry, even when nutritional conditions deteriorate.

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Goats, camels and donkeys are the traditional livestock of man in the extreme deserts of the Middle East [1]. They are his livelihood, they provide him with protein-rich food in the hostile environment and carry his loads across rugged terrain.

The harsh environment in which these animals are raised is characterized by sparse precipitation (annual average of about 25 mm), intense solar radiation, high ambient temperature (daily maximum temperature well above 40°C) and low relative humidity. Water sources are few and several days' walking distance apart [2].

The vegetation in these areas is extremely meagre. Following a short rainy season, the pasture dries out and its quality deteriorates. To meet their energy requirements, livestock graze unselectively and consume mostly dry plant material that, during most of the year, is both high in fibre and low in protein [3, 4]. Shortage of both water and adequate food imposes various degrees of stress on the water and energy metabolism of desert livestock and may hamper production and even threaten life in the hot and dry deserts. To varying degrees these two deficiencies characterize vast arid zones in most continents [5, 6].

Compared with the extensive reports regarding the variety of ways in which desert animals cope with a shortage of water [7-10], only little is known about the ways in which large desert herbivores manage to balance their energy expenditure and meet their nutritional requirements [11]. Our recent studies were, therefore, aimed at investigating the capacities of desert livestock to economize energy metabolism and to use low quality feed efficiently under extreme desert conditions.

Digestive efficiency, energy metabolism and nitrogen balance were assessed in three species:

- (1) *The black Bedouin goat* — a ruminant capable of maintaining productivity in the harshest parts of the desert [2].
- (2) *The camel* — a non-ruminant which possesses a foregut fermentation compartment and which is the desert environment's most characteristic representative [7].
- (3) *The donkey* — an equid with a hindgut fermentation site and known for its modest nutritional requirements. In the arid, hot areas of North and East Africa the ancestors of this species are still found in the wild [12].

All the animals included in our study were tested on wheat straw (35% crude fibre, 5% crude protein), which is considered inadequate to support any domesticated ruminant [13]. For control, before each trial on wheat straw all the animals were tested on alfalfa hay, a high quality roughage that contained 25% crude fibre and 18% crude protein. Adequate time (4 to 8 weeks) was allowed for all the animals to adjust to each experimental diet.

Throughout the study the animals were maintained in unshaded pens (maximum daily temperature: 35°C; relative humidity: 60 to 80%; maximum solar radiation: 0.959 W/cm²). Goats were maintained in metabolism cages. Camels and donkeys were equipped with special bags for collecting faeces. Foley catheters were employed for gravimetric urine collection.

When on alfalfa hay, all three species consumed virtually the same amount of feed on a mass specific basis (Table I). Their gain in digestible energy was similar. When tested on wheat straw, the gross energy intakes in the goat and the camel were 64 and 76%, respectively, which were lower than those recorded on alfalfa hay. In the donkey, however, the gross energy intake on wheat straw was only 25% lower than that recorded on alfalfa hay and amounted to twice the intake on wheat straw recorded in the goat and three times that recorded in the camel (all calculated on a mass specific metabolic basis). It was only in the goat that a slight loss (2% of initial mass per week) of body mass was recorded on wheat straw. It has, however, been demonstrated in a previous study [14] that goats given water only once every four days showed improved utilization of low quality feed, enabling them to maintain a constant mass when fed low quality feed.

Hindgut fermenters such as the donkey are known to have less restrictions on the flow of particulate digesta along their gastrointestinal tract [15, 16]. As a result they can maintain a higher rate of low quality roughage consumption without filling the gut to an extent that will hamper further intake of feed, as is often experienced by ruminants.

In addition to its high gross energy intake, the digestibility of low quality roughage in the donkey was higher than that recorded in the Bedouin goat but not significantly lower than the one recorded in the camel (Table I). Its gain in digestible energy when on wheat straw is twice that of the goat and about three times that of the camel.

It has been suggested that hindgut fermenters possess a less potent cellulolytic microbial population [17]. The results we have demonstrated in the donkey, an equid indigenous to the desert, seem to challenge the general validity of this suggestion.

The animals' requirements for metabolizable energy were assessed in our study as the resting oxygen consumption rate. According to Brody [18] and Blaxter [19] the metabolic rate measured as oxygen consumption at rest and under standardized conditions [20] accounts for most (85%) of the energy required by the animal for maintenance. Until a more direct measure of the metabolic rate of free ranging animals is available (currently being tested in our laboratory) changes in oxygen consumption may be considered to be indicative of the changes in the animals' requirements for metabolic energy. When fed alfalfa hay, oxygen consumption was lowest in the goat and highest in the donkey. Although all the animals were not under the standardized conditions required for measuring the 'basal metabolic rate' [20], the measured value of oxygen consumption in the goat when fed alfalfa hay was similar to the one expected from Kleiber's most famous equation. In the camel it was 32% higher and in the donkey it was over 50% higher than the value expected (Fig. 1). When fed on wheat straw, oxygen consumption in the goat amounted to 66% of the value recorded when tested on alfalfa hay. In the donkey it dropped to 52% of the value recorded on alfalfa hay and in the camel it was only 43% of the value recorded in the animal when fed alfalfa hay. Oxygen consumption in the goat and the camel was 34 and 43%, respectively, lower than expected, while in the donkey it was only 19% lower than expected (Fig. 1). Lowering the metabolic rate to the extent demon-

TABLE I. FEED CONSUMPTION, DRY MATTER DIGESTIBILITY AND O₂ CONSUMPTION OF BEDOUIN GOATS, DONKEYS AND CAMELS MAINTAINED ON EITHER A HIGH (ALFALFA HAY) OR LOW (WHEAT STRAW) QUALITY ROUGHAGE

Animals	Diet	Feed consumption (kJ/kg W ^{0.75} per day)	DM digestibility (%)	O ₂ consumption (L O ₂ /kg W ^{0.75} per day)
Goats	Alfalfa hay	1496	62.8	17.4
	Wheat straw	538	46.6	10.6
Donkeys	Alfalfa hay	1514	68.4	25.2
	Wheat straw	1137	49.5	13.1
Camels	Alfalfa hay	1137	68.0	21.5
	Wheat straw	376	50.3	9.3

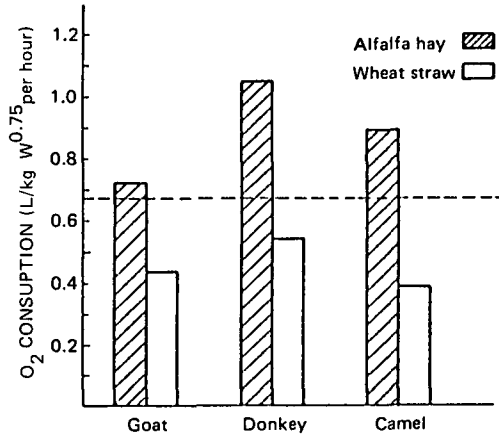


FIG. 1. Resting metabolic rate of goats, donkeys and camels fed alfalfa or wheat straw. Broken line shows expected value for basal metabolic rate.

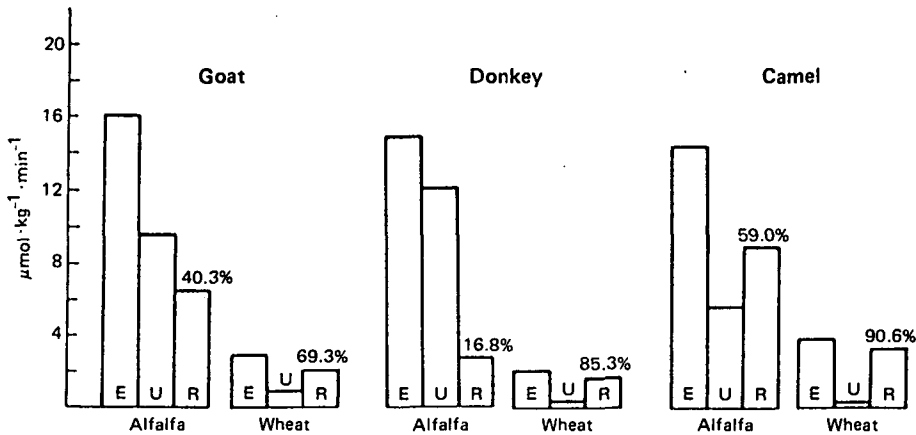


FIG. 2. Urea kinetics in goats, donkeys and camels fed either alfalfa hay or wheat straw.

strated in the goat and the camel may play a major role in helping these animals balance their energy metabolism when nutritional conditions deteriorate. On low quality feed, the donkey consumed more gross and digestible energy than the two other species. It seems, therefore, that under adverse nutritional conditions the requirements for metabolic energy are challenged in the donkey to a lesser degree than in the other two species.

In most herbivores' urea, the main nitrogenous end-product of protein metabolism (that is discharged from the liver into the blood) is partially transferred

from the digestive tract. There it supports the growth of the microbial population, thereby determining the efficiency of digestion of cell wall materials [21]. Microbial proteins may also be hydrolysed and provide the host animal with additional amino acids. In many herbivores this mechanism is instrumental in helping maintain a balanced nitrogen metabolism on low protein intake.

Recycling of urea was first described in the camel [22] and was interpreted as an important adaptation to life in the desert. Urea recycling occurs in herbivores, but seems to be more extensive in those indigenous to the desert environment [23–27]. The rate of urea synthesis (E), urea excretion (U) and urea recycled (R) in goats, donkeys and camels tested on alfalfa hay and wheat straw are presented in Fig. 2.

In our study the camel's kidney seems better able to conserve the urea synthesized in the liver than the other species. When on wheat straw it excreted only 8% of the urea synthesized. In the Bedouin goat and the donkey when fed the low protein diet urine excretion amounted to 32 and 14% of the entry rate, respectively. The camel, when on wheat straw, recycled 90.6% of the urea entry rate, the goat 69.3% (of its urea entry rate) and the donkey 85.3%. On a weight specific basis the camel recycled considerably more urea than either the Bedouin goat or the donkey. Even on high quality feed such as alfalfa hay, the urea recycled by the camel and the Bedouin goat amounted to 59 and 40% of the entry rate, respectively. The urea recycled in the donkey when on alfalfa hay was less than one-third of the value recorded in the camel.

The donkey is capable of achieving relatively high rates of urea recycling. In ponies on diets low in protein urea recycling amounted to 65% of the total urea entry rate; on a high protein diet it amounted to 38% of the entry rate [24]. The high rate of urea recycling observed in the donkey may still be of high nutritional significance, even though microbial nitrogen is unavailable to the host animal [17].

Most of the dietary nitrogen in equids is absorbed in the proximal parts of the digestive tract. Thus, the prime contribution of recycled urea to equids and presumably to other hindgut fermenters is to increase the amount of nitrogen available for the hindgut microorganisms, thereby increasing their fermentative capacity.

In supporting human life in the extreme desert for several millenia Bedouin livestock have acquired not only the capacity to cope with a shortage of water but they also seem capable of balancing their energy metabolism and meeting their nutritional demands during prolonged droughts when feed quality deteriorates. In the Bedouin goat it has been demonstrated [14] that when facing a simultaneous deficiency of water and adequate food the herbivores' capacity to cope with both deficiencies may even improve. In the desert when water becomes scarce adequate food is also at a premium. The ways in which herbivores cope with these two problems have largely been studied under laboratory conditions. Extension of the conclusions for free ranging animals will only be possible by employing techniques that require minimum interference with the animals' natural behaviour. Simultaneous measurement of both water and energy turnover using the double-labelled water technique [28, 29] seems to be the best approach.

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EFFECT OF GENOTYPE ON THE PROTEIN AND ENERGY REQUIREMENTS OF GROWING CATTLE*

P. SUSMEL, B. STEFANON, E. PIASENTIER

Facoltà di Agraria,
Istituto di Produzione Animale,
Università di Udine,
Pagnacco, Italy

Abstract

EFFECT OF GENOTYPE ON THE PROTEIN AND ENERGY REQUIREMENTS OF GROWING CATTLE.

The effect of energy and protein intake on energy retention and lean production in cattle is discussed. The differences between genotypes in body gain, protein and fat retention are summarized. Interactions between some breeds and diets are described in order to assess the energy retention and daily gain efficiency. For a reliable comparison between breed, sex and feeding regimes it is necessary to refer to age at puberty. The possibility of extending the main energy and protein systems to other breeds and genotypes is also investigated.

1. INTRODUCTION

The efficiency with which energy and protein of feed are used by different animals varies widely. It is well known that monogastric animals are more efficient converters of feed, but such animals compete with man for feed sources. Even more efficient are fish, e.g. trout produce 8 to 10 g of protein/MJ gross energy (GE) and carp, if fed vegetable protein, give 4 to 5 g of protein/MJ GE [1, 2]. Herbivores and ruminants continue to play a primary role in providing protein in all countries, and particularly in developing countries the lower feed efficiency of ruminants is more than counterbalanced by the lower energy cost of providing and keeping a suitable artificial environment for more productive but very capital-intensive species. This paper will deal with the possibility of fitting feed sources and different genotypes of cattle in a more efficient way.

2. ENERGY AND PROTEIN UTILIZATION IN GROWING ANIMALS

The efficiency with which a growing animal converts feed energy and protein into meat depends on the way in which the feed is partitioned into energy retention (ER) in the body as fat and protein and heat production (HP). The growth of different

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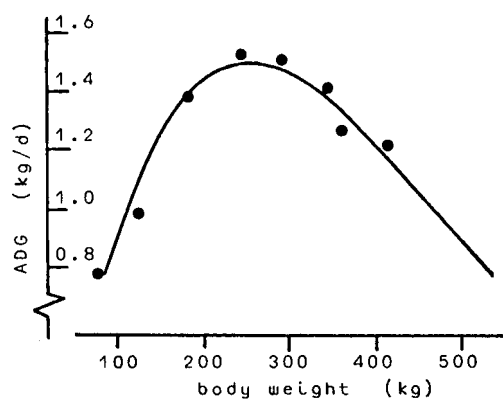
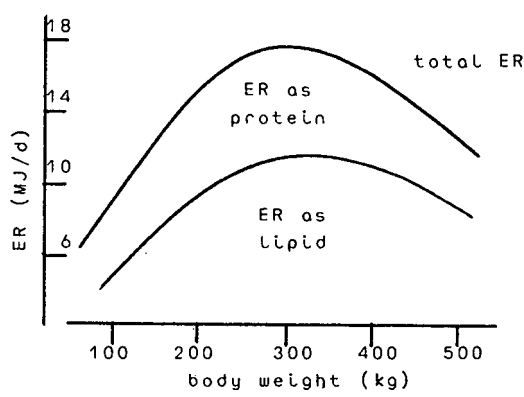


FIG. 1. Daily gain in growing and fattening early maturing bulls fed a high energy diet.



ADG=Average Daily Gain, ER=Energy Retention

FIG. 2. Energy retention in growing and fattening early maturing bulls fed a high energy diet.

body organs and tissues follows sigmoid curves which have an asymptote at maturity; the S curve of live weight (LW) in cattle has an inflection point around puberty, when the daily gain reaches its maximum value (see Fig. 1). Also, body composition varies continuously during growth and is influenced by endogenous hormones, the levels of which also change until the animal reaches sexual maturity.

Androgens promote skeletal growth and protein synthesis of muscles, which contain specific receptors [3-5]. Also, oestrogens exert an anabolic effect in ruminants; as the presence of specific receptors for these hormones in muscles is still uncertain [6, 7], they more probably act indirectly by stimulating the secretion of

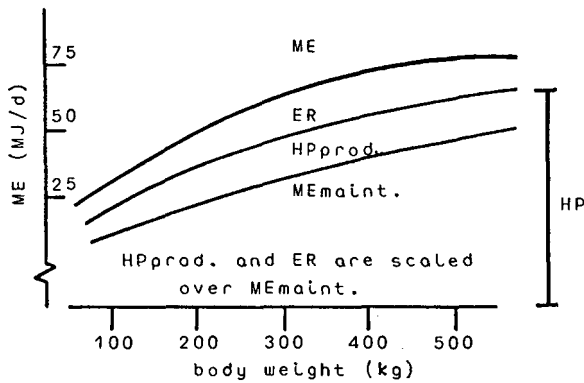


FIG. 3. Energy utilization in growing and fattening early maturing bulls fed a high energy diet.

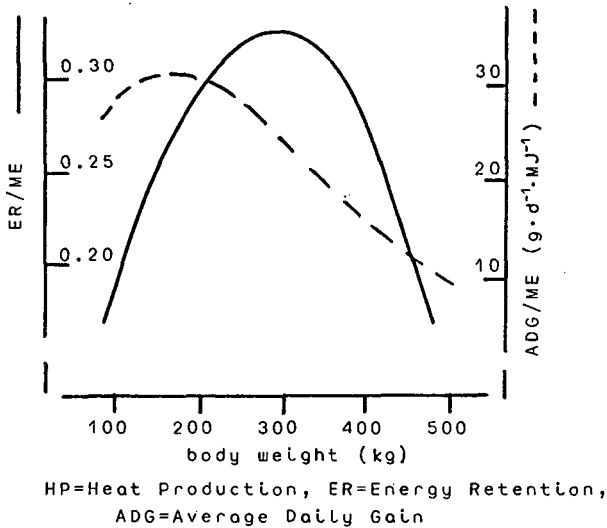


FIG. 4. Energy efficiency in growing and fattening early maturing bulls fed a high energy diet.

anabolic hormones, e.g. growth hormone (GH) and somatomedins (SM) [8]. GH enhances protein deposition and promotes glucose mobilization from tissues [6]. The positive correlation between GH and skeletal muscle weight gain [9] and on cartilage growth is probably mediated by the liver which, under GH stimulation, produces SM [4-7]; in fact, GH receptors in muscles have not yet been identified. Thyroid hormones (T3 and T4) are involved in many metabolic functions and, directly or indirectly, contribute to the normal growth of animals [10, 11]. Other hormones, apart perhaps from insulin, do not seem to stimulate tissue deposition directly [7]. Variations in the levels of hormones during the period up to puberty and the relation-

ship between these levels and growth are difficult to investigate since concentrations are largely unsteady due to episodic releases. Evaluation requires a multifactorial approach as hormones have complementary effects. Nevertheless, blood testosterone and oestrogen levels increase from birth to puberty [12–15], while GH concentrations decline with age [16, 17]. A partial correlation with body gain has been shown for testosterone [12] and for GH [9, 17]. On the other hand, evidence of the role of gonadal hormones on growth derives from the widespread use of their synthetic analogues as growth promoters [18]. From the above, hormones play a significant part in determining the efficiency with which energy and protein are retained by growing animals.

During continuous growth ER reaches a peak which varies according to energy and protein availability, sex and genotype. It also depends on the potential and composition of LW gain, and hence also on hormonal secretion. Research on energy and protein partitioning and utilization by growing animals should therefore more frequently involve concurrent determination of circulating blood hormones and metabolites. Consequently, radioimmunoassay techniques could be usefully associated with nutritional trials.

To introduce the effect of genotype on energy and protein requirements a useful first step is to consider how energy and protein retention vary with age. In Figs 1 to 4, the growth and energy utilization by growing and fattening bulls of early maturing breeds are described. Protein and fat body gains were obtained by applying the equations reported by Robelin and Geay [19] to the results of our own experiments [20]; the requirements are from the Agricultural Research Council [21].

Daily gain continues to increase up to 250 to 300 kg LW (Fig. 1), or about 9 months of age, which corresponds to puberty [22]. ER reaches a peak at about 35% of mature LW (Fig. 2), when the energy retained as protein reaches its highest value and does not exceed 10 to 15% of the metabolizable energy (ME) intake. Thereafter, the total HP, including maintenance, begins to increase progressively and at maturity tends to approach the ME intake (Fig. 3). Thus, the ER:ME ratio, after having reached a value of about 30 to 35% when animals are growing faster, subsequently diminishes steeply (Fig. 4).

The inflection point of energy efficiency of average daily gain (ADG) occurs earlier. As reported by Kielanowski [23] and Geay [24], the efficiency of ME for fat deposition is higher (0.56 to 0.76) than that for protein synthesis (0.20 to 0.34), but the energy content of fatty tissues is about 39.3 MJ/kg, while that of lean is 7.1 MJ/kg (23.1 MJ/kg for protein) [21]. Thus, as the fat contribution to LW gain increases with age, the energy efficiency of LW gain remains relatively constant during the early stage of growth, and then falls continuously (Fig. 4). At puberty the animal gains weight faster, and although feed efficiency is high it is not as great as ER:ME, which reaches a peak. This results from hormonal factors which at puberty act in favour of a higher rate of gain.

Protein seems to be a less important limiting factor than energy in growing animals. Lean contains no more than one-third protein and the adsorbed amino acids

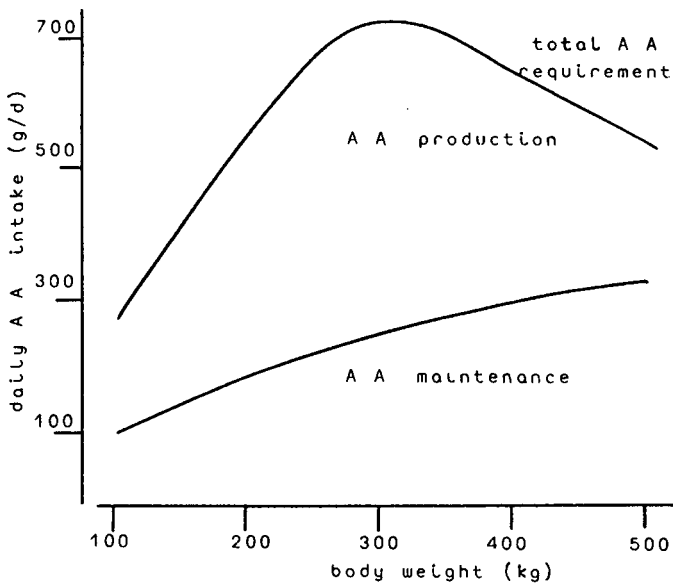


FIG. 5. Amino acid requirements in growing and fattening early maturing bulls fed a high energy diet.

(AA) are fixed in the body with an efficiency of about 0.63 [25]. The AA requirements (as AA adsorbed in the small intestine, defined as PDI by French scientists) of early maturing bulls [19, 20], evaluated according to Rohr and Geay [25], are indicated in Fig. 5. The total requirement shows a peak corresponding to the highest ADG (Fig. 1), when the demand for protein accretion contributes about 70% to the total requirement. When fat deposition increases, the total daily needs of AA begin to fall. Rumén microbes can thus largely meet the demands of a growing animal. The partition of protein deposition changes when the animal approaches puberty and, under the effect of gonadal hormones, deposition in muscles takes priority over that in other tissues.

From the above-mentioned review of Robelin and Geay [19] it is evident that protein retention in the body and carcass is affected by genetic potential, and for all breeds and classes of weight the body protein retention is equal to 0.82 FFDM (fat-free dry mass).

An evaluation of the results obtained in different situations may be useful in order to ascertain the interaction between the expression of genetic potential and the protein and energy availability. Unfortunately, very few studies have been conducted that allow a comparison from which the effects of genotype and nutrition can be interpreted without bias. Generally, we can refer to LW gain over a long period, less frequently to carcass composition; the main problems derive from the difficulty of comparing energy and protein allowances calculated using different methods and

TABLE 1. PERFORMANCES OF PURE-BREED BULLS [26-46]

	Chianina, Romagnola	Charolais, Limousin	Aberdeen Angus, Hereford (steers)	Saler	Simmental, Brown Swiss	Friesian
Initial LW (kg)	303	274	194	339	177	263
Final LW (kg)	566	538	418	559	482	453
Nutritive level	1.98	1.93	2.08	2.02	2.10	2.06
ADG (kg)	1.23	1.21	1.00	1.17	1.14	1.11
Average DM intake (g/LW)	90	87	97	95	91	96
Dressing (%)	0.645	0.672	0.656	0.652	0.631	0.626
Daily carcass gain (kg)	0.70	0.81	0.61	0.67	0.72	0.70
Daily lean gain (kg)	0.50	0.55	0.42	0.46	0.48	0.43
NE efficiency						
Daily LW gain (g/MJ)	13.3	12.3	12.5	12.5	11.8	11.6
Carcass gain (g/MJ)	7.6	8.3	7.6	7.2	7.6	7.4
Lean gain (g/MJ)	5.3	5.6	5.3	5.0	5.1	4.6

units, and from the choice of final LW, without any attention to the degree of maturity. Finally, the adoption of feeding planes often leads to discontinuous growth and makes results difficult to interpret. Besides, in Europe and the United States of America, while one can find at least 100 different breeds or types of cattle, only a few of these have been subjected to intensive research.

3. ENERGY AND PROTEIN UTILIZATION BY DIFFERENT GENOTYPES

The results obtained with bulls of different breeds are summarized in Table I. These data are compiled from trials reported in the literature [26–46]. Store calves were intensively raised using maize-silage-based diets fed *ad libitum* and, except for the Salers, were slaughtered at 35 to 40% of mature LW. With a nutritive level of about twice maintenance, energy and protein allowances should not be a limiting factor for daily gain; thus, these data should represent the genetic potential of the different breeds. As expected, daily gains are lower in steers than in late maturing animals; the differences are about 19%. If the carcass and the lean gain are compared, the Charolais bulls grow 25% faster than steers. In terms of the energy efficiency of daily gains there is a similar trend, but due to the different carcass and fifth-quarter composition, the Friesian, dual-purpose (Simmental and Brown Swiss) and local breeds (Saler) perform worse than steers. In these types of animals, the body and carcass rates of gain and the relative efficiencies of gain reflect the different ways of maturing. Recently Robelin and Geay [19], summarizing different experiments with steers and early and late maturing bulls, derived some allometric equations from which it appears that there are extreme differences between very early and late maturing animals in tissue contribution to ER (Fig. 6). It also appears that at the same LW there are large variations in body composition which still remain when comparisons are made at the same percentage of mature LW. Thus, to compare performances simply on the basis of percentage of mature LW does not avoid a certain bias.

As reported before, at puberty and if the animal is properly fed, the rate of gain declines, the composition of the gain changes, and the protein accretion shifts from body organs to skeletal muscles. Bulls of different breeds may reach puberty at different percentages of mature LW. Webster [47], comparing Hereford and Aberdeen Angus bulls, observed that the former reached 50% of mature LW earlier than the latter (341 versus 452 days), which, however, were substantially fatter; for this reason the author distinguishes fast and early maturing animals. If one takes into consideration the data presented by Lunstra et al. [48], which showed that puberty in Aberdeen Angus occurred earlier (256 days) than in Hereford (326 days), it is evident that the rate of gain of the former begins to fall much earlier than when 50% of mature LW has been reached. The conclusion is that in order to obtain comparable information it is preferable to refer to the age of puberty instead of to a proportion of mature LW. The main difference between these animals is that French and Italian

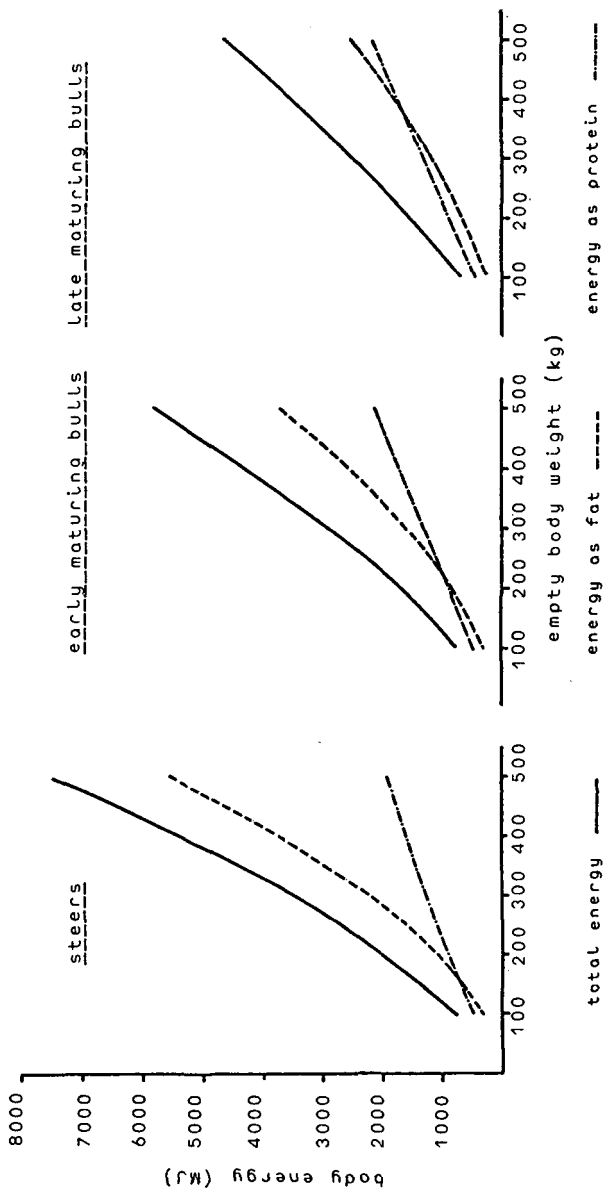


FIG. 6. Energy stored in the body as fat and protein in growing and fattening steers and bulls.

TABLE II. EFFECT OF ENERGY INTAKE ON LW, CARCASS AND LEAN GAIN IN BULLS OF DIFFERENT BREEDS [28, 59-61]

	Charolais		Federal German Simmental		Friesian	
	High	Medium	High	Medium	High	Medium
Nutritive level	1.99	1.54	1.96	1.55	1.88	1.63
ADG (g/d)	1224	1035	1127	909	1085	1005
Carcass gain (g/d)	746	566	623	479	561	501
Lean gain (g/d)	547	413	389	338	332	357
<i>ME efficiency</i>						
LW gain (g/MJ)	14.2	15.0	14.2	14.5	14.0	15.0
Carcass gain (g/MJ)	8.7	8.3	7.4	7.6	7.5	8.3
Lean gain (g/MJ)	6.4	6.0	4.8	5.4	4.3	5.4

breeds were initially selected for draught; the choice made was for bulls with delayed puberty, as they continued to grow for a longer period, in order to attain higher body mass and LW. When beef production began to be more important animals were chosen for higher LW at 18 and 12 months, for feed efficiency and for leanness; actually, the Italian and French breeds are now much more precocious than before. The English breeds were, on the other hand, initially selected for higher gains and feed efficiency to a given and higher degree of fatness. As the production of leaner gain implies a lower energy intake, another important consequence was that the DM intake per kilogram of metabolic weight of continental beef breeds is 10 to 15 % lower than that of English breeds [26, 49]. These differences may be related to the hormonal balance, but so far few attempts have been made to determine the levels of hormones directly connected with growth (GH, SM and gonadal hormones) in different breeds during the pre- and post-pubertal periods.

Recently, however, some results on the hormone levels of different breeds kept under similar conditions have become available. Keller et al. [17], bleeding Hereford and Aberdeen Angus bulls and heifers at 5, 8 and 11 months of age, observed a significantly higher GH plasma level in the latter, which until puberty also showed a higher rate of gain. The reduction of daily gain and GH plasma level with age ($P < 0.01$), evident in both breeds, was more accentuated in the more precocious Aberdeen Angus bulls from 5 to 9 months of age. Ohlson et al. [50] comparing Simmental and Hereford bulls, observed higher ADG and GH levels at 8 months of age in the continental breed.

The problem of beef production may be regarded from two different points of view: that of meeting the market demand for quality, and that of trying to maximize

TABLE III. LW GAIN EFFICIENCY WITH INCREASING NE INTAKE [62-67]

Breed	Average daily gain (g/d)				NE intake (MJ/d)			NE/kg ADG			Energy units	Refs
	Low	Medium	High		Low	Medium	High	Low	Medium	High		
Italian Friesian	894	972	987		6.17	7.09	8.78	6.98	7.29	8.81	SFU ^a	[62]
Italian Friesian	944	1067	1130		5.41	6.54	7.44	5.73	6.13	6.58	SFU	[63]
Italian Friesian	1002	1033	1125		5.54	5.80	7.33	5.53	5.61	8.52	SFU	[64]
Aberdeen Angus, Hereford and Shorthorn	1000	1110	1130		43.83	50.26	55.48	43.83	45.30	49.10	NE (ME)	[65]
Hereford	700	810	850		36.77	44.04	50.92	52.53	54.37	59.90	NE	[66]
Charolais × Hereford	950	1010	1100		56.39	61.89	71.89	59.27	61.28	65.35	NE	[67]
Aberdeen Angus, Hereford and Shorthorn crosses	1060	1120	1180		43.83	50.26	55.48	41.34	44.90	47.00	NE (ME)	[65]

^a Scandinavian feed unit.

TABLE IV. EFFICIENCY OF LW GAIN WITH INCREASING NE INTAKE IN BEEF PRODUCTION [60, 68-78]

Breed		Average daily gain (g/d)		Relative values (high/low × 100)	
		Low	High	NE intake	NE efficiency
Beef breeds	(A)	1099	+ 117	113.9	99.1
Dual-purpose and beef × dairy crosses	(B)	1171	+ 61	114.4	92.4
Dairy breeds	(C)	939	+ 90	119.6	91.9
English beefs and steers	(D)	715	+ 117	125.0	91.6

(A) Marchigiana [68], Charolais [69-71], Piedmont [72]

(B) Saler [70], Majne Anjou [73], Normandy [73], Limousin [74], Saler $\times$ Charolais [75], Charolais $\times$ Saler [70], Charolais $\times$ French Friesian [76]; dual-purpose and Charolais crosses [71]

(C) Israeli Friesian [77], Italian Friesian [60], Federal German Friesian [71], Federal German Friesian and Federal German Red and White [71]

(D) Hereford [78], Aberdeen Angus and crosses [71], Aberdeen Angus crosses [71].

the energy efficiency of daily gain. The first problem is typical of developed countries, where taste prevails over efficiency. In countries where the main problem is to produce cheaper beef protein using fibrous feeds, it is necessary to maximize feed efficiency and use of animal resources. Webster [51] pointed out that for a better comprehension of differences in daily gain efficiency among genotypes it is necessary to look carefully at the HP. A positive correlation between HP and protein synthesis was shown by Webster et al. [52] and Reeds et al. [53]. A high HP in leaner animals was also observed by Webster et al. [54], who compared bulls and steers at the same energy intake. The same explanation was evoked in the experiments held at the Institut national de recherches agronomiques (INRA) which showed an effect of breed and sex on overall energy efficiency (k_{pt}); at the same percentage of mature LW early maturing animals were more efficient than late maturing ones and females more so than intact males [28, 55]. This general trend is also confirmed by the comparisons made between normal and double-muscled bulls of continental beef breeds. The double-muscled Charolais is characterized by a higher muscle and protein deposition ($\approx 17\%$), lower DM intake ($\approx 17\%$) and ER ($\approx 6\%$) [56]. Similar information has been obtained in very late maturing tropical animals such as Brahman and Africander, but this seems to be an adaptation to the extreme climate, as these animals are said to have lower energy requirements for maintenance and do not modify appetite with hot temperature [57, 58]. This confirms that breeds which are able to convert more energy into protein are energetically less efficient. On the other

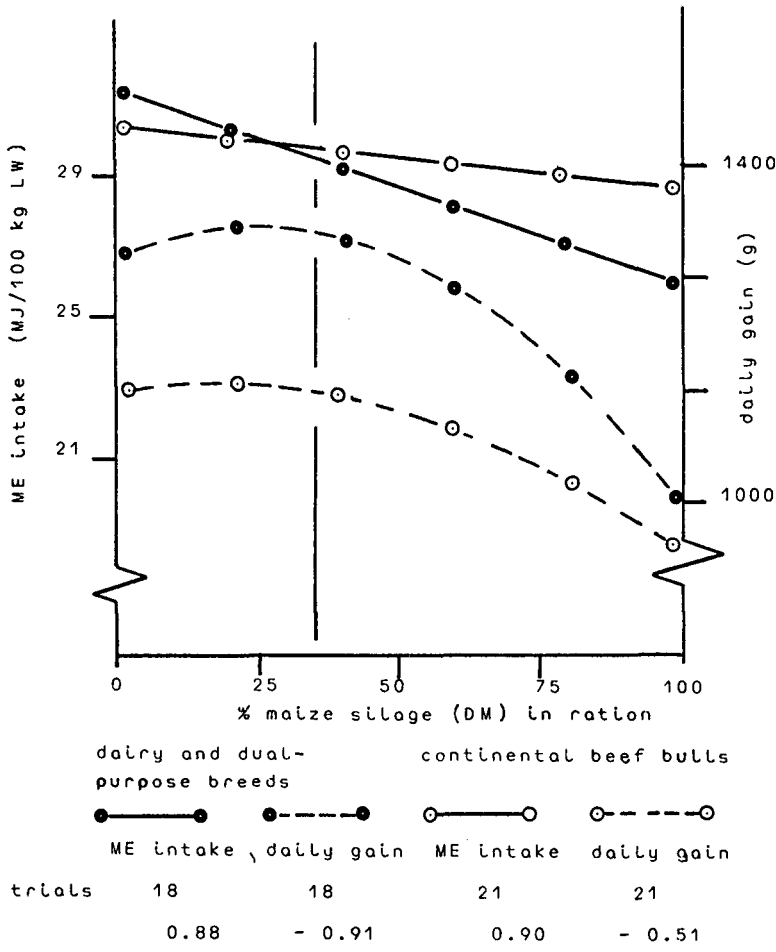


FIG. 7. Daily gains (g) and ME intake observed with rations containing growing levels of maize silage [59].

hand, the daily gain and energy efficiency of LW gain are generally higher in the leaner breeds; the consequence is that these animals, if properly fed, can compensate for the higher HP due to lean gain and convert feed more efficiently. Until puberty, animals of all breeds show higher protein deposition. Thereafter, carcass protein accretion in early maturing animals remains constant for a certain period, while in late maturing bulls the proportion of muscle in daily gain seems to increase [19]. It is then convenient, also with early maturing bulls, to postpone slaughter for a certain period after puberty. Carcass fat increases and feed efficiency declines, but more lean carcass can be produced with the same animal.

The overall feed efficiency of LW and lean gain depend upon the interaction between genotype and nutritive level. As reported in Table II [28, 59–61], early maturing animals show a higher lean gain and convert energy more efficiently when they receive a moderate nutritive level, while to take advantage in an efficient way of late maturing animals it would be necessary to use higher nutritive levels. The same results were obtained by Frisch and Vercoe [57, 58] when comparing British and tropical breeds. From most of the results obtained with different breeds and diets, when LW gains were over 0.6 kg/d an increase of energy intake corresponded to a decrease in efficiency of LW gain. This is well documented in Table III, which is a compilation of reported data [62–67] from the few experiments conducted with three increasing nutritive levels under homogeneous conditions. Whatever the breed and the energy system of reference, for an increase in energy intake a reduction in the overall efficiency of LW gain is observed; also, small increases lead to reduced utilization. To indicate the breed effect, the results have been summarized of some trials where bulls or steers of different breeds were given two nutritive levels [60, 68–78]. From Table IV it appears that only for bulls of continental beef breeds is there no reduction of efficiency with increasing LW gain. With other animals an energy surplus lowers overall energy efficiency.

In conclusion, the ration given to earlier maturing animals must be adapted to the goal: higher productive efficiency or faster gain. In late maturing animals it is more convenient to apply high nutritive levels. If one compares breeds in the same environment using one diet, the stratification of some performances may change. In a trial with the same breeds as those illustrated in Table I fed *ad libitum* with a diet containing 37% DM of maize silage (about 10.7 MJ/kg), dual-purpose breeds and Charolais performed better than Limousin and Italian beef breeds; the higher LW gains and feed efficiency resulted from the higher DM intake of the former breeds. Dual-purpose breeds eat 10 to 15 g/kg $LW^{0.75}$ more than other breeds [26]. This confirms, as reported in Fig. 7, that dual-purpose and dairy animals, compared with beef ones, maximize ME intake with diets containing 35 to 40% maize silage on DM [59]. Since late maturing continental and tropical animals show a modest intake, to feed an adequate nutritive level is practically impossible without using concentrate.

It is evident from the data of Table II that by manipulating feeding regimes it is impossible to substantially modify the beef performances of different breeds without penalizing the efficiency to an even larger extent. Experience shows that to change the characteristics of a breed by selection is a long-term process. It is universally accepted that it is much more convenient to try to modify age at puberty by cross-breeding. The results of many trials demonstrate that it is possible to obtain a large variety of classes of maturation [79–81]. It has to be stressed that the choice of sire breeds should be made very carefully, because the productive and reproductive performances of cross-bred animals change according to the production system. The available information is sparse regarding trials conducted to evaluate the effect of protein on different genotypes. Examining experiments where protein level varies together with energy it is very difficult to observe any trend. Recently Smith et al.

TABLE V. THEORETICAL PROTEIN REQUIREMENTS FOR GROWTH OF DIFFERENT GENOTYPES

	ADG (kg/d)	Tissue protein synthesis (g/d)	UDP ^a (g/d)	PDI ^b (g/d)
Late maturing bulls	1.20	270	1397	676
Early maturing bulls	1.15	190	1088	536
Steers	1.10	170	965	475

^a Endogenous N requirement: 368 mg N/kg LW^{0.75} per day; undegraded dietary protein = 0.326 net protein requirement [86].

^b Maintenance N requirement: 3.25 g PDI/kg LW^{0.75} per day; tissue protein PDI = 0.66 [25].

[82] and Holzer et al. [83], with two trials, provided further evidence of the interaction between protein quality and energy from different sources of carbohydrates. The results confirm that NPN is utilized as well as fish meal if energy support is adequate. From these authors it seems that with early maturing animals it is useless to add a high degradable protein or NPN above 12 g crude protein/MJ ME. There are also some results which indicate that late maturing bulls such as Simmental [84] or Charolais and Limousin [85] have higher protein requirements than Friesian. Using the data of protein body synthesis and the indications from the ARC [86] and Rohr and Geay [25], an idea can be obtained about the relative protein demand of different types of animals (see Table V); on average, protein requirements of early and late maturing animals are, respectively, 10 to 15% and 40 to 50% higher than those of steers.

4. SEX EFFECTS ON BEEF PRODUCTION

The sex of an animal affects the growth pattern and efficiency to a larger extent than breeds. From the results reported in Table VI [20, 80, 87–89] it is evident that differences between sexes involve mainly final LW, LW gain and carcass composition. In our four experiments the gain of males from birth to six months of age was only 11% higher than that of females and feed conversion was practically the same; the difference increased in the second part of the experiment, because females were slaughtered 65 days before males. DM intakes of heifers were 8 to 10% higher than those of bulls. Robelin and Geay [19] reported that, at the same carcass weight or

empty body weight, heifers have 20 to 60% more adipose tissue than bulls. In another experiment with male and female crosses [90], by reducing the difference between the final LW larger differences were obtained in LW gain and energy efficiency between the two sexes. A similar trend can be observed in the other two experiments reported in Table VI; it is evident that when females were slaughtered at higher LW, they had carcasses 60% fatter than the males. It can be concluded, therefore, that for a sound evaluation of the effect of the sex of the animal on growth and efficiency it is necessary to take into account the different ages at puberty of the males and females. By raising and slaughtering bulls and heifers with reference to age at puberty, carcass composition should be almost the same; differences, however, remain for DM intake and for daily gain. Also, in this case some differences in hormone concentrations were found. Keller et al. [17], Reynert et al. [91] and Kertz et al. [92] found a higher GH level in males, and suggested that testicular hormones exert a more effective stimulation to GH secretion than ovarian steroids.

Turton [93] revised the effect of castration on males. From Table VI it is evident that daily gain and ME efficiency remain lower in steers independent of intake. As also stated by Harte [94] and Price and Yeates [95], steers profit only slightly from higher energy intakes. Furthermore, steers produce fatter carcasses than males at the same carcass or empty body weight, but the increase in adipose tissues content (10 to 45%) is lower than that of females.

In terms of energy efficiency of LW gain, apart from the conflicting views on ME requirements for maintenance [24, 54, 96], it can be concluded that when slaughtered at the same LW males are generally more efficient than steers, and the difference in favour of males increases at higher LW or LW gain. In this case the different growth rate potential may also be related to higher GH plasma levels in entire males, as reported by Davis et al. [7].

According to Lofgreen and Garrett [97] steers are more efficient than heifers; the difference is smaller with lower rates of gain and at higher LWs. This last point is somewhat conflicting if one attempts to obtain a general trend of LW efficiency from interactions between breed or sex and LW gain or energy intake.

In fact, trying to generalize from the different results of reported papers and taking into account the hormonal characterization of breeds, an increase of efficiency from early to late maturing animals and from higher nutritive level or LW gain to lower ones can be observed (see Fig. 8(a)). The energy intake difference affects efficiency more in early maturing animals.

Considering sex in terms of precociousness, it appears from the results of studies on males and females that the nutritive level affects LW efficiency in the same way as breed (see Fig. 8(b)). The trend of efficiency of LW according to the nutritive level for females and steers, derived from Lofgreen and Garrett's requirements (Ref. [97], black arrows, Fig. 8(b)), contrasts with the more general assumption. On the other hand, all the energy systems report an increasing efficiency for different breeds or sex when the nutritive level rises which, as already pointed out, is not confirmed by experimental trials with growing animals and in practice.

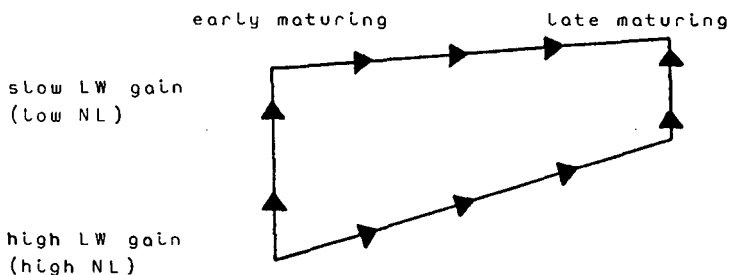
TABLE VI. EFFECTS OF DIFFERENCES IN SEX ON THE PRODUCTIVE PERFORMANCES (INTACT MALES (B) VERSUS HEIFERS (H) VERSUS STEERS (S)) [20, 80, 87-89]

Breed	Sex	LW		ADG	DM intake (g/LW ^{0.75})	ME efficiency (g/MJ)	Dressing (%)	Lean (%)	Fat (%)	Bone (%)
BC [20]	B	Initial	Final	1.26	95	15.1	0.628	0.643	0.193	0.165
	H	223	480							
LM × (CH × M) [80]	B	201	341	1.08	103	14.6	0.619	0.617	0.220	0.163
	H	—	550							
LM × (CN × M) [80]	B	—	400	0.96	95	19.9	0.648	0.719	0.099	0.152
	H	—	400							
H [87]	B	—	550	1.02	103	21.2	0.668	0.715	0.104	0.148
	H	—	440							
CH [88]	B	231	505	1.56	118	14.9	0.569	—	—	—
	S	231	499							
CH [88]	B	291	451	1.50	110	16.9	0.563	—	—	—
	S	268	446							
CH [88]	B	290	583	1.36	113	13.2	0.595	—	—	—
	S	269	578							
BF [89]	B	—	—	1.00	—	17.3	0.577	0.769	0.067	0.163
	S	—	—							

BC = various breeds and crosses (220 males and 243 females); LM = Limousin; CH = Charolais; M = Maremmana; CN = Chianina; H = Hereford;
 BF = British Friesian.

→ → → increasing efficiency

(a) breed



(b) sex

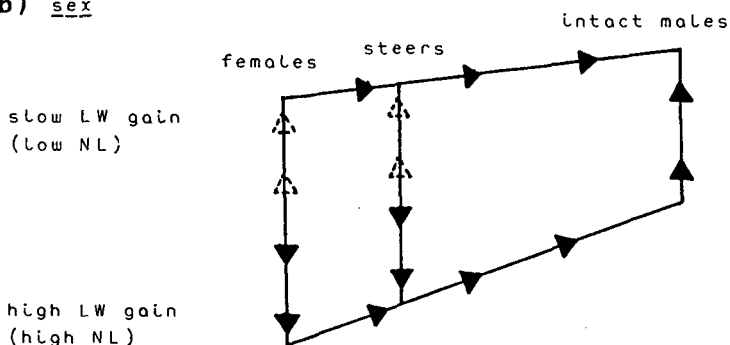


FIG. 8. Trend of energy efficiency for different genotype and LW gain.

5. USE OF ENERGY AND PROTEIN SYSTEMS

A reliable energy system has to take into account the main factors which affect feed requirements: (a) nutritive level; (b) breed; (c) sex; (d) feed quality. It is accepted that in growing and fattening animals there is a curvilinear relationship between ER and energy intake, but the different energy systems do not reflect this trend. The general curve of efficiency must also work with the increasing nutritive levels and not only with age. None of the following energy systems include any correction for the nutritive level [98, 99]: (a) Scandinavian feed unit (SFU, Denmark and Sweden); (b) net energy for fattening (NEF, German Democratic Republic);

(c) starch equivalent (SE, Federal Republic of Germany); (d) metabolizable energy (ME, United Kingdom); (e) net energy for fattening (NEF, USA). The French system (UFV) [100], the Dutch system (VEVI) [101] and the Swiss system (NEW) [102] take into consideration the effect of the plane of nutrition, making reference to an animal production level (APL) of 1.5; more recently, the ME system [21] also introduced a correction for the level of feeding between maintenance and twice-maintenance. If we also apply the data from the systems in which the effect of the plane of nutrition is included to the performances reported in Tables III and IV it can be observed that the efficiency of feed energy for LW gain, including maintenance, increases, while the observed trend is the opposite. So energy systems do not reflect the law of diminishing returns or the equation of Mitscherlich, which works in practice.

There are many justifications for the observed disagreements such as differences in models, methodology and animals. However, the conclusion is that the systems were elaborated mainly on a country basis and to extend the field of application of one system too greatly may cause uncontrolled bias.

Major problems arise in less intensive production systems where the main part of the consumed feed is used for maintenance. As reported by Geay et al. [55], Geay [24] and Frisch and Vercoe [58], it seems that the later the animals mature, the lower the maintenance requirement per kilogram of metabolic LW. This partly explains why natural selection in countries where feed resources availability is discontinuous or limited leads to leaner and later maturing cattle with lower maintenance requirements. The only system which takes breed differences into account is the French one, where the requirements were not calculated in a factorial way but used only one equation with two components. The first varies with metabolic weight and the second with the energy content of gain; the coefficients are different for dairy, dual-purpose and beef animals and also for sex. In this way the maintenance requirement, as part of the total, varies with class of breed.

All the main systems link the efficiency of ME utilization for maintenance and growth to the metabolizability of the diet; thus, this allows relatively safe application to different feeding systems.

The protein systems based on crude or digestible protein are unsatisfactory. Attention is now given to a new approach in which bypass and microbial proteins are considered separately for evaluation of feed requirements. The tuning of a similar system requires many experimental results which are as yet not available. Only INRA [100] and the ARC [86] have developed new methods for evaluating the protein requirements of ruminants. The main difference between the two systems is that the former is based on *in vitro* protein solubility, while the latter takes into account the rumen degradability of protein. These two systems should give a better estimate than the factorial approach based on crude and digestible protein, but this requires further confirmation.

At present, it is impossible to test different energy and protein systems on local breeds or other bovidae. In fact, information on growth potential and daily gain com-

position is completely lacking. To have a rough idea about the interaction between feed and animal in tropical environments, one may apply the reported data for fat and lean deposition in late maturing bulls to an animal of a tropical breed with a low feed intake (90 to 100 g/kg of metabolic LW) [57]. If the animal ingests an average tropical forage (120 g CP or 80 g PDI and 9.0 MJ ME/kg DM) the protein and energy content can efficiently satisfy only lean gain; little energy is available in the ration to allow fat deposition, even if maintenance requirements are relatively low.

If we try to change the breed characteristics by introducing early maturing types the crosses will probably not be able to show a higher appetite due to the hot climate [57, 58]. There may, therefore, also be scope to optimize the quality of the ration.

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ANIMAL REPRODUCTION
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EFFECTS OF FEEDING SALT-TOLERANT GRASSES ON THE REPRODUCTIVE EFFICIENCY OF DWARF GOATS

S.A. KHANUM, M. ALI, S.H. MUJTABA NAQVI
Nuclear Institute for Agriculture and Biology,
Faisalabad, Pakistan

Abstract

EFFECTS OF FEEDING SALT-TOLERANT GRASSES ON THE REPRODUCTIVE EFFICIENCY OF DWARF GOATS.

Salinity of soil and groundwater is a major agricultural problem facing several countries. Numerous approaches have been made to alleviate this situation. A biological approach has been developed at our Institute whereby salt-tolerant plants that are more suited to the environment have been grown rather than reclamation of land for conventional crops. *Kallar* grass (*Leptochloa fusca*) has been grown on such lands using brackish water for irrigation, thus producing large amounts of biomass. Since this biomass is not usually used as forage it was necessary to evaluate its effects on the growth and reproduction of farm animals. Buffalo, cows and goats are important for providing milk and meat in Pakistan. Goats are more important in arid and semi-arid areas. Dwarf goats were selected for the study because of easier handling, a shorter maturity period, better breeding, etc. One group of goats was contained and fed only *Kallar* grass throughout the year. (This grass is green in summer and, although it is a perennial, it dries in winter.) Another group was allowed to free graze an area where, in addition to *Kallar* grass, some other wild species were also available. The effects of this forage were evaluated by recording the weight of the animals, pregnancies, lamb survival and hormone levels. It was found that when dry grass was the only source of nutrition the animals lost weight and reproduction was also affected; even though conception did take place lactation was reduced, the animals had difficulty in delivery and the lambs were born weak and did not survive. The progesterone and oestradiol levels and patterns were normal. The study concludes that dwarf goats can survive even when fed dry, low protein grass and that their body weight increases when the grass is green. They can, therefore, be raised on *Kallar* grass if kept for meat purposes; however, if they are to be maintained for breeding some supplementary fodder is desirable. Poor nutrition did not affect the hormone levels and patterns.

1. INTRODUCTION

Salinity is one of the major agricultural problems facing Pakistan. However, the various methods that have been tried to alleviate this situation have all proved to be very expensive. Our institute has developed a biological approach to the problem whereby plants that are more suited to the environment have been grown rather than reclamation of land for conventional crops. We can now produce large amounts ($40 \text{ t} \cdot \text{ha}^{-1} \cdot \text{a}^{-1}$) of biomass on highly saline land using brackish subsoil water for irrigation.

Since the plants that grow on such lands are not usually used as forage it was necessary to evaluate their fodder value and the feeding effects on the health and reproduction of farm animals.

One of the highly salt-tolerant plants selected for introduction into salt-affected lands is *Leptochloa fusca*, commonly known as *Kallar* (meaning salt) grass. The studies described here were carried out to evaluate *Kallar* grass as fodder.

In Pakistan most of the milk is produced by buffalos, followed by cows and goats. While the buffalo and cow are raised in areas where fodder is plentiful, the goat is preferred in arid, semi-arid and salt-affected areas because it can survive and remain productive even in areas that are overgrazed and eroded. The goat has a greater digestive capacity and adaptability to adverse environments and it has a different gastrointestinal physiology than other domestic animals [1].

Dwarf goats were selected for the study because they are easier to handle, they have a shorter maturity period, they breed better than other goats and they are used to improve meat production. The paper describes the data obtained on the effects on growth and reproduction in dwarf goats after being fed *Kallar* grass.

It is known that nutrition is one of the modulating factors for the reproductive function of an animal [2]. The adverse effects of poor nutrition on farm animals and humans have been well documented. Poor nutrition causes changes in skeletal growth and development of the foetus in ewes [3] and heifers with severely checked growth are less fertile [4]. Trace elements and protein deficiencies caused by poor nutrition result in early abortions in goats [5]. Nutritional stress causes ovine pregnancy toxemia characterized by hypoglycaemia, hyperketonia and elevated levels of free fatty acids [6]. In humans severe preclampsic toxemia and low fasting glucose levels are associated with low birth weight, prematurity and still births [7]. While these studies indicate the effects of malnutrition, other studies confirm the positive effects of good nutrition, e.g. better lactation in well nourished sheep [8].

The cause/effect situation can be elucidated by hormone analysis. Progesterone assay has proved particularly valuable in assessing the fertility situation in herds, in addition to farmers' observations and veterinary care [2].

In evaluating *Kallar* grass as a fodder, its effects on growth and reproduction have been evaluated by recording data on live body weight changes, hormonal levels (progesterone and oestradiol 17 β), live births and lamb survival.

2. MATERIALS AND METHODS

Eight female dwarf goats of varying ages weighing between 11 and 21 kg were randomly divided into two groups of four each, regardless of their fertility status and body weight. Each group also contained one healthy fertile male. One group was allowed to graze freely in an area where *Kallar* grass and several other plant species

were available, while the other was kept in a paddock (33 to 66 ft)¹ and was fed only *Kallar* grass. *Kallar* grass is a perennial with a maximum photosynthetic activity in mid-summer when it is lush green and succulent, but becomes drier and harder in winter with a decreased nitrogen content.

The experimental animals were kept under these conditions for 1½ years, during which time data were collected for three 40-day periods each in winter, autumn and summer. Blood samples from the jugular vein of each animal (control and contained) were collected daily during the 40-day sampling periods; daily weight records were also maintained, as was the incidence of parturition and survival of the kid. Blood samples were allowed to clot overnight at 4°C. Serum was separated by centrifugation at 2000 rev/min for 10 minutes and stored at -20°C until assayed. Progesterone and oestradiol 17β were determined by the radioimmunoassay method [9, 10]. The antiserum raised in rabbit was supplied by Sigma (USA); the (1, 2, 6, 7)³H-progesterone and (2, 4, 6, 7)³H-oestradiol were supplied by Amersham (UK), with a specific activity of 1 mCi/mL.² The extraction recovery of tracers was 80 to 95%. Reagent grade diethylether (BDH) was used as an extraction solvent for extraction of the required hormone. All dilutions were made in a 0.01 M-Na-phosphate buffer, pH 7.2 to 7.4, containing 0.1% gelatine. Dextran-coated charcoal was used for the separation of bound and unbound fractions. The purified progesterone and oestradiol 17β used in this study were supplied by Steranti (USA). The practical detection limit of the method was 8 pg, as read in the standard curve.

3. RESULTS

Table I shows the body weight records of the free grazing control group and the contained group of animals fed only *Kallar* grass during the three 40-day periods in winter (January-February), autumn (October-November) and summer (April-May). In winter the contained group lost weight significantly ($P < 0.05$) after the first two weeks of the experiment period, while the control group gained ($P < 0.05$) weight. During the autumn and summer data collection periods the contained animals showed a slight but not significant decrease in weight. Surprisingly, there was a decrease in weight in the control animals; however, in summer it increased significantly ($P < 0.05$).

The progesterone levels in blood serum during the winter are shown in Fig. 1(w). Three animals in each of the two groups showed an anoestrous state, maintaining lower basal levels of the hormone ranging between 0.1 and 1.5 ng/mL. Two animals from the control group were pregnant and their levels of hormones kept fluctuating between 3 and 9 ng/mL, but normal parturition occurred

¹ 1 ft = 3.048×10^{-1} m.

² 1 Ci = 3.70×10^{10} Bq.

TABLE I. EFFECTS OF FEEDING *Kallar* GRASS ON THE BODY WEIGHT OF GOATS DURING DIFFERENT SEASONS

Groups	Winter	Autumn	Summer	Mean	LSD
<i>24 Days</i>					
Control	+ 8.52	— 5.08	— 2.69	+ 0.25	3.30
Contained	— 6.99	— 5.51 (5.72)	— 3.48	— 5.33	
Mean	+ 0.765	— 5.23	— 3.1		4.04
Difference	+ 15.51	+ 0.43	+ 0.79		8.09
<i>32 Days</i>					
Control	+ 4.17	— 3.27	+ 5.32	+ 2.07	3.5
Contained	— 11.21	— 1.37 (6.15)	— 0.27	— 4.28	
Mean	— 3.52	— 2.32	+ 2.53		4.35
Difference	+ 15.38	— 1.9	+ 5.59		8.70
<i>40 Days</i>					
Control	+ 9.68	— 2.75	+ 6.99	+ 4.64	3.94
Contained	— 9.42	+ 0.14 (6.82)	— 1.35	— 3.54	
Mean	+ 0.13	— 1.31	+ 2.82		NS
Difference	+ 19.1	— 2.89	+ 8.34		9.64

Numerals in brackets represent the probability factor.

at the end of the gestation period and healthy kids were produced. In the contained group three animals were pregnant and the hormone levels ranged from 4 to 10 ng/mL, but all the animals aborted at different stages of pregnancies. A pre-abortion decrease in hormone levels occurred in two goats, while in one goat the concentration of hormone did not change appreciably before abortion and miscarriage occurred when the level of progesterone was 4.3 ng/mL. After abortions the concentration decreased to 1.4 ng/mL and remained at the levels of 0.3 to 1.8 ng/mL in the postpartum period in all animals. Figure 1(a) presents the pattern of serum progesterone levels during autumn. In the control group one of the animals maintained lower levels of progesterone throughout the sampling period (0.4 to 1.6 ng/mL), thus showing acyclicity. All other animals from both groups were pregnant and the levels of hormone changed between 4 and 10 ng/mL (7 ± 3). In the control group two of the animals completed their gestation term during the sampling period; the progesterone levels declined significantly during the last few days of gestation and the

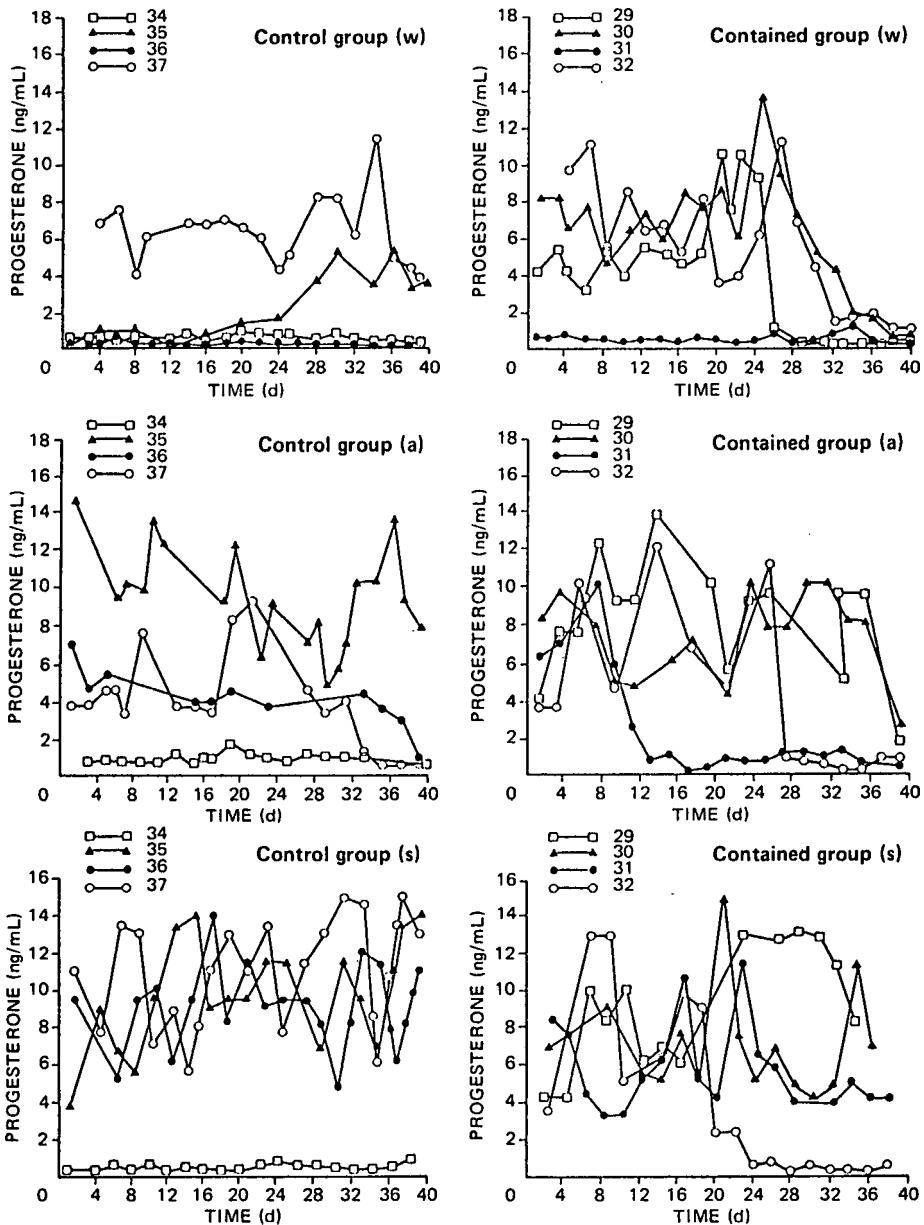


FIG. 1. Levels of progesterone in goat serum over a period of 40 days in three different seasons: winter (w), autumn (a) and summer (s). The control group was free grazing, while the contained group was fed only Kallar grass.

concentration fell to 1 ng/mL on the day of parturition. One of the animals exhibited normal parturition after a few months of the sampling period. All five kids produced by the control animals were normal and healthy. In one of the animals from the contained group the serum progesterone level declined to 10 ng/mL and then to 1 ng/mL, at which point the animal aborted. The three other animals produced live kids but all but one were too weak and did not survive for more than a day or two; most of the contained animals showed complications at the time of parturition and required help in delivery.

The pattern of serum progesterone levels during summer is shown in Fig. 1(s). All the animals were at the same state of fertility as those in the autumn experiment (three fertile, one infertile). In the control group the infertile animal maintained progesterone levels in the range of 0.2 to 0.8 ng/mL, while the other animals showed higher concentrations of progesterone (6 to 14 ng/mL, 10 ± 4) and pregnancy was maintained in each case, resulting in normal parturition and survival. The progesterone concentration in the contained group remained between 4 and 10 ng/mL (7 ± 3). Parturition occurred in one of the contained animals on day 23 of the sampling period and the prepartum decrease in progesterone levels reached 9 and then 1.5 ng/mL. During the postpartum period the levels remained in the range of 0.2 to 0.8 ng/mL. After a few days of the sampling period one of the contained animals gave birth to premature dead kids, while in others parturition occurred at term but the kids were too weak and could not suckle. Two survived but died later (3 to 4 months) of unknown causes.

Oestradiol 17β showed considerable variations but in most animals a rise in level at the end of pregnancy was quite evident (Fig. 2(w,a,s)). In winter the hormone levels in pregnant animals from the control and contained groups were in the range of 40 to 250 pg/mL and 100 to 270 pg/mL, respectively; however, the hormone levels of anoestrous animals from the control group were extraordinarily high (80 to 300 pg/mL) compared with all the other anoestrous contained animals (10 to 80 pg/mL). In autumn the levels of oestradiol 17β in the control and contained pregnant animals were in the range of 90 to 190 pg/mL and 40 to 150 pg/mL, respectively, and fell to 10 to 30 pg/mL after parturition. In summer the levels were 50 to 140 pg/mL in pregnant animals from the control group and 70 to 160 pg/mL in pregnant contained animals; they fell to 40 pg/mL after parturition in all the animals.

4. DISCUSSION

During the three seasons of the experiment the control animals showed a weight loss in autumn and the contained animals lost weight in winter. The *Kallar* grass used as the sole source of nutrition for the contained group is a perennial which is green and succulent during summer and dries in winter, which also affects the protein content. Although no data have been recorded on the amount of intake of fodder by either group of experimental animals, it was observed that dry *Kallar* grass was not

palatable to the goat. The loss in weight in the contained animals in winter can thus be ascribed mainly to the quality of the fodder. The observation that the free grazing controls lost weight in autumn may be due to the fact that during this season the summer fodder was drying up and the winter fodder was not yet ready in the fields.

As to the effects of nutrition on reproduction, all parturitions in the control animals were normal and produced healthy lambs in all seasons, while the contained animals were severely affected in winter; they not only lost weight but all the pregnancies resulted in miscarriages. In summer and autumn, though, there was a marked improvement in their health and they maintained their pregnancies and produced live lambs; however, most did not survive, mainly because they were born weak and there was too little lactation to feed them. Most of these animals required assistance at the time of delivery, perhaps due to a deficiency in foetal fluids [11]. Maternal nutritional levels are known to influence foetal development, viability, birth weight, etc. [12] and reduction in dietary energy supply during pregnancy is also known to result in lower levels of glucose and some essential amino acids in ewe blood. It has been reported that the viability of kids after parturition is dependent on increased levels of corticosteroids in the foetal plasma during the last 7 to 10 days before parturition. The corticosteroids are believed to induce several functions in the foetus for extra uterine survival [13]. Further studies are warranted to establish which factors are mainly responsible for the death of several newborn animals.

Conception rates seemed to be high in both groups in autumn and summer, while in winter some animals showed anoestrus, confirmed by hormonal analysis (Figs 1 and 2). The goat is a seasonal breeder, with a maximum sexual activity in March–April and September–October and a lesser one in January–February. The seasonal variation in conception may thus be due to variation in sexual activity rather than nutritional factors.

The progesterone pattern (Fig. 1), i.e. an increase in maternal plasma progesterone concentration during pregnancy followed by a marked decline before parturition, that was observed in both groups of animals in this study was normal and was similar to that reported by others [14, 15]. Since no effort was made to synchronize conception in all the animals, the levels of the two hormones studied varied in each animal, according to its reproductive stage. However, the sudden surge and drop in hormone levels in some cases cannot be explained with the available data.

Wastney et al. [6] have reported on the effects of undernourishment and stress in sheep. Early abortion caused by deficiencies in protein, phosphorus, magnesium and copper has also been documented [5]. Similarly, there are other reports relating nutrition to reproductive efficiency [2–8]. The present study indicates that the deleterious effects of poor nutrition do not affect the hormone levels. The apparent relationship between multiple pregnancies and maternal plasma levels of progesterone [15] was not observed in this study.

The prepartum decline in serum progesterone levels observed in this study (Fig. 1) is similar to that reported by others [15, 16]. However, in some cases the progesterone levels were still elevated (4.3 ng/mL) and progesterone withdrawal did

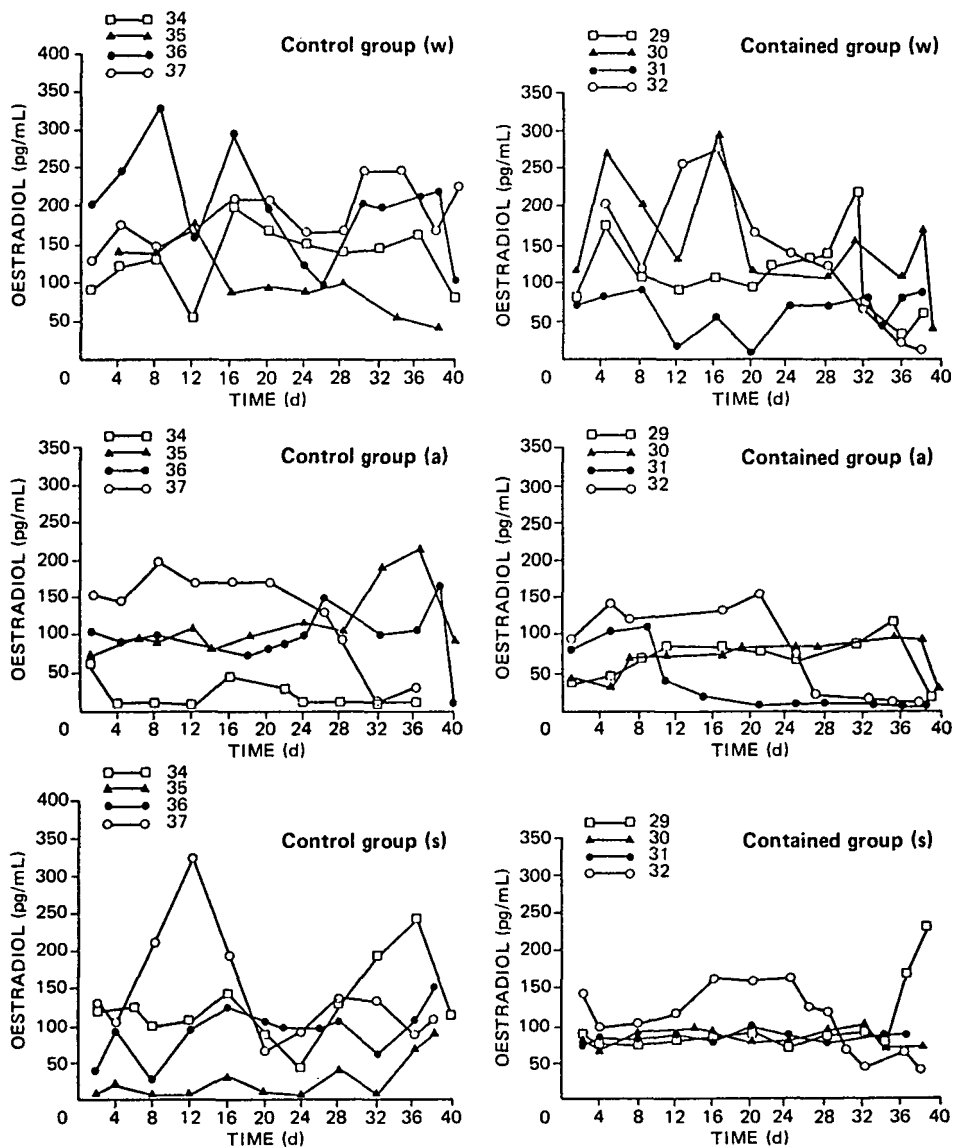


FIG. 2. Levels of oestradiol 17 β in goat serum over a period of 40 days in three different seasons: winter (w), autumn (a) and summer (s). The control group was free grazing, while the contained group was fed only Kallar grass.

not appear to be completed by the time of foetal delivery. Changes in serum progesterone levels, leading to normal parturition [17] and spontaneous abortion, have been reported [18, 19]; the decrease in progesterone levels associated with abortion or parturition is believed to be due to, among other things, the luteolytic activity of increasing prostaglandin secretion during this period. It has also been reported [20] that complete progesterone withdrawal does not appear to be associated with the development of uterine activity in the goat; no significant correlation was found between the pattern of maternal progesterone concentration or oestrogen and uterine activity. In humans, progesterone levels do not fall before labour commences [19]; however, in the cow they do [21]. In the case of sheep, there is some variability, while some ewes experience a decline in circulating concentrations of progesterone well before parturition occurs and others do not [22]. The possible involvement of a progesterone withdrawal at the initiation of parturition thus appears to be equivocal in a number of species [20] and the present study confirms that the goat is no exception.

It has been reported that oestradiol 17β , and not oestradiol 17α , appears to be effective in stimulating the release of prostaglandin in the periparturient period in the goat. However, less oestradiol 17β was found in the plasma of the late pregnant goat than other oestrogens [17, 23, 24]. Catchpole [25] has reported that the level of oestradiol showed a gradual increase in the first half of the pregnancy, and achieved a peak of 600 pg/mL a few days before parturition. Rawlings and Ward [11] have shown that concentrations of oestrogens in the goat changed significantly during the last phase of pregnancy; the values recorded one day before delivery were significantly higher than any other values. Although the levels of oestradiol 17β observed in this study (Fig. 2) were lower than those reported elsewhere, the pattern was similar to that found by others [11, 25] for oestradiol and other oestrogens.

Anoestrous animals in the control group showed higher values for oestradiol 17β (80 to 300 pg/mL) than all the others. The observation cannot be explained with the available data. However, excessive oestrogens in cows, resulting in an insufficient release of LH and a consequent failure to ovulate, have been reported as a result of ovarian cysts.

The goat is basically not a grass eating animal. Feeding it only with *Kallar* grass, particularly when it was hard and dry in winter, and containment in a paddock amounted to very severe stress. However, the data presented here indicate that the dwarf goats used in this study can survive these stresses; while these animals lost weight in winter when there was the severest nutritional stress, they regained it in summer. However, the effects on reproduction, particularly in winter, were deleterious. Some supplementary fodder is therefore desirable if the goats are to be kept for breeding. However, for meat purposes goats can be maintained on *Kallar* grass fodder alone.

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CONDUITE DE LA REPRODUCTION DES PETITS RUMINANTS DANS LES DIFFERENTES PARTIES DU MONDE

J. THIMONIER, M. TERQUI, P. CHEMINEAU

Station de physiologie de la reproduction,
Institut national de recherches agronomiques,
Nouzilly, France

Abstract-Résumé

MANAGEMENT OF REPRODUCTION OF SMALL RUMINANTS IN VARIOUS PARTS OF THE WORLD.

Small ruminants from middle and high latitudes have a limited breeding season beginning in summer or autumn. Their reproductive capacity is thus lowered unless techniques for inducing oestrus and ovulation are used. The photoperiod is the main trigger (proximal factor) of reproductive activity in both male and female. The pineal gland is implicated in the response to photoperiod treatment because of the diurnal rhythm in the secretion of its hormone, melatonin. The combination of photoperiod treatment and administration of melatonin opens up new prospects for breeding period control. Small ruminants from the tropical and subtropical zones, on the other hand, are less seasonal in their reproductive activity. Other environmental factors (relationship between individuals: males-females and females-young, nutritional level, temperature, etc.) appear to have a regulatory role in this activity (termination or initiation). Alteration of these factors (male effect, for example) can improve breeding period control.

CONDUITE DE LA REPRODUCTION DES PETITS RUMINANTS DANS LES DIFFERENTES PARTIES DU MONDE.

Les petits ruminants originaires des latitudes moyennes et élevées ont une saison de reproduction d'une durée limitée débutant en été ou en automne. Leurs possibilités de reproduction sont donc réduites s'il n'est pas fait appel à des techniques d'induction de l'œstrus et de l'ovulation. La photopériode est l'entraîneur principal (facteur proximal) de l'activité de reproduction chez le mâle et la femelle. La glande pinéale est impliquée dans la réponse à des traitements photopériodiques par le rythme nycthéméral de sécrétion de son hormone, la mélatonine. L'association de traitements photopériodiques et d'administration de mélatonine ouvre de nouvelles perspectives pour le contrôle de la période de reproduction. Les petits ruminants originaires des zones intertropicales et subtropicales sont, au contraire, caractérisés par un moindre saisonnement de leur activité de reproduction. D'autres facteurs de l'environnement (relations entre individus: mâles-femelles et femelles-jeunes, niveau alimentaire, température, etc.) semblent avoir un rôle modulateur de cette activité (arrêt ou déclenchement). La modification de ces facteurs (effet mâle, par exemple) permet de mieux contrôler les périodes de reproduction.

INTRODUCTION

Sous les latitudes moyennes et élevées, les petits ruminants ont une saison de reproduction d'une durée limitée. En général, elle débute à la fin de l'été ou au début de l'automne et se termine en hiver ou au début du printemps [1]. Cependant, il existe des différences raciales importantes. En France, les races ovines rustiques du Massif Central débutent leur saison quelques jours après le solstice d'été et la terminent quelques jours après le solstice d'hiver [2]. Au sein d'une même race, les variations individuelles sont tout aussi grandes: certaines brebis de la race Préalpes-du-Sud n'ont pratiquement pas d'anœstrus; d'autres ont, au contraire, une saison sexuelle très restreinte [3].

Le taux d'ovulation subit également une variation: il est maximum au milieu de la saison sexuelle [4].

La saisonnalité n'est pas un phénomène limité à la femelle. Ainsi, chez le bélier Ile-de-France, le poids testiculaire varie de 200 g au printemps à 280–300 g à l'automne [5]; l'efficacité de la spermatogénèse est plus faible au printemps qu'à l'automne: respectivement $9,3 \times 10^6$ et $12,2 \times 10^6$ spermatozoïdes produits par gramme de testicule [1]. De ce fait, la production spermatique quotidienne par testicule, mesurée par cannulation du rete testis varie de 1×10^9 au printemps à $4,8 \times 10^9$ à l'automne chez le bélier Ile-de-France [6]. L'amplitude des variations est moindre pour d'autres races mais elle est toujours significative. Chez les caprins mâles, la production spermatique appréciée par des récoltes au vagin artificiel subit également des variations saisonnières marquées [7].

Sous des latitudes plus faibles, un allongement de la saison sexuelle est observé [8]. Cependant, transportés dans les zones intertropicales, les petits ruminants originaires des zones tempérées conservent leur saisonnalité [9, 10].

En revanche, dans l'ensemble du monde intertropical et subtropical, la plupart des auteurs décrivent généralement les races locales de petits ruminants comme susceptibles de se reproduire toute l'année. Ainsi, maintenues non gravides, les chèvres créoles de Guadeloupe [11], les brebis Peulh du Niger [12], les brebis à queue fine de Java [13] ne présentent pas d'anœstrus marqué bien que des périodes de moindre activité soient observées. En Amérique tropicale, on cite plusieurs races d'ovins crineux et de caprins locaux dont les femelles sont capables de manifester des œstrus et d'être fécondées quelle que soit la saison de l'année [14]. En Afrique tropicale et en Asie du Sud-Est, il semble en être de même [15, 16].

Cependant, même pour ces races, des périodes préférentielles de reproduction, variables d'une année à l'autre et d'une région climatique à une autre, semblent exister puisque la répartition des mise-bas au cours de l'année n'est pas uniforme.

Que ce soit sous des latitudes moyennes ou élevées ou dans les régions tropicales, la reproduction des petits ruminants est sous l'influence de plusieurs facteurs de leur environnement. Certains d'entre eux sont entraîneurs de l'activité sexuelle; ils la déclenchent ou l'arrêtent; d'autres ne sont que des modulateurs de l'intensité de l'activité sexuelle.

1. LA PHOTOPERIODE, ENTRAINEUR DE L'ACTIVITE SEXUELLE CHEZ LES PETITS RUMINANTS

La plupart des études effectuées de par le monde ont été réalisées avec des races originaires des zones tempérées et ayant une saison de reproduction d'une durée limitée. Les données relatives aux races originaires des zones intertropicales sont très restreintes.

1.1. Mise en évidence

Le rôle de la photopériode comme entraîneur de l'activité sexuelle est démontré chez la femelle et le mâle par plusieurs types d'expériences (fig. 1):

- l'inversion artificielle du cycle photopériodique annuel entraîne le déplacement de la période d'activité sexuelle [17] ou de croissance testiculaire [18];
- l'utilisation de rythmes photopériodiques semestriels, reproduisant en 6 mois les variations normalement annuelles de la photopériode, induit chez la brebis 2 saisons de reproduction [19] et, chez le bélier, 2 périodes d'activité spermatogénétique [20, 21] par an;
- l'alternance tous les 3 mois de jours longs et de jours courts entraîne, chez la brebis, l'alternance des périodes d'activité et d'inactivité ovariennes; l'activité débute 50 jours après le passage jours longs-jours courts et s'arrête environ 1,5 mois après le changement jours courts-jours longs [22, 23]; chez le bélier, l'alternance tous les 3 mois de jours longs et de jours courts entraîne l'alternance des périodes de croissance (jours courts) et de régression testiculaires (jours longs) [24].

Ces différentes expérimentations mettent en évidence l'aptitude des petits ruminants à mesurer la durée du jour et à interpréter ses variations.

1.2. Lecture de la durée du jour

1.2.1. Mise en évidence

Des travaux récents [25] démontrent que les ovins mesurent le temps à l'aide d'un rythme endogène circadien de photosensibilité. Ainsi, au cours de la journée, il existe une phase pendant laquelle les ovins sont sensibles à la lumière. La photopériode agirait non par la durée totale d'éclairement quotidien, mais par la coïncidence ou non de la lumière avec la phase photosensible. L'aube servirait de point de repère et d'entraîneur pour le rythme circadien de photosensibilité comme le montrent les expériences suivantes:

- l'arrêt de l'activité ovarienne de brebis soumises à 1 heure de lumière donnée 16 à 17 heures après le début d'une phase claire principale de 7 heures se produit en même temps que celui de brebis maintenues sous un jour long (16 heures de lumière) [23] (fig. 2);

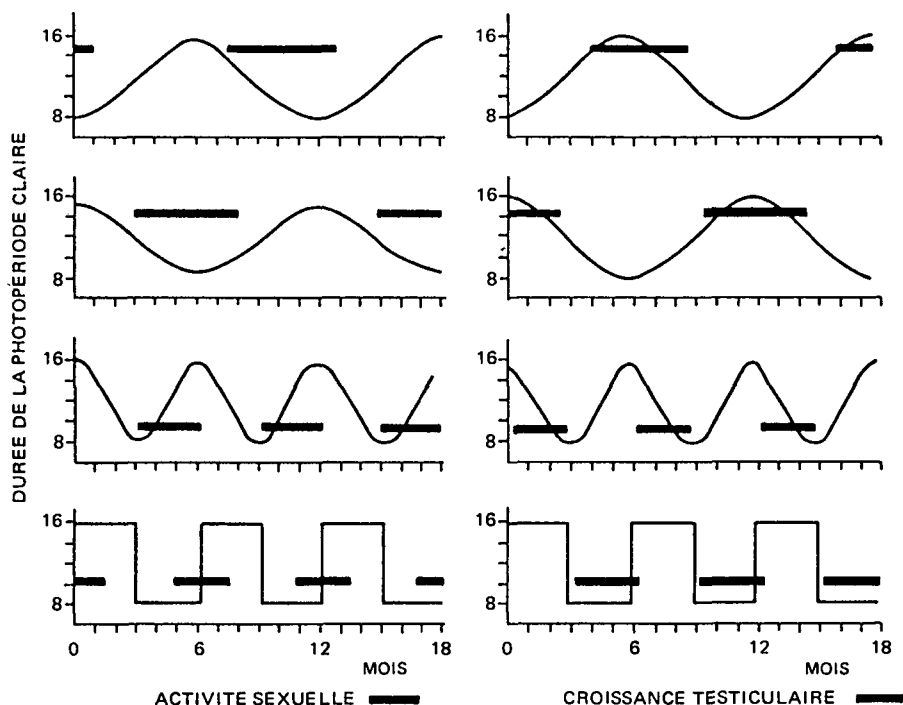


FIG. 1. La photopériode, entraîneur de l'activité sexuelle chez les ovins mâles et femelles:

Femelles	Mâles
[3] Variations normales de la photopériode	[18]
[17] Inversion du rythme annuel	[18]
[19] Rythme semestriel	[21]
[23] Alternance de jours longs et courts	[24]

— l'évolution du volume testiculaire est identique chez des béliers soumis (fig. 3), soit à un régime de période semestrielle reproduisant en 6 mois les variations normalement annuelles de la photopériode, soit à un régime de même période mais dont la photopériode claire est donnée en 2 fractions, une phase principale de 7 heures dont le début suit l'aube du régime précédent et un «flash» de 1 heure dont l'arrêt correspond au crépuscule du même régime [26].

1.2.2. Implication de la glande pinéale et de son hormone, la mélatonine

Chez les ovins l'ablation de la glande pinéale empêche la réponse des animaux mâles [27] et femelles [28] à des traitements photopériodiques. La sécrétion de

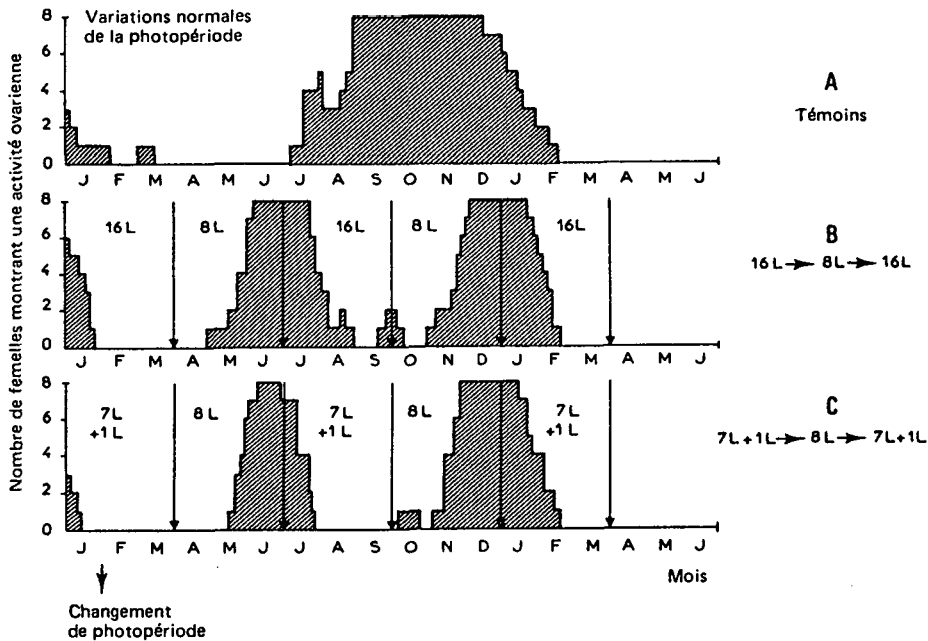


FIG. 2. Activité ovarienne chez des brebis Ile-de-France soumises [23]:

A: aux variations normales de la photopériode;

B: à l'alternance tous les 3 mois de jours longs (16L) et de jours courts (8L);

C: à l'alternance tous les 3 mois de 8 heures de lumière en 2 fractions (7L-9D-1L-7D) et 8 heures de lumière en 1 seule fraction (8L).

mélatonine, hormone de la pinéale est élevée pendant la phase nocturne et c'est par la durée de sécrétion au cours du nyctémère que se manifestent les effets inducteurs ou inhibiteurs de l'activité sexuelle dus à la photopériode [29] (fig. 4).

1.2.3. Etat réfractaire

Le maintien de brebis sous des jours courts n'empêche pas l'apparition d'une période d'anœstrus et leur maintien sous des jours longs n'empêche pas l'apparition de l'activité ovarienne [30].

Des béliers maintenus sous des jours longs ou sous des jours courts présentent également des périodes de croissance et de régression testiculaires [31]. Il s'établirait donc un état réfractaire aussi bien aux jours longs qu'aux jours courts [32, 33] bien que le rythme de sécrétion de la mélatonine soit entraîné par celui de la photopériode.

De même, l'administration de mélatonine (mimant des jours courts) aboutit à l'établissement d'un état réfractaire chez la femelle [34] et chez le mâle [35].

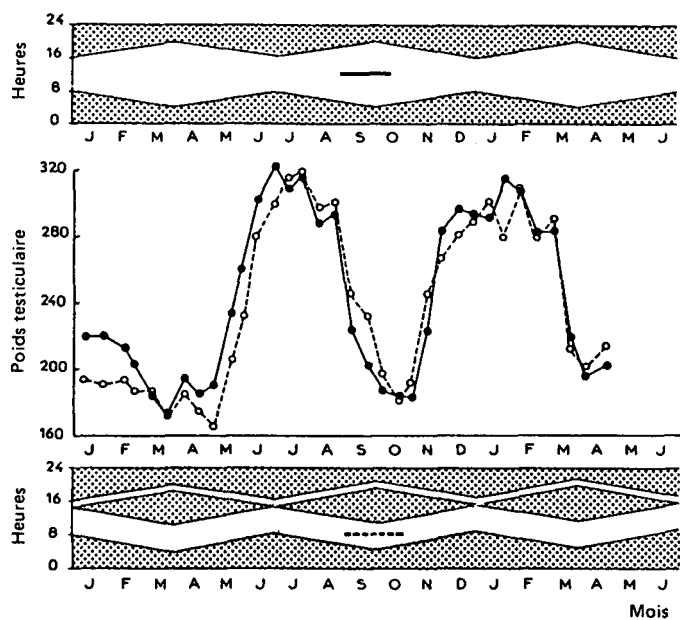


FIG. 3. Evolution du poids testiculaire chez des béliers Ile-de-France soumis à des rythmes semestriels [23, 26].

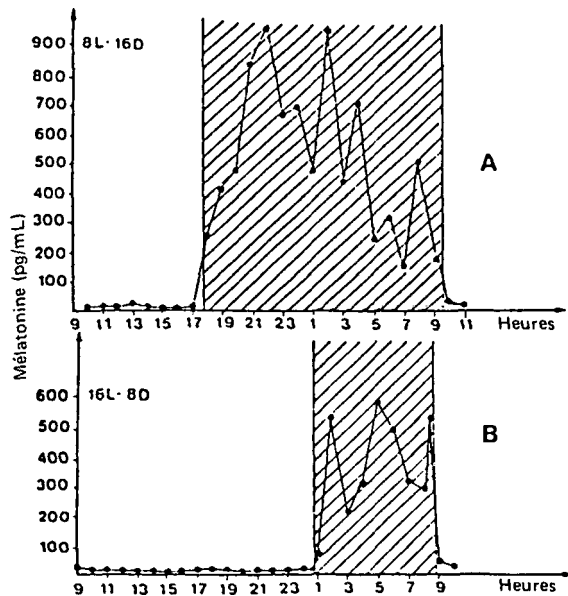


FIG. 4. Evolution de la concentration plasmatique de mélatonine au cours du nyctémère:

A: jour court: 8L-16D;

B: jour long: 16L-8D.

Source: Ravault et coll., non publié.

1.3. La photopériode et l'activité sexuelle chez les petits ruminants originaires des zones intertropicales

Le rôle d'entraîneur de la photopériode pour l'activité de reproduction chez les petits ruminants originaires des régions intertropicales est loin d'être démontré. Cependant, les chèvres naines africaines ont une saison de reproduction débutant à la fin de l'été ou en automne lorsqu'elles sont maintenues sous des latitudes moyennes [36, 37]. Leur activité ovarienne n'a cependant pas été étudiée. En revanche, les caractéristiques spermatiques des boucs, apparemment de la même race, ne montrent pas de variations saisonnières marquées en République fédérale d'Allemagne [38].

Alors que toutes les femelles Border Leicester $\times$ Merinos présentent anœstrus et arrêt de l'activité ovulatoire cyclique, les brebis à queue fine de Java conservent une activité ovarienne cyclique après maintien sous des jours longs (16 heures de lumière) pendant 6 mois [13] révélant une moindre sensibilité aux changements photopériodiques.

En fait, les données expérimentales sont insuffisantes pour juger de la sensibilité à la photopériode des petits ruminants originaires des régions intertropicales. L'étude de la variabilité intraspécifique de la photosensibilité et de son déterminisme génétique devrait apporter une meilleure compréhension des mécanismes physiologiques responsables de la saisonnalité.

2. LES FACTEURS MODULATEURS DE L'ACTIVITÉ DE REPRODUCTION CHEZ LES PETITS RUMINANTS

2.1. Température

Expérimentalement, il a été démontré que des brebis maintenues sous des températures peu élevées pendant la période estivale débutent leur saison de reproduction plus tôt que celles soumises aux températures habituelles à cette saison [39, 40]. Sous les latitudes moyennes, la température ne semble pas être un facteur limitant de l'activité sexuelle des femelles des petits ruminants. Cependant, une corrélation positive existe entre la température moyenne au mois de juillet et la date du début de la saison de reproduction chez les brebis Clun [41]. Chez les béliers, le pourcentage de spermatozoïdes anormaux augmente en été pendant la période des fortes chaleurs [42]. Suivant l'intensité et la durée du stress thermique, la morphologie des spermatozoïdes et/ou la spermatogénèse sont perturbées [43]. La survie des embryons résultant des fécondations par les spermatozoïdes de béliers ayant subi un stress thermique est réduite [44].

De même, des brebis soumises à un stress thermique dans les heures qui précèdent ou suivent l'œstrus ont une fertilité altérée [45]. Dans les conditions naturelles, les variations thermiques diurnes atténuent cependant les conséquences dramatiques des stress thermiques sur la mortalité embryonnaire [46].

2.2. Niveaux alimentaires et nutritionnels

Les effets d'un niveau alimentaire peuvent se manifester à long, moyen et court terme [47]. Ainsi, le niveau alimentaire pendant l'hiver et l'été a une influence sur le pourcentage de brebis présentant des œstrus à l'automne suivant [48] et une sur-alimentation passagère augmente la fertilité et le taux d'ovulation [49, 50]. La réponse peut être rapide comme dans le cas d'un apport de lupin [51], avant même que soit observée une augmentation de poids.

La sous-alimentation, qui retarde la croissance corporelle, diffère l'apparition de la puberté chez l'agnelle; lorsqu'elle est suffisamment importante pour provoquer un arrêt de croissance, elle peut repousser l'apparition de la première ovulation à la saison sexuelle suivante [52].

Les variations des disponibilités alimentaires pourraient rendre compte des variations de fertilité observées d'une année à l'autre, même sous des climats tempérés, et expliquer la répartition non uniforme des mise-bas dans les régions à régime pluviométrique très variable au cours d'une année ou d'une année à l'autre.

L'analyse des résultats de Cognié et al. [53] montre qu'il existe des corrélations de rang significatives entre la pluviométrie du mois qui précède la fécondation et le pourcentage mensuel de fécondations ou la prolificité chez le Cabrit créole dans certaines régions de Guadeloupe. Une corrélation de rang significative entre la pluviométrie du mois qui précède la fécondation et la prolificité a été retrouvée avec la même race [54].

2.3. Relations entre individus

2.3.1. Relations mères-jeunes

Dans les conditions normales d'élevage, les petits ruminants des zones tempérées n'effectuent en général qu'une seule mise-bas annuelle compte tenu de l'interférence entre anœstrus post-partum et anœstrus saisonnier [55].

En revanche, dans les zones intertropicales et pour les femelles des races locales, l'intervalle entre 2 mise-bas est souvent inférieur à 1 an aussi bien chez les ovins que chez les caprins [14, 15, 56].

La reprise de l'activité sexuelle post-partum est, cependant, dépendante, dans ces zones, de la saison de mise-bas probablement par l'intermédiaire des conditions d'alimentation pré- et post-partum. Ainsi, les intervalles mise-bas-fécondation et mise-bas-première ovulation sont significativement plus courts lorsque les chèvres créoles mettent bas en fin de saison sèche qu'aux autres périodes de l'année [54, 57].

En fait, il existe une grande variabilité quant aux intervalles entre mise-bas d'une race à l'autre, au sein d'une race ou d'un individu à l'autre au sein d'un même troupeau.

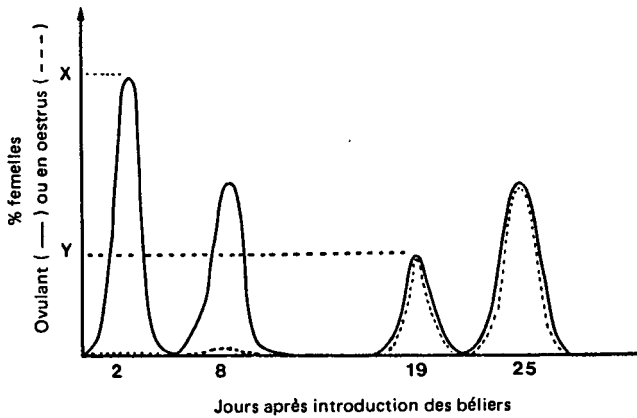


FIG. 5. Effet mâle chez la brebis [60]. L'ovulation est induite dans les 3 jours suivant l'introduction des béliers. Les pourcentages de femelles ovulantes (X) et ayant un premier cycle ovarien de durée normale (Y) dépendent de l'intensité de l'œstrus. Chez les brebis, l'ovulation induite n'est pas accompagnée d'œstrus.

Le tarissement des femelles, lié à une mortalité élevée [15, 56], dans les régions intertropicales peut expliquer partiellement la rapidité de la reprise d'activité sexuelle post-partum.

2.3.2. Relations mâles-femelles

Il est connu que l'introduction de mâles dans un troupeau de femelles [58] induit l'ovulation, la cyclicité ovarienne et comportementale. Toutefois, la réponse à cet «effet mâle» dépend de l'intensité de l'œstrus [59-61] (fig. 5). Inversement, la réponse à l'effet mâle permet de mesurer l'intensité de l'œstrus.

Avec les races utilisées sous les latitudes moyennes, une réponse ne peut être obtenue que pendant des périodes limitées, en général juste avant (3 semaines à 1 mois) le début normal de la saison sexuelle.

En revanche, avec les races originaires des latitudes plus faibles, cet effet mâle semble efficace sur des périodes plus longues [60, 62], à condition que le niveau alimentaire ne soit pas un facteur limitant.

Bien que des femelles gardées non gravides ne présentent que de très faibles variations du comportement sexuel cyclique ou de l'activité ovarienne, il existe dans un troupeau toujours des femelles en inactivité ovarienne (femelles prépubères, allaitantes, sous-alimentées, etc.). Dans ces conditions, l'effet mâle reste un moyen efficace pour induire une période de reproduction quelle que soit la saison, comme cela a été montré chez la chèvre créole [59].

Il semble donc que les petits ruminants aient développé sous des latitudes différentes des stratégies différentes pour contrôler leur reproduction.

Sous des latitudes moyennes, malgré la domestication, les variations photopériodiques sont restées le facteur proximal le plus important permettant aux petits ruminants de déclencher leur activité de reproduction [1]. En revanche, dans les régions intertropicales, il semble que d'autres facteurs beaucoup plus variables (température, niveau alimentaire, relations entre individus) puissent déclencher ou retarder les périodes de reproduction.

Ces observations ne sont pas sans conséquences sur la conduite de la reproduction dans les différentes parties du monde.

3. CONSEQUENCES PRATIQUES: CONDUITE DE LA REPRODUCTION DANS LES DIFFERENTES PARTIES DU MONDE

Avec les races saisonnées sous les latitudes moyennes et élevées, une période de reproduction débutant en été ou au début de l'automne, correspondant à des mises-bas en fin d'hiver ou au début du printemps est la règle générale dans la plupart des pays. Les éleveurs savent préparer leurs animaux (mâles et femelles) à la lutte pour augmenter fertilité et prolificité, soit par une complémentation passagère, soit par modification des chargements au pâturage. Ils peuvent bénéficier pour l'élevage des jeunes des conditions favorables du printemps. De plus, chaque pays, chaque région ont su développer des races particulières souvent bien adaptées à leur environnement. Pour des raisons économiques, dans certains pays, des programmes de sélection pour avancer le début de la saison de reproduction sont mis en place [63].

La compréhension des mécanismes physiologiques au cours du cycle œstral a permis de concevoir des traitements d'induction de l'œstrus qui grâce à leur simplicité (éponges vaginales [64, 65]) ont connu et connaissent dans certains pays un développement important: en France, environ 20% des brebis du troupeau national reçoivent de tels traitements.

Le développement de cette technique pose, cependant, deux problèmes principaux:

- celui de l'insémination des femelles en œstrus,
- celui de la scission du troupeau en 2 groupes (femelles gravides et non gravides), compte tenu de l'absence de retours en œstrus chez les femelles non gravides.

Plusieurs solutions sont proposées: insémination artificielle et diagnostic plus ou moins précoce de gravidité ou de non gravidité [66, 67]. Le traitement photopériodique des mâles producteurs de semence est par ailleurs utilisé [18] pour faciliter les inséminations artificielles à contre saison.

Le plus faible saisonnement de certaines races [8, 60] dont la race Mérinos [68] sous des latitudes plus faibles permet des luttes naturelles à contre saison en bénéficiant de l'effet mâle qui induit une période d'activité cyclique. Le succès de ces luttes est souvent variable, fonction de l'intensité de l'anœstrus qui dépend des facteurs environnementaux. Une lutte de rattrapage est souvent nécessaire. Dans certains cas,

par un choix judicieux des périodes de lutte, il est possible de réaliser 3 agnelages en 2 ans.

Les données récentes sur le rôle de la photopériode pour la mesure de la durée du jour, l'implication de la glande pinéale et de son hormone, la mélatonine, ouvrent de nouvelles perspectives. Ainsi, il est possible d'avancer le début de la saison de reproduction par administration de mélatonine [69]. L'induction d'une activité ovarienne cyclique à contre saison pose des problèmes pratiques plus importants dans la mesure où des jours longs seraient nécessaires pour rendre efficace l'administration de mélatonine.

Avec les races peu saisonnées des zones intertropicales, si les techniques hormonales d'induction et de synchronisation de l'œstrus se révèlent au moins aussi efficaces, elles restent souvent coûteuses.

Les éleveurs doivent apprendre à mieux bénéficier du potentiel élevé de reproduction en modifiant également les facteurs environnementaux: choix des meilleurs parcours, modification des relations mâles-femelles ou mères-jeunes. Ceci impose quelques contraintes pour aboutir à une augmentation de la productivité. Il reste à savoir si ces contraintes sont acceptables et peuvent s'insérer dans la conduite plus générale des troupeaux dans ces zones souvent difficiles.

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FISIOLOGIA DE REPRODUCCION DE LA ALPACA*

J. SUMAR, M. GARCIA

Instituto de Investigaciones Tropicales

y de Altura (IVITA),

Universidad Nacional Mayor de San Marcos,

Lima, Perú

Abstract-Resumen

REPRODUCTIVE PHYSIOLOGY OF THE ALPACA.

The alpaca and llama are considered to be seasonally reproductive (from December to March) and given good feeding conditions are reproductively active throughout the year. The males reach puberty at the age of about 2 years when they are free from peno-preputial adhesions. Puberty in the females is affected by body weight and may be reached at the age of 12 months. The females do not have sexual cycles but follicular waves, therefore oestrus varies from 2 to 36 days. Copulation takes place in a seated position and lasts from 5 to 50 minutes; ovulation is induced by coitus, hCG, GnRH and the semen of the alpaca and the bull. 'Spontaneous' ovulation has been observed at the height of the reproductive season and is caused by mechanisms which are not well defined. Multiple ovulation occurs in 10% of females but the number of multiple births is very low. The corpus luteum (CL) forms after mating, attaining maximum size and having the greatest secretory activity on day 9 (14.0 nmol/L); it lasts for the 11 months of gestation. If there is no pregnancy, shrinkage of the corpus luteum begins between days 10 and 13 and the progesterone levels fall rapidly. The incidence of embryonic mortality during the first two months of gestation is high. Gestation lasts approximately 342 days, and 93.5% of births occur during the daytime when the environmental conditions are favourable for the newborn animal. The placenta is diffuse and epitheliochorial. Uterine involution is completed within 20 days after birth. Intra- and inter-specific artificial insemination tests have been carried out with intra-uterine implantation of semen. Embryonic transplant tests have also been conducted.

FISIOLOGIA DE REPRODUCCION DE LA ALPACA.

La alpaca y la llama son consideradas de reproducción estacional (diciembre a marzo), y bajo buenas condiciones de alimentación muestran actividad reproductora todo el año. Los machos alcanzan la pubertad a la edad aproximada de 2 años, cuando están libres de las adherencias pene-prepuciales. En las hembras el peso corporal influye la pubertad, que puede alcanzar a los 12 meses. Estas no tienen ciclos sexuales, pero sí ondas foliculares, por lo que el celo es variable de 2 a 36 días. La cópula se realiza en posición sentada y dura entre 5 a 50 minutos; la ovulación es inducida por el coito, hCG, GnRH y semen de alpaca y toro. Se han observado ovulaciones 'espontáneas' en el apogeo de la estación de reproducción, causadas por mecanismos no bien determinados. La ovulación múltiple se produce en el 10% de hembras, pero el número de partos múltiples es muy raro. Después de la monta se forma el cuerpo luteo (CL), que alcanza el mayor tamaño y actividad secretoria el 9° día (14,0 nmol/L) y dura los 11 meses de gestación; en ausencia de preñez la regresión del CL se inicia entre los días 10 y 13, cayendo rápidamente los niveles de progesterona. La incidencia de mortalidad embrionaria durante los dos primeros meses de gestación es alta. La gestación dura aproximadamente 342 días y el 93,5% de

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los partos ocurre durante las horas del día, cuando las condiciones ambientales son favorables para el recién nacido. La placenta es difusa y epiteliocorial. La involución uterina es completa a los 20 días postparto. Se han realizado ensayos de inseminación artificial intra e interespecífica con deposición intrauterina del semen. También se han llevado a cabo ensayos de transferencia de embriones.

1. INTRODUCCION

La crianza de alpacas es una actividad de gran importancia para miles de familias de campesinos que habitan en los Andes Altos, donde tienen muy limitadas alternativas para subsistir, debido principalmente a las limitaciones que les impone la ecología de las grandes alturas, además de las restricciones económicas propias de los países en desarrollo donde viven.

El Perú, Bolivia, Chile y Argentina poseen el 95% del total de camélidos sudamericanos existentes en el mundo. Las alpacas, además de producir valiosa y fina fibra para el mercado nacional e internacional, producen carne, que es la única fuente de proteína animal de que disponen los campesinos en esa zona, y pieles, que casi en su totalidad se usan artesanalmente, dando mano de obra a gran número de habitantes que de otra manera se verían forzados a emigrar a las grandes ciudades con los problemas sociales que esto lleva consigo.

Los sistemas existentes de crianza y manejo son extrapolaciones de técnicas de crianza desarrolladas para otras especies animales, especialmente para los ovinos, aunque ahora se sabe que las características reproductoras de los camélidos son muy diferentes de las del ovino y por lo tanto requieren un manejo adecuado. En una encuesta realizada muy recientemente en fundos alpaqueros de la zona sur del Perú se encontró que las tasas de fertilidad aún dejaban mucho que desear (55—60%), que la mortalidad de crías estaba por el 35% y la producción de fibra así como las ganancias de peso vivo no estaban en el nivel deseado. Esta situación se debe en parte a la falta de mecanismos de difusión y capacitación de los conocimientos adquiridos en los últimos 25 años de investigación y, por otra parte, a la falta de conocimientos más avanzados sobre fisiología, salud, nutrición, etc. que posibiliten el establecimiento de una base necesaria para el desarrollo de una tecnología de crianza alpaquera.

Se trata de presentar aquí los resultados de la investigación en reproducción de alpacas realizada en los últimos 25 años y principalmente llevada a cabo en la Estación Principal de Altura La Raya del IVITA, Universidad Nacional Mayor de San Marcos. Estos resultados se han publicado en diferentes revistas nacionales y extranjeras con el propósito de que el lector interesado conozca la especial fisiología reproductora de la alpaca. No pretendemos que esta revisión bibliográfica sea completa, pero llenará el vacío de información que existe al respecto.

2. BASES ANATOMICAS DE LA REPRODUCCION EN LA ALPACA

2.1. Anatomía de la hembra

2.1.1. Ovario

Por lo general es de forma globosa irregular, semejando en algunos casos al ovario de la cerda, especialmente cuando contiene numerosos folículos en desarrollo. La extremidad tubo-ovárica (bursa) cubre totalmente al ovario. El ovario izquierdo es ligeramente más pesado ($2,40 \pm 1,34$ g) que el derecho ($1,87 \pm 0,94$ g) cuando no contienen un cuerpo lúteo activo, en cuyo caso el peso se incrementa entre un 60 y 100%. Las dimensiones son las siguientes: largo, $1,6 \pm 0,3$ cm y ancho, $1,1 \pm 0,2$ cm.

Cuando los folículos alcanzan un diámetro mayor a 4 mm, así como el cuerpo lúteo, éstos se proyectan sobre la superficie del ovario en forma muy prominente y son fácilmente identificables. Folículos entre 5 y 12 mm son considerados normales, mientras que estructuras mayores se consideran patológicas.

2.1.2. Tubos uterinos

Llamados también oviductos, son largos y sinuosos, de un largo promedio de $20,4 \pm 4,2$ cm, suspendidos en el mesosalpinx. El istmo, que mide unos 2—3 mm de diámetro, termina en el cuerpo uterino y en forma de una papilla que tiene un esfínter bien definido. Es imposible lavar el tubo uterino desde el cuerpo uterino, pero sí es posible hacerlo en la dirección opuesta.

2.1.3. Utero

El útero de la alpaca es bifurcado (*uterus bipartitus*), con dos cuernos uterinos separados, y la mucosa es lisa. El largo del cuerno izquierdo es ligeramente mayor ($7,9 \pm 1,3$ cm) que el derecho ($7,4 \pm 0,9$ cm). El cuerpo del útero es relativamente pequeño ($3,05 \pm 0,71$ cm). La cervix está formada por 2 a 3 anillos o pliegues anulares. En cierta medida, el útero de la alpaca se parece al de la borrega, aunque en ésta los cuernos uterinos se van adelgazando a medida que se acercan a la unión útero-tubárica. Ambos lados del útero están sujetos a las paredes abdominales y pélvicas por el ligamento ancho.

2.1.4. Vagina

Mide aproximadamente unos $13,4 \pm 2,0$ cm de largo y tiene un diámetro de $3,4 \pm 0,7$ cm. La túnica mucosa presenta pliegues longitudinales.

2.1.5. Vulva

La hendidura vulvar tiene una dirección ventro-caudal de aproximadamente 3,0 cm de longitud. Los labios vulvares, apenas prominentes, son de bordes redondeados, generalmente pigmentados y con escasa pilosidad. La comisura labial dorsal, ligeramente redondeada, está a unos 2—3 cm del orificio anal. La comisura labial ventral es aguda y termina en una corta proyección cónica.

2.2. Anatomía del aparato genital del macho

2.2.1. Testículos

En un animal adulto, los testículos se encuentran en un escroto no penduloso, sin cuello definido, formando un abultamiento sub-anal, tal como sucede en cerdos, roedores y carnívoros. El peso promedio de un testículo completamente desarrollado es de aproximadamente 18 g; existe una variación considerable en el tamaño de los testículos y el peso vivo del animal; el peso de los testículos varía entre 0,02 y 0,03 % del peso vivo. Los reproductores se seleccionan por el tamaño testicular, como sucede en otras especies domésticas, asumiéndose que existe una relación directa entre el tamaño testicular y la producción espermática. Para el animal adulto mayor de tres años, las medidas y pesos promedio de los testículos figuran en el Cuadro I.

Se considera que a los 5 años de edad, el macho se encuentra desarrollado completamente, con un peso promedio de 62,5 kg. Al nacimiento o poco tiempo después, los testículos ya deben encontrarse en las bolsas escrotales, pequeños, flácidos y de un tamaño de 0,5 cm; al año de edad, y cuando se hace la primera selección para reproductores, ambos testículos deben encontrarse en las bolsas escrotales de un tamaño promedio de $1,5 \times 0,40$ cm.

Los testículos están localizados en las bolsas escrotales, en tal forma que su diámetro mayor es el oblícuo, con una orientación dorso-caudal y ventro-cranial. Normalmente ambos testículos son del mismo tamaño, turgentes a la palpación y se mueven libremente en el escroto.

2.2.2. Epidídimo

Es similar al de otras especies, estando constituido por cabeza, cuerpo y cola. Con práctica es posible palpar y diferenciar estas tres regiones. El conducto deferente es muy delgado en su inicio (2 mm) y luego de ingresar en la cavidad pélvica, termina cerca del cuello de la vejiga, donde llega a tener un grosor de 3 mm, constituyendo lo que en otras especies sería la ampolla del deferente, sin llegar a tener las características de esta estructura. El largo total del conducto deferente es de más o menos 40 cm.

CUADRO I. TAMAÑO Y PESO DE
TESTICULOS NORMALES DE
ALPACAS ADULTAS

	Testículo izquierdo	Testículo derecho
Largo ^a (en cm)	3,98 ± 0,40	—
(rango)	(3,2—4,8)	—
Ancho ^a (en cm)	2,59 ± 0,31	—
(rango)	(1,9—3,2)	—
Peso (en g)	17,72 ± 3,65	17,78 ± 3,69
(rango)	(13,15—28,0)	(13,5—28,0)

^a Se consideró solamente el largo y ancho del testículo izquierdo por no existir diferencias mayores entre ambos testículos.

2.2.3. Glándulas sexuales accesorias

La próstata tiene la forma de una H y se encuentra ubicada dorsal y lateralmente sobre el cuello de la vejiga. Las glándulas bulbouretrales son ovoides, pequeñas, ubicadas lateralmente a la uretra en la salida pélvica. Esta especie, igual que el perro, carece de vesículas seminales.

2.2.4. Pene y prepucio

Es de tipo fibroelástico, llegando a medir 35 a 40 cm en erección. Describe, como en el toro, la “S” peniana, con la primera y segunda inflexión localizadas por delante de los testículos, en lugar de estar por detrás como en el toro. La forma del glande es de gancho curvo a la derecha, al cual sobrepasa un pequeño “proceso uretral” de más o menos 1 cm de largo. El pene está dirigido hacia adelante, al contrario de lo que se dice a menudo. El forro prepucial es triangular y pende a manera de una teta grande, con el orificio prepucial localizado en el ángulo libre del triángulo, lo que hace que cuando orina el chorro se orienta hacia atrás, como en la hembra, particularidad ésta que ha servido para que se tejan una serie de fábulas sobre esta especie animal. Durante la erección, el forro prepucial triangular se endereza para adelante, gracias a los músculos protractores, y la verga se adelanta sobre el vientre como sucede en los otros rumiantes.

3. FISIOLOGIA REPRODUCTORA

3.1. Reproducción en el macho

3.1.1. Pubertad

Al nacimiento (peso promedio de 7,5 kg) el pene se encuentra completamente adherido al prepucio. Estas adherencias desaparecen gradualmente a medida que el animal desarrolla e inicia la producción de testosterona. A la edad de un año y con un peso promedio de 35 kg, los machos muestran un interés sexual por las hembras; sin embargo, a esta edad sólo aproximadamente un 8% de los machos muestran una completa liberación de las adherencias pene-prepuciales, que les permitirá realizar la cópula. A la edad de dos años y con peso promedio de 50 kg, cerca del 70% de los machos ya no tienen las adherencias pene-prepuciales y cuando llegan a los tres años, todos, es decir el 100%, están completamente libres de las adherencias (Fig. 1).

Una pubertad más temprana en los machos es una característica deseable en los programas de selección genética; los futuros reproductores, al ser seleccionados al año de edad, preferentemente no deben tener las adherencias pene-prepuciales. Una práctica general en la mayoría de las explotaciones alpaqueras es comenzar a usar a los machos a los tres años de edad.

3.1.2. Comportamiento sexual

Los estudios y observaciones de campo revelan un comportamiento sexual muy típico y especial en las alpacas. Cuando se unen machos y hembras para el empadre, los machos comienzan el cortejo corriendo detrás de la primera hembra que encuentran, al azar; si la hembra está en celo, permitirá que el macho la monte y luego tomará la posición de sentada o posición prona para efectos del apareamiento. La cópula puede durar de 5 a 50 min.

Cuando la cópula rebasa los 20 minutos, la hembra, sin interrumpir el acto, puede adoptar la posición de cúbito lateral por unos minutos, para luego tomar la posición prona.

Durante la cópula, el macho emite un sonido gutural característico, mientras la hembra permanece silenciosa. Con frecuencia se observa que una o más hembras se sientan junto a la pareja en cópula, como un signo de estar en celo franco.

Al iniciarse la estación de monta, y cuando se unen machos y hembras por primera vez, se observa una gran actividad sexual en el rebaño, llegando los machos a copular hasta 18 veces al día; posteriormente su actividad sexual disminuye. Esto parece deberse a los siguientes factores:

- 1) Establecimiento de un status social con la formación de una jerarquía donde existen machos dominantes y machos dominados.
- 2) Cansancio físico o agotamiento sexual. Sucede entonces que, ya sea por el establecimiento de una jerarquía o el cansancio físico, o una combinación de

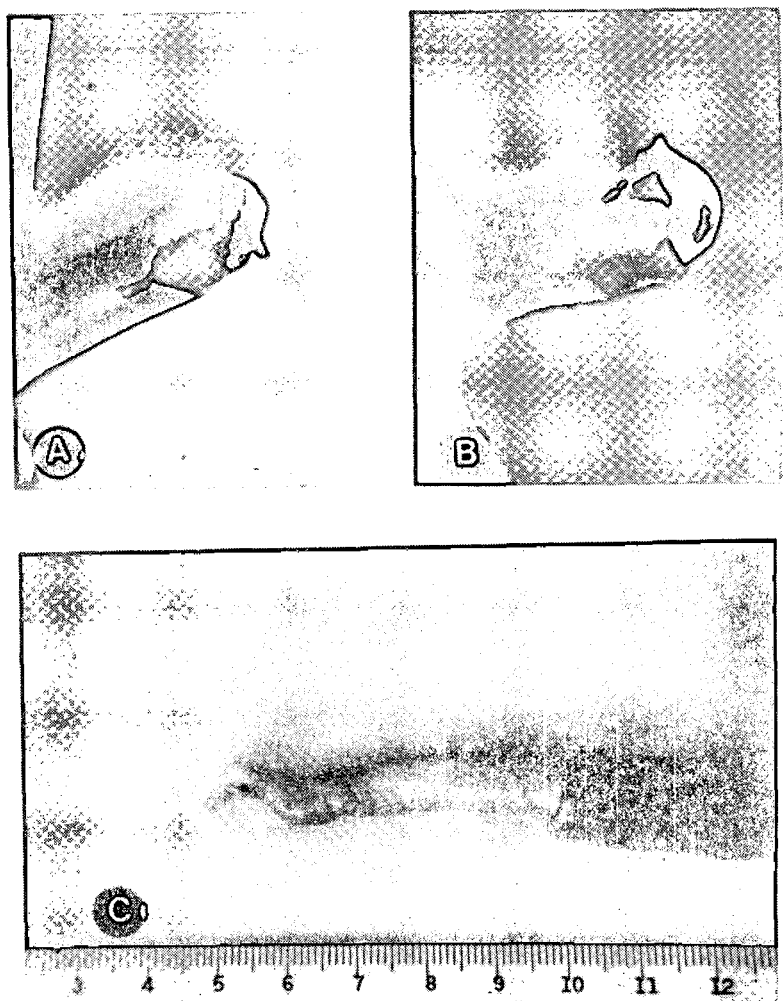


FIG. 1. Diferentes etapas de la separación pene-prepucio en alpacas: A) adhesión completa; B) separación parcial; C) separación completa.

ambos, las cópulas son muy infrecuentes en el rebaño después de unos días de iniciado el empadre, a pesar de haber hembras en celo, y como consecuencia la fertilidad es baja. Si cambiamos los machos, se renovará la actividad sexual en el rebaño, cubriéndose aquéllas que muestran celo por primera vez o retornan al celo, y así se incrementa notablemente la fertilidad del rebaño.

La Fig. 2 nos muestra el efecto del cambio de machos sobre la actividad sexual del rebaño. Por ejemplo, los machos A-B-C, cuya actividad copulatoria con el grupo I de hembras disminuyó considerablemente hasta casi desaparecer

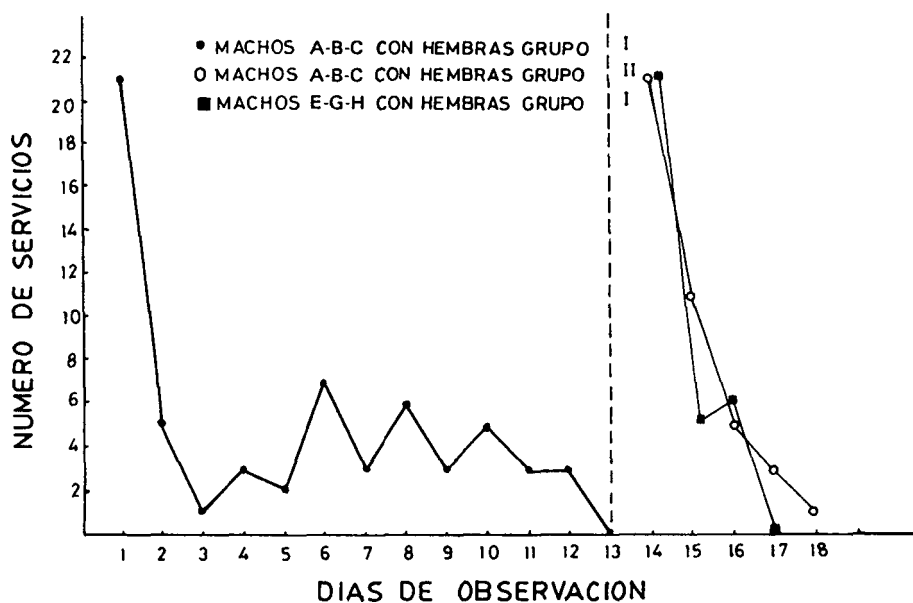


FIG. 2. Efecto del cambio de machos sobre la actividad sexual del rebaño.

el día 13 del empadre, se incrementó bastante al juntarse con otro rebaño de hembras (grupo II) el día 14. Similarmente, la actividad de las hembras del grupo I, que había disminuido con los machos A-B-C, se elevó considerablemente cuando se les unió con los nuevos machos E-G-H, lo que demuestra claramente el efecto renovador del cambio de machos.

El conocimiento de la conducta sexual y la necesidad de cambiar los machos semanalmente ha dado lugar al desarrollo de un sistema de empadre alterno, acorde a la fisiología reproductora de la alpaca, que da resultados de fertilidad satisfactorios cuando se trabaja con cientos o miles de hembras.

El Cuadro II nos muestra el efecto del empadre alterno sobre la natalidad de alpacas de una empresa en el Dpto. de Puno. En los 6 años previos a la introducción del nuevo sistema de empadre (1966-1971), la natalidad osciló entre un 51,2% y un 63,1%. En 1972, cuando se introdujo el empadre alterno, la natalidad se incrementó hasta el 81,0%.

3.1.3. Producción de semen

La colección de semen en la alpaca es complicada por la posición que adoptan los animales durante la cópula (Fig. 3) y por el tiempo que dura ésta (5 a 50 min). Mogrovejo [1] reportó en 1952 la colección de semen de 50 machos por medio de fundas vaginales que eran retiradas de la vagina después de la cópula. San Martín

CUADRO II. EFECTO DEL EMPADRE
ALTERNO SOBRE LA NATALIDAD EN
ALPACAS DE UNA COOPERATIVA
PERUANA

Año	Natalidad (%)	Tipo de empadre
1966	53,1	T
1967	59,0	T
1968	51,2	T
1969	54,4	T
1970	63,1	T
1971	61,0	T
1972	81,0	EA

T = Tradicional.

EA = Empadre alterno.

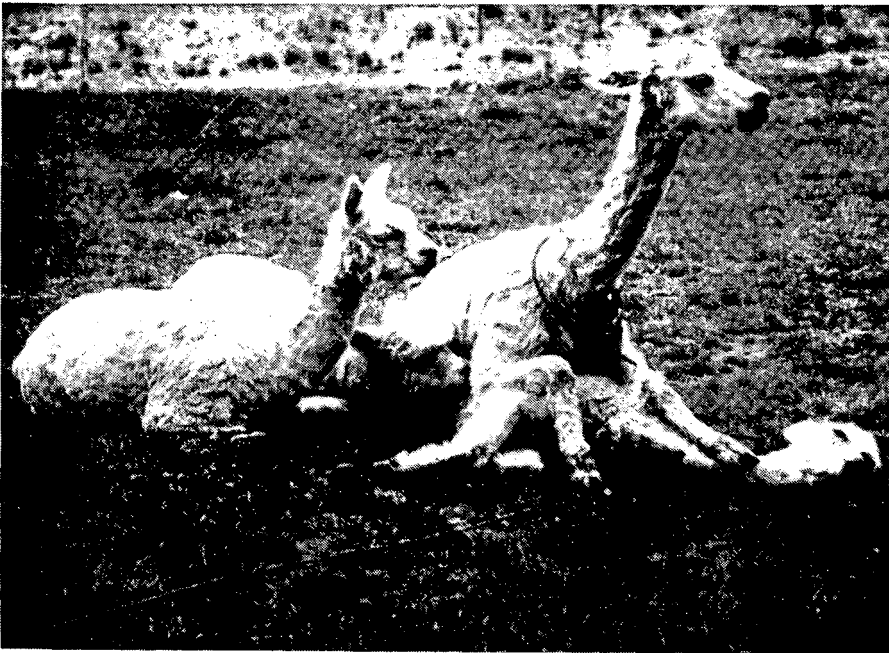


FIG. 3. Servicio natural en alpacas. Nótese dos hembras en celo que se sientan al lado de la pareja en cópula.

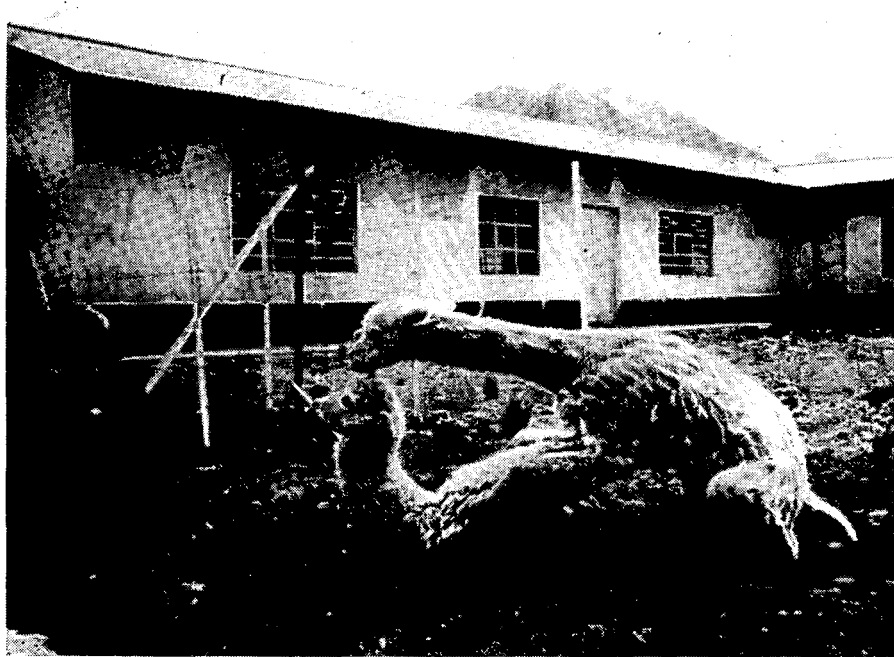


FIG. 4. Colección de semen por vagina artificial, colocado dentro del maniquí.

et al. [2] usaron esponjas vaginales para colectar el semen de 190 machos; los que no tenían espermatozoides en la primera colección fueron chequeados posteriormente hasta 3 veces, encontrándose hasta un 8% de machos azoospermicos. Sin embargo, ambas formas de colectar el semen son cuestionables, pues interfieren con la cópula normal y muestran alta contaminación con materiales extraños. El volumen del eyaculado es muy variable, fluctuando entre 0,4 y 6,6 mL.

El uso de un electroeyaculador fue reportado por Fernández-Baca y Calderón [3] y parece ser más conveniente que los métodos de las fundas o esponjas vaginales; sin embargo, durante las descargas eléctricas, el semen puede contaminarse con orina y su concentración es muy variable, fluctuando entre 1000 y 255 000 espermatozoides por mm^3 .

Recientemente, Sumar y Leyva [4] reportan el uso de una vagina artificial colocada dentro de un maniquí (Fig. 4), obteniendo así hasta 12,5 mL de semen de buena calidad, con concentraciones que superan los 600 000 espermatozoides por mm^3 y con muy buena motilidad.

El semen de la alpaca es altamente viscoso, siendo muy difícil separar los espermatozoides del plasma seminal por centrifugación, así como estimar la concentración espermática por medios convencionales y frotices y coloraciones. Se están ensayando diversas enzimas que, sin hacer daño al espermatozoide, produzcan la licuefacción

del plasma y su mejor manejo en el laboratorio. Además, es importante anotar que debido a la alta viscosidad del plasma seminal, el movimiento de los espermatozoides es muy lento, si lo comparamos con el de otras especies domésticas como el bovino y ovino; muchas publicaciones no toman en cuenta este hecho e indican pobre motilidad en general del semen de la alpaca.

Evidencias obtenidas en el uso de la fístula uretral [5] así como por medio de la vagina artificial [4] indican que la eyaculación es un proceso continuo, sin fracciones y con una calidad de semen uniforme desde el inicio hasta el final de la cópula. Asimismo, recientes estudios indican que la deposición del semen es intrauterina [6].

3.2. Reproducción en la hembra

3.2.1. Pubertad

A los doce meses de edad, la mayoría de las hembras vírgenes muestran receptividad sexual al ser puestas en contacto con el macho; sin embargo, la actividad ovárica se inicia a los diez meses, cuando los ovarios muestran folículos de 5 mm o mayores. El plano de nutrición, como sucede en otras especies, parece influir notablemente en el inicio de la pubertad. Novoa et al. [7] mostraron que en las hembras vírgenes la actividad sexual, seguida de ovulación y fertilización, ocurre una vez alcanzado cierto grado de desarrollo corporal el cual, bajo las condiciones actuales de crianza (doce a catorce meses de edad), se obtiene cuando mejora la disponibilidad de los alimentos, lo que a su vez depende de una mayor precipitación pluvial y mejora en la temperatura ambiental.

El Cuadro III nos muestra la receptividad sexual, la ovulación y la fertilización de las hembras antes y después del destete.

En un estudio de la evaluación del peso corporal al empadre, sobre la capacidad reproductiva de 280 hembras de 1 año de edad, se encontró que existe una asociación altamente significativa ($P > 0,001$) entre el peso corporal al empadre y el porcentaje de natalidad [8]. Por cada kg de incremento en peso corporal hubo un incremento del 5% en el porcentaje de natalidad, y por encima de los 33 kg de peso vivo al empadre el porcentaje de hembras vacías es relativamente independiente del peso corporal, mientras que por debajo el porcentaje se incrementa notablemente. Posteriormente se evaluaron las campañas de estas hembras en relación con su primer parto y el peso de las crías fue similar al de las de hembras adultas, así como no hubo detención en el crecimiento y producción de las madres.

Bajo los actuales sistemas de alimentación y crianza sólo un 50% de las primerizas alcanza un peso superior a los 33 kg en el momento del empadre; con mejores condiciones alimenticias y de manejo, hasta un 80% de las primerizas alcanza y supera los 33 kg.

Un retardo en la pubertad de los animales de granja en la zona tropical del mundo ha sido atribuido a una lenta tasa de crecimiento, y se ha sugerido que la pubertad llega con un cierto umbral de peso corporal [9]. En las actuales condiciones

CUADRO III. RECEPTIVIDAD SEXUAL, OVULACION Y FERTILIZACION DE HEMBRAS ANTES Y DESPUES DEL DESTETE

Edad (meses)	N° de expuestas al macho	Conducta en presencia del macho			Rechazaron	Sacrificadas	Ovularon	Fertilizadas
		Aceptaron	Forzadas					
7	34	0	0		34	3	0	0
8	8	0	0		8	3	0	0
9	8	0	0		8	3	0	0
10	8	0	0		8	3	0	0
11	8	0	2		6	3 ^a	0	0
12	8	5 ^b	0		3 ^c	4	2	2
13	13	13	0		0	3 ^d	3	3

^a Incluye a las 2 hembras tomadas a la fuerza.

^b 3 fueron sacrificadas y las 2 restantes dejadas para estudiar sobrevivencia embrionaria.

^c 1 fué sacrificada, comprobándose que no ocurrió ovulación.

^d Las 10 restantes se dejaron para estudiar sobrevivencia embrionaria.

Fuente: Novoa, C. et al. [7].

CUADRO IV. TASAS DE OVULACION Y FERTILIZACION EN DIFERENTES MESES DEL AÑO (1970)^a

Mes	Hembras servidas	Ovularon		Fertilizadas		Ovulaciones múltiples
		Número	%	Número	%	
Mayo	10	8	80	5 ^b	83	1
Junio	10	8	80	7	88	1
Julio	9	8	89	4 ^b	67	1
Agosto	15	9	60	8	89	1
Septiembre	15	10	67	5 ^c	83	1
Octubre	13	9	69	7 ^d	88	1
Noviembre	10	9	90	8 ^d	100	0
Diciembre	10	9	90	6	67	0
Total	92	70	76	50	71	6

^a Computado como porcentaje de hembras con por lo menos un óvulo en división, sobre el total de hembras que ovularon y de las que se recuperó óvulo.

^b No se recuperaron óvulos de dos hembras, una vírgen y una adulta en mayo, y de dos adultas en julio.

^c No se recuperaron óvulos de cuatro hembras, una vírgen y dos adultas.

^d No se recuperó óvulo de una hembra adulta.

Fuente: Fernández-Baca, S., et al. [13].

de manejo se acostumbra empadrear a las hembras a los 2 años de edad, lo cual puede ser reflejo de una inadecuada alimentación y manejo.

3.2.2. Estación de reproducción

Estudios efectuados por San Martín [10] en alpacas criadas en su habitat natural del Altiplano Sur del Perú muestran una estacionalidad reproductora que se extiende de diciembre a marzo; esta época coincide con los meses más abrigados del año, lluviosos y con abundancia de forraje verde. También, en las explotaciones grandes y en las comunidades campesinas donde machos y hembras se encuentran juntos todo el año, los nacimientos ocurren entre los meses de diciembre a marzo. Esta estacionalidad reproductiva se observa también en los camélidos silvestres, la vicuña y el guanaco [11, 12].

Sin embargo, cuando las hembras son mantenidas separadas de los machos y se permite el servicio una sóla vez por mes, hembras y machos muestran actividad sexual durante todo el año, como lo refleja el Cuadro IV, donde las tasas de ovulación y fertilización, así como los de sobrevivencia embrionaria, no fueron influenciadas

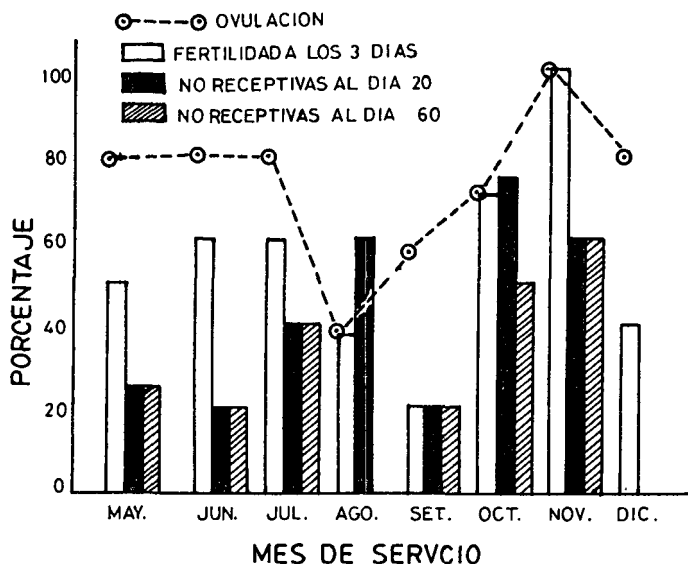


FIG. 5. Tasas de ovulación y fertilidad a los 3, 20 y 60 días post-coito en hembras vírgenes.

significativamente por la estación del año, puesto que los valores mostrados son similares a los observados durante la estación normal de monta (diciembre a marzo).

Sin embargo, se observó una disminución de las tasas de ovulación a partir de agosto. Esta disminución fue mucho más manifiesta y alcanzó significado estadístico en las hembras vírgenes de poco más de un año de edad. Como quiera que la disminución de las tasas de ovulación ocurrió durante los meses de mayor sequía y por consiguiente de menor disponibilidad de alimento, es probable que se debiera a la disminución de los niveles de ingestión de energía y proteína (Figs. 5, 6).

Otras observaciones experimentales muestran que la continua asociación de hembras y machos inhibe la actividad sexual de los machos hasta casi anularla [13]. No se conocen los factores que son responsables de este efecto inhibitor en condiciones naturales y qué factores son los que la restauran. Factores ambientales, así como estímulos visuales y olfatorios, pueden estar influenciados a través del sistema nervioso en la conducta sexual. En relación a la estación de monta de camélidos cautivos en Zoológicos, Schmidt [14] manifiesta que el guanaco y la vicuña muestran una bien definida estación de monta que corresponde exactamente con lo que sucede en el estado salvaje, mientras que la llama se reproduce continuamente, con un pico de mayor reproducción, que corresponde a lo que sucede en su habitat natural.

Parecería ser que ciertos factores ambientales, como la disponibilidad de alimentos abundante y verde, constituyen importantes estímulos que desencadenan la actividad reproductora.

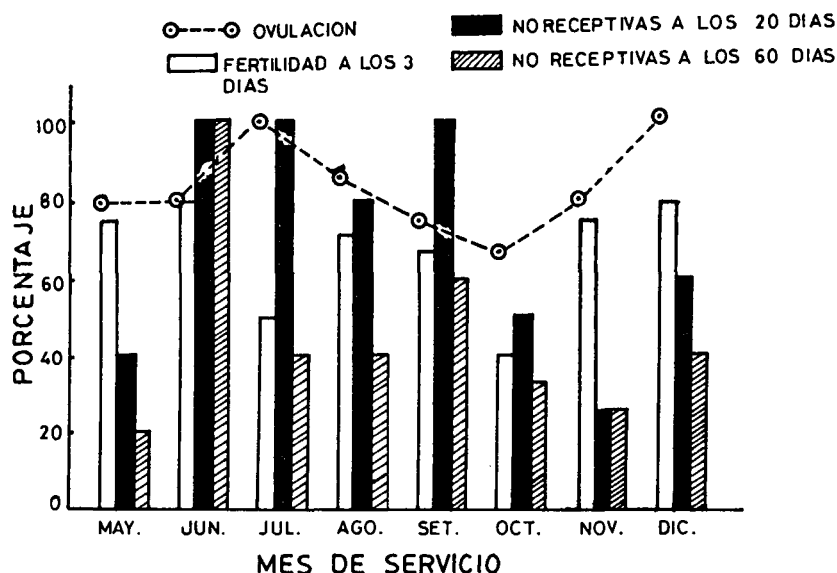


FIG. 6. Tasas de ovulación y fertilidad a los 3, 20 y 60 días post-coito en hembras multíparas.

3.2.3. Celo y ovulación

La hembra alpaca no presenta ciclos estruales como en otras especies domésticas. Durante la estación reproductora y en ausencia de macho permanece en un estado de celo continuo que puede llegar a los 21 días e inclusive a los 36 días, con periodos cortos de no aceptación que pueden durar hasta 48 horas [2].

Los signos o síntomas de celo en la alpaca no son tan fácilmente visibles y definidos como los de la vaca o la yegua, existiendo una gran variación individual en las diferentes manifestaciones del celo.

Recientemente, Sumar y Bravo (datos no publicados), usando laparoscopia, demostraron que no había relación directa entre la imagen ovárica (presencia de folículos de tamaño ovulatorio) y el grado de receptividad sexual; muchas hembras sin la presencia de folículos ovulatorios mostraban signos de receptividad cuando eran enfrentadas al macho. Los autores anteriormente citados manifiestan que parecería que existen "ciclos foliculares" en la alpaca, de 8 a 12 días de duración.

La hembra alpaca en celo muestra un comportamiento especial ante la presencia del macho. Puede asumir la posición de sentada cuando se le acerca el macho o puede acercarse a una pareja en cópula y sentarse muy cerca de ella. Otras veces, el macho puede perseguir a la hembra durante corto tiempo hasta que la monta, para inmediatamente sentarse e iniciar la cópula. También es frecuente ver a hembras en celo montar a otras hembras.

CUADRO V. NUMERO DE HEMBRAS EN CELO QUE RESPONDIERON A VARIAS CLASES DE ESTIMULOS DE MONTA

Tipo de estímulo	N° grupo	N° que ovularon	% de ovulación	N° con óvulos divididos	N° con ovulación múltiple
1. No servidas	20	1	5	0	0
2. Montadas solamente	13	2	15	0	0
3. Montadas solamente + I.A.	9	3	33	2	0
4. Servicio interrumpido ^a	10	6	60	4	1
5. Servicio estéril	22	17	77	0	2
6. Servicio estéril + I.A.	21	18	86	3 ^b	1
7. Servicio simple	44	36	82	30	3
8. Servicio múltiple	10	7	70	6 ^c	0
9. 750 U.I. hCG	10	10	100	0	1
10. 750 U.I. hCG + I.A.	18	18	100	4	4

^a Ovulos de tres animales no recuperados.

^b Ovulo de un animal, no recuperado.

^c El servicio fue interrumpido aproximadamente a los 5 min de iniciado.

Fuente: Fernández-Baca, S., et al. [16].

Dado que las hembras al inicio de la estación de monta muestran un continuo desarrollo folicular y, por lo tanto, una receptividad continuada, se puede esperar que la mayoría de las hembras del rebaño estará en celo, siempre y cuando las hembras no hayan sido expuestas al macho o a otros estímulos capaces de inducir la ovulación.

La alpaca, así como el camello, coneja, gata, hurón, mapache y otros, muestra ovulación inducida o refleja. En la alpaca, la ovulación ocurre 26 horas después de la monta con macho, y 24 horas después de la inyección endovenosa de hCG [2]. Según el autor de esta Ref., San Martín, una dosis de 10 U.I. de hCG endovenoso fue suficiente para inducir la ovulación en uno de dos animales. Un miligramo de hormona luteinizante (NHI-LH-SII OVINE) fue efectivo para inducir la ovulación, así como los factores de liberación (Gn RH) (Buserelein-Hoechst) a la dosis de 4—8 mg [15].

El estímulo necesario para la descarga de la hormona LH y subsecuente ovulación es la introducción del pene; el estímulo de la monta sola, sin introducción del pene resulta en una baja tasa de ovulación [16]. Alrededor del 20% de las hembras no ovula en respuesta a una simple o múltiple monta con machos enteros o vasectomizados, debido probablemente a una deficiencia en la hormona luteinizante de la pituitaria, ya que una simple inyección intramuscular de 750 U.I. de hCG en alpacas en celo indujo la ovulación en todos los casos, como se ve en el Cuadro V.

Las fallas en la ovulación pueden ser un factor importante que contribuye a la baja eficiencia reproductora en las condiciones actuales de manejo en las explotaciones alpaqueras. Sumar y Bravo (datos no publicados) encontraron que, en hembras adultas, un 38% ovulaba tardíamente (después de 30 h del servicio y hasta las 72 h) y que un 8,1% no ovulaba; en primerizas, un 36% ovulaba tardíamente y un 35% no ovulaba.

El porcentaje de ovulaciones múltiples después de la monta natural es variable, fluctuando del 1,7% al 8,6%. El uso de gonadotropinas coriónicas duplica el porcentaje de ovulaciones múltiples (Cuadro VI).

A pesar de la existencia de ovulaciones múltiples para la alpaca, nunca se ha reportado en el Perú nacimientos múltiples, aunque la doble gestación ocurre ocasionalmente durante los primeros 40 días de gestación [15]. La literatura mundial reporta un sólo caso de nacimientos de mellizos en llamas, en los Estados Unidos [17].

Ovulación espontánea en alpacas ha sido observada por Fernández-Baca [18] en cerca del 5% de las hembras. Fenómeno similar es reportado por Leyva (comunicación personal), quien encontró hasta un 40% de ovulación espontánea durante el apogeo de la estación reproductora, bajando a un 10% durante la época de no estacionalidad reproductora. En cualquiera de los casos, no se descarta el efecto estimulante de los machos vasectomizados durante el “chequeo de celo” sobre la ovulación.

Muy recientemente, Ríos et al. [19] demostraron que algún substrato químico presente en el semen de la alpaca y toro es capaz de inducir la ovulación sin necesidad de la cópula, constituyendo, al igual que el camello bactriano [20], un nuevo

CUADRO VI. ACTIVIDAD OVÁRICA, SEGUN VARIOS AUTORES

Autores	Año	Número de animales	% ovulación		
			Ovario izquierdo	Ovario derecho	Ambos ovarios
San Martín, M., et al. ^a	1967	52	61,5	38,5	—
Calderón, W., et al. ^b	1968	71	47,8	42,2	9,8
Calderón, W., et al.	1970	31	67,7	25,8	6,5
Fernández-Baca, S., et al. ^c	1970	118	49,6	50,4	—
Fernández-Baca, S., et al.	1972	70	62,8	28,6	8,6
Fernández-Baca, S., et al.	1973	928	47,4	50,9	1,7

^a Después de monta natural o hCG. Los autores indican ovulación de ambos ovarios en 2 hembras de monta natural y en 3 de hCG.

^b En 37 animales la ovulación se indujo con hCG.

^c 12 hembras tuvieron ovulación múltiple; 10% de las hembras después de monta natural y 20% con hCG. 11 hembras produjeron 2 óvulos y 1 hembra 3 óvulos.

mecanismo de ovulación en estos mamíferos. Aún se desconoce la naturaleza química del sustrato seminal que induce la ovulación, así como los mecanismos anatómo-fisiológicos de transferencia hacia el ovario.

La actividad ovárica diferencial en relación con el ovario que ovula, derecho o izquierdo, se da en el Cuadro VI, que es un examen de la literatura existente sobre el tema. La interpretación no es fácil debido a que algunos autores usaron dos métodos de inducción de ovulación (monta natural y hCG), tiempos de cópula no estandarizados, cantidad y ruta de las gonadotropinas diferentes, número diferente de animales, épocas distintas, etc. Sin embargo, el método más confiable es el de Fernández-Baca et al. [21], donde se examinaron en camal los órganos de 928 alpacas gestantes y donde se encontró que el ovario derecho era ligeramente más activo (50,9%) que el izquierdo (47,4%) y había un 1,7% de ovulaciones múltiples.

3.2.4. Formación y función del cuerpo lúteo

Tras la ovulación se forma un cuerpo lúteo que a los 8 días muestra el mayor tamaño (16 mm) y actividad secretoria [18]. En ausencia de preñez, la actividad del cuerpo lúteo, medida por los cambios de tamaño y actividad secretoria, inicia su declinación el día 13 aproximadamente, mostrando los ovarios nuevos folículos y la hembra pronto retorna al celo. La regresión del cuerpo lúteo es completa alrededor del día 18 después de la monta estéril. En el pasado se creía que las cópulas estériles eran seguidas por un período más o menos largo de pseudopreñez.

Es especialmente importante notar la disminución de la concentración y de la progesterona total tanto en el tejido luteal como en la sangre periférica, del día 8 al 13, lo que sugiere que una gran disminución en tamaño e incluso la mayor disminución en actividad secretoria ocurrieron durante esos 5 días, que constituyen un período crítico en la vida del cuerpo lúteo [18].

3.2.5. Niveles de progesterona en la leche después del servicio estéril

Recientemente se han medido los niveles de progesterona en la leche de alpacas lactantes, después del servicio estéril (con macho vasectomizado), por el método de radioinmunoanálisis, usando ^{125}I , con los resultados que se exponen más abajo. Los niveles de progesterona son indetectables hasta el 5° día después de la cópula, alcanzando su mayor concentración en el 9° día y llegando a niveles basales el 11° día después de inducida la ovulación. Las hembras que fallaron la ovulación muestran niveles basales de progesterona. (Cuadro VII.)

Los resultados nos indican que la fase luteal después del servicio estéril es más corta que en otras especies domésticas mayores, así como los niveles totales de progesterona; estos resultados son muy similares a los encontrados en la llama [22].

CUADRO VII. NIVELES DE PROGESTERONA EN LA LECHE, DESPUES DEL SERVICIO ESTERIL EN ALPACAS^a

Días ^b	nmol/L
1	0
2	0
3	0
4	0
5	0
6	5,1 ± 3,3
7	9,4 ± 7,3
8	10,1 ± 5,4
9	14,3 ± 9,2
10	4,3 ± 4,9
11	0
12	0
13	0
14	0
15	0
16	0

^a Promedio de progesterona en las hembras que ovularon.

^b Día 1 = día del servicio.

3.2.6. Mecanismos de regresión del cuerpo lúteo en la alpaca

En la actualidad se está poniendo mucha atención en los mecanismos de regresión del cuerpo lúteo (luteolisis) en todas las especies domésticas. La caída rápida en los niveles de progesterona en el tejido luteal y en el plasma circulante el día 13 en alpacas no preñadas sugiere una interferencia con la actividad del cuerpo lúteo entre los días 8 y 13. Esto se debería ya sea a la acción de una activa sustancia luteolítica, a la rápida caída de un agente luteotrófico, o a una combinación de ambas [16, 18].

Uteros unicornios son encontrados en camal o al sacrificio de hembras infértiles, y como regla se encuentra uno o dos cuerpos lúteos ipsilaterales al cuerno faltante, lo que sugiere la participación del cuerno del útero en la regresión del cuerpo lúteo.

Recientemente se ha estudiado el efecto del útero en la función del cuerpo lúteo [23]. La eliminación del cuerno uterino izquierdo de hembras con un cuerpo lúteo en el ovario izquierdo prolongó significativamente la vida del cuerpo lúteo en

CUADRO VIII. TASAS DE PREÑEZ A DIFERENTES ESTADIOS DE LA MONTA, Y LOCALIZACION DE LOS EMBRIONES

Días después del servicio	Número de animales	Número que ovularon	Preñez		% embriones en el cuerno izquierdo
			Número	%	
3	20	16	14 ^a	70	56
28—31	20	—	7 ^b	35 ^c	87
40—45	20	—	7	35 ^c	71
87—95	20	—	8	40 ^c	100 ^c

^a Un total de 16 óvulos se recuperaron de 14 hembras.

^b Un total de 8 embriones fueron recuperados. Dos embriones vivos de 31 días se encontraron en un animal, ambos en el cuerno izquierdo; dos cuerpos lúteos se encontraron en el ovario derecho.

^c Diferencia significativa ($P < 0,05$) de los valores para el tercer día.

Fuente: Fernández-Baca, S., et al. [18].

3 de 4 alpacas, evidenciada por los altos niveles de progesterona plasmática, la presencia de un largo cuerpo lúteo y la falta de receptividad sexual. Igualmente, la eliminación del cuerno uterino derecho en hembras con cuerpo lúteo en el ovario derecho también prolongó la vida del cuerpo lúteo, aunque el efecto no fue tan marcado como en el caso anterior. Todo esto indica que la regresión del cuerpo lúteo en la alpaca no preñada está bajo la influencia del útero. Sin embargo, parece haber una diferencia en el efecto luteolítico entre los cuernos uterinos derecho e izquierdo, sugiriendo que la actividad luteolítica del cuerno uterino derecho es local, mientras que la del cuerno uterino izquierdo tiene efectos locales y sistemáticos [23].

3.2.7. Mortalidad embrionaria

En un estudio conducido por Fernández-Baca et al. [18], el 70% de los óvulos recuperados tres días después de la monta con machos enteros estaba fertilizado, pero solamente un 50% sobrevivió más allá de los 30 días de gestación (Cuadro VIII).

La mortalidad embrionaria durante los primeros meses de gestación es más alta en alpacas que en otras especies domésticas y representa un serio problema de reproducción de esta especie.

3.2.8. Función del cuerpo lúteo durante la preñez

Sumar y Leyva [8] estudiaron el papel que desempeña el cuerpo lúteo en el mantenimiento de la preñez en la alpaca; el estudio incluyó la eliminación quirúrgica del ovario conteniendo el cuerpo lúteo o la enucleación del mismo. En todos los

CUADRO IX. LARGO DE GESTACION EN LAS RAZAS DE ALPACA SURI Y HUACAYA

Raza	Número de hembras	Período de gestación (días)	
		Rango	Media $\pm$ S.E.
Huacaya	169	325—358	341,6 $\pm$ 0,105
Suri	137	326—361	345,3 $\pm$ 0,401

animales intervenidos desde el 1^{er} mes de gestación hasta el 10^o ocurrió el aborto. El grupo de control o testigo continuó normalmente gestando. A los 11 meses de gestación (casi a término) se producía el nacimiento de la cría, pero con pesos bajos y alta mortalidad. Todo parece indicar que en la alpaca el cuerpo lúteo es la mayor fuente de progesterona a lo largo de los 11 meses que dura la gestación en esta especie.

3.2.9. Diagnóstico de gestación

La conducta de la hembra preñada frente al macho es un buen método de diagnóstico de preñez. Se ha observado, sin excepción, que las hembras que mostraron comportamiento de celo 20 o más días después del servicio estaban vacías y que todas las hembras que rechazaron al macho estaban o, más probablemente, habían estado preñadas. Algunas hembras que rechazaron al macho no estuvieron preñadas, debido posiblemente a la mortalidad embrionaria sin regresión del cuerpo lúteo. Este método funciona bien durante la estación reproductora y cuando los machos muestran un buen líbido, pero no así fuera de la estación reproductora, cuando los machos no muestran interés por las hembras.

Por medio de la palpación rectal en hembras adultas se puede diagnosticar preñez a partir de los 35 días, teniendo cuidado en palpar el útero delicadamente así como el ovario, por la presencia del cuerpo lúteo. Este método no puede usarse en alpacas primerizas o adultas de poco desarrollo y requiere alguna experiencia.

Recientemente hemos probado el método de ultrasonido (Scanopreg) con muy buenos resultados, entre los 70 y 120 días de gestación. Se está trabajando en otros métodos de diagnóstico, como los químicos, biológicos e inmunológicos.

Actualmente y en la mayoría de las explotaciones alpaqueras, el diagnóstico de gestación se realiza a los 8 meses más o menos por el sistema de balotaje o palpación externa.

CUADRO X. RELACION ENTRE LA LOCALIZACION DEL CUERPO LÚTEO Y DE LOS EMBRIONES EN LA ALPACA

Localización del cuerpo lúteo	Número de animales	Localización del embrión	
		Cuerno derecho	Cuerno izquierdo
Ovario derecho	472 (50,9%)	12 (2,5%)	460 (97,5%)
Ovario izquierdo	440 (47,4%)	3 (0,7%)	437 (99,3%)
Ambos ovarios	16 (1,7%)	0	16 (100%)
Total	928 (100%)	15 (1,6%)	913 (98,4%)

Fuente: Fernández-Baca, S., et al. [21].

3.2.10. Gestación

San Martín et al. [2], en un estudio que incluía 306 alpacas de las razas Suri y Huacaya y donde se registraron los apareamientos y los partos, dan las cifras que se presentan en el Cuadro IX.

Casi todos los fetos se encuentran localizados en el cuerno uterino izquierdo, aunque los óvulos provienen indistintamente del ovario derecho o izquierdo (Cuadro X), lo que indicaría que los embriones que se originan en el lado derecho tienen que migrar al izquierdo para sobrevivir [21].

No se conocen las causas de esta migración interna ni el porcentaje, pequeño, de embriones que se implantan en el cuerno uterino derecho, ambiente no propicio para el desarrollo embrionario. Estudios recientes sobre el efecto luteolítico diferencial de los cuernos uterinos derecho e izquierdo indican que la actividad luteolítica del cuerno uterino derecho es solamente local mientras que la actividad luteolítica del cuerno uterino izquierdo es local y sistemática [23].

3.2.11. El parto en la alpaca

Los nacimientos de alpacas ocurren solamente durante las horas más abrigadas del día, comenzando con un pequeñísimo número a las 5 de la mañana con un pico alrededor de las 9 (23,85% de los casos) y terminando a las 5 ó 6 de la tarde. No se han observado nacimientos entre las 7 de la tarde y las 4 de la mañana, período del día en que las temperaturas están generalmente bajo cero durante todo el año, incluyendo el verano (Sumar y Leyva, datos no publicados). Esto significa que un 93,5% de los nacimientos ocurre de las 7 de la mañana a la 1 de la tarde, lo que muestra una admirable adaptación de estos animales al ambiente alto andino (Fig. 7). Esto

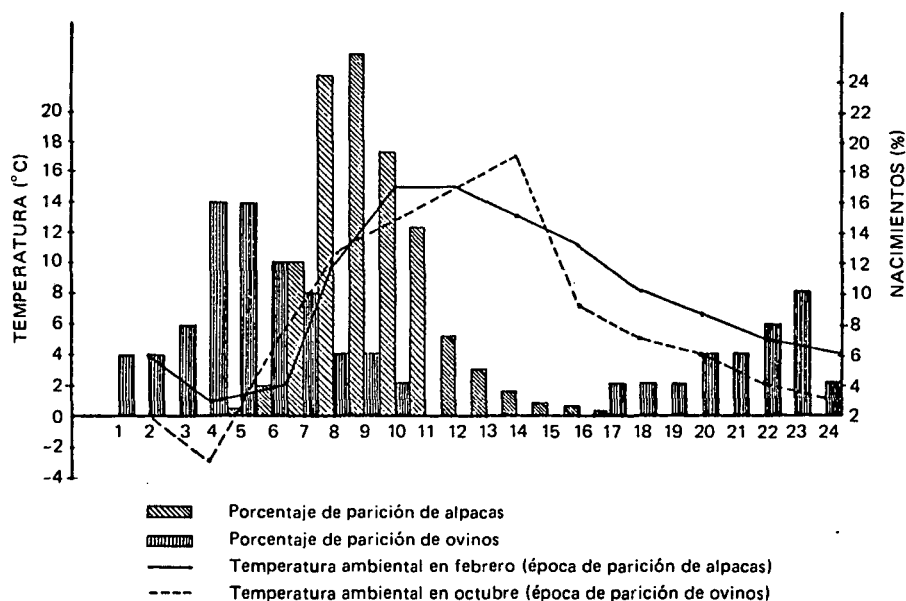


FIG. 7. Histograma que muestra el porcentaje de parición en alpacas y en ovinos en relación a la hora del día, en zonas ecológicas alto-andinas muy similares.

contrasta con lo que sucede con el ovino, por ejemplo, cuya mayoría de partos tiene lugar durante las horas de oscuridad y con temperaturas generalmente bajo cero, lo que causa gran mortalidad entre los corderos. En otros ungulados domésticos los partos se producen también en las horas de oscuridad [24].

Otra de las características de la alpaca es que no lamen a sus crías y nunca se ha visto la placentofagia. Las alpacas son excelentes madres, incluso las primerizas, y muy rara vez abandonan a sus crías aun cuando estén malnutridas.

El parto en la alpaca, comparado con otras especies domésticas, no presenta complicaciones, se produce fácilmente y toma un promedio de 3 horas y 30 minutos desde el inicio de los primeros síntomas hasta la expulsión de la placenta. El tiempo promedio de duración en minutos para las tres etapas del parto se puede observar en el Cuadro XI.

3.2.12. Post-parto en la alpaca

Hasta el cuarto día después del parto la hembra es submisiva a ser servida por el macho, pero no está en celo; para ello se requiere la completa regresión del cuerpo lúteo, el crecimiento folicular y la involución del útero. A los 5 días posteriores al parto, algunas hembras muestran aceptación al macho y si son servidas hay posibilidades de que ovulen y sean fertilizadas, pero el útero aún contiene loquios.

**CUADRO XI. TIEMPO PROMEDIO DE DURACION EN MINUTOS PARA
LOS TRES ESTADIOS DEL PARTO DE HEMBRAS ALPACA
MULTIPARAS Y PRIMERIZAS**

	Primer estadio	Segundo estadio	Tercer estadio
Múltiparas	87,54 ± 67,26	24,73 ± 16,18	80,76 ± 38,83
Primerizas	101,52 ± 77,59	24,52 ± 12,80	77,13 ± 38,61

A los 10 días post-parto, los folículos tienen entre 8 y 10 mm, el cuerpo lúteo de la preñez anterior ha regresionado considerablemente y el útero ha involucionado, pesando sólo la quinta parte del peso a las 24 horas de parida. A los 20 días ya hay involución completa (Fig. 8). Se recomienda servir a las hembras nuevamente después de 15 días del parto para obtener resultados satisfactorios de natalidad [25].

3.2.13. Placenta

La placenta en la alpaca, igual que en otros camélidos, es difusa y epiteliocorial. El epitelio coriónico se inserta dentro de los villi o pliegues no ramificados, los cuales están yuxtapuestos a las ondulaciones correspondientes del epitelio uterino, y la aposición o interfase neonatal consiste en una intrincada interdigitación de los microvillis del feto y de la madre. En gestación avanzada, ambos, epitelio coriónico y uterino, están profundamente dentados por los capilares placentarios, de tal manera que la mínima distancia intercapilar a través de la comunicación extendida y difusa puede ser tan pequeña como 2 micras. Esta distancia parecería mucho menor que la encontrada en la placenta epiteliocorial de cualquier otra especie de ungulados domésticos en gestación avanzada y puede ser una de las varias adaptaciones a las grandes alturas [26].

3.2.14. Inseminación Artificial (I.A.)

Se han realizado varios estudios sobre la factibilidad de la inseminación artificial y se han ensayado también algunos cruces interespecíficos, como el de la alpaca × vicuña (F1 = paco-vicuña) y el de vicuña × llama (F1 = llama-vicuña). El primer intento ha sido la inseminación de hembras alpacas con semen de vicuña y con semen de paco-vicuña macho, producto de un cruzamiento natural [27]. El semen fue obtenido por la técnica de electroeyaculación descrita por Fernández-Baca y Calderón [3]. En esta primera prueba, 42 alpacas hembras fueron inseminadas intracervicalmente con semen puro recién colectado. Solamente una cría nació del

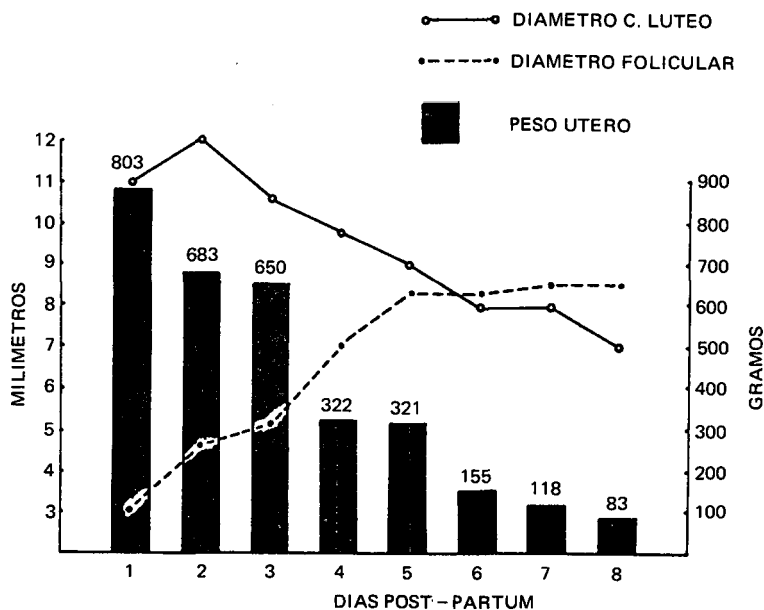


FIG. 8. Cambios en el diámetro del cuerpo lúteo y folículos y peso del útero después del parto en la alpaca.

total de inseminaciones hechas con el semen de paco-vicuña, es decir, la cría tenía solamente 1/4 de vicuña y 3/4 de alpaca. Estos pobres resultados se atribuyeron a la pobre calidad del semen.

Calderón et al. [28] realizaron un estudio para determinar el tiempo más oportuno para inseminar a la hembra, después de inducida la ovulación —ya sea con vasectomizados o hCG, encontrándose las mayores tasas de óvulos fértiles entre 35 a 45 horas. En este estudio las tasas de fertilización fueron mayores usando machos vasectomizados que hCG para inducir ovulación. En este mismo estudio la inseminación fue intrauterina por rectopalpación.

Más recientemente, Leyva et al. [29] inseminaron alpacas y llamas con semen de vicuña y paco-vicuña con una tasa total de natalidad del 38%. Cuando el semen de vicuña se usó en llamas hembras se obtuvo una natalidad del 16,7% y en alpacas hembras se obtuvo un 22,2%. Cuando se usó el semen de paco-vicuña en llamas hembras, la tasa de natalidad fue del 60% y en alpacas hembras del 31,1%. En este estudio las tasas de natalidad fueron mejores cuando se usó hCG (750 U.I.) para inducir la ovulación.

Los resultados de la I.A. son todavía bajos y se puede deber, entre otros, a fallas de ovulación, muerte embrionaria y otros factores. Es interesante anotar que, bajo condiciones de monta natural con un sólo servicio, las tasas de natalidad son muy bajas (Sumar, datos no publicados).

Los camélidos sudamericanos tienen una ventaja sobre otras especies en el uso de la inseminación artificial. Dado que las alpacas hembras están en celo continuado durante la estación reproductora, la ovulación puede inducirse con un macho vasectomizado o hCG, seguido de inseminación intrauterina.

3.2.15. Transferencia de embriones

Novoa y Sumar [30] reportaron por primera vez la colección de óvulos después de la ovulación natural o superovulación. Los óvulos fueron colectados por laparotomía y lavando los oviductos con suero sanguíneo homólogo a 37°C. El lavado fue hecho del oviducto hacia el cuerno uterino, ya que no es posible pasar fluidos del útero hacia el oviducto debido a la papilla en la unión útero tubárica. Los óvulos fueron recogidos 3 días después del servicio, puesto que en este período los óvulos se encuentran aún en el oviducto. Para la superovulación se usó PMS diariamente durante tres días, seguido de una inyección de 750 U.I. de hCG para inducir la ovulación, e inmediatamente el servicio con macho entero. Este procedimiento dió un promedio de 3 óvulos por colección, mientras que el número de cuerpos lúteos fue de 6 en promedio. Debido al gran volumen de líquido usado para colectar los óvulos del útero, muchos de éstos se pierden en la colección.

Posteriormente se hicieron ensayos de transferencia de embriones en alpacas [31] usando 44 receptores y 11 donantes. Los óvulos fueron colectados quirúrgicamente y fueron tranferidos a las receptoras. Cuatro hembras quedaron preñadas con un nacimiento normal y tres abortos avanzados. Estos pobres resultados se deben en parte a las dificultades de encontrar un medio de transferencia adecuado o a otros requerimientos fisiológicos.

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USE OF RADIOIMMUNOASSAY METHODS FOR STUDIES ON THE REPRODUCTION OF BUFFALOES*

B.M.A.O. PERERA

Department of Veterinary Clinical Studies,
Faculty of Veterinary Medicine
and Animal Science,
University of Peradeniya,
Peradeniya, Sri Lanka

Abstract

USE OF RADIOIMMUNOASSAY METHODS FOR STUDIES ON THE REPRODUCTION OF BUFFALOES.

The water buffalo is an important livestock resource of many tropical and subtropical regions of the world. In Asia it has a crucial role in rural farming systems, providing draught power, meat and milk for small farmers. However, productivity is often limited by many adverse factors which depress reproductive efficiency. Research is therefore necessary to identify the constraints under each specific situation and to generate appropriate methods for overcoming them. Nuclear techniques such as radioimmunoassay for measuring reproductive hormones, when used in combination with clinical and ethological studies, have proved valuable in understanding the reproductive biology of buffaloes. Changes occurring in the major steroid hormones and prostaglandins during the oestrous cycle, pregnancy, parturition and the postpartum period have recently been characterized. The long calving intervals, which constitute the major reproductive constraint, have been shown to be mainly due to prolonged postpartum ovarian inactivity or acyclicity. This is influenced by a variety of managerial, nutritional and climatic factors, among which intensity of suckling, usage for draught and season of calving appear to be specifically important. Nevertheless, it has been established that buffaloes can have optimum fertility with short acyclic periods under certain village conditions and are therefore not biologically limited in this respect. Further studies are therefore needed to clarify the relative importance of the factors delaying postpartum ovarian activity under specific small farm conditions at the village level. Although hormone assays and hormone treatments have been used as management and therapeutic aids in improving reproductive efficiency in certain situations, their applicability at the wider field level is severely limited. The need, therefore, is to study methods of manipulating the management practices which influence fertility by judiciously combining field surveys with clinical methods and hormone assays. It would then be possible to devise economically feasible and socially acceptable management systems that would permit the full reproductive potential of the buffalo to be achieved.

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1. INTRODUCTION

The population of water buffaloes (*Bubalus bubalis*) in the world is about 150 million. They are widely distributed in tropical and subtropical regions, with over 80% of the population located in Asia. Other areas with significant populations include the Mediterranean region, South America and Australia.

Water buffaloes have been classified into two main groups: the river type and swamp type [1], based on their phenotypic characters and wallowing habits. It has been found that they also differ in their chromosome complements, the former having 50 and the latter 48 [2]. The indigenous buffaloes of Sri Lanka (Lanka buffaloes), although classified phenotypically as belonging to the swamp type, have been found to be karyotypically similar to the river type [3].

The river types include the specialized dairy breeds of India and Pakistan, and the breeds used as dual-purpose milk and meat producers in Egypt, southern European and West Asian countries and Latin America. The swamp types, which are found in South-East Asian countries and in China, are raised by small-scale rural farmers for draught purposes in the cultivation of crops. In many situations they are also utilized for subsidiary purposes such as milk, meat or manure.

When raised under modern systems of management and nutrition, with adequate measures for controlling diseases and parasites, buffaloes have been found to perform exceptionally well. However, under the majority of small farm situations in developing countries, several constraints operate to limit production and reproductive efficiency. It is therefore necessary that studies be undertaken to determine the more important problems under each specific situation, and to formulate practically applicable and economically feasible methods for improving productivity under small farm conditions. Such programmes would have a significant effect in improving the living conditions of millions of poor marginal farmers who are the mainstay of food production in most developing countries within the tropics.

This paper presents a brief review of the current knowledge on reproductive endocrinology of the female water buffalo, together with an appraisal of the gaps in knowledge and a consideration of the potentially useful applications of hormone measurement for improving reproductive performance.

2. MEASUREMENT OF HORMONES

Reproductive efficiency is a major determinant of productivity in all livestock operations. To improve reproductive performance a thorough understanding of the physiological processes underlying these functions is essential. Studies aimed at providing this knowledge have been greatly facilitated by the advent of methods for accurately measuring the minute quantities of reproductive hormones present in blood and other body fluids [4, 5]. Of the many methods available, the technique of

radioimmunoassay (RIA) is the most widely used at present. In certain situations enzymeimmunoassays (EIA) appear to hold great potential, being quicker and requiring less expensive instrumentation.

In the buffalo, measurement of steroid hormones presents no difficulty since assays developed for other species can be directly applied. For protein hormones such as gonadotrophins, however, the non-availability of purified buffalo hormones has necessitated the adoption of heterologous assay systems. It should be remembered that such assays must be rigorously validated and that the results should be interpreted in terms of relative rather than absolute hormone concentrations. A further consideration is the endogenous pattern or profile of a particular hormone in peripheral blood; this may have characteristic random or cyclic fluctuation which may be episodic or diurnal in nature. This should be considered in selecting the frequency of sampling, as well as in interpretation of the observed results.

In spite of the above limitations, hormone measurements can provide valuable insights into the basic reproductive biology of the water buffalo if used judiciously and in combination with clinical methods such as rectal palpation of internal genitalia and behavioural observations.

3. STUDIES ON REPRODUCTION

3.1. Puberty

Buffalo heifers of the river type are said to attain puberty at about 15 to 18 months of age [6], whereas swamp types appear to take longer. However, the studies reported to date have not provided accurate measurements of this parameter and the ways in which genotype, nutrition and management influence it. On the other hand, more data are available on the age at first calving under a variety of situations in many countries; a wide range of 32 to 64 months has been reported, with mean values of around 45 months for river types and 55 months for swamp types [7].

The physiological events and hormone changes occurring around the time of puberty have not been characterized in the buffalo heifer.

3.2. Oestrous cycle

In both river and swamp type buffaloes the oestrous cycle has a mean duration of 21 days [6]. The duration of oestrus, albeit less overt than in cattle, ranges from 15 to 27 hours, with a mean duration of 20 hours [8, 9]. Ovulation occurs about 24 to 48 hours after onset of oestrus, or 6 to 21 hours (mean 14) after the end of oestrus [8].

Progesterone concentration is basal (<0.2 ng/mL in jugular venous plasma) from about 2 to 3 days before onset of oestrus until 3 to 4 days after oestrus. Significant elevation of progesterone (>0.5 ng/mL) occurs after days 4 to 6 of the cycle

and continues progressively until peak levels (1 to 2.5 ng/mL, which are significantly lower than those in cattle) are reached between days 10 and 16 of the cycle [8–11]. Higher luteal phase values (around 4 ng/mL) have been reported in some studies [12], but it is not yet clear whether this is due to differences between breeds or methodologies. If the animal has not conceived at the previous ovulation the concentration declines sharply 3 to 5 days before the next oestrus.

Progesterone concentration in milk parallels that in plasma, being two- to six-fold higher in whole milk [13], while the levels in fat-free milk are similar to those in plasma [14]. Plasma oestradiol 17β concentration remains at low levels of around 3 to 7 pg/mL during the luteal phase and is elevated to 10 to 15 pg/mL at oestrus in the swamp buffalo [8, 9], while the respective concentrations in river buffaloes appear to be about double these values [15]. The characteristic second peak of oestradiol, which occurs around day 6 in cattle [16], has not been reported in buffaloes.

Luteinizing hormone (LH) concentration is below 5 ng/mL during the luteal and pre-oestrus phases of the cycle. It rises to a pre-ovulatory peak (> 30 ng/mL) at the commencement of oestrus and returns to basal levels after 7 to 12 hours [8, 9]. The interval from the LH peak to ovulation is around 27 hours. The pattern of tonic pulsatile LH release, and the frequency and amplitude of such episodic peaks at different stages of the oestrous cycle, have not been investigated in buffaloes.

The pattern of secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can be assessed by measuring its chief metabolite, 13,14-dihydro-15 keto $PGF_{2\alpha}$ (PGFM), in jugular blood. The concentration of PGFM around the time of luteolysis is about 900 pg/mL, while basal levels at other stages of the cycle are around 250 pg/mL [17]. Injection of natural $PGF_{2\alpha}$ or its analogues in the presence of a corpus luteum (CL) causes rapid luteolysis, with a decline in progesterone to basal levels followed by follicular growth, oestrus and ovulation [10]. Thus, oestrus synchronization regimes developed for cattle have found useful practical applications in the buffalo as well, permitting preplanned breeding and fixed-time insemination [18, 19].

Progesterone measurement can be used as a means of confirming oestrus, on the basis that levels in blood and milk should be basal at the time of service [11]; high concentrations indicate errors in heat detection. This is a useful method for assessing the extent of lost inseminations in field AI programmes. Further application of progesterone measurement would be for confirming clinical diagnoses of pathological conditions such as cystic ovaries and in monitoring their response to different treatments.

3.3. Pregnancy

The mean duration of pregnancy is 310 days in the Murrah and Surti breeds, 317 days in Egyptian and indigenous Lanka buffaloes, and 320 to 340 days in swamp buffaloes [20]. Progesterone remains elevated at luteal phase levels throughout pregnancy [11]. Oestrone-sulphate increases gradually during the first three months, and is significantly higher than in non-pregnant animals for about 120 to 150 days of gestation [21].

Progesterone concentration in blood or milk at 20 to 23 days after service can be used as an early test for pregnancy, with an accuracy of over 95% for negative diagnoses (low progesterone), whereas positive diagnoses (high progesterone) have an accuracy of around 70% only [22]. The false positives are usually those with embryonic death, cystic ovaries, pyometra or services during the luteal phase. Measurement of oestrone-sulphate after 150 days affords a more accurate positive test for pregnancy. However, it is of limited practical value since rectal palpation is simpler and cheaper and can be done with accuracy from 50 to 60 days of gestation.

Sequential measurements of progesterone and oestrone are useful in studies on embryonic and foetal mortality, but it should be appreciated that early embryonic death (before days 14 to 16) cannot be detected by these methods.

3.4. Parturition

The process of parturition and the temporal changes in hormones appear to be similar in cattle and buffaloes. In buffaloes oestrone levels increase from around 200 pg/mL two weeks before parturition, reach peak concentrations of 400 to 750 pg/mL during the last days of pregnancy and decline to basal levels (<100 pg/mL) after delivery of the foetus [23]. Progesterone levels also decline abruptly on the day of calving. The concentration of PGFM in jugular blood shows elevations from 15 days before parturition, with further increases occurring during the last 3 days of pregnancy. The peak levels then decline gradually after calving, reaching baseline levels at 15 to 20 days postpartum [23].

As in cattle, corticosteroids can be used for induction of parturition during the last month of pregnancy in buffaloes [24]. This finds practical application in certain disorders of pregnancy and, in more intensive systems of management, for preplanned calvings.

3.5. Postpartum period

During the postpartum period the animal has to recover from the effects of the preceding pregnancy and parturition and return to its normal status of fertility. This involves involution of the uterus and re-establishment of cyclic ovarian activity. The facility with which these are achieved has an important bearing on the 'open period' or the interval between calving and subsequent conception, which is the major determinant of the calving interval. Since shorter calving intervals yield more calves and lactations during the lifetime of an individual animal, the open period should be as short as possible if high reproductive efficiency is to be achieved.

Clinically, involution of the uterus is usually completed at an average of 28 to 39 days [14, 25]. As in cattle, the blood concentration of PGFM remains elevated for 10 to 20 days postpartum [23], indicating release of PGF_{2α} from the involuting uterus. However, its significance in relation to normal and delayed involution has not been studied in buffaloes.

After calving the ovaries remain inactive for a variable period. Prolonged acyclicity has been found to be the major cause of long calving intervals under village small holder conditions [26] as well as on certain institutional farms [27]. The first postpartum ovulation is often delayed for more than 100 days in river types [13, 27] and swamp types [25], although in some situations this may occur as early as 20 to 30 days after calving [14]. This variation is reflected in the interval taken for the first postpartum elevation of progesterone, which may occur between 60 and 200 days in river buffaloes [13, 27]. In a herd of Malaysian swamp buffaloes, 32% of the animals had a mean interval of 96 days to the first progesterone elevation, while the remainder took longer than 150 days [25]. In a study of Lanka buffaloes under free range village conditions [14], albeit at a location selected for its high reproductive efficiency on the basis of a large-scale field survey [26], the first progesterone elevation was found to occur between 27 and 95 days (mean 55 days). Palpation of the first CL was recorded at a mean interval of 50 days, with over 70% of the herd receiving a first service before 60 days postpartum. In many cases, the first indication of onset of cyclic activity was mating by bulls running with the herd, together with concurrent follicular growth in the ovaries. In such cases the first palpable CL and first elevation of progesterone were recorded after the first service; in the majority of these luteal activity persisted and the animals were subsequently confirmed pregnant. In a few cases, however, a minor elevation of progesterone of short duration together with a palpable CL preceded first service. This study [14] confirms that buffaloes are capable of early resumption of ovarian activity under certain conditions. Furthermore, a high proportion of the first postpartum ovulations appear to be accompanied by oestrus, albeit detected only by the bull, and the first CL seems to be fully functional and capable of supporting a pregnancy. The incidence of shortened first cycles, commonly seen in cattle and characterized by a low progesterone concentration, appears to be low in buffaloes [13, 14]. In some studies, however, reproductive events around the time of commencement of cyclicity have been less regular. Instances where the first ovulation was silent in 20 to 30% of animals [25], or where 28% of all oestrous periods were anovulatory [28] have been reported.

Many factors clearly influence postpartum ovarian activity. Suckling has a major effect in prolonging the acyclic period [29] and temporary weaning has been shown to stimulate ovarian activity [30]. In a survey of village buffaloes, those which were used for milk and therefore subjected to limited suckling by calves were found to have higher reproductive efficiency than those allowed *ad libitum* suckling [26]. The same study revealed longer calving intervals in herds where females are used for draught purposes compared with those in which only males are used. The season of calving also markedly influences the interval from calving to conception [14, 31]; those calving during seasons which are climatically and nutritionally more favourable have shorter open periods than those calving at adverse times of the year. It now remains to be determined how these factors exert their effects on postpartum ovarian activity and fertility so that methods for overcoming these limitations can be developed.

Current knowledge on postpartum gonadotrophin patterns is limited; most studies have relied on sampling frequencies which are inadequate. Plasma LH concentration is known to be higher on day 21 postpartum than on the day of calving [32], while the ability of the pituitary to respond with LH release to an injection of exogenous gonadotrophin-releasing hormone (GnRH) is apparently restored by day 30 [30]. However, detailed studies on the pattern of tonic LH secretion at different stages postpartum are lacking. It is as yet unknown whether establishment of a particular pulsatile pattern of LH, as described in cattle [33], is an essential prerequisite for commencement of ovarian activity in the buffalo. If so, LH profiles would be useful as markers in studies comparing the effects of different factors on hypothalamo-hypophyseal activity during the postpartum period.

4. CONCLUSIONS

Measurements of reproductive hormones have contributed to a better understanding of the reproductive patterns in buffaloes. However, many gaps still exist in our knowledge of the ways in which environmental factors such as climate, nutrition and management influence the hypothalamo-hypophyseal-gonadal axis, and the ways in which genotype and environment interact to determine overall reproductive efficiency. When viewed on a national scale in most Asian countries, the overall fertility of buffaloes is poor. The major limiting factors are late age at first calving and long calving intervals, leading to fewer offspring and lactations during the reproductive life of each individual. Both of these relate to control of ovarian activity, the former at the onset of puberty and the latter during the postpartum period. There is a real need for basic research in this area, in particular to clarify the role of gonadotrophins in initiating follicular growth and the feedback effects of gonadal steroids in initiating and sustaining cyclic activity. Hormone measurement has a pivotal role in providing insights to these physiological processes but, to be of value, it must be combined with clinical and behavioural observations.

At present there are several possible practical applications of hormone measurement, that of progesterone being the most useful. It should be appreciated, however, that these methods are too demanding in time, expertise and expense to be directly applicable at the field level, especially under small farm conditions. Their value lies in research tools, aimed at identifying the constraints to reproductive efficiency under the systems of buffalo production prevailing at specific locations. Similarly, although methods of treatment with hormones and drugs may be effective and practicable under improved systems in institutional farms, their applicability in rural small holdings is severely limited. It should also be remembered that any attempts to improve buffalo productivity, to be of significance in the wider national context, should involve the small holder segment.

Applied research aimed at improving fertility through reduction of the postpartum acyclic period should therefore concentrate on the effects of manipulating

management practices. Evidence available to date indicates that changes in the intensity and pattern of suckling, exclusion of females for draught, and providing locally available crop residues and cheap mineral supplements merit further study. Finally, the methods found to be effective in improving fertility should be evaluated in different genotypes under varied conditions to determine practical applicability, economic feasibility and social acceptability within the rural farming systems.

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REPRODUCTIVE PERFORMANCE OF INDIGENOUS CATTLE IN MALAYSIA*

H.S. TAN, H. KASSIM, T.K. MAK
Faculty of Veterinary Medicine and
Animal Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia

Abstract

REPRODUCTIVE PERFORMANCE OF INDIGENOUS CATTLE IN MALAYSIA.

Postpartum ovarian activity in indigenous Kedah-Kelantan cattle was monitored by progesterone radioimmunoassay to study the fertility of suckled cows exposed to natural mating. In the university herd, postpartum reproductive performance was monitored over two breeding periods. In one period, 34 suckled cows were bled weekly just before mating, with 70.6% showing detectable ovarian activity by 60 days postpartum. Cows bred at a mean ($\pm$ SD) interval of 20.1 ± 6.2 days ($n = 8$) postpartum conceived at 51.1 ± 23.7 days, with 87.5% conception, while cows bred at 49.1 ± 7.1 days ($n = 17$) conceived at 78.5 ± 14.7 days, with 100% conception. In another breeding period when 37 suckled cows were bled twice weekly after calving, 21, 81.1 and 91.9% had resumed ovarian activity by 30, 60 and 90 days postpartum, respectively. The mean intervals to first oestrus and ovulation were 37.7 ± 18.3 and 43.0 ± 17.2 days, respectively. For animals bred at 25.8 ± 3.7 days ($n = 12$), the time to conception was 56.1 ± 20.8 days, with 83.3% conception. When mated at 40.6 ± 8.0 days ($n = 35$), the mean interval to conception was 64.3 ± 15.2 days postpartum, with 88% conception. In spite of calf suckling, high fertility to natural mating was observed by 90 days postpartum. Comparative studies were also conducted in a commercial herd ($n = 90$) and smallholder cattle ($n = 73$). Fertility after oestrus synchronization and AI was also investigated ($n = 70$). A single prostaglandin (PG) injection was given to 30 cycling animals, which were then inseminated either after detection of standing oestrus (GI) or at fixed times of 72 and 96 hours (GII). Another 40 cows were given a double PG injection (GIII) or PRID treatment (GIV), with insemination at 72 and 96 hours and 48 and 72 hours, respectively. Blood was collected twice weekly to monitor the efficacy of treatment and fertility. Based on standing oestrus, 80, 100, 100 and 95% of the animals in groups I to IV were synchronized, with mean times to oestrus of 86.9 ± 17.4 , 83.3 ± 29.2 , 66.5 ± 20.4 and 61.0 ± 13.0 hours, respectively; conception rates were 25, 33.3, 50 and 35%, respectively. Plasma progesterone profiles revealed that low conception to AI was associated with fertilization failure, although early embryonic mortality, anovulation and asynchrony between AI and oestrus were also detected.

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1. INTRODUCTION

Despite the importation of several breeds of exotic cattle, indigenous Kedah-Kelantan (KK) cattle still form the bulk of the cattle population in Malaysia, comprising over 80% of the national cattle population. These Zebu-derived cattle constitute an important and major source for local beef cattle production. They are owned predominantly by smallholder farmers to whom they are valuable for meat production and draught purposes. Although these indigenous animals have been in existence for a long time, it is relatively recently that a concerted attempt has been made to assess their inherent characteristics and performance [1-3]. Basic studies to identify and characterize the important attributes that have enabled these indigenous cattle to be well adapted to the hot, humid tropical environment are lacking.

Studies were therefore undertaken to evaluate the reproductive performance of KK cattle using plasma progesterone radioimmunoassay. This involved assessment of the postpartum ovarian function and fertility after natural mating in suckled cows, since postpartum anoestrus, especially in beef cattle, can be a major contributing factor to reduced reproductive efficiency. The fertility of KK cows to AI after synchronization of oestrus was also investigated, since any effective national cross-breeding beef programme depends on these indigenous cattle. The potential of KK cattle remains to be exploited, especially in the context of expanded ruminant production, in order to meet the increasing demand for beef in Malaysia.

2. MATERIALS AND METHODS

2.1. Experimental animals

Most of the animals used were obtained from the KK beef herd on the university farm. The suckled cows, 4 to 8 years old, were maintained on cultivated pastures of *Setaria splendida*, *Panicum maximum* and *Bracharia decumbens*. Mineral licks and water were available ad libitum. Seasonal mating was practised with cows mated to the bull twice a year for two months from April to May and October to November. Calves were normally weaned at 6 months of age. A comparative study on postpartum ovarian activity was also conducted on suckled KK cows in a commercial herd as well as on those belonging to smallholder farmers. The animals on the commercial farm were maintained similarly on improved pastures with open season mating, and calves were weaned at 6 to 7 months of age. The animals belonging to smallholder farmers were managed traditionally, with extensive-type grazing along roadsides and near rubber or oil-palm estates and no specific weaning period.

2.2. Oestrous cycle

Five postpartum cows with ovarian activity were bled daily by jugular venipuncture from oestrus for 3 to 4 weeks to monitor peripheral changes in the plasma progesterone level in relation to the oestrous cycle. Blood was collected in heparinized test tubes, the samples were immediately chilled in ice, then centrifuged and the plasma stored at -20°C until analysis.

2.3. Postpartum ovarian function

To monitor the onset of postpartum ovarian activity and fertility to natural mating, 71 suckled KK cows were selected from the university herd. In one experiment, 34 cows at different postpartum periods were introduced to a KK bull during October and November. Blood samples were collected weekly from 1 week before until 3 weeks after the breeding period. At mating, 8, 17, 5 and 4 cows were within 11 to 30, 31 to 60, 61 to 90 and 91 to 120 days postpartum, respectively. Subsequently, another experiment was conducted during April and May on 37 suckled cows that were introduced to the bull before 60 days postpartum. These consisted of 12 and 25 cows within 15 to 30 and 31 to 60 days postpartum, respectively, at mating. Blood samples were collected twice weekly from calving up to 3 weeks after the breeding period to monitor more precisely the onset of postpartum ovarian activity. Rectal examinations were also performed to determine uterine involution and to diagnose pregnancy. Oestrus detection was by visual observation twice daily for $\frac{1}{2}$ hour in the first experiment and by a chin-ball device attached to the bull in the second experiment.

Postpartum ovarian activity was also evaluated in 90 suckled cows from a commercial herd by collecting two consecutive blood samples at an interval of 10 days. Similarly, blood samples were also collected from 73 suckled cows belonging to smallholder farmers to assess ovarian activity in relation to the postpartum interval. Progesterone levels ≥ 1 ng/mL in one or two blood samples were taken as indicative of ovarian activity. The pregnancy rate at 120 days postpartum was assessed by rectal palpation.

2.4. Oestrus synchronization

To evaluate oestrus and fertility after oestrus synchronization, 70 postpartum animals with ovarian activity per rectum were selected from the university farm. In one experiment, 30 cows with palpable corpus luteum were given a single injection of 25 mg prostaglandin ('Lutalyse', Upjohn) and divided equally into two groups. In one group (GI), AI was performed about 12 hours after detection of standing oestrus, while in the other group (GII) the animals were inseminated at fixed times of 72 and 96 hours after treatment, regardless of oestrus manifestation. In another experiment, 40 cows were similarly divided into two groups. One group (GIII) was given a double

injection of prostaglandin, 10 days apart, and inseminated at 72 and 96 hours after the second injection; the other (GIV) was treated with a progesterone-releasing intravaginal device (PRID, Abbots) for 11 days and inseminated at 48 and 72 hours after PRID removal.

All animals were painted to enable easy identification from a distance and oestrus observation was carried out for 2 hours, at 07:00, 10:00, 14:00 and 17:00 hours daily. Tail painting and a penile-deviated bull carrying a chin-ball marker were used as additional aids. The oestrous signs manifested were scored and the time to onset of standing oestrus was recorded. Rectal palpation was done before insemination to determine uterine tone, amount and quality of vaginal mucus, as well as closer examination of the vulva for signs of swelling, erythema and fluid. Observations for return to oestrus were continued for the next 4 weeks after insemination. Blood was collected twice weekly for the duration of both experiments to monitor the efficacy of treatment and the conception rate after service.

2.5. Hormone analysis

The plasma progesterone concentration was determined by radioimmunoassay as described previously [4], with a slight modification. Instead of extraction with petroleum ether, danazol was used to displace progesterone from binding proteins in unextracted plasma. A volume of 50 μL of plasma was incubated at room temperature with 200 ng danazol for 3 hours before the addition of 100 μL of 1, 2, 6, 7- ^3H -progesterone (Radiochemical Centre, Amersham, United Kingdom) containing 20 000 dis/min in assay buffer followed by 100 μL of diluted antiserum (1:2500) against 11 α -hydroxyprogesterone hemisuccinate-BSA-conjugate.¹ After overnight incubation, the unbound hormone was removed with 200 μL of dextran-coated charcoal. The supernatant containing the antibody-bound hormone was then counted in a liquid scintillation counter (Packard 300 C). The standard curve ranged from 25 to 1000 pg per assay tube and all determinations were performed in duplicate. The progesterone content in the unknown samples was calculated using a logit-log program on a Zilog (Model 280-R10) computer. The sensitivity of the assay was 0.30 ng/mL. The intra-assay coefficients of variation of quality control samples at three levels were 12.7, 7.5 and 10.8%, while the inter-assay coefficients of variation were 19.7, 10.2 and 16.8%, respectively.

3. RESULTS

3.1. Oestrous cycle

The mean ($\pm$ SD) length of the oestrous cycle was found to be 19.9 ± 2.5 days ($n = 26$). The levels of plasma progesterone over an oestrous cycle are shown in

¹ 60 dis/min = 1 Bq.

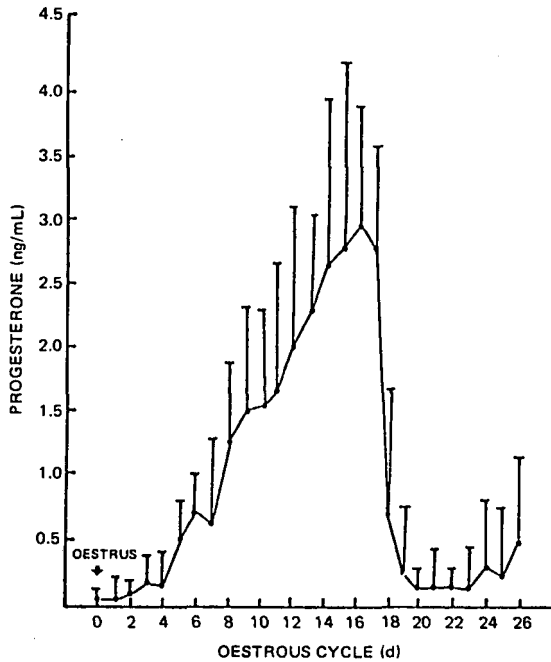


FIG. 1. Mean ($\pm$ SD) plasma progesterone levels during the oestrous cycle in KK cows.

Fig. 1. Progesterone values were low or undetectable during oestrus and an increase in level was observed by day 5, reaching peak values around days 14 to 16. A marked decline was observed after day 16 or 17, reaching basal or undetectable levels by the next oestrus.

3.2. Postpartum ovarian function

Based on rectal palpation, the mean ($\pm$ SD) interval from calving to complete uterine involution in suckled KK cows was 23 ± 6 days ($n = 37$). Representative plasma progesterone profiles of suckled cows which conceived to a bull during the early postpartum period are shown in Fig. 2. Using a plasma progesterone level ≥ 1 ng/mL, the percentage incidences of suckled cows with detectable ovarian activity at commencement of the first breeding period (October to November) were 25, 70.6, 80 and 75% for animals within 11 to 30, 31 to 60, 61 to 90 and 91 to 120 days postpartum, respectively (Table I). Although resumption of postpartum ovarian activity was detected in many animals at 60 days, only 35% ($n = 40$) of the oestrus detected by progesterone profiles were reported by the stockman. Good fertility was observed in this suckled group, with an overall conception rate of 97%

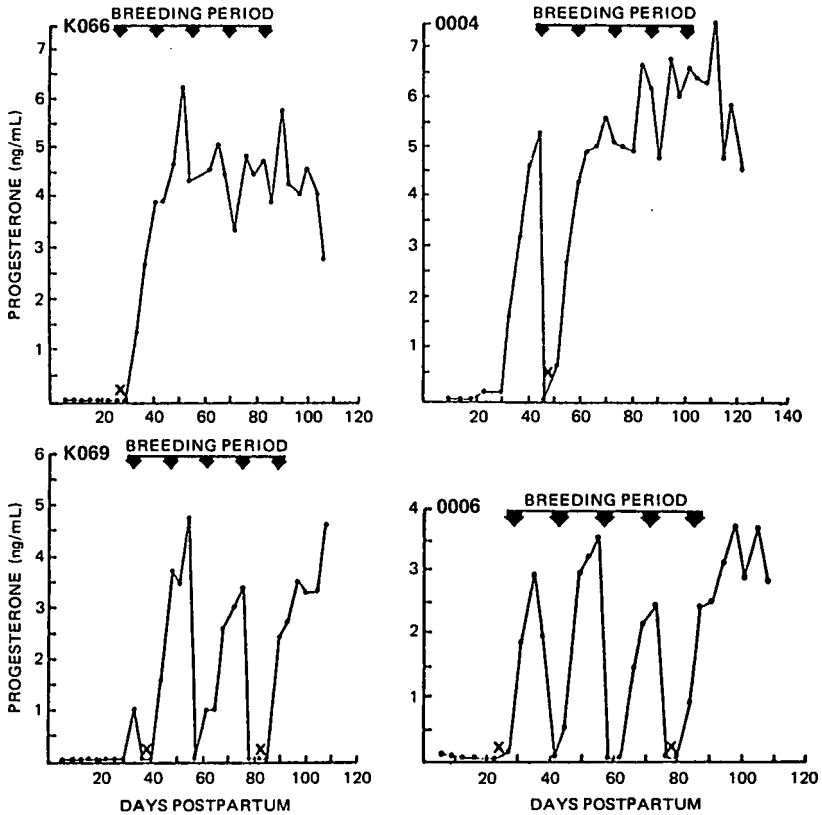


FIG. 2. Representative plasma progesterone profiles in KK suckled cows that conceived to natural mating (X = oestrus detected by chin-ball marks).

($n = 34$) detected from plasma progesterone profiles. For animals bred before 30 days postpartum, the mean interval to conception was 51.1 ± 23.7 days, with a conception rate of 87.5%. For animals mated between 31 and 60 days postpartum, the mean interval to conception was 78.5 ± 14.7 days, with 100% conception. Pregnancy rates based on rectal palpation two months after the breeding period indicated that two animals (5.9%) had early embryonic mortality. All other cows maintained their pregnancy to term, as indicated by the calving rates shown in Table I.

During the second breeding period, resumption of postpartum ovarian activity was detected in 32, 81.1, 91.9 and 91.9% of the suckled cows by days 30, 60, 90 and 120 days postpartum, respectively (Table II). In contrast to visual observation during the previous experiment, 83.1% ($n = 65$) of the oestrus from plasma progesterone profiles were detected by the bull based on chin-ball marks. The overall mean intervals from calving to first oestrus and ovulation were 37.7 ± 18.3 days and 43.0 ± 17.2 days, respectively, with conception at 61.7 ± 17.2 days. The mean

TABLE I. FERTILITY OF SUCKLED KK COWS MATED WITH A BULL DURING THE FIRST BREEDING PERIOD (OCTOBER–NOVEMBER)

	Postpartum period			
	D11–D30	D31–D60	D61–D90	D91–D120
Ovarian activity (%)	25(8)	70.6(17)	80(5)	75(4)
Calving-to-breeding interval (d) ^a	20.1 ± 6.2	49.1 ± 7.1	76.2 ± 1.8	114.8 ± 3.5
Calving-to-conception interval (d) ^a	51.1 ± 23.7	78.5 ± 14.7	107.2 ± 19.6	130.5 ± 4.0
Conception rate (%)				
Progesterone assay	87.5	100	100	100
Rectal palpation	75	100	100	75
Calving rate (%)	75	100	100	75

^a Mean ± SD.

Numerals in brackets denote number of animals.

interval to conception at 56.1 ± 20.8 days for animals mated before 30 days postpartum was comparable to that obtained during the first breeding period, with a conception rate of 83.3%. Of the 32 animals that became pregnant, 25, 50, 21.9 and 3.1% conceived at the beginning of the first, second, third and fourth postpartum oestrous cycles, respectively. The overall conception rates by progesterone assay and rectal palpation were 86.5 and 81.1%, respectively. Animals that did not conceive had true anoestrus, repeat breeding or early embryonic mortality (Fig. 3).

The percentage incidence of suckled KK cows with ovarian activity in three locations, based on progesterone determinations at different postpartum periods, are shown in Table III. A higher percentage ($P < 0.01$) of animals was found to exhibit ovarian activity before 30 days postpartum in the commercial herd compared with smallholder farms, with the university herd in between. A significant difference ($P < 0.05$) was still observed between commercial and smallholder cows from 31 to 60 days postpartum. However, by 90 days postpartum there was no significant difference, with over 85% of cows showing ovarian activity in the three herds. A lower pregnancy rate ($P < 0.05$) was detected in smallholder cattle at 120 days postpartum than in the university or commercial herd.

TABLE II. FERTILITY OF SUCKLED KK COWS MATED BEFORE 60 DAYS POSTPARTUM DURING THE SECOND BREEDING PERIOD (APRIL-MAY)

	Postpartum period		Overall
	D15-D30	D31-D60	
Ovarian activity (%)	32.4(37)	81.1(37)	-
Calving-to-breeding interval (d) ^a	25.8 ± 3.7 (12)	40.6 ± 8.0 (25)	35.8 ± 9.8 (37)
Calving-to-first oestrus (d) ^a	31.6 ± 12.0	40.4 ± 20.2	37.7 ± 18.3
Calving-to-first ovulation (d) ^a	37.3 ± 11.7	45.6 ± 20.0	43.0 ± 17.2
Calving-to-conception interval (d) ^a	56.1 ± 20.8	64.3 ± 15.2	61.7 ± 17.2
Conception rate (%)			
Progesterone assay	83.3	88	86.5
Rectal palpation	83.3	76	78.4
Calving rate (%)	83.3	76	78.4

^a Mean ± SD.

Numerals in brackets denote number of animals.

3.3. Oestrus synchronization

Based on chin-ball marks and/or tail paint removal, 80, 100, 100 and 95% of the animals in groups I, II, III and IV, respectively, were detected in standing oestrus by day 6 post-treatment (Table IV). The retention rate of PRID in group IV was 100%, with 90% of the animals showing a foul-smelling vaginal discharge at the time of PRID removal. The mean (± SD) time to onset of standing oestrus after treatment was significantly different ($P < 0.05$) after a single prostaglandin (PG) compared with a double PG injection or PRID treatment. Spontaneous vaginal mucus discharge was detected in 8.3, 13.3, 20 and 21% of the animals in standing oestrus in groups I to IV, respectively. Upon rectal manipulation during AI, the percentage with vaginal mucus discharge increased to 58.3, 46.7, 50 and 94.7% for the four groups, respectively.

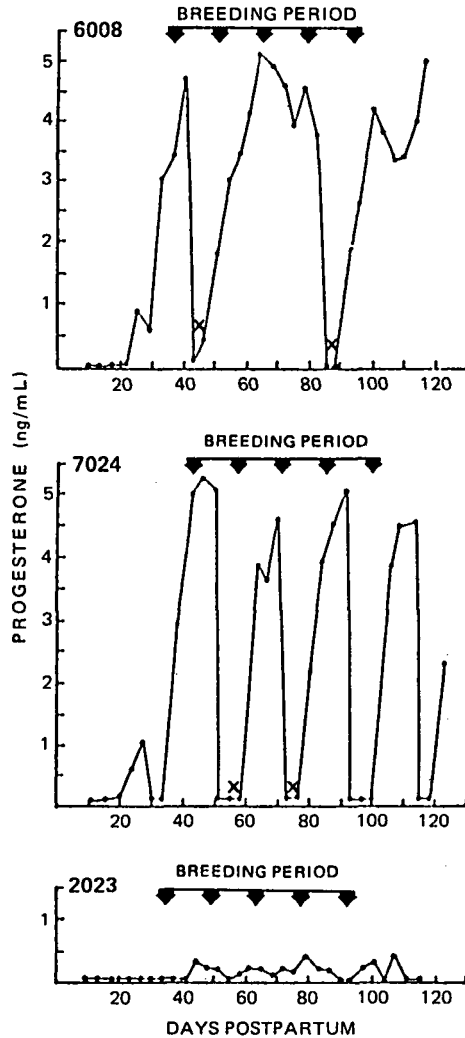


FIG. 3. Plasma progesterone profiles in KK cows that showed early embryonic mortality (6008), repeat breeding (7024) and true anoestrus (2023) during breeding (X = oestrus).

Plasma progesterone profiles confirmed that all the animals had ovarian activity before treatment, with good agreement between response to treatment and onset of oestrus in most cases. In groups I and II, plasma progesterone showed a rapid decline to basal levels after a single prostaglandin injection, except for three (20%) animals in group I where luteolysis was incomplete and standing oestrus was not detected. In group III, the first prostaglandin injection caused luteolysis in 55% of the animals and 85% showed elevated progesterone levels by the time of the second injection.

TABLE III. COMPARISON OF POSTPARTUM OVARIAN ACTIVITY AND FERTILITY OF KK COWS ON THREE FARMS

Location	Per cent ovarian activity			Per cent pregnant ^a
	D11-D30	D31-D60	D61-D90	
University	31.1 ^b (45)	77.8 ^{b,c} (54)	90.5 ^b (42)	80.1 ^b
Commercial	59.4 ^c (32)	89.7 ^b (29)	93.1 ^b (29)	74.4 ^b
Smallholder	16.7 ^b (24)	63.6 ^c (33)	87.5 ^b (16)	37.0 ^c

^a By 120 days postpartum.

^{b, c} Means with different superscripts in the same column differ significantly ($P < 0.05$).
Numerals in brackets denote number of animals.

Insertion of PRID maintained plasma progesterone levels during treatment, followed by a rapid and consistent decline to basal levels after withdrawal. Basal progesterone levels after PG and PRID treatment corresponded well with observations of synchronized oestrus. From the progesterone profiles, most animals ovulated post-treatment, with high progesterone levels maintained at or beyond 3 weeks after AI in pregnant cows (Fig. 4). In non-pregnant animals, a fall in progesterone levels to basal values by 3 weeks post-insemination was again detected. Although good synchrony of oestrus was achieved, the conception rates from progesterone profiles were only 25, 33, 50 and 35% for groups I to IV, respectively. Lower conception rates were subsequently obtained by rectal palpation in groups I, III and IV, which suggest early embryonic mortality. Asynchrony between AI and oestrus was detected in 20, 10 and 5% of the cows in groups II, III and IV, respectively.

4. DISCUSSION

Being indigenous to Malaysia, KK cattle are completely adapted to the hot, humid tropics. Any parameter of the adaptability of these animals can be considered as a standard for comparative performance of imported exotic breeds in the tropics. The reproductive performance of these cattle is, therefore, of interest since they constitute an important and major component of the cattle population in Malaysia. The present study indicates that the indigenous KK cow is very fertile under well managed conditions, with a high conception rate to natural mating. Plasma progesterone levels during the oestrous cycle were lower than those reported in *Bos taurus* cattle, and

TABLE IV. PERCENTAGE OESTRUS AND CONCEPTION RATE AFTER PROSTAGLANDIN AND PRID TREATMENT IN KK COWS

	GI—single PG (AI—oestrus)	GII—single PG (AI-72 and 96 h)	GIII—double PG (AI-72 and 96 h)	GIII—PRID (AI-48 and 72 h)
Oestrus (%)	80(15)	100(15)	100(20)	95(20)
Hours to onset of oestrus ^a	86.9 ± 17.4 ^b	83.3 ± 29.2 ^b	66.5 ± 20.4 ^c	61.0 ± 13.0 ^c
Mean cycle length post-treatment (d) ^a	19.6 ± 1.3 ^b	20.2 ± 1.6 ^b	20.0 ± 1.7 ^b	20.0 ± 2.2 ^b
Conception rate (%)				
Progesterone assay	25 ^d	33.3	50	35
Rectal palpation	16.7 ^d	33.3	47.4 ^e	20

^a Mean ± SD.^{b,c} Means with different superscripts on the same row differ significantly ($P < 0.05$), except ^d and ^e where $n = 12$ and 19 , respectively.

Numerals in brackets denote number of animals.

are in conformity with those observed in *Bos indicus* females [5,6]. In both postpartum studies in the university herd it was observed that ovarian activity was re-established at 60 days postpartum in many animals, in spite of calf suckling. It was interesting to observe that conception occurred as early as 30 to 60 days postpartum when suckled cows were mated to the bull.

In beef cattle, long intercalving intervals are usually caused by postpartum anoestrus and mean postpartum intervals in suckled beef cows generally ranged between 46 and 168 days [7]. In Brahman cross-bred cattle, long postpartum anoestrus was shown to account for 70% of the failure to maintain a 12-month calving interval [8]. Although postpartum anoestrus is also a major cause of poor reproductive performance in imported cross-bred cattle in Malaysia [9], the current finding that KK cattle have a relatively short postpartum anoestrous period, in spite of calf nursing, may be a unique physiological trait of indigenous cattle in the hot, humid tropics.

Several factors can contribute to postpartum anoestrus in beef cows and suckling by the calf appears to be a major contributing factor [10–13]. For example, suckling cows experienced regular ovarian cycles only at 14 weeks postpartum in contrast to 10 to 33 days in non-suckling cows [10]; thus, calf removal hastens the resumption of postpartum ovarian activity [12]. Whether the early resumption of postpartum ovarian activity and the subsequent fertility observed in KK cows were related to a

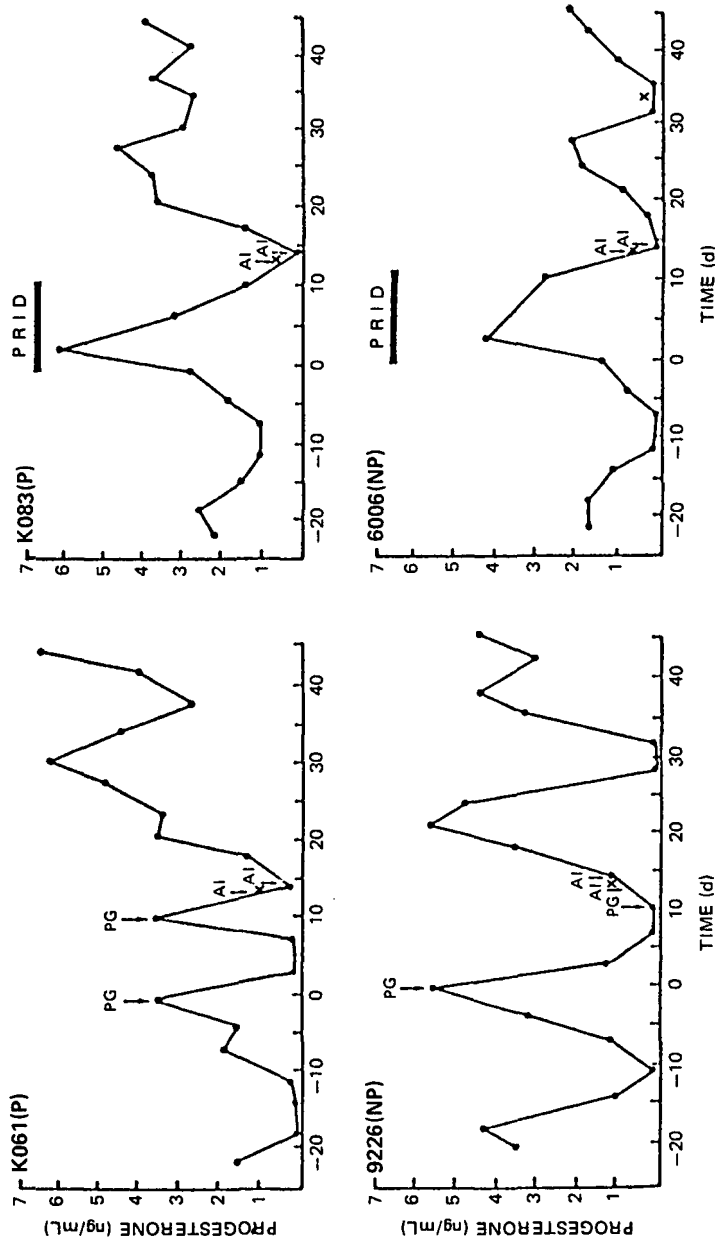


FIG. 4. Plasma progesterone profiles in pregnant (P) and non-pregnant (NP) KK cows to AI after a double prostaglandin injection or PRID treatment (X = oestrus).

lower intensity or frequency of suckling needs to be evaluated. The mean calving interval of the university KK herd has been reported as 367 ± 39.8 days [2]. In a commercial herd, calving intervals as low as $10\frac{1}{2}$ months have been detected [14].

When postpartum ovarian function was compared between farms, a significant difference was detected in ovarian activity before 60 days postpartum between the commercial herd and smallholder cattle. However, by 90 days the difference in ovarian activity between farms was not significant, although it was still slightly lower in smallholder cattle. In contrast, a significantly lower pregnancy rate was found in smallholder cattle by 120 days postpartum compared with the university or commercial herd. Although better management practices and improved nutrition were responsible for the higher performance on the latter farms, observations also suggest that the availability of the services of bulls can be a major constraint in smallholder cows. Since fertility and overall reproductive performance are improved by early resumption of ovarian cycles [15, 16], the delayed onset of postpartum ovarian function may also be a contributory factor to lowered reproductive performance in smallholder cattle.

Problems associated with the detection of oestrus are the greatest deterrent to the use of AI, especially in beef cattle, unless fixed time insemination can be used to achieve good conception. Several constraints exist before the quality of superior sires can be disseminated effectively by AI to local cattle in tropical countries [17]. Two major constraints are poor heat detection and a low conception rate. Although the present study showed that problems of oestrus detection can be overcome by oestrus synchronization with either prostaglandin or PRID, the conception rates were low in comparison with natural mating. Careful selection of animals with luteal activity was probably responsible for the high percentage of animals responding to treatment compared with a random cycling herd [18–20].

Before AI can be implemented effectively under field conditions, the causes of low conception after oestrus synchronization have to be elucidated. In Brahman-cross-bred or Zebu cattle, fertility after prostaglandin treatment has also been poor [21, 22]. The time to the start of oestrus and to ovulation after PG injection has been found to be 24 hours longer in Brahman than in other breeds [8]. In the present study, the time to oestrus after a single PG injection was significantly longer than after double injection. However, no significant difference in fertility was found between animals inseminated after observed oestrus versus fixed times. Also, no significant improvement in fertility was observed after PRID compared with PG treatment. Plasma progesterone profiles revealed that a low pregnancy rate was associated predominantly with fertilization failure, although early embryonic mortality, anovulation and asynchrony between AI and induced oestrus were detected in a few cases. Although poor conception after oestrus synchronization may have an endocrine basis [23], treatment with HCG or GnRH during the post-insemination period in synchronized beef cows did not affect the conception rate consistently [24–25]. Use of exogenous hormones to improve fertility after oestrus synchronization in KK cattle, however, remains to be evaluated.

In conclusion, further studies are required to elucidate the functional relationship between the hypothalamus, pituitary and ovaries during suckling in KK cows and to characterize the physiological attributes that enable this breed of cattle to reproduce efficiently under well managed tropical conditions. More research is also required to improve fertility after oestrus synchronization and AI before superior genetic stock can be disseminated effectively to improve local cattle. Indigenous cattle in the tropics definitely deserve more attention, since they are completely adapted to the hot, humid environment.

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STRICTURES ON REPRODUCTIVE PERFORMANCE ON SMALL- TO LARGE-SCALE DAIRY FARMS IN KENYA*

S. GOMBE, H.B. O'HARA
Reproductive Biology Unit,
College of Biological and Physical Sciences,
University of Nairobi,
Nairobi, Kenya

Abstract

STRICTURES ON REPRODUCTIVE PERFORMANCE ON SMALL- TO LARGE-SCALE DAIRY FARMS IN KENYA.

Poor reproductive performance in dairy cattle in Kenya, although known to exist, has not been adequately documented. Three large-scale farms were chosen from Agricultural Development Corporation (ADC) farms in western Kenya. Four medium-scale and three small-scale farms were chosen from central Kenya (around Nairobi). Blood, and later milk, were taken on days 1, 15, 17, 21 and 24 from the onset of heat to monitor progesterone and oestradiol. Modes of heat detection and insemination and return to heat were recorded. Similarly, body scores, incidence of disease, abortions and cullings were recorded. In large-scale management systems heat detection was made twice a day by herdsmen, while on medium-scale farms both herdsmen and bulls were used to detect heat. On small-scale farms owner/herdsmen provided observations on heat. Service was by AI on large- and small-scale farms and mixed on medium-scale farms. AI was usually carried out within 6 to 12 hours, although it could be delayed up to 16 hours. In all management systems body scores were good (3 to 5) and no major diseases were found. Heat detection was satisfactory. However, the quality of AI was very variable. In large- and small-scale farms 30% of the cattle conceived at first service, as opposed to 70% in medium-scale management. The number of services/conception was high in large-scale (3.5) compared with medium-scale systems (1.7). Large-scale farms kept their cows for a longer parity than medium-scale (4 to 5 versus 3 to 4), while better selection for milk yield (16 kg/d) was found in medium-scale management than in large-scale (10 kg/d). It is concluded that the current extensive grazing management system in the dairy industry in Kenya offers a cheap and effective method of husbandry, but that poor AI is a major constraint on reproduction. An interim solution could be mixed use of bulls and AI, where the latter cannot be delivered regularly. Better selection of cows and stricter culling would also improve productivity and therefore should be encouraged.

1. INTRODUCTION

To increase milk production in the country the policy of the Kenyan Government has been to encourage small farmers to adopt imported dairy breeds. On the whole, the smallholder preferred the Ayrshire breed because it requires less food.

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Most smallholders obtained their cows from the culled animals of medium- or large-scale farmers. Automatically, this poor selection places the smallholder at a production disadvantage, apart from the other management problems which are common to all the farmers.

Hansel and Alila [1] reviewed some of the postpartum causes of low productivity in cattle in the tropics and identified poor oestrus detection as one of the major constraints on reproduction. Similar findings have also been reported by Casida and Wisnicky [2] and by numerous other scientists studying cattle under other climatic conditions [3, 4].

The present investigation was undertaken to document the low productivity of imported dairy cows in Kenya, with a view to establishing that they in fact hinder realization of the government's policy to increase the milk yield in the country.

2. MATERIALS AND METHODS

2.1. Breed

There are two major imported dairy breeds in Kenya: Friesian and Ayrshire. Of the two, Ayrshire is the breed preferred by small- and medium-scale dairy farmers because it is cheaper to maintain, whereas the larger Friesian, even though it yields much more milk, is expensive to keep and tends to suffer environmental stress much more readily than the Ayrshire breed. Therefore, Friesians are mainly raised by large-scale farmers in the higher and cooler altitudes of the Kenya Highlands. The Ayrshire was chosen for our investigation as it is the most popular breed among small- and medium-scale farmers and because it is also used by some large farmers.

2.2 Farms

The large farms in the Kitale areas of western Kenya were chosen because of the availability of good, reliable records on government Agricultural Development Corporation (ADC) farms. Kitale lies at an altitude of some 6000 ft and is close to the foothills of Mount Elgon (14 000 ft) at the Kenya-Uganda border.¹ The farms are typical highland savannah with wide open expanses of grassland interspersed with natural (acacia) and planted (eucalyptus) trees; the weather is mild throughout the year.

The medium and small farms were chosen from around the Nairobi area at an altitude of 5000 ft, with low hills and valleys but comparable grassland and tree topography. Nairobi is bounded by low tropical savannah on the eastern side and the

¹ 1 ft = 3.048×10^{-1} m.

Aberdare ranges on the western side. Like Kitale, the temperature ranges between 15 and 27°C throughout the year; cooler temperatures are experienced in the rainy months of June to August and warmer temperatures pertain to the dry months of December to March.

2.3. Management

On the large-scale Kitale farms cattle were grazed extensively throughout the year; this was supplemented by silage feeding in the drier periods. A unique provision was the placement of tree shelters for cattle during the hot weather. On medium-scale farms, extensive grazing was also the major form of management, with some hay and concentrates provided, although this varied from farm to farm. On small farms there was very little grazing land. Although there was some grazing on uncultivated grass ledges, the most frequent practice was to cut green grass, maize stalks or any other herbage and feed it to the cattle in their sheds, which amounts to zero grazing.

2.4. Herd size

By definition, large-scale farmers were those with more than 50 dairy cattle, medium-scale farmers had 10 to 50 and small-scale farmers less than 10. These divisions more or less correspond to the form of management practised. Three large-scale, four medium-scale and three small-scale farms were chosen for our investigations.

2.5. Data collection

On all the farms, the farmers and herdsman were interviewed with regard to the modes of heat detection, incidence of diseases, causes of culling, modes of insemination and management. Experimental dairy cattle were identified and their body scores recorded as well as their reproductive histories. To date, 48 cows on large-scale, 31 on medium-scale and 13 on small-scale farms have been examined.

Initially, blood was collected from cows on days 1 (oestrus), 15, 17, 21 and 24. Later milk was substituted for blood. Because of the distances involved, the collection tubes were left with the farmers with the request to collect samples on the stipulated days. In retrospect, this proved to be unsatisfactory as the compliance of the farmers/herdsman was erratic, which led to incomplete data. However, sufficient data were collected to enable meaningful analyses to be made.

2.6. Heat detection and insemination

On the large- and small-scale farms heat detection was done by herdsman and owner/herdsman, respectively. On the former farms, this was done twice daily, in

the morning and evening at milking times, while in the latter it was less regular and depended on chance observations. On the large farms cows mounting others or standing to be mounted were regarded as being on heat, while on the small farms vaginal discharge, lowing and restlessness were taken as signs of heat. On the medium-scale farms the behavioural signs of heat were often supplemented by the use of bulls to detect cows in oestrus.

On the small- and large-scale farms insemination was strictly by AI, often within 6 to 12 hours of oestrus, but it was sometimes delayed up to 16 hours. On medium-scale farms AI was supplemented by bull service in three out of four farms whenever the AI was late.

2.7. Diseases, abortions, cullings

No reproductive diseases were recorded on any of the farms visited. However, one case of abortion did occur on one of the large ADC Kitale farms prior to the investigations, the cause of which was not determined.

The most frequent reason for culling on the large ADC Kitale farms was because of repeat breeding. When a cow had been served (AI) six or more times without conception it was culled at 4 to 5 parities. The best of the medium-scale farmers culled the cows routinely after they had given birth three or four times. None of the small farms we visited had culled any cows, even though many were obviously old.

It is important to stress that none of the cattle we saw were in poor condition. On a scale of 0 to 5 the majority were in the range 3 to 5 (where 0 represents thin, emaciated with ribs showing and 5 represents fat, well rounded and no ribs showing). These body scores were taken during the rainy season. Nonetheless, when the farms were visited recently (1 March, 1986) during the dry season the cattle were still found to be in good body condition.

2.8. Plasma and milk progesterone profiles

Plasma progesterone was measured by radioimmunoassay routinely in our laboratory using the methodology and matched reagents supplied by the World Health Organization (WHO 1982). Briefly, 200 μL of plasma was extracted with 10 volumes of diethyl ether dried in vacuo and redissolved in 2000 μL of phosphate-buffered saline. Five hundred microlitres were mixed with 100 μL antiserum and 100 μL H^3 -progesterone (10 000 counts/min) and incubated overnight at 4°C. The free tracer was separated from the bound by dextran-coated charcoal (200 μL of 0.6%) and centrifuging at 500 g for 10 min. The supernatant was decanted into scintillation vials containing 5 mL toluene/PPO and counted in a PRIAS scintillation counter.

Milk progesterone was similarly assayed, except that shorter incubation periods (2 h) at 4°C and smaller incubation volumes (300 μL) were used. The milk samples

were preserved in sodium azide at room temperature until taken to the laboratory where they were kept at 4°C.

In all cases the extraction efficiency was >95%. The inter-assay coefficient of variation was <15% and the intra-assay coefficient of variation was <5%. The assay sensitivity was 0.05 nmol/L.

3. RESULTS

3.1. Management

Without exception the farmers looked after their cattle well. Even the small-scale farmer with his limited means went to great lengths to cut grass to feed to his cattle. It should be noted, however, that he does not make hay for feeding during the dry months and is therefore forced to buy hay (which is expensive) or feed his cattle all manner of refuse (dry maize stalks, cabbage rejects, etc.) which has an inadequate nutritional value. This inevitably leads to low milk production, but since he does not keep records, no quantitative values can be arrived at.

The small-scale farmer must also be encouraged to buy younger and more productive cattle than is currently the practice and these animals should be more readily available to him at prices within his financial capability. Dependence on culled stock from large- or medium-scale farmers obviously militates against his productivity, despite his conscientiousness. At the moment the limited number of dairy Ayrshires makes it difficult to solve this problem. It must, however, be tackled if the small-scale farmer is to be convinced that keeping fewer but more productive animals is to his advantage.

The medium-scale farmers showed the best motivation, with an excellent understanding of animal husbandry. They not only fed their animals well during wet and dry seasons, but also showed better selection of high yielding dairy cattle, which they kept for as long as they were highly productive and thereafter got rid of. They kept good records, conscientiously looked for heat and kept bulls on stand-by to make sure that the animals were served on time.

Although the large-scale farms were well managed, the managers tended to leave the day to day contact with the animals to the herdsmen. Since the herdsmen worked in rotation, observations of heat were irregular. Indeed, on one of the three farms examined the pregnancy rate was only 10% even though the overall for the three farms was 30%.

3.2. Heat detection and service

There was no problem in correct heat detection on medium-scale farms as this was assisted by bulls. There was also no servicing problem on these farms. Indeed, 70% of the animals on the medium-scale farms conceived at first service and no cow

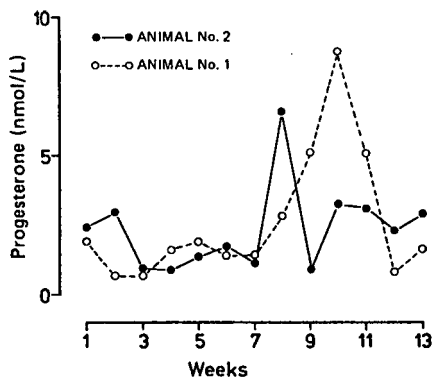


FIG. 1. Progesterone profiles on a small-scale farm.

was served more than twice before conception. The mean service/conception ratio was 1.7.

However, the reverse was true on the large- and small-scale farms, where only 30% of the cows conceived at first service. On the large farms the mean service/conception ratio was 3.5. The major reason for culling on these farms was repeat breeding, with some cows being served (by AI) more than six times. Because of this very poor record these farms have now introduced their own AI system as opposed to the national AI scheme.

Heat detection by small-scale farmers was equally poor. As can be seen in Fig. 1, several cycles occurred in two animals, without the owners being aware that they had taken place. Service was entirely by AI from the national service. However, because of logistical problems this service is not always timely and is sometimes not carried out at all when needed.

3.3. Diseases, abortions, cullings

Although no attempt was made to select well maintained farms, on all 10 farms examined no incidence of disease related to reproduction was found; however, the major disease found in all management systems was mastitis. Evidently the farmers are very aware of the importance of keeping their herds healthy. There was one incidence of abortion on a large ADC Kitale farm, but this occurred before our investigations. The cause was unknown and may not have been related to reproduction. Nonetheless, the possibility of brucellosis cannot be ruled out as it has been known to occur in Kenya.

Culling practices were variable. On large farms culling was carried out at about 4–5 parities, the major reason being repeat breedings. On the medium-scale farms examined the cows were culled at 3 to 4 parities to get younger and more productive

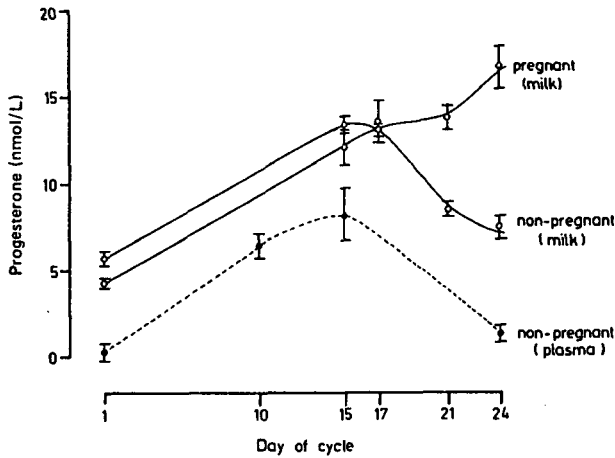


FIG. 2. Milk and plasma progesterone profiles in Ayrshire cattle.

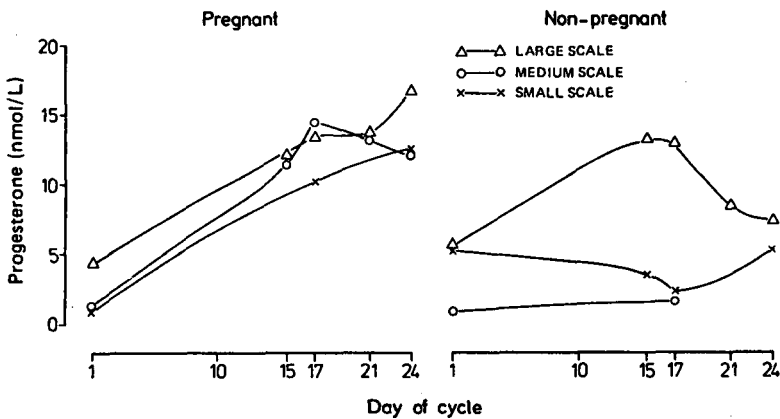


FIG. 3. Milk progesterone profiles.

cows. None of the three small-scale farms we investigated had culled their cows. As stated earlier, these farmers tended to procure their cows from those culled from large- or medium-scale farms.

3.4. Plasma and milk progesterone profiles

Figure 2 shows that the milk and plasma progesterone profiles were qualitatively the same, even though the milk values were five times higher. At oestrus the plasma progesterone was 1 nmol/L, rising to 8 nmol/L at day 15 and thereafter

declining to 2 nmol/L on day 24 in animals which did not become pregnant. The comparable values for milk progesterone were 5, 15 and 7 nmol/L, respectively. When the animals became pregnant the milk progesterone increased to 17 nmol/L between days 15 and 24.

Figure 3 compares the milk progesterone profiles in the three management systems. It can be seen that on medium-scale farms (where oestrus detection was best) very low oestrus progesterone was found (< 1 nmol/L). This contrasted with the higher values (> 5 nmol/L) found in the large- and small-scale management systems, where oestrus detection was poorer.

4. DISCUSSION

The outstanding problems encountered in these investigations were poor heat detection and poor insemination. As indicated in the results found on one small-scale farm, the progesterone profiles clearly indicated that the cows were cycling, yet the owners did not detect the heat periods. This must be partly due to the fact that small-holders usually have other jobs and keep the cattle only as a sideline. Therefore, they are not with the animals long enough to detect signs of heat, particularly if these occur at night or when they are at work.

However, even when heat was detected, the conception rate was unacceptably low (30%). This is directly attributable to the vagaries of the national AI system. Often the services are not delivered on time (due to lack of transport) and sometimes not at all. Yet the use of AI is officially encouraged in order to improve the productivity of the national herd.

Similar problems were encountered on the large government-run ADC farms at Kitale. Although heat detection was better, the conception rate at first service was only 30% and the service/conception ratio was as high as 3.5. The managers of these farms correctly diagnosed that the problem lies with the poor national AI system and have now instituted their own on-farm AI system.

Medium-scale farmers have also realized the inadequacies of the national AI system. Because they can afford it, they keep their own bulls and consequently have no problems with heat detection and early service.

The conclusion is, therefore, that until there is a fast and reliable national AI service, groups of small-scale farmers should have access to communal bulls within easy reach. With the conscientiousness that they show towards maintaining and feeding their cows, there is no doubt that they would similarly look after an expensive bull very well.

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ASSESSMENT OF THE REPRODUCTIVE EFFICIENCY OF *Bos indicus* CATTLE IN THE TROPICAL AREAS OF MEXICO*

C.S. GALINA, A. DUCHATEAU,
R. NAVARRO-FIERRO

Departamento de Reproducción,
Facultad de Medicina Veterinaria y Zootecnia,
Universidad Nacional Autónoma de México,
Mexico, D.F., Mexico

Abstract

ASSESSMENT OF THE REPRODUCTIVE EFFICIENCY OF *Bos indicus* CATTLE IN THE TROPICAL AREAS OF MEXICO.

Poor bovine reproductive efficiency in tropical climates could explain why areas with these conditions account for only 50 and 25% of the world's beef and milk production, respectively. The marked influence of season on reproduction in the tropics is reflected in reduced oestrus detection and calf survival, as well as later onset of ovarian activity during the winter months under range conditions. Lack of knowledge of some of the basic mechanisms that regulate reproductive physiology in farm animals in the tropics has restricted use of techniques such as artificial insemination, thereby limiting genetic progress. From our data we have detected differences in oestrous behaviour, hormonal levels during the oestrous cycle and fertility when comparing our results with the information already available for bovines in temperate areas.

The reproductive capacity of *Bos indicus* cattle, the most popular breed in tropical areas of the world, has been studied extensively and it is generally agreed that their performance is poor compared with *Bos taurus* cattle raised in more temperate environments [1].

There are only a few reports published in scientific journals concerning the reproductive efficiency of cattle raised under Mexican conditions, particularly in tropical areas where average annual temperatures are around 20°C, rainfall is 1200 mm and most of the pluvial precipitation occurs from June until October. The majority of studies are available only as undergraduate theses which are not easily accessible to the scientific community in general. Table I contains a summary of information for some of the areas in which *Bos indicus* type cattle are the predominant species [1-6]. The calving interval has been found to be approximately 15 to 16 months, and the calving index in the order of 50 to 60%.

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TABLE I. SELECTED INFORMATION ON CALVING INTERVAL AND AGE AT FIRST CALVING FOR SOME CATTLE BREEDS REARED IN THE TROPICAL AREAS OF MEXICO

No. of animals and location	Breed	Calving interval (d)	Age at first calving (d)	Reference and year
380 Veracruz	Indobrazil	530 $\pm$ 147		[2] 1982
	Brahman	505 $\pm$ 164		
	Brown Swiss	518 $\pm$ 162		
	Charolais	458 $\pm$ 142		
664 Veracruz	Brahman	451	1376	[3] 1978
	Charolais	522	1060	
	Ch $\times$ Br	414	1059	
	$\frac{1}{4}$ Ch $\times$ $\frac{3}{4}$ Br	465	1152	
250 Veracruz	Gyr	Jan.-Mar. 486 Apr.-Jun. 516 Jul.-Sep. 522 Oct.-Dec. 363		[4] 1986
	Indobrazil	Jan.-Mar. 450 Apr.-Jun. 435 Jul.-Sep. 393 Oct.-Dec. 438		
1626 Tamaulipas	Brahman	< 360 (40.5%) 361-480 (41%) 481-600 (10.6%) 601-730 (6.4%) > 731 (1.3%)	< 730 (28.3%) 731-900 (44.7%) 901-1080 (13.8%) > 1081 (13.4%)	
368 Veracruz	Indobrazil	488		
263 110 248 126 Veracruz	Indobrazil	544		[6] 1983
	Gyr	546		
	Brown Swiss	611		
	Br $\times$ Ib	565		

Increasing interest in the use of artificial insemination in *Bos indicus* cattle in Mexico has led to examination of some of the physiological problems adversely affecting fertility. Of primary importance is the nutritional quality of the feedstuffs available in the tropics. Many authors consider that the soil, and consequently the grass, in these areas is deficient in phosphorus, which could affect fertility [7]. Furthermore, forage quality varies not only from one area to another but also from year to year, which results in many animals in the dry season being dependent on poor quality feed at a time when more energy is required to compensate for the extreme conditions. In some areas the cold is intensified by the rainy north winds that prevail in the Gulf of Mexico during November and December.

In contrast to *Bos taurus* cattle, whose reproductive ability is adversely affected by higher temperatures [8], *Bos indicus* reproduce better during the hot summer months [9]. This latter seasonal effect has been well documented regarding some basic physiological mechanisms such as oestrus expression [10]. Thus, in winter it is more difficult to detect oestrus in cycling cows, delayed or anovulatory cycles occurring with a 30% incidence [11], and when prostaglandin $F_{2\alpha}$ is used to synchronize oestrus the corpus luteum seems to be more refractory, as evidenced by a subsequent less sharp decline in progesterone [12].

In the light of this evidence it is not surprising to find that in Mexico there is a marked seasonal calving peak of 40% during the spring, even when the bulls are kept with the cows for the whole year [2]. Furthermore, only 5% of the herd is normally detected in oestrus during the months of January and February, as opposed to 40% in the summer months [4]. Fertility after AI is also affected by season. In a large retrospective study fertility rates as low as 31% were found for the winter compared with 49% for the summer [13]. This trend is more evident following a synchronization programme where fertility in the winter months can be as low as 8% in contrast to 47% in the summer [12]. Similar results were also found in larger field trials [14, 15].

This seasonal trend could be the result of an evolutionary mechanism causing *Bos indicus* cattle to produce their young when environmental conditions are propitious for survival, both of the dam and the offspring [1].

Conception rates are also affected by variations in the time of onset of the rainy season [16, 17]. This variation in rainfall from one year to another complicates the reproductive management of the herd, especially when AI is contemplated. An extensive field trial (following $PGF_{2\alpha}$ injection) was carried out in a year when the onset of the rainy season was delayed; this led to poor reproductive performance (19% conception rate) [18], whereas in a similar study slightly better fertility (28%) was found in a year when the rainfall was more evenly distributed [19].

An even more difficult problem arises from the fact that heifers born during the height of the dry season will achieve first calving much later than calves born before or at the beginning of the rainy season [1, 17]. Consequently, these first-calf heifers tend to lose a year in their calving pattern and will eventually end up calving in the spring.

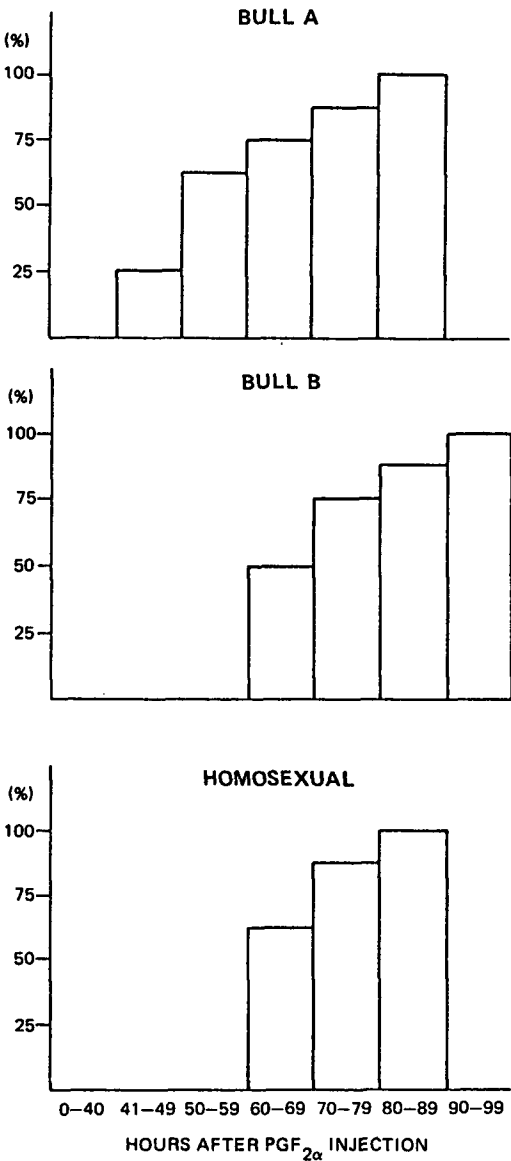


FIG. 1. Cumulative percentages of cows detected in oestrus following PGF_{2α} injections using bulls A and B and/or homosexual interactions as detectors.

TABLE II. COMPARISON OF OESTROUS RESPONSES DURING THREE CONSECUTIVE PROSTAGLANDIN-INDUCED PERIODS OF HEAT AS DETECTED BY BULL A (EXPERIMENT 1), BULL B (EXPERIMENT 2) AND/OR HOMOSEXUAL INTERACTIONS (EXPERIMENT 3);
+ OESTROUS BEHAVIOUR: - NO OESTROUS BEHAVIOUR

Cow	Bull A	Bull B	Homosexual
1	+	+	+
2	+	+	+
3	-	+	+
4	+	+	+
6	+	+	+
9	-	-	+
11	+	-	+
13	+	-	+
15	-	-	-
16	-	-	-
21	+	+	+
25	-	-	-
30	+	+	+
31	-	+	+
33	+	+	+
	60%	60%	80% ^a

^aDifference not significant at $P > 0.05$.

As there are proven meteorological influences on reproduction in *Bos indicus* cattle, research directed towards developing suitable techniques to enhance reproduction under tropical conditions must take this fact into consideration. However, there are also certain physiological characteristics of *Bos indicus* cattle that should be borne in mind, especially when AI programmes are considered. These cattle are difficult to detect in oestrus, probably because their highly organized social hierarchy is disturbed by different management techniques such as constant moving around, or introduction of a teaser bull not known to the herd, or by removing them from their natural environment when the AI programme is in progress [20, 21].

TABLE III. DISTRIBUTION OF NUMBER OF MOUNTS BY BULLS A AND B AND HOMOSEXUAL INTERACTIONS DURING THREE-HOURLY PERIODS OF OBSERVATION FOLLOWING CONTINUOUS OBSERVATIONS FOR 100 HOURS AFTER $\text{PGF}_{2\alpha}$ INJECTIONS IN ZEBU CATTLE (Numerals in bold type denote when the most consistent sexual activity was recorded)

Period (h)	Bull A	(%)	Bull B	(%)	Homosexual	(%)
0-3	79	(12.5)	73	(9.9)	99	(19.8)
3-6	11	(1.7)	22	(2.9)	112	(22.4)
6-9	78	(12.4)	145	(19.7)	25	(5.0)
9-12	46	(7.3)	171	(23.2)	11	(2.2)
12-15	28	(4.4)	96	(13.0)	1	(0.2)
15-18	89	(14.1)	115	(15.6)	65	(13.3)
18-21	159	(25.2)	84	(11.4)	83	(16.6)
21-24	141	(22.3)	28	(3.8)	104	(20.8)
Total	631		734		500	

Orihuea [20] consecutively synchronized, through $\text{PGF}_{2\alpha}$ injections, three oestrous cycles in a group of cows and thereafter continuously followed each synchronization for 100 hours by detecting oestrus signs through use of two different bulls (bulls A and B; experiments 1 and 2, respectively) coupled with homosexual interaction observations. In experiment 3, homosexual interaction alone, in the absence of a bull, was recorded. In the first experiment, bull A started to mount cows 40 hours after injection of $\text{PGF}_{2\alpha}$ and ceased this activity after 90 hours. In contrast, bull B started to mount at 60 hours and ceased 100 hours after the $\text{PGF}_{2\alpha}$ injection. Similar results were obtained for experiment 3, when only homosexual interaction was recorded. However, the distribution for 'when cows entered oestrus' was different (for details see Fig. 1). Furthermore, the intensity of mounting behaviour towards a particular cow in oestrus varied between the two bulls; almost 20% of the cows had homosexual behaviour in the presence of the bull but they were not mounted by the male and another 20% showed no oestrous behaviour at all (Table II).

Since the length of time and the frequency of observations for signs of oestrus vary from farm to farm, accuracy of oestrus detection also varies. Table III summarizes the intensity of mounting behaviour as a function of the time of day for the above experiment. As can be seen, if a farmer attempts to detect cows in oestrus following $\text{PGF}_{2\alpha}$ synchronization without using a bull as marker, he will have a

difficult time as cows show riding behaviour mostly at night; also, depending on the bull the mounting intensity will vary. Similar results have been reported previously by Orihuela et al. [22]. In addition, the sexual behaviour of cows in oestrus is not well marked. On average, *Bos indicus* cattle will receive one mount per hour in oestrus, whereas *Bos taurus*, under the same conditions, will carry out 2.8 mounts per hour [23]. This behaviour appears to be a consistent feature of *Bos indicus* cattle, even in the presence of a bull [22].

Unfortunately, oestrus detection following $\text{PGF}_{2\alpha}$ in Zebu cattle remains to be accomplished, since in an ongoing experiment we have failed to recover fertilized eggs from the oviduct in cows not showing standing oestrus, which agrees with a previous observation that fertility following predetermined AI after synchronization in Zebus has been as low as 6% [14].

Although it is a well known fact that a cow suckling a calf has a marked anoestrous period, particularly if the cow has calved in the middle of the dry season [24], little research has been carried out on the physiological mechanisms of lactational anoestrus in *Bos indicus* cattle. It has, however, been shown that when suckling is restricted renewal of ovarian activity occurs sooner than in cows whose calves have unrestricted suckling [2, 24]. However, there are few studies which have attempted to determine if the problem is due to lack of detectable oestrous behaviour or to the fact that the cows do not cycle at all. The former assumption is more likely, as there is good evidence that significant concentrations of progesterone, indicative of the presence of an active corpus luteum, are detected in the peripheral circulation as early as 40 days postpartum [25]. Furthermore, Escobar and co-workers [2] showed that when suckling of the calves was restricted to once daily, the appearance of the first corpus luteum was detected by rectal palpation at 94 days and was not significantly different to a group of cows with no suckling restrictions (101 days); however, the days from calving to first AI were significantly different (121 versus 173 days), indicating the difficulties in oestrus detection in the latter group.

More information is needed with regard to the onset of ovarian activity in *Bos indicus* cattle, since cows that do not become pregnant within the first 120 days postpartum are likely, after calving in the spring, to have a prolonged open period due to the effect of then having to conceive in the difficult time of the year. This effect is even more marked in heifers calving for the first time [26].

Controversy exists in the literature concerning the endocrinology of the oestrous cycle in *Bos indicus*. Whereas some researchers indicate that they are inherently different from *Bos taurus*, for example in their LH peak at oestrus and response to oestrogens [9], others believe that the environment is probably the main cause of these apparent differences. When these two types of cattle were compared for LH peak at oestrus under the same tropical conditions, no differences were found [27, 28].

If environment is the cause of the differences, then more studies of a regional nature comparing *Bos taurus* with *Bos indicus* need to be carried out, as well as research to determine if the behaviour of the different breeds of *Bos indicus* will vary

according to the suitability of the environment. It is important to remember, however, that Indian breeds have the ability to adapt well to adverse conditions, whereas European cattle have difficulty in even surviving.

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INTELLIGENT USE AND POTENTIAL ABUSE OF HORMONE ASSAYS IN ANIMAL PRODUCTION RESEARCH*

G.J. KING

Department of Animal and Poultry Science,
Ontario Agricultural College,
University of Guelph,
Guelph, Ontario, Canada

Abstract

INTELLIGENT USE AND POTENTIAL ABUSE OF HORMONE ASSAYS IN ANIMAL PRODUCTION RESEARCH.

Immunoassays provide sensitive and specific techniques to measure very low concentrations of hormones in biological fluids. Use of these procedures has provided comprehensive plasma profiles for most hormones and contributed to a better understanding of reproductive function. One future challenge for basic research is the development of new methods to monitor and quantitate target organ response rather than continuing to determine concentrations. In applied or production-oriented projects the difficulties and expenses associated with most hormone assays are usually not justified. One exception is sequential monitoring of plasma or milk progesterone concentrations in twice weekly or weekly samples which can be used to detect the occurrence of sexual maturity, the time of first postpartum ovulation, the true incidence of anoestrus, whether individual females have regular ovarian cycles, and as an early test for pregnancy. Past results indicate that most healthy, adequately fed dairy cows in temperate regions ovulate during the first three or four weeks postpartum. Under intensive management systems the major problem in reproductive management of dairy cattle is oestrus detection. In contrast, many beef cows have not ovulated by 60 days postpartum, so prolonged anoestrus is a serious problem in suckled cows. Very little is known about nutrition, disease or climatic stress effects or interactions on reproductive performance in tropical environments. Regular progesterone concentrations from individual cows, combined with condition scores, management evaluation and breeding plus health records should accurately define the current reproductive performance, potential and problems. Once the major factors limiting reproductive efficiency have been defined, research projects can concentrate on the development of practical solutions. Sequential progesterone assays can then be incorporated into experimental procedures to objectively determine ovarian response.

1. INTRODUCTION

Immunoassays to determine hormone concentrations are frequently used in animal reproduction studies, with the expectation that the results will assist in determining reproductive status or help to explain function. Since most organs or tissues

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of the reproductive system may produce endocrine secretions, the number of hormones that could be measured is large. In general, circulating patterns for reproductive hormones in domestic animals at all stages of reproductive activity can be predicted from the literature. Thus, continuing to sample and perform assays simply to determine the precise concentration of gonadotrophins, steroids or prostaglandins throughout the oestrous cycle or pregnancy does little more than satisfy an investigator's curiosity. Before immunoassays are used a researcher should have a specific objective or hypothesis and an experimental design which requires specific immunoassay results to assist in reaching definitive conclusions. Otherwise, assay data may confuse rather than clarify the investigation.

The immunoassay of samples collected at frequent intervals will continue to be important in fundamental research projects or for evaluating an animal's response to environmental factors, treatments or disease. However, endocrinologists now recognize that for most hormones it is target organ response to changes in pulse frequency and magnitude that is biologically important, rather than the mean plasma values. Thus, a future challenge for basic research is to develop new methods to monitor and quantitate target organ response and to correlate these with fluctuating plasma concentrations of individual hormones.

Production-oriented or applied projects should concentrate on determining major constraints that limit reproductive efficiency. Once these are known emphasis should be placed on developing practical solutions for major problems and modifying existing technology to suit local conditions. Any success in reducing the interval from birth to sexual maturity and from one parturition to the beginning of the next pregnancy will increase the number of females producing offspring in any particular year. To be of practical significance, this must be accomplished through better feeding practices, improved animal health and greater management expertise, but without a substantial increase in operating expenses. Certain immunoassays, if intelligently used, can be valuable in conducting research to develop improved livestock production systems in all environments.

2. ASSESSMENT OF REPRODUCTIVE STATUS — IMMUNOASSAYS IN PRODUCTION RESEARCH

Good reproductive performance is essential for efficient livestock production, so improvement programmes should increase reproductive efficiency to the extent that this can be justified economically. Female domestic mammals must grow to sufficient body size to reach sexual maturity, initiate oestrous cycles, ovulate and be mated by fertile males or inseminated with viable semen at the proper time to have any chance of conception. Perhaps, the basic goal of all production-oriented animal reproduction research projects should be to increase the proportion of females that have oestrous cycles and get pregnant in a reasonable time. Investigations in this area usually require some method to assess ovarian activity. This can be determined by

visual observation for signs of behavioural oestrus alone or combined with detection aids. Casual observation may be adequate for some applications, but individuals may have silent oestrus or the signs may be missed. The detection efficiency can be improved by combining visual observations with tail paste, heat mount detectors or androgenized animals with chin-ball markers, but some cycling animals will still remain undetected [1].

Another method to assess reproductive status is by rectal palpation. In practice, the value of this procedure is governed by the presence of records, the frequency of examination and the palpator's skill or experience. Many cows can be classified accurately into acyclic, cycling, pregnant or abnormal categories with a single examination if a comprehensive breeding and health history is available. With no records even the most experienced theriogenologists would be uncertain about precise classification for a substantial number of animals. Repeated examinations at regular intervals could be used but these must be conducted by professionals, are time consuming and may be impractical if large distances are involved. Palpation is subjective and even experienced individuals are not always accurate. When a number of trained veterinarians examined cows immediately before slaughter for the presence of corpora lutea, the recorded palpation results did not always agree with those from physical examination after slaughter [2]. Also, the agreement between positive milk progesterone pregnancy diagnosis at 23 days and subsequent rectal examination of pregnant cows increased as the time from insemination to palpation was extended. The difference was significant up to 50 days and milk progesterone results were more accurate prior to that date [3].

A more objective evaluation of ovarian activity is provided by monitoring changing concentrations of various hormones. This could be done through detecting the short pre-ovulatory surge of LH or oestrogen. Similarly, the concentration of 15-keto-13,14-dihydro-prostaglandin F_{2a} could be monitored throughout the post-partum period and oestrous cycles to indicate when luteolysis occurred. These techniques would require twice daily or more frequent sampling to be certain peaks were detected, so the labour and expense involved would preclude use with larger numbers or in field studies. In addition, current immunoassay methods for these hormones can only be applied to plasma or serum.

In cows with normal ovarian activity, plasma or milk progesterone concentrations are at or near basal values during the follicular-phase week and elevated for the two-week luteal phase. Concentration changes in samples collected twice weekly or even less frequently provide a very convenient indicator of ovarian activity. The sequential monitoring of progesterone can be used to detect sexual maturity, post-partum and subsequent ovulations, the proportion of cycling females in a group, whether inseminations are performed during the follicular phase, and as an early test for pregnancy. Relatively infrequent sampling is sufficient to illustrate the functional status of the reproductive system and ELISA or RIA kits for plasma and milk are readily available. Thus, progesterone immunoassays can be used on large numbers of animals in controlled experiments or field studies.

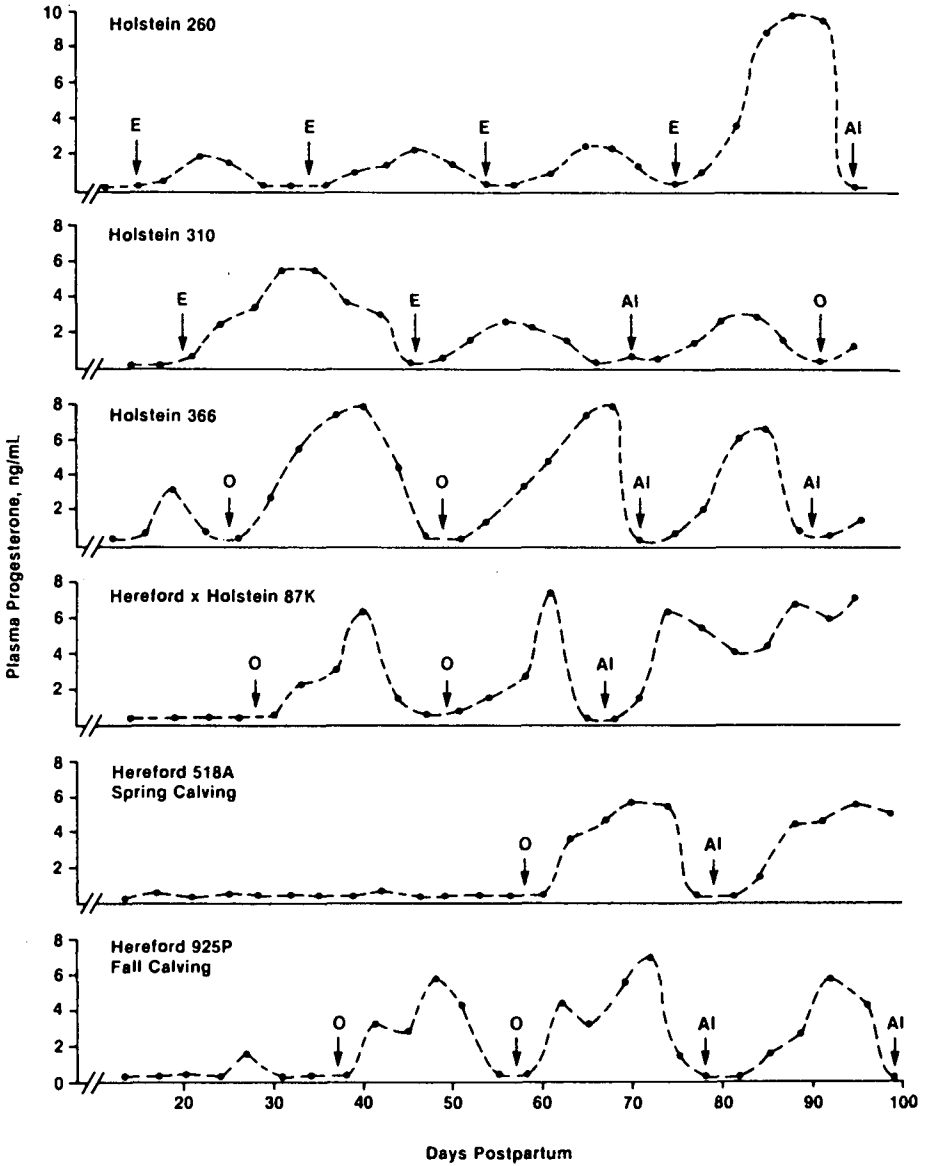


FIG. 1. Sequential plasma progesterone profiles for apparently normal Holstein dairy cows milked twice daily and for suckled Hereford beef cows. The variation in cycle lengths and progesterone concentrations within and between cows is illustrated. Arrows with O denote predicted ovulations, with E show days when heat signs were observed and with AI indicate time of insemination.

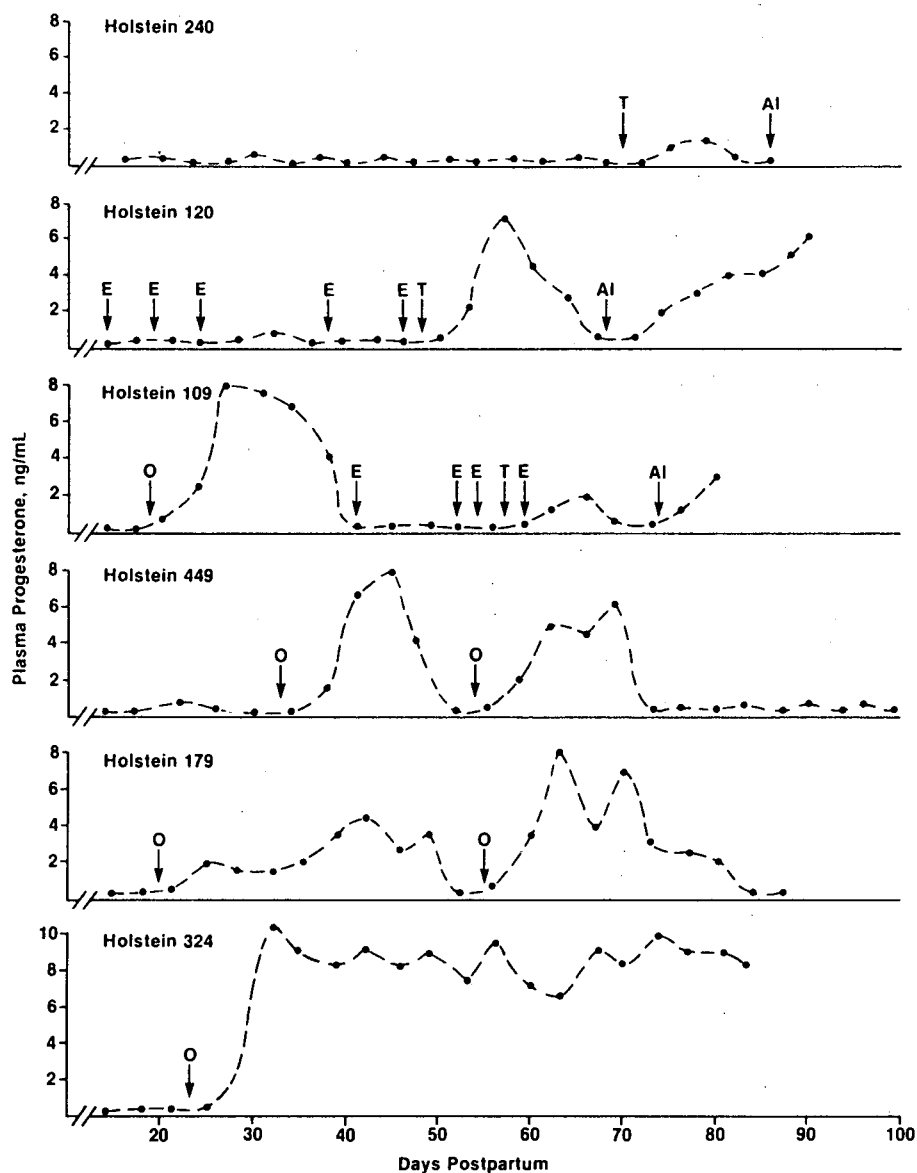


FIG. 2. Unusual plasma progesterone profiles observed in dairy cows during early lactation. Ovarian cysts were diagnosed in each of the top four cows. No pathology was evident in the bottom two animals. Arrows with E show days when oestrous signs were observed, O indicates predicted ovulation, T denotes treatment and AI the day of insemination.

3. POSTPARTUM PLASMA PROGESTERONE PROFILES

Plasma progesterone profiles in normal cows will vary considerably between animals and between successive cycles of an individual (Fig. 1). The maximum plasma concentration during the luteal phase may range from around one to above 10 ng/mL, while values in milk fat can be much higher. Holstein cow 260 recorded three cycles with very low luteal-phase progesterone concentrations, followed by a cycle with very high values. Holstein 310 did the opposite, recording higher values during the first postpartum cycle than in either of the next two luteal phases. In contrast, No. 366 had relatively uniform maximum luteal-phase concentrations for each of her first three cycles. Progesterone profiles following an unsuccessful mating can be seen after the first inseminations for cows 366, 310 or 925P and after successful matings in cows 518A and 87K.

A series of unusual or abnormal profiles are illustrated in Fig. 2. Cows 240 and 120 both formed ovarian cysts before or shortly after parturition. Numbers 109 and 449 also had cystic ovaries, but the progesterone profiles indicated that these females had one or two ovulations followed by formation of a normal corpus luteum before cyst expression. Cystic ovarian degeneration can apparently form at any stage of reproductive life and is one of the most common causes of infertility in dairy cattle. The clinical syndrome may include nymphomania (cows 120 and 109) or anoestrus (cows 240 and 449). All these animals were diagnosed early in the postpartum period and conceived after treatment [4].

Cow 324 ovulated early in the postpartum period and the resulting corpus luteum was retained for over 100 days. Most similar cases of prolonged luteal function resulted from metritis or related uterine pathology, but no diagnosis could be made in this cow. Oestrus finally occurred on day 130. Cow 179 also had a prolonged luteal function after each of her first two ovulations. Several other prolonged cycles were recorded in individual cows but, since none of the animals had been inseminated before the cycles and most conceived after them, they were considered unusual rather than abnormal [4].

The occasional dairy cow and a substantial number of beef cows had long intervals from calving to first ovulation and progesterone was basal throughout this period. The corpus luteum of pregnancy must regress before parturition so it does not secrete any progesterone during the postpartum period. Metabolites or other cross-reacting compounds will, however, produce falsely elevated values for 5 to 14 days postpartum in some assay systems.

4. PRACTICAL APPLICATIONS — DAIRY COWS

4.1. Postpartum ovarian activity in totally confined dairy cows

Cows housed under total confinement in a temperate climate throughout the year in free (n = 36) and tie (n = 33) stall areas were studied during the first 75

to 100 days after calving. All cows moved through the holding area and milking parlour twice daily. Routine oestrus detection was part of the normal duties of the herdsmen moving cows into the holding area. Cows in the free stall area were continually observed by a closed circuit television system connected to a slow speed videorecorder. All signs of oestrus and related behaviour occurring anytime during the day or night were observed when the recording was replayed. In addition, blood samples were collected twice weekly from all cows and assayed for progesterone to determine when ovulation occurred and oestrous behaviour should have been detected. Thus, it was possible to compare the functioning of the reproductive system and behaviour of animals under continual observation (free stalls) with those under normal management (tie stalls).

The average time from calving until the ovaries began to function or reproductive cycles commenced, as determined by the progesterone assay, was approximately three weeks for both free and tied cows. Considerable variation between individual cows was observed in both groups, with some animals starting as early as 10 to 15 days after calving and most cycling by five weeks postpartum. Once the ovaries began to function, the cycle continued in a normal regular pattern in almost all cows (Table I). Of the 69 cows studied, 57 showed apparently normally reproductive activity and all of these had at least two cycles during the observation period. Failure to initiate oestrous cycles shortly after parturition was obviously not a problem in these dairy animals [5].

4.2. Oestrous behaviour in totally confined dairy cows

Only half of the continuously observed free stall cows showed oestrous signs associated with the first ovulation, but detectable signs were present at almost all later ovulations (Table I). There was no reason to assume that behavioural signs would not

TABLE I. OCCURRENCE OF POSTPARTUM OVULATIONS AND PERCENTAGES DETECTED IN OESTRUS BY CONTINUOUS OR CASUAL OBSERVATION FOR TOTALLY CONFINED DAIRY COWS IN A TEMPERATE CLIMATE

	Postpartum ovulation		
	First	Second	Third
Days postpartum	23.1 $\pm$ 12.2 ^a	44.4 $\pm$ 13.2	63.7 $\pm$ 10.1
Per cent detected, continuous observation	50	94	100
Per cent detected, casual observation	20	44	64

^a Distribution skewed, median days to first ovulation = 19.5.

TABLE II. OESTROUS BEHAVIOUR IN TOTALLY CONFINED DAIRY COWS

	No. of cows in or near oestrus		
	One	Two	Three
Mounts/cow in oestrus	11	37	51
Coming into oestrus (mounting) (h)	2.5 ± 1.1	5.5 ± 1.4	9.8 ± 2.6
True oestrus (standing) (h)	7.5 ± 2.4	7.8 ± 1.4	10.1 ± 2.4
Going off (mounting) (h)	0.2 ± 0.1	4.5 ± 1.6	3.9 ± 1.8
Total mounting-mounted (h)	10.2	17.8	23.8

be present in the same proportion of tie stall cows (i.e. 94 and 100% at the second and third ovulations), but the herdsmen were unable to detect these in many instances (Table I) [5].

Observations on the continuously observed free stall animals revealed that the mutual mounting of cows in the group is the most obvious and reliable indicator of oestrus. However, the occurrence of mounts (combining the mounting of other animals by cows going through oestrus and the mounting of cows in oestrus by herd-mates) showed considerable variation from cow to cow. The most obvious factor contributing to this variation was the relationship between the number of cows in or close to oestrus at any particular time (Table II). Thus, the presence of other animals that were sexually excited influenced the duration and intensity of signs for an individual cow. Also, the time intervals for true or standing oestrus recorded for these cows on slatted floors in a total confinement system were shorter than generally assumed. Mounting was most frequent during the night, especially between 22:00 and 04:00 hours, with the least activity observed in the late afternoon [6].

4.3. Postpartum ovarian activity in pastured dairy cows

Sequential plasma progesterone monitoring and regular observations for oestrous behaviour were conducted in Jamaica on 25 Holstein cows (Canadian origin) at a commercial dairy farm and on 31 Jamaica Hope cows at the government research farm. These cows were on improved pastures throughout the year, received adequate amounts of a balanced dairy ration and were dipped for tick control at 7- to 14-day intervals. The regular daytime observations for oestrous signs were supplemented by twice nightly observation periods (23:00 and 04:30 hours).

The intervals from parturition to first, second and third ovulations, as determined by plasma progesterone monitoring, are presented in Table III. Although Jamaica Hope cows ovulated significantly later than Holsteins, animals in both breeds should have cycled one or more times before 60 days postpartum. The Holsteins kept

in this semi-tropical environment and exposed to temperatures above their comfort zone for some hours on most days of the year were able to initiate ovarian activity and be ready for rebreeding as quickly as their Canadian counterparts. The satisfactory results with Holsteins and Jamaica Hope cows suggest that much of the prolonged postpartum acyclicity and poor reproductive performance frequently encountered when *Bos taurus* animals are introduced into tropical environments probably result from management deficiencies, disease and malnutrition rather than a basic inability of the reproductive system to function in the warmer climate. The night-time observations increased the numbers of cows detected in oestrus and mated between 60 and 80 days after calving, but some cows that ovulated and should have been mated were still missed [7].

4.4. Practical significance of dairy cattle studies

The results obtained with dairy cows indicate that the reproductive system can work properly soon after calving in most cows under total confinement in temperate regions or on tropical pastures. Unfortunately, in high producing cows the period when oestrous signs are demonstrated is short and many mounting-mounted interactions occur at night when no one is present. Failure to detect the first or second ovulation would not be too serious, since these should occur earlier than the dairyman would normally wish to rebreed if cows are properly fed and managed. However, the third ovulation generally occurs near or after 60 days and cows should be serviced by that time. The herdsmen were detecting only 64% of the normal cycling cows at the third ovulation (Table I), so a substantial number of animals were missed and rebreeding was delayed. The conclusion was that failure to rebreed at the desired time usually resulted from the inability of herdsmen to detect oestrus rather than because cows failed to cycle or had silent heats [5].

If most animals are to be successfully mated in time to maintain a 12- to 13-month calving interval, efficient oestrus detection must be part of the regular

TABLE III. OCCURRENCE OF POSTPARTUM OVULATIONS AND PERCENTAGES DETECTED IN OESTRUS FOR PASTURED HOLSTEIN AND JAMAICA HOPE COWS IN A TROPICAL ENVIRONMENT

	Postpartum ovulation		
	First	Second	Third
Holstein, days postpartum	19.8 ± 8.8	39.4 ± 9	58.6 ± 9.2
Jamaica Hope, days postpartum	28.7 ± 15.6	46.9 ± 13.6	66.3 ± 12.6
Per cent detected, Holstein	19	62	58
Per cent detected, Jamaica Hope	43	55	73

management routine. The amount of time herdsmen can devote to this is limited, so observation periods should coincide with the time when sexual activity is highest. A 20-min observation period late in the evening and again just before the morning milking would be the minimum time required. Do not waste time checking for oestrus at feeding or when any equipment is operating, since cows will be distracted and may not interact sexually.

Both free and tied cows become more excitable and restless as they pass through oestrus. Any animals that show increased activity or a change in normal behaviour should be checked carefully for clear mucous discharge and an enlarged reddened vulva plus other definite signs of oestrus when first noted and again at subsequent observation periods. Ideally, only animals that stand firmly and accept mounting by herdsmates should be inseminated. However, on slatted floors or whenever the footing is poor, cows not in or near oestrus may be reluctant to mount others. Thus, any female that persistently attempts to mount should be considered in oestrus. If this practice is followed it may result in the occasional animal being inseminated when she is just coming into oestrus. Any cow still showing strong signs on the day following mating should be re-inseminated.

Detecting oestrus and proper timing of insemination in continuously tied cows is extremely difficult. If those cows between 50 and 100 days fresh can be placed in a box stall or other loafing area between the evening and morning milkings they will have opportunity to demonstrate sexual behaviour. If these cows are watched in the late evening and before the morning milking, oestrus detection efficiency will increase and pregnancy rates should improve.

Heat mount detectors, tail paste or altered animals with chin-ball markers can increase detection efficiency when cows are free to engage in mounting-mounted interactions. These are only aids, however, and cannot completely replace the eyes of a knowledgeable observer. To improve reproductive performance it is essential that routine detection observations become a part of the normal work schedule.

5. PRACTICAL APPLICATIONS — BEEF COWS

5.1. Postpartum ovarian activity in totally confined beef cows

Pure-bred Hereford ($n = 105$) and beef X dairy cross-bred ($n = 142$) cows were housed year round in open-fronted pole barns with concrete floors. Balanced rations, formulated entirely from stored feeds, were fed and regularly adjusted to provide requirements for growth, stage of gestation or lactation and season. Calvings occurred from March to June (spring) or August to November (autumn) and calves remained with the cows throughout the experimental period. Blood samples were collected twice weekly from all cows, commencing shortly after parturition and continuing until three weeks after insemination or until at least 100 days postpartum. Plasma progesterones were determined and sequential values plotted graphically for

all cows. Eight-week rebreeding periods commenced in late June or November when most of the cows from the previous calving group were at least seven weeks postpartum. Herdsmen were responsible for oestrus detection and all cows observed in oestrus during these periods were inseminated.

Postpartum ovulations occurred significantly earlier in cross-bred than in pure-bred cows (38.1 ± 18.5 versus 58.1 ± 21.8 days; $P < 0.01$) and in autumn compared with spring calving cows (32 ± 13.9 versus 59.1 ± 20.3 days; $P < 0.001$). Correlation between the calendar date of calving and the interval to first ovulation was significant for spring ($r = -0.38$, $P < 0.01$) but not for autumn calving animals. Since cows were confined and received a balanced ration of stored feeds throughout the year, photoperiod alone or interacting with temperature rather than nutritional factors would be the probable cause of delayed ovarian activity in spring calvers [8].

5.2. Reproductive efficiency of beef cows

Reproductive performance was assessed during the period when samples were collected and the responses for pure-breds versus cross-breds and spring versus autumn calvers were compared. The difference with the greatest practical significance was that a higher proportion of the late summer/autumn calving animals were pregnant by day 100 postpartum ($P < 0.01$), indicating that under eastern Canadian conditions reproductive performance was superior in later calving beef cows [8].

The entire series of progesterone graphs and breeding results were examined to determine whether cows that had ovulated before the commencement of the rebreeding period had a better chance of being detected for mating and becoming pregnant

TABLE IV. EFFICIENCY OF OESTROUS DETECTION AND PREGNANCY RATE TO INSEMINATIONS AT FIRST OVULATION DURING THE REBREEDING PERIOD FOR SUCKLED COWS THAT HAD OR HAD NOT OVULATED PREVIOUSLY

	Ovulations before rebreeding period	
	Yes	No
No. of cows	149	94
Per cent detected	80	63
Pregnant to first AI		
Per cent of detected cows	69	51
Per cent of total cows	55	32

at the first ovulation during the rebreeding period. The effect of previous ovulation or no ovulation before the rebreeding period on the efficiency of oestrus detection is presented in Table IV. Significantly more cows that had ovulated previously were detected at the first ovulation in the rebreeding period and the response was consistent for both spring and autumn calving females. This indicates that silent heat or weak oestrous signs may be associated with the first ovulation in some beef cows, but most show detectable signs at subsequent oestrous periods [9].

Since individual progesterone profiles were available for all cows, it was also possible to compare pregnancy results obtained when the first artificial insemination service was given at the first or at a subsequent ovulation. The initial insemination was performed at the first ovulation in 59 cows and 30 of these conceived to this mating for a pregnancy rate of 51%. By contrast, when the first service was performed at later ovulations, 125 out of 149 cows conceived for a single mating pregnancy rate of 69%. Therefore, better reproductive efficiency was observed with cows that ovulated at least once before the rebreeding period commenced because both the efficiency of oestrus detection and pregnancy rates were significantly higher ($P < 0.05$, Table IV) [9].

5.3. Practical significance of beef cow studies

In most north temperate regions, beef cow calvings usually spread from January to July or August (winter to mid-summer), with only 70 to 80% of the mature females weaning a calf each year. Increasing the calving rate and reducing the calving season would result in larger numbers of more uniform calves weaned. Even a modest improvement in reproductive efficiency, if achieved through practical and inexpensive means, should improve profits. Under eastern Canadian conditions, with adequate nutrients provided throughout the year, beef cows that calved in the late spring, summer or early autumn ovulated sooner and had better pregnancy rates than cows that calved in late winter to mid-spring [8]. Beef cows that ovulated one or more times before the next rebreeding period have a greater chance of being detected in oestrus in the first few weeks and higher pregnancy rates from first mating [9]. The results suggest that adjusting the calving-rebreeding season or any other treatments that will reduce the interval from calving to initiation of oestrous cycles should increase reproductive efficiency.

Cow-calf operators wishing to use AI face special problems, since most beef cows in oestrus do not show as many mounting-mounted interactions as dairy cows. Also, any distractions such as feeding, other chores or even the presence of an observer will inhibit sexual behaviour. The best time to detect oestrous signs in beef cows is during the hour immediately after sunrise or just before sunset. The observer must approach the cows without upsetting them and remain until well after they get over their concern about his or her presence. The various heat detection aids work very well in beef herds when used by conscientious herdsmen, but these are not substitutes for good management.

The prolonged postpartum anoestrus in early calving beef cows is related to suckling intensity and the problem is often confounded by underfeeding. Limiting the suckling period to only a portion of the day may help to shorten the interval from calving to first oestrus, but additional labour and facilities would be required to separate cows and calves each day. Early weaning of calves immediately before start of the rebreeding period could also be effective for first calf heifers and late calving cows, but this would also require extra labour for calf care. Short-term calf removal for 48 h between 40 to 65 days postpartum has substantially increased the number of females showing heat in the few days immediately after removal, without significantly affecting milk production or calf growth rate. Thin cows may not respond to the short-term calf removal stimulus, but results can be improved if the cows are provided with a higher nutrient intake for about two weeks (flushing) before separation. Flushing plus short-term weaning may be a useful management system in many regions. The major criterion for evaluating any of these management systems would be if more cows became pregnant in a shorter time. The regular collection of plasma or milk samples from treated animals and subsequent immunoassay for progesterone would add substantial information on how and when the cows responded. Assaying for other hormones in these samples would be of doubtful value.

6. OESTROUS CYCLE LENGTH

The normal bovine oestrous cycle is 21 ± 4 days, but many cows return in shorter or longer intervals [10]. The first corpora lutea formed after parturition in 23 cows lasted for normal ($n = 7$), shortened ($n = 13$) or lengthened ($n = 3$) periods, with luteal phases of 7.3 ± 3 days for short and 13 ± 2.1 days for normal cycles [11]. Thus, twice weekly sampling should detect most short luteal phases and the postpartum plasma progesterone profiles for monitored dairy [5] and beef [8] cows were examined to determine the incidence of short, normal and long oestrous cycles. An interval of 17 to 25 days between successive oestruses or predicted ovulations was considered normal. Results were tabulated only for animals apparently free of ovarian or uterine pathology and not mated at the first or second ovulation.

The occurrence of short, normal and long intervals between first and second ovulations for dairy and beef cows ovulating during various intervals after parturition are presented in Tables V and VI. The proportions of normal intervals were lowest in cows ovulating quickly after parturition. The percentages of both short and long cycles decreased as the postpartum interval lengthened and uterine involution progressed [12]. In this study, a single elevation above 1 ng/mL was not considered evidence of a true luteal phase, so very short cycles which could result from partially luteinized follicles [13] may not be included.

Dairy cows had a mean interval of 21.4 ± 6 days ($n = 57$) between the first and second ovulation and 22.4 ± 3.5 days ($n = 51$) between the second and third.

TABLE V. OCCURRENCE OF FIRST POSTPARTUM OVULATION AND INTERVAL BETWEEN FIRST AND SECOND OVULATIONS IN DAIRY COWS

First ovulation (days postpartum)	Cumulative (%)	Interval between 1st and 2nd ovulation					
		< 17 days		17-25 days		> 25 days	
		(No.)	(%)	(No.)	(%)	(No.)	(%)
< 20	56	8	25	18	56	6	19
21-40	89	2	10.5	15	79	2	10.5
41-60	94	0		4	80	1	20
> 60	100	0		1		0	
Total		10	17.5	38	66.7	9	15.8

TABLE VI. OCCURRENCE OF FIRST POSTPARTUM OVULATION AND INTERVAL BETWEEN FIRST AND SECOND OVULATIONS IN SUCKLED BEEF COWS

First ovulation (days postpartum)	Cumulative ^a (%)	Interval between 1st and 2nd ovulation					
		< 17 days		17-25 days		> 25 days	
		(No.)	(%)	(No.)	(%)	(No.)	(%)
< 20	13	8	25	20	62.5	4	12.5
21-40	44	13	17	55	71	9	12
41-60	59	6	16	30	79	2	5
> 60	62	3	12.5	20	83	1	4
Total		30	17.5	125	73.1	16	9.4

^a Total cows in sample = 274. Most beef cows with first ovulation after 50 days were inseminated and excluded from interval tabulations.

Most of the second cycles were within the 17- to 25-day range, but one cow had a short interval of 16 days, five returned in 26 to 29 days and one took 39 days. For beef cows the respective intervals were 20.1 ± 5.2 days for first ($n = 171$) and 21 ± 3.1 days for second ($n = 101$) cycles. Almost all the second cycles in beef cows were normal, with the five exceptions returning in 10, 27, 28, 28 and 39 days.

A substantial number of first cycles was short but almost as many were long (Tables V and VI). The proportion of second cycles outside 17 to 25 days was

reduced to 14% in dairy and 5% in beef cows, resulting in smaller variance associated with the mean. Most of the aberrant second cycles were long rather than short. Since none of the cows had been inseminated, early embryonic mortality would not be a possible cause of these extended cycles [12].

7. SAMPLING FREQUENCY

Daily or even more frequent sampling is essential to clearly define the plasma profiles of most hormones and similar numbers of progesterone determinations are necessary to accurately quantitate luteal function and to detect subtle response to treatments. Under field conditions or in trials with large numbers of animals it is inconvenient or impossible to handle daily collections. However, precise or quantitative results are not usually required. A decision must be made on sample frequency which will minimize field and laboratory work but still yield sufficient results for decision making. Generally, it is only necessary to differentiate basal from elevated values and the selected frequency will depend on the specific objective. If it is simply to determine the proportion of animals in the luteal phase of their cycle at the time of insemination, only one sample collected at the time of breeding is required. Similarly, the progesterone concentration in one sample collected around three weeks after mating could yield a definite diagnosis of non-pregnancy if it was low, or a presumptive diagnosis of pregnancy if elevated. More confidence of pregnancy would be provided by a basal value in a sample collected at insemination and a second sample with high progesterone three weeks later [3]. To determine approximately when animals reach sexual maturity, weekly or even biweekly samples should suffice.

Probably the most common use for sequential progesterone sampling is to monitor ovarian activity. A combination of results for plasma progesterone determinations performed throughout the oestrous cycle by competitive protein binding [14–17] or radioimmunoassay [18–24] yielded a basal or follicular phase period of 7.0 ± 0.9 days ($n = 202$ cycles). Thus, twice weekly sampling should be sufficient to detect the first postpartum ovulation and all subsequent follicular and luteal phases.

The milk progesterone profiles obtained from the daily sampling of 30 cycling cows were used as a basis to further examine sampling frequency effects [25]. There was no difference between ability to predict when animals ovulated between daily and every fourth day values. If every seventh sample only was used over a three-week period, 20 of the 30 cows could accurately be divided into follicular and luteal phases and the approximate time of oestrus predicted. If the single weekly sampling was extended to five weeks, the reproductive status of all animals could be properly determined. In production-oriented field projects, weekly sampling of substantial numbers over relatively long periods should provide an accurate picture of the reproductive status and indicate whether acyclicity is a problem.

8. CONCLUSIONS

The reproductive component of any livestock improvement programme should attempt to develop a practical livestock management system, based primarily on locally available resources, that will produce more offspring per year. Once the existing resources and current practices have been evaluated, it should be possible to decide where immediate priorities belong and design specific projects with clearly defined objectives. Occasionally, assaying for hormones other than progesterone can be justified but, unless sampling is frequent, these usually produce non-essential or inconclusive data. For most production-oriented projects, immunoassays for hormones other than progesterone are usually unnecessary.

Several immunoassay systems to accurately determine progesterone concentrations in blood or milk are currently available and can be performed in most laboratories. If samples are collected from individual females weekly or twice weekly the results can be readily interpreted and usually provide conclusive data on reproductive status. The progesterone profiles can be used in monitoring studies to determine the incidence of acyclicity and how season, nutrition or disease affect reproductive function. Regular progesterone concentrations from individual cows, combined with condition scores, management evaluation, breeding and health records, should accurately define the current reproductive performance, potential and problems. Once the major factors limiting reproductive efficiency have been defined, further research can concentrate on the development of practical solutions. Sequential progesterone assays can then be incorporated into experimental procedures to objectively determine ovarian response to individual treatments and to assist in understanding the relationships between animals and their environment.

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ENZYME- AND RADIOIMMUNOASSAY TECHNIQUES FOR HORMONE DETERMINATION IN LIVESTOCK*

D.F.M. van de WIEL, W. KOOPS, E. VOS

Research Institute for Animal

Production Schoonoord,

Zeist, Netherlands

Abstract

ENZYME- AND RADIOIMMUNOASSAY TECHNIQUES FOR HORMONE DETERMINATION IN LIVESTOCK.

Hormone determination in livestock is an important research tool to improve both production and reproduction of domestic animals. For instance, measurement of growth hormone, prolactin, thyroid hormones, insulin, glucocorticoids, androgens, etc. is used to evaluate traits for meat and milk production, whereas measurement of luteinizing hormone, testosterone, progesterone, oestrone sulphate, etc. is used to evaluate reproductive capacity. Techniques for measurement of hormones are based mainly on the principle of immunoassay, and a wide variety of labelling substances is used as markers for end-point determination. Radioactive isotopes are probably the most widely used markers, but other substances such as enzymes, fluorochromes, chemiluminescent precursors, etc. are increasingly gaining acceptance as valuable alternatives. Currently, practical application of hormone measurement in livestock is limited mainly to animal reproduction, and the hormones most frequently measured are progesterone and oestrone sulphate. Many attempts have been made to improve the sensitivity, speed and reliability of the assay systems for these hormones. An increasing number of assays is based on the enzyme immunoassay (EIA) technique, and many further simplifications of these assays are currently being developed. This is reflected in the rapid increase of 'test-kits' being brought on to the market by pharmaceutical companies, with the ultimate aim of providing the farmer with 'cow-side' tests which can be applied on the farm. Future trends in immunoassay will most likely proceed in two directions, with the development of easy to apply 'dry chemistry systems' on the one hand, and highly sophisticated and easy to automatize biosensor systems for in vivo hormone measurement on the other.

1. INTRODUCTION

Hormone determinations in livestock are increasingly used to achieve improvement of both production (milk, meat, wool, etc.) and reproduction (offspring). Conventional ways of (genetic) improvement of livestock are based on selection and cross-breeding programmes. These programmes have proved to be very effective, but in general they are time consuming and expensive. Furthermore, heritability estimates for fertility traits are generally very low. For these reasons, much of the current research is directed towards the development of alternative methods of selection,

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which are based on the correlation between biochemical markers such as hormones, enzymes, metabolites, etc. and economically important traits. Much of this type of research has initially been directed to the study of blood types and protein polymorphisms, in which markers are most likely linked to the action of a single gene or a few genes only. These marker genes may be coupled to other genes which are relevant to economically useful traits, and therefore can be used, in principle, as a basis for selection. It seems likely, however, that other quantitative physiological traits in blood, which are not necessarily coupled to the action of single genes, can also be used for selection purposes [1]. Some hormones considered to be possible markers for production traits are: thyroid hormones, growth hormone, insulin, prolactin, corticosteroids and androgens [2-5].

For fertility traits, very few reports exist on hormones as possible (genetic) markers [6-8]. However, hormone research in the field of reproduction has had a very important impact in terms of an improved understanding of the physiology and pathophysiology of reproduction, and of the therapeutic and/or biotechnological use of hormones. Typical examples of these hormones are prostaglandins, GnRH, PMSG, FSH, LH, HCG, gestagens, oestrogens, androgens, either alone or in combination with each other. Recent reviews in the area of physiology and manipulation of the oestrous cycle have been published by Peters [9] and Voss and Holtz [10].

An interesting experimental approach might also be selection (by classical means) for biological extremes of economically important traits, as suggested by Land [11], followed by investigation of the possible physiological correlates of these extremes. Once these correlates, such as critical hormonal levels or profiles, have been identified, they may be applied in large-scale selection programmes. A successful example of this type of approach is the elimination of stress susceptibility and poor meat quality in pigs by selection on the physiological response after halothane application [12].

An important step towards an improved understanding of reproductive physiology was the introduction in the early 1960s of highly specific and sensitive radioimmunoassay (RIA) techniques. RIA and other related techniques nowadays allow measurement of hormones and other substances with a sensitivity down to the level of attograms (10^{-18} g) [13].

2. IMMUNOASSAY TECHNIQUES FOR DETERMINATION OF HORMONES

Radioactive isotopes in RIA methods have allowed great sensitivity because they provide a clear and distinct signal for detection, with a very high signal-to-noise ratio. Use of radioactive isotopes, however, also has some disadvantages, such as:

- (1) Limited shelf-life and stability of radiolabelled compounds
- (2) Disposal of radioactive waste

- (3) Need for expensive counting equipment and for regular servicing
- (4) Need for well-trained personnel and a specialized isotope laboratory
- (5) Potential health hazard from radiation when the isotope is produced
- (6) Increasing legislative restrictions on the use of radioactive materials.

For these and other reasons, many attempts have been made to develop non-isotopic labels, such as enzymes, fluorochromes, fluorogens, chemiluminescent precursors, substrates, cofactors, and many others [14–17]. The ideal characteristics of a diagnostic test as listed by Charan and Gautam [18] are speed, sensitivity, specificity, safety, potential for automation, broad applicability and potential for field use. Furthermore, the test should be cheap, and the test constituents should have a long shelf-life. These characteristics seem to be met to a large extent by the technique of enzymeimmunoassay (EIA), although this may depend on the nature of the analyte, the sample composition, and the conditions under which the assay has to be performed [19].

Especially in tropical and developing countries where electrical supplies and instrument maintenance facilities may be unreliable, non-isotopic and especially EIA techniques seem to be the method of choice [13, 20, 21].

3. ENZYMEIMMUNOASSAY TECHNIQUES

In this paper enzymeimmunoassay techniques are defined as immunological techniques in which enzymes are used to determine antibodies, antigens or haptens. This usually means that an enzyme-labelled immunoreagent is used, and that its presence and level can be monitored by means of coloured, fluorescent or chemiluminescent substrates or by changes in pH or conductivity. Nomenclature in non-isotopic immunoassays is, unfortunately, very confusing. Sometimes EIA methods are not classified according to the enzyme label used but to the signal detected, e.g. fluorescence, chemiluminescence, etc. Therefore, occasionally the same nomenclature is used for assays in which an enzyme acts, for instance, on a fluorescent substrate, and for assays in which a fluorescent group has been coupled to an immunoreactant. Enzymeimmunoassays with a colorimetric end-point do have a great advantage over other assay techniques because they offer the possibility of visual reading of the results. It seems likely that, for this reason, the EIA method will ensure its continuance into the future as a rather simple field test [22]. In this paper emphasis is mainly placed on those EIA methods which have a colorimetric end-point and which, in principle, can be used under field conditions with very limited laboratory facilities.

For a general description of the enzymeimmunoassay technique, its various types and the different purposes for which it has been used, several review papers are available [18, 23–25]. EIA methods are usually subdivided into homogeneous (= non-separation) and heterogeneous (= separation) assays. In homogeneous assays

TABLE I. DIFFERENT TYPES OF ENZYMEIMMUNOASSAY TECHNIQUES

Homogeneous	EMIT ^a (drugs, T ₄ , cortisol, IgG)	EEIA ^f (proteins)
	ECIA ^b (IgG, morphine)	EIIA ^g (IgG)
	EMMIA ^c (T ₄)	
	PGLIA ^d (haptens, antigens)	
	SLFIA ^e (haptens, antigens)	
Heterogeneous	comp. EIA ^h (haptens, antigens)	IEMA ^k (hCG, hLH, hTSH)
	DASP-EIA ⁱ (haptens, antigens)	Competitive sandwich ELISA ^l
	AMETIA ^j	Sandwich antigen, or antibody, ELISA ^l

^a EMIT = enzyme-multiplied immunoassay test.

^b ECIA = enzyme-channelling immunoassay.

^c EMMIA = enzyme-modulator-mediated immunoassay.

^d PGLIA = prosthetic group label immunoassay.

^e SLFIA = substrate-labelled fluorescent immunoassay.

^f EEIA = enzyme-enhancement immunoassay.

^g EIIA = enzyme-inhibition immunoassay.

^h EIA (comp.) = competition type of enzymeimmunoassay.

ⁱ DASP-EIA = double-antibody solid-phase EIA.

^j AMETIA = antibody-masking enzyme tag immunoassay.

^k IEMA = immunoenzymometric assay.

^l ELISA = enzyme-linked immunosorbent assay.

the enzyme activity is dependent on the antigen-antibody binding reaction. In heterogeneous assays the enzyme activity is not influenced by the binding reaction and therefore a separation of bound and free analyte is necessary, similar to the procedure in conventional RIA methods. A summary of the most popular EIA systems is given in Table I. The optimal type of assay to be used may vary with the type of analyte and the composition and source of the sample. Typical examples of analytes which have been measured by each type of assay are given in Table I (between brackets).

4. MONITORING OF FERTILITY BY MEASUREMENT OF PROGESTERONE AND OESTRONESULPHATE

Measurement of progesterone and, to a lesser extent, of oestrone sulphate has found practical application as a method for improving reproduction in farm animals. For instance, diagnosis of (non-)pregnancy and confirmation of (non-)oestrus is possible on the basis of progesterone concentrations in body fluids. This is because progesterone levels are low around the time of ovulation and high during the luteal phase of the oestrous cycle or during pregnancy. Measurement of progesterone

by RIA has been used for early diagnosis of (non-)pregnancy in cattle [26, 27], pigs [28, 29], buffaloes [30], goats [31, 32], for confirmation of oestrus in cattle [33, 34] and buffaloes [35] and for monitoring of fertility in cattle [36–42], goats [43], buffaloes [44] and horses [45]. In the pig, increased plasma levels of oestrone sulphate (E_1S) early after conception can also be used for pregnancy diagnosis, whereas in cows, sheep, goats and buffaloes increased levels of E_1S have been shown to be indicative of pregnancy during the second half of gestation (as reviewed by van de Wiel [46]).

5. ENZYMEIMMUNOASSAYS OF PROGESTERONE AND OESTRONESULPHATE

Applications of EIA techniques for the measurement of steroid hormones (especially progesterone and oestrone sulphate) in animal reproduction have been reviewed recently by Peters et al. [47] and van de Wiel [46].

5.1. Progesterone EIA

Different EIA methods for milk-progesterone measurement have been compared by Rattenberger [48] and Drew [49]. Initially, progesterone EIA methods were developed for use in humans [50–52]. To facilitate collection of biological samples, special assays have been developed for progesterone measurement in human saliva [53–56].

EIA methods for progesterone measurement in cows' milk have been developed by several groups. The assay initially developed by Arnstadt and Cleere [57] has led to two different types of assay, both of which are now available as commercial kits. A 'coated tube' kit is produced by Biolab GmbH (Munich, Federal Republic of Germany). It has been tested on different types of samples [58], under different environmental conditions [59, 60], and in different species such as mares [61] and cattle [62–64]. A 'coated microtitre plate' kit (Reprostrip) is produced by Noctech Ltd (Galway, Ireland) and has been tested under field conditions [65, 66] in bovine milk and in bovine or equine plasma [67], and in bovine milk and serum samples without extraction [68].

Another microtitre plate EIA has been developed independently by Sauer et al. [69–71] and has been applied in AI programmes by Foulkes et al. [72]. This EIA is also produced commercially as a kit (Ovucheck) by Cambridge Veterinary Sciences (Cambridge, UK).

A double antibody progesterone EIA has been developed by a Japanese group. This test has been used for pregnancy testing by measurement of progesterone in bovine serum [73] and milk [74–76]. The test has also been applied for differential diagnosis of cystic ovaries [77].

Two other EIA methods for progesterone measurement in large domestic animals have been developed and reported by research groups in the USA. Chang and Estergreen [78] developed a paper-fibre solid-phase tube assay which can be applied to unextracted milk, and Munro and Stabenfeldt [79] developed a microtitre plate assay for progesterone measurement in plasma (after extraction) in the sheep, cow, horse, goat, dog and cat.

We in our laboratory have developed a non-extraction type microtitre plate EIA for the measurement of progesterone in milk or plasma which also allows visual reading of the results [80, 81]. This assay has been applied for monitoring fertility in the water buffalo [30, 82, 83], cattle [81], goats [84] and mares [85]. The same assay, except for the antibody used for coating of the plates, has been applied to the measurement of progesterone in porcine plasma [86].

Commercially available progesterone EIA kits not mentioned above are produced by Hoechst/IQ-BIO (enzynost), Immucell (bulls-eye test) and Pitman-Moore (progestassay).

5.2. Oestrone sulphate EIA

Measurement of E_1S has been used for pregnancy diagnosis in dairy cows [87], sows [88], goats [89] and mares [90]. Tube-type EIA methods have been described for E_1S measurement in porcine plasma after extraction [91–93]. A microtitre plate EIA for E_1S measurement in porcine plasma has been reported by van de Wiel [46], and a similar assay for unextracted cows' milk has been described by Power et al. [94].

6. CURRENT AND FUTURE DEVELOPMENTS

Reviews on current and future developments have been described among others by Ekins [13] and Bosch [16]. These developments can be divided according to the particular (combinations of) components of the assay system, such as monoclonal antibodies, dry chemistry systems, antibody probes and biosensors. Other new developments are in the field of new methods for end-point detection, such as pulsed light time resolved fluorescence, bio- and chemiluminescence and sol particle immunoassays.

6.1. Monoclonal antibodies (for a review: see Siddle [95])

Monoclonal antibodies (MCAs) have the unique advantage that they are monospecific and can be produced in indefinite quantities. This is important, for instance, in large-scale routine immunoassays, where a constant quality of antibodies and an uninterrupted supply over a prolonged period of time are required in order to make results comparable over laboratories and over time. MCAs also have the

advantage that they can be produced by immunization against impure, or even unknown, antigens. An important methodological aspect in setting up immunoassays with MCAs is the screening method for selection of the best producing cell-line. It is our experience that MCAs can behave very differently when applied in different assay systems [96]. This means that the MCAs producing cell-lines should preferably be screened by the same method as the method ultimately to be used in the diagnostic test. A very elegant application of MCA has been the use of two different antibodies in a 'tandem-sandwich' ELISA, which allows very specific measurement of large protein hormones, such as HCG, LH, FSH, etc. Some disadvantages of MCAs compared with polyclonal antibodies are generally lower affinity and the fact that they do not always form precipitates with antigens.

6.2. Antibody probes and biosensors

Antibody probes and biosensors are based on the principle that immobilized antibodies can be used to specifically measure analytes in biological samples independently of the sample volume. This principle will most likely lead to the development of in vivo 'immunometer' techniques for the measurement of analyte concentrations in particular sites within the body, without the necessity of sample removal ('ambient analyte immunoassay'). A further possibility is the simultaneous measurement of many analytes in the same sample by the introduction of a small probe with an appropriate array of antibody spots each of differing specificity ('hormone profile immunometer'). Ultimately, this may enable the construction of artificial endocrine systems capable of sensing and responding to changing hormonal environments in selected body fluids [13].

To a certain extent this idea has already been realized by the development of 'biosensors'. These are very small pieces of silicon (field effect transistor = FET) coated with (bio)chemically sensitive material, such as antibodies. After binding of the antibodies with the analyte in the sample, the sensor material undergoes an electrical change which is amplified by the FET to a signal that can be measured. A more advanced type of these biosensors is the ion-sensitive FET (ISFET) which can be miniaturized (to a few square millimetres) and therefore can be applied as an implant [97].

6.3. Dry chemistry systems

Immunochemical test strips have the potential of allowing rapid quantitative analysis in non-traditional testing environments such as a milking parlour. Enzymeimmunoassays are particularly well suited for test strip applications because great signal amplification is possible and because of the ease with which the chromophoric products of enzymes can be detected, either visually or with simple instrumentation. Ideally, dry chemistry systems should be 'one step assays' in which, for instance, wetting of an especially prepared paper strip with the biological sample

results in a visually detectable signal, corresponding quantitatively to the concentration of the analyte to be measured. A paper strip assay system has been developed, for example, for measurement of theophylline [98]. Further developments in this field might include dry solid-phase reagent systems such as multilayer film elements [99]. In principle, dry chemistry systems can also be based on the technique of EMIT [24] or SPIA [100], and may very well be applied to the measurement of hormones such as progesterone. Work on the development of dry chemistry systems for measurement of progesterone is currently in progress [101].

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POSSIBLE WAYS OF IMPROVING ENZYMEIMMUNOASSAY (EIA) TECHNIQUES AND THEIR APPLICATION IN ANIMAL PRODUCTION

H.H.D. MEYER

Institut für Physiologie,
Süddeutsche Versuchs- und Forschungsanstalt
für Milchwirtschaft,
Technische Universität München,
Freising-Weihenstephan,
Federal Republic of Germany

Abstract

POSSIBLE WAYS OF IMPROVING ENZYMEIMMUNOASSAY (EIA) TECHNIQUES AND THEIR APPLICATION IN ANIMAL PRODUCTION.

To achieve reliable EIA techniques as alternatives to RIA procedures for evaluation of endocrine events in reproduction or for control of residues of anabolic agents, several investigations were performed using the microtitration plate technique. In association with progesterone determination in skimmed milk, kinetic studies were carried out. The speed of the immune reaction is proportional to the concentration of ligands (hormone, tracer, antibody). Therefore, equilibrium of immune reactions is reached later if a progesterone assay is done with skimmed milk instead of final stripping, which has a high but variable fat content and hence a higher progesterone content. A former incubation technique with direct coating of the hormone-specific antibody on to the wells was compared with a new second antibody technique. In the latter technique an excess of affinity purified sheep IgG (anti-rabbit-IgG) is first coated onto the wells, followed by the addition of hormone plus tracer and hormone-specific antibody from rabbits. Variabilities of colour readings were reduced in the new technique (3.2% versus 11.4%) and about 10 to 30 times less antibody was required. The second antibody technique was used for the determination of several hormones (progesterone, trenbolone, diethylstilbestrol, 19-nortestosterone and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ = PGFM). Comparison of alkaline phosphatase (AP) and horseradish peroxidase (HRP) showed that a similar sensitivity was obtainable with both enzymes. However, the phosphatase system was more practicable as the nitrophenylphosphate solution is stable for several days and phosphatase is insensitive to light and aspirin (stabilizer in prostaglandin assays), whereas peroxidase is inactivated by both and the substrate solution is stable for only a few hours. A direct test for PGFM was established using 2 to 20 μ L of plasma per well. Unspecific interferences could be avoided only if 2 μ L/well were analysed. A detection limit of ~ 2 fmol/well (1000 fmol/mL) enables demonstration of PGFM peaks connected with luteolysis.

1. INTRODUCTION

There are five main uses to which hormone analysis can be put in animal production using several radioimmunoassays (RIAs), namely for mapping of physiological profiles, elucidation of pathological deviations, control of therapeutic and

biotechnical measures, evaluation of traits for selection and control of exogenous hormonal agents, which includes pharmacokinetics and residue studies in edible tissues [1]. Besides the vast importance of hormone analysis for scientific investigations, only a few hormones have been approved as useful parameters for practical veterinary and agricultural requirements. For these purposes non-isotopic procedures are demanded, especially to avoid the disadvantages of RIAs that are connected with the handling (and disposal) of radioactive-labelled hormones. Although the first EIAs for haptens were described as early as 1972 [2], test procedures providing sufficient practicability and reliability have only been achieved stepwise. Particularly for progesterone determination in milk to control ovarian function in cows, tube tests [3, 4] were developed; these were followed by assay systems on microtitration plates [5–9]. The latter technique, which has also been used for determination of oestrone sulphate for pregnancy control in cattle [10] and the boar taint compound 5- α -androst-16-en-3-one [11], offers several advantages: solid-phase separation of bound and unbound tracers is very practical; the amount of reagents and waste is largely reduced; the tests are always clearly arranged and easy to survey; colorimetric evaluation of the results is completed within a few minutes; also, several commercial kits are already available.

Although most of these tests work quite well, there is still a need to improve the techniques. 'Head/tail' phenomena within the test [7] cause reduced reproducibility and unknown factors in milk or plasma have an effect on the total binding of the tracer. In the following section we describe the investigations that were carried out to improve EIA techniques with a view to widening their field of application.

2. RESULTS AND DISCUSSION

2.1. Limitations of EIA due to the kinetics of immune reactions

The immune reaction follows a 'second order rate equation': $d[AH]/dt = [A] \cdot [H] \cdot k$, where $d[AH]/dt \equiv$ reaction speed; $[A] \equiv$ concentration of antibody; $[H] \equiv$ concentration of hormone; $k \equiv$ rate constant. The rate constant depends on temperature (T), molecular weight (MW), etc.: $k = f(T, MW, \text{etc.})$ or $k \sim 1/\sqrt[3]{MW}$. Increasing the MW causes reduced diffusion rates and lower reaction speeds. Therefore, the equilibrium of the immune reaction is always reached more rapidly using RIA than it is using EIA, since the molecular weight of ^3H -labelled hormones is much lower than that of enzyme-labelled hormones. According to the first equation the concentrations of ligands (hormone, tracer, antibody) also influence the time required to reach equilibrium. For this reason equilibrium of the immune reaction in the progesterone assay is reached later in skimmed milk than in final stripping, which contains ten times more progesterone (Figs 1, 2). (The assay of skimmed milk offers reproducible progesterone values independent of the variations in fat content of the milk samples [9, 12–14]. The immune reaction is not completed after

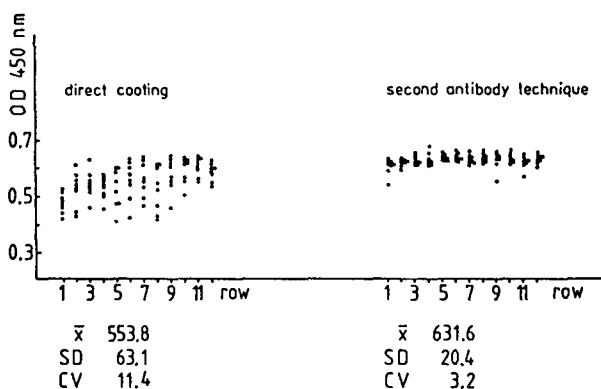


FIG. 1. Variabilities of different EIA techniques.

Direct coating:

Progesterone antibody was purified by salt precipitation, directly coated onto the wells and then washed; tracer (progesterone-11 α -HS-HRP) was added to all the wells without the standard or sample containing the hormone and incubated for 2 h at 37°C. After washing and enzyme reaction, the optical densities were measured.

Second antibody technique:

Affinity purified sheep IgG (anti-rabbit-IgG) was coated and then tracer and progesterone antibody were added. Further steps as for direct coating.

120 min at 37°C using 20 μ L skimmed milk per well (Fig. 1) and about 40 min are required for colour production. If 20 μ L of final stripping per well are analysed, antibody and tracer concentrations are elevated to the same extent and equilibrium is almost reached after 30 min at 22°C (Fig. 2). Since more enzyme is also bound, only 10 min are required for colour production and the whole test is completed in less than 1 hour.

It is possible to speed up the reactions if even higher amounts of antibody, enzyme and milk from samples of final stripping are used and the procedure is also practicable for single samples if test-tubes and stoppers with coated sticks are utilized. Hence, it was possible to elaborate a 'pocket test' that can be completed within 15 min [15].

2.2. Second antibody technique

Separation of bound and unbound tracers in microtitration plate EIA is commonly done by a solid-phase technique after coating the specific antibody directly onto the wells. This technique does not always afford sufficient reproducibility and storage of coated plates is difficult. To overcome these problems a second antibody technique was developed: sheep IgG (anti-rabbit-IgG) was coated onto the wells, the sample or standard containing the hormone plus the tracer were added and the immune reactions started after dispensing the hormone-specific antibody raised in

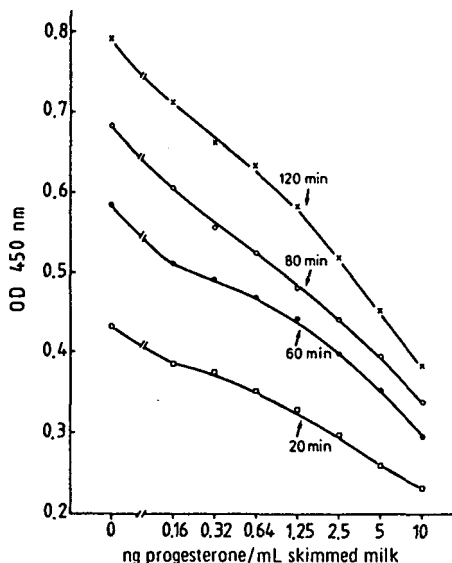


FIG. 2. Time dependency of the immune reaction of a direct progesterone assay with 20 μ L of skimmed milk at 37°C.

rabbits. After incubation overnight, the plates were washed and evaluated after the enzyme reaction had taken place. The first trials using the ovine antisera directly or after simple purification by salt precipitation resulted in insufficient IgG binding because of the limited binding capacity of the microtitration plate wells. Therefore, the quality of sheep IgG was improved by affinity chromatography: serum from a sheep that had been immunized with rabbit IgG was stirred overnight with rabbit IgG-agarose. The gel was transferred to a column, washed and then eluted with 2M NaSCN; 0.5 μ g affinity isolated sheep IgG were coated per well. This was about ten times more than that required for total binding of the hormone-specific rabbit antibody; minor variations in coating were negligible. Figure 3 shows the variabilities of colour readings after immune reaction with tracer (without standards) in all 96 wells of the plate. In the former technique the readings of the wells on the outer rim of the plate often deviated from those of the inner wells. The second antibody technique largely overcomes this problem in that there was a reduction in the variability of the readings from 11.4 to 3.2%. Coated plates can now be stored at 0°C for two weeks or frozen at -20°C for a few months without losing precision. Compared with RIA or the former EIA technique only 3 to 10% of the hormone-specific antibody were required. We tested the usefulness of Protein A instead of affinity purified sheep IgG, i.e. 200 ng of Protein A (Sigma Chemical Company, St. Louis, USA; Product P 6650) were coated per well; the results were similar if the analysed sample did not contain any additional IgG. However, the quality of commercial affinity puri-

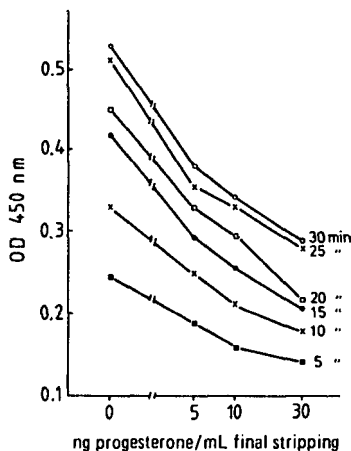


FIG. 3. Time dependency of the immune reaction of a direct progesterone assay with 20 μ L of final stripping at 22°C.

fied sheep IgG (anti-rabbit-IgG) was inadequate for our purposes. Besides, for progesterone the improvement discussed above was introduced to establish reliable microtitration plate EIAs for total physiological oestrogens and the anabolic compounds diethylstilbestrol, trenbolone and 19-nortestosterone (details will be published elsewhere).

2.3. Comparison of the tracer enzymes alkaline phosphatase (AP) and horseradish peroxidase (HRP) and development of a microtitration plate EIA for a prostaglandin $F_{2\alpha}$ metabolite

PGF_{2 α} , which is of interest in the reproductive endocrinology of ruminants, is released from the endometrium but is metabolized [16] during the first passage through the lungs. The first metabolite 13,14-dihydro-15-keto-PGF_{2 α} (PGFM) can be measured in the peripheral circulation [16]. For determination of prostaglandins, aspirin is added to the plasma to prevent further metabolisms. Since aspirin completely destroys HRP, the assay can only be done with AP, which is insensitive to aspirin. HRP, AP and β -Galactosidase are the preferred enzymes for EIA. In our experiments preparation of hormone-enzyme conjugates was always done by the mixed anhydride procedure [17, 9]. HRP contains only a few unblocked amino groups [18, 19] and therefore preparation of HRP-steroid conjugate does not seem to be critical, since only a few hormone molecules per enzyme molecule are bound, even with an excess of hormone. The conjugated tracer can be prepared with a constant quality. AP contains much more accessible amino groups and it was possible to couple up to 80 mol PGFM/mol AP, resulting in unsuitable and insensitive tests. However, we found standard curves with a good sensitivity if only 2 mol PGFM/mol

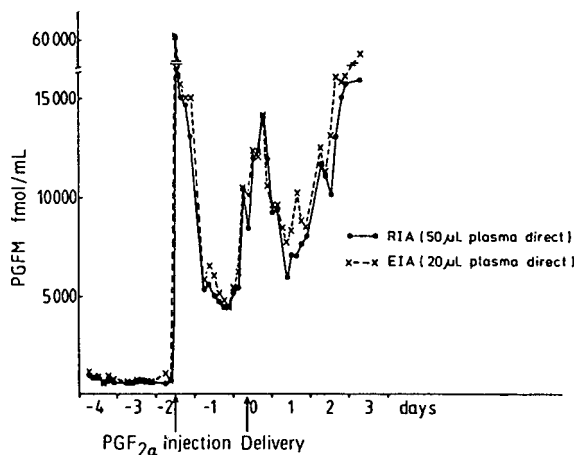


FIG. 4. Concentrations of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in blood plasma samples taken from the jugular vein of a cow peripartum after application of $PGF_{2\alpha}$.

enzyme were bound. With such a low coupling rate a similar sensitivity was revealed by both HRP and AP tracer enzymes. Unfortunately, the coupling rate to AP was not very reproducible in our experiments and the production of AP-hormone conjugates with a constant quality remains to be developed.

On the other hand, AP has several advantages: it is insensitive to light, temperature and oxidizing or hydroxylating agents, in contrast to HRP. Also, the substrate solution for AP remained stable for up to a week, whereas the substrate solution for HRP can only be utilized for a few hours. Using PGFM-AP as a tracer we developed an EIA with an absolute detection limit of ~ 1 fmol/well in buffer and ~ 2 to 4 fmol/well after addition of 20 μ L of PGFM-free plasma. In the direct test, the practical detection limit amounted to ~ 100 fmol/mL plasma, which is sufficient. The good coincidence of the results measured in blood plasma samples from a cow peripartum by direct RIA (50 μ L plasma/tube) and direct EIA (20 μ L plasma/well) is demonstrated in Fig. 4. The results of EIA and RIA were similar in samples from most of the animals, but in samples from a few cows there was a total deviation in the results. The nature of this unspecific effect in EIA could not be identified, but it could be avoided if less plasma per well was analysed. By measuring 2 μ L plasma/well the results of EIA and RIA were always identical, but the practical detection limit of EIA was reduced to 1000 fmol/mL. This is sufficient to monitor peaks in connection with luteolysis but minor variations cannot be detected. Unspecific effects in EIA can also be reduced by extraction of the prostaglandins from the plasma or other clean-up steps.

3. CONCLUSIONS AND OUTLOOK

To date, reliable EIA methods have been established which are ready to replace RIAs under certain conditions, but each hormone requires the development of special systems. Improvement of the sensitivity — which may even be much superior to RIA — is possible, but optimal antibody and enzyme qualities are a prerequisite and they must be matched. Further improvements in sensitivity will be possible using enzymatic amplification systems or by the enzymatic production of fluorescent markers.

Unspecific interferences during the immune reactions seem to be greater in EIA than in RIA. Particularly milk and plasma interfere in direct tests and therefore their reliability remains a problem. Unspecific effects could be reduced by appropriate buffers or small volumes, but further investigations are necessary. After prior clean-up steps the reliabilities of EIA and RIA are similar. Depending on the aims of the tests sometimes a decision has to be taken as to whether a more precise result or a quicker estimation is demanded.

The former higher variability of EIAs could be overcome by an incubation technique involving an affinity purified second antibody. Nevertheless, to obtain good reproducibility, precise laboratory work is necessary as well as practical experience with the very small volumes to be handled in microtitration plate EIA.

We are still at the beginning of the development of EIA techniques but further research will certainly provide more improvements in the practicability and reliability of assays and hence a wider application of EIA techniques in future.

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SOME ASPECTS OF THE POSSIBLE ROLES OF DISEASES IN ALTERING REPRODUCTIVE PERFORMANCE*

H. KINDAHL**, N. CORT**, G. FREDRIKSSON**,
L.-E. EDQVIST***, G. STABENFELDT⁺

**Department of Obstetrics and Gynaecology

***Department of Clinical Chemistry

College of Veterinary Medicine,
Swedish University of Agricultural Sciences,
Uppsala, Sweden

⁺Department of Reproduction,
School of Veterinary Medicine,
University of California,
Davis, California,
United States of America

Abstract

SOME ASPECTS OF THE POSSIBLE ROLES OF DISEASES IN ALTERING REPRODUCTIVE PERFORMANCE.

Intravenous infusion of endotoxins causes a rapid and pronounced release of prostaglandin $F_{2\alpha}$ in the mare, cow, goat and sow. In the doe a marked relationship exists between the dose of endotoxin administered and the amount of prostaglandin released. Endotoxin-mediated prostaglandin release can affect luteal function, causing shortening of the oestrous cycle or abortion in pregnant animals.

1. INTRODUCTION

Various diseases can alter the reproductive function, e.g. through changing the oestrous cycle length, or causing foetal resorption or abortion. Several infectious agents located in the female genital tract cause specific reproductive diseases which directly influence reproductive function. There is, however, general evidence to support the concept that diseases primarily located in organs other than the genital tract can influence reproduction in a more indirect way. This seems to be particularly valid for Gram-negative bacteria, since one possible effect on reproductive function seems to be mediated through endotoxins.

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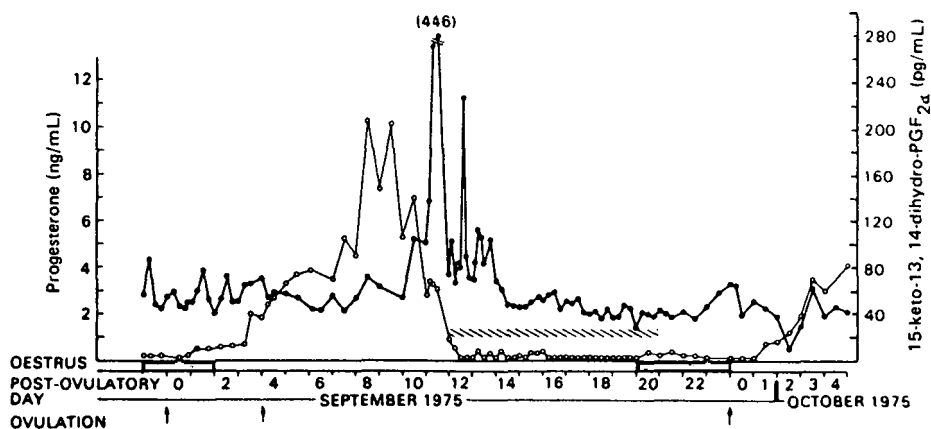


FIG. 1. Premature release of $\text{PGF}_{2\alpha}$ and induction of luteolysis in a mare elicited by an acute inflammatory response as part of a spontaneously induced endometritis. Levels of 15-keto-13,14-dihydro- $\text{PGF}_{2\alpha}$ (●—●) and progesterone (○—○) in the jugular vein. Black horizontal bar = sexual receptivity; arrows = days of ovulation; \\\ = cervico-vaginal discharge (from Ref. [1]).

The aim of this paper is to discuss the possible links between bacterial infections or experimentally administered endotoxin and subsequent endocrine changes affecting the reproductive function in non-pregnant and pregnant animals.

2. EFFECT OF BACTERIAL INFECTIONS

2.1. Non-pregnant animals

Uterine infections are capable of changing oestrous cyclicity. Shortening of the oestrous cycle has been observed in mares with spontaneously or experimentally infected uteri [1, 2]. Two mares with spontaneous uterine infections have been followed with frequent peripheral blood samples analysed for progesterone and 15-keto-13,14-dihydro- $\text{PGF}_{2\alpha}$ (the main circulating metabolite of $\text{PGF}_{2\alpha}$). In normal mares the luteolytic release of $\text{PGF}_{2\alpha}$ is initiated on days 14 to 15 after ovulation. In the two infected mares premature release of prostaglandins started about 5 days before this time (Fig. 1 [1]) and was associated with a rapid decline in progesterone levels. This release of prostaglandin also preceded the appearance of clinical signs in the form of purulent cervico-vaginal discharge. The reason for the premature prostaglandin release is not known, but it could tentatively be attributed to a cellular response due to the presence of bacteria. This cellular response may be a function of both the cellular destruction or a repair process. It is known from studies in cattle that cellular repair following necrotizing endometritis caused by an intra-uterine iodine infusion is associated with prostaglandin release [3].

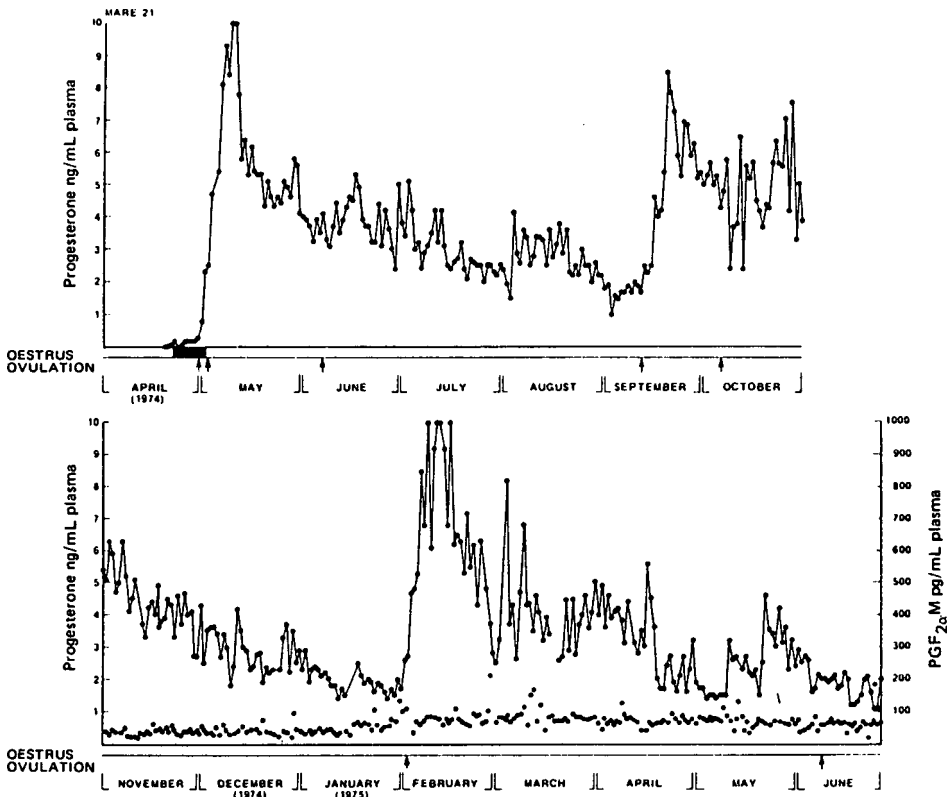


FIG. 2. Persistence of the luteal phase (as indicated by progestin concentrations, (●—●)) in a mare with pyometra in conjunction with extensive tissue damage of the endometrium. The luteal phase persisted via the occurrence of occasional, sporadic ovulations coupled with corpus luteum formation. Note the absence of any significant and sustained release of PGF_{2α} (as determined by 15-keto-13,14-dihydro-PGF_{2α} concentrations (● ● ●)). Black horizontal bar = sexual receptivity; arrows = days of ovulation (from Ref. [4]).

In cases of chronic uterine infections in the mare the oestrous cycles are in most cases prolonged [4]. Most likely the bacterial infection causes progressive destruction of the endometrial elements with little or no concomitant repair, thus reducing the capacity for synthesizing prostaglandins in quantities required for luteolysis. In general, we have found that the greater the disruption of endometrial mucosal surface the lesser the capability of the mare for controlling cyclic ovarian activity through prostaglandin-mediated lysis of the corpus luteum [4]. In an extreme case, luteal activity was prolonged for at least 425 days (Fig. 2 [4]).

It is likely that similar mechanisms to those described above for the mare also exist in the cow. In the cow, postpartum prostaglandin release has been studied in

animals with normal puerperia and in animals with positive bacteriological findings in the uterus.

Ruminants have elevated levels of $\text{PGF}_{2\alpha}$ during several weeks postpartum [5–8]. $\text{PGF}_{2\alpha}$ is secreted from the uterus and plays a role in uterine involution. A significant negative correlation has been demonstrated between the duration of elevated levels of prostaglandins postpartum and the time required for completion of uterine involution [5]. Furthermore, the time for completion of uterine involution can be shortened by about one week after massive doses of exogenous prostaglandins [9]. Cows with persistent uterine infections postpartum have a longer duration of elevated levels of prostaglandins. There is also an opposite relation between the time of elevated levels of prostaglandins and uterine involution compared with cows with uncomplicated puerperia [5, 10]. It is likely that the bacterial infection and toxins produced by these bacteria stimulate the uterus to an increased production of prostaglandins which is superimposed on the physiological one.

In normal cows, ovulations seldom start before prostaglandin levels return to basal concentrations [7, 8]. It is possible that the high postpartum prostaglandin levels serve as a uterine signal, preventing a too early onset of cyclicity. Uterine infections in cows are most prevalent during the immediate postpartum period, after which the incidence is reduced. Onset of cyclicity is usually delayed until the time when the cow has cleared herself from the infection and when prostaglandin levels are low. However, in some cows with documented uterine infection, ovarian cyclicity started relatively early (Fig. 3 [10]). The onset of sexual functions coincided with a temporary decrease in prostaglandin levels, which could be explained either by a temporary relief in the infection or more probably by destruction of the endometrium and prostaglandin-synthesizing cells, especially as the infectious agents found were *Corynebacterium pyogenes* and *Fusobacterium necrophorum*. When the first luteal phase started, prostaglandin levels rose again. These continuous and somewhat higher concentrations were, however, not sufficient to cause luteolysis. The oestrous cycle following this early ovulation was prolonged and was at this stage terminated by exogenous administration of $\text{PGF}_{2\alpha}$. Otherwise, this condition would most probably have developed into pyometra, with prolonged luteal function.

2.2. Pregnant animals

In pregnant animals few endocrinological studies have been performed to follow the hormonal, including prostaglandin, changes after bacterial infections. Baetz and co-workers [11] exposed pregnant cows at 4 to 5 months of gestation to *Sarcocystis cruzi* or *Campylobacter foetus* and analysed PGF and its metabolite in jugular vein plasma. About 50% of the animals aborted because of the treatment. Concentrations of the prostaglandin metabolite increased tenfold 24 to 48 hours before abortion or parturition. The infected animals had a prostaglandin release pattern that was indistin-

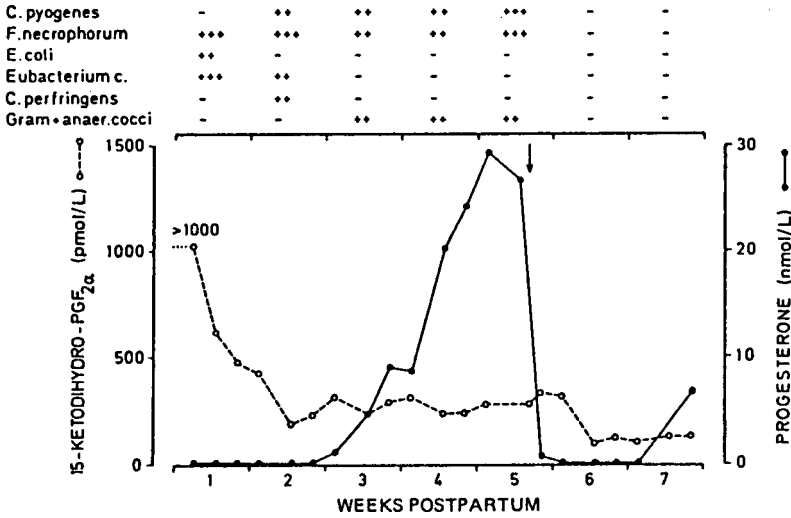


FIG. 3. Intrauterine bacterial findings and levels of 15-keto-13,14-dihydro-PGF_{2α} (○—○) and progesterone (●—●) during 7 weeks postpartum in one cow. (Luteolytic treatment (see text) is indicated by the vertical arrow.) Bacteriological cultures were made from uterine biopsies taken once per week; — indicates no, + scanty, ++ moderate and +++ heavy growth of bacteria [10].

guishable from that in cows with normal parturition, except that the rise in prostaglandin metabolite levels may have started 1 or 2 days earlier in the affected animals.

3. EFFECTS OF ENDOTOXINS ON THE OESTROUS CYCLE AND PREGNANCY

Endotoxins are a part of the cell wall of Gram-negative bacteria. The structure of endotoxins from different types of bacteria and the biological effects are similar. Endotoxins seem to be a very potent biological compound in releasing prostaglandins. They most likely activate the whole arachidonic acid cascade, generating prostaglandins and related compounds of different types. In these studies endotoxins (lipopolysaccharide) from *Salmonella typhimurium* have been administered intravenously to animals. They have been followed with clinical examinations and blood chemistry. To monitor the luteal function, progesterone levels have been analysed in peripheral plasma [12]. For studying prostaglandin release, the main plasma metabolite of prostaglandin F_{2α}, 15-keto-13,14-dihydro-_{2α}, has been measured [12].

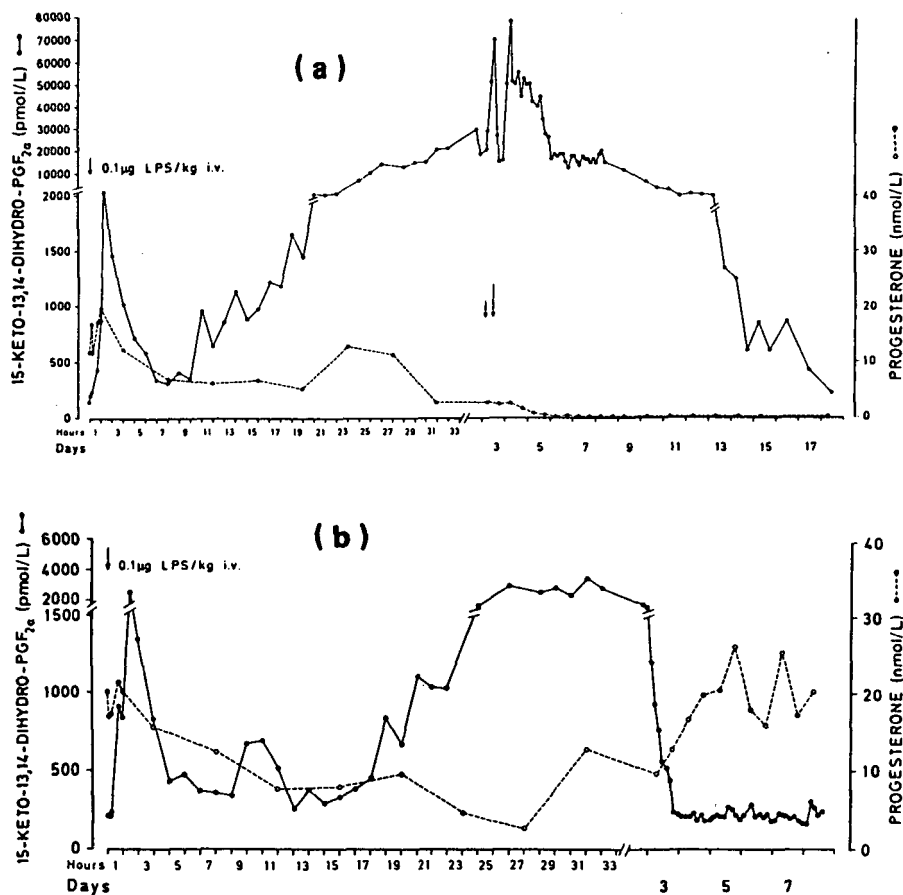


FIG. 4. Peripheral blood plasma levels of 15-keto-13,14-dihydro-PGF_{2α} (●—●) and progesterone (O—O) in goats pregnant for 3 months. The goats received endotoxin (LPS; lipopolysaccharide) injections into the jugular vein (0.1 μg/kg). (a) An animal aborting after a total dose of 3.0 μg, the second solid arrow indicates the time of abortion; (b) an animal which maintained pregnancy after a total dose of endotoxin (5.0 μg) (from Ref. [13]).

3.1. Doe

Endotoxin was injected intravenously to does with normal oestrous cycles or those that were pregnant [3]. Different doses of the endotoxin were tested and it was found that doses varying from 0.5 to 5 μg/kg body weight in cyclic animals initiated a release of prostaglandin F_{2α} in a dose-dependent way (the coefficient of correlation was 0.87). When endotoxin was injected in doses of about 1.8 μg/kg, the subsequent release of prostaglandin F_{2α} initiated a premature luteolysis and the animals

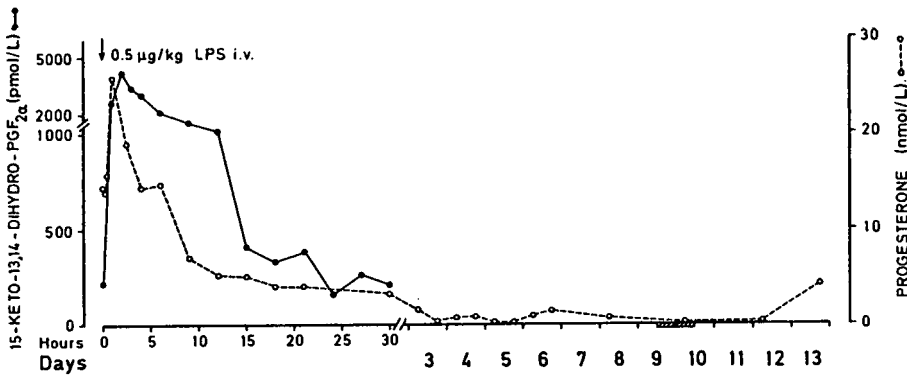


FIG. 5. Peripheral blood plasma level of 15-keto-13,14-dihydro-PGF_{2α} (●—●) and progesterone (○—○) in a cycling cow. Solid arrow indicates the time of endotoxin injection into the jugular vein (0.5 μg/kg) on day 8 of the oestrous cycle; horizontal hatched bar denotes the time of rectally determined ovulation (from Ref. [14]).

came into heat. When smaller doses of endotoxins were injected a transient depression of progesterone levels was monitored.

In pregnant does, 0.1 μg/kg of the endotoxin induced abortion in two out of three animals (see Fig. 4(a)) [13]. The endotoxin induced a prostaglandin release, which resulted in a decrease in progesterone levels. Abortion occurred about 1 to 2 days after the injection of endotoxin, concomitantly with a secondary rise in prostaglandin metabolite levels. The non-aborting animals also showed a direct response in prostaglandin levels as well as a secondary rise associated with a temporary drop in progesterone levels. With recovery of the progesterone levels, the prostaglandin levels were reduced and pregnancy was maintained (see Fig. 4(b)) [13]. This could be described as an endocrine 'battle' that occurred between the prostaglandin and progesterone functions.

3.2. Cow

Different doses of endotoxin (0.5, 2.5 and 5.0 μg/kg body weight) were administered to cows with regular oestrous cycles [14]. No relation was observed between the endotoxin dose and the subsequent prostaglandin response, as has been reported in the goat, and it seemed more likely that the response was more individual. Endotoxin was injected during the luteal phase and caused either a complete luteolysis or a temporary drop in progesterone levels (Fig. 5 [14]). The changes in leucocytes and serum calcium levels were pronounced. The total leucocytes were reduced by 80% and the serum calcium levels by 20 to 25%. No studies have been performed in pregnant animals, but it is likely that results in the cow would be similar to those in the goat.

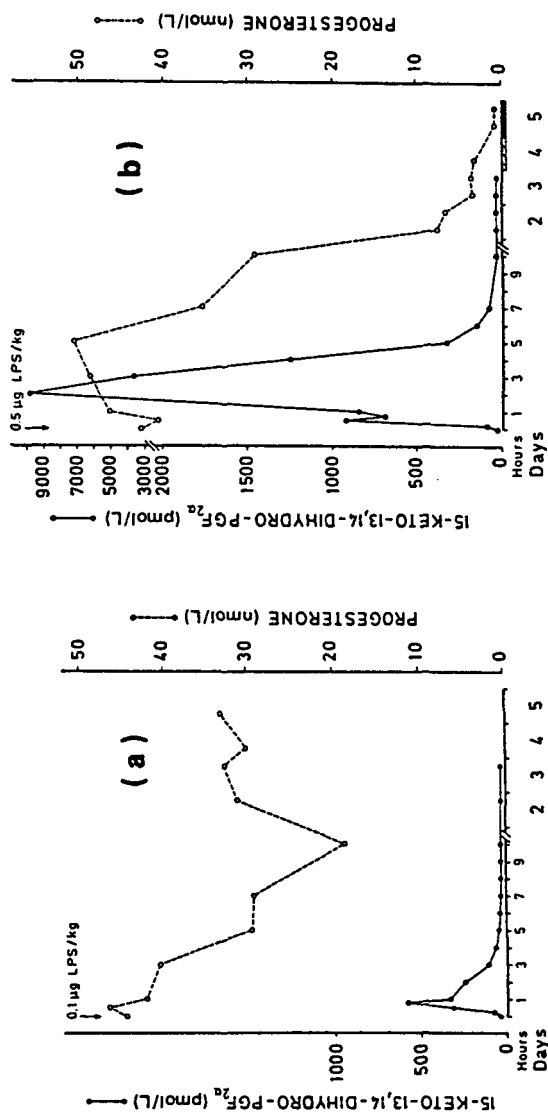


FIG. 6. Peripheral blood plasma levels of 15-keto-13,14-dihydro-PGF_{2α} (●—●) and progesterone (○—○) in two mares. Solid arrows indicate the time of endotoxin injections into the jugular vein. (a) Mares receiving a low dose of endotoxin (0.1 µg/kg) and (b) a higher dose (0.5 µg/kg). In (b) the horizontal hatched bar indicates the time of pro-oestrus and the black bar the time of oestrus (from Ref. [15]).

In cattle, several frequently occurring diseases located in, for example, the gastrointestinal tract and udder are caused by Gram-negative bacteria. Thus, if endotoxin generated from such infections comes in contact with prostaglandin-producing cells, the above clinical effects (hypocalcaemia, leucopenia, altered oestrous cycle length, abortion) may occur.

3.3. Mare

An intravenous injection of endotoxins in the mare caused a rapid and pronounced release of $\text{PGF}_{2\alpha}$, as indicated by increased levels of 15-keto-13,14-dihydro- $\text{PGF}_{2\alpha}$ [15]. The response in prostaglandin release was not dose-dependent as in the goat, but rather more individually related as described in the cow. Injection of endotoxin can result in a temporary progesterone decline (Fig. 6 (a)) or a complete decline indicative of luteolysis and a subsequent shortening of the oestrous cycle (Fig. 6 (b)).

Preliminary results from ongoing studies in pregnant mares indicate that very early pregnancy stages are extremely sensitive to endotoxins and that foetal resorption or abortion is induced. It seems that the sensitive period for endotoxin-induced abortions follows the period of spontaneously occurring foetal deaths.

3.4. Sow

A clinical and endocrine study was conducted to investigate the luteolytic and abortifacient effects of endotoxins in sows [16–18]. The endotoxin was injected in doses of 2.0 to 3.0 $\mu\text{g}/\text{kg}$ body weight into cycling or 60 to 100 day pregnant sows. The induced endotoxaemia was manifested by blood circulation shock, fever, leucopenia, etc. A massive release of prostaglandin $\text{F}_{2\alpha}$ followed endotoxin administration. In cycling animals this release was not capable of altering oestrous cyclicity or changing the progesterone levels [17]. These results are in agreement with the findings that the corpora lutea in the non-pregnant pig are not sensitive to exogenous prostaglandin $\text{F}_{2\alpha}$ or its analogues [19]. The effect of endotoxin on the course of gestation provided results of three categories when injected at days 60, 80 or more than 80 days of gestation:

- (1) A rapid decrease of progesterone and abortion within 48 hours (Fig. 7 [17])
- (2) A transient decrease of progesterone and maintenance of gestation with no damage to the foetuses
- (3) A transient decrease of progesterone but fatal effect of the endotoxin on 1 or 2 foetuses, causing another massive release of $\text{PGF}_{2\alpha}$ and termination of gestation 15 to 20 days later (Fig. 8 [18]).

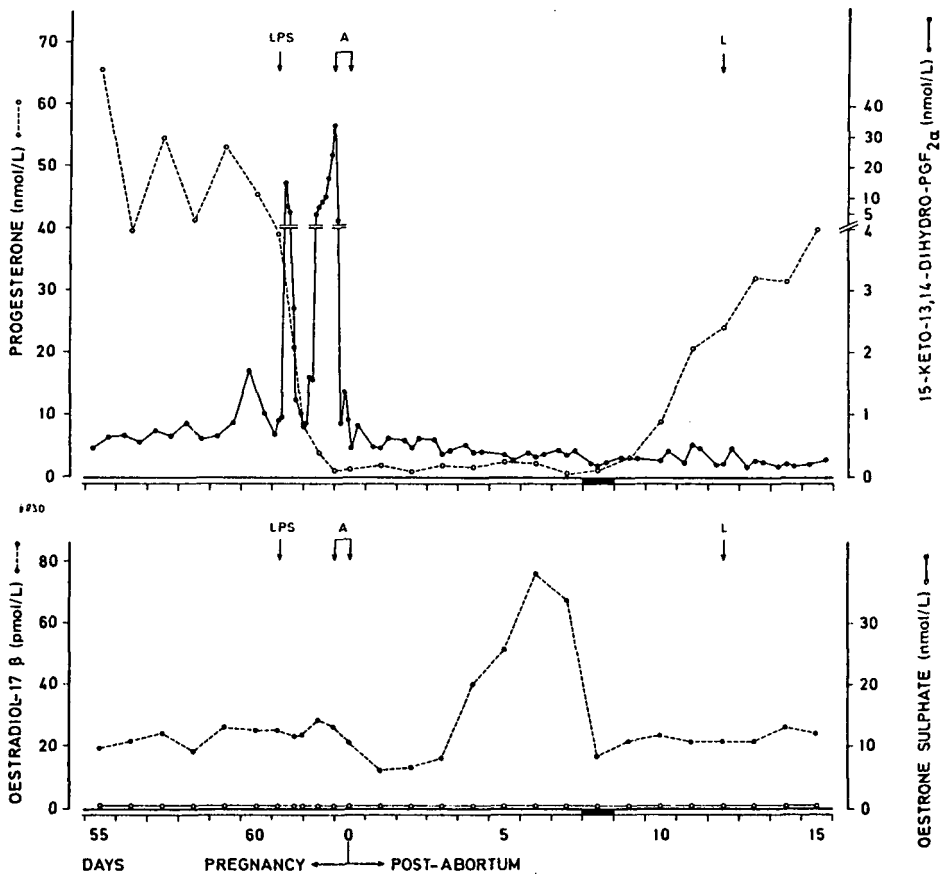


FIG. 7. Plasma levels of progesterone, 15-keto-13,14-dihydro-PGF_{2α}, oestradiol-17β and oestrone sulphate after 2.0 μg/kg body weight of endotoxin (LPS) in a pregnant gilt. Arrows mark injection (LPS), abortion (A) and laparoscopy (L). The horizontal black bar marks oestrus (from Ref. [17]).

Abortions according to item (1) were caused by the endotoxin only when injected at day 60 of pregnancy. The animals injected at day 80 or later of pregnancy behaved according to items (2) or (3) above. Additional studies in different periods of gestation are desirable for a more complete picture of the endotoxin's effect.

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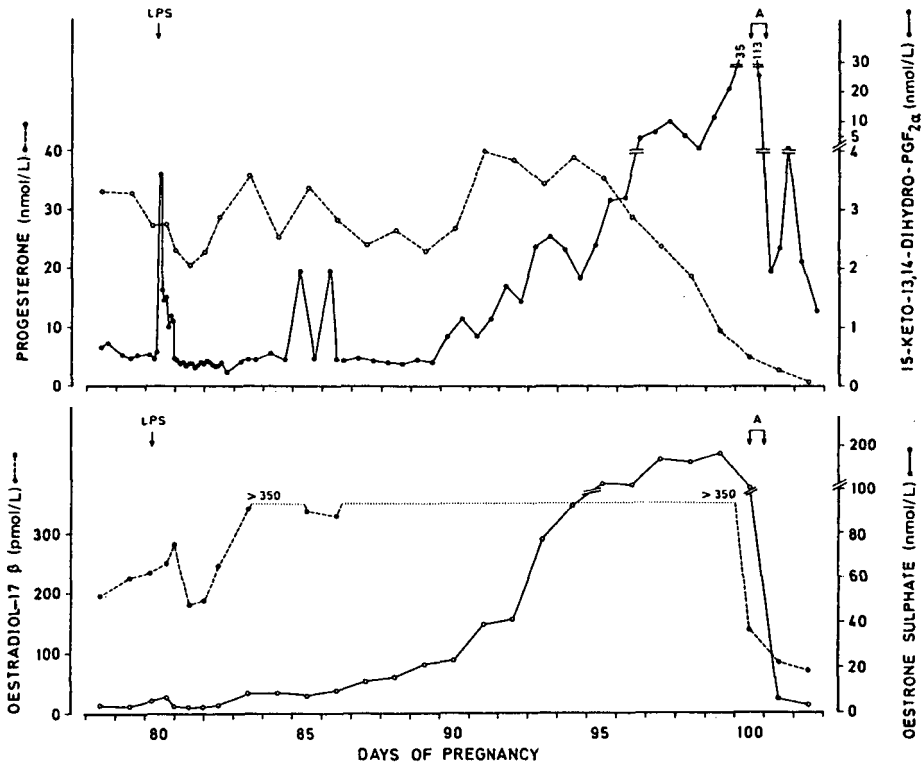


FIG. 8. Plasma levels of progesterone, 15-keto-13,14-dihydro-PGF_{2α}, oestradiol-17β and oestrone sulphate after 2.0 μg/body weight of endotoxin (LPS) in a gilt. Arrows mark injection (LPS) and abortion (A) (from Ref. [18]).

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IMPACT OF ANIMAL DISEASES ON LIVESTOCK PRODUCTIVITY AND ECONOMIC LOSSES

Y. OZAWA

Animal Health Service,
Animal Production and Health Division,
Food and Agriculture Organization
of the United Nations,
Rome

Abstract

IMPACT OF ANIMAL DISEASES ON LIVESTOCK PRODUCTIVITY AND ECONOMIC LOSSES.

The most serious impact of animal disease on livestock productivity in developing countries derives from its effect on overall livestock production and trade development rather than from the direct losses it causes. The global importance of major infectious diseases such as foot and mouth disease, rinderpest and African swine fever is reviewed. The impact of major livestock diseases in tropical Africa on livestock productivity and economic losses is analysed, and the importance of in-depth analysis of the disease impact on livestock and rural development is stressed. Lack of diagnostic facilities that are needed to acquire reliable information on the distribution of disease is often a major constraint to cost-benefit analysis of control options. However, enough evidence exists to substantiate the fact that improved disease control is a prerequisite for progress towards increased productivity based on the adoption of more intensive production systems and use of animals of improved genotype. Veterinary services in developing countries are at various stages of development, and the priority order of infrastructure, manpower and technological development for disease control programmes should be carefully planned and be based on socio-economic, cost-benefit and feasibility studies.

1. INTRODUCTION

Rational decisions on policies for the control of animal diseases require evaluation of the cost of each alternative programme and the benefits which can be expected from its implementation. Application of this economic analysis approach has already provided the information that benefit-cost ratios of 5:1 are not exceptionally high in animal health programmes.

However, assessment of the impact of animal diseases on livestock productivity and economics would be very misleading if it were based solely on our knowledge of the distribution, incidence, morbidity and mortality of diseases, even if this knowledge were complete. It is necessary, therefore, to place the disease impact in perspective at the outset. A fuller understanding of the role of disease in determining production systems is needed in order to improve the approach to control needs and to provide more realistic orientation to livestock development programmes.

1.1. Overall perspective

The most serious impact of animal disease on livestock productivity in developing countries derives from its effect on overall livestock production and trade development rather than from the direct losses it causes. In West Africa progress in genetic improvement for milk production is thwarted by trypanosomiasis and streptothricosis and in East Africa by East Coast fever (ECF); progress in pig production is precluded by African swine fever (ASF). Actual losses from ECF, trypanosomiasis and ASF are of less economic significance than the fact that livestock raising and animal traction are not possible in large areas because of these diseases.

The fact that overgrazing is the most serious problem confronting livestock production in Africa and elsewhere is increasingly being recognized. Some observers have reached the simple conclusion that animal disease control programmes such as the JP15 rinderpest campaign have constituted a major causal factor. However, one must go deeper into this problem for a fuller understanding of cause and effect. An underlying reason for trying to maximize herd buildup is recognition of the high disease risk and the desire to ensure against this by the 'safety in numbers' strategy. Other fundamental factors such as disappearance of traditional grazing lands under the plough and the universal tendency to overexploit a no-cost resource have to be recognized. However, it must also be understood that traditional attitudes to livestock numbers, vis-à-vis livestock quality, will not change until owners are confident that satisfactory protection against disease is assured.

Another aspect of disease which requires mention is that of definition. If disease is defined as any state of departure from good health, i.e. if consideration of parasite infestations is included as well as the nutritional deficiencies, metabolic disorders and endocrine dysfunctions which impinge seriously on growth and reproduction performance particularly in tropical environments, then we are dealing with a much wider impact than that attributable only to diseases of high mortality caused by pathogenic organisms. Coverage of the impact of some of the major epidemic diseases is restricted in this paper, but recognition is given to the serious effects of conditions such as chronic parasitic diseases, nutritional diseases and various forms of reproductive wastage.

2. SOME DISEASES OF GLOBAL IMPORTANCE

Foot and mouth disease (FMD), rinderpest and ASF are three major infectious diseases of livestock of economic importance. All have trade implications, often causing economic catastrophes in different parts of the world.

2.1. Foot and mouth disease

The economic losses caused by FMD may not be realized in many developing countries until such time as the disease causes serious problems, namely production

losses in dairy herds, the inability to use draught animals during ploughing seasons or the trade suspensions that would result.

A study carried out by Ellis and Putt [1] on FMD control in different production systems in Kenya indicated that the greatest improvement had been shown in dairy production. Overall, the campaign has proved to be highly successful in economic terms, with a benefit-cost ratio of 4.55:1. The study indicates the potential economic benefits resulting from the control of FMD in some areas of the country in spite of the high costs involved. Emphasis on mass vaccination is obviously correct when the disease incidence is high, but when it has been reduced to a low level FMD vaccination campaigns become extremely costly and very difficult to implement. In such cases, more selective, efficient and cost-effective methods of control should be sought.

The benefits accrued from FMD mass vaccination were not clearly recognized in Europe until the 1960s when annual mass vaccination of the entire cattle population was adopted in important exporting countries such as France, Belgium, the Federal Republic of Germany and Switzerland and in major importing countries such as Italy. Changing from a mass vaccination policy to a strategic one, which will eventually lead to a slaughtering policy, requires in-depth studies, including cost-benefit analysis.

In developing countries where FMD is enzootic, long-term control planning is essential, based on the most cost-effective ways and means. A prerequisite to any FMD control programme is a clear knowledge of the distribution of various types of FMD viruses in the area or country and the epidemiology of the disease.

2.2. Rinderpest

Rinderpest is a highly contagious viral disease of ruminants, particularly cattle and buffaloes. In the past it has caused devastating losses in Asia, the Near East and Africa. In newly infected regions mortality rates are usually high (up to 90%), but in enzootic regions they are usually low (as low as 10%).

Direct losses caused by rinderpest before the development of effective vaccines against the disease were extremely high. At the turn of the century, when the veterinary services of developing countries were still in their embryonic stage of development, rinderpest spread freely and resulted in a 90% mortality rate in most areas. In fact, until 1950 rinderpest killed some two million cattle annually in Asia, Africa and some parts of Europe.

In the 1950s effective rinderpest vaccines were developed and applied in many countries, markedly reducing the incidence of the disease. In the 1960s a tissue-culture vaccine was developed and used in Africa during the Joint Project 15 (JP15) campaign. It is inexpensive and extremely effective when properly applied. In fact owing to the effectiveness of this vaccine rinderpest is now considered the easiest infectious disease of cattle to control or eradicate.

The vaccine, however, is heat labile and its potency can easily be lost unless it is kept refrigerated. It is, therefore, important to monitor the quality of the vaccine used, either by testing samples kept in the field or by examining the serological response in vaccinated animals. It is our estimate that more than 50% of the tissue-culture rinderpest vaccine used in Africa during the past five years did not have the minimum potency required when it was actually administered in the field.

Incomplete vaccination campaigns will result in livestock owners being continuously threatened by the periodic recurrence of rinderpest. The disease can be transmitted from one country to another, or even from one continent to another, through the movement of infected animals, including certain wildlife. Therefore, if a rinderpest campaign is to be launched it should aim at eradicating the disease in order to justify the high costs. To achieve this the campaign should be followed by extensive disease surveillance to eliminate all the residual foci of the disease. This means that efficient and reliable diagnostic networks must be established throughout the country, using the most simple methods of diagnosis.

2.3. African swine fever

ASF is a very infectious disease which poses the most serious threat to the pig industry worldwide as it can be transmitted by infected pigs or pork products. Until 1957 the disease was only found in Africa; then it appeared for the first time in Portugal. In 1960 the virus was reintroduced into Portugal and it subsequently spread throughout the country and then into Spain. ASF became enzootic in the Iberian Peninsula and resulted in serious economic losses. Direct and indirect losses caused by the disease in Spain alone were estimated at over US \$2 billion.¹

In 1978, ASF suddenly surfaced in Malta and Sardinia (Italy). In the same year, ASF was diagnosed in Brazil, the Dominican Republic and Haiti. It continued its spread from Haiti and appeared in Cuba in 1980. The estimated economic losses caused by ASF in Brazil, Malta and the Dominican Republic are shown in Table I. More recently, ASF has been detected in Italy (near Torino) and Belgium; thus, the pig industry in Europe is once again being threatened.

Despite years of research work on ASF, no reliable vaccine for the disease has been developed. Also, the signs of infection are similar to those of classical swine fever, thus creating difficulties in differentiating between them. Attention should be paid to the fact that ASF became widespread and resulted in economic catastrophes in Malta, Sardinia, Brazil, the Dominican Republic and Haiti due to delays in diagnosis or to misdiagnosis.

As there is no vaccine or treatment yet available for ASF, it should be stressed that all countries in which the swine industry is of economic importance should make

¹ 1 US billion is a thousand millions.

TABLE I. ECONOMIC LOSSES CAUSED BY INFECTIOUS DISEASES

Country (year of outbreak)	Estimated losses
<i>Foot and mouth disease</i>	
United Kingdom (1967/68)	46.5 million pounds sterling
France (1981)	20.5 million French francs
Denmark (1982)	484 million Danish krone
Netherlands (1983/84)	1.0 million US \$
Greece (1984)	1.0 million US \$
<i>African swine fever</i>	
Spain (1960-80)	2 billion US \$
Malta (1978-82)	29.5 million US \$
Dominican Republic (1978-82)	14.5 million US \$
Brazil (1978-84)	226.8 million US \$
<i>Rinderpest</i>	
Nigeria (1980-84)	404 million US \$ (animal losses only)
<i>Newcastle disease (VVND)</i>	
United States of America (1974-84)	16.8 million US \$ (direct costs)
<i>Avian influenza</i>	
United States of America (1984)	63.2 million US \$

every effort to prevent the entry of the ASF virus or ASF-infected materials. It should also be stressed that they should be able to make differential diagnosis of ASF without depending on the services of diagnostic laboratories abroad. For this reason, a standard ELISA kit was developed for ASF diagnosis by the Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Instituto Nacional de Investigaciones Agrarias (INIA), Madrid, Spain, with the aim of enabling national veterinary laboratories to diagnose ASF without having to import infectious antigens.

2.4. Other infectious diseases

There are several other infectious diseases which may cause serious direct and indirect economic losses if they are introduced into countries in which they do not normally exist. Examples of these diseases and the resulting economic losses are shown in Table I.

3. TROPICAL AFRICA

Although the African continent has enormous potential for livestock development, programmes to increase milk and meat production through more intensive systems have had a very limited impact. There are several reasons for this, of which socio-economic factors in pastoral areas and the lack of trained personnel are probably the most important. However, another major problem is animal disease.

The control of three groups of diseases utilize a major part of the resources of the African veterinary services, namely major infectious diseases, tsetse flies and trypanosomiasis, and ticks and tick-borne diseases. These groups are of major importance in that successful control is considered to be an essential prerequisite for the development and intensification of livestock production.

3.1. Major infectious diseases

Rinderpest, contagious bovine pleuropneumonia and FMD have been and still are the three major infectious diseases of cattle in Africa. Although the incidence of these diseases has declined gradually in many countries in Africa as veterinary services have developed, they still cause catastrophic losses in countries where difficulties in maintaining self-reliance in veterinary services are prevalent.

Between 1977 and 1981 the yearly average mortality rate of the cattle population in Africa was estimated at approximately 10% because of these infectious diseases. In addition to these direct losses, other indirect losses such as movement restrictions, prohibition of livestock sales and trade, and various forms of production losses could result.

3.1.1. *Rinderpest*

Rinderpest, which once wiped out almost the entire bovine population when it first entered Africa in the late 19th century, has been and still is the most devastating infectious disease of cattle and buffaloes in Africa. To combat this disease the comprehensive JP15 campaign was organized in 1962. The financial support provided by various donors between 1963 and 1973 was estimated at over US \$20 million.

The effectiveness of the vaccination campaign was clearly demonstrated in 22 countries after the vaccination of over 70 million cattle. With the exception of a few

countries the disease was eradicated, for example, in 1972 rinderpest was eradicated in Nigeria. A cost-benefit analysis of the campaign in that country carried out by Felton and Ellis [2] concluded that the campaign led to a net benefit of 758 127 pounds sterling, a benefit-cost ratio of 2.48:1. These authors further concluded that in Nigeria the JP15 campaign had not contributed to overstocking in the drought period but had resulted in an improved herd structure.

However, the serious weaknesses in the campaign were inadequate follow-up of residual foci of the disease and deficient vaccination of the annual increase of young stock. As a result, sporadic outbreaks of rinderpest occurred in Mali, Mauritania and Senegal in 1978 and 1979. In 1980 the disease spread to Burkina Faso, Niger, Benin and Nigeria. This situation again called for international action, and an emergency campaign was conducted in 1981 with the financial support of the FAO and the European Economic Community (EEC). In 1982, rinderpest in Sudan began to spread to its neighbouring countries, Chad and Egypt, and in 1983 the disease caused serious losses in Chad, the Central African Republic, Cameroon and Nigeria. To cope with the situation the FAO, EEC and other donors continued emergency campaigns in infected countries, and this has led to an improvement in the situation since the peak incidence in 1983.

It is difficult to estimate the overall economic losses caused by rinderpest outbreaks in countries in West and Central Africa during this period. The most serious losses occurred in Nigeria when the country was invaded by rinderpest from both its western and eastern borders. According to Nawathe and Lamorde [3] the direct losses caused by animal deaths are estimated at US \$404 million.

Meanwhile, various international organizations (FAO, EEC, the Organization of African Unity and the Office international des épizooties) have convened several meetings since November 1981 with a view to organizing yet another long-term international campaign against rinderpest in Africa.

Previous experience in Africa, however, posed a very fundamental question on the part of many donors. Is it worth while repeating a rinderpest vaccination campaign in Africa? The simple answer is 'No', unless the strategies are changed and the veterinary services of each country can be strengthened so as to enable them to prevent reinvasion on their own.

In 1983, when rinderpest was widespread in Africa, the FAO carried out a survey on the diagnostic capabilities of African countries. It showed that only a few laboratories were capable of diagnosing rinderpest, and that the number of diagnostic specimens submitted by field veterinarians was only about 30 specimens per year in the whole of Africa.

This clearly indicated that laboratory diagnostic services for rinderpest did not exist or function in most countries in Africa at that time. It was also recognized that the techniques should be simplified and diagnostic services should be decentralized to avoid delays in decision making.

Improved diagnostic services are just one of several key factors which are essential for the success of any rinderpest campaign. Other key factors include effi-

cient disease surveillance and reporting systems, improvement of vaccine production and delivery systems, improved knowledge of the epidemiology of rinderpest and, last but not least, improvement in the efficiency of vaccination teams and recording systems.

3.1.2. African swine fever

In 1984 the swine population in Africa was approximately 11 million, which represents only 1.4% of the world's swine population. The reason for this is obvious — there are too many constraints, especially diseases such as ASF, trypanosomiasis, classical swine fever, etc.

Several attempts to establish pig and pork industries in Africa by introducing exotic breeds failed because of inadequate knowledge of these diseases, in particular ASF. Recent outbreaks of ASF in São Tomé and Príncipe (1979), Cameroon (1983), and Rwanda (1983/84) resulted in catastrophic losses, since the producers and the veterinary services of these countries did not have sufficient knowledge of the disease. It is to be stressed that many indigenous breeds of pig in Africa are resistant to many diseases and that in some cases they may act as the reservoirs of diseases. ASF is one such disease.

On the other hand, in some other African countries such as Kenya, Zambia, Côte d'Ivoire and a few other countries where ASF is known to be enzootic in some areas, pig industries have been maintained with low economic losses. The key to success in the swine and pork industries in Africa would be to acquire knowledge on the exact distribution of ASF and trypanosomiasis in areas or countries and on the epidemiology of these diseases. All swine farms should be well protected by double fencing and stockowners and veterinarians should know how to diagnose and deal with the disease in the event of an outbreak because there is no effective vaccine.

3.2. Trypanosomiasis

Trypanosomes are protozoan parasites which cause disease in man and all species of domestic livestock except poultry. Although non-tsetse-transmitted trypanosomes can cause losses in domestic livestock, by far the most important constraints to livestock production are caused by trypanosomes transmitted by tsetse flies, which occur only in Africa.

Estimates of the potential for agricultural development of the tsetse-infested areas vary. Fischer et al. [4] estimated that some 37 countries were affected and that the total land area infested was some 1085 million hectares and contained an estimated 55 million livestock units in 1975. They assessed the potential for livestock in the area as being between 169.4 and 418.9 million units, depending on the assumptions used. These figures illustrate the magnitude of the problem and the potential that exists should satisfactory cost-effective solutions be found to control the disease. It should be added, however, that much of the land infested by tsetse flies has a rela-

tively high cropping potential and it is not envisaged that all cleared areas will be used for livestock production only.

The main constraints encountered by livestock producers can be broadly summarized as follows:

- (1) In high trypanosomiasis risk areas keeping of most domestic ruminant species is precluded, except under close veterinary supervision.
- (2) In medium trypanosomiasis risk areas, land resources may, in the absence of control measures, be used on a limited and seasonal basis at those times of year when tsetse fly populations, and the consequent risk of trypanosomiasis, are diminished.
- (3) In low risk areas, domestic ruminants can be kept permanently, even in the absence of control measures, although mortality and morbidity losses will result.

These constraints mean that the development and intensification of livestock production in tsetse-infested areas become extremely difficult in the absence of adequate control measures. This is particularly so if such intensification involves the implementation of breed improvement programmes, since the animals involved generally have a higher susceptibility to trypanosomiasis.

Constraints on rural development also arise as a result of the absence of livestock from infested areas; this may discourage agricultural settlement. Draught animals are particularly susceptible to trypanosomiasis and may need close veterinary supervision, even in low risk areas.

There are also many constraints for the control of trypanosomiasis and tsetse flies. First, no vaccine is available to protect animals from trypanosomiasis and the phenomenon of antigenic variation exhibited by trypanosomes means that development of a vaccine is unlikely in the foreseeable future. Tsetse control by application of insecticides may involve the selective application of persistent insecticides either by ground spraying, or by helicopter, or repeated applications of non-persistent insecticides of ultra-low volume by fixed wing aircraft. Methods of vector control involving bush clearance and/or the destruction of game carriers have largely been abandoned, either on economic or conservationist grounds.

Apart from technical considerations, the main constraints experienced by veterinary services have been financial and organizational. Tsetse control programmes are expensive and a high degree of organization is involved. Extensive surveying of infested areas has to be carried out before control operations commence. Spraying can only be conducted at certain times of the year and, if interrupted through shortage of essential inputs, may have to be repeated. Spraying operations have to be closely monitored and cleared areas have to be protected against reinvasion and constantly resurveyed.

Research is being conducted into ways of overcoming these problems. Among them is the development of improved tsetse traps and the development of slow release insecticides in the form of animal implants, long acting drugs and pour-on synthetic, pyrethroid-based, non-systematic insecticides which offer hope of ways in which tsetse populations can be reduced at a reasonable cost. The release of sterile male

tsetse flies is another solution to the problem, but the high costs involved in installation of the necessary rearing facilities mean that use of this method will probably remain limited or complementary to other methods.

Chemotherapeutic and chemoprophylactic drugs have been used with success. However, their utilization is dependent on adequate veterinary supervision. Unless this is assured, drug-resistant strains of trypanosomes will appear and the protection afforded will break down. Drugs are often used, however, on a widespread basis, and drug resistance is becoming a common phenomenon. The problem is compounded by the fact that cross-resistance to different types of drugs frequently occurs and also that the range of trypanocidal drugs currently available is limited. Furthermore, drug regimes are most effective in low risk situations and become increasingly costly and more difficult to implement as the trypanosomiasis risk increases. This limits their usage in high risk areas of good development potential. It is obvious that economic and organizational constraints, coupled with the lack of adequate diagnostic facilities to monitor their application and detect drug resistance, have hampered the successful implementation of drug regimes in many instances.

An entirely different approach is the use of trypanotolerant breeds of livestock. However, various constraints also need to be overcome before this approach can be widely adopted. First, the availability of these animals is limited; second, the mechanism of tolerance is still imperfectly understood; third, although the productive potential of these breeds is higher than was originally thought, the situations which would profitably benefit from their introduction need to be more clearly defined.

Recent trends raise hopes for the development of low cost methods of tsetse and trypanosomiasis control, but these methods are still in the developmental stage. However, to be economically successful in the long term eradication must be followed by improved land use on a sustainable basis to avoid overgrazing and land degradation.

3.3. Ticks and tick-borne diseases

The catastrophic losses in livestock in Africa, which were caused by *Theileria parva* East Coast fever (ECF) in the early twentieth century, resulted in the introduction of disease control measures based primarily on the control of the vector tick.

The strategy employed in many countries of East, Central and Southern Africa was close interval dipping to prevent transmission of *Theileria parva* and thus control of ECF. Eradication of ticks was impossible in the presence of alternative hosts for ticks, although ECF appears to have been eradicated from large parts of Southern Africa. In those countries where ECF has been eradicated and in many where ECF has not occurred, close interval dipping is practised, although it is not always justified.

This strategy has contributed significantly to the economic prosperity of livestock industries in some countries, but eradication of ECF from Southern African states did not reduce the need for strict tick control.

In recent years, improvement in our knowledge of the epidemiology of tick-borne diseases and of tick ecology and population dynamics as well as an increased incidence of acaricide resistance have resulted in a reassessment of the continued validity of this strategy. The costs of close interval dipping as practised in East Africa and elsewhere (up to twice per week) impose an enormous burden on state and producer alike, and this frequency of tick control is becoming less justifiable on economic grounds. The scientific and economic realities of our time have already forced changes in tick and tick-borne disease control policies. These changes reduce dependence on the chemical control of ticks and increase exploitation of the natural resistance of cattle to ticks and the enzootic stability to disease in some countries; in others these changes will continue to be modified.

The main elements of emerging policies related to tick control are: (1) control of ticks only when economically justified by increased productivity; (2) strategic dipping programmes based on knowledge of the population dynamics of the major tick species, thus reducing the frequency, and cost, of acaricide application and the risk of development of acaricide resistance; (3) a fuller exploitation of the ability of indigenous cattle to develop resistance to ticks; and (4) the development of new methods for the application of acaricides which reduce the labour costs involved (boluses, ear tags, pour-ons, etc.).

Emerging new policies related to the control of tick-borne diseases are: (a) recognition and exploitation of enzootic stability to tick-borne diseases in endemic disease situations; (b) immunization of susceptible cattle against babesiosis, anaplasmosis and heart water in marginal tick areas or when introducing susceptible cattle to tick-infested areas; and (c) using ECF (*T. parva*), as until the methods of immunization currently being introduced are fully tested, tick control will have to be applied together with immunization; this applies particularly to areas in which exotic breeds are being used.

Application of these principles has already started in *Boophilus* spp. areas of other continents and strategies are being refined as technical knowledge and experience increase. In the ECF areas of Africa, where not only *Rhipicephalus appendiculatus* (the vector of ECF) but also a large number of other ticks parasitize cattle and transmit diseases, this process is only just starting. Thus, in Burundi a pilot strategic tick control programme will be started during 1986, while similar strategies are being tested in Zambia. The programme in Burundi, if successful, will reduce the frequency of dipping to one-third or one-quarter of the current policy and thus save 75% of the costs of acaricides; it is expected to achieve better control of tick populations.

In the lowveld areas of Zimbabwe relaxation of tick control has been introduced which will save Z \$4 million in the first year and will result in an annual saving in labour costs of up to US \$30 per head. The latter saving will allow use of labour in more productive activities.

Research into the control of ticks and tick-borne diseases has concentrated largely on attempts to find more cost-effective methods and on development of more

efficient vaccines and drugs. In the future there is some hope that bio-engineered vaccines for ECF, babesiosis, anaplasmosis and ticks may become available. Application of the currently available methods of vaccination against babesiosis, anaplasmosis and heart water, coupled with the development of new strains of cattle which are relatively resistant to tick infestation, will help exotic breeds of cattle to survive in Africa.

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DIAGNOSIS OF VIRAL DISEASES USING ELISA TECHNIQUES

Current status and future prospects

M.H. JEGGO

Animal Virus Research Institute,
Pirbright, Surrey, United Kingdom

Abstract

DIAGNOSIS OF VIRAL DISEASES USING ELISA TECHNIQUES: CURRENT STATUS AND FUTURE PROSPECTS.

Use of enzyme-linked immunosorbent assays (ELISAs) for the detection of virus or anti-viral antibody has now become commonplace in diagnostic laboratories. These assays offer considerable advantages to the virologist over previously used tests because of their simplicity, lack of tissue culture requirements and ease of standardization. The direct ELISA is now seldom employed and indirect ELISAs using sandwich and competition procedures enable the versatility of ELISA to be fully exploited. Improvements in test procedures and reagents continue to increase the sensitivity and speed of the assays and utilization of monoclonal antibodies has resulted in greatly improved specificity with their use in competition assays, reducing the need for antigen purity. The versatility of new enzyme systems, with their increased sensitivity, improved conjugation and more distinctive colour changes, further increases the flexibility of this test system. In assay development and standardization use of sophisticated laboratory equipment is essential. The automatic ELISA microplate reader and high speed centrifuge are necessary tools for the correct establishment of a diagnostic ELISA. Once this step is completed standardized reagents are required in only small amounts and the test can be easily adapted for use in simply equipped laboratories using by-eye reading. This system of virus diagnosis has immense potential in the developing world. The cost and supply of reagents, difficulties of maintaining tissue culture and the complexity of equipment for other techniques for virus diagnosis are problems that have resulted in limited achievements in this area. ELISA offers a solution to most of these problems and perhaps its greatest prospect lies in the development of assays specifically designed for use in developing countries.

1. INTRODUCTION

After the introduction of enzyme-linked immunosorbent assays (ELISAs) in 1972 [1] the mass of published work employing this technique indicated clearly its many advantages to the diagnostician, epidemiologist and research worker in the biological field.

In the area of diagnostic virology, both for antigen detection and antibody determination, its use is widespread, although the reasons for its preferential selection are many and varied (Table I). In animal disease diagnosis laboratory confirmation using ELISA is now available for nearly all virus infections and in the majority of cases

TABLE I. ADVANTAGES OF ELISA OVER OTHER ANTIGEN/ANTIBODY DETECTION SYSTEMS

<i>Sensitivity</i> — Concentration as low as 1 to 10 ng/mL detected
<i>Speed</i> — Results in 1 to 24 h
<i>Scale</i> — Many hundreds of samples can be readily processed; ideal for national campaigns
<i>Specificity</i> — Can easily differentiate subtypes or immunoglobulin subclasses
<i>Easily quantifiable</i>
<i>Easily standardized</i>
<i>Low cost</i> — Efficient use and long shelf-life of reagents
<i>Relatively simple</i>
<i>Does not need live antigen</i> — Important in exotic virus detection systems

ELISA compares favourably with alternative procedures. As an example, comparative work carried out on methods employed in the diagnosis of African swine fever [2], both for antigen (Table II) and specific antibody (Table III) detection, illustrates its choice as the most suitable test.

In the mid-1970s the high sensitivity and relative novelty of ELISA were considered a handicap to the diagnostician and the test was mainly used in the area of research. However, in the last ten years, with ever increasing use of the test and the advent of new technologies (e.g. monoclonal antibodies), ELISA is now able to offer the diagnostician rapid and simple tests to give information hitherto unobtainable using other methods. Thus, we can now obtain details of viral subtypes or antibody subclasses using well characterized reagents and on a large scale. ELISA now forms the basis of many national eradication and control programmes (e.g. Aujeszky's disease and Visna-Maedi in the United Kingdom) and with the development of internationally accepted kits it is central to several multinational research projects [2].

Early ELISAs relied on direct conjugation of specific antigen or antibody but requirements for improved sensitivity, simplicity and cost reduction have favoured the introduction of indirect systems [3], although which system is chosen will vary depending on the diagnostician's requirements.

2. INDIRECT ELISA

In a simplified form the main stages in indirect ELISA are as follows: adsorption of antigen to the solid phase; wash; addition of antibody — incubation; wash; addition of enzyme-labelled antiglobulin — incubation; wash; addition of enzyme substrate; determination and expression of results.

TABLE II. DIAGNOSTIC TESTS FOR DETECTING AFRICAN SWINE FEVER ANTIGENS

Property	Enzyme-immunoassay	Haemadsorption	Direct fluorescence	Radio-immunoassay	Complement fixation	Immuno-peroxidase	Electron microscopy
Sensitivity	High	High	Moderate	High	Low	Moderate	Low
Specificity	High	High	High	High	Low	High	Low
Reproducibility	High	High	Low	High	Low	Moderate	Low
Ease	Easy	Easy	Moderate to easy	Hard	Hard	Moderate to easy	Hard
Cost	Low	Moderate	Low	High	Low	Low	High
Use in field	Yes	No	No	No	Yes	No	No
Stability of reagents	Good	Poor	Good	Poor	Poor	Good	—
Training required	Low	Moderate	Low	High	Moderate	Moderate	High
Interpretation of results	Easy	Hard	Hard	Easy	Easy	Easy	Hard
Automation	Yes	No	No	Yes	Yes	No	No
Safety	Some hazard	Safe	Safe	Hazard	Safe	Safe	Safe

TABLE III. DIAGNOSTIC TESTS FOR DETECTING ANTIBODIES AGAINST AFRICAN SWINE FEVER

Property	Enzyme-immunoassay	Immune electro-osmophoresis	Indirect immunofluorescence	Radio-immunoassay	Complement fixation
Sensitivity	High	Low	High	High	Low
Specificity	High	Low	High	High	Low
Reproducibility	High	High	High	High	High
Ease	Easy	Easy	Easy	Hard	Hard
Cost	Low	Low	Low	High	Low
Use in field	Yes	Yes	No	No	Yes
Stability of reagents	Good	Good	Good	Poor	Good
Training required	Low	Moderate	Moderate	High	Moderate
Interpretation of results	Easy	Moderate	Easy	Easy	Moderate
Automation	Yes	Moderate	No	Yes	Yes
Safety	Some hazard	Safe	Safe	Some hazard	Safe

The indirect ELISA comes in several forms, including sandwich and competition assays; details of these have been described previously [3]. In essence though, what makes ELISA such an excellent system is: (a) the ability of the antigen or antibody to bind to a solid matrix, and (b) use of an enzyme label to detect antibody-antigen binding. It is important, therefore, to consider developments in these two areas in evaluating the current status of ELISA in disease diagnosis.

2.1. Solid-phase assay development

Solid-phase immunoassay has progressed rapidly owing to the easy separation of the bound and free label [4]. Most of the solid matrices are based on the simple adsorption of antigens or antibodies to plastic surfaces. In this, the 96-well microtitre plate has been paramount in popularizing ELISA. It is relatively cheap, allows the testing of large numbers of samples, requires only small amounts of reagents per well and allows a degree of mechanization to occur. A recent study of the stability and binding characteristics of antibody to a polystyrene surface [5] has, however, highlighted the pitfalls of these systems and the need to identify optimum protein concentrations of antibody to avoid antibody-to-antibody linking following saturation of the polystyrene surface. It is clear that each system must be evaluated separately to determine suitable storage times and conditions to obtain minimum desorption.

Even so, it is not always possible to overcome non-specific adsorption of immunoreagents, or the desorption of antibody-antigen from the solid phase. To reduce these problems the covalent bonding of antigens or antibodies has been studied in relation to the preparation of solid immunosorbents [6, 7]. Recently, a system was described [8] employing amino-Dylark balls cross-linked via glutaraldehyde to specific immunoglobulin which appears to avoid problems of desorption. Although not a practical solution for the routinely used diagnostic ELISA this system may indicate the way forward.

Problems of conformational changes of antigen following binding to PVC plates or when using trapping monoclonal antibody have resulted in the development of an amplified liquid-phase ELISA [9] in which the first encounter of antigen and specific antibody occurs in the liquid phase (Fig. 1). The complex is then transferred to a solid-phase detection system. This procedure allows optimal antigen-antibody binding and thus recognition of viral epitopes to be most confidently predicted.

2.2. Enzyme conjugates

Two conjugates, horseradish peroxidase and alkaline phosphatase, are widely employed; each has its own distinct advantages (Table IV). Recently, however, a number of new detection systems have emerged and although not yet in widespread use they offer some advantages over the existing systems. For example, beta-lactamase (penicillinase E.C.3.5.2.6.) has a number of characteristics that make it a desirable choice as a label [10]. It has a relatively low molecular weight (24 000),

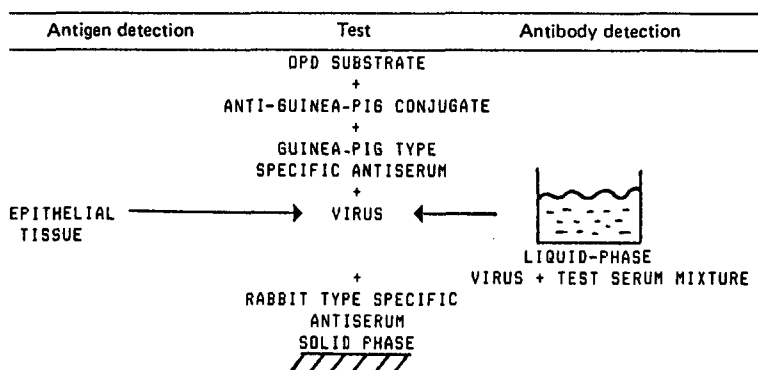


FIG. 1. Foot and mouth disease: ELISA tests.

TABLE IV. COMPARISON OF ALKALINE PHOSPHATASE AND HORSERADISH PEROXIDASE AS ENZYME LABELS

	Advantages	Disadvantages
<i>Alkaline phosphatase</i>	Stable Simply and conventionally linked to proteins by glutaraldehyde bridge Exhibits linear reaction kinetics	Expensive Poor conjugation to Protein A
<i>Horseradish peroxidase</i>	Cheap Can be conjugated by many methods due to steric conformation Wide range of chromogenic substrates available Simply linked to Protein A by periodate oxidation	Non-linear kinetics Poorly linked by one-step glutaraldehyde procedures to proteins

a high turnover rate, is readily available in a highly purified form and is totally absent from mammalian tissues. The activity of this enzyme can be detected by a number of procedures, including an iodometric method, using chromogenic substrates, measurement of fluorescent end-products, measurement of penicilloic acid-iodine complexes or measurement of pH changes [10, 11].

2.3. Methods of amplification

Although the need for high sensitivity in disease diagnostics may not be of paramount importance, amplification of ELISA can enhance both the sensitivity and speed of this test; of the several systems described [12], the avidin-biotin system is most widely used. This relies on the extraordinarily high binding affinity of avidin (obtained from egg white) for biotin (a vitamin) and the ability of four molecules of biotin to bind one molecule of avidin. The outcome is amplification without loss of sensitivity. In some diagnostic tests this degree of amplification is essential if the test is to meet statutory requirements [13]. Several recently developed alternative assays, including chemluminescence, bioluminescence and fluorescence measurement [14], do offer increased sensitivity (of 10- to 100-fold) over the standard ELISA [15].

3. USE OF MONOCLONAL ANTIBODIES

In considering recent developments perhaps the most valuable has been the availability of monoclonal antibodies (Mabs) against specific viral antigens for use in ELISA. These antibodies can be used as highly specific reagents in competition ELISA, both for antigen and antibody detection at the group, type and subtype specific levels.

In all aspects of virology, from studies on virus functions [16] to large-scale epidemiological surveys [17], use of Mabs with ELISA has enabled major advances to take place. In three virus infections in particular, namely influenza [18], polio [19] and rabies [20], the progress forwards using this system has been startling. In foot and mouth disease a similar approach is proving to be equally successful [21].

Monoclonals have been successfully used not only in the test itself but also in reagent preparation. The ability to simply and rapidly purify biological reagents used in ELISA by employing such columns as AFFI-GEL 10 and AFFI-GEL 15¹ covalently linked to specific Mabs has removed problems of extraneous cross-reactions and improved sensitivity through greater separation of positive and negative reactions.

Although Mabs production is a complex and time-consuming task, once available they offer a specific, totally standard and relatively cheap reagent and their use in ELISA can only become more widespread.

¹ BioRad Laboratories, Richmond, California, USA.

4. COMPUTERIZATION AND AUTOMATION

Computerization and automation of ELISA has been of major importance. It has greatly improved the speed of test development and the processing of results and it encourages use of this test in national disease control campaigns.

The microcomputer, combined with a multichannel spectrophotometer, has enabled large amounts of data to be analysed rapidly, with a range of statistical tests being applied [22]. The results can then be simply displayed in any one of a number of ways for evaluation. Indeed, a number of ELISA equipment manufacturers now offer a range of computer hard- and software specifically designed for use with their equipment.

Although complete automation of ELISA is not yet available, the 96-well microtitre plate, multichannel pipette, automatic plate washer and multiscan reader have all enabled the processing of large numbers of samples to be carried out quickly and cheaply. This has been a major factor not only in the widespread use of ELISA, particularly in large-scale epidemiological studies, but also for other specialized tasks such as the evaluation of large numbers of hybridomas.

5. SPECIFIC DISEASES

To illustrate in more detail how these developments in ELISA are being used and to indicate some of the future prospects for the test, its use in a number of specific exotic virus infections is described.

5.1. Foot and mouth disease (FMD)

In FMD diagnosis speed is often of the essence and although the previously used complement fixation test (CFT) met this requirement it had many limitations. ELISA is now used for both antigen and antibody detection, although the tests differ significantly.

5.1.1. *Antigen detection*

ELISA used for antigen detection (Fig. 1) is based on the method described by Hamblin et al. [23], but now uses rabbit anti-FMD virus antisera bound to the solid-phase plates. Epithelium samples have been examined using both ELISA and CFT [24] and in no case did routine CFT type samples which were not typed by ELISA (Table V). The advantages of ELISA over traditional CFT are as follows:

- (1) Specifically types samples containing live or inactivated virus and/or 12S viral subunits
- (2) Pro- and anti-complementary effects are irrelevant

TABLE V. COMPARATIVE FOOT AND MOUTH DISEASE VIRUS TYPING BY COMPLEMENT FIXATION AND ELISA

	Direct CF (%)		Direct ELISA (%)	
Positive typing	16	23	52	75
Anti-complementary	12	17		
Negative or inconclusive	41	60	17	25
<i>Total</i>	69	100	69	100

- (3) Greater sensitivity (20 to 100 times)
- (4) Heterologous and non-specific reactions are of low order, removing large numbers of doubtful reactors
- (5) Only small quantities of reagents are required, thus standardized reagents may easily be established for all typing laboratories.

5.1.2. Antibody detection

In antibody detection the virus neutralization (VN) test has been the accepted test for many years. However, although theoretically sensitive, specific and easy to perform, it requires cells for virus growth and these can vary in sensitivity or fail to grow. Further, the assay requires 3 days before reading. A rapid, amplified, liquid-phase ELISA is now used for quantification of anti-FMD virus antibodies and for screening of sera from animals before import or export [25].

The assay is similar to the typing ELISA, but test sera are incubated in the presence of a constant dose of clarified tissue culture virus in a carrier plate (Fig. 1). Unreacted antigenic sites on the virus in the serum-virus mixture are then detected by ELISA. Comparison of VN and ELISA results gives good correlation (> 0.9), but the advantages of ELISA are as follows:

- (1) Tissue culture cells are not required and the assay is thus unaffected by the biological variables inherent in the use of live cells
- (2) Assay is complete in 24 hours
- (3) Each test has an in-built control from which antibody titres are calculated, so removing the need for test repeats
- (4) Since ELISA will function with inactivated viruses it can be used in laboratories normally not authorized to use live FMD virus

- (5) Since good correlation is obtained between the VN test and ELISA, workers using either test can compare results.

5.1.3. Epitope detection

Using both monoclonal and polyclonal sera against FMD virus in a liquid-phase ELISA, possible neutralizing epitopes on the virus have been identified [9]. Although much work still needs to be done, progress in this direction, combined with cloning and sequencing of the virus, is most likely to result in an effective synthetic polypeptide vaccine against FMD.

5.2. Rinderpest

The recent re-emergence of rinderpest in Africa and the initiation of the Pan-Africa Rinderpest Campaign (PARC) has highlighted the need for a rapid screening test for antibodies to rinderpest, both for disease monitoring and vaccination status evaluation. Virus neutralization is time consuming, expensive and difficult to perform in many developing countries. Indirect ELISA [26] offers a practical solution to the monitoring of the vaccination programme envisaged through PARC. In Tanzania problems of high background activity have already been found to be negligible in practice and the test is performing well under field conditions. Of particular interest is the ELISA/neutralization test for detection of the antibody or virus [26]. As rinderpest virus infection of cell culture does not result in detachment of infected cells, the test cannot be read by viral staining of the remaining viable cells and must therefore be read microscopically. This is time consuming and requires a skilled worker to discriminate between the virus-specific cytopathic effect and non-specific cytotoxicity. Use of ELISA to detect viral antigen at this stage removes the need for microscopic examination. The advantages of this test over the standard indirect ELISA are that it only detects neutralizing antibody and that it could be used for examining exotic animal sera where non-specific levels in negative sera are not known or where anti-species sera are not available.

5.3. Bluetongue

In bluetongue (BT) detection of group-specific precipitating antibody by the agar gel test has been the accepted import-export test. Although this test has problems of cross-reactions with related orbiviruses, it has been difficult to improve due to problems associated with virus purification. Production of a monoclonal antibody directed against the group-specific protein (P7) and its use in a blocking ELISA [27] has created a test of high sensitivity and specificity which can readily be standardized and thus is an ideal alternative to the agar gel test. Further, this test is quantitative, unlike the agar gel test, and thus can provide vital antibody development data in epidemiological sentinel herd studies [27].

TABLE VI. ADVANTAGES OF USING ELISA IN DEVELOPING COUNTRIES

<i>Simple</i> — Simple step-by-step procedures required in execution of the test
<i>Inexpensive</i> — High dilution of reagents and microplate usage drastically reduce the need for hard currency purchase
<i>Non-sterile</i> — Problems related to laboratory and reagent sterility avoided
<i>Easily standardized</i> — Enables comparisons and therefore checks to be made between laboratories
<i>Low equipment needs</i> — Little expensive equipment required; good electricity supply not essential; for many tests by-eye reading will suffice
<i>Exportability</i> — Tests can be developed in high technological laboratories and then sent with reagents in small highly concentrated amounts to less advanced laboratories

5.4. African swine fever

The last disease to be considered is that of African swine fever (ASF). As indicated earlier, ELISA is the test of choice both for antibody and antigen detection. However, the success of ELISA in this disease lies in its standardization. Using international funds, a standard test was developed and distributed to laboratories working on this disease. The results obtained from its use were then correlated to finally produce an international test kit, fully accepted by all, with results which could be relied on as being comparable [2].

6. FUTURE PROSPECTS

A number of future developments in the use of ELISA have already been eluded to and this section considers its potential for use in the developing world.

The needs of diagnostic and research laboratories in developing countries are in many cases specifically met by ELISA (Table VI). This test system offers a method of introducing into these laboratories a level of accuracy, reproducibility and standardization not previously possible and at a cost that is realistic. ELISA is uniquely placed to enable rapid progress to be made, not only in the area of virus diagnosis but also in all aspects of disease investigation and control.

Initial test development may require a high level of expertise, expensive equipment and not easily obtainable reagents. However, once standardized, these tests can easily be exported to less advanced countries.

Each country needs to develop a central pool of experts proficient in the use of ELISA who can give advice on the development of specific tests. These experts will require training and need to have access to the reagents required. Once established, however, they can then act as a focus for the development of ELISA in their countries.

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RECENT DEVELOPMENTS IN THE DIAGNOSIS OF INFECTIOUS AND PARASITIC DISEASES OF FARM ANIMALS

R.H. JACOBSON

New York State College of Veterinary Medicine,
Cornell University,
Ithaca, New York,
United States of America

Abstract

RECENT DEVELOPMENTS IN THE DIAGNOSIS OF INFECTIOUS AND PARASITIC DISEASES OF FARM ANIMALS.

Considerable scientific effort devoted to enzyme-based immunoassays during the last decade has provided a large array of diagnostic tests. These have proved extremely helpful in disease control and eradication schemes. In the continuing search for improvement in sensitivity, specificity and practicality of enzyme-linked immunosorbent assays (ELISAs), certain reagent and methodological limitations and concerns have emerged. Antigen purity, epitope density and immobilization of antigens on the solid phase are variables which continue to be potentially troublesome for each new assay that is developed or transferred to a simpler technology. Experience with two ELISAs used daily in the New York State Diagnostic Laboratory at Cornell University over the last three years has revealed practical problems which resulted in very different resolutions. Use of an adequate system of reagent control and data reduction methods proved critical in understanding the variables in each assay. Development of ELISAs thus requires fairly sophisticated and complex instrumentation, often with computer control, in order to preclude future problems with the assay. Such thoroughness yields dividends in future routine use of the assay or in transfer of the assay to more simplified 'kits' for use in the field. New non-isotopic serodiagnostic technology other than ELISA is currently being developed. An example of such a system is a fluorochrome-based quantitative fluorometric immunoassay system which we evaluated using African swine fever (ASF) as a model. The ASF assay showed good sensitivity and specificity, and was simple and conducive to field use via portable instrumentation. Although the instrumentation is equivalent in cost to the less expensive colorimeters, the reaction vessels are more costly than microtitre plates. This exemplifies a significant problem which needs to be overcome if transfer of non-isotopic assays to developing countries is to be cost effective. Because such innovations are not commercially viable ventures, industry will probably be of limited assistance in fulfilling the need. Thus, the responsibility may fall on international agencies to provide support to reference and developmental laboratories (such as the IAEA Laboratory at Seibersdorf) in order to facilitate timely development and transfer of validated simple non-isotopic assays for disease diagnosis to developing nations.

1. INTRODUCTION

Infectious and parasitic diseases continue to be an important cause of high morbidity and mortality in livestock worldwide. Animal production is restrained, particularly in developing countries where environmental conditions often

favour survival of infective organisms and methods of disease diagnosis and control remain limited. A principal difficulty is the lack of diagnostic techniques which are practical for use where lack of finances, manpower and technical resources are limiting factors [1]. Accurate diagnosis and epidemiological studies are essential in determining the prevalence and significance of disease. Control programmes utilizing serological techniques are a principal means through which this can be accomplished.

Until recently, immunoassays were impractical for use in developing countries. Complement fixation, serum neutralization, haemagglutination and a variety of other assays have been technically difficult and expensive, thus limiting their usefulness to well equipped laboratories; such assays have been the main serological tools available to veterinary medicine. During the last decade, however, rapid progress has taken place in the development of serotest technology. The result is unique assay systems which show considerable promise for widespread and practical application in disease diagnosis. Some of these assays are improvements of the more conventional tests, while others rely on new technology. Although transfer of this newer technology to developing countries is not free of problems, the difficulties do not seem insurmountable, as has been the case with existing technology.

2. MODERN IMMUNOASSAYS

The new generation of immunoassays is based on amplified indicator systems which greatly improve sensitivity and provide the advantage of significantly increased sample throughput. Although radioimmunoassay (RIA) has enjoyed unparalleled success as a highly sensitive test system, particularly as used in reproductive biology, its acceptance in routine infectious or parasitic disease diagnosis has been limited. Enzymeimmunoassays (EIAs), however, involve principles similar to those of RIA (summarized in Ref. [2]), but have a greater potential of fulfilling the needs in animal disease diagnosis because of their reduced costs, ease and breadth of application, and simplicity. There is a massive amount of literature available on EIA describing assays, which now rival isotope-based systems for their potential sensitivity, specificity, precision and accuracy [3, 4]. Although commercial availability of these assays is now being realized and some massive screening programmes have been implemented, many of the developments reported have yet to be translated into practical applications from the highly specialized research laboratory or clinical settings [5]. When they do become available, they will primarily be used for diagnosing companion animal diseases, where the return on company investment is greater. However, the current trends in industry to ever improve and simplify the technology have raised expectations that the technology will also become available to farm animal disease detection. It is our purpose in this brief review, therefore, to discuss some of the recent developments that reveal both the strengths and weaknesses of enzyme-linked immunosorbent assays (ELISAs) and to describe some variant approaches to enzyme-based assays which are currently in the formative

stage. Because the strengths of enzyme-based technology are being presented in other papers in these Proceedings [6–8], we will emphasize the potential limitations of and some difficulties with the enzyme-based immunoassays. We will also describe a fluorometric-based assay [2] in a study on African swine fever serodiagnosis.

2.1. ELISA

Since its introduction nearly 15 years ago, ELISA, or some variant of it, has been applied to virtually every infectious agent of consequence in veterinary medicine. Innumerable review articles, books, symposia and scientific papers have been devoted to the subject. The search for improvements in the technology continues, with emphasis placed on sensitivity, specificity and practicality [9]. The components of ELISA which most affect these characteristics are the solid phase upon which the reactants are bound, the nature and quality of the reagents (antibodies, enzyme labels and their substrates), the method of detection (instrumentation), assay set-up parameters and attendant data reduction methods.

2.1.1. *Antigens and monoclonal antibodies in ELISA*

The method of antigen preparation, the chemical composition of the antigens and the manner in which they are immobilized for use in detection of antibody can cause considerable variation in ELISA results [10, 11]. Mixed or crude antigens may present significant sensitivity and specificity problems due to competition between proteins and other macromolecules for adsorption sites on plastic surfaces [10]. Also, if only a small portion of the antigen preparation is specific for the antibody to be detected, steric hindrance from the irrelevant antigenic material reduces assay sensitivity. The epitope density of the antigen bound to the solid phase is also important in order to alleviate false negative results. Yet, if too much antigen is adsorbed to the solid phase, a prozone effect occurs.

Specificity is in part dependent upon the purity of the antigen used; crude heterogeneous antigenic mixtures are obviously at greater risk of introducing cross-reactivity in ELISA than are highly selected and purified homogeneous antigens. Chemical purification of antigens has significantly alleviated specificity problems in ELISA. Even so, contamination with cross-reacting moieties may pose problems. Recombinant DNA technology thus provides an excellent alternative for producing highly characterized and homogeneous antigen preparations which theoretically could be exquisitely specific. Emphasis on vaccine development via this technology will provide antigens which should also be useful in diagnostic tests for detecting specific antibodies. Mapping of antigenic sites is important in this process [12]. This technology will help to improve ELISAs by providing well characterized and highly specific antigens that can detect subpopulations of specific antibodies.

Another approach for reducing background in antibody detection assays, and thus increasing specificity and sensitivity, is to immobilize specific antigens to a

monoclonal antibody which was previously bound as the first layer on the solid phase in an ELISA system. This approach has been used to great advantage for detecting specific antibody to bovine leukaemia virus (BLV) [13]. Following immobilization of monoclonal antibody, a crude BLV antigen (which is relatively easily prepared) was added to the well. The relevant group-specific antigens (gp 51) were then captured by the monoclonal; other non-specific antigens including tissue culture contaminants did not bind and were eliminated in the subsequent washing step. Antigen bound to the monoclonal was presented conformationally, such that binding of specific antibody from the test serum was favoured. The efficiency of the antigen was also improved. Compared with the crude viral preparation, gp 51 antigen presented (via a monoclonal antibody bridge) not only improved specificity but also significantly increased sensitivity [13].

Monoclonal antibodies have also proved to be useful in affinity columns for isolating antigens which lend greater specificity to an ELISA, as exemplified in detection of the antibody to *Trichinella spiralis* in swine [14]. What are the limits of monoclonals? They are generally of lower affinity than polyclonal antibodies and thus may not be efficient in affinity columns; likewise, they may not be efficient as capture molecules. In the BLV instance cited above [13], the most efficient binding of antigen to the immobilized monoclonal required over 24 hours at 4°C. Possibly, if several monoclonals, specific for several epitopes, were used the overall avidity of the group of monoclonals would increase, thus enhancing the antigen capture process.

2.1.2. Solid phase for immobilization of antigens

The solid phase to which antigens are bound is generally polystyrene or polyvinyl. The process is adsorption primarily by electrostatic and hydrophobic forces. Unfortunately, high buffer pH, in which the antigen is usually diluted, does not always enhance antigen adsorption. The best method and diluent composition are usually determined empirically. What is needed is covalent binding of antigen to a transparent solid phase not unlike that which is done using nylon and other opaque/translucent materials. This would preclude the current difficulty of desorption of the antigen, which may result in a prozone effect. Furthermore, antigens which are only reluctantly adsorbed to plastic (and thus heighten variability) could be bound with greater uniformity and probably greater epitope density via covalent linkages.

2.1.3. Configuring samples in microtitre plates

Placement of replicates in microtitre plates has become important with the recognition that 'edge effects' occur periodically, resulting in significant within-plate variation [15, 16]. If one is reducing the ratio method based on one or two controls located in the same area of the microtitre plate, the results could be highly errant. Although this source of variation has been reduced as manufacturers have made the

microtitre plates more uniform, a potential problem still exists, particularly when small differences in results could influence interpretation of the data.

2.1.4. Instrumentation and data reduction

Colorimeters are now being produced which are less expensive than models of several years ago and provide more options for the user. Although still more expensive than manual gamma counters, lower cost, hand-held colorimeters should soon become available. Development of tests with full evaluation of all the test parameters requires a more complex assay system. Because of highly time- and environment-dependent factors in ELISA, a well characterized assay that has reduced systematic and random error factors to a minimum is virtually impossible without using fairly complex and sophisticated instrumentation.

Computerization is extremely useful and, in fact, a virtual necessity if one is to fully and accurately assess all the variables in a typical ELISA test development. Because the concentration/reactivity of reagents used in the ELISA are functionally interdependent, a multifactorial box titration with several replicates of each combination of reagents all tested in the same run is highly desirable. This may require upwards of 500 to 1000 test wells in a complex array of reagent dilutions. With a computerized ELISA which controls important time-dependent steps in the assay and automatically acquires and reduces the data statistically, one can often determine the best combination of reagents in one or two runs, each of which takes one day.

In our experience, sources of error in ELISAs remain undetected because of inadequate controls, insufficient monitoring of the variables and questionable assumptions. For example, within- and between-run variations are often not reported in ELISA papers. Without this knowledge, no certainty exists regarding uniformity of antigen binding, accuracy in pipetting of test serum, conjugate and substrate, and susceptibility of that test system to 'edge effects', all of which significantly affect precision. Although essentially the same difference in absorbance readings may occur between a negative and positive serum control in each of several developmental runs, one cannot assume that all the test samples will behave in the same way, either within or between runs. Several hundred test sera may be required to provide statistical validity to the assay and to determine the number of replicates required for each test sample. Our experience with ELISA development of a feline coronaviral antibody test [17] has taught us these lessons; although we have run the assay as a service function on nearly a daily basis for over three years, we are still obliged to use five replicates of each test sample and five different control samples (negative and four positive samples of increasing titre), each replicated five times for establishing a reproducible standard curve; all these samples are run *both* on a coronaviral preparation (positive antigen) and on a tissue culture control for a total of 10 replicates of each sample. The need for such an extensive panel of controls and replicates stems from variability associated with lack of antigen-binding efficiency/epitope density on the plastic surface and the variability in background levels test sera. Although

day-to-day reproducibility is uniquely difficult for this assay, it is essentially overcome by use of controls as well as instrumentation which attends to each step of the assay. The same kind of problem may occur with other antigens and sera; of greatest concern is that such problems may never be discovered for lack of daily controls. We would have been unaware of the degree of variation over time had we not included the replicates and the five controls for establishing a standard curve for each run.

Finally, the standard curve is essential; a single serum control upon which to normalize data would have been totally inadequate in this assay. For unknown reasons, one of the controls periodically deviates significantly from the standard curve (generated by exponential regression of observed to expected values). This deviation occurs despite: (a) extreme care afforded our aliquoted pools of control sera, (b) reduction of systematic error through automated sample, conjugate and substrate delivery done by instrumentation, and (c) substrate addition and three colorimeter readings all timed and initiated under computer control (kinetics ELISA). Should the assay have been validated on the basis of one or even two controls, the amount of error of the type we have controlled would be unknown. Similarly, for each daily run, one would not know if controls one or two were performing properly. At least three controls are required for statistically approved accuracy in each day's run.

In contrast to the troublesome (and probably worst-case) coronaviral assay, our antibody test for feline toxoplasmosis is very stable. The daily coefficient of variation (CV) for five sample replicates is always very low (about 2 to 5% maximum). Replicates are thus less important in this assay. The serum controls virtually always perform well (the correlation coefficient for five control observed versus expected values is usually greater than $r = 0.990$ and often approaches 1.0), and no negative antigen control is required due in part to the degree of antigen purity and optimal epitope concentration on the plastic. Even so, occasionally we have a serum control value go awry; with four other controls, one can legitimately discard the outlier (Dixon standard gap test) and use the nomograph.

Once the characteristics of an assay are fully understood through rigorous developmental protocols, transfer of the test to a simpler field-adapted system can be carried out with confidence. Having identified the sources of error, one at least has an opportunity to modify the technology to overcome the problem. Thus, fairly sophisticated instrumentation should be placed in any reference laboratory, not only for development of assays but also for highly controlled monitoring of their performance.

2.2. Objective immunofluorescence assay system

A system has recently been described which utilizes a fluorochrome rather than an enzyme as the marker [18]. This simple, portable and relatively stable assay system (Microbiological Associates, Bethesda, USA), which provides quantitative results, exemplifies the kind of technology that is a candidate for use in developing

countries. Basically, the system consists of a fluorimeter, twelve 30 μL capacity wells evenly spaced in a row of a 15 cm ruler-like strip called a 'Track', and a special coating of the wells which, upon rehydration, swells into a three-dimensional colloidal gel-like matrix that serves as the solid phase. The added surface area of the matrix allows binding of about ten times more antigen than polystyrene, thus allowing use of undiluted test serum; whole blood can also be used.

A typical test involves rehydration of the antigen-containing matrix (antigen previously dried onto the matrix), addition of three control sera (negative, low and high positive) in the first three wells, and test sera in the remaining wells.

A study was conducted to determine the feasibility of this system for detecting antibody to African swine fever (ASF). Using VP-73 as the coating antigen and a panel of 130 well characterized swine sera, the Track system was compared with the results of indirect immunofluorescence, immunoelectro-osmophoresis and ELISA. Variability within and between runs was low ($\text{CV } 5.0 \pm 3.0$ and 6.7 ± 2.1 , respectively) [18]. With a sensitivity of 97.8% and a specificity of 98.5%, the ASF serology on the Track system modelled a promising approach for use in developing countries.

2.3. The 'IDEAL' system

Another variant of the enzyme-based assays called the 'immunodiagnostic enzyme-activated luminescence' (IDEAL) system will soon be available (Microbiological Associates). The Track system is utilized with a fluorophore (rather than a fluorochrome), characterized by a large Stokes shift and an efficient yellow-orange fluorescence, is catalysed by horseradish peroxidase. Not enough is yet known of the system's performance characteristics to ensure that the increased sensitivity (Microbiological Associates data) is accompanied by overall assay efficiency. Furthermore, the Track system costs may be prohibitive (the current cost of the instrument for use in developing countries is about equivalent to that of a simple colorimeter and the Tracks cost more per well than microtitre plates). Its attraction is durability, potential for developing reagents that are more stable than ELISA reagents under tropical applications, simplicity and portability (can be operated from a car battery via the cigarette lighter connection).

3. CONCLUSIONS

Throughout this Symposium, we will be hearing of innovations in ELISA technology which speak strongly for its future in the diagnosis of livestock diseases worldwide [6-8]. The technology has improved considerably over the last 10 years. The great amount of research being conducted in the area virtually assures continued adaptation of reagents, new reaction matrices and modified procedures which will bring stability to the system without jeopardizing its major strength, that of inherent sensitivity. With the concurrent development of monoclonal antibodies for capture

ELISA and eventually with the products of genetic engineering, the degree of specificity we do not yet have (but wish we had) in many assays will become commonplace. As the hardware is improved and simplified, costs associated with testing animals for diagnostic, epidemiological and surveillance purposes will most likely recede, while the degree of sophistication required to provide an efficient assay will be maintained.

At present, development of individual assays requires fairly sophisticated instrumentation for full evaluation of all test parameters. Although the IAEA Laboratory at Seibersdorf will be invaluable as a resource station where standardized reagents can be produced and disseminated, of equal importance will be its role in validating assays via fairly sophisticated equipment and then developing and transferring the technology through simple, user-friendly, field-adapted kits. The commercial incentive for marketing the Track and most ELISA systems for food and fibre animal-related serodiagnosis in developing nations is virtually non-existent. Adaptations from commercial serodiagnostic technology available in industrialized countries (companion animal test kits, etc.) and competition among commercial enterprises will no doubt continue to aid, albeit partially and indirectly, the efforts of international agencies such as the IAEA, Food and Agriculture Organization of the United Nations, World Bank and others who are searching for ways of improving the health, productivity and vitality of livestock and human populations in developing countries. In the foreseeable future, however, the responsibility for developing the specific technology applicable to the need will probably have to come from the agencies themselves.

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DETECTION OF ANTIBODIES AGAINST THE ORTHOPOX VIRUS CAMELI IN SERA OF EAST AFRICAN DROMEDARIES USING TWO DIFFERENT ELISAs

E. MUNZ, E. KROPP,
W. PFAHLER, M. REIMANN
Institut für Vergleichende Tropenmedizin
und Parasitologie,
Tierärztliche Fakultät,
Universität München,
Munich, Federal Republic of Germany

Abstract

DETECTION OF ANTIBODIES AGAINST THE ORTHOPOX VIRUS CAMELI IN SERA OF EAST AFRICAN DROMEDARIES USING TWO DIFFERENT ELISAs.

The suitability of using two different enzyme-linked immunosorbent assays (ELISAs) for the detection of immunoglobulin G antibodies against the camel pox virus has been tested by examining 297 sera of dromedaries from Kenya, Somalia and Sudan. The ELISAs were based on an indirect method of antibody detection. One technique used direct conjugation of enzymes to antispecies-antibodies (C-EL), the other a biotin-avidin amplification system (BA-EL). Using the conventional technique (C-EL) on 196 sera of dromedaries from different ranches in Kenya during the period 1981 to 1984 we determined a high prevalence of antibodies, with titres of up to 1:4096. A comparison made for the years 1981 to 1984 showed a slight decrease in antibody titres. Five animals suffering from acute camel pox showed titres between 1:8192 and 1:32768; eight serum samples from Somalia, collected during an outbreak of camel pox, showed titres of 1:2048 to 1:8192. A high prevalence of antibodies was also found in 93 samples from Sudan; 95% of the animals showed titres of between 1:128 and 1:4096. Differences due to sex or age could not be determined. According to these results, camel pox appears to be endemic in the areas investigated. To test the applicability of the new BA-EL method, 60 camel sera were investigated. It was found that the sensitivity and specificity of this technique seem to be superior to those of the standard ELISA. The advantage of BA-EL is that it avoids direct conjugation of enzymes to antispecies-globulins which, in the case of exotic animals, are not commercially available. Both ELISAs can be regarded as suitable for serological screening tests and for rapid serological differentiation of orthopox and parapox virus infections, also in camels.

1. INTRODUCTION

One of the most important infectious diseases of camels is camel pox (CP), caused by the orthopox virus cameli. Kenya, Somalia, Sudan, the Near East and some Asian countries are regarded as the main endemic areas of CP, although no exact data are available [1]. On the other hand, CP caused by a parapox virus that is very difficult to differentiate from the orthopox virus cameli infections has been observed.

Knowledge of the aetiology, prevalence and distribution of these diseases is therefore a prerequisite to control CP or to apply prophylactic measures. We investigated the applicability of a conventional ELISA (C-EL) and a biotin-avidin amplified ELISA (BA-EL) for the detection of antibodies against the orthopox virus cameli in sera of East African dromedaries (Kenya, Somalia, Sudan) and the suitability of these methods for aetiological and epidemiological surveys.

2. MATERIALS AND METHODS

2.1. Antigen

Gross purified camel pox virus antigen derived from orthopox virus cameli type CP-1 was obtained by propagation in Vero cell cultures, freeze-thawing, sonification and subsequent centrifugation [2].

2.2. Sera

We received 13 sera containing virus-neutralizing antibodies as positive controls and 182 test sera from animals on different farms in Kenya. The details are given in Table I. Apart from the Korbessa farm, CP has been observed on all other farms from 1980-1981 until 1984.

TABLE I. ORIGIN, TIME OF BLEEDING AND
NUMBER OF SERA OF DROMEDARIES
FROM KENYA

Origin	Date of bleeding	No. of sera
Ol Maisor	Aug. 1981	20
Ol Maisor	Aug. 1982	20
Ol Maisor	Mar. 1983	20
Ol Maisor	Aug. 1984	20
Ol Maisor	Nov. 1984	5
Kisima	Mar. 1982	20
Kisima	Mar. 1983	20
Galana	Jan. 1981	20
Ngare Ndare	Oct. 1982	20
Korbessa	1982	17

Eight serum samples were obtained from animals being raised in the Makenere district of Somalia. At the time of bleeding two animals suffered from severe CP and six others showed no symptoms. Ninety-three samples were obtained from the Tambul and Rufa'a districts of Sudan. Four blood samples taken from camels which were born and raised in zoos in the Federal Republic of Germany served as negative control sera.

2.3. Antispecies globulin

Anti-camel immunoglobulins derived from rabbits (RIGs) were lyophilized after ammonium sulphate precipitation. They were subsequently dissolved in sterile aq.dest.

2.4. Performance of the C-EL technique

We used a 0.1M sodium carbonate-bicarbonate buffer, pH 9.6, as coating.

The washing and diluent buffer was PBS (pH 7.2) containing 0.05% Tween 20. This buffer was supplemented with 5% foetal calf serum (fcs) for diluting conjugates and antispecies globulins. The ELISAs were carried out in flat-bottomed, 96-well microtitre plates (Immunolon M 129, Dynatech, Plochingen, FRG) which were washed thoroughly between all working steps. CP-antigen was diluted 1:20 for coating (100 μ L/well).

The plates were incubated for 3 hours at 37°C and overnight at 4°C. Serial twofold diluted test sera (100 μ L/well) were added to the wells and incubated for 1 hour at 37°C. Negative, medium and high positive sera served as controls; 100 μ L of RIGs (diluted 10^{-4}) were added and incubated. Then sheep anti-rabbit globulin (conjugated with horseradish peroxidase) was added (100 μ L/well). These plates were incubated at 37°C for 1 hour and finally 100 μ L of freshly prepared 5-amino-2-hydroxybenzol acid (400 mg in 500 mL aq.dest.) were added.

After a reaction time of 60 min at room temperature, the optical density was measured at 450 nm. Those dilutions were regarded as positive which had extinction values twice as high as those of the mean ($\bar{x}$) of the negative sera in their corresponding dilutions 1:32 to 1:128.

For serum dilutions of more than 1:128 we used the same cut-off limit as that used for 1:128 dilutions. In addition, sera were only considered positive if their reactions with antigen-produced optical densities which were at least 1.5-fold higher than those obtained with mock antigen.

2.5. Performance of the BA-EL technique

RIGs were dialysed for 24 h at 4°C against two changes of 0.1M NaHCO₃. After dialysis the protein concentration was adjusted to 1 mg/mL. Biotinyl-N-hydroxysuccinimide ester (E-Y Laboratories, San Mateo, USA) dissolved in

dimethyl formamide (50 mg/mL) with a molar ratio of 1:1 was added to the free amino groups of protein. Calculations were based on the estimated value of 90 free amino groups per immunoglobulin G molecule. After 2 h at room temperature the reaction was stopped by adding 0.5% 2-amino ethanol. The protein solution was dialysed for 24 h at 4°C with two changes of PBS, mixed with equal volumes of glycerol and stored at -20°C until used at a final concentration of 3 µg/mL. Avidin horseradish peroxidase conjugate (Sigma Chemical Company, St. Louis, USA) was used in a 1:2000 dilution of a 1 mg/mL stock solution.

A 0.06M carbonate-bicarbonate buffer (pH 9.6) was used as coating. Camel sera were diluted in PBS (pH 7.4) containing 0.05% Tween 20 and 5% fcs. The same buffer containing 1% normal rabbit serum instead of fcs was used to dilute biotinylated RIG. AvPO was diluted in a 0.05M phosphate buffer (pH 8.0) containing 0.5M NaCl and 1% Tween 20. 0-phenylenediamine acted as the enzyme substrate at a concentration of 0.4 mg per millilitre of 0.1M citrate-phosphate buffer (pH 5.0) activated with 0.01% H₂O₂. Washing procedures were carried out with the buffers used in the corresponding working steps. Coating was performed as described above. The plates were sequentially filled (100 µL/well) and incubated (1 h at 37°C) with diluted sera RIG and AvPO.

Finally, activated enzyme substrate was applied (100 µL/well). The degree of orange colouring was measured after 30 min at 492 nm. Between all working steps the plates were washed, as described above. Sixty different camel sera, which had been already tested with C-EL, were retested at a single dilution level (1:100) with BA-EL. The specific antibody activity of a sample was estimated by the ratio of its mean optical density measured in blocks of four replicate wells and the cut-off limit was represented by the mean optical density value of the negative control plus three standard deviations [3].

3. RESULTS OBTAINED WITH C-EL

3.1. Antibodies against CP in sera of dromedaries from Somalia

All sera had high positive titres. Sera from animals suffering from severe symptoms of CP had titres up to 1:8192.

3.2. Antibodies against CP in sera of dromedaries from Kenya

Table II summarizes the results obtained from 182 sera. The antibody prevalence in animals on the Ol Maisor farm had a decreasing tendency from August 1981 until August 1984. This may indicate that during this period no outbreak of CP had occurred. However, we assume that there was an acute outbreak of CP in November 1984 on this farm as sera from five diseased animals kept in a separate herd had titres of 1:8192 (1×), 1:16384 (3×) and 1:32768 (1×).

TABLE II. ANTIBODIES AGAINST CP IN SERA OF DROMEDARIES FROM KENYA

Origin	Date of bleeding	No. of sera with titres of 1:							
		:32	:64	:128	:256	:512	:1024	:2048	:4096
Ol Maisor	Aug. 1981	8	1	4	4	1	2	0	0
Ol Maisor	Aug. 1982	8	2	5	5	0	0	0	0
Ol Maisor	Mar. 1983	12	4	3	1	0	0	0	0
Ol Maisor	Aug. 1984	5	13	2	0	0	0	0	0
Galana	1981	8	4	4	1	2	1	0	0
Ngare Ndare	1982	7	0	1	2	4	5	0	1
Korbesa	1982	2	0	1	3	6	4	1	0
Kisima	Mar. 1982	2	1	1	5	7	2	1	1
Kisima	Mar. 1983	1	1	5	7	4	2	0	0

TABLE III. DISTRIBUTION OF ANTIBODY TITRES IN SERA OF 93 DROMEDARIES FROM SUDAN

Origin	Total	No. of sera with titres of 1: ^a							
		:32	:64	:128	:256	:512	:1024	:2048	:4096
East of the Blue Nile	60 (100)	2 (3.3)	3 (5)	5 (8.3)	21 (35)	20 (33.3)	5 (8.3)	4 (6.6)	0 (0)
West of the White Nile	33 (100)	0 (0)	0 (0)	1 (3)	9 (27.2)	11 (33.3)	5 (15.1)	4 (12.1)	3 (9.1)

^a Numerals in brackets are percentages.

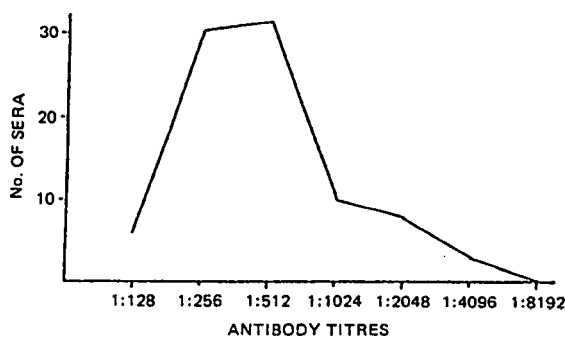


FIG. 1. Distribution of antibody titres of positive sera from Sudan.

We were able to demonstrate a high prevalence of antibodies against the CP virus in dromedaries from Kenya. In 1982, 72% of the samples were positive, 19% doubtful and 9% negative. Differences between male and female animals were not found, but two calf sera had antibody titres of 1:128 and 1:512.

3.3. Antibodies against CP in sera of dromedaries from the Sudan

The results are summarized in Table III and Fig. 1. There were no major differences in the prevalence of antibodies against CP between the two regions where the animals had been bled, but the percentage of positive sera (95 to 97.8%) was surprisingly high.

4. RESULTS OBTAINED WITH BA-EL

The sensitivity of the BA-EL technique was quantified by comparison of the results with the titres found in C-EL using antispecies globulin enzyme conjugates. A calibration curve was prepared which enabled assignment of the matching end-point titres to antibody activities measured at the 1:100 dilution level.

There was good correlation between the values obtained with the two different methods. On average, the titres detected by BA-EL were 16-fold higher than those determined by C-EL. The variation coefficient was 1.81. On the other hand, three of 16 sera in which low titres of 1:32 and 1:64 were detected by C-EL proved to be negative when using BA-EL. For all other sera, however, this technique gave consistently higher values than C-EL.

5. DISCUSSION

Hitherto, neutralization tests have been used for demonstration of antibodies against the orthopox virus cameli, but these tests are not very suitable for large-scale serological surveys. We developed C-EL and BA-EL to demonstrate antibodies in sera of dromedaries against camel pox virus and tested the applicability of these methods for sero-epidemiological surveys. However, these techniques were severely hampered by the difficulties in obtaining commercially manufactured conjugated or non-conjugated anti-camel immunoglobulins. Therefore, we used rabbit-anti-camel globulin produced in our laboratory and commercially available anti-rabbit conjugate in a sandwich technique. Reactions in C-EL were regarded as positive if their extinction values were significantly higher than those of the mean of the negative control sera. Tests with mock antigen served as further controls for the specificity of C-EL.

Procurement of truly negative sera caused some problems. Sera of dromedaries from countries where CP is endemic sometimes produced low titres in the C-ELs, although they had been found to be negative in the serum neutralization tests. There-

fore, we preferred to use sera of dromedaries which had been born and raised in zoos in the Federal Republic of Germany as negative controls, and regarded all those sera which exceeded a cut-off limit of dilutions of up to 1:64 as doubtful positive.

A high prevalence of antibodies against CP was demonstrated by both methods in sera of dromedaries from Somalia, Kenya and Sudan. All sera (eight) from Somalia and up to 95% of 93 sera from Sudan were found by C-EL to be positive, mostly with titres between 1:128 and 1:4096.

In Kenya the percentage of positive sera between different farms varied considerably. On one specific farm (Ol Maisor) a definite decrease of antibody titres was detected between 1981 and 1984. It may be concluded that during this time no outbreak of CP had occurred. Two calf sera from the Kisima farm proved to be positive. It is not known if these animals had had CP or if these antibodies indicated passive immunity.

Using serum neutralizing tests a high prevalence of antibodies in sera of dromedaries from northern Kenya has been reported [4]; Davies therefore believes that CP is endemic in Kenya. Our results support this, although it is not possible to compare our results directly with those of Davies, as we do not know when his samples were taken. Based on our findings it may be assumed that CP is also endemic in Somalia and Sudan. In comparison with C-EL the increased amplification of BA-EL makes detection of highly diluted antibodies feasible and therefore results in a higher sensitivity and an improved specificity due to the minimized background reactions of low concentrated antibodies. An additional advantageous side-effect is the lower consumption of reagents. The reproducible and simple technique of labelling proteins with biotin proved to be particularly useful in testing the sera of exotic animals because species-specific conjugated proteins are not commercially available. Furthermore, cross-linking of proteins does not occur and a surplus of unreacted biotin is easily removed by dialysis.

We believe that both the ELISA techniques described are very useful methods for performing sero-epidemiological surveys for the detection of antibodies against the orthopox virus cameli. These tests can also be applied to differentiate serologically between infections of orthopox and parapox viruses. It has been found that parapox virus infections in dromedaries are sometimes clinically very similar to orthopox virus infections [1].

As the application of electron microscopic investigations to differentiate between the two diseases based on the different morphology of orthopox and parapox viruses is not possible everywhere, it is believed that a relatively quick differentiation can also be achieved using the ELISA techniques.

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USO DE LA TECNOLOGIA DE RECOMBINACION DEL ADN EN EL DIAGNOSTICO DE ENFERMEDADES INFECCIOSAS VIRALES

J. MORENO-LOPEZ

Section of Virology,
Department of Veterinary Microbiology,
Faculty of Veterinary Medicine,
Swedish University of Agricultural Sciences,
Uppsala, Suecia

Abstract-Resumen

USE OF RECOMBINANT DNA TECHNOLOGY IN THE DIAGNOSIS OF VIRAL INFECTIONS.

Traditionally, diagnosis of a viral infection is based on the detection of viruses or viral antigens in clinical material or on demonstration of specific antigens in the serum of patients. The viruses can be detected by isolation in the cellular cultures or by electron microscopy. The latter method also makes it possible to identify hitherto unknown viruses. The techniques used for the specific identification of nucleic acid from clinical material have been adapted to the identification of viruses and other microorganisms. This was made possible by application of the recombinant DNA technology. A combination of techniques such as molecular cloning, digestion with restriction enzymes and hybridization of nucleic acid has been introduced in diagnosis laboratories. Under suitable conditions, a single-stranded nucleic acid will always form a double strand when they are complementary. This reaction is known as 'hybridization'. Molecular hybridization is visualized using radioactive nucleic acid, known as a 'probe', after its autoradiographic exposure. This method is extremely sensitive and capable of detecting less than one viral genome per cell; it also makes it possible to differentiate between closely related nucleic acids. This technique has been applied to detect and classify a large number of microorganisms. The authors describe the theoretical basis of nucleic acid hybridization, its application in the diagnosis of certain infectious diseases and some of the techniques involved.

USO DE LA TECNOLOGIA DE RECOMBINACION DEL ADN EN EL DIAGNOSTICO DE ENFERMEDADES INFECCIOSAS VIRALES.

Tradicionalmente, el diagnóstico de una infección viral se basaba en la detección de virus o antígenos virales en material clínico o en la demostración de antígenos específicos en el suero de pacientes. Los virus pueden detectarse mediante el aislamiento en cultivos celulares o mediante el microscopio electrónico. Este último método nos permite además identificar virus hasta ahora desconocidos. Los métodos para la identificación específica del ácido nucleico a partir de material clínico se han convertido en instrumentos para la identificación de virus y otros microorganismos. Esto ha sido posible gracias a la aplicación de la tecnología de recombinación del ADN. Una combinación de técnicas tales como el clonaje nuclear, la digestión con enzimas de restricción y la hibridación del ácido nucleico ha sido introducida en los laboratorios de diagnóstico. En condiciones apropiadas, una cadena única de ácido nucleico formará una cadena doble, siempre y cuando sean complementarias. Esta reacción se conoce con el nombre de "hibridación". La hibridación molecular se visualiza utilizando ácido nucleico radiactivo, conocido con el nombre de "sonda" o "probe", después de su exposición autorradiográfica. Este método es extremadamente sensible y capaz de detectar menos de un genoma viral por célula,

pudiendo además diferenciar entre ácidos nucleicos estrechamente relacionados. Esta tecnología ha sido aplicada para la detección y clasificación de un sinnúmero de microorganismos. En este trabajo se darán a conocer las bases teóricas de la hibridación del ácido nucleico, su aplicación en el diagnóstico de algunas enfermedades infecciosas y la descripción de algunas técnicas.

1. INTRODUCCION

El diagnóstico de enfermedades infecciosas se basaba en sus comienzos en las observaciones de síntomas clínicos o en las autopsias. Los métodos de laboratorio como complemento para el diagnóstico de enfermedades microbiales tuvieron que esperar el advenimiento del microscopio y de técnicas inmunológicas que pudieran detectar pequeñas cantidades de antígenos o anticuerpos.

La inoculación en animales, en huevos embrionados y el desarrollo de sistemas de cultivos celulares han sido y son los instrumentos utilizados en el diagnóstico de enfermedades virales. Tales métodos facilitan el cultivo de virus y, en el caso de cultivos celulares, nos permiten observar el efecto citopático del virus.

Los métodos serológicos convencionales, tales como neutralización por suero, fijación del complemento, inmunofluorescencia, inhibición de la hemoaglutinación etc., son métodos tediosos o de sensibilidad limitada y por lo mismo poco aconsejables para un diagnóstico rápido de rutina.

Los métodos de Radio Inmuno Ensayo (RIA) o Enzima Inmuno Ensayo (EIA), este último más conocido como ELISA, han revolucionado prácticamente el diagnóstico en microbiología. Estos son métodos altamente específicos y sensibles, que permiten la detección directa de microorganismos en muestras clínicas o su utilización en el diagnóstico serológico.

La técnica de RIA exige equipos y reactivos algo sofisticados, mientras que la de EIA permite su realización en laboratorios modestamente equipados.

El advenimiento de los llamados anticuerpos monoclonales ha venido a contribuir al mejor desarrollo de estas técnicas, y los llamados hybridomas se han convertido en fuentes interminables de reactivos altamente específicos.

Por otra parte, técnicas simples y sensibles derivadas de la tecnología de recombinación del ADN han hecho posible el desarrollo de métodos de diagnóstico, basados en la hibridación del ácido nucleico [1]. Estos métodos se convertirán ciertamente en un complemento para los inmunoensayos.

2. LA HIBRIDACION DEL ACIDO NUCLEICO

La cadena doble del ADN esta compuesta de cadenas complementarias de polinucleotidos, fácilmente separables por acción de la temperatura o el aumento de pH. Esta separación o desnaturación es reversible y, en condiciones apropiadas, las dos cadenas simples de ADN podrán reasociarse y formar la cadena doble, idéntica

a la original. Este fenómeno es específico y, debido al emparejamiento natural de las bases que conforman esta cadena, la adenina se reasociará con la timidina y la guanina con la citosina. Esta reacción es estable y se la conoce con el nombre de *hibridación*, técnica que ha sido utilizada desde hace más de 20 años para el estudio de la expresión y estructura de genes celulares y virales. La complementación del ácido nucleico no es absoluta y, a pesar de que se tolera cierto grado de *mal* emparejamiento, tendrá lugar la formación de la doble cadena. Esto quiere decir que cadenas de ácidos nucleicos no idénticos, pero relacionados, podrán participar en la reacción de hibridación. El resultado es lógicamente menos estable a la acción de la temperatura y a la concentración iónica. Esta propiedad es la base para un método que diferencia entre híbridos *bien* o *menos bien* emparejados.

El principio para el uso práctico de la técnica de hibridación es simple: en condiciones apropiadas de temperatura y pH, las dos cadenas complementarias de polinucleótidos formarán una cadena doble o híbrida. La presencia de ácido nucleico en un espécimen clínico se demuestra generalmente después de la extracción de ADN mediante técnicas convencionales, que incluyen la digestión proteolítica y la extracción repetida del ADN con fenol y cloroformo. Un problema práctico en la reacción de hibridación es la competencia. El ADN de la muestra en suspensión, una vez desnaturado, tratará de reasociarse consigo mismo, mientras tenga lugar la hibridación con el ácido nucleico radiactivo denominado "sonda" o "probe". Este problema ha sido solucionado inmovilizando el ADN en una matriz sólida, generalmente una membrana o filtro de nitrocelulosa. De esta manera se evita la auto-reasociación, permitiendo la hibridación de la "sonda" con la cadena simple de nucleótidos expuesta en la membrana. La "sonda" radiactiva se utiliza en este caso para detectar la presencia de ácido nucleico específico a la muestra inmovilizada. La reacción de hibridación puede ser medida en un contador de centelleos o visualizada mediante autorradiografía.

La tecnología de recombinación del ADN ha facilitado el desarrollo de los métodos de hibridación. El ácido nucleico puede ser clonado o subclonado en un vector bacterial para así lograr grandes cantidades. Es posible asimismo seleccionar clones que representan partes únicas o comunes de un genoma y que permitan la diferenciación entre microorganismos estrechamente relacionados. Una nueva tecnología ha permitido construir equipos con capacidad de sintetizar cadenas de ácido nucleico de hasta más de 50 nucleótidos, abriendo las posibilidades para diseñar "sondas" específicas basadas en secuencias previamente establecidas.

Para la detección del ácido nucleico, las llamadas "sondas" deben marcarse a un alto nivel de especificidad radiactiva. Los métodos *in vitro* son los más utilizados y el llamado método de "nick translation" es el más conveniente, lográndose "sondas" de doble cadena de ADN con actividad específica de más de 10^8 cpm/ μ g.

El isótopo ^{32}P es el más utilizado, aunque existen precursores de nucleótidos marcados con ^3H que se prefieren menos debido a su desfavorable límite de detección. Más recientemente, nucleótidos marcados con ^{35}S o ^{125}I con un término de vida medio mucho más largo han sido introducidos como alternativas para ^{32}P .

Como es lógico, las “sondas” radiactivas son inconvenientes para su utilización en el diagnóstico de rutina y, por lo mismo, se tiende a reemplazarlas por “sondas” no radiactivas. Varias de ellas ya se han desarrollado [2-5]. Las más utilizadas son las basadas en nucleótidos biotinizados y sintetizados enzimáticamente, que son detectadas con una enzima conjugada con avidina en combinación con un sustrato apropiado. Sin embargo, a pesar de su potencial práctico, las “sondas” no radiactivas han demostrado menor sensibilidad que las “sondas” marcadas con ^{32}P .

Durante los últimos años, las técnicas de clonaje molecular y de recombinación del ADN han permitido aplicar la hibridación del ácido nucleico para el diagnóstico de enfermedades. Varias técnicas han sido desarrolladas y entre ellas cabe destacar la siguiente.

2.1. Hibridación “spot” en filtro

Esta es una manera, tal vez la más simple, de detectar el ácido nucleico de un microorganismo en una muestra clínica. Utilizando métodos convencionales se extrae el ADN de la muestra y, una vez desnaturalado, se inmoviliza en una membrana de nitrocelulosa. Su presencia se detecta utilizando “sondas” radiactivas. Para la detección de un sinnúmero de microorganismos se han utilizado como “sondas” ácidos nucleicos (ADN o ARN), secuencias clonadas, ARN sintetizado o bien oligonucleótidos sintéticos. Secuencias de Epstein Barr Virus (EBV) en células linfoblastoides inmovilizadas y desnaturaladas *in situ* fueron detectadas por primera vez por Brandsma y Miller [6]. Variaciones en este método permitieron luego detectar EBV directamente en aspirados nasofaríngeos. Ståhlhandske y Pettersson [7], en Uppsala, utilizaron la hibridación “spot” para detectar Herpes simplex (HSV) en células infectadas y en muestras clínicas. La “sonda” consiste en un fragmento clonado de HSV-1 ADN que permite diferenciar células infectadas con HSV-1 y HSV-2. La hibridación con el serotipo heterólogo es menos estable en condiciones estrictas, permitiendo así la tipificación de HSV.

Otros investigadores han utilizado este método para detectar citomegalovirus humano en muestras de orina. El nivel de detección fue de aproximadamente 5 pg de ADN viral [8-10]. Papillomavirus, el agente etiológico de las verrugas, es un virus que aún no se ha logrado cultivar *in vitro*, por cuya razón es difícil obtener cantidades suficientes de antígenos para diseñar inmunoensayos de rutina. Este virus, que está muy distribuido en la naturaleza, ocasiona tumores benignos en la mayoría de especies animales, incluyendo al hombre. Estos tumores benignos, en ciertas condiciones, pueden transformarse en malignos. De especial interés es el hecho de que papillomavirus tipos 16 y 18 están comúnmente asociados con tumores genitales malignos en humanos [11, 12]. La hibridación del ácido nucleico en estos casos ha permitido un diagnóstico y diferenciación de alrededor de 34 diferentes tipos de papillomavirus hasta ahora observados en el hombre. La identificación y clasificación de papillomavirus en diferentes especies animales ha sido posible gracias a las técnicas de hibridación del ácido nucleico.

Otra importante aplicación se ha logrado en el diagnóstico del virus de la hepatitis B (HBV). Varios investigadores [13, 14] han utilizado la hibridación en "spot" para detectar secuencias específicas de HBV en la sangre, permitiendo su aplicación directa en un filtro de nitrocelulosa, sin previa purificación del ADN. La sensibilidad del método es comparable a las obtenidas en inmunoensayos.

En el Departamento de Microbiología Veterinaria de Uppsala se están desarrollando técnicas basadas en la hibridación del ácido nucleico para el diagnóstico del virus de la pseudorabia porcina (PRV) y del virus de la diarrea viral bovina (BVDV). Para detectar la presencia de PRV ADN en órganos de cerdos natural y experimentalmente infectados, utilizamos "sondas" radiactivas derivadas de varios fragmentos del genoma. El método de hibridación "spot" permite detectar por lo menos 20 pg de ADN viral. El tiempo necesario para la realización de esta técnica es de alrededor de 24 horas. Métodos similares han sido desarrollados por otros investigadores [15-17]. El diagnóstico de BVDV, basado en la hibridación del ácido nucleico, es un proyecto de actual prioridad.

En la literatura encontramos muchas aplicaciones en el diagnóstico bacteriológico y parasitológico. Este método se ha utilizado para la detección de *E. coli* y especialmente para la detección de genes que determinan su patogenicidad [18, 19]. El diagnóstico de enfermedades de transmisión sexual, *Chlamydia trachomatis* [20, 21] y *Neisseria gonorrhoeae* [22] es otro ejemplo.

2.2. Detección de ARN

Las moléculas de ARN a diferencia de las de ADN tienen menor afinidad para adherirse al filtro de nitrocelulosa y además son más sensibles a la acción de nucleasas y cambios de pH. Sin embargo, el ARN de enterovirus fue detectado por Hyypiä et al. [23] utilizando una "sonda" clonada y derivada de la región no estructural del genoma de coxsackie virus B3. Esta "sonda" hibridiza además con ADN de diferentes serotipos de poliovirus, echovirus y coxsackievirus tipo B.

Utilizando una "sonda" de ARN obtenida mediante transcripción in vitro Flores et al. [24] detectaron rotavirus ARN en muestras fecales. Esta técnica demostró ser tan sensible como ELISA. Otro ejemplo lo tenemos en la detección del "potato spindle tuber viroid" [25]. Los viroides carecen de proteínas, y por lo mismo no pueden detectarse mediante inmunoensayos.

La sensibilidad de la técnica de hibridación del ácido nucleico puede incrementarse, por ejemplo, utilizando secuencias repetidas de un gene. Franzen et al. [26] lograron detectar secuencias de *Plasmodium falciparum*. Secuencias repetidas correspondientes al 1% del genoma total fueron utilizadas para detectar la presencia de parásitos en pacientes con malaria. La técnica es simple, y el ADN se extrae de una pequeña muestra de sangre. Con este método fue posible detectar aproximadamente 25 pg de *P. falciparum* ADN.

La lista es larga, pero se debe mencionar que métodos similares han sido desarrollados para la detección de *Trypanosoma cruzi* [27] y *Leishmania* [28], e

igualmente un método comercial para detectar infecciones de mycoplasmas en cultivos celulares. Finalmente, he aquí a continuación algunas variantes de la hibridación del ácido nucleico.

2.3. Hibridación in situ

Esta técnica detecta secuencias en frotos, secciones histológicas o en colonias de bacterias. El procedimiento es similar al descrito anteriormente, y tiene la ventaja de excluir la extracción de ADN. En el caso de secciones histológicas, el material puede ser estudiado paralelamente en el microscopio.

2.4. Hibridación en "sandwich"

Este método se basa en la utilización de dos diferentes reactivos de ácido nucleico, derivados del organismo que va a ser identificado. Uno de ellos se inmoviliza en un filtro de nitrocelulosa, mientras que el otro, conjugado con un isótopo, funciona como "sonda" que se fijará al filtro solamente cuando exista en él el ácido nucleico correcto. Este método es apropiado cuando no se exige pureza en el material que va a ser analizado.

2.5. Hibridación según el método de Southern

En esta variante, el ADN celular o viral se fragmenta gracias a la utilización de las llamadas enzimas de restricción. Estas enzimas reconocen determinadas secuencias de nucleótidos, introduciendo cortes en la cadena del ADN. Los fragmentos se separan electroforéticamente en un gel de agarosa y luego se transfieren a una membrana de nitrocelulosa. Esta es la técnica denominada "Southern blot" [29]. La hibridación con una sonda radiactiva y la subsecuente exposición autorradiográfica revelará los fragmentos específicos que contengan secuencias complementarias a la "sonda" utilizada. En condiciones óptimas, pueden identificarse específicamente cantidades equivalentes a 1 pg de ADN de papillomavirus en 20 μ g de ADN celular, obtenido de la biopsia de un tumor. La fragmentación del ADN permite identificar secuencias específicas de ADN que diferencian a los 34 tipos de papillomavirus humano hasta ahora descritos.

3. CONCLUSIONES

La recombinación del ADN aplicada a la hibridación del ácido nucleico es la base de una tecnología desarrollada y utilizada en las ciencias biológicas. Su campo de aplicación varía desde la investigación básica molecular hasta el diagnóstico de enfermedades infecciosas y hereditarias.

La hibridación es un método extremadamente sensible, capaz de detectar por lo menos un genoma viral por célula y de diferenciar ácidos nucleicos estrechamente relacionados.

Los métodos de clonaje molecular permiten la obtención de "sondas" específicas en grandes cantidades. Sin embargo existe un problema que debe ser resuelto: el isótopo comúnmente utilizado es el ^{32}P , que tiene corta actividad radiactiva y por lo mismo no es apropiado para su uso rutinario en laboratorios de diagnóstico. Es obvia la necesidad de "sondas" no radiactivas que puedan ser manejadas sin precauciones particulares de seguridad. Estas "sondas" existen, pero requieren una mayor familiarización. El futuro de esta tecnología depende en alto grado del desarrollo de tales "sondas".

Generalmente, los resultados se obtienen dentro de las 24 horas pero, una vez optimizada la técnica, el tiempo se reducirá a pocas horas. Los procedimientos de hibridación son relativamente fáciles y, sin duda, la automatización también hará su ingreso. La hibridación es una técnica nueva que está incrementando el arsenal de métodos para la detección de componentes estructurales de microorganismos a partir de especímenes clínicos.

Si bien esta tecnología no está en camino de reemplazar a los inmunoensayos, sin duda constituye ya un complemento de mucho valor para el diagnóstico de enfermedades.

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STUDIES ON AUJESZKY'S DISEASE USING RECOMBINANT DNA TECHNOLOGY

H.-J. RZIHA, T.C. METTENLEITER,

N. LUKÁCS, C. SCHREURS,

V. OHLINGER, G. WITTMANN

Bundesforschungsanstalt für Viruskrankheiten der Tiere,

Tübingen, Federal Republic of Germany

Abstract

STUDIES ON AUJESZKY'S DISEASE USING RECOMBINANT DNA TECHNOLOGY.

The paper summarizes the results of studies carried out on the occurrence and physical state of Pseudorabies virus (PRV; Suid herpes virus 1) DNA in latently infected swine. During latency (between 7 and 52 weeks post-infection) 0.05 to 0.3 genome copies per cell were detected in different neural areas and extraneural cells of swine. During the latent state the predominant form of the PRV genome was found to represent linear, unit-length DNA. In some cases, two types of viral DNA alteration were found in the latently infected organ cells: (a) deletion of about 750 base pairs had occurred in the unique short region of PRV DNA, and (b) the abundant presence of limited portions of the viral genome. Results are also presented on the influence of a PRV glycoprotein (gI) in possibly controlling viral virulence. Avirulent PRV vaccine strains were shown not to express gI or its precursor protein. Analysis of the gI expression was further extended to other virus isolates obtained in vitro and in vivo. These data show a highly variable expression of gI in PRV, both qualitatively and quantitatively, leading to a loss of in vitro neutralization, with anti-gI monoclonal antibodies in some cases.

1. INTRODUCTION

Pseudorabies virus (PRV), the causative agent of Aujeszky's disease, represents a member of the *Herpesviridae* family (Suid herpes virus 1) and contains a linear double-stranded DNA of about 94 million dalton in size.¹ The viral genome is composed of two sets of unique sequences, unique long and unique short regions, separated by inverted repeat regions (Fig. 1), leading to the existence of two isomeric forms of the DNA molecule [1]. PRV can infect a wide range of animals, and natural infection of, for example, cattle and swine leads to great economic losses in animal husbandry. Differences exist between virus strains with regard to the severity of clinical symptoms in swine. In most animals the disease is nearly always fatal; however, swine can survive the infection with increasing age, consequently leading to the establishment of latency. Latently infected swine must be regarded as the main reservoir of PRV. Existing vaccines against PRV can reduce the severity of clinical

¹ 1 dalton $\equiv$ 1 u (unified atomic mass)
 $\approx 1.66 \times 10^{-27}$ kg.

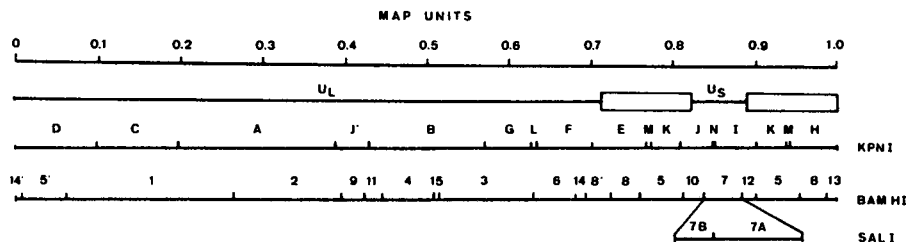


FIG. 1. Genomic map of PRV strain Phylaxia. Boxes represent inverted repeat regions, which separate the genome into unique long (U_L) and unique short (U_S) regions.

symptoms, but they cannot prevent virus replication in the infected animal nor can they prevent latent PRV infection in swine. Thus, there is a requirement for evaluation of more efficient and safer vaccines against PRV.

This paper summarizes the results of studies carried out on (a) the occurrence, physical state, and possible activity of the PRV genome during latency, and (b) the expression of PRV glycoproteins of different virus strains and isolates. The possible role of viral glycoproteins in the expression of PRV virulence is discussed. Since herpes viral glycoproteins are known to play an important role in eliciting immune response in the infected host, these studies are devoted to elucidating the role of PRV glycoproteins in virus neutralization and protection against PRV infection.

2. MATERIALS AND METHODS

2.1. Animal experiments

Four to 6 week old swine were intranasally infected with the virulent PRV strain *Phylaxia* and analysed at different times post-infection for clinical symptoms, virus-specific neutralizing serum antibodies and the presence of infectious virus, as described elsewhere [2, 3]. Organ samples were snap-frozen in liquid nitrogen immediately after removal, and stored frozen until used for nucleic acid analysis.

2.2. DNA preparations

Cellular DNA was obtained from lysed organ cells by repeated phenol-chloroform extraction [3]. Viral DNA was purified from extracellular virions by two cycles on CsCl^- or sodium-iodide density gradients [3, 4].

Restriction enzyme (Bam HI or Sal I) fragments of PRV DNA were cloned in plasmid pBR325 and recombinant plasmid DNA was obtained by standard procedures [5, 6]. Some of the Bam HI fragments used were kindly provided by T. Ben-Porat (Vanderbilt University, Nashville, USA).

2.3. In vitro labelling of DNA

Viral DNA or recombinant plasmid DNA was nick translated with DNA-Polymerase I (Boehringer, Mannheim, FRG) in the presence of ^{32}P - or tritium-labelled deoxynucleotides (Amersham Buchler, Braunschweig, FRG), as described in Refs [3, 7].

Oligo-labelled probes were obtained after excising the viral DNA insert from recombinant DNA with restriction enzyme and purification in low melting agarose gels [8].

Biotinylation of the cloned PRV fragments was achieved by nick translation in the presence of biotin-11-dUTP (BRL/GIBCO, Karlsruhe, FRG).

2.4. Hybridization procedures

Frozen thin sections of organ tissues were hybridized in situ principally according to Haase et al. [9] as described in Ref. [3].

Restriction enzyme-cleaved DNA was transferred to nitrocellulose filters (Schleicher & Schüll, Dassel, FRG) after separation in 0.7% agarose gel according to Southern [10] and Wahl et al. [11] and hybridized under stringent conditions at 52°C [3].

For dot blot hybridization, DNA samples were alkali-denatured at heat (80°C, 10 min), neutralized and 0.05 mg of each sample spotted onto a nitro-cellulose filter using Hybri-Dot manifold (BRL/GIBCO, Karlsruhe, FRG). Hybridization with radioactive probes was as described above for Southern blotting; biotinylated probes were annealed in the same buffer at 45°C. The filters hybridized with biotinylated probes were developed using the DNA detection system of BRL/GIBCO with the modifications recently published in Ref. [12].

2.5. Viral RNA

Isolation, Northern blotting, hybrid selection and in vitro translation of late PRV RNA isolated from infected cells have recently been described in detail in Ref. [6].

2.6. Analysis of PRV glycoproteins

Identification and characterization of the PRV major glycoproteins and their precursor polypeptides were achieved by radioimmuno-precipitation and acrylamide gel electrophoresis or Western blotting using monoclonal antibodies or specific polyclonal antisera [6, 13].

2.7. Virus neutralization assays

About 50 plaque-forming units of PRV were mixed with tenfold dilutions of heat-inactivated ascite fluid of monoclonal antibodies in the presence of complement; neutralization was assessed as plaque reduction to controls without antibody [14].

Protection assays were performed in 6 week old C57/Bl10 mice. Diluted monoclonal antibodies were inoculated (intraperitoneal) and challenge infection with the strain *Phylaxia* was done 24 hours later (intramuscular). These tests were kindly performed by N. Visser and D. Lütticken (Intervet International, Boxmeer, Netherlands).

3. RESULTS AND DISCUSSION

3.1. Latency of PRV

One important prerequisite for controlled studies on latent PRV infection is to determine at which time after infection latency can be established in swine. Operationally, we defined the latent state as long-term infection of the animals, during which time the infectious virus cannot be recovered. Upon examination of more than 20 pigs infected with PRV, we found that other than in a very few rare cases the infectious virus could no longer be detected beyond 7 to 11 weeks post-infection. However, latent PRV was readily reactivated by immunosuppressive treatment of latently infected swine, which was also the case for animals vaccinated with an inactivated vaccine (Nobivac) previous to challenge infection [2, 15]. In the present study, animals which had not experienced immunosuppression and did not display virus recovery were defined as latently infected between 7 and 52 weeks post-infection.

As a screening assay for detection of PRV DNA in organ cells, dot blot hybridization was performed using radioactive oligo-labelled or biotinylated probes. The limit of detection was less than 0.1 genome equivalent per cell using ³²P-oligo-labelled probes, and about 0.1 genome copies with biotinylated probes. One representative hybridization is shown in Fig. 2. During latency the amount of detectable PRV DNA sequences was found to range between 0.05 and 0.3 genome copies per cell, as determined by dot blot hybridization, reassociation kinetic experiments [3] and Southern blotting. After in situ hybridization of the thin sections of different organs, only a very few isolated cells were found to harbour viral DNA (Fig. 3). Thus, we conclude that more than one genome copy of PRV might persist in the latently infected cell.

Our data clearly demonstrate that upon a single exposure by PRV to the oropharynx, the natural route of infection, latency can be established at different sites. (1) During latency PRV DNA could be demonstrated both in the peripheral and the central nervous system of swine. After testing more than 20 latently infected

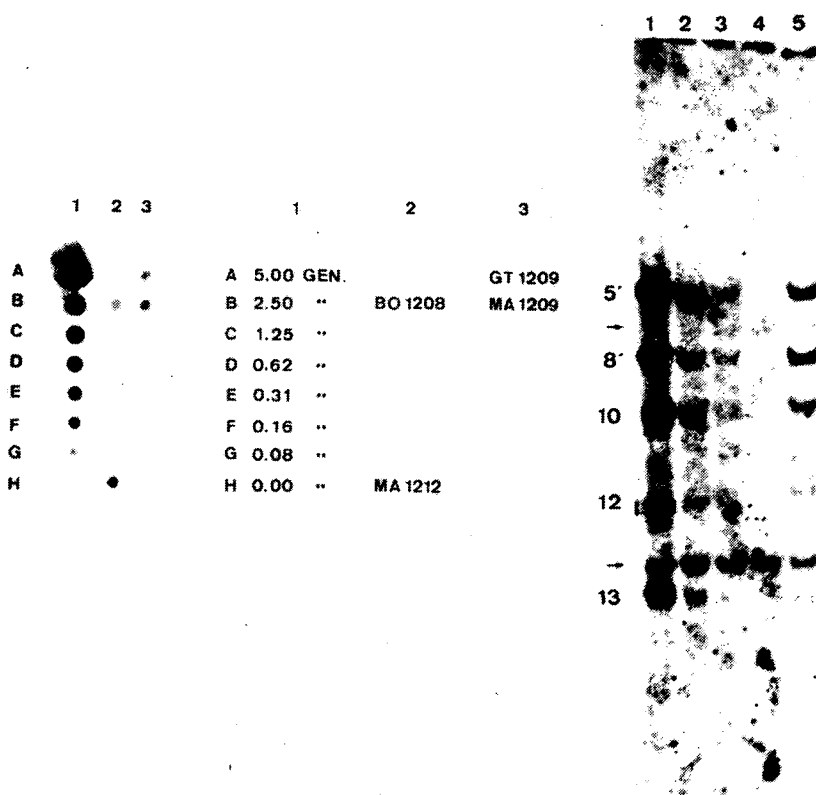


FIG. 2. Detection of viral DNA sequences in latently infected organ tissues. Left part: dot blot hybridization; in row 1A to 1H a reconstruction experiment is shown; the indicated amounts of PRV genome equivalents per 0.05 mg DNA were mixed together with DNA from uninfected macrophages. BO1208: olfactory bulb of an animal, 36 weeks post-infection; MA1212: adherent macrophages, 46 weeks post-infection; GT1209: trigeminal ganglia, 25 weeks post-infection; MA1209: adherent macrophages, 25 weeks post-infection. Oligo- ^{32}P -labelled probe was used for hybridization. Right part: Southern blot hybridization after Bam HI digestion of MA1209-DNA (0.02 mg; lane 5). Lanes 1-4 show reconstruction controls containing 5 (lane 1), 1 (lane 2), 0.2 (lane 3), or 0 (lane 4) genome equivalents. The arrows indicate unspecific cross-hybridization with cellular DNA sequences. The specific PRV DNA fragments are designated.

pigs, the viral genome was detected in nearly 30% of cases in the trigeminal ganglia, medulla oblongata and olfactory bulb, about 20% in the brain stem, and less frequently in different parts of the spinal cord. (2) More recent results obtained by DNA hybridization showed the presence of PRV DNA also in cells of the haematopoietic and lymphoid system of latently infected swine (e.g. Fig. 2). One of the most salient findings was that nearly 50% of the latently infected pigs were found

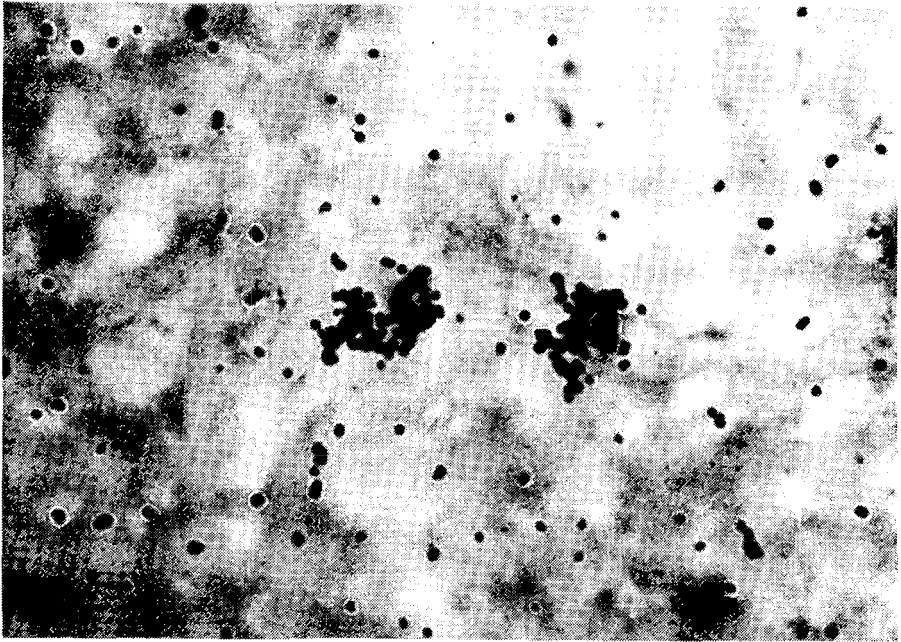


FIG. 3. *In situ* cytohybridization. Thin section of spinal cord, 13 weeks post-infection, was hybridized with ^3H -labelled PRV DNA; exposure was for 6 weeks. Note the accumulation of specific grains over two cell nuclei, indicating the presence of PRV DNA.

to harbour the viral genome in the bone marrow, and about 20% of the animals displayed PRV DNA in macrophages and lymphocytes and in the thymus. However, the significance of these results for the latent state of PRV needs clarification. Current studies focus on the possible involvement of those extraneural cells in virus reactivation. If the persistence of PRV DNA in extraneural cells, especially in blood cells, could be unequivocally correlated with the latent state, this might also have important implications for the detection of carrier animals. Previous experiments have indicated a spread of PRV to various organs via the haematogeneous and lymphatic pathways [16]. In porcine thymocytes we have demonstrated a subset of cells which are permissive for *in vitro* PRV infection (this work is in preparation).

That trigeminal ganglia represent a major site for latent PRV is well established. Our data now demonstrate that PRV can also persist in a similar frequency in additional neural sites, independent of the time of established latency following experimental intranasal infection.

The physical state of PRV DNA during latent infection was further analysed by Southern blotting. The results [3] have shown that latently infected organ cells harbour linear, unit-length PRV DNA. The existence of circular, episome-like or concatemeric viral DNA represented the exception in our studies [3]. However, in

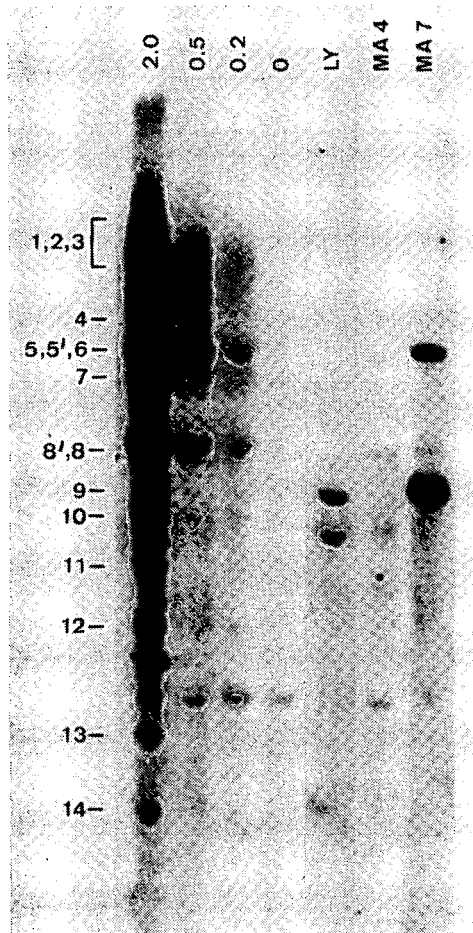


FIG. 4. Southern blot hybridization. Detection of limited portions of PRV genome in lymphocytes (LY) and macrophages (MA) at 36 and 45 weeks post-infection after Bam HI digestion and annealing with ^{32}P -labelled PRV DNA. Reconstruction controls containing the indicated amounts of PRV genome copies are shown in the first lanes; viral fragments are numbered on the left.

some cases the latently infected organs displayed an altered PRV genome. (a) Deletion of DNA sequences, about 750 base pairs in size, occurred in Bam HI fragment 10, which is located in the unique short region of the genome (Fig. 1). The significance of this alteration for the latent state of PRV remains speculative. However, one has to take into account that this part of the viral genome is highly active during permissive virus replication, including the expression of structural genes coding for PRV glycoproteins, as described below. (b) More recent data finally demonstrated that in both neural and extraneural sites of some latently infected pigs detection of the complete viral genome was not possible. In these cases (Fig. 4)

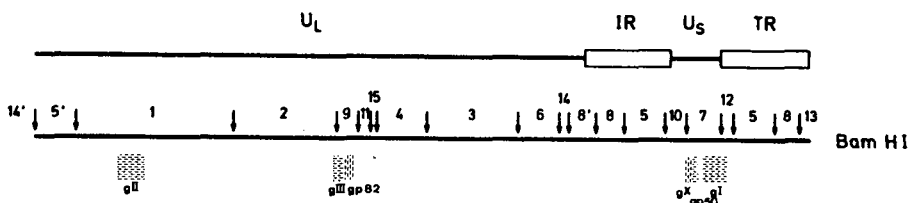


FIG. 5. Genomic location of structural genes coding for PRV glycoproteins (data published recently [6, 17-21]).

virus-specific DNA sequences homologous to limited regions of the PRV genome were found in abundant copy number. Further detailed studies have to be performed to clarify if these viral DNA sequences may originate from, for example, the presence of defective PRV genomes, and if these might influence the latent state or virus reactivation. However, preliminary results indicated the presence of virus-specific transcripts and antigen(s) in these latently infected cells.

In conclusion, using the most sensitive nucleic acid hybridization techniques and recombinant DNA technology, investigations on the latent state of PRV in its natural host enable not only the detection and determination of PRV distribution in swine organ tissues, but will also help to answer questions concerning the establishment, maintenance and reactivation of latent PRV.

3.2. Expression of PRV glycoproteins

As already mentioned, there is a need for more potent and better characterized vaccines against PRV. At the current stage of herpes virus research there is hope that recombinant vaccines expressing viral glycoproteins might be suitable vaccines. Therefore, our studies focused on the identification, characterization and expression of PRV glycoproteins (gp). In Fig. 5 [6, 17-21] the known map locations of some PRV gp are shown. We demonstrated that monoclonal antibodies against gI are able to neutralize PRV in vitro in the presence of complement [14], whereas monoclonal antibodies against gII neutralize PRV with and without complement.

As published earlier [22-24], certain attenuated, avirulent PRV strains display a similar deletion of DNA sequences in the unique short region of the genome. Furthermore, it was reported that restoration of these deleted DNA sequences by wild-type virus DNA can at least partially restore the virulent character [25, 26], and that replacement of the unique short region and parts of the repeat region of virulent PRV by the corresponding DNA region of an avirulent virus strain resulted in reduced virulence of the hybrid virus [27]. Recently, we demonstrated that some avirulent PRV strains fail to express the gI and another 40 000 dalton polypeptide [28], as shown in Fig. 6. After in vitro translation of late viral RNA obtained from cells infected with the virulent strain *Phylaxia* the 80 kilodalton precursor protein of

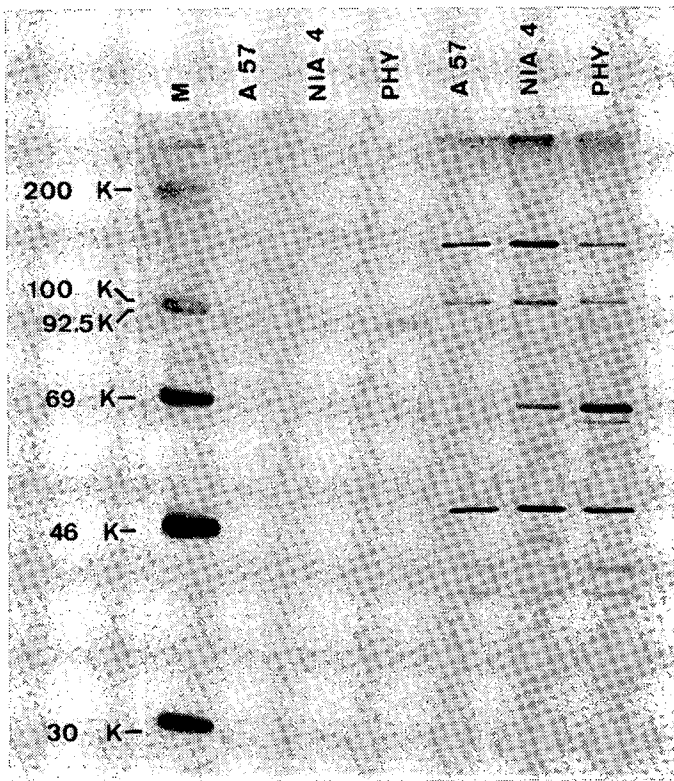


FIG. 6. Expression of *gI* in different PRV strains. In vitro translational products of late viral RNA obtained from vaccine strains A57 (Bartha derivative) and NIA 4, or from wild-type strain Phylaxia (PHY) were separated in 10% SDS-PAGE before (last three lanes) or after immunoprecipitation with anti-*gI* monoclonal antibody. Lane M shows molecular weight markers with the indicated sizes in kilodalton. Note the absence of *pgI* in the vaccine strains.

gI (*pgI*) was precipitated with monoclonal antibodies directed against *gI*. In contrast, upon translation of mRNA from the avirulent vaccine strains (Fig. 6), no *pgI* was detectable. Failure of the *gI* expression in these vaccine strains could be explained by the existence of a deleted *gI* gene, which also affected the transcriptional pattern of this genomic region of PRV [28]. More recent results [14] have revealed a highly variable expression of *gI*, both qualitatively and quantitatively. It became obvious from these results that certain PRV strains represent a heterogeneous population of virions with respect to *gI* expression. As demonstrated in Fig. 7, single plaque isolates of strain Ka can or cannot express wild-type *gI/pgI*. Furthermore, viral variants were found to express a truncated *pgI* and *gI* molecule. These variants, obtained both in vitro and in vivo, displayed neither a deletion of DNA sequences in the *gI* gene nor detectable altered *gI*-specific transcripts. However, all those variants which

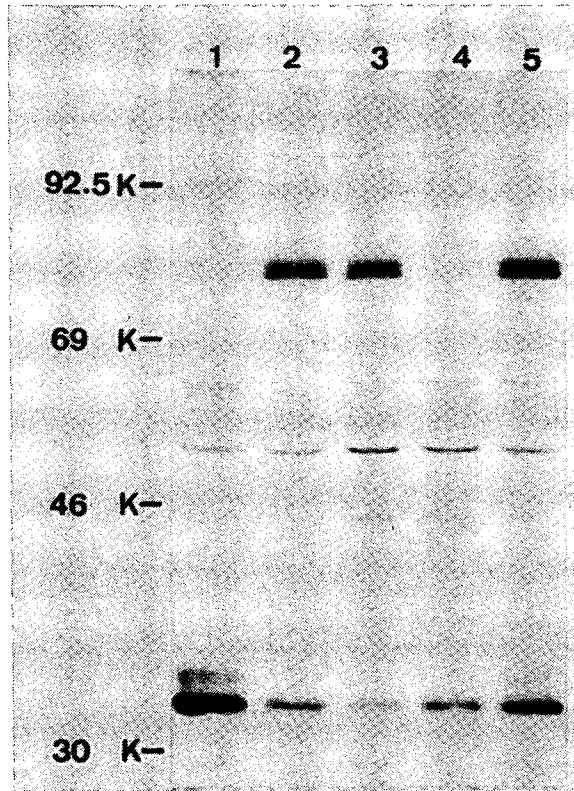


FIG. 7. Variable gI expression. Late viral RNA was isolated from single plaque isolates of strain Ka (lanes 1–5), *in vitro* translated, immunoprecipitated with anti-gI monoclonal antibody, and separated in 10% SDS-PAGE. Molecular weight markers are indicated on the left. Note missing pgI in isolates in lanes 1 and 4, in contrast to isolates in lanes 2, 3 and 5 displaying wild-type pgI.

expressed no detectable or altered pgI/gI were not neutralized by different monoclonal antibodies directed against gI (Fig. 8). To examine the stability and virulent character of these PRV variants, further *in vitro* and *in vivo* studies are currently being carried out.

In conclusion, besides other viral genes, gI can be suspected to play a role in the expression of PRV virulence. A relationship between gI expression and virus release from the infected cells has recently been reported [26]. Furthermore, it remains necessary to clarify the possible influence of gI expression in cell tropism and attenuation of PRV. From the practical point of view, application of an ELISA assay for detection of wild-type infected or vaccinated animals [29] is of interest. Using this assay the absence or presence of gI-specific serum antibodies of animals

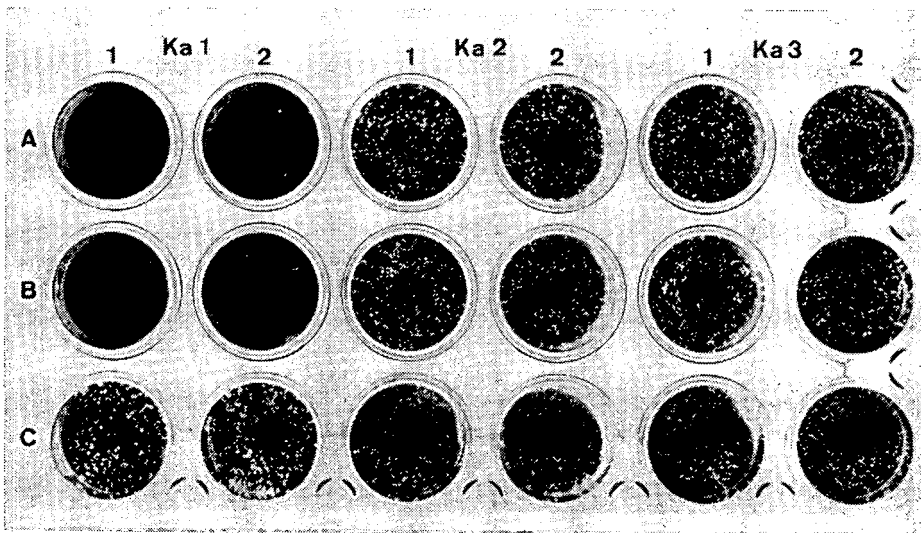


FIG. 8. Correlation between *gI* expression and *in vitro* neutralization with anti-*gI* monoclonal antibodies. Results of a plaque reduction assay are shown after reaction of single plaque isolates of strain Ka (Ka1, Ka2, Ka3) with two different anti-*gI* monoclonal antibodies (lane 1: 3/6; lane 2: 1/14). Ascites fluid was diluted 1:100 (A) or 1:1000 (B); untreated controls are shown in C.

suspected to be infected with PRV can be determined. However, results obtained with this test must be interpreted carefully, because of the described variable *gI* expression in different PRV strains and isolates.

For evaluation of a subunit or recombinant vaccine against PRV, *gI* does not seem to be a good candidate. However, data on *gI* and its probable relationship to virulence might be of particular importance for the construction of live vaccines, which should not revert to virulence *in vivo*.

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APPLICATION OF NUCLEAR AND RELATED TECHNIQUES TO THE DIAGNOSIS AND CONTROL OF TICK-BORNE PARASITIC DISEASES OF LIVESTOCK*

I.G. WRIGHT, C.A. SCHUNTNER,
B.V. GOODGER
Long Pocket Laboratories,
Division of Tropical Animal Science,
Commonwealth Scientific and Industrial
Research Organization,
Indooroopilly, Queensland,
Australia

Abstract

APPLICATION OF NUCLEAR AND RELATED TECHNIQUES TO THE DIAGNOSIS AND CONTROL OF TICK-BORNE PARASITIC DISEASES OF LIVESTOCK.

Conventional serodiagnosis has, until recently, been based mainly on agglutination and/or indirect fluorescent antibody (IFA) techniques. Such techniques are, in general, insensitive and/or relatively non-specific for diagnosing long-term infections. Solid-phase adsorption of semi-defined antigens on plastic matrices and detection of antibodies with sensitive marker systems (radioimmunoassay (RIA) and enzymeimmunoassay (EIA)) have now been applied to the detection of antibodies to some of these diseases. In particular, an RIA utilizing the lysate of *Babesia bovis*-infected erythrocytes as antigen has been developed. Less than 2% false positive and no false negative reactions have been detected. The sensitivity of this test is shown by its ability to detect antibodies in animals 4 years after they had received a single infection. An RIA of similar sensitivity has also been developed for detection of *A. marginalis* antibodies. This test differs from the *B. bovis* test in that it requires simultaneous testing of pre-absorbed sera with antigens derived from both infected and non-infected erythrocytes from the same host. Sera from singly infected cattle are all positive after 2 years and 85% are still positive after 3 years. Naturally infected animals under constant infection are all positive after 3 years. There are no false negatives and 2% false positive reactions using this test. Control of two tick-borne haemoparasitic diseases by vaccination (*B. bovis* and *B. bigemina*) has been largely restricted to one country, Australia. This vaccine has a number of disadvantages, which include thermolability and reversion to both virulence and tick transmission. The problem of inducing a stable avirulent state has been attempted in four *Babesia* species: *B. bigemina*, *B. bovis*, *B. divergens* and *B. major*. In general, irradiating live organisms with 350 Gy gamma irradiation from a ^{60}Co source induced a stable avirulent organism which, upon infection of susceptible animals, resulted in a mild disease; these animals were subsequently strongly protected against virulent homologous or heterologous challenge. The new direction for production of vaccines suitable for widespread dissemination is in the field of synthetic vaccines produced by either genetic engineering or peptide synthesis. Already a number of protective antigens have been purified from *B. bovis* and one of these has also been shown to be common to *B. bigemina*. Use of nuclear techniques in the isolation of these antigens and in their subsequent production by recombinant DNA techniques is discussed.

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1. INTRODUCTION

Although there are three major tick-borne haemoparasitic genera affecting livestock, this review will discuss only two of them, *Babesia* and *Anaplasma*, which, because of their mutual tick vectors in most countries, generally occur in association with one another. This association is prevalent in much of Africa, South-East Asia, Central and South America as well as northern Australia. In Central and South America about 300 million cattle are found in *Boophilus microplus* endemic regions. *A. marginale* is also found in the United States of America, where a number of different vectors have been identified. The only accurate estimates of the economic impact of these parasites were made in a 1972 estimate for *A. marginale* which appeared in the USA and which in 1986 terms would cost US \$300 million/annum [1], while in Ireland the mildly pathogenic *B. divergens* was estimated in 1983 to cost the industry US \$14 million [2]. It is generally accepted that in Central and South America and in Australia *B. bovis* is by far the most economically important parasite, followed by *A. marginale* and then *B. bigemina*. Long-term studies on disease outbreaks in Australia (where widespread vaccination against *B. bovis* and *A. marginale* but only limited vaccination against *B. bigemina* is carried out) show that 74% are due to *B. bovis*, 16% to *A. marginale* and 10% to *B. bigemina*, even though the latter two parasites have a much higher transmission rate than *B. bovis*. The picture is less clear in Asia and parts of Africa where *B. bigemina* is thought to be more pathogenic than *B. bovis*. However, in both these continents babesiosis and anaplasmosis are overshadowed by the highly pathogenic *Theileria* species *T. annulata* and *T. parva* in Asia and Africa, respectively. Very little is known about ovine or caprine babesiosis and anaplasmosis except that they are thought to be very widespread in southern Europe, Asia, the Middle East and along the Mediterranean coastline where 300 to 400 million sheep and goats are found. *B. ovis*, which has many of the biological characteristics of *B. bovis*, appears to be highly pathogenic. Even less is known about porcine babesiosis, except that it appears to be widely distributed in Africa.

The diagnosis and control of these diseases are restricted largely to the USA where a dead vaccine against anaplasmosis is used, and to Australia where live blood vaccines are used to control both babesiosis and anaplasmosis. Even in these countries serological diagnosis is not widely used, diagnoses being largely dependent on the use of blood smears and/or post-mortem material such as brain and kidney smears. It is only recently that highly sensitive enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) tests suitable for large-scale testing have been developed. Consequently, the status of tick fever and the relative importance of the various organisms are not known in many countries. Without such serodiagnostic techniques control cannot be attempted. It is the purpose of this review to outline recent advances in serodiagnostic tests and the development of experimental live and dead vaccines. Unfortunately, all relate to tick fever of bovine origin as no research effort has as yet been directed at the ovine, caprine or porcine species.

2. SERODIAGNOSIS

Serological tests must meet a number of criteria. They must be highly specific, highly sensitive tests enabling long-term infections to be diagnosed, and must be relatively quick and inexpensive to perform. To date, most serological tests for tick fever have suffered from one or more shortcomings. Usually they are relatively insensitive and/or non-specific such as the indirect immunofluorescent antibody (IFA) test [3] for *B. bigemina* and the latex agglutination test (LAT) for *B. bovis* [4]. They do not diagnose long-term infections (complement fixation (CF) test for *B. bovis* or *B. bigemina*) [5], or are deficient for both criteria (card test (CT) and CF tests for *A. marginale*) [6]. The indirect haemagglutination (IHA) test for *B. bovis* did, however, meet most of these criteria. Unfortunately, it was a difficult test to perform [7].

Recently, tests aimed at meeting the required criteria have been developed. These tests depend on using solid-phase adsorption of antigen on a plastic matrix, the reaction with it of a test serum and finally detection of the immobilized test sera with a suitable antiserum conjugated with either an enzyme or radiolabel marker. These markers are highly sensitive, enabling very low levels of primary antibody to be detected.

2.1. Babesiosis

The first ELISA test for diagnosing bovine babesiosis utilized crude antigens from either *B. divergens* or *B. major*, and were compared with the CF and IFA tests [8, 9]. In all instances positive titres were observed within 13 days of infection, but whereas the CF test was negative after 216 days both ELISA and IFA gave positive results. The authors concluded, however, that the ELISA was superior to the IFA test because it was less subject to operator error and fatigue.

More recently, an EIA for detection of *B. bovis* antibodies was developed by Barry et al. [10]. This test, when compared with IFA, had 95% agreement and was shown to be more sensitive. As with the ELISA test for *B. divergens*, antibody was detected 14 days post-infection (DPI) and for at least 268 DPI. This test utilized a crude antigen preparation from *B. bovis*-infected erythrocytes concentrated by differential lysis in hypotonic saline solution using a modification of the method of Mahoney [11]. Antigen was used routinely at a 1/400 dilution (150 to 200 µg protein/mL), and antibody at 1/100. A rabbit anti-bovine IgG antisera conjugated with alkaline phosphatase at a dilution of 1/250 or 1/500 was used to detect reactions. Cross-reactions between *B. bigemina*, *Theileria* spp., *A. centrale* or negative sera were not significant under these test conditions, but at lower dilutions the data showed a distinct lack of specificity.

A markedly improved ELISA for detection of *B. bovis* antibodies has now been developed [12]. This test, using a semi-defined antigen preparation at dilutions of 1/500 to 1/1000 and antisera at 1/1000, was highly specific in differentiating positive

from negative sera. This selectivity was optimized by the prior absorption of test sera with a pool of normal erythrocyte lysate obtained from at least six normal calves at a dilution of 1/50 at 4°C overnight. This absorption reduced cross-reactivity with membrane isoantigens, which is an inherent problem with red-cell-derived antigens [13]. A series of different enzyme conjugates including alkaline phosphatase were tested and it was found that horseradish peroxidase (HRP) was the most suitable due to its relative cheapness and the fact that it could be used at higher dilutions (1/500) than the other enzymes. The great advantage of this test was its ability to differentiate between positive and negative sera up to 4 years after a single infection. The discrimination ratio between positive and negative sera (cut-off point being the mean absorbance of 100 negatives + 2 SD) up to 2 years after a single infection was about fivefold, and even after 4 years was at least twofold at a serum dilution of 1/1000. No false positive or false negative values were obtained. Three of 47 *A. marginale* sera gave positive values, while none of 40 *B. bigemina* sera were positive. This test could discriminate between antibodies from a number of different *B. bovis* strains, which makes it suitable for use in field situations both in Australia where it was developed and overseas, as there is a body of evidence that Australian strains cross-react with those from other continents [14, 15].

Radioimmunoassays of similar sensitivity have also been developed for detection of *B. bovis* antibodies [16, 17]. Of these tests, the former was conducted under conditions similar to those of the EIA of Barry et al. [10], while that described by Wright [17] was performed under comparable conditions to the ELISA described by Waltisbuhl et al. [12]. The RIA described previously by Wright [17] was more sensitive than that of Kahl et al. [16] in that antibody was used at a standard dilution of 1/800 and antigen of 1/250 to 1/400. The sera were pre-absorbed with normal bovine stroma (1/50) at 4°C overnight prior to use to reduce cross-reaction with erythrocyte isoantigens. No false negative and 2.7% false positive results were obtained from testing 906 *B. bovis*, *B. bigemina*, *T. mutans*, *A. marginale* or negative sera. The test was able to detect *B. bovis* antibodies up to 5 years after a single infection and was not strain specific. This test has more recently been used in South America with a similar degree of specificity [18]. Like the ELISA test, due to the high degree of specificity and reproducibility, this RIA has great potential for the diagnosis of *B. bovis* infections under field conditions.

Attempts to develop ELISA or RIA tests to diagnose *B. bigemina*-infected animals have run into difficulties. Strong cross-reactions of *B. bigemina* antigens with *B. bovis* positive sera have been demonstrated by immunoblotting and by RIA. These antigens vary in size from > 200 to < 20 kilodalton and some reactions are also demonstrable with negative sera.¹ Fractionation using HPLC, or affinity chromatography using lectins, antibodies or hydrophobic ligands have not improved specificity. A recent report [19] that antigenic fractions isolated by Sephadex G200

¹ 1 dalton = 1 u (unified atomic mass unit)
≈ 1.66 × 10⁻²⁷ kg.

chromatography are specific for *B. bigemina* positive sera is probably inaccurate for all the reasons stated above. These authors tested one positive and one negative serum and no *B. bovis* or *A. marginale* positive sera. Currently, although it is not highly specific, the IFA test to diagnose *B. bigemina* positive sera remains the test of choice. Limited testing of an RIA method suggests that *B. bovis* cross-reaction can be reduced although, as yet, not sufficiently to diagnose mixed infections.

2.2. Anaplasmosis

Very little application of either ELISA or RIA techniques to the detection of anaplasmosis has been made. Two papers using an ELISA to detect *A. marginale* antibodies have been published [6, 20]. The first test utilized a crude antigen at 1:40 dilution, and an HRP-Protein A conjugate as a marker system. Sera were negative at greater than 1:80 dilution. This test has a number of major shortcomings. First, it is very insensitive, as evidenced by the high concentration of antigen and antibody necessary to obtain a positive result. However, its major drawback and almost certainly the reason for its insensitivity is the fact that Protein A, when used at pH 7.6 as in this test, will not cross-react with either bovine IgM or IgG1 [21]. These immunoglobulin isotypes have been shown to be the sero-reactive antibodies in *A. marginale* infections, whereas IgG2 is not [22]. Protein A reacts avidly only with bovine IgG2, and weakly or not at all with IgG1 and IgM. Murphy et al. [22] showed that soon after infection only IgM antibodies were demonstrable, but during the carrier phase the peak of activity had shifted to IgG1 class antibodies. Similar results have been obtained in bovine babesiosis [23]. In the second test [6], a rabbit anti-bovine IgG HRP conjugate was used but it, too, was very insensitive.

Recently, both ELISA and RIA tests for the detection of *A. marginale* antibodies have been developed at the Division of Tropical Animal Science, CSIRO, Australia [24]. Both tests are essentially the same and require the simultaneous testing of sera with antigen preparations derived from the same animal both before and after infection. This use of 'positive' and 'negative' antigens derived from the same animal compensates for the cross-reactions that occur between sera and incompatible blood group antigens. As with the *B. bovis* test described previously, sera are also pre-absorbed with a 1/50 dilution of a pool of normal erythrocyte lysate to further reduce non-specific reactions due to erythrocytic isoantigens. Both tests are performed with a standard antigen dilution of 1/400 (150 to 200 µg protein/mL) and a primary antibody dilution of 1/800. The conjugated second antibody (HRP or ¹²⁵I) is used at a dilution of 1/400 for ELISA or the equivalent of $\approx 200\,000$ counts/min per well for RIA. No false negative and 2% false positive reactions were obtained with both tests. Sera obtained from animals 3 years after a single infection are all positive. About 2% of either *B. bovis* or *B. bigemina* positive sera are also positive. These tests are a marked improvement on existing tests, which rely on particulate antigen (CF and the CT tests) and are also generally negative after 1 to 2 years, even though animals are in the carrier phase of infection. The detection of antibody for at least three years

after a single infection makes both the ELISA and RIA tests suitable for the screening of animals being imported into tick-fever-free countries or regions. A defined *A. marginale* antigen might eliminate some cross-reactions without recourse to a 'negative' antigen approach.

3. VACCINES

Attempts to improve existing vaccines against cattle *Babesia* spp. and *Anaplasma* have concentrated on both live and dead vaccines. Unfortunately, as with serology, no comparable effort has been made to develop either live or dead vaccines against *Babesia* spp. of non-bovine hosts. This review will summarize the developments from the various groups which have utilized gamma irradiation to produce stable avirulent replicating vaccines.

3.1. Irradiation

Three species of bovine *Babesia* have been subjected to numerous trials using gamma irradiation. These three species, *B. bovis*, *B. divergens* and *B. major*, have been attenuated with 240 to 350 Gy gamma irradiation and inoculated into susceptible calves and adult cattle. Animals generally only underwent a mild disease vis-à-vis non-irradiated organisms and upon subsequent challenge with virulent heterologous parasites were totally protected. Irradiation doses above 400 Gy were lethal to the parasites and poor protective immunity was induced. Below the optimal level very little attenuation occurred and cattle underwent severe reactions. These data are summarized in Table I [25–29]. Long-term studies with *B. bovis* showed that the organism did not revert to virulence after 12 months in a carrier animal and could not be transmitted by the tick vector, even though the virulent parent strain under the same conditions maintained both its virulence and its ability to be transmitted by its vector. Strong protective immunity was maintained 12 months after a single infection with irradiated organisms [28, 30, 31].

With *B. bigemina*, however, the situation is less clear. In one study successful attenuation was achieved after irradiation at 240 Gy [29]. However, this protection was only demonstrated in splenectomized calves which were challenged four weeks later using the non-irradiated parent strain. More recently, work funded by the IAEA has been conducted in our laboratory [32]. It was found that 300 Gy was completely lethal to parasites, whereas 200 Gy had no effect; only at 250 Gy was any effect observed. Two distinct strains were used and while reactions were mild upon initial infection with irradiated blood, subsequent challenge with either virulent homologous or heterologous organisms was invariably fatal. There was strong evidence from these studies that selection of strains was important. One of the strains selected was from a clinically affected cow in an epidemic area. This animal was almost certainly infected with only one or a small number of ticks, which would indicate a relatively

TABLE I. SUMMARY OF BOVINE *Babesia* spp. WHICH HAVE BEEN SUCCESSFULLY ATTENUATED WITH GAMMA IRRADIATION

Parasite	Irradiation dose range (Gy)	Surviving animals (%)	Refs
<i>B. divergens</i>	240–300	100	[25, 26]
<i>B. major</i>	240–300	100	[27]
<i>B. bovis</i>	350	100	[28]
<i>B. bigemina</i>	240	100	[29]

homogeneous parasite population, whereas the other strain was isolated from a carrier animal in an endemic area where multiple infections and hence in all likelihood multiple parasite populations would be probable. The population that was derived from the endemic area was less pathogenic than that from the epidemic area. The data suggested that selection for a less virulent subpopulation was occurring in the endemic strain, whereas irradiation was having a sublethal effect only on the epidemic strain, i.e. the latter were not being attenuated, merely that the apparently homogeneous population was being reduced in numbers. This reduction in numbers resulted in a delay in onset of parasitaemia, but not in the eventual need for chemotherapy.

Unfortunately these results are inconclusive, and as yet no satisfactory, stable, avirulent but protective strain of *B. bigemina* has been produced.

Similar inconclusive results have been obtained for *A. marginale* attenuation. Initial work in our laboratory showed that 900 Gy induced attenuation and strong immunity to virulent challenge; however, subsequent work was unable to confirm this. Collaborative studies between Colombian and CSIRO scientists are being funded by the IAEA to further explore this area as it is imperative that for the foreseeable future a replicating vaccine be developed that will induce a strong protective response to *A. marginale* [33]. Existing *A. centrale* vaccines are not in widespread usage and there is concern that they do not induce a satisfactory degree of cross-protection against *A. marginale*, especially in young animals [34].

3.2. Dead vaccines

For a dead vaccine to be effective it must: (a) induce non-strain specific protection, (b) be non-labile, (c) give protection for at least 12 months, preferably after one injection, (d) be free of contaminating antigens and infectious organisms (e.g. blood group antigens, viruses), and (e) be cheap to manufacture. Current dead vaccines meet some of these criteria, notably item (a), but most of the others have yet to be

satisfied. A further advantage of dead vaccines is their inability to disseminate the infectious virulent organism in the environment, a major drawback of current live vaccines where reversion to both virulence and tick transmission occurs after passage of the relatively avirulent organism into cattle [35]. This drawback does not occur with irradiated *B. bovis*, although to date no use has been made of this property for a live vaccine.

The efficacy of utilizing crude extracts of *B. bovis* as a protective immunogen has been demonstrated by Mahoney [36] and Mahoney and Wright [37]. Since then considerable effort has gone into subfractionating the organism to identify protective antigens. This identification relies heavily on use of nuclear and related techniques.

It is essential that antigens be selected for in vivo protection rather than for magnitude of immunological reaction in vitro. This is dependent upon the testing of antigens in protective experiments using the definitive host because suitable animal models are unavailable for all except *B. divergens*, which has been adapted to hamsters [38]. This makes testing both expensive and laborious and because of the facilities necessary for maintaining cattle or sheep under tick-free conditions it restricts the number of laboratories able to enact such research. This has resulted in fragmented research efforts, mainly in Australia, South Africa and the USA, with no overall co-ordination. Nevertheless, considerable progress has been made in identifying protective antigens from *B. bovis* and also many sero-dominant antigens have been identified in in vitro tests which have been shown to be non-protective by in vivo testing. All protective antigens identified to date are minor components which are difficult to isolate. Extensive use has been made of nuclear and related techniques, both to assess the immune response in vaccinated animals and for use in immunoblotting and autoradiography to analyse antigens during purification steps. Such purification steps are multifaceted and include various chromatography, electrophoresis and isoelectric focusing steps. Numerous forms of chromatography have been used to purify antigens, including affinity chromatography using mono- and polyvalent sera, lectins, proteinase inhibitors and hydrophobic and hydrophilic ligands. Incorporation of specific radiolabelled amino acids, such as methionine and histidine, and carbohydrate and lipid moieties have also been used to determine the presence or absence of specific chemical characteristics of the antigens. These markers, if present, can then be visualized by autoradiography of electropherograms. Immunoblotting with mono- and polyvalent antisera using either radioisotopes or enzyme marker systems is a very sensitive means of determining the immunogenic purity of an antigenic preparation. However, extreme care must be taken with interpretation of data as cross-reactions with antigens can occur as a result of red cell isoantigens and also auto-antibodies resulting from non-specific inflammation. Extensive absorption of test sera with disrupted normal red cells derived from a number of calves is necessary to reduce cross-reactions due to erythrocyte isoantigens. It is also necessary to absorb antigen preparations with a broad spectrum of normal sera and/or sera derived from non-specific inflammation to reduce these non-specific antibody reactions [39].

Four distinct protective antigens have now been isolated from *B. bovis* using these techniques, two of them, designated 15B1 [40, 41] and protease [42], to near purity. A third fraction, designated β [43], which is of higher molecular weight, is a complex of four components and is currently being further fractionated, while a fourth, designated 'heparin', is impure. All these antigens are common to a number of strains of *B. bovis*, which makes them ideal candidates for synthesis by recombinant DNA techniques as commonality is an important criterion for a synthetic vaccine. Indeed, one, 15B1, is also present in *B. bigemina* and in view of the cross-protection induced by this organism against subsequent challenge with *B. bovis* [44] may well be a key component in a multivalent vaccine. This aspect is currently being investigated. Likewise, monoclonal antibodies against protective *B. bovis* antigens are being used to screen other important *Babesia* spp., where possible, to determine whether commonality and hence potential cross-protection may exist. This aspect, however, is severely hampered first by the problem of quarantine laws and second by the death of well characterized strains of many *Babesia* spp.

In all likelihood a 'cocktail' vaccine of a number of protective antigens will be necessary to afford protection of the same magnitude as that induced by natural infection. Crude preparations are as effective as natural immunity [37], but to date single pure antigens have been shown to be not quite as effective. As the immunizing dose of purified antigens is only of the order of 10 to 20 μg or less per 400 to 500 kg animal, it remains to be seen whether higher doses of single antigens can give protection as strong as natural immunity. Synthesis of such amounts is now theoretically possible using either peptide synthesis of important epitopes or by reproducing part or all of the protein by gene splicing techniques. Such technologies can theoretically produce the large amounts of antigen necessary for universal control of tick fever, something that is not at present feasible.

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EFFECTS OF PATHOGENIC TRYPANOSOMES ON THE MAMMALIAN REPRODUCTIVE SYSTEM

A.G. LUCKINS, C. LLEWELYN

Centre for Tropical Veterinary Medicine,
University of Edinburgh,
Edinburgh

C.D. MUNRO

Royal (Dick) School of Veterinary Studies,
University of Edinburgh,
Edinburgh

M. MURRAY*

Department of Veterinary Medicine,
University of Glasgow Veterinary School,
Glasgow

United Kingdom

Abstract

EFFECTS OF PATHOGENIC TRYPANOSOMES ON THE MAMMALIAN REPRODUCTIVE SYSTEM.

Trypanosomiasis is known to cause infertility and abortion in susceptible livestock in tsetse-infested areas and there are reports that trypanosomiasis can exert long lasting effects on ovarian function. Although there is considerable evidence for disruption of the oestrous cycle in the female, the causes of these changes are not fully understood. The aim of the present investigation therefore was to study the pathogenic effects of infection with *Trypanosoma congolense* on the ruminant reproductive system with particular emphasis on the hormonal control of oestrus. In particular, the effects of infection were monitored with specific regard to the reproduction of plasma progesterone and the sexual behaviour of the animals. Initially, experiments were carried out using goats infected by the bites of *Glossina morsitans* carrying *T. congolense*. To establish chronic infections it was necessary to administer subcurative doses of diminazene aceturate to the infected animals on a number of occasions during the experiment. Control, uninfected but drug-treated goats showed regular oestrous cycles during the experiments, with cycle lengths of between 19 and 23 days. In contrast, in the infected goats, three out of four animals did not show regular oestrous cycles. The periods without oestrus activity varied between 53 and 97 days. Determination of plasma progesterone levels suggested that in these animals the luteal phase of oestrus was prolonged, indicating a persistent corpus luteum. In further experiments using Boran cattle, a group of 22 animals were selected, 12 of which were infected with *T. congolense* IL1180. The packed cell volume in infected cattle fell to <20% and most of the animals showed loss in body weight. Two animals died and of the remainder five were treated with trypanocidal drug 60 days after infection and five 90 days after infection. In the remaining ten infected cows, nine stopped cyclical activity: in five there was a prolonged luteal phase with elevated progesterone levels and in the remainder progesterone levels fell to basal values for up to 100 days. After treatment these infected cows

* Present address: International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

resumed cyclical activity and within three or four cycles were cycling normally. These results have confirmed that trypanosomes adversely affect ovarian activity but it is not known if more long-term damage might have resulted which could affect subsequent fertility.

1. INTRODUCTION

Animal trypanosomiasis is a disease complex caused by a number of different species of parasitic haemaprotozoa which affects many species of domesticated livestock and wildlife. In Africa, the major causes of this disease are *Trypanosoma congolense*, *T. vivax* and *T. brucei*. These three species are cyclically transmitted by flies of the genus *Glossina*, the tsetse fly. Tsetse flies infest approximately 10 million km² of Africa and their presence precludes or seriously inhibits the development of livestock in large areas of the African continent. Beyond the northernmost limits of the range of the tsetse fly trypanosomiasis caused by *T. evansi* is a serious problem in camels. This species of trypanosomes, which is transmitted non-cyclically by the bite of, for example, *Stomoxys* or *Tabanus* has spread beyond Africa into the Middle East, Asia and South-East Asia and is also found in South America. Although this species tends to be less pathogenic in bovidae than the tsetse-transmitted species, it does cause epidemic outbreaks of disease in cattle and buffalo and it is highly pathogenic in horses. As mentioned, *T. vivax* has also spread beyond the confines of Africa to South America, where it is reported from a number of countries, including Brazil, Colombia and Venezuela, where it is a problem in cattle and buffalo. In North and South America, *T. vivax* is a non-cyclically transmitted parasite carried by biting flies.

Although there is no precise information about the impact of trypanosomiasis on livestock production in Africa or other regions of the world, it is well recognized that the disease may cause death after acute or chronic infection. The clinical disease exhibited by animals infected with trypanosomes varies considerably in severity and duration, ranging from acute infections with death occurring in a few months to conditions in which low numbers of parasites are present in the bloodstream and the animals may survive for many months [1]. A number of factors may influence the outcome of infection, including the species and strain of the parasite involved, the condition of the animals, their plane of nutrition and the degree of stress to which they are subjected such as heavy work of draught animals or trekking of trade cattle.

The pathogenesis of disease caused by *T. congolense*, *T. vivax* and *T. brucei* is similar for all three species: extensive tissue and organ damage occur, affecting the heart, skeletal muscles, central nervous system, endocrine organs and the reproductive tract [1, 2]. A major feature of trypanosomiasis in domesticated livestock is anaemia. This is manifested by a drop in packed cell volume (PCV) of as much as 50% within 4 to 6 weeks of infection and a further progressive decline to values as low as 0.10 to 0.15 L/L; death can then occur quite rapidly. In some

animals with such low PCVs their condition stabilizes and they maintain a low level PCV for long periods. Parasites become difficult to detect in cattle during this phase of infection and animals sometimes respond only poorly to chemotherapy. Chronic anaemia due to trypanosomiasis is common in field situations and may be difficult to diagnose due to the absence of circulating parasites. Loss of appetite associated with pyrexia is common but animals usually eat throughout infection. Animals lose body condition and become weak, and if they survive they remain unproductive; the growth of young animals is also stunted. This long-term effect of trypanosomiasis is important as it causes losses in production due to poor growth, weight loss, low milk yield, reduced capacity for work, infertility and abortion. The work done in this last area, i.e. the effects of trypanosomiasis on the reproductive potential of ruminants, is reported here.

2. EFFECTS OF INFECTION WITH *T. congolense* ON THE OESTROUS CYCLE IN GOATS

Initial studies on the effects of trypanosomiasis on the ruminant reproductive cycle were carried out using British white goats infected with *T. congolense*. Eight doe goats were monitored throughout one breeding season to establish patterns of behavioural activity associated with oestrus and to determine changes in plasma progesterone levels during the cycle. Throughout the breeding season the goats were housed with a vasectomized male, which was then removed. At the beginning of the following breeding season, the male was reintroduced into the group of does to initiate reproductive cycles. After the goats had completed one to three oestrous cycles, four animals were infected with *T. congolense* TREU 1457.

Each goat was infected by allowing *Glossina morsitans* carrying *T. congolense* TREU 1457 to feed on the shaved flanks of the animals. Daily observations of parasitaemia were made by phase contrast microscopy, PCV was determined twice weekly and rectal temperatures were recorded daily. The clinical condition was recorded and the goats were weighed weekly. *T. congolense* TREU 1457 runs a rapidly fatal course in goats, causing death within 30 to 40 days. To prolong infection, therefore, infected goats were treated with subcurative doses of diminazene aceturate (Berenil, Farbwerke Hoechst AG) when their clinical condition had deteriorated and PCVs were ≤ 0.15 L/L. Control, uninfected goats were treated at the same time.

For progesterone determinations heparinized blood samples were collected daily by jugular venepuncture and placed on ice. Plasma progesterone concentrations were measured using an extracted double-antibody radioimmunoassay with ^{125}I progesterone 11α -glucuronide tyramine as tracer (Amersham International) and antiserum raised against progesterone 11α -hemisuccinate-bovine serum albumin [3, 4].

TABLE I. TIMES OF TREATMENT AND DOSAGE OF BERENIL ADMINISTERED TO GOATS INFECTED WITH *T. congolense* TREU 1457

Goat No.	Dosage of Berenil (mg/kg) ^a		
	0.75	1.00	2.00
	Days of treatment		
1	33	37, 57	59, 87, 88, 100
2	33, 46	58	
3	29	41, 57	59, 81, 92
4	38		63, 75

^a The dosage of Berenil was increased during the course of the experiment, since the low initial dose rate became less effective.

Trypanosomes were first detected in the peripheral blood 14 days after infection. The number of parasites fluctuated throughout the infection, often disappearing after trypanocidal drug treatment, but all animals harboured trypanosomes throughout the duration of the experiment. PCVs fell rapidly from pre-infection values of 0.25 to 0.15 L/L by day 30 post-infection. Rectal temperatures showed periodic increases, usually associated with peaks in the numbers of parasites in the blood. Animals were clinically treated on a number of occasions (Table I). One goat died in spite of treatment, while in the remaining three animals the PCVs and haemoglobin remained at low levels until 50 days after infection and then began to return to pre-infection levels. Three of the infected goats progressively lost weight during the experiment, one showed no weight change and uninfected goats increased in weight.

The length of the oestrous cycle in normal goats was approximately 20 days. Plateau plasma progesterone levels were 8.95 ± 0.28 ng/mL (Mean $\pm$ SD). One of the infected goats continued to cycle throughout infection, while the other three exhibited a persistent corpus luteum 28 to 30 days after infection (Table II). In these goats, mean progesterone concentrations were approximately half those of normal goats. Regression of the corpus luteum occurred in one goat 53 days after infection, after which this animal completed normal oestrous cycles. In the other two infected goats the corpus luteum persisted and progesterone levels remained elevated throughout infection.

TABLE II. DURATION OF OESTROUS CYCLE IN NORMAL GOATS AND GOATS INFECTED WITH *T. congolense* TREU 1457

Goat No.		Duration of inter-oestrus period (d)
1	<i>T. congolense</i> infected	20, 53, 6, 20
2	<i>T. congolense</i> infected	22, 59
3	<i>T. congolense</i> infected	20, 97
4	<i>T. congolense</i> infected	17, 20, 23, 20, 20, 17
5	Control uninfected	25, 32, 22, 22, 35
6	Control uninfected	19, 20, 20, 20, 19, 19
7	Control uninfected	20, 19, 20, 20, 23, 19
8	Control uninfected	19, 19, 19, 19, 19, 19

3. EFFECTS OF INFECTION WITH *T. congolense* ON THE OESTROUS CYCLE IN BORAN CATTLE (*Bos indicus*)

Although there was experimental evidence that trypanosome infections disrupted the oestrous cycle in goats, to control infections it was necessary to administer subcurative doses of Berenil in order to prolong the life of the goats. To extend these observations a second experiment using Boran cattle was initiated. For the experiment the cows were not treated with subcurative doses of drug to prolong infection but, when the clinical signs of disease became too severe, some cows were given a single curative dose of Berenil. The remaining animals were cured 90 days after infection and observations on all animals were continued for up to 90 days post-treatment. Twenty-two primiparous Boran cows were used, commencing approximately five months after calving. All cows had resumed normal cyclicity by the start of the experiment. The animals were divided into groups of 10 and 12 matched with respect to weight, body condition and time taken to return to cyclicity postpartum. The group of 12 were infected with *T. congolense* IL1180 by the bites of *G. morsitans*. The other group of 10 served as controls and the two groups were housed together throughout the experiment. Plasma samples were collected at 2-day intervals, parasitaemia was assessed using jugular blood, PCV was measured and rectal temperatures were recorded. Animals were weighed once weekly. Plasma progesterone values were determined using similar assay techniques to those described above.

TABLE III. ALTERATIONS IN CYCLICAL STATUS IN BORAN COWS FOLLOWING INFECTION WITH *T. congolense* IL1180 AND TREATMENT WITH BERENIL

Cow No.	Alteration in cyclical status	% body weight change post-infection	Time of treatment (d)	Onset of cyclicity post-treatment (d)	Cycle lengths after treatment (d)
1	Acyclic	-14	60	42	20, 19, 19
2	Acyclic	-5	60	35	23, 19, 19
3	Acyclic	-3	90	21	16, 21, 19, 17
4	Acyclic	-6	90	73	15
5	Acyclic	-6	90	35	20, 25, 19
6	Persistent corpus luteum	-5	60	23	21, 22, 31
7	Persistent corpus luteum	-21	60	33	16, 18, 21, 24
8	Persistent corpus luteum	-3	60	72	19
9	Persistent corpus luteum	-8	90	45	19, 24
10	Cyclic throughout experiment	-1	90	45	19, 24

Trypanosomes appeared in the peripheral blood 11 to 15 days after infection; thereafter, parasites were present in low numbers throughout the duration of the experiment, with a fluctuating parasitaemia, until the cattle were treated with trypanocidal drug. After this drug treatment using 10 mg/kg Berenil, trypanosomes disappeared from the blood within 48 hours and could no longer be detected during routine microscopical examination. PCVs fell in the infected group to < 0.25 L/L within 19 to 45 days after infection. By 60 days post-infection PCVs were ≤ 0.20 L/L. At this time seven cows that were severely affected by the disease were treated with Berenil but two, with PCVs < 0.15 L/L, died in spite of treatment. Five matched control cows were also treated at this time. The remaining infected cows and their controls were treated 90 days after infection. Following treatment, PCVs gradually rose, reaching approximately 90% of pre-infection values by 90 days after treatment.

Infected cows showed pyrexia, with marked fluctuations in temperature throughout infection. After treatment, temperatures stabilized rapidly. Weight loss was first recorded 20 days after infection, and thereafter in most infected animals a progressive loss in body weight of 4 to 20% of the pre-infection values took place (Table III). However, after treatment weight gain commenced within 14 days and by day 90 post-treatment the animals had returned to within 96% of their weight at the commencement of the experiment. Control animals showed little change in weight.

All cows exhibited one or two oestrous cycles before infection. Control, uninfected animals exhibited regular oestrous cycles of 19 to 26 days in length throughout the period of observation. Plasma progesterone values showed regular cyclical changes ranging from basal progesterone levels of < 0.5 ng/mL to plateau levels of approximately 5 ng/mL (Fig. 1). Cyclical changes in nine infected cattle ceased between days 34 to 67 after infection. Five cows became acyclic, with prolonged basal progesterone levels, and four cows showed evidence of persistent luteal tissue, since their progesterone levels remained at high levels for extended periods after the basal period of basal progesterone. One infected cow continued to cycle throughout infection (Table III). All cows that had stopped cycling resumed cyclicity within 21 to 73 days after treatment. Most of them showed normal cycle lengths within the first two cycles after treatment (Table II). In the four cows with high levels of progesterone, the progesterone fell to basal levels in 3 to 15 days; two resumed cyclicity immediately after regression of the corpus luteum and completed three cycles before termination of the experiment. The other two cows became acyclical following regression of corpus luteum, with basal progesterone levels persisting for 30 and 50 days, respectively, before resumption of cyclicity (Fig. 2). One cow which had been acyclic showed a rise in progesterone after drug treatment, then it went on to complete a series of cycles; however, the remaining four animals continued to be acyclical for 24 to 66 days before resuming normal activity (Fig. 3).

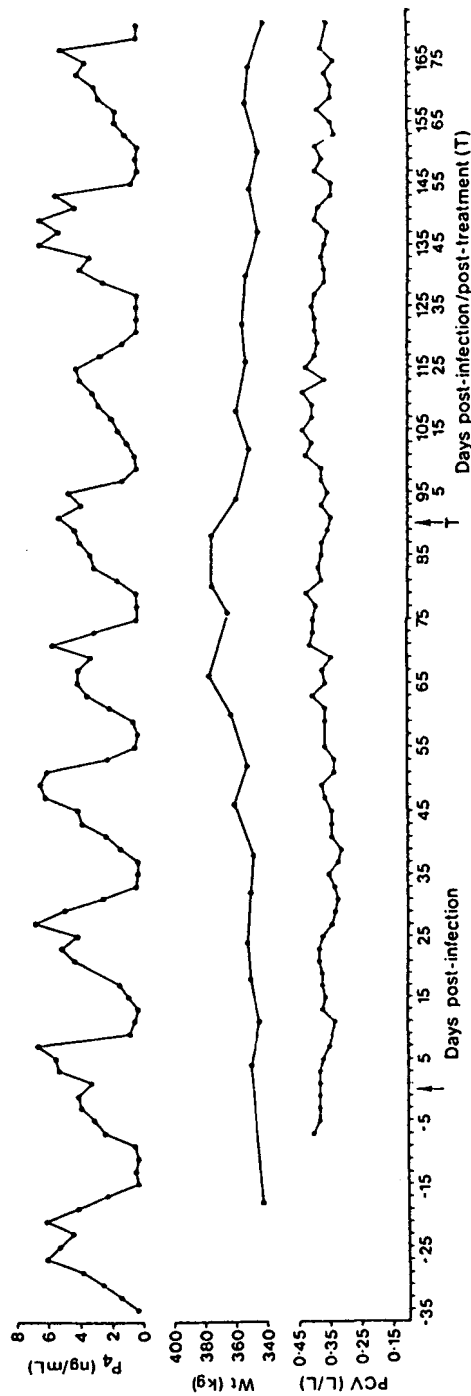


FIG. 1. Progesterone levels, weight measurements and PCV determinations in a control, uninfected Boran cow (T = time of treatment with 10 mg/kg Berenil).

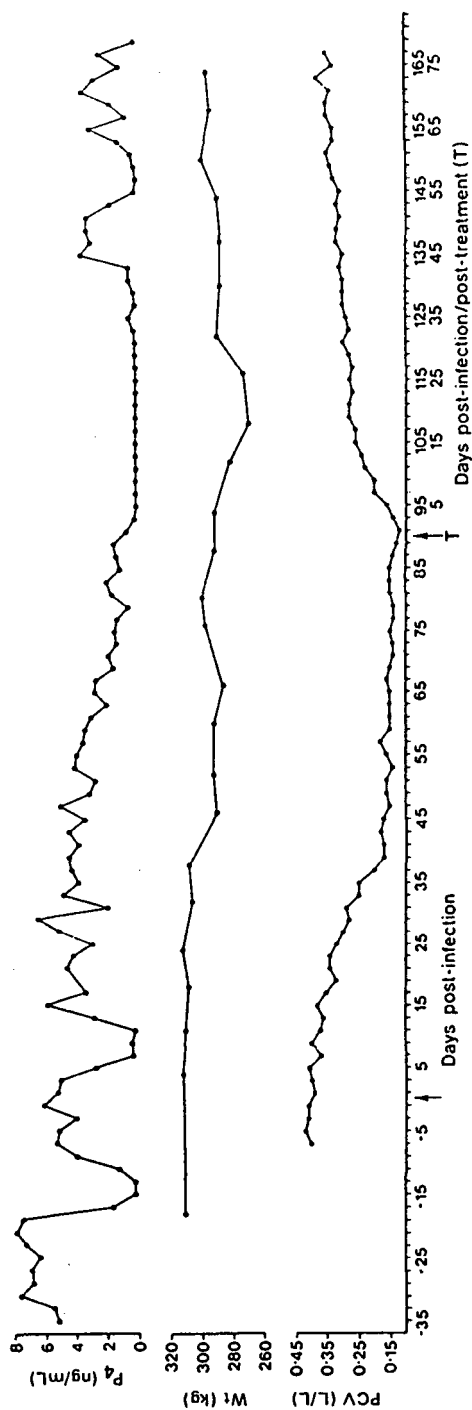


FIG. 2. Progesterone levels, weight measurements and PCV determinations in a Boran cow which showed prolonged, high levels of progesterone following infection with *T. congolense* IL1180 (T = time of treatment with 10 mg/kg Berenil).

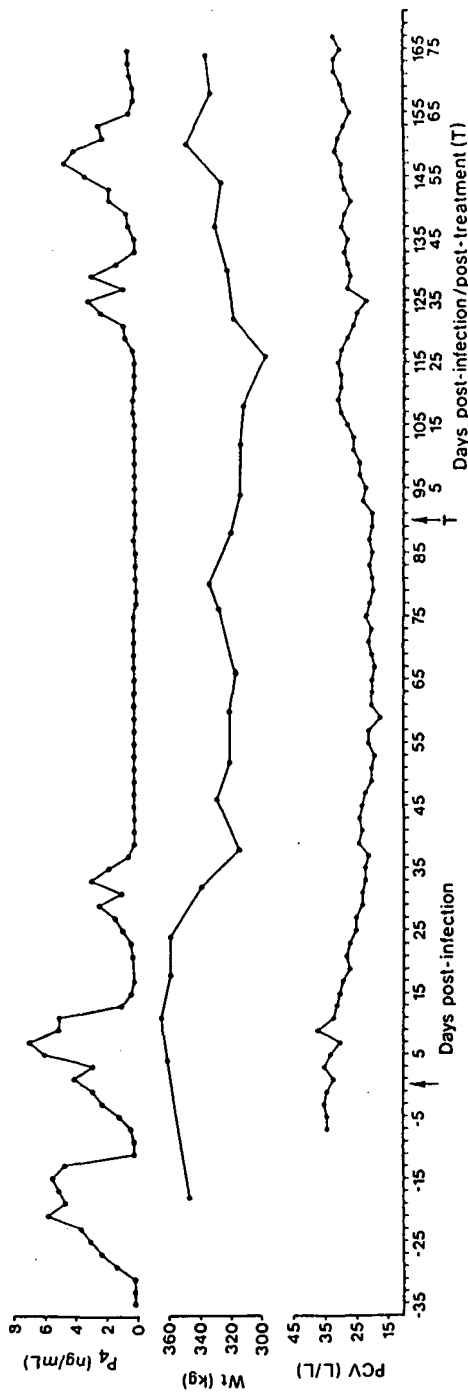


FIG. 3. Progesterone levels, weight measurements and PCV determinations in a Boran cow which became acyclic following infection with *T. congolense* IL1180 (T = time of treatment with 10 mg/kg Berenil).

4. DISCUSSION

In man, endocrine dysfunction is a major clinical feature of sleeping sickness caused by *T. rhodesiense* or *T. gambiense* [5]. Thus, sterility, menstrual disorders such as amenorrhoea, high abortion rate, premature birth and still births are frequently associated with trypanosomiasis [5]. Trypanocidal drug treatment brings about a return to normal function. In ruminants, extensive lesions of the pituitary glands have been described [6, 7] which could lead to defective hormonal secretions and hence to reproductive disorders. In the field there are numerous reports, some anecdotal, on the occurrence of abortions, infertility and long inter-calving periods [8, 9]. There is also some experimental evidence that reproductive function may be affected to a greater or lesser extent depending on the species of trypanosome causing disease [10]. Thus, N'dama cows infected with *T. vivax*, *T. congolense* or *T. brucei* were observed to commence oestrus activity 3½ months, 9 months and 16 months, respectively, after trypanosomes had been cleared from the blood [10]. Our own work has complemented this report because of the detailed information on ovarian activity provided by the plasma progesterone estimations. There are a number of findings from our studies which could have important implications on our understanding of the pathogenesis of the animal trypanosomiasis and their effects on livestock production:

- (1) Infection with *T. congolense* causes disruption of normal cyclical ovarian activity.
- (2) Current concepts of the relationship between the magnitude of weight loss and disruption of cyclical ovarian activity in *Bos taurus* cattle cannot explain the altered cyclical ovarian status found after *T. congolense* infection.
- (3) The occurrence of acyclicity after trypanosome infection suggests some disruption of the normal pattern of pituitary gonadotrophin release or a lack of ovarian responsiveness to such stimuli.
- (4) Persistence of the corpus luteum in these non-pregnant animals was an unexpected finding in these studies. Previously, these changes were considered to be associated with an abnormality of the uterus. Persistence of the corpus luteum indicates that the pituitary is still providing some gonadotrophins as the corpus luteum in the cow requires continuous trophic stimulation to function. This may mean that synthesis and transport of PGF_{2α} fails or that there is a lack of responsiveness of the corpus luteum.
- (5) The pattern of response following infection was similar to disruption of the oestrous cycle associated with subnutrition; however, following treatment resumption of cyclical ovarian activity could not simply be explained in terms of improvement in body weight and condition. This suggests that a possible mechanism for the alterations in oestrous cycles in animals infected with trypanosomes is caused by a disruption of the normal pattern of LH release.

We therefore conclude that infection with *T. congolense* affects the oestrous cycle by a specific effect on the endocrine mechanisms regulating cyclical ovarian

activity. The impact of trypanosomiasis on cattle which have survived the disease is not known. Even in trypanotolerant cattle such as the N'dama of West Africa, which are able to survive trypanosome challenge which would kill Zebu cattle, there is evidence to indicate that the effects on the reproductive system may be a serious impediment on their productivity [10, 11]. Since breeding of trypanotolerant cattle is often advocated as a means of improving livestock production in the tsetse-infested areas of Africa, a more detailed study on the effects of trypanosomiasis on the reproductive system of this breed is essential. Our findings are also of relevance in the field of chemotherapy. Treatment is ideally administered on a herd basis but, in practice, this does not always happen and individual animals may go undiagnosed and untreated. If treatment intervals are too long it is possible that although animals may be clinically healthy and parasitologically negative their reproductive function could still be impaired. Although infertility and long calving intervals have been associated with trypanosomiasis, the evidence is poorly documented. To determine whether inadequacies in administration of trypanocidal drugs or a residual effect of trypanosome infection are responsible for the effects on reproduction there is a need to gather more epidemiological data and comprehensive information on productivity in cattle exposed to tsetse risk in different ecological situations and under different management systems.

ACKNOWLEDGEMENTS

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SUR LES MECANISMES DE LA DIMINUTION DE LA PRODUCTIVITE DU MOUTON INFESTE PAR LES PARASITES DE L'ABOMASUM*

A. DAKKAK

Département de parasitologie et des
maladies parasitaires,

Institut agronomique et vétérinaire Hassan II,
Rabat, Maroc

Abstract-Résumé

MECHANISMS CAUSING REDUCED PRODUCTIVITY IN SHEEP INFESTED WITH ABOMASAL PARASITES.

Only disorders which affect digestive tract function and their effects on productivity are discussed, following a review of various studies on disorders affecting protein, lipid, glucide and mineral metabolism. The parasites reduce the secretion of acid by the infested mucosa, as indicated by increased pH and decreased K^+ and Cl^- ion concentration in the abomasal contents. The reduction in acid secretion has direct effects, including disordered protein metabolism and bacterial complications in the intestine, as well as indirect effects resulting from stimulated gastrin secretion, which gives rise to hypersecretion of water and electrolytes by the liver, the pancreas and the proximal section of the intestine along with decreased absorption by the ileum and a reduction in pancreatic secretion. The infestation also causes increased permeability of the abomasal mucosa. This has been demonstrated by measuring Na^+ and Ca^{++} ion concentrations in the abomasal contents, where they are increased, and by measuring plasma loss through the mucosa. Moreover, study of gastrointestinal motility, duodenal passage and intestinal transport shows that the infestation stimulates motility, which increases throughput and shortens transit time. These motor disorders provide a partial explanation for the poor digestion of foodstuffs and malabsorption of nutrients. Disorders of digestive tract function are not caused only by parasitic destruction of abomasal cells and glands, the causes may also be biochemical; prostaglandins play an important causative role in the development of these disorders since the inhibition of prostaglandin synthesis eliminates the increase in potential difference across the abomasal wall which is concomitant with parasitism and benefits the parasite, whereas *in situ* administration of prostaglandin precursors restores the integrity of the abomasal mucosa after aggravation of the disorders caused by the parasites and their subsequent expulsion.

SUR LES MECANISMES DE LA DIMINUTION DE LA PRODUCTIVITE DU MOUTON INFESTE PAR LES PARASITES DE L'ABOMASUM.

On ne traite ici que des troubles affectant le fonctionnement du tractus digestif et de leurs conséquences sur la productivité. Ces troubles sont présentés après une synthèse de différentes études consacrées à ceux qui affectent le métabolisme des protéines, des lipides, des glucides et des minéraux. L'infestation provoque une diminution de la sécrétion acide de la muqueuse parasitée, diminution indiquée par l'augmentation du pH et la diminution des concentrations en ions K^+ et Cl^- dans le contenu abomasal. Cette diminution de la sécrétion acide a des conséquences directes, dont des troubles du

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métabolisme des protéines et des complications bactériennes au niveau intestinal, et indirectes par la stimulation de la sécrétion de la gastrine provoquant une hypersécrétion de l'eau et des électrolytes par le foie, le pancréas et la partie proximale de l'intestin associée à une diminution de leur absorption au niveau de l'iléon et à une diminution des sécrétions pancréatiques. L'infestation provoque aussi une augmentation de la perméabilité de la muqueuse abomasale. Celle-ci a été mise en évidence par les mesures des concentrations en ions Na^+ et Ca^{++} dans le contenu abomasal où elles augmentent, et par les fuites plasmatiques qui se produisent au niveau de la muqueuse. Enfin, l'étude de la motricité gastro-intestinale, du débit duodénal et du transit intestinal montre que cette infestation provoque une stimulation de la motricité associée à une augmentation du débit et à une accélération du transit. Ces troubles moteurs expliquent en partie la mauvaise digestion des aliments et la malabsorption des nutriments. Le déterminisme des troubles du fonctionnement du tractus digestif n'est pas seulement lié à la destruction des cellules et des glandes abomassales par les parasites. Il est aussi de nature biochimique, les prostaglandines jouant un rôle important dans la genèse de ces troubles, puisque l'inhibition de leur synthèse supprime l'augmentation de la différence transmural de l'abomasum concomitante du parasitisme et profite aux parasites, alors que l'administration in situ de leurs précurseurs restaure l'intégrité de la muqueuse abomasale après aggravation des troubles induits par les parasites suivie de l'expulsion de ceux-ci.

1. INTRODUCTION

Il est actuellement bien établi que les infestations par les nématodes qui se développent dans l'abomasum (*Haemonchus contortus*, *Ostertagia circumcincta* et *Trichostrongylus axei* principalement) sont à l'origine d'importantes pertes économiques pour l'élevage ovin. Ces pertes concernent la mortalité mais aussi, et surtout, le retard de croissance et la faible productivité.

Contrairement aux publications relatives aux aspects parasitologiques, cliniques, lésionnels et immunologiques de ces helminthoses, le nombre de celles consacrées chaque année aux mécanismes de la diminution des productions des animaux atteints est encore limité. La raison principale de ce défaut nous paraît être liée aux difficultés pour les chercheurs d'une double polarisation parasitologique et physiologique. Cette double polarisation est, en effet, nécessaire à la fois pour:

- l'étude des mécanismes de la baisse du rendement des animaux parasités, qui ne peut être abordée que par des appréciations qualitatives et quantitatives non seulement des actions spoliatrices des vers mais aussi, et surtout, des troubles du fonctionnement de l'organe hôte et leurs conséquences sur la transformation des aliments en nutriments;

- l'étude du déterminisme même de la genèse de ces troubles.

Ce mémoire n'a pas la prétention de traiter de l'ensemble des mécanismes par lesquels les infestations de l'abomasum diminuent la productivité du mouton. Son seul objet est de présenter une synthèse relative aux troubles du fonctionnement du tractus digestif, à leur déterminisme et à leur conséquences sur cette productivité.

2. TROUBLES DU METABOLISME DES PROTEINES, DES LIPIDES, DES GLUCIDES ET DES MINERAUX

2.1. Baisse de l'efficacité de la digestion

A part celle due à *H. contortus*, les infestations de l'abomasum par les trichostrongylidés s'accompagnent d'une diminution de l'appétit chez le mouton [1, 2]. Cependant, il a été constaté que celle-ci ne peut, à elle seule, expliquer l'importance de la diminution de la productivité [1, 3, 4]. Mieux encore, au cours de l'haemonchose, considérée comme l'une des plus graves de ces helminthoses, l'appétit est conservé [5, 6]. Plusieurs travaux ont montré que la baisse de l'efficacité de la digestion joue un rôle prépondérant [7-12]. La part de cette baisse dans la diminution de la productivité des animaux a été évaluée à 20-30% dans l'infestation par *O. circumcincta* [10, 11] et à 10% dans celle par *T. axei* [13, 14].

2.2. Métabolisme des protéines

C'est surtout au cours de l'infestation par *O. circumcincta* que ce métabolisme a été le mieux étudié. Il a été démontré que, dans ce cas, l'efficacité de la digestion des protéines subit une diminution, variable selon la gravité de l'affection, de 20 à 30% [2, 11, 12, 15]. D'autre part, les animaux infestés éliminent plus de matières azotées dans leurs urines [1] et dans leur fèces [15] que leur «pair fed». Chez le mouton infesté par *H. contortus*, Stewart [16] avait démontré dès 1934 la diminution de la digestion des protéines. Plus récemment, Dargie [7] a montré que cette infestation s'accompagne d'une augmentation de l'excrétion d'azote dans les fèces et dans les urines. Enfin, Spedding [13] a observé que chez le mouton infesté par *T. axei*, la digestion des protéines n'est pas affectée mais leur absorption diminue. Ross et ses collaborateurs [17] ont démontré de leur côté une hypoprotéïnémie chez les animaux infestés par ce parasite.

2.3. Métabolisme des lipides

Les seuls travaux consacrés aux lipides concernent leur dépôt et le taux des corps cétoniques dans le sang. Chez les animaux infestés par *O. circumcincta*, le dépôt des matières grasses est inférieur de 16 à 21 grammes par jour par rapport aux «pair fed» [10, 15]. D'autre part, Bennett et al. [9] ont montré que l'infestation des brebis par *H. contortus* provoque une augmentation des corps cétoniques dans leur sang.

2.4. Métabolisme des glucides

L'efficacité de l'utilisation de l'énergie fournie par la ration alimentaire a été étudiée chez le mouton infesté par *O. circumcincta*. Elle subit dans ce cas une baisse

qui a été évaluée à 30% environ [10, 15], dont 5 à 14% ont été attribués à une diminution de la digestibilité [15] et 20% environ à un défaut d'absorption [18]. D'autre part, alors que Horak et al. [4] ont montré une hypoglycémie au cours de cette infestation, Bennett et al. [9] ont, par contre, observé une hyperglycémie lors de l'infestation par *H. contortus*.

2.5. Métabolisme des minéraux

Bien que des troubles du métabolisme phosphocalcique aient été mis en évidence chez le mouton infesté par *H. contortus* par Shearer et Stewart dès 1933 [19], le métabolisme des minéraux n'a retenu l'attention des chercheurs ni dans le cas de l'infestation par ce même parasite, ni lors de celle par *T. axei*. D'importants travaux leur ont, par contre, été consacrés lors de l'infestation par *O. circumcincta*. Il a été démontré, en effet, que cette infestation s'accompagne d'une diminution de l'absorption du fer [20] alors que la digestibilité du calcium et du phosphore alimentaires diminue de 15% [15]. De même, les dépôts du calcium et du phosphore dans le corps des animaux diminuent de 11%, 25% et 75% chez des moutons recevant 1000, 3000 et 5000 larves par jour respectivement [21]. Sykes et ses collaborateurs [11] ont évalué cette diminution à 40–52 g de calcium et 30–35 g de phosphore selon le degré d'infestation. Les examens histologiques et chimiques de tibias, vertèbres et côtes ont permis à ces auteurs de montrer que les animaux infestés étaient atteints d'ostéoporose.

3. LES TROUBLES DU FONCTIONNEMENT DU TRACTUS DIGESTIF ET LEURS CONSEQUENCES

Les troubles du fonctionnement du tractus digestif chez le mouton infesté par *H. contortus* [22] ou *O. circumcincta* [23] ont récemment fait l'objet de synthèses rassemblant les données actuellement disponibles relatives à ce sujet.

3.1. Perméabilité et activité sécrétoire de la muqueuse abomasale

3.1.1. Activité sécrétoire

Les troubles de la sécrétion acide ont été mis en évidence par les mesures du pH du contenu abomasal et des concentrations en ions Cl^- , HCO_3^- et K^+ dans celui-ci, eu égard à leur rôle dans la formation de l'acide chlorhydrique. Ainsi, il a été démontré que l'infestation par *H. contortus* s'accompagne d'une augmentation de la concentration en ions HCO_3^- dans les 30 min qui suivent l'administration des larves infestantes dans le rumen [24, 25] et du pH à partir du 4^e jour de l'infestation [6, 26], alors que les concentrations en ions Cl^- et K^+ diminuent à partir du 3^e jour de celle-ci [6, 24, 26]. La figure 1 représente l'évolution du pH et des

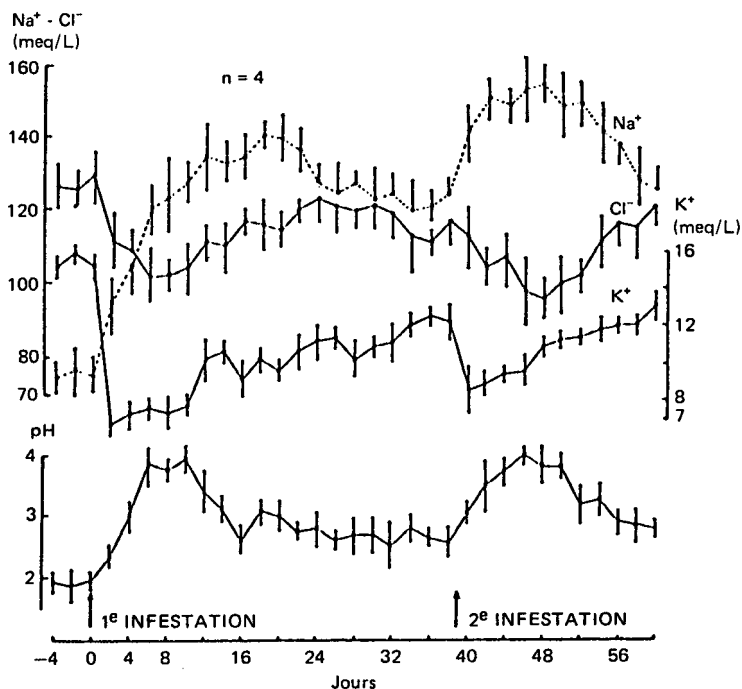


FIG. 1. Modifications biochimiques du contenu abomasal indiquant des troubles de sécrétion acide (augmentation du pH et diminution des concentrations en ions K^+ et Cl^-) et une perméabilité anormalement augmentée de la muqueuse abomasale (augmentation de la concentration en ions Na^+) chez le mouton infesté par *Haemonchus contortus* [6].

concentrations en ions Cl^- et K^+ au cours de deux infestations expérimentales consécutives à 39 jours. De même, l'infestation par *O. circumcincta* provoque une augmentation du pH à partir du 8^e jour de l'infestation [15, 27–29] et une diminution des concentrations en ions Cl^- et K^+ à partir du 6^e jour de celle-ci [27–29]. L'évolution de ces trois paramètres chez le mouton infesté expérimentalement avec 150 000 larves est représentée dans la figure 2. Enfin, les mêmes effets sont observés en cas d'infestation par *T. axei*; mais l'augmentation du pH et la diminution de la concentration en ions K^+ (la seule ayant été mesurée) ne sont significatives qu'à partir du 10^e jour de l'infestation [1, 17].

De nombreux troubles du fonctionnement du tractus digestif peuvent résulter de cette diminution de la sécrétion acide. Celle-ci peut en effet provoquer:

a) une stimulation des sécrétions abomasales en général [30] et celles du pepsinogène et de la pepsine [31] ainsi que celle de la gastrine [32] qui peut elle-même induire une hypersécrétion de l'eau et des électrolytes par le foie, le pancréas et la partie proximale de l'intestin associée à une diminution de leur absorption au

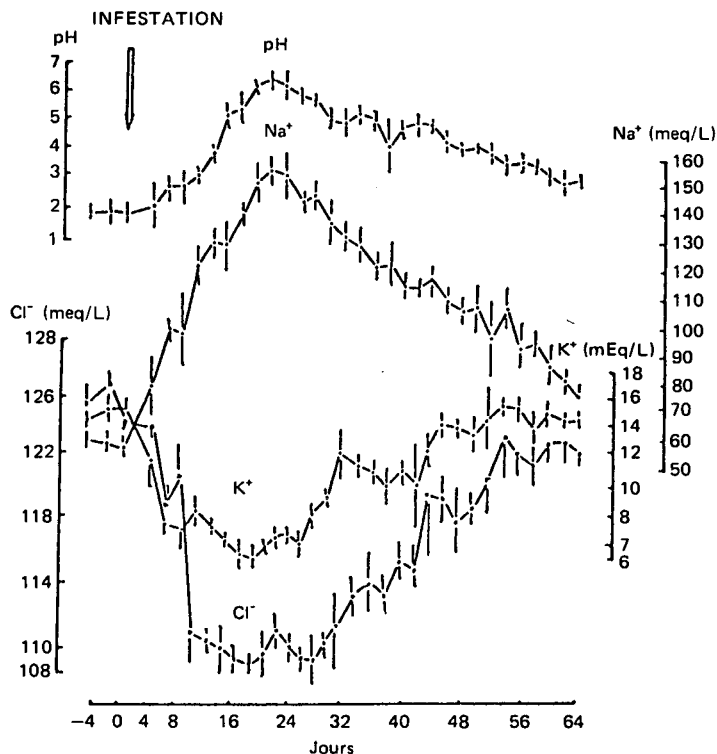


FIG. 2. Modifications biochimiques du contenu abomasal indiquant des troubles de la sécrétion acide (augmentation du pH et diminution des concentrations en ions Cl^- et K^+) et une perméabilité anormalement augmentée de la muqueuse abomasale (augmentation de la concentration en ions Na^+) chez le mouton infesté par *Ostertagia circumcincta* [26].

niveau de l'iléon [33], une stimulation de la motricité de l'intestin et une inhibition de celle du rumen et du réticulum [34];

b) une diminution des sécrétions pancréatiques et intestinales intervenant dans la digestion des protéines [8];

c) des troubles de la motricité gastro-intestinale avec un renforcement des contractions de la région antrale de l'abomasum, une diminution de l'intensité des contractions du réticulum et de l'omasum [30] et une accélération du transit des digesta dans le duodénum [30].

Les conséquences de ces troubles sur la productivité des animaux sont rassemblées dans la figure 3.

3.1.2. Perméabilité de la muqueuse abomasale

Pour apprécier la perméabilité de la muqueuse, les paramètres suivants ont été étudiés: les concentrations en ions Na^+ et Ca^{++} dans le contenu abomasal dont

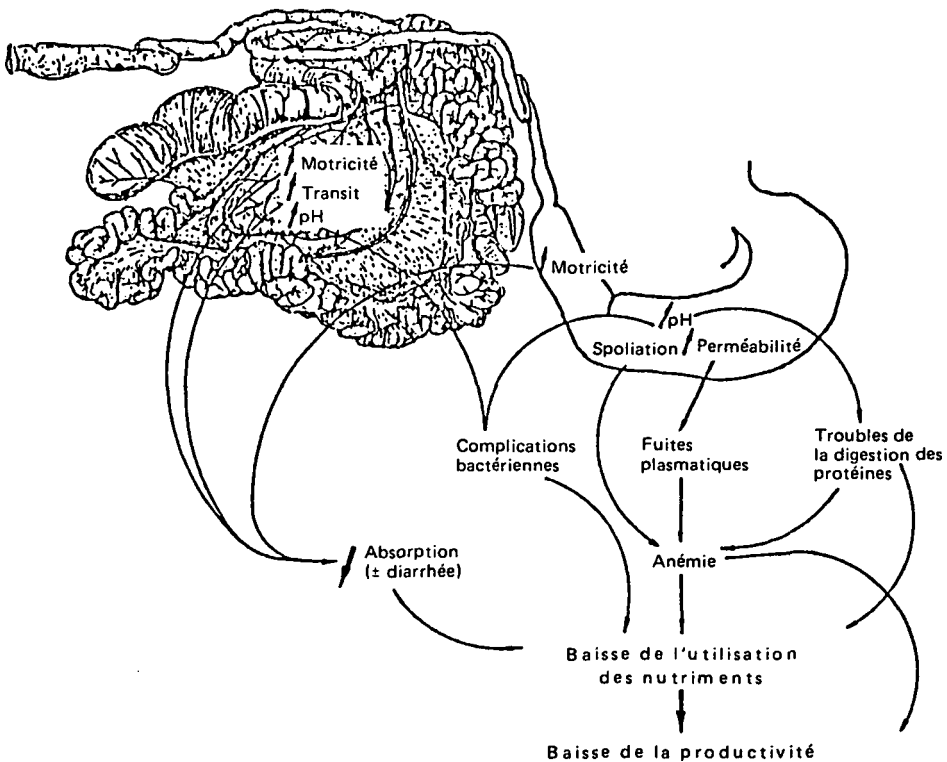


FIG. 3. Quelques effets des troubles de fonctionnement du tractus gastro-intestinal chez le mouton infesté par les parasites de l'abomasum.

l'augmentation indique des fuites des liquides interstitiels [35, 36], le taux du pepsinogène sérique qui augmente si la muqueuse est endommagée [37], et enfin les fuites plasmatiques elles-mêmes au niveau de celle-ci.

Il a été démontré qu'une augmentation importante de la concentration en ions Na^+ se produit dans le contenu abomasal à partir du 4^e jour de l'infestation par *H. contortus* [6, 26], du 6^e jour de celle par *O. circumcincta* [15, 27–29] et du 18^e jour de celle par *T. axei* [1, 17]. L'évolution de cette concentration est représentée dans la figure 1 au cours de l'infestation par *H. contortus* et dans la figure 2 au cours de celle par *O. circumcincta*. La concentration en ions Ca^{++} n'a été étudiée que dans le cas de l'infestation par *O. circumcincta* au cours de laquelle elle subit une importante augmentation à partir du 8^e jour [29]. Ces infestations s'accompagnent également d'une augmentation du taux du pepsinogène sanguin à partir du 5^e jour dans le cas de *H. contortus* [6, 26] et de *O. circumcincta* [15, 27, 28] et du 24^e jour dans celui de *T. axei* [17]. Enfin, les fuites plasmatiques ont été démontrées chez le mouton infesté par *H. contortus* [7, 38] ou par *O. circumcincta* [15, 28, 39] grâce à l'utilisation des techniques de marquages

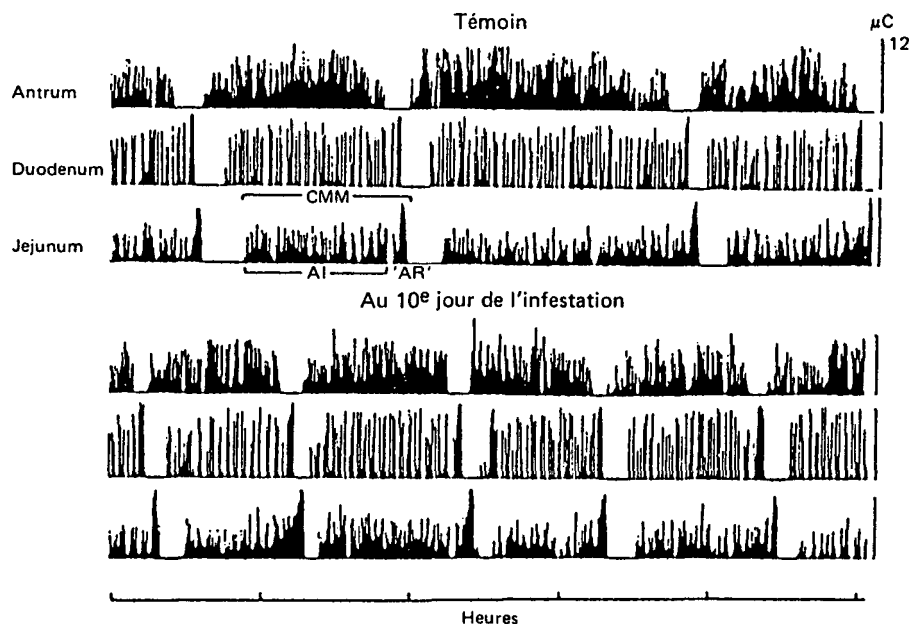


FIG. 4. Réduction de la durée des cycles moteurs (électromyographie) de l'antrum, du duodénum et du jéjunum chez le mouton infesté par *Haemonchus contortus* [46].

radioactifs. Selon Symons et Steel [40], la détermination des protéines plasmatiques dans les fèces des animaux ne reflèterait pas l'importance des fuites au niveau de l'abomasum puisque 80% de celles-ci sont réabsorbés au niveau de l'intestin.

3.2. Motricité du tractus gastro-intestinal

Bien que la motricité du tractus gastro-intestinal commande le transit des digesta dans celui-ci [41, 42] dont dépend en grande partie l'utilisation des nutriments [43, 44], peu de travaux lui ont été consacrés.

L'étude de la motricité gastro-intestinale par électromyographie nous a permis de montrer que l'infestation par *H. contortus* provoque, au niveau abomasal comme au niveau duodénal et jéjunal, une augmentation de la fréquence des cycles moteurs (fig. 4) avec une réduction de leur durée (fig. 5) [45, 46]. Des études concomitantes du débit et du temps de transit des digesta dans le duodénum nous ont permis, d'autre part, de constater que cette stimulation de la motricité gastro-intestinale est associée à une augmentation du premier paramètre et à une diminution du deuxième (fig. 5) [46]. De même, l'infestation par *O. circumcincta* s'accompagne d'une importante augmentation du débit duodénal et d'une accélération du transit des digesta dans l'intestin (fig. 6) [47]. Enfin, Bueno et ses collaborateurs [48] ont montré que l'infestation mixte par *T. axei* et *Chabertia ovina* provoque une désor-

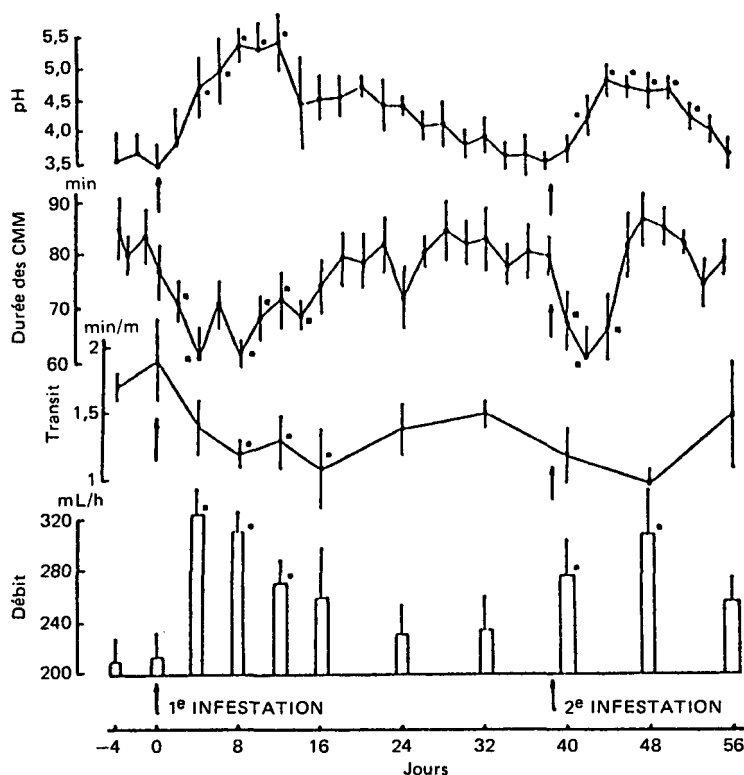


FIG. 5. Augmentation du pH du contenu abomasal, du débit duodénal et de la fréquence des complexes myoélectriques (CMM) migrant sur le duodénum chez le mouton infesté par *Haemonchus contortus* [45].

ganisation du profil moteur de l'intestin associée à une accélération du transit des digesta dans celui-ci.

4. LE DETERMINISME DES TROUBLES DU FONCTIONNEMENT DE L'ABOMASUM

Malgré l'importance des troubles du fonctionnement de l'abomasum et de leurs conséquences sur celui du tractus digestif en entier, leur déterminisme n'a pas bien retenu l'attention des chercheurs. En effet, on se contente d'évoquer, chaque fois qu'il en est question, la destruction des cellules et des glandes par les parasites. Cette hypothèse purement mécanique, bien fondée sans doute, ne peut cependant en exclure d'autres et tout particulièrement une hypothèse biochimique. Les interactions hôte-parasite peuvent, en effet, se manifester par la stimulation ou l'inhibition de la

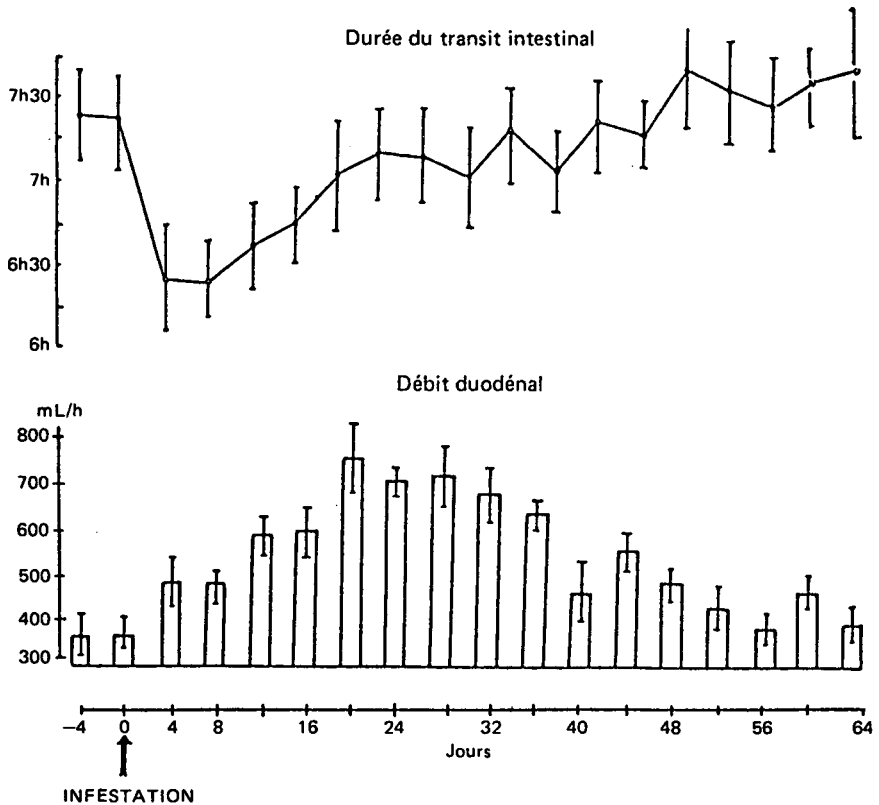


FIG. 6. Augmentation du débit duodénal et diminution du temps de transit des digesta (phase liquide) dans l'intestin chez le mouton infesté par *Ostertagia circumcincta* [47].

synthèse de substance(s) chimique(s) pouvant avoir des effets importants sur le fonctionnement du tractus digestif.

4.1. Insuffisance de l'hypothèse purement mécanique

Trois expérimentations nous ont permis de démontrer l'insuffisance de l'hypothèse purement mécanique. D'abord, la mesure en continu de la différence du potentiel transmurale de l'abomasum (DDP), qui est actuellement reconnue comme le moyen le plus sensible pour apprécier l'état d'une muqueuse digestive et son activité sécrétoire [49, 50], a montré que l'intégrité de la muqueuse abomasale est modifiée à partir des 10 ou 15 min qui suivent l'arrivée des larves infestantes dans le rumen (fig. 7) lors de l'infestation par *H. contortus* [51] ou par *O. circumcincta* [52]. Ensuite, l'étude de la cinétique de l'arrivée des larves dans l'abomasum après leur administration dans le rumen a montré que les premières d'entre elles (moins de trois)

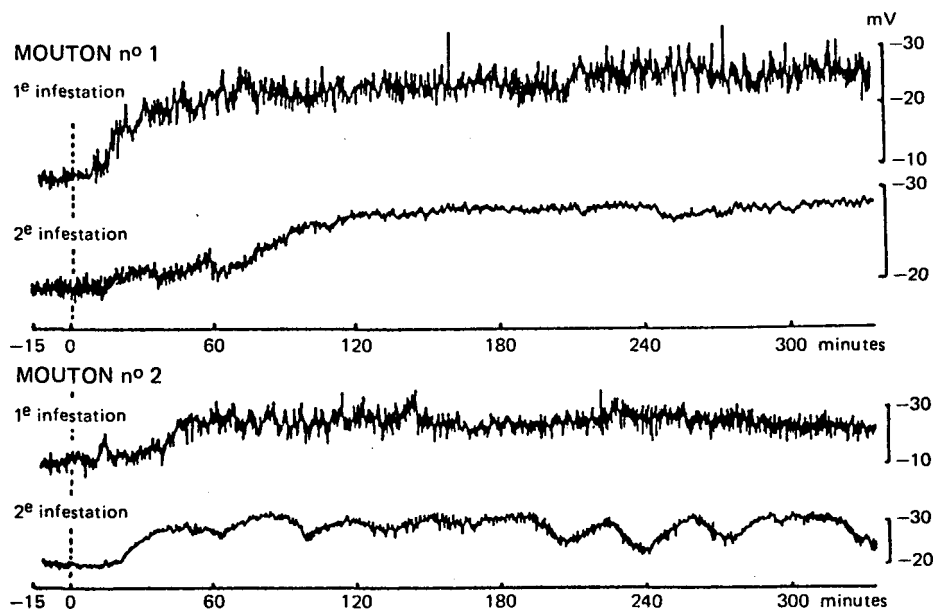


FIG. 7. Augmentation de la différence transmurale de l'abomasum (enregistrement direct) chez le mouton infesté par 25 000 larves de *Haemonchus contortus* directement administrées dans le rumen [51].

y arrivent entre la 20^e et la 30^e min [53]. Ainsi, l'intégrité de la muqueuse abomale est donc affectée alors que moins de trois larves y sont arrivées et probablement même avant. Enfin, le traitement anthelminthique de moutons infestés expérimentalement par *H. contortus* ou *O. circumcincta* depuis 28 jours et chez lesquels sont mesurés la DDP en continu ainsi que le pH et les concentrations en ions Na^+ , K^+ et Cl^- tous les jours, a permis de constater un retour à la normale de tous ces paramètres en moins de 48 heures [52]. Ce délai est trop court pour une éventuelle restauration des cellules et des glandes qui auraient été détruites par les parasites.

4.2. Hypothèse biochimique: rôle des prostaglandines

L'augmentation de la DDP observée au cours de l'infestation de l'abomasum par les nématodes s'oppose à sa diminution, voire même à son annulation, qui se produit dans les cas d'ulcères [49, 50]. Associée à l'augmentation du pH et des concentrations en ions K^+ et Cl^- , cette augmentation indique une stimulation du transport des ions HCO_3^- [54] que nous avons démontrée par ailleurs [25]. Ceci nous a suggéré l'intervention des prostaglandines de la série E (PGE) qui sont de puissants stimulants de ce transport [55, 56]. Ces substances inhibent, par ailleurs, la sécrétion acide par la muqueuse gastrique [57, 58], stimulent la motricité de

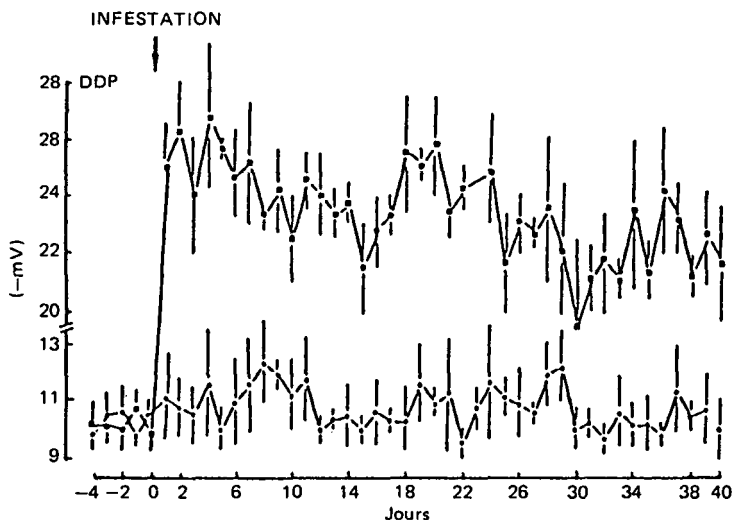


FIG. 8. Modifications de la différence du potentiel transmurale de l'abomasum chez le mouton infesté par 15 000 larves de *Haemonchus contortus* et recevant une administration intraveineuse quotidienne d'aspirine (●—●) ou pas (■—■) [62].

l'intestin, accélèrent le transit des digesta dans celui-ci [59, 60] et induisent une hypergastrinémie [59]. Ces effets sur le fonctionnement du tractus digestif rappellent ceux qui accompagnent le parasitisme de l'abomasum. Ainsi, l'hypothèse qu'une stimulation de la synthèse des PGE accompagne ces infestations devient justifiée. Ceci nous a incité à étudier les effets de ces substances et ceux de l'inhibition de leur synthèse sur la muqueuse abomasale parasitée et sur les parasites eux-mêmes.

Chez les animaux infestés et soumis à une inhibition de la synthèse des prostaglandines (PG) grâce à une administration intraveineuse quotidienne d'aspirine [61], la DDP montre des fluctuations mais ne subit pas de modifications significatives (fig. 8). D'autre part, le développement des parasites, qui atteignent leur maturité sexuelle plus précocement et dont la population est plus importante que chez les animaux n'ayant pas reçu d'aspirine, est favorisé [62].

Au contraire, l'application intra-abomasale de l'acide arachidonique, principal précurseur des PG [63], à des animaux infestés par *H. contortus* ou *O. circumcincta* aggrave les troubles du fonctionnement de l'abomasum provoqués par ces parasites avant de les supprimer dans les 48 heures qui suivent. Elle entraîne en même temps l'élimination d'une proportion importante de la population parasitaire [64].

Ainsi, la destruction des cellules et des glandes par les parasites semble jouer, dans le déterminisme des troubles du fonctionnement du tractus digestif, un rôle secondaire par rapport à celui joué par des médiateurs chimiques, dont les prostaglandines. Les résultats présentés ici montrent que l'intervention de ces substances est mise en jeu dès les premiers contacts (dans le rumen) entre l'hôte et le parasite. Cette

intervention semble faire partie de l'ensemble des réactions développées par l'organisme contre le parasite. Cependant, cette réaction, sûrement néfaste pour celui-ci, a des «effets secondaires» qui ne sont pas toujours favorables à l'hôte.

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USE OF RADIOISOTOPIC METHODS IN STUDIES ON THE PATHOGENESIS OF GASTROINTESTINAL HELMINTH INFECTIONS OF SHEEP

P.H. HOLMES

Department of Veterinary Physiology,
University of Glasgow Veterinary School,
Glasgow, United Kingdom

Abstract

USE OF RADIOISOTOPIC METHODS IN STUDIES ON THE PATHOGENESIS OF GASTROINTESTINAL HELMINTH INFECTIONS OF SHEEP.

Domestic ruminants, including sheep, are frequently infected with a variety of gastrointestinal helminth parasites. As a result their productivity in terms of survival, live weight gain, wool and milk production and reproductive performance can be seriously depressed and cause major economic losses. The mechanisms by which parasites cause impaired productivity have become the subject of intensive study and radioisotopic techniques have played a key role in these studies. Despite the variety of different gastrointestinal parasites which infect sheep, the clinical signs and changes in haemological and blood biochemical indices show a number of similarities. In particular, it has been found that a reduction in the concentration of serum albumin is a frequent finding and anaemia also commonly occurs. The development of simple radioisotopic techniques to measure albumin and red cell breakdown and turnover rates using ^{125}I -albumin, $^{51}\text{CrCl}_3$, ^{59}Fe -transferrin and ^{51}Cr -red cells has enabled accurate quantitation of kinetic changes associated with hypoalbuminaemia and anaemias in a variety of gastrointestinal helminth infections of sheep. As a result of these studies it is apparent that the gastrointestinal loss of blood proteins is a major factor in the pathogenesis of such infections. More recent experiments using cannulated sheep and flow marker techniques have attempted to quantify the amount of lost protein which is reabsorbed from the gastrointestinal tract. Associated changes in protein metabolism have been examined using radiolabelled amino acids to examine tissue protein synthesis in parasitized animals. From such studies it is apparent that there is a net movement of amino acid nitrogen from muscle to the liver and intestines which decreases the availability for growth and milk and wool production. Resultant changes in body composition have been investigated by standard methods of carcase analysis and, recently, by use of neutron activation analysis.

1. INTRODUCTION

Gastrointestinal helminth parasites of sheep are a major cause of economic loss throughout the world, principally as a result of infections with various trichostrongyle species (*Ostertagia* spp., *Trichostrongylus* spp. and *Haemonchus* spp.) and liver flukes (*Fasciola* spp.). The economic impact of these parasites is brought about by losses at times through mortality but more commonly through inferior weight gains,

impaired wool and milk production and poor reproductive performance. To these factors must be added the costs of anthelmintics and veterinary care. Helminth parasites therefore pose a major threat to livestock production and, understandably, many aspects of their control and pathogenesis have been the subject of intense study. In recent years there has been a considerable increase in our knowledge of the pathogenesis of such infections, especially in terms of alterations in host function (i.e. pathophysiology) caused by various gastrointestinal helminths, and radioisotopic methods have played a major role in these studies.

2. EFFECTS ON PRODUCTIVITY

Gastrointestinal helminths can have a range of effects on animal production, depending on the weight of infection and the age, and the nutritional and immunological status of the host.

Change in body weight of the host is the most common criterion for judging the effects of parasites on animal production and reductions in live weight gain of 20 to 60% have been recorded in lambs (e.g. Refs [1-3]).

In addition to changes in body weight, alterations in body composition also occur and these can be of considerable importance in judging the economic impact of gastrointestinal parasitism. Many authors have found that in infected animals the percentage of water was higher and the deposition of fat, protein and skeletal calcium and phosphorus lower than in worm-free controls (e.g. Refs [1, 2]). However, there are major technical difficulties associated with body composition studies in ruminants. Frequently it is necessary to restrict analysis to indicator joints. Alternatively, the whole body or carcass may be homogenized and aliquots taken for chemical analysis. Unfortunately, problems arise since suitable aliquots are difficult to analyse by chemical methods. A new approach which overcomes many of these problems is to use neutron activation analysis. This technique has been developed over the past two decades for analysing the living human body. It provides unique information about changes in body composition in health and disease and has contributed significantly to medical research [4]. In essence it is a two-step process, activation followed by detection. Activation is achieved by neutron irradiation of the body. During neutron irradiation atomic nuclei can undergo reactions which produce new isotopes. Very often these product isotopes are radioactive and can be detected by their characteristic emissions. Generally, analysis is done by comparing the activity in the subject with that of a standard 'phantom' containing known amounts of the elements in question. Radioactivity measurement is usually achieved by delayed analysis in which the subject is quickly transferred after irradiation to a detector system. Such systems normally use NaI scintillation crystals to quantify the induced radioactive products by their gamma ray emissions. Neutron activation has recently been applied to the measurement of total N, Ca, P, Na and Cl in sheep carcasses with considerable success [5]. It also offers the possibility of conducting serial

whole body analyses on live animals. With the development of simpler equipment this somewhat specialized technique could become more widely available.

It has been recognized for some time that gastrointestinal parasites can adversely affect wool production. The interrelationship between wool production and the level of exposure to trichostrongyle infections has recently been studied in weaner lambs. The threshold level of exposure for impairing wool growth was found to be between 950 and 3000 *T. colubriformis* larvae/week, with the maximal effect occurring during the first 12 weeks [6]. In the case of *O. circumcincta* an intake of more than 1200 larvae/week was required to produce significant effects [7]. When lambs were concurrently infected with *T. colubriformis* and *O. circumcincta*, production losses were exacerbated and wool growth was reduced by up to 66% [8].

Liver fluke infections can also cause significant reductions in wool growth [9]. The effects on wool production are correlated with the adult fluke burden and may be affected by as few as 30 adult *F. hepatica*. Not only is the quantity of wool reduced but the quality is also decreased.

In the recent past there has been considerable interest in the possible effects of gastrointestinal helminths on milk production. Most studies have been conducted in dairy cattle but a few experiments have been carried out on housed sheep to evaluate the impact of nematode parasites. In one study milk production was reduced by 17% in ewes infected with *O. circumcincta* [10]. In another recent experiment ewes infected with *H. contortus* showed a marked weight loss and gave 23% less milk than control animals, despite a similar feed intake [11]. Reductions in milk production in sheep at pasture exposed to *O. circumcincta* have also been reported [12].

It is generally accepted that gastrointestinal helminths impair reproductive performance, although the precise effects are largely unknown due to the absence of suitable studies.

Gastrointestinal parasites are associated with a range of clinical signs, including failure to gain weight or weight loss, poor bodily condition, inappetence and often diarrhoea.

Reduction in voluntary food intake, or inappetence, is an important feature of parasitism and is widely recognized as a major factor in the pathogenesis of such infections. The degree of inappetence can vary considerably. In moderate to heavy infections, food intake can be reduced by 20% or more but in lighter infections it may be less obvious. The degree of inappetence has been shown to vary not only with the level and duration of parasitism but also with the level of protein nutrition [13, 14]. Despite the obvious importance of inappetence in parasitized animals, it is still not known why it occurs. Numerous explanations have been suggested, although none of them is entirely satisfactory. A review highlighting the limited state of current knowledge on this topic has recently been presented by Symons [15].

There are a number of characteristic changes in blood constituents, the most notable being hypoalbuminaemia and, commonly, a depression in the serum total protein concentration. In severe cases of hypoalbuminaemia facial oedema may develop as, for example, in lambs infected with *H. contortus* [16]. This parasite also

causes anaemia, which can be severe and is the major factor in the pathogenesis of haemonchosis. Abomasal parasitism is associated with characteristic increases in the concentration of pepsinogen in the blood and this finding has become the basis of a useful diagnostic test for abomasal trichostrongylosis.

3. ENDOGENOUS LOSSES OF PROTEINS

A distinctive feature of gastrointestinal parasitism is the consistent association with losses of protein into the gastrointestinal tract. The proteins represent plasma (and red cells), exfoliated epithelial cells and mucus. Most studies have been concerned with measurements of plasma protein and red cell losses into the gastrointestinal tract and radioisotopic techniques have been an invaluable aid in these investigations.

The rationale and applications of radioisotopic techniques to the study of red cell and plasma protein metabolism in helminth diseases of sheep have been reviewed by Dargie [17]. In essence, radioisotopic techniques permit detailed evaluation of anaemia and hypoalbuminaemia by providing data on pool sizes and distribution, rates of turnover and sites of loss.

Two radioisotopes, ^{51}Cr and ^{59}Fe , have proved to be particularly useful in establishing how helminth parasites such as *F. hepatica* and *H. contortus* cause anaemia. Red cells labelled with ^{51}Cr can be used to measure the circulating red cell volume, red cell survival and the losses of red cells into the gastrointestinal tract. Following intravenous injection of ^{59}Fe as ferric citrate, it is possible to obtain direct estimates of erythropoiesis from the rate of disappearance of ^{59}Fe from the plasma and the appearance of ^{59}Fe in circulating red cells. When such techniques were applied to the study of sheep infected with *F. hepatica* or *H. contortus*, it was found that red cells labelled with ^{51}Cr disappeared more rapidly from the circulation than in uninfected controls and the rate of removal correlated closely with the parasite burden [18, 19].

Furthermore, the shortened red cell survival in parasitized animals could be accounted for by the appearance of radioisotopes in the faeces, indicative of the loss of red cells into the gastrointestinal tract as a result of haematophagic activity by the parasites. Additional support for the view that the anaemia is primarily due to the feeding activities of the parasites has come from studies of the relationship between the onset of blood loss and the development of clinical anaemia following infection [20, 21] and from observations that blood loss drops dramatically following anthelmintic therapy [22, 23].

Studies using ^{59}Fe demonstrated that, in the face of the high levels of blood loss into the gastrointestinal tract, erythropoiesis was usually increased to up to three times the normal. Possible exceptions are in chronic infections in which, as a result of continued red cell losses and severe inappetence, an iron deficiency may develop [3, 24].

Gastrointestinal helminth infections are associated with changes in plasma protein concentrations. These changes are generally dose-related and in moderate to heavy infections take the form of pronounced hypoalbuminaemia accompanied by an increase in one or more of the globulins.

Plasma proteins, like other body proteins, are in a state of dynamic equilibrium between synthesis and catabolism and evaluation of these processes in parasitized animals has been greatly assisted by the use of radioisotopic techniques. The majority of studies have utilized albumin labelled in vitro with radioiodine (^{125}I or ^{131}I). Such preparations can be used to measure both body pool sizes and the catabolic rate of albumin.

The results of such studies in sheep infected with fascioliasis [17, 22], haemonchosis [24, 25] and ostertagiasis [26, 27] were remarkably similar. In all the infected sheep, which were hypoalbuminaemic, the most significant feature was their high fractional catabolic rate of albumin relative to control sheep (i.e. two to three times the normal levels). There was also an alteration in the distribution of albumin between the intra- and extravascular pools, with reductions in the former being much less than in the latter.

One possible cause of the hypercatabolism of albumin is loss of plasma proteins into the gastrointestinal tract. Unfortunately, radioiodinated protein cannot be used to measure protein losses into the gut because of breakdown and absorption of the label. For this reason other markers, such as ^{51}Cr (as $^{51}\text{CrCl}_3$) or radioiodinated PVP, have had to be used since these preparations are not subject to the same restraints as iodine-labelled plasma proteins and can be used to quantify protein losses into the gastrointestinal tract.

Such preparations have been used in large numbers of studies in sheep with gastrointestinal helminths and they have all consistently demonstrated that large quantities of plasma proteins are lost into the gastrointestinal tract in experimentally [17, 22, 24, 26] and naturally infected sheep [27].

In most experimental studies on the pathophysiology of ovine helminthiasis, parasite-naïve lambs have been used and the possible influence of immunity to reinfection following repeated challenge has not been a complicating factor. However, there have been indications from several studies that older animals previously exposed to field [28] and experimental [29] trichostrongyle infections, though showing low or negative faecal egg counts and low worm burdens, suffer production losses when under subsequent larval challenge. In Anderson's studies where the larval challenge was primarily with an abomasal parasite, *Ostertagia circumcincta*, the plasma pepsinogen levels were also markedly elevated. The latter finding may be of significance since earlier studies have indicated that elevated plasma pepsinogen values reflect histopathological changes in the host associated with increased permeability of the parasitized abomasal mucosa which permits increased diffusion of pepsinogen into the blood and a leakage of plasma proteins into the gastrointestinal tract [26, 30]. It is therefore possible that the production losses reported could be the result of plasma protein losses into the gastrointestinal tract.

This hypothesis was examined by Yakoob et al. [31] in ewes which had previously grazed contaminated pasture. It was confirmed using radioisotopic techniques that immune ewes challenged with a mixed trichostrongyle infection (90% *O. circumcincta*) showed marked pathophysiological disturbances and, in particular, a doubling of plasma pepsinogen levels, albumin catabolism and losses of plasma proteins into the gastrointestinal tract. If larval challenge was continued, these effects could persist for several months [32].

The results of experiments with the intestinal nematode *T. colubriformis* have been inconclusive. In studies by Barger and Southcott [29], wool growth was reduced by larval challenge of naturally immune sheep. However, studies in which lambs had been made resistant to infection by vaccination with gamma-irradiated larvae failed to demonstrate any effects on productivity as a result of continuous larval challenge. Nevertheless, the onset of challenge of immune sheep with *T. colubriformis* larvae has been shown to be associated with a transient rise in enteric losses of protein [33, 34].

These initial experiments indicate that the response of immune sheep to larval challenge varies with the species of parasite and the method of immunization. Further studies are required to elucidate the mechanisms involved, which may be associated with an increased permeability of the mucosa created by an immune reaction induced by the ingested larvae. A possible basis for this lesion may be the development of a hypersensitive state in the mucosa, as previously demonstrated by the leak of plasma proteins during the self-cure reaction of rats infected with the nematode *Nippostrongylus brasiliensis* [35].

It is also important to investigate the interrelationships between nutrition and the development of immunological responses to gastrointestinal helminths. For example, it has recently been demonstrated that lambs given a high protein diet and subjected to repeated infections are more likely to develop resistance to reinfection than animals on a low protein diet [36]. Preliminary results suggest a similar effect in lambs continuously infected with *T. colubriformis* and given a high or low plane of nutrition [37].

4. FATE OF PROTEINS LOST INTO THE GASTROINTESTINAL TRACT (GIT)

One important question arising out of these studies is — what is the fate of protein lost into the GIT? It should be appreciated that measurements using radioisotopically labelled proteins, because of enzymic action in the GIT, do not show the amount of blood protein lost from the body into the faeces but only give the amount lost into the gastrointestinal tract. Similarly, nitrogen balance studies, although clearly demonstrating that reduced nitrogen retention is a characteristic effect of helminth infections, do not permit direct assessment of the levels of endogenous protein loss or the fate of proteins within the tract. Such information can only be obtained from

animals with in-dwelling gastrointestinal cannulae. Some recent experiments using cannulated sheep and flow-marker techniques have attempted to quantify the amount of lost protein which is reabsorbed from the gastrointestinal tract. One study by Poppi et al. [38] in lambs infected with *T. colubriformis*, in which digestion and absorption of protein were estimated by the disappearance of ^{35}S -labelled microbial protein, suggested that plasma protein only accounted for one-fifth of the nitrogen lost into the small intestine, i.e. 1 g N of an estimated total of 5 g. Of this, only 60% was judged to have been absorbed further down the small intestine. In contrast, a study by Rowe et al. [39] using sheep infected with *H. contortus* and sheep 'sham' parasitized by infusing a similar amount of blood (200 mL/d) into the abomasum as that lost by infected sheep showed that the blood loss accounted for virtually all the increase in N flow into the duodenum and this was all absorbed by the end of the ileum. The difference in the results of these two studies is partly explained by the different locations of the parasites, namely small intestine and abomasum, and the fact that *H. contortus* infection results in the loss of whole blood, but may also be due to differences in technique. Both studies have provided useful new data on the consequences of endogenous loss but both require confirmation using larger numbers of animals at different stages of infection.

The importance of sloughed cells and mucin in endogenous protein loss is still a matter of speculation. In both *T. colubriformis* and *T. vitrinus* infections of lambs [40, 41] the elongated crypts contain increased mitotic figures which are indicative of increased cell proliferation. Furthermore, it has been found that ^3H -thymidine uptake by the abomasal mucosa is increased in sheep infected with *H. contortus* [39] which, again, is indicative of increased cell turnover. Another possible source of endogenous protein loss is an increase in mucus production, and proliferation of goblet cells at the site of infection has been observed with ostertagiasis [42, 43]. Since it is thought that mucus is neither digested nor absorbed in the small intestine, this may form a major source of extra ileal nitrogen in sheep with intestinal infections. However, although goblet cell hyperplasia has been observed in some lambs infected with *T. vitrinus* [44], it is not commonly reported in cases of intestinal helminthiasis of ruminants.

5. PROTEIN DEPOSITION AND TISSUE METABOLISM

Attempts to evaluate protein deposition and tissue metabolism in parasitized hosts have been limited. One approach has been to examine feed utilization in infected animals. For example, in lambs chronically infected with *O. circumcincta* Sykes and Coop [2] and Coop et al. [45] demonstrated a significant reduction in the deposition of fat and protein in infected animals compared with pair-fed controls.

A series of studies by Symons and his co-workers to examine tissue protein synthesis using ^{14}C -L-leucine and ^{14}C -L-tyrosine in parasitized animals is particularly relevant. Early experiments indicated that protein synthesis was reduced in skeletal

muscle of pair-fed controls as well as infected sheep [46]. However, in later experiments with tyrosine the fractional synthetic rate (FSR) and protein synthesis/d by the semi-tendinosus muscle and kidney cortex were reduced, and the rate of protein synthesis by the liver was increased (in infected animals but not in pair-fed controls), although the level of inappetence was much less than in the earlier studies [47]. It has also been demonstrated that protein synthesis by homogenates of wool follicles of sheep with trichostrongylosis was depressed by over 50% [46].

Measurements of protein synthesis by the gastrointestinal tract have, for technical reasons, so far been restricted to guinea-pigs infected with *T. colubriformis*. In such animals the amount of protein synthesis/d was increased in both the small and large intestine [48]. It is important that this finding is confirmed in ruminants, as these sites of increased protein synthesis may be an important cause of reduced nutrient utilization by infected sheep and cattle.

As a result of such studies, Symons [15] concluded that, due to inappetence, gastrointestinal losses of protein and increased rates of gastrointestinal tissue protein metabolism, there is a net movement of amino acid nitrogen from muscle, and possibly skin, to the liver and gastrointestinal tract which decreases the availability for growth and milk and wool production. However, it is still not possible to construct accurate balance sheets of protein synthesis in parasitized ruminants nor is there any detailed information on the underlying mechanisms, including hormonal, which may be responsible for the changes in protein synthesis.

6. MINERAL METABOLISM

Radioisotopic techniques have also played a part in examining changes in mineral metabolism in parasitized ruminants. A number of workers have demonstrated that skeletal growth and mineralization are impaired in sheep infected with gastrointestinal helminths [1, 49–51].

There are several possible causes for impaired skeletal growth in parasitized sheep and these appear to be related to the level and site of infection.

In lambs infected with the abomasal parasite *O. circumcincta* the skeletal changes have been attributed to deficiencies of protein and energy induced by the parasite leading to a matrix osteoporosis [51]. The results of experiments using ^{45}Ca and ^{32}P in infected sheep fitted with abomasal and ileal cannulae support this view, since neither absorption nor secretion of phosphorus and calcium in the digestive tract was found to be affected [52].

In sheep infected with *T. colubriformis* and other intestinal nematodes, reduced bone matrix synthesis due to an induced protein deficiency probably also occurs but it is exacerbated by a reduction in absorption of P [1, 52, 53]. There are also increased losses of endogenous P and Ca as a result of intestinal parasitism [52]. The net effect of this is to induce a P deficiency, leading to a reduced flow of salivary P and a reduction in the plasma concentration of P. The effect on Ca metabolism was found to be limited to an increase in endogenous faecal excretion.

7. WATER AND ELECTROLYTE BALANCE

Diarrhoea is commonly reported in parasitized ruminants and especially in those at pasture. In experimental infections increases in the water content of the faeces leading to frank diarrhoea have been reported in moderate to heavy infections of all the important gastrointestinal helminths except *Haemonchus* spp. and *Fasciola* spp. In abomasal infections with *Ostertagia* spp. the onset of diarrhoea has been found to coincide with the maturation of larvae into young adults. This also corresponds with the time that many of the pathophysiological changes such as inappetence, plasma protein losses and negative nitrogen balance are particularly pronounced [26, 54]. Experiments with tritiated water (TOH) have shown that water turnover is also markedly altered at this stage of infection [55].

Various other studies, some using TOH, have demonstrated that the water retention of parasitized ruminants is commonly higher than in parasite-free controls [16, 56, 57]. Such changes in water retention clearly demonstrate that tissue loss attributable to parasitic infections cannot be reliably determined from changes in body weight alone.

8. GENERAL CONCLUSIONS

Gastrointestinal helminths are a major cause of impaired productivity in sheep. Radioisotopic techniques have played a major role in identifying many of the important factors in the pathogenesis of such infections. In particular, they have been instrumental in identifying increased losses of plasma proteins into the gastrointestinal tract as a consistent and essential part of the pathogenic process.

Radioisotopic methods have also been essential in examining changes in protein digestion and synthesis, mineral metabolism and water balance in parasitized animals.

However, many aspects of the pathogenesis of gastrointestinal helminths still require examination and there is no doubt that radioisotopic techniques will continue to play a major role in such investigations.

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USE OF ISOTOPIC METHODS IN NUTRITION $\times$ PARASITE INTERACTIONS*

D.P. POPPI

Department of Animal Sciences,
Lincoln College,
University College of Agriculture,
Canterbury, New Zealand

Abstract

USE OF ISOTOPIC METHODS IN NUTRITION $\times$ PARASITE INTERACTIONS.

The mechanisms by which parasites influence host metabolism are reviewed and the use of various isotopic techniques in elucidating this is illustrated. Emphasis is placed on identification and quantification of the pathways of altered metabolism, knowledge of which enables speculation on the nutritional strategies to minimize the effects of infection on the host. Most data of this type have been derived from lambs infected with *T. colubriformis*. These studies have observed the depression in N and energy retention in infected animals, with increased urinary and sometimes faecal N excretion. This has been associated with increased flows of N from the ileum of infected animals, not related to malabsorption of protein but to increased endogenous protein loss into the small intestine. This was largely in the form of mucus and sloughed epithelial cells rather than plasma protein. Diversion of protein synthesis away from muscle, bone and wool synthesis to liver and gut tissue synthesis has been demonstrated. The cause of reduced energy retention in infected animals is not clear and the results are conflicting, indicating either a reduction in energy digestibility or a depression in the use of metabolizable energy for growth. The same approach has been used to study aspects of calcium and phosphorus metabolism. *T. colubriformis* infection appears to have a major effect on phosphorus absorption and recycling via the saliva. Nutrition and body condition of the host markedly influence parasite establishment and pathogenicity, with better nutrition and body condition being of positive benefit in minimizing the effects of infection. Recently, supplementation of infected animals with protein or phosphorus has resulted in positive growth responses, illustrating that it is possible to manipulate the growth of infected animals by specific nutrient manipulation of the diet.

1. INTRODUCTION

Internal parasites occur in animals worldwide and cause large losses in production. Subclinical parasitism probably accounts for greater production losses than clinical levels of parasitism, but little work has been directed at the former aspect [1-3].

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A number of recent reviews has extensively covered the effects of internal parasites on the host [1-4]. This review concentrates on the mechanism by which the parasites affect host metabolism and outlines the quantitative changes that occur, particularly in lambs exposed to *T. colubriformis*. It also concentrates on the effects of subclinical infections. Identification and quantification of the pathways of altered metabolism are important and use of isotopes to determine this approach is essential. No attempt has been made to describe the techniques, as they have been adequately described in previous papers and reviews [5, 6]. Knowledge of the pathways of altered metabolism enables assessment of the opportunities for nutritional or other manipulation of the adverse effects of parasitism.

2. INTAKE

A major effect of parasitism is to cause varying degrees of anorexia in the host [3, 7]. This occurs particularly when large numbers of larvae are administered to the host over a short time period, a usual experimental approach in early studies. Of more interest is the effect of low continuous larval intakes. Intake depression follows the time course pattern outlined in earlier studies using large single larval intakes, but depression is often slower to develop and quite variable between animals. Figure 1 [8] illustrates the variation apparent in lambs exposed to *T. colubriformis* (2500 larvae/d). Such variation introduces difficulties in experimental design when

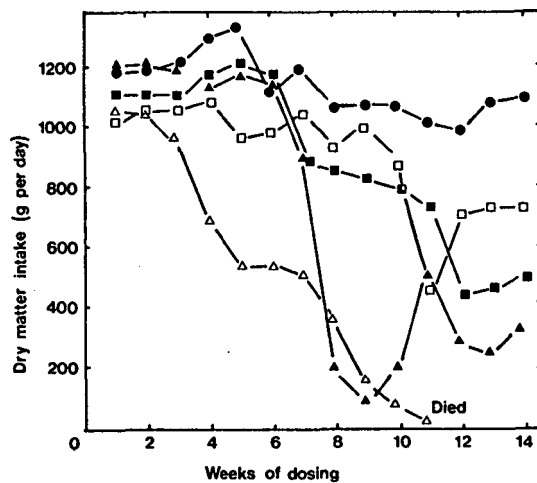


FIG. 1. Individual food intakes of five infected lambs [8].

studying nutrition $\times$ parasite interactions, but more importantly it markedly affects the degree to which production is depressed in a flock, and hence makes detection more difficult. Second, it complicates management in that growth of individuals is variable, which in turn complicates forward planning related to feed supply and slaughter times.

The cause of intake depression is not clear. Hormones, in particular cholecystokinin (CCK) and gastrin, have been implicated in intake depression associated with *T. colubriformis* and *O. circumcincta* [9, 10]. Symons and Hennessy [9] have shown that CCK infusion can depress intake and that CCK levels are elevated in infected lambs; they concluded that this was a major factor involved in anorexia associated with parasitism. The precise role of CCK in intake regulation in non-infected animals is not clear and the site of release and action of CCK is also debatable [11]. Similar detailed work does not appear to have been done for other hormones, but Titchen [10] has drawn attention to the changes in a range of hormones, e.g. gastrin and somatostatin, with the possible effects on intake, digestive tract motility and metabolism. Use of radioimmunoassay techniques and isotopically labelled compounds in determining concentration and metabolic action in these studies has been well documented [6, 12].

Pain or neural feedback mechanisms do not appear to have been studied. Animals experimentally infected with parasites often grind their teeth. Such behaviour is associated with pain after abdominal surgery. In this regard studies by Gregory et al. [13] have indicated interference with digesta clearance in the proximal intestine of lambs exposed to a low continuous larval intake of *T. colubriformis*, with the duodenum and proximal jejunum constantly distended with digesta. This observation also appears to occur with other mammalian species infected with intestinal parasites [7]. Such a phenomenon would be expected to activate CCK production, but could also give rise to increased neural discharges either contributing to satiety [14] or indicating pain. Disturbances in digestive tract motility patterns with resultant increased digesta flow occur with clinical infections of *H. contortus* in sheep [15]. Titchen and Anderson [16] have outlined the possible neural and hormonal pathways involved in ostertagiasis.

Alternative theories for the observed intake depression have been proposed. Sykes [3] has postulated that intake depression may be related to nutrient requirement in that parasitism induces a calcium and/or phosphorus mineral deficiency, with a concomitant effect on skeletal development. He proposed that lack of skeletal development influences nutrient requirement and hence intake by infected animals. Keymer et al. [17] have proposed that 'learned taste aversion' may contribute to anorexia. In their experiments rats exposed to *N. brasiliensis* infection while on a diet with a particular flavour subsequently rejected that diet in favour of another when offered free choice, the implication being that they associated the period of infection with food flavour. The extent to which this contributes to diet selection and food intake in parasitized ruminants is not known, but there is evidence for taste aversion in lambs on roughage diets which are unbalanced in amino acid composition [18].

3. PROTEIN METABOLISM

Effects on animal metabolism other than those caused by differences in intake can be examined by pair feeding control and parasitized animals. With regard to protein metabolism these studies have generally shown an increase in urinary N excretion and variable effects on faecal N excretion associated with parasitism [1, 3]. Sykes and Coop [19, 20] and Coop et al. [21], using the comparative slaughter technique, have shown that these changes are associated with a depression in body protein and fat deposition. Such results indicate on a gross basis what is happening to nutrient metabolism in a parasitized animal but give no indication of the mechanism of the altered metabolism, e.g. is it associated with changes at the tissue level or in alterations in digestion and absorption? Roseby [22] made the first attempt to quantify the changes in N metabolism associated with parasitism and although his estimates were limited by the techniques and data available at the time the approach was sound and is recommended. Nolan [23] has outlined in more detail the quantitative pathways of N metabolism in non-infected animals.

The role of parasitism on digestive tract metabolism and body protein turnover are examined in the following subsections, largely using examples from animals infected with *T. colubriformis*.

3.1. Site of protein digestion

Increased urinary N could arise from a shift in the site of digestion from the small intestine to the large intestine, with subsequent absorption of NH_3 and excretion in the urine. Animals fistulated at the abomasum or proximal duodenum and the terminal ileum may be used to partition digestion. Digesta flow and any constituent nutrient can be measured by reference to dual phase markers, ^{51}Ch -EDTA and ^{103}Ru -phenanthroline [24, 25]. Other rare earth markers can be used [26–28].

Using these techniques it has been shown that abomasal parasitism was associated with increased N flows into the duodenum of approximately 6 g N/d (*H. contortus* [29] and 3 to 8 g N/d (*O. circumcincta* [30]). In the case of *H. contortus* infection this did not result in increased ileal N flows [29], but in *T. colubriformis* infection either alone or in combination with abomasal infection an increase of up to 2.5 g non-ammonia N(NAN)/d [31] and an increase from 0.4 to 5 g N/d were observed to flow past the ileum [32–34]. The order of the increase is similar to that observed in urine and faecal N excretion.

Increased ileal flows of NAN and N could arise from malabsorption in the small intestine and/or increased endogenous loss into the small intestine.

3.2. Protein absorption in the small intestine

Because of the massive villous atrophy which occurs in the small intestine, particularly at the site of parasite habitation, it might be thought that malabsorption

TABLE I. TRUE DIGESTION AND ABSORPTION OF PROTEIN IN THE SMALL INTESTINE OF CONTROL AND INFECTED ANIMALS^a

Parasite/Protein	Weeks post-infection	Control	Infected	Refs
Whole small intestine				
<i>T. colubriformis</i>				
¹⁴ C chlorella protein	—	0.65	0.65	[35]
<i>T. colubriformis</i>				
³⁵ S rumen bacteria	5–6 11–12	0.71 0.69	0.71 0.66	[32, 37]
<i>T. colubriformis</i>				
³⁵ S rumen bacteria	12–13 18–20	0.72 ^b 0.72 ^b	0.64 ^c 0.66 ^c	[33]
Sections of small intestine				
<i>T. colubriformis</i> + <i>O. circumcincta</i>				
¹²⁵ I albumin				[36]
1/5 of distance along small intestine	6 17	0.06 0.11	0.01 0.12	
Whole small intestine	6 17	0.89 0.88	0.86 0.86	

^a Different superscripts denote significant differences ($P < 0.05$).

occurs under parasitism. Most techniques employed to measure partitioning of digestion and absorption only measure net absorption, but these changes can be influenced by endogenous loss. The technique of injecting a labelled protein source and an indigestible marker immediately before the absorption site (e.g. abomasum or proximal duodenum) and collecting digesta just distal to the site (e.g. terminal ileum) enables true digestion and absorption to be calculated by examining the change in ratio of the markers at the two sites [32, 33, 35, 36].

Such a procedure has shown that malabsorption is not a problem under these forms of parasitism, although in one report [33] a depression in true protein absorption in infected animals was observed (Table I [32, 33, 35–37]).

The type of protein source used influences the absorption values obtained (Table I). Carbon-14 chlorella protein [35] and ^{35}S bacterial protein [32, 33] have been used on the basis that this protein represents the major protein type entering the small intestine. However, since malabsorption appears not to be a problem under parasitism, emphasis has shifted to endogenous protein. For this reason Bown et al. [36] used ^{125}I albumin and obtained a much higher value of absorption than bacterial protein (0.87 versus 0.7) but one which agreed with independent estimates in normal animals of total endogenous protein absorption [38]. The actual values are important to calculate estimates of endogenous protein lost into the tract; these are discussed in subsection 3.3.

The data of Bown et al. [36] are important because for the first time they looked at mixed nematode infections and the possibility that concurrent abomasal and intestinal parasitism, with associated elevated abomasal pH, may have an enhanced detrimental effect on absorption. Clearly this was not the case (Table I).

Because of the intestinal damage and villous atrophy the question arises as to whether there is a distal shift in absorption away from the parasitic area towards the ileum. This was examined by Bown et al. [36] who used animals fistulated at the abomasum, one-fifth of the way along the small intestine and at the terminal ileum (Table I). It was observed that even in control animals the duodenum and proximal jejunum were not important for protein absorption, so it is not surprising that parasitism has little effect on protein absorption. Such an effect may be inferred from the studies of Ben-Ghedalia et al. [39] who observed that the mid-region of the small intestine was the major absorption site as it was associated with the appropriate digesta pH for protease activity. These results also help explain the lack of an effect with *H. contortus* infections and clearly shift the emphasis to endogenous protein loss as the source of the extra ileal NAN and N flow.

3.3. Endogenous protein loss into the digestive tract

Quantifying endogenous protein loss has always been a difficult task. In studies on parasitism, plasma and whole blood loss into the digestive tract have been examined in detail because of the associated clinical signs of anaemia and hypoalbuminaemia.

Such studies have generally used one of two techniques. Intravenous injection of $^{51}\text{CrCl}_3$, with subsequent recovery in the faeces, has given estimates of total plasma protein loss into the digestive tract [40]. The $^{51}\text{CrCl}_3$ molecule associates almost exclusively with the plasma proteins and not with the red blood cells [41]. Alternatively, intravenous injection of iodinated albumin (^{125}I or ^{131}I) can provide information on albumin catabolism and net loss of albumin [5]. Estimates of red blood cell loss into the gut can be obtained from faecal excretion of ^{51}Cr -labelled red blood cells obtained by using anionic hexavalent chromate as the label because it adsorbs strongly to red blood cells [41]. Iron-59 can be used to estimate erythropoiesis [5]. Abbott et al. [42] illustrated in their study on haemonchosis in Merino

TABLE II. CALCULATION OF EXTRA ENDOGENOUS PROTEIN LOSS INTO THE DIGESTIVE TRACT OF LAMBS INFECTED WITH *T. colubriformis* [32, 37]

Extra ileal NAN (g/d)	Extra endogenous protein N loss ^a (g/d)	Extra plasma protein N loss ^b (g N/d)	Extra mucus and sloughed epithelial cells ^c (g N/d)
0.7-2.2	5.4-16.9	0.9-1.5	4.5-15.4

^a Assuming absorption co-efficient of endogenous protein of 0.87 (extra ileal NAN)/0.13.

^c By difference (a - b) and assumed to be mucus and sloughed epithelial cells.

and Scottish Blackface lambs how these techniques can be used to examine the pathophysiology of infection.

In terms of examining N transactions in infected animals it is the true loss or exchange which occurs rather than the net loss that is important. Various studies have shown the large two- to threefold increase in plasma protein loss observed under parasitism (Table II [32, 37] and Ref.[1]), the level of which is dependent on the parasite species and level of larval intake [43-45]. Blood loss under haemonchosis is also increased, markedly increasing protein loss into the gut sixfold [1].

Using data on ileal NAN flow and true absorption of endogenous protein, the total endogenous protein loss may be estimated (Table II). It is apparent that plasma endogenous protein loss is the minor source of endogenous loss into the gut and that other sources, presumably mucin and sloughed epithelial cells, are more important. Quantifying these sources directly has proved elusive. Qualitative estimates of increased cell proliferation along the full length of the small intestine, not just at the site of infection, have been derived by noting the increased mitotic rate of crypt cells [46] but no quantitative estimates are available. Rowe et al. [29] used tritiated thymidine to quantify increased abomasal cell wall turnover in lambs infected with *H. contortus*. Mucin loss has also proved difficult to quantify and it has been suggested that the cyst(e)ine component is poorly absorbed [4, 38], but no data are available to validate this. If it is low, then the calculations relating to endogenous loss may be over-estimates as they rely on a relatively high absorption coefficient for endogenous protein (Table II).

3.4. Consequences of protein exchange down the digestive tract

The consequences of these exchanges down the digestive tract are that protein absorption from the diet is not affected by parasitism, but the increased endogenous protein loss into the tract and its subsequent resorption have increased the endogenous

protein recycling (deamination in the tract and synthesis at the cell level) by approximately 0.4 to 1.2-fold (assuming 12.6 g endogenous protein N absorbed per day in control animals [38] and using the values from Table II). This inevitably requires extra energy. A second consideration is that since the endogenous protein is not completely reabsorbed approximately 0.13 of that going into the tract is irreversibly lost to the large intestine, where it may be excreted in the faeces or deaminated to NH_3 and eventually excreted in the urine. The extra N flow from the ileum of infected animals approximately equals the extra faecal and/or urinary N excretion. These values approximate the decrease in body protein deposition in pair-fed control and infected animals. This implies that in net terms absorbed amino acids are being directed away from muscle, bone and wool synthesis to mucus, gut and plasma protein synthesis to replace the small proportion irreversibly lost.

3.5. Protein turnover

These increases in endogenous loss into the gut and the subsequent increased amounts absorbed imply that there is greater protein recycling. Whether this leads to increased protein synthesis and turnover in the whole body is not clear.

Early studies in this area [47] were confounded by problems with the technique [48, 49], but they nevertheless indicated that liver protein synthesis was increased and that muscle protein synthesis decreased under parasitism. Using better procedures, it was found that total body protein synthesis was increased under parasitism and that this occurred particularly in the gut tissues [4, 50]. The quantitative estimates of protein synthesis and turnover, in relation to gut tissue, are still fraught with technical difficulty [48, 49], but in comparisons between infected and control animals they probably give reasonable estimates of the proportional differences and in most cases this is all that is required to ascertain the consequences of parasitism.

This area is an important one to determine the metabolic effects of parasitism, but it has not received its deserved attention.

4. ENERGY

The consequence of increased protein recycling in the gut and increased body protein synthesis must inevitably be increased heat production and reduced energy retention. Sykes and Coop [19, 20] and Coop et al. [21], in comparative slaughter trials, determined that the gross efficiency of use of metabolizable energy (ME) for growth was reduced by 42% in animals infected with *T. colubriformis* and, depending on larval intake, up to 30% in animals infected with *O. circumcincta*. Sykes [3] further calculated on the basis of estimates of endogenous protein loss that this recycling could account for the depression in use of ME.

In contrast to these results MacRae et al. [51] using closed circuit calorimeters have found no evidence to support increased heat production under parasitism. Parasitism altered ME supply in their study by increasing faecal energy losses.

The results relating to energy expenditure under parasitism have suffered from a lack of concurrent studies on protein turnover. Until this is done the reasons for the discrepancy between the two techniques used to estimate energy retention will remain elusive. Such studies should also examine the obligatory requirement for gluconeogenesis from amino acids under parasitism and it is interesting to note that Roseby [52] using ^{14}C glucose and ^3H glucose observed a greater entry rate and recycling of glucose in sheep infected with *T. colubriformis*.

5. MINERALS

The role of parasitism on mineral metabolism has been extensively reviewed [1, 2]. Recent studies have found how some of these effects originate [8, 53]. As with protein metabolism the approach of quantifying exchanges down the digestive tract is the important first step. Techniques developed to study mineral metabolism in normal animals are used.

When using dual-phase markers [24], both the above studies have observed that there is little effect on Ca absorption. However, absorption differences in the order of 1 g Ca/d need to be detected to account for the body Ca changes observed by Sykes and Coop [19], but it is unlikely that the technique has sufficient accuracy to detect this. In contrast, the additional procedure used by Wilson and Field [53], namely that of intravenous ^{45}Ca injection and sampling in digesta and faeces, quantifies net endogenous loss and allows estimates of true absorption of exogenous Ca. However, the precision is only as accurate as the flow data. Using this procedure both *O. circumcincta* and *T. colubriformis* were observed to result in a small increase in faecal endogenous loss.

Phosphorus recycling and absorption in the digestive tract have been shown to be markedly depressed under parasitism. Using the same approach as that used in quantifying N exchange in the gut, and that outlined above for Ca, Wilson and Field [53] and Poppi et al. [8] demonstrated that *T. colubriformis* markedly depressed P absorption and salivary P recycling (Table III). Thus, the effect of *T. colubriformis* infection was to induce a P deficiency, with the associated effects on Ca retention. This induced P deficiency may be a contributing factor to the observed intake depression.

Another technique used to study mineral metabolism in normal animals, that of intravenous injection of a labelled marker, e.g. ^{45}Ca ^{32}P , and determination of body pool sizes and exchanges by using a computer program, SAAM [54], does not yet appear to have been extended to parasitized animals. This would be an important addition to the study on exchanges occurring in the digestive tract.

TABLE III. PHOSPHORUS FLOW DOWN THE DIGESTIVE TRACT OF LAMBS INFECTED WITH *T. colubriformis*

	Intake (g/d)	Duodenum (g/d)	Ileum (g/d)	Saliva P ^a (g/d)	Absorption before ileum ^b (g/d)
Control ^c	5.7	12.4	4.2	6.7	1.5
Infected	5.8	8.9	6.1	3.1	-0.3
Control ^d	7.6	12.4	5.8	4.8	1.8
Infected	7.6	9.9	7.7	2.3	-0.1

^a Endogenous addition assumed to be all saliva P (P intake — duodenal P flow).

^b Derived from P intake — ileal P flow.

^c From Ref. [53].

^d From Ref. [8].

6. PHYSIOLOGICAL STATE AND LEVEL OF NUTRITION

This is a largely ignored area of study, although it is generally accepted that the physiological state and level of nutrition influence the effect of the parasite on the host [1]. Many early experiments were general observations rather than controlled experiments [55], but Coop and Field [56] and Abbott et al. [57, 58] have outlined a series of experiments in which the content of only one nutrient was manipulated. For the first time this approach allows meaningful interpretation of the nutrition $\times$ parasite interaction.

There is also confusion in interpreting studies on the effect of the physiological state and level of nutrition on host response to parasite infection. Some studies have used different levels of nutrition before inducing parasitism and these have been termed nutrition $\times$ parasite interaction studies. However, in many of these studies it is difficult to determine whether the physiological state (body condition) was also markedly changed before the imposition of infection with an influence on the host response to the nutrition $\times$ parasite treatment proposed [55, 57–71]. Coop and Field [56] present a clear demonstration of animals starting at the same physiological state and showing a parasite $\times$ nutrient (phosphorus) interaction, in this case the benefit of high P levels in the diet. Experiments in which the body condition or physiological state are altered before experimental treatment examine the physiological state $\times$ levels of nutrition $\times$ parasite interactions. This applies to body weight and in extreme cases to the effects of pregnancy and lactation [72].

Body condition and/or low levels of nutrition have been shown to be important in the establishment and effect of the parasite on the host, with increased susceptibility being associated with low body condition and/or level of nutrition [1, 57, 58, 61, 62, 64].

Interpretation of experiments of this type require data on parasite establishment and maintenance, immunological response of the host and the quantitative metabolic consequences of infection. Until recently [56-58, 61, 62, 64] this was not always done.

7. NUTRITIONAL MANIPULATION

The above studies have generally examined gross changes in nutrient concentration and digestibility but they have difficulties in interpretation because of the changes in the spectrum of nutrients. The pathophysiological studies on protein, energy and mineral metabolism imply that there are ways of manipulating host response by altering specific nutrient supply. Elucidating whether this occurs is important because it indicates alternative mechanisms to minimize the effects of parasitism. Second, it places more emphasis on quantifying the nutrient supply from various forage types. Much of this is known for formulated diets but for herbages grazed in situ there is a paucity of data [73, 74]. Thus, the differences in protein supply to the intestines of animals grazing legume pastures compared with grass pastures [73-75] may be more important if these animals are infected with parasites. Similarly, if the protein

TABLE IV. N RETENTION IN CONTROL LAMBS, INFECTED LAMBS (3000 *T. colubriformis*/d) AND INFECTED LAMBS (3000 *T. colubriformis*/d) INFUSED INTRAGASTRICALLY WITH CASEIN (50 g/d) OR GLUCOSE (70 g/d)
Values were obtained in a comparative slaughter trial lasting 12 weeks (Bown et al. [76])

Treatment	Control	Infected	Infected + protein	Infected + glucose
N intake ($g \cdot W^{-0.75} \cdot d^{-1}$)	1.27	1.3	1.83	1.31
N retention ($g \cdot W^{-0.75} \cdot d^{-1}$)	0.09	0.02	0.10	0.05

supply to the intestines is shown to be important in host response to parasitism, then improving this aspect by plant breeding may have greater consequences than at first realized by using conventional non-parasitized animals in forage evaluation.

Recently Bown et al. [76], in a comparative slaughter trial, evaluated the importance of protein or energy in host response to parasitism. What was important was the approach. The treatments were devised after detailed studies on the effects of parasites on N and energy metabolism. As outlined in the earlier sections, it appears that parasites exert a major effect on the host by diverting amino acids away from muscle, bone and wool synthesis to plasma, gut and mucus protein synthesis. Since protein absorption was not impaired it was postulated that supplying extra protein (50 g casein/d) to the intestines could enhance muscle, bone and wool synthesis. The possibility that the energy costs of increased protein synthesis were involved was examined by glucose supplementation (70 g glucose/d). All supplements were infused via an abomasal catheter. The results are seen in Table IV [76] and clearly show that the protein supply to the intestines rather than their energy content has by far the greater effect on energy and N retention.

Practically, these results may be achieved by supplementation or by the use of forage types supplying high levels of protein to the intestines. Use of complete intragastric infused animals [77] would greatly enhance these studies if it could be shown that the parasites established in a normal manner and had the usual effects on the host.

8. CONCLUSIONS

Until recently the study of nutrition $\times$ parasite interactions monitored gross responses in the host, with little quantification of the pathophysiological effects of infection. Application of radioisotope techniques, designed to study protein and mineral digestion, absorption and metabolism in uninfected animals, has enabled the metabolic and nutritional consequences of parasite infection on the host to be quantified. What is important in this approach is identifying the metabolic pathways of interest and quantitatively determining the consequences of infection.

Such information enables identification of those nutrients, the manipulation of which might be expected to improve the growth rate of infected animals.

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ANIMAL NUTRITION
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IMPORTANCE DES RESSOURCES FOURRAGERES POUR LES PAYS EN DEVELOPPEMENT ET CONTRAINTES ACTUELLES DANS LEUR UTILISATION

R. SANSOUCY, P. AURIOL

Service de la production animale,
Division de la production et de la santé animales,
Organisation des Nations Unies pour l'alimentation
et l'agriculture (FAO),
Rome

Abstract-Résumé

IMPORTANCE OF ANIMAL FEED RESOURCES IN DEVELOPING COUNTRIES AND CURRENT CONSTRAINTS ON THEIR UTILIZATION.

Inadequacy and poor utilization of animal feed resources are the main limiting factors on animal production in most developing countries. The bulk of ruminant foodstuffs consists of poor quality fodder, such as pasture and rangeland grass, crop residues and fibrous by-products. It is very rarely possible to use cereals as supplements, since most developing countries at present need to import cereals for human consumption. This has not prevented many development agencies from recommending that cereals and other concentrate feeds be imported to boost monogastric production, reproducing the model developed for temperate countries where there is a cereal surplus. In most cases, the types of feed available locally do not allow high levels of individual animal performance. However, this does not mean that it is impossible to improve animal production in developing countries. During the last two decades, much research on animal nutrition has been successfully conducted and the results are being applied in practice. The most significant of these concern the utilization by animals of sugar-cane and its by-products, the use of non-conventional animal feeds and the treatment of straw and other fibrous materials. In all cases, supplements are required. Livestock feeding systems could also be improved if it were possible to use more of those by-products which are at present exported. New livestock feeding strategies must be developed based on currently or potentially available local resources. It is more logical and profitable for developing countries to adapt animal production systems to available feed resources than vice versa.

IMPORTANCE DES RESSOURCES FOURRAGERES POUR LES PAYS EN DEVELOPPEMENT ET CONTRAINTES ACTUELLES DANS LEUR UTILISATION.

L'insuffisance des ressources fourragères et leur mauvaise utilisation constituent les principaux facteurs limitant la production animale dans la plupart des pays en développement. La masse des aliments consommés par les ruminants consiste en fourrages grossiers tels que prairies permanentes et parcours, résidus de récoltes et sous-produits fibreux. La possibilité d'utiliser des céréales comme complément de la ration est très limitée car la plupart des pays en développement sont actuellement obligés d'importer des céréales pour l'alimentation humaine. Cela n'a pas empêché de nombreuses agences de développement de recommander l'importation de céréales et d'autres aliments concentrés pour l'accroissement de la production des monogastriques, selon le modèle mis au point par et pour les pays tempérés bénéficiant d'un surplus de céréales. Dans la plupart des cas, le type d'aliments disponibles localement ne permet pas de hautes performances individuelles. Toutefois cela ne signifie pas qu'il

ne soit pas possible d'améliorer la production animale des pays en développement. Au cours des deux dernières décennies, de nombreuses recherches sur la nutrition animale ont été effectuées avec succès et leurs résultats sont en cours d'application dans la pratique — les résultats les plus significatifs sont ceux concernant l'utilisation par les animaux de la canne à sucre et de ses sous-produits, l'emploi d'aliments du bétail non conventionnels et le traitement de la paille et d'autres produits fibreux. Dans tous les cas, il est essentiel de distribuer un aliment complémentaire. Les systèmes d'alimentation pourraient aussi être améliorés s'il était possible d'utiliser davantage les sous-produits actuellement exportés. De nouvelles stratégies alimentaires doivent être imaginées qui reposeront sur les ressources présentes ou potentielles disponibles localement. Il est plus logique et profitable pour les pays en développement d'adapter les systèmes de production animales aux ressources fourragères disponibles et non l'inverse.

1. LES RESSOURCES FOURRAGERES DISPONIBLES

L'insuffisance des ressources fourragères, tant en qualité qu'en quantité, et leur mauvaise utilisation sont reconnues comme les facteurs les plus limitants de la production animale dans la plupart des pays en développement. Faire un inventaire exhaustif de ces ressources constituerait un exercice intellectuel passionnant mais d'utilité incertaine compte tenu du manque de statistiques fiables et de la variation considérable d'une région à l'autre, d'une année à l'autre et des multiples autres facteurs qui interviennent dans l'appréciation de ces ressources. Il serait nécessaire de diviser les pays en développement selon des critères géographiques, climatiques, démographiques, sociologiques et économiques en différentes régions homogènes. Des études plus ou moins approximatives ont cependant été réalisées [1-3]. Elles donnent une idée de l'importance relative des différentes ressources fourragères à l'échelle mondiale et pour certaines régions regroupées conventionnellement.

1.1. Pâturages

Il n'existe pas de véritable inventaire des pâturages dans les pays en développement. D'ailleurs le concept même de pâturages recouvre une extrême variété de situations. C'est ainsi qu'il n'y a pas de comparaison possible entre un hectare de pâturage de la pampa humide d'Argentine et un hectare de pâturage de la région sahélienne! De plus, certains territoires qui sont classés comme forêts sont souvent pâturés, au moins pendant une partie de l'année.

Les pâturages constituent évidemment la part essentielle des ressources fourragères consommées par les ruminants dans la plupart des pays. Il est toutefois impossible de déterminer de façon raisonnablement précise leur production annuelle, en particulier dans les zones arides et semi-arides. Une approximation peut consister dans le rapprochement entre les surfaces estimées des prairies permanentes et de la population de ruminants (tableau I).

TABLEAU I. SURFACES ESTIMEES DES PRAIRIES PERMANENTES ET POPULATION DE RUMINANTS DANS LES PAYS EN DEVELOPPEMENT (1983)

	Prairies permanentes (millions ha)	Nombre de ruminants (millions unités animales) ^a	Charge animale (unité/ha)
Afrique	629	115	0,18
Amérique Latine	550	270	0,49
Proche-Orient	267	66	0,25
Extrême-Orient	35	307	8,77
Pays d'Asie à planif. centrale	410	86	0,21
Total pays en développement	1891	844	0,45
Pays développés	(1265)	(416)	(0,33)

^a 1 unité animale = 1 ruminant de 500 kg de poids vif à l'entretien.

Source: d'après [4].

TABLEAU II. PRODUCTION DE FOURRAGES VERTS DANS 90 PAYS EN DEVELOPPEMENT EN 1980^a

	Production de matière sèche (millions tonnes)	Superficie (millions ha)	Rendement de matière sèche (tonnes/ha)
Afrique	5,1	1,1	4,7
Extrême-Orient	53,3	10,8	4,9
Amérique Latine	53,3	12,6	4,3
Proche-Orient	19,7	2,7	4,7
90 pays en développement	131,4	27,2	4,8

^a Exprimée en matière sèche.

Source: d'après [5].

TABLEAU III. PAILLES ET TIGES DE CEREALES DANS LES PAYS EN DEVELOPPEMENT

	Blé	Riz	Maïs	Total
	(en millions de tonnes)			
Afrique	5	6	32	43
Amérique Latine	15	17	112	144
Proche-Orient	31	5	10	46
Extrême-Orient	107	359	168	634 ^a
Total pays en développement	158	387	322	867

^a Dont 326 pour la Chine et 132 pour l'Inde.

Source: d'après [2].

TABLEAU IV. UTILISATION DES ALIMENTS CONCENTRES

	Pays développés			Pays en développement		
	1972-74 (10 ⁶ t)	1983 (10 ⁶ t)	Augmentation (%/an)	1972-74 (10 ⁶ t)	1983 (10 ⁶ t)	Augmentation (%/an)
Céréales	430	470	1,1	55	120	8,0
Issues de meunerie	35	40	0,5	45	65	3,7
Tourteaux	45	70	4,7	15	30	6,6
Autres concentrés ^a	80	135	5,3	40	40	0,0
Total concentrés	590	715	2,0	155	255	5,0

^a Incluant manioc, mélasse, légumineuses, farines de viande, etc.

Source: d'après [2].

Ce tableau met en évidence les grandes variations régionales. Il illustre le fait que la population de ruminants de l'Extrême-Orient dépend essentiellement de ressources autres que les pâturages. Elle montre aussi la faible productivité des pâturages d'Afrique et du Proche-Orient due à des conditions climatiques défavorables. Encore faut-il noter que de nombreux pâturages de ces dernières régions sont surpâturés pendant une grande partie de l'année.

TABLEAU V. UTILISATION DU MANIOC ET DE LA MELASSE
PAR LES ANIMAUX

	1972—1974 (10 ⁶ t)	1983 (10 ⁶ t)	Augmentation (%/an)
Manioc			
Pays en développement	13,5	12,7	—0,6
Pays développés	6,3	16,7 ^a	10,2
Mélasse			
Pays en développement	2,7	4,2	4,5
Pays développés	7,7	9,8	2,4

^a 28,9 en 1982, soit une augmentation moyenne annuelle de 18,4% de 1973 à 1982.

Source: d'après [8].

1.2. Cultures fourragères

La superficie des cultures fourragères est restée extrêmement réduite dans les pays en développement en dépit d'efforts considérables de recherche et de vulgarisation (tableau II). Selon une étude de la FAO [5], ces cultures se sont toutefois développées ces dernières années, en particulier dans des pays tels que la Chine, l'Inde et ceux du Proche-Orient. Dans ces derniers, il y a en effet une tendance croissante à introduire des cultures de légumineuses fourragères à la place de la jachère dans le but d'améliorer la fertilité des sols et d'accroître en même temps la production fourragère.

Dans les pays en développement les cultures fourragères sont souvent en concurrence avec les cultures vivrières pour la terre, la main-d'œuvre, les fertilisants ou l'eau. Même les légumineuses fourragères, intéressantes pour leur capacité d'amélioration des sols pourraient aussi bien être remplacées par des légumineuses directement consommables par l'homme.

1.3. Résidus de récolte et sous-produits agro-industriels

Les quantités de résidus de récolte et surtout de sous-produits agro-industriels sont plus faciles à estimer. Les premiers sont de loin les plus importants en volume. Différentes estimations [2, 6] ont montré que si tous les résidus et sous-produits lignocellulosiques étaient utilisés en alimentation animale, ils permettraient de couvrir environ les deux tiers des besoins en matière sèche de l'ensemble des ruminants. Les pailles et tiges de céréales représentent à elles seules plus des trois quarts de l'ensemble, soit un tonnage considérable (tableau III).

Ce tableau montre toute l'importance des résidus de récolte pour les ruminants dans la région de l'Extrême-Orient par opposition à la faible disponibilité en pâturages (tableau I). En fait dans certaines zones les ruminants vivent essentiellement de ces résidus complémentés par très peu de fourrages verts et/ou de concentrés.

Les sous-produits agro-industriels, à part la bagasse de canne à sucre qui se rapproche davantage des résidus de récolte, sont souvent de valeur nutritive supérieure, qu'ils soient riches en énergie comme la mélasse, ou riches en matières azotées et en énergie comme les tourteaux d'oléagineux. Ils sont largement utilisés pour la fabrication des aliments composés (tableaux IV et V) et font l'objet d'un commerce intense, une part très importante étant exportée vers les pays développés. Les pays en développement qui produisent de 60 à 70 millions de tonnes de tourteaux sur une production mondiale d'environ 100 millions de tonnes, soit 60 à 70%, n'en consomment que 30% de ce total, et ce malgré une population animale supérieure.

1.4. Aliments concentrés

L'expansion rapide et l'intensification des systèmes de production, particulièrement pour les volailles et les porcs, dans les pays en développement a entraîné une demande accrue en aliments concentrés dans ces pays. Il y a lieu toutefois de préciser que 14 pays seulement consomment 80% des concentrés utilisés dans les pays en développement; ce sont pour la plupart des pays ayant un niveau de revenu relativement élevé. En 1983, les pays en développement ont consommés environ 25% des aliments concentrés consommés dans le monde (tableau IV).

L'augmentation très rapide de l'utilisation des aliments concentrés dans les pays en développement reflète d'une part le faible niveau de départ et d'autre part une tendance à copier des systèmes de production conçus dans et pour les pays développés. Ceci est d'autant plus frappant que ce sont les céréales, dont les pays en développement sont importateurs, qui ont connus la plus forte augmentation. Ceci pose le problème de la compétition entre l'utilisation de celles-ci pour nourrir l'homme ou nourrir les animaux [7].

Dans le même temps l'utilisation des concentrés moins traditionnels tels que le manioc a diminué dans ces mêmes pays qui en sont pourtant les producteurs alors qu'elle s'est considérablement accrue dans les pays développés pourtant importateurs (tableau V). L'utilisation de la mélasse a augmenté plus rapidement dans les pays en développement, mais reste à un niveau faible.

2. LES CONTRAINTES

Les contraintes en matière de ressources fourragères dans les pays en développement ont été évoquées dans de nombreux articles, conférences ou livres. Elles sont aujourd'hui assez bien connues. Elles ont en général surtout été

considérées sur le plan de la technologie. En fait elles sont, elles aussi, tributaires des facteurs géographiques, climatiques, démographiques, sociologiques et économiques que l'on peut en simplifiant qualifier de facteurs d'environnement.

2.1. Les facteurs d'environnement

Ces facteurs, qui sont trop souvent oubliés, conditionnent en fait la réussite ou l'échec de toute technique dans un pays donné, et pas seulement dans les pays en développement! C'est pour les avoir trop souvent ignorés ou sous-estimés que les efforts de «transfert de technologie» ont échoué. Il ne sera pas possible de considérer ici en détails ces facteurs, seuls quelques exemples seront donnés pour illustrer ce problème très important.

Dans l'ensemble des pays en développement, la population humaine a connu un accroissement considérable au cours des 25 dernières années (plus de 70%). Dans la même période, le nombre (exprimés en unités animales) d'animaux monogastriques a augmenté de 160% et celui des ruminants de 30 à 40% (figure 1). Les prairies permanentes, principales ressources fourragères dans ces pays, n'ont pas progressé. Ceci a entraîné une pression accrue sur les autres ressources fourragères.

Les systèmes très intensifs de production animale ont été mis au point aux États-Unis et en Europe, pays caractérisés par:

- des excédents céréaliers importants
- un coût de la main-d'œuvre très élevé
- une main-d'œuvre formée et qualifiée
- un coût de la mécanisation relativement modeste
- un environnement technologique adapté
- une disponibilité permanente de tous les intrants
- une disponibilité permanente de tous les services d'appui
- des facilités d'accès au crédit bancaire
- des facilités d'importation des produits déficitaires
- un pouvoir d'achat du consommateur élevé et une relation directe entre qualité et prix.

Rares sont les pays en développement où l'ensemble de ces conditions sont satisfaites et où de tels systèmes sont adaptés. Les installations d'élevage dit «industriel» qui se sont multipliées, en particulier dans le secteur avicole, ont peut-être pu permettre de résoudre l'approvisionnement en viande de volaille des grandes cités. Mais elles ont maintenu la dépendance des pays en développement vis-à-vis de l'extérieur. En effet, avec l'importation de cette technologie, il a aussi fallu importer tous les ingrédients qui l'accompagnent: des animaux hautement sélectionnés pour des performances individuelles très élevées, des équipements et machines sophistiqués, et jusqu'à la quasi totalité des ressources alimentaires nécessaires pour maintenir ces systèmes de production, en particulier des céréales pour permettre à ces animaux d'exprimer leur potentiel génétique.

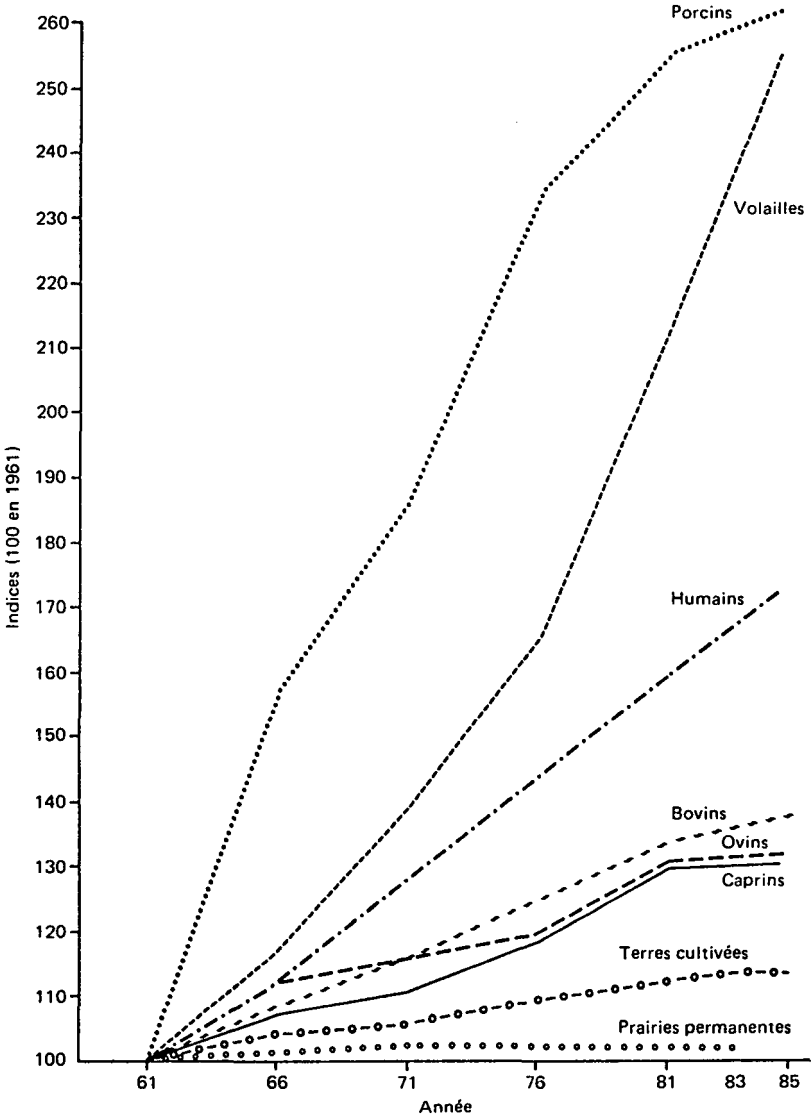


FIG. 1. Evaluation des populations humaine et animale sur 25 ans dans les pays en développement.

Dans la région sahélienne soumise régulièrement à des sécheresses sévères, il existe un système de production traditionnel bien établi qui a survécu à des millénaires et pour lequel aucune alternative viable n'a pu encore être offerte. Les contraintes climatiques et sociologiques sont extrêmement rigoureuses et il est très difficile d'introduire des innovations technologiques sans détruire le fragile équilibre naturel. Les éleveurs de cette région doivent en permanence se tenir prêts pour lutter contre les effets de la sécheresse [9]. Aucun transfert de technologie n'a pu à ce jour apporter la moindre solution au problème des ressources fourragères limitées de cette région. Toute possibilité d'introduction de nouvelle technique, en particulier dans le domaine de l'alimentation des animaux, se heurte aux ressources trop limitées des éleveurs, à leurs habitudes ancestrales et au milieu physique hostile. Par exemple, l'établissement de cultures fourragères qui a parfois été suggéré se trouve en concurrence aiguë avec les cultures vivrières, elles-mêmes insuffisantes, pour l'utilisation des terres, de l'eau et de la main d'œuvre. Une gestion améliorée des pâturages se heurte aux aléas climatiques, aux problèmes de propriété des terres, aux grands mouvements de transhumance des éleveurs venant parfois de pays voisins en quête de pâturages de saison sèche ou simplement d'eau pour l'abreuvement de leurs troupeaux, à la pression croissante de la population humaine et animale, etc.

En ce qui concerne l'utilisation de nombreux résidus de récolte et sous-produits agro-industriels, les contraintes sont également multiples:

- éloignement entre le lieu de leur disponibilité et le lieu où se trouvent les animaux potentiellement utilisateurs;
- coût extrêmement élevé des transports;
- saisonnalité de leur production;
- travail nécessaire pour la collecte et/ou la conservation;
- utilisations concurrentielles;
- exportations des sous-produits agro-industriels de meilleure valeur nutritive (par exemple tourteaux et mélasse).

2.2. Les facteurs technologiques

A toutes ces contraintes s'ajoutent des facteurs technologiques divers qui entravent la production ou l'utilisation des ressources fourragères. Ces facteurs concernent essentiellement la valeur nutritive de ces aliments.

Les aliments disponibles pour les animaux dans les pays en développement peuvent se classer en deux grandes catégories:

- ceux qui constituent la ration de base des ruminants, qui sont riches en fibre et qui proviennent essentiellement des pâturages pauvres et des résidus de récolte.
- ceux qui peuvent être utilisés comme compléments pour les ruminants et les monogastriques, ou comme base de la ration pour ces derniers; ce sont principalement les sous-produits agro-industriels (les sons, les tourteaux et la mélasse, et le manioc).

Les aliments lignocellulosiques ne peuvent être utilisés que par les ruminants. Les principales limitations des résidus de récolte sont leur faible digestibilité entraînant une ingestion et une valeur énergétique insuffisantes, et leur pauvreté en matières azotées et en minéraux essentiels. Ils doivent être nécessairement complémentés pour être valorisés valablement par les animaux. Dans certains cas, ils peuvent subir un traitement pour accroître leur digestibilité.

Les fourrages tropicaux eux-mêmes ont une teneur en fibres supérieure à celle des fourrages tempérés au même stade végétatif. Ils sont aussi plus pauvres en azote et en phosphore. Il en résulte qu'ils ont une ingestibilité et une digestibilité plus faibles [10]. Ceci explique les faibles performances obtenues au pâturage par les animaux, tout au moins au niveau des performances individuelles. Ces faibles performances individuelles sont toutefois compensées par les rendements par hectare très élevés obtenus à partir de ces fourrages.

3. STRATEGIES POUR AMELIORER L'UTILISATION DES RESSOURCES FOURRAGERES DANS LES PAYS EN DEVELOPPEMENT

Compte tenu de la situation décrite dans les deux chapitres précédents, il est indispensable de définir une stratégie adaptée aux problèmes spécifiques des pays en développement. Une telle stratégie pourrait se résumer au titre d'un livre récent [11]: *Adapter les systèmes d'élevage aux ressources fourragères disponibles* (Matching livestock systems with available feed resources).

Cette stratégie, contrairement à la stratégie classique, a pour but la valorisation maximum des ressources fourragères localement disponibles. En fait la stratégie classique, tout en se prétendant «moderne», se basait essentiellement sur le «transfert de technologie». Elle se limitait à copier des systèmes de production établis dans des circonstances complètement différentes comme montré dans le chapitre précédent (para. 2.1). Elle a aussi eu pour conséquence d'empêcher les chercheurs nationaux de s'attaquer aux véritables problèmes de leurs pays et d'identifier des thèmes de recherche adaptés.

3.1. Définir de nouveaux critères de productivité

Dans les pays en développement où la main-d'œuvre est souvent abondante et relativement bon marché, et, par contre, où les équipements importés sont chers et les pièces de rechange pas toujours disponibles, il n'y a pas lieu de maximiser la production par travailleur à l'aide d'équipements.

Lorsque la base de l'alimentation des ruminants est constituée par des pâturages pauvres très extensifs, ou de la paille, ou des résidus de récolte qui ne peuvent être valorisés autrement, la productivité par unité fourragère n'a pas beaucoup de signification. Ce qui par contre peut en avoir beaucoup est la valorisation du complément

alimentaire distribué qui coûte cher à l'unité mais peut parfois permettre une amélioration considérable de la production.

Lorsqu'un pays doit importer des céréales à un prix élevé, pour nourrir ses habitants, et que celles-ci sont subventionnées pour les rendre accessibles aux plus pauvres, il n'est pas raisonnable de développer des systèmes d'alimentation animale basés sur ce type d'aliments, pour nourrir des animaux qui servent à la consommation des classes à plus haut revenu [7]. Dans ces circonstances une telle politique a également pour effet de décourager le développement des ressources fourragères locales qui sont artificiellement concurrencées par ces subventions. Par ailleurs, est-il nécessaire de rappeler que le modèle «maïs-soja» a été développé aux Etats-Unis, pays excédentaire en ces deux produits qui y sont largement disponibles à des prix relativement bas? Il n'est pas transposable dans la très grande majorité des pays en développement déficitaires en ces deux produits. Plutôt que de rechercher les performances animales individuelles élevées, il faut alors rechercher la production globale maximum à partir des ressources existant localement.

3.2. Baser l'alimentation sur les ressources fourragères localement ou potentiellement disponibles

Comme indiqué dans le premier chapitre la ressource la plus abondante dans la plupart des pays en développement est constituée par les aliments lignocellulosiques généralement de faible valeur nutritive. Ils ont par contre l'avantage d'être relativement bon marché surtout quand ils sont disponibles sur la ferme. Ceci favorise l'élevage des ruminants qui peuvent les digérer grâce à la fermentation dans le rumen, sans entrer en concurrence avec l'homme qui est incapable de les utiliser directement. Pour en tirer le meilleur parti, il est toutefois nécessaire de corriger les déficiences de ces aliments qui seuls ne peuvent même pas assurer l'entretien des animaux, et parfois à peine leur survie.

Dans les pays tropicaux humides, il est possible aussi d'encourager le développement de cultures à haut potentiel de production de biomasse tels que la canne à sucre ou le manioc. Ce sont des cultures à plusieurs fins pouvant être utilisées en partie pour l'alimentation humaine et l'alimentation animale, et aussi comme source de combustible.

Elles permettent à l'agriculteur-éleveur de produire à la ferme même l'essentiel des aliments pour ses animaux, ruminants comme monogastriques, limitant sa dépendance vis-à-vis de l'extérieur aux seuls compléments nécessaires pour valoriser la ration de base.

3.3. Complémenter les aliments pauvres ou déséquilibrés par des suppléments appropriés (compléments «catalytiques»)

En ce qui concerne les ruminants, le principe de cette complémentation se résume en deux règles essentielles [12]:

a) d'abord satisfaire les besoins des microorganismes du rumen afin d'assurer une fermentation efficace et un bon rendement des aliments de base dans le rumen (aboutissant essentiellement aux protéines microbiennes et aux acides gras volatils);

b) compléter et équilibrer les produits de la digestion fermentative avec des aliments qui échappent à la fermentation dans le rumen (principalement des protéines «protégées»).

Dans la pratique il est recommandé d'apporter par ordre de priorité:

1) De l'azote fermentescible (urée) utilisable par les microbes du rumen. Ceci est nécessaire pour assurer que le niveau d'ammoniac dans le liquide du rumen atteigne 150 mg/L; c'est le niveau permettant une bonne utilisation de la cellulose et un bon développement des microorganismes. Il est nécessaire d'apporter en même temps les minéraux essentiels (sodium et phosphore). Cette simple complémentation peut permettre non seulement de maintenir les animaux mais peut aussi assurer un léger gain de poids [13, 14].

2) Une petite quantité (10 à 20% de la ration) d'un fourrage de bonne qualité, de préférence une légumineuse. Bien que le mécanisme exact de l'action de ces compléments ne soit pas bien connu, leur efficacité a été mise en évidence depuis longtemps, par exemple chez les troupeaux entretenus sur des pâturages pauvres, mais disposant d'arbres fourragers sur leurs parcours. Ceci a également été démontré en station de recherche avec de l'herbe jeune [15] en complément de sous-produits fibreux (pulpe de sisal).

3) Des protéines non dégradées au niveau du rumen. Celles-ci peuvent être fournies par des tourteaux d'extraction, du son, de la farine de viande ou de poisson. Des apports en faibles quantités de ces compléments peuvent avoir des effets considérables sur les performances des animaux [16].

Un exemple de complémentation adaptée pouvant être employée par des petits éleveurs est constitué par les blocs de mélasse-urée, que ce soit pour la production laitière pour des animaux nourris à base de paille de riz [17] ou pour des troupeaux maintenus sur pâturages pauvres de fin de saison sèche [18].

3.4. Traiter les résidus fibreux

Depuis de nombreuses années, différents traitements ont été employés afin d'améliorer la valeur alimentaire des produits fibreux, principalement la paille [19, 20]. En particulier, les traitements aux alcalis se sont révélés les plus pratiques. Récemment, ce sont surtout les traitements au gaz ammoniac [21] et à l'urée (utilisé comme générateur d'ammoniac) qui ont été popularisés [14, 22]. Ceci est dû à leur facilité d'emploi et à l'apport d'azote qu'ils assurent en même temps qu'ils améliorent la digestibilité et l'ingestibilité de la paille.

Tandis que le traitement au gaz ammoniac peut s'effectuer dans des fermes disposant de grandes quantités de paille dans les pays où ce gaz est disponible, le traitement à l'urée est particulièrement bien adapté aux petits fermiers. Le traitement à l'urée s'est révélé légèrement plus efficace que la seule supplémentation à l'urée dans un essai effectué avec de la paille de blé [23].

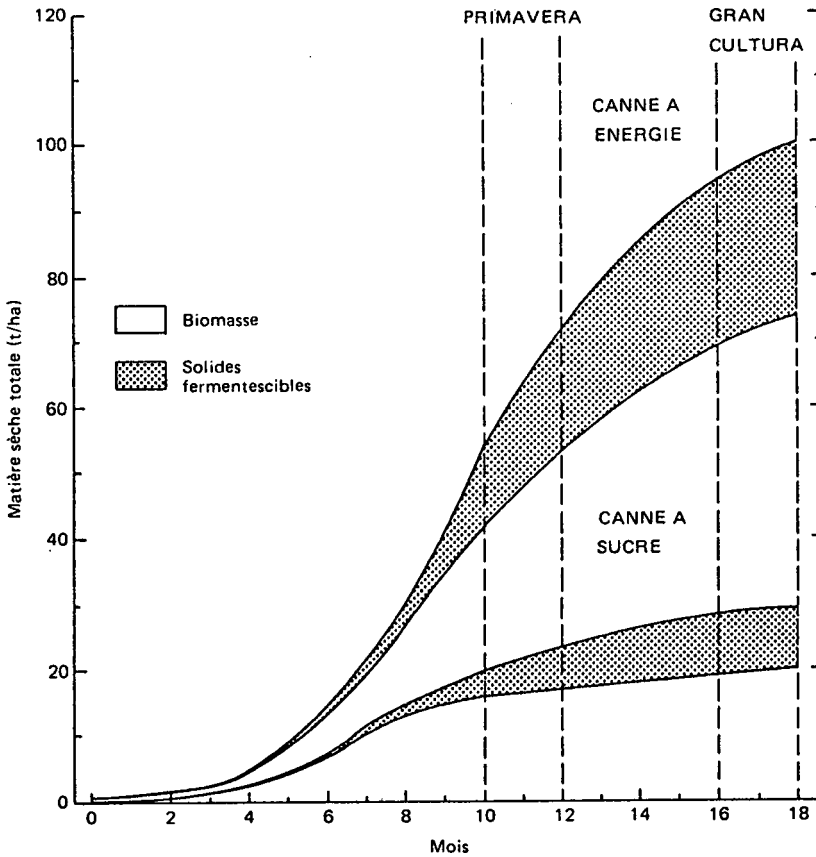


FIG. 2. Rendements comparés de la canne à sucre et de la canne à énergie à Puerto Rico [25].

3.5. Fractionner les produits celluloseux

Le concept de fractionnement est utilisé depuis fort longtemps ne serait-ce que pour les céréales où l'on sépare la paille du grain ou pour la canne à sucre où l'on sépare le jus de la bagasse. Ce même concept peut être développé pour l'alimentation animale [24]. On sélectionne les plantes à plus haut potentiel de production de biomasse, telles que la canne à sucre. A Puerto-Rico, la sélection de cette plante comme productrice de biomasse et non plus comme productrice de saccharose a donné des résultats étonnants [25], atteignant des rendements de 100 tonnes de matière sèche par hectare (figure 2). Dans ces conditions, la canne à sucre pourrait bien devenir la plante fourragère de l'avenir dans les pays tropicaux humides. Ceci pourrait constituer une alternative à la crise très grave qui secoue actuellement l'industrie sucrière dans de nombreux pays en développement exportateurs de sucre.

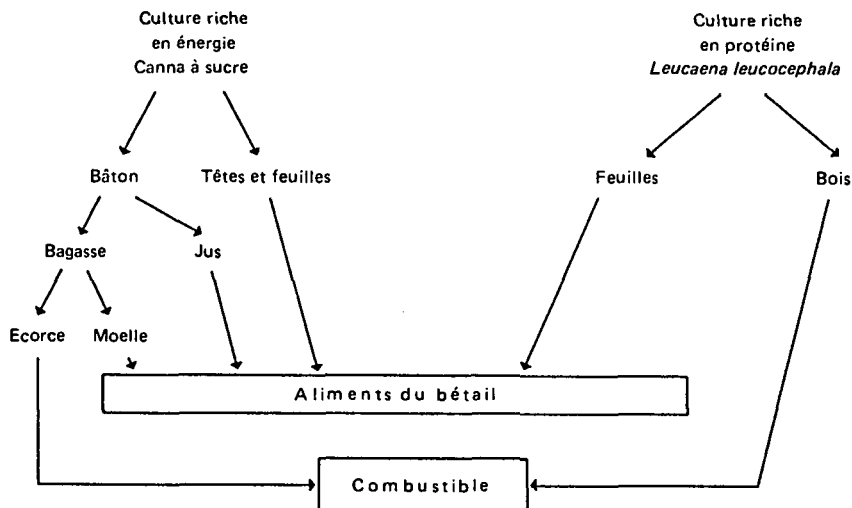


FIG. 3. Exemple de fractionnement de la biomasse pour la production d'aliments et de combustible. (D'après [24].)

Les têtes de canne peuvent être données aux ruminants directement ou après conservation sous forme d'ensilage. La séparation du jus de la bagasse peut être effectuée au niveau du petit paysan en utilisant des moulins artisanaux traditionnels, ou à grande échelle dans une usine sucrière. Le jus est distribué comme aliment énergétique aux monogastriques ou aux vaches laitières. La bagasse fraîche peut être distribuée à des chèvres qui en consomment la partie moëlleuse. L'écorce restante est utilisée comme combustible. Le fourrage et les protéines peuvent être apportés par des feuilles de *Leucaena*, le bois servant également de combustible (figure 3). Les travaux récents effectués en République Dominicaine [26] ont montré que le jus de canne à sucre pouvait remplacer avantageusement les céréales dans l'alimentation des porcs.

3.6. Être attentif aux nouvelles possibilités offertes par la biotechnologie

Les progrès réalisés au cours de ces dernières années dans le domaine de la biotechnologie concernent aussi la nutrition animale et tout particulièrement les ruminants. Ils peuvent s'appliquer aux fourrages et à leur utilisation par les animaux.

Dans le domaine des fourrages, l'obtention de variétés à très haut potentiel de production de biomasse et de sucres fermentescibles a été mentionnée précédemment pour ce qui concerne la canne à sucre qui est en train de se transformer en «canne à énergie». On peut envisager également des variétés de céréales orientées vers une production de biomasse plus importante (variété de maïs-fourrage, riz produisant

d'avantage de paille) ou plus riches en protéines, ou avec des grains dépourvus génétiquement de balles.

La digestibilité de certains sous-produits ligno-cellulosiques peut être améliorée par bio-dégradation préalable à leur mise à disposition des animaux. Elle peut également l'être par manipulation de l'écosystème microbien du rumen [27] par l'utilisation d'ionophores (Monesin, Lasalocid), la défaunation des protozoaires, ou la manipulation génétique des microorganismes du rumen.

4. CONCLUSION

La situation des pays en développement en ce qui concerne les ressources fourragères est très variable d'un pays et d'une région à l'autre en fonction de nombreux facteurs liés aux conditions naturelles et au milieu humain.

Il est impossible d'effectuer un inventaire suffisamment réaliste des ressources fourragères obtenues à partir des pâturages dans l'ensemble des pays en développement. Les résidus de récolte et surtout les sous-produits agro-industriels sont plus faciles à estimer. L'utilisation des aliments concentrés est assez bien connue. Dans l'ensemble, la plupart des pays sont dans une situation déficitaire en fourrages. Cependant autant que la disponibilité en aliments, c'est aussi leur faible valeur alimentaire qui s'oppose à l'obtention de performances individuelles élevées des animaux.

Les contraintes ne sont pas seulement d'ordre technologique. Celles liées à l'environnement sont nombreuses et probablement plus difficiles à surmonter car plus complexes.

La stratégie pour améliorer l'utilisation des ressources fourragères dans les pays en développement peut se résumer à la formule suivante: «adapter les systèmes d'élevage aux ressources fourragères».

Ceci nécessite la définition de nouveaux critères de productivité basés sur la valorisation des ressources locales existantes ou potentielles, et non sur la recherche de hautes performances individuelles des animaux. Dans ce contexte, la complémentarité de la ration de base a un rôle extrêmement important à jouer. L'utilisation de compléments dits «catalytiques» peut transformer une ration de survie ou d'entretien en une ration de production.

Dans certaines circonstances l'utilisation de traitements simples mais efficaces peut améliorer considérablement la valeur alimentaire des produits fibreux. La séparation de la partie cellulosique de la partie riche en glucides fermentescibles est une autre technique prometteuse. Enfin les nouvelles techniques de la biotechnologie laissent entrevoir des possibilités d'amélioration considérables tant au niveau des aliments eux-mêmes que de leur transformation par l'animal.

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PROTEIN METABOLISM AND UTILIZATION DURING UNDERNUTRITION IN RUMINANTS

E.R. ØRSKOV, F.D. DeB. HOVELL

Rowett Research Institute,
Bucksburn, Aberdeen,
United Kingdom

Abstract

PROTEIN METABOLISM AND UTILIZATION DURING UNDERNUTRITION IN RUMINANTS.

Recent advances made in the understanding of protein nutrition in ruminants are discussed, with particular emphasis placed on the implications for low-level feeding systems. It is shown that protein supplements normally given when the rumen outflow rate is low are virtually completely degraded, leaving a very constant protein:energy ratio to be absorbed by the animals even if the dietary crude protein is varied, since the microbial protein produced is directly related to the energy fermented. When ruminants are given a maintenance energy diet the microbial protein produced is insufficient to meet the protein demand for tissue maintenance in young ruminants; consequently, they will lose protein and live weight. If the animals are given less than energy maintenance they will lose still more protein. The consequences for subsequent compensatory growth are discussed in detail. Using the intragastric nutrition technique it has been shown that ruminants can attain a protein balance by being given only the protein required for maintenance when body fat is available as a source of energy. The ability to manipulate fat stores as an energy source by use of protein is discussed in detail for both growth and feed conservation.

1. INTRODUCTION

Of the many aspects of ruminant nutrition in which the dietary supply of nutrients is modified as a result of intervention of rumen microbes, protein quality and protein supply certainly play a very important role. While dietary energy is also substantially modified, it is limited by the thermodynamic conditions imposed by anaerobiosis [1]. The capture of energy from carbohydrates into energy utilizable by the host animal can vary because of differences in methane production, but even so, the proportion of fermented energy captured as volatile fatty acids (VFAs) probably varies no more than 70 to 85% [1, 2]. While this variation is undoubtedly important, the dietary protein intake relative to the supply made available for absorption post-ruminally can vary several-fold. Thus, diets fed at a low level of nutrition (containing either 10 or 20% crude protein) resulted in the same amount of protein reaching the small intestine as a consequence of microbial action [3]. Indeed, diets which are virtually protein free can supply substantial amounts of microbial protein to the small intestine because of urea recycling via the rumen. The difficulties in predicting the supply of protein made available to the host animal have led to much debate and to

the evolution of several different models. For instance, systems of protein evaluation were made originally using digestible crude protein as a method for assessing requirement. This is now known to bear little relation to true protein supply.

1.1. Development of new methods for the study of protein utilization in ruminants

This paper is concerned with protein supply and utilization during undernutrition, and in this respect two important developments during the past two decades have led to a much clearer understanding of the issues relating to protein supply during undernutrition. The first is the ability to cannulate ruminants post-ruminally; the

TABLE I. EXAMPLES OF TABULATION OF DEGRADABILITY AT DIFFERENT FRACTIONAL OUTFLOW RATES OF PROTEIN SUPPLEMENTS AND OTHER SMALL-PARTICLE FEEDS

Protein source	Degradability of fractional outflow rates (k)		
	0.02	0.05	0.08
Meat and bone meal	0.52	0.45	0.41
Fish meal — herring type (stale at processing)	0.62	0.52	0.48
Fish meal — herring type (well preserved)	0.23	0.22	0.22
Cotton seed meal	0.81	0.70	0.63
Linseed meal	0.78	0.59	0.46
Soyabean meal	0.81	0.63	0.50
Groundnut meal	0.87	0.74	0.64
Sunflower meal	0.82	0.66	0.55
Beans (<i>Vicia faba</i>)	0.91	0.83	0.77
Protected rape seed meal	0.41	0.30	0.24
Rape seed meal	0.87	0.78	0.72
Dried lucerne	0.83	0.70	0.62
Sugar-beet pulp	0.64	0.50	0.45
Ground peas	0.89	0.80	0.74
Barley	0.83	0.75	0.69
Brewers grain (dried)	0.78	0.70	0.64

From Ørskov [7].

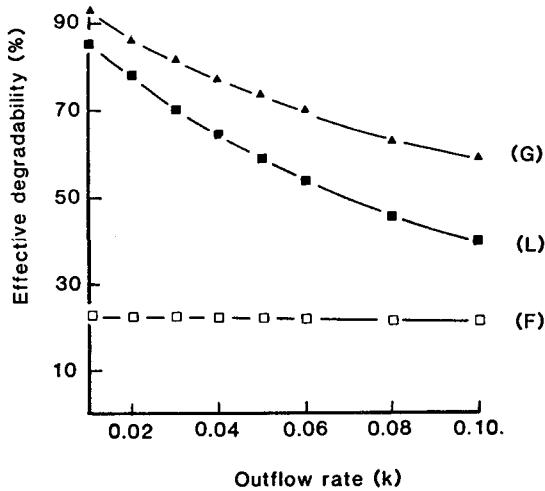


FIG. 1. Effect of outflow rate on effective degradability of fish meal (F) linseed meal (L) and groundnut meal (G).

second, the ability to nourish ruminants by total intragastric nutrition, thus abolishing the microbes altogether. The first development led to the recognition that the amount of microbial protein produced when nitrogen was not limited was related to the amount of carbohydrate fermented in the rumen (see reviews in Refs [4–6]) and that dietary protein was degraded by the rumen microorganisms to a varying extent, dependent on the source of the protein and the time it remained in the rumen. Thus, protein supplements could be virtually completely degraded or degraded by less than 30%.

Table I [7] illustrates how the rate of outflow can influence the degradation of protein. This is also graphically illustrated in Fig. 1 for three different protein supplements. As can be seen, if the outflow rate is about 1 to 2% per hour, as is the case in many low-level production systems, both the vegetable protein and protein contained in roughages are almost completely degraded. Therefore, for practical purposes, low-level feeding results in the protein supply made available to the animal being virtually all of microbial origin, and is thus related to the energy fermented. Under such circumstances attempts to increase protein supply by supplementation can only be successful when a protein source of low degradability such as fish meal or meat and blood meal is used. Alternatively, protein supplements can be made less degradable by chemical treatment, or the rumen itself can be bypassed using the oesophageal groove reflex [8], which is the method developed by evolution.

1.2. Production of microbial protein and the requirements necessary for tissue maintenance

Having ascertained that microbial yield is related to energy fermented in the rumen, it is now important to ask how the microbial protein produced relates to the maintenance requirement for protein of the host animal. This appears to be a very simple question that can be answered by straightforward experimentation, but it has in fact proved very difficult to solve. In part, this has been due to the old concepts of digestible crude protein and the origin of metabolic faecal N (MFN). It was quickly recognized that the faeces contained a large proportion of N not of dietary origin. However, it was difficult to ascertain whether this was due to a dietary cause related to level of intake or to an obligatory animal component. This appeared to be further complicated by the fact that MFN increased with food intake. On the other hand, endogenous urinary N (EUN) was considered to be representative of the true maintenance requirement of the animal. When it was recognized that MFN in fact consisted almost entirely of indigestible microbial cell walls originating mainly from the rumen, the Agricultural Research Council (ARC) [5] made the assumption that tissue maintenance could be taken as EUN provided microbial protein was expressed on the basis of net protein or amino acid supply, i.e. digestibility of the microbes should be allowed for.

Development of the intragastric nutrition technique [9] changed many of these concepts. A normally fed ruminant will maintain microbial fermentation in the rumen. If the rumen environment is deficient in N (for the microorganisms), there is a net flow of N from the blood (via saliva and direct absorption) to microbial N, with the consequence that N which would be excreted in urine as part of the EUN is excreted in faeces as microbial debris. The intragastric nutrition technique provides energy as VFAs infused directly into the rumen. There is no fermentation and hence no microbial N. Thus, for the first time it is possible to nourish ruminants on completely protein-free nutrients, so enabling true endogenous loss to be determined.

Of the many values recently determined in our laboratory for cows, calves and sheep of different ages, a mean value for EUN of $350 \text{ mg/kg } W^{0.75}$ has been derived. This value is not constant, but most of the values fall between 300 and $400 \text{ mg/kg } W^{0.75}$. The values are highest for younger, faster growing animals and lowest for mature, slower growing animals. Often the amount of faecal loss of N truly of metabolic origin (gut and enzyme debris) amounts to no more than about one-tenth of the total endogenous loss.

Having ascertained the true net losses, it was then possible to evaluate the microbial contribution to the supply of protein for maintenance. If it is assumed that ruminants require between 350 and $450 \text{ kJ/kg } W^{0.75}$ per day for energy maintenance, then a simple calculation will show whether provision of this amount of metabolizable energy (ME) by rumen fermentation is associated with a supply of microbial amino acids that is sufficient to meet the net protein losses of the host animal at maintenance. The amount of microbial N synthesized was calculated by the

TABLE II. EFFECT OF ENERGY INTAKE ON ADEQUACY OF MICROBIAL PROTEIN FOR TISSUE MAINTENANCE (see text for details)

Energy input (kJ/kg $W^{0.75}$)	225	350	450
Microbial N produced (mg N/kg $W^{0.75}$)	299	465	598
Microbial amino acids (mg N/kg $W^{0.75}$)	239	372	478
Absorbed amino acids (mg N/kg $W^{0.75}$)	203	316	405
Net supply of amino acid (mg N/kg $W^{0.75}$)	162	253	324

Tissue maintenance 300–400 mg net amino acid N/kg $W^{0.75}$.

ARC [6] (average value from many trials) to be 1.33 g N/MJ of ME. Using the values derived by Storm et al. [10] (amino acid content of microbial N to be 0.80 total N, a digestibility in the small intestine of microbial amino acids of 0.85, and an efficiency of utilization of absorbed amino acids of 0.80 (giving $0.80 \times 0.85 \times 0.80 = 0.54$)), it is possible to calculate whether and to what extent the tissue maintenance requirement in normally fed animals is met by the contribution of microbial protein. Table II shows the microbial protein supply, with two estimates of energy requirement for maintenance and tissue maintenance varying from 300 to 400 mg/kg $W^{0.75}$. It can be seen that while mature animals or animals excreting around 300 mg N/kg $W^{0.75}$ are close to meeting their required supply of microbial protein, younger animals are not. They will therefore have a negative protein balance at energy maintenance if microbial protein forms the sole supply. As is also shown in Table II, this is aggravated still further if the animals are given less than their needs for energy maintenance. In this case they will lose protein, and the lower the energy intake the more negative the protein balance. The longer the animals are undernourished and the more severe the undernourishment, the greater the depletion of protein.

This observation has enabled a better understanding of the nature of the compensatory growth observed in protein-deficient animals in earlier trials [11]. As has been pointed out, the relationship between energy intake and microbial protein supply in normally fed ruminants means that energy undernutrition is always associated with protein undernutrition. The precision offered by the total infusion technique has enabled us to study protein depletion and repletion in detail [12]. In this study, lambs

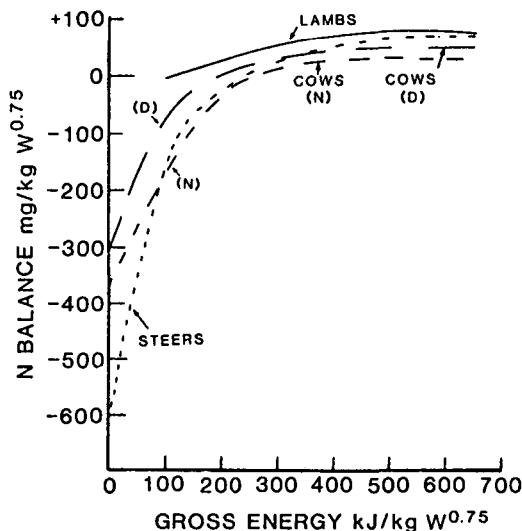


FIG. 2. Effect of infusions of proteins (first 100 kJ of ME) followed by a mixture of VFAs on N balance in lambs, steers, depleted cows (D) and normal cows (N).

entirely nourished by intragastric infusion of nutrients were slowly depleted of body protein over a period of about one month (protein given to about 0.7 maintenance), and then rapidly repleted. (The amount of protein given during repletion was about 5 to 6 times maintenance.) The nitrogen lost was replaced within days, but an enhanced rate of N accretion continued for about a month (compared with protein accretion at a similar level of nutrition before depletion). Thus, compensatory growth after a period of protein depletion comprised two stages: first, a very rapid replacement (repletion) of the protein lost during depletion, followed by a period of enhanced protein accretion — true compensatory growth.

1.3. Protein utilization during undernutrition

Another question which arose from the principle illustrated in Table II was, what would happen to protein utilization if it is given to animals well below energy maintenance? Would the protein be used as a source of energy or as a source of amino acids? The results of experiments reported by Hovell et al. and Ørskov et al. [12–14] with cows, steers and lambs are illustrated in Fig. 2. The first increment of energy shown in the figure (up to about 100 kJ/kg $W^{0.75}$) consisted of casein alone to supply a little more than the estimated protein needed for tissue maintenance. The remaining increments consisted of progressive increases in the amount of a mixture of VFAs. The results are quite conclusive. The animals achieved a positive protein balance only when protein was given. In other words, body fat was effectively used

as a source of energy, and protein utilization remained efficient substantially below energy maintenance. It is thus possible that body fat is simply an alternative to dietary energy. This concept gave rise to a more practical trial [15] in which lambs were given alkali-treated straw for 92 d, either with or without a supplement of fish meal. The purpose was to increase the post-ruminal supply of amino acids. The gain or loss in protein and fatty tissue was estimated by comparative slaughter (see Table III [15]). The lambs given straw alone, to an estimated half energy maintenance, lost both fat and protein. When the protein supplement (fish meal) was given, only body fat was lost, whereas body protein increased. The treatment in which straw was given at the high level together with the protein supplement achieved a more than 4 kg increase in empty body weight, yet there was a loss of fatty tissue. Thus, fat and protein deposition were negatively correlated, although this is in contrast to the conventional belief that fat and protein are normally positively correlated [16]. The results conform better with the model proposed by Black and Griffiths [17].

The results also indicate how the concepts of host animal nutrition have been constrained by the fact that the supply of microbial protein normally results in a relatively constant ratio of protein:energy supply.

Even with an alteration in the supply of crude protein, the effective protein:energy ratio does not often change markedly because of degradation in the rumen. With the better understanding we now have and a better knowledge of the protein supplements which are relatively undegraded in the rumen, it is possible to achieve greater changes in the effective protein:energy ratios made available to the host animal and thus to manipulate body fat stores as a source of energy.

TABLE III. EFFECT OF FISH MEAL SUPPLEMENTS TO STRAW DIETS FOR LAMBS ON BODY GAIN AND COMPOSITION

Treatment	Intake (kJ/kg W ^{0.75})	Live weight gain (kg)	Empty body gain (kg)	Body fat (kg)	Body protein (kg)
Low straw	285	— 4.32	— 5.05	— 3.53	— 0.47
Low straw + fish meal	307	0.29	0.64	— 1.53	0.48
High straw	466	0.08	— 0.08	— 1.40	— 0.14
High straw + fish meal	488	6.22	4.18	— 0.90	0.89

From Fattet et al. [15]

1.4. Depletion of body protein during fasting

The ability to manipulate energy and protein supply independently has also led to other developments. Work in our laboratory has shown that N excretion is usually 25 to 40% higher in fasting ruminants than it is if the animals are given protein-free energy to supply energy maintenance. The reason appears to be that some generation of glucose or glucose precursors is necessary when almost only fat is being oxidized; this is not surprising. What is more surprising is that very little gluconeogenic energy is required to reduce fasting N excretion to a level similar to that of N excretion at energy maintenance. While propionic acid is, as would be expected, more effective in reducing fasting N excretion than acetic acid [18], unpublished observations from our laboratory showed no difference in N loss between a mixture containing 75, 15 and 10% acetic, propionic and butyric acids and a mixture containing 45, 45 and 10%, respectively, even when these mixtures were given to supply only about one-fifth of energy maintenance. This indicates that only small amounts of glucogenic materials are required to reduce fasting N excretion levels comparable to those of animals at energy equilibrium but given no protein (basal N loss).

1.5. Implications for low-level feeding

The knowledge derived from these experiments has interesting implications for low-level nutrition systems. In fact, if body fat is thought of as an energy source it is perhaps questionable if it is correct to refer to low-level energy nutrition of fat animals if their external food source is providing less than is required to maintain energy balance. It is possible to efficiently utilize this internal food source as a source of energy even for growth provided sufficient protein is supplied from an external source. Whether such a policy is practical in commercial terms will depend on the cost of the benefits derived. Thus, it is probable that normally it would be of little value to supply protein during low-level external nutrition in cases where the animals are allowed to graze again in the spring or following a dry season. The animals' ability to compensate when the external supply of nutrients and thus also protein are increased is well known. The results previously referred to [12] have shown that if sufficient protein is provided the period of repletion is very short and presumably the compensatory period could also be minimized. During repletion and compensation, rates of gain in cattle of 2 to 3 kg/d and in lambs 500 to 700 g/d are not uncommon.

The ability to utilize the fat stores of animals as a deliberate management policy may be of interest in areas in the world with large seasonal fluctuations in food supply. It is possible to imagine systems in which animals could be largely dependent for a considerable period upon their own fat reserves for energy without losing weight or even gaining weight provided protein was given. This would be of some interest, for example, in cattle destined for slaughter during the dry period. If, however, the animals are to be allowed to graze again in the following spring or rainy season, then the compensatory growth would probably make protein feeding during

the winter or dry season uneconomical. If such a system is to be successful it is important to develop the means by which supplementary protein or amino acids could be made to leave the rumen without being degraded. In this regard a great deal of work is already in progress for application to high producing animals. However, it is quite possible that the greatest application of protected protein will be for periods of low-level feeding using fat reserves both for growth and milk production. In so doing we would be manipulating a system refined by millions of years of evolution.

The better understanding we now have of protein metabolism during undernutrition needs to be further explored by experimentation in areas with large fluctuating supplies of nutrients. It may well be that animals capable of accumulating the maximum amount of fat when the quality of grazing is good are also those animals which should be selected for. The information now available on protein suggests that it is possible to manipulate efficiently the use of body-stored fat as a source of energy for growth when the external supply of energy is low.

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METABOLIC RESPONSES TO SUPPLEMENTATION IN GROWING RUMINANTS CONSUMING LOW DIGESTIBILITY FIBROUS DIETS*

J.V. NOLAN**, G.J. LEE***,
D.W. HENNESSY⁺, R.A. LENG**

**Department of Biochemistry, Microbiology
and Nutrition,
The University of New England,
Armidale, New South Wales

***Agricultural Research Station,
Trangie, New South Wales

⁺ Agricultural Research Station,
Grafton, New South Wales
Australia

Abstract

METABOLIC RESPONSES TO SUPPLEMENTATION IN GROWING RUMINANTS CONSUMING LOW DIGESTIBILITY FIBROUS DIETS.

Appropriate feeding strategies for ruminants consuming fibrous diets depend on an understanding of how supplements alter the array of absorbed (metabolizable) nutrients, both through their effects on the ratios of the end-products of rumen fermentation and by providing nutrients that escape unfermented from (or bypass) the rumen. The metabolic interdependence of these absorbed nutrients means that the requirements for each nutrient cannot be simply predicted; the requirement for any one nutrient depends on the availability of and metabolic needs for other nutrients. Imbalances in the rate of supply of nutrients reduce their efficiency of utilization. Animals on fibre-based diets appear to utilize absorbed nutrients (e.g. acetate and amino acids) less efficiently than animals given concentrates. Less glucogenic precursors (e.g. propionate, starch, amino acids) are available to these animals and so they may not synthesize adequate amounts of glucose and reduced NADP to enable acetate to be converted to long chain fatty acids and fat; their inability to clear acetate may lead to an increase in blood acetate concentration that could inhibit feed intake. The objective of a feeding strategy for ruminants on fibrous feeds should therefore be either to increase the propensity for glucogenesis, or decrease the animal's metabolic requirement for glucose by providing dietary lipid, or to provide alimentary starch, or combinations of these. Protein supplements which provide dietary amino acids for absorption directly, and which may increase the total availability of nutrients through their effects on ruminal fermentation, are therefore likely to be effective supplements for ruminants on fibrous feeds.

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1. INTRODUCTION

In many countries native pasture or hays or straws which have low digestibility and low protein content are the major feed resources available for ruminants, and farmers and nutritionists are faced with the problem of how to obtain acceptable levels of production using these feeds as the basal diet.

Clearly, the primary objective of any feeding strategy should be to provide an appropriate amount and balance of absorbed nutrients to enable their efficient use in the metabolism and synthesis of tissues or products. Meeting these objectives requires consideration of the following:

- (1) How to optimize microbial activity in the rumen in order to maximize feed intake and digestibility
- (2) How to provide additional nutrients to complement the products of fermentative digestion and thereby provide an array of absorbed nutrients that matches the animal's requirements for the particular stage of growth or type of production.

A general consideration is that intake and digestibility of the basal feed (which is the least expensive dietary ingredient) should be maximized, while the levels (and therefore costs) of the more expensive supplements are kept low in order to optimize economic returns.

In practice, for ruminants consuming mature roughages, supplements containing protein that escapes ruminal fermentation (bypass protein) often provide the most effective means of promoting efficient growth and production (e.g. see review by Leng et al. in Ref. [1]). In this paper we attempt to explain why this is so. We suggest that absorbed amino acids derived from protein entering the intestines are metabolically 'flexible', not only correcting essential amino acid insufficiencies but also providing ATP when oxidized and glucogenic amino acids that can be synthesized into glucose, which is essential for some functions in the body. One essential function of glucose metabolism is to provide reduced NADP for lipogenesis in adipose tissue. Thus, protein and fat deposition during growth compete for glucogenic amino acids. The importance of an appropriate amino acid or protein:energy (P:E) ratio in the absorbed nutrients for efficient production has been stressed previously (e.g. Ørskov [2], Leng et al. [1]), but in this review the need for adequate amounts of 'glucogenic energy' in the total energy as postulated by Leng and Preston [3], Leng et al. [1], Preston and Leng [4], MacRae and Loble [5] and Preston and Leng [6] is emphasized. New supporting evidence is given for the importance of an adequate supply of glucose to enable efficient energy retention.

Protein supplementation provides a convenient and appropriate way of providing both amino acids and glucose precursors but, in addition, protein supplements may also affect rumen fermentative activity by providing ammonia (and peptides and amino acids) for use by rumen microbes [7] and could increase fibre digestion and thus feed intake when the nitrogen (N) content of the basal feed is low.

This review is concerned mainly with the use of supplements to ensure efficient fermentative digestion and an appropriate balance of metabolizable nutrients for tissue synthesis in growing ruminants, but similar arguments apply to foetal growth and milk production.

2. OPTIMIZATION OF FERMENTATIVE ACTIVITY IN THE RUMEN

Microorganisms in the rumen require fermentable substrates (cellulose, hemicellulose, starch, sugars), a source of nitrogen (N) as ammonia, minerals, and perhaps some vitamins. In practice, readily fermentable substrate, ammonia and sulphur (as $S^{=}$ ion) are most likely to limit rumen fermentation in animals on fibrous forages. During fermentation, organic matter (OM) is either converted to volatile fatty acids (VFAs), CO_2 and CH_4 (with production of ATP) or synthesized into microbial OM (with utilization of ATP). The efficiency of utilization of ATP for cell OM synthesis will be reduced if certain essential nutrients (i.e. ammonia, sulphur, etc.) for synthesis of cellular macromolecules are in short supply.

Studies of the kinetics of ^{15}N -ammonia in the rumen (see Nolan and Leng [7], Leng and Nolan [8]) have provided valuable information on the extent of degradation of dietary protein, and have identified ammonia as the major N source for the mixed microflora in the rumen. It is not entirely clear from these studies, however, what fraction of the dietary protein is degraded and used locally by the microflora associated with feed particles rather than first entering the ^{15}N -labelled ammonia pool in rumen fluid.

Despite conservation of endogenous urea by the kidney and its transfer to the rumen and hydrolysis to ammonia, animals on low N forages (i.e. < 20 g N/kg digestible organic matter) do not have sufficient ammonia in the medium to support maximum fermentative activity or efficient microbial cell synthesis; animals on such diets benefit from supplementation with urea (Leng and Nolan [8]).

If microbial cell synthesis is inefficient because of a deficiency of ammonia (Schaefer et al. [9]), increasing the ammonia concentration (e.g. by supplementing with urea or protein meals) will increase the yield of cells. In addition, it will concomitantly decrease the production of VFAs and gases per unit of organic matter fermented, thereby altering the balance of nutrients in the end-products of fermentative digestion. The increase in feed intake in response to urea supplementation (see Campling et al. [10], Leng et al. [1], Boniface et al [11]) is therefore probably due both to an increase in digestibility of the diet OM and also to a more appropriate P:E ratio and therefore glucogenic energy:total energy in the absorbed nutrients.

3. OPTIMIZATION OF THE BALANCE OF METABOLIZABLE NUTRIENTS

The discussion in Section 2 indicates that there is potential for manipulating the balance of individual nutrients available to the animal by altering the nature of fermentative digestion, or by supplying bypass nutrients, or both. The principal nutrients of interest in this context are: VFAs including acetic (Ac), propionic (Pr) and butyric (Bu) acids; microbial and dietary amino acids; dietary lipids; and with some diets, glucose from dietary starch.

VFAs are absorbed from the rumen in proportion to their molar proportions in rumen liquor (see Leng [12]) which are typically (Ac:Pr:Bu) 70:20:10 for animals on roughage-based diets, and 60:30:10 for most concentrate-based diets. With increases in the efficiency of microbial cell production (g cells/kg OM fermented) that may result, for example, from correcting a deficiency of ammonia or sulphur in the rumen, the ratio of microbial cells to total VFAs (and thus microbial protein and lipid in the products) of fermentative digestion increases (Leng [13]). Ruminants on roughage diets absorb negligible quantities of glucose and are almost entirely dependent on gluconeogenesis, whereas animals given grain-based diets may obtain some of their glucose requirements by absorption of glucose from the small intestine; these diets also tend to promote higher rates of production of Pr in the rumen.

In practice it is difficult to identify the primary limiting nutrient and so determine a strategy for correcting any imbalance. The requirement for individual nutrients depends on the availability of and metabolic needs for other nutrients. If total non-protein-energy intake is below requirements but protein absorption exceeds requirements, energy in any form (VFA, or lipid, or starch) could increase the rate of tissue growth, but efficiency of energy use will be low if these latter nutrients are not available in balanced amounts. If essential amino acids are insufficient to meet the losses during obligatory protein turnover, plus the amounts needed for protein accretion, only additional supplies of digestible protein to the small intestine will improve growth (see Egan [14]). However, protein supplements that are suitable for achieving this (e.g. oil-seed meals, fishmeal, rice polishings) also contain long chain fatty acids (LCFAs) and glucose as well as amino acids. The LCFAs are efficiently absorbed but the fates of glucose and amino acids are uncertain and this hinders interpretation of the feeding trials. Moreover, glucogenic amino acids can be a source of extra glucose. The presence of a surplus of digestible protein may therefore reduce the requirement for other glucogenic precursors, whereas absorption of LCFAs may, as discussed in Section 4, reduce the requirement for glucose (for glycerol phosphate synthesis) and for oxidation (to provide NADPH).

4. GLUCOSE — A LIMITING NUTRIENT?

For many years scientists have recognized that glucose could be a limiting nutrient for ruminants (Lindsay [15], Annison and White [16], Leng et al. [1]). This

is especially likely in ruminants on low digestibility fibrous feeds that promote low Pr:Ac ratios and probably also low efficiency of microbial cell (and therefore protein) production in the rumen; it is less likely in animals consuming fresh pasture or concentrates when Pr:Ac ratios are generally higher, and often, because of higher intakes, there is also potential for protein and starch to escape fermentation. Under these conditions amino acids and glucose are unlikely to be in short supply.

In technologically advanced countries, where the majority of basic nutritional studies with ruminants have been carried out, experiments have generally been undertaken with animals on high digestibility, high protein forages or grain-based concentrates. However, recognition by Preston and co-workers [17] that the feeding practices of temperate countries were generally inappropriate for tropical developing countries led to the development of feeding strategies which emphasized the utilization of locally available resources such as molasses and sugar-cane. Experience in the use of these feeds led these workers to recognize that animals need to have a balance of certain important nutrients, particularly glucose, to optimize the efficiency of utilization of feeds for production (Leng and Preston [3], see also Preston and Leng [4]). Growth trials with cattle or sheep on low protein feeds that were high in sugar (Preston and Willis [18]), starch (Ørskov [2]) or fibre (Leng et al. [1]) had highlighted the value of bypass protein sources and supplements such as rice polishings that contained protein, starch and lipid (Leng and Preston [3]). Development of feeding strategies for using sugar-based diets refocused attention on glucose as a potentially limiting nutrient (Leng and Preston [3], Leng et al. [1], Preston and Leng [4]).

The need for a balanced supply of glucogenic substrate relative to lipid and amino acids has also been discussed by Kronfeld [19] for the lactating animal, and MacRae and Lobley [5] have emphasized the important role of glucose for the growing animal on fibrous diets. In addition the high rates of utilization of available glucose in pregnancy (Setchell et al. [20]) and requirements for working muscle (Pethick et al. [21], see also Leng [22]) is further evidence of the critical role of glucose in ruminant production.

5. GLUCOSE AND LIPID REQUIREMENTS FOR GROWTH

As animals grow to maturity they deposit both fat and protein with the proportion of protein relative to fat declining with age. As they approach mature weight, there is a greater proportion of LCFA relative to the protein needed for tissue deposition.

Lipids are readily hydrolysed to LCFAs in the rumen, they are largely hydrogenated but are otherwise unaltered when they pass out of the rumen. LCFAs are efficiently absorbed (see Thornton and Tume [23]) and may be efficiently used for lipogenesis in adipose tissue (Lindsay [24]). Mature roughages, however, generally have a low lipid content (ca 2% of dry matter (DM)) compared with fresh leafy

forage (ca 10% of DM) and ruminants fed mature roughage absorb only small quantities of LCFAs; they therefore rely on Ac and Bu, which are the only other potential precursors for LCFA synthesis. In tissues this synthesis requires NADPH, which arises from glucose oxidation. This increases the need for glucose that must (on these diets) be supplied by gluconeogenesis. Possible ways of ameliorating this problem are to supplement the animal with dietary lipid to reduce the need for glucose, or to provide additional glucose or its precursors.

In ruminants, adipose tissue is the major site of LCFA synthesis (Ingle et al. [25]), and it is accepted (see Bauman [26]) that Ac is the major precursor (Hanson and Ballard [27]). Glucose is the major precursor of the glycerol 3-phosphate needed for glyceride synthesis during lipogenesis, as the enzymes for utilizing glycerol are absent from adipose tissue; 14 mol NADPH (generated by oxidation of glucose via the pentose phosphate pathway in the cytosol of adipocytes) are needed for each mole of palmitate synthesized (Vernon [28]). If glucose were the sole source of NADPH and glycerol phosphate, then 89 g glucose would be oxidized for the synthesis of 100 g triglyceride (Leng [22]).

Some sparing of glucose use for NADPH formation would be possible if NADPH is produced from Ac via the NADP-isocitrate dehydrogenase which is present in adipose tissue of ruminants (Ingle et al. [25]). Nevertheless, if glucose availability is limited by insufficiency of ruminal Pr and glucogenic amino acids, low NADPH availability in adipose tissue could restrict LCFAs and fat synthesis from Ac. The necessity to maintain blood Ac within physiological limits could promote feedback on appetite and restrict feed intake (Leng [29]), or the Ac load might be reduced by 'futile', heat-producing cycles (MacRae and Lobley [5]). This heat production lowers the partial efficiency of use of Ac above maintenance for growth and fat deposition (K_g).

Ruminal Pr is a major precursor of glucose and the suggestion of glucose-NADPH-limited lipogenesis is in accord with the conclusion of Blaxter [30], based on earlier calorimetric studies, that the lower K_g in ruminants on forage-based diets is related to the higher molar proportion of ruminal Ac relative to Pr, and with the lower efficiency of use of Ac in tissues compared with Pr (Armstrong and Blaxter [31]). However, results of feeding trials with cattle (Rook et al. [32]) and lambs (Ørskov and Allen [33]), and more recent studies (Ørskov et al. [34]) with lambs nourished entirely by intra-abomasal infusion of casein and intraruminal infusion of mixtures of VFAs varying in molar proportions (Ac:Pr:Bu) from 75:15:10 to 45:35:20 showed that Ac can be used with efficiencies consistent with the biochemical processes by which it is metabolized.

It has been suggested (Hovell et al. [35], MacRae and Lobley [5], Gill et al. [36], Preston and Leng [6]) that these apparently contradictory results can be reconciled when the glucose-producing potential of the diets is considered. A mathematical model that simulates the metabolism of the major absorbed nutrients in ruminants on the basis of currently accepted stoichiometry and enzyme kinetics has been used by Gill and co-workers [36] to evaluate this suggestion. The model predicts that partial

TABLE I. EFFECT OF COMPOSITION OF DIET (LUCERNE HAY VERSUS HAY CONCENTRATE (30:70)) ON EFFICIENCY OF RETENTION OF METABOLIZABLE ENERGY PROVIDED BY INTRARUMINAL INFUSIONS OF ACETATE AND ON NITROGEN RETENTION IN MATURE, NON-PREGNANT COWS [37]

		Hay		Concentrate	
		Control	+ Acetate (increment) ^a	Control	+ Acetate (increment) ^a
Gross energy intake	(MJ/d)	163	8.1	103	11.4
Metabolizable energy	(MJ/d)	68	8.1	66	10.4
Heat production	(MJ/d)	67	5.9	59	3.3
Energy stored	(MJ/d)	0.8	2.2	6.7	7.2
N intake	(g/d)	240		152	
N balance	(g/d)	+12	-14	+8	+10

^a The increment occurring as a result of intraruminal infusion of acetate solution rather than water.

efficiency of Ac utilization will increase with increasing glucose supply, and at constant amino acid availability efficiency will be positively related to the rate of generation of NADPH.

The effect of diet composition, and therefore the proportions of absorbed nutrients, on the efficiency of use of Ac is apparent in the study of Tyrell et al. [37] (Table I). The results show that in mature, non-lactating cows infused Ac is used much more efficiently, and N retention is increased, with diets that provide more glucogenic precursors. Eskeland et al. [38] also obtained higher N retention in lambs when Pr (or Bu) rather than Ac was infused intravenously. It is therefore possible that both fat and protein are deposited less efficiently when glucose availability is limited.

Direct absorption of glucose, or absorption of its precursors (ruminal propionate or amino acids), appears to promote more efficient use of nutrients for live weight gain. When steers were given maize concentrate in place of pelleted lucerne of similar metabolizable energy content, they grew faster and fat deposition and lipogenic enzyme activities were enhanced [39]. It is likely that the grain increased the ruminal Pr:Ac ratio and some starch and LCFAs escaped to and were absorbed from the small intestine, augmenting the glucogenic potential or decreasing the need for glucose.

ME was used more efficiently in mature fattening animals given maize-forage diets than in those given barley-forage diets (Thomson [40]), probably because, as suggested by Preston and Leng [6], more maize- than barley-starch escaped to the

small intestine. Corbett et al. [41] showed that ME in spring-harvested grass was used more efficiently by sheep than autumn-harvested grass, and that the higher efficiency was associated with higher molar ratios of Pr:Ac in the rumen. More recently, similar differences were found between spring and autumn pasture material, but with no differences in molar ratios of rumen VFAs (Ribeiro et al. [42]). However, there was evidence in this study that 25% more crude protein was absorbed from the small intestine of the sheep on the spring-harvested material. Thus, availability of both propionate and protein may affect the efficiency of nutrient utilization.

The metabolic roles of glucose for growth, pregnancy, lactation or work have been discussed in detail by Preston and Leng [6]. Points relevant to growing ruminants can be summarized as follows.

Glucose absorption is negligible in ruminants consuming mature forages and the potential for gluconeogenesis from propionate and amino acids is low. Glucose is therefore a potentially limiting nutrient. At the same time, because dietary LCFA supply is low, the need for glucose (to supply glycerol and NADPH to enable fat synthesis in adipose tissue and for essential functions) is increased. The animal may have to utilize amino acids to synthesize glucose and so maintain essential functions; this will reduce the potential for protein deposition and will decrease N retention, and may also increase the circulating levels of ketogenic amino acids. The latter will tend to increase the entry of Ac into a pool already enlarged by absorption of ruminal Ac from VFAs with a high Ac:Pr ratio, and by the reduced (glucose-NADPH-limited) propensity for Ac removal by conversion to LCFA. The Ac load may result in reduced appetite and feed intake by the animal. Futile heat-producing cycles may be invoked to reduce the load, but the resulting heat production (especially in animals in hot environments) could further inhibit feed intake.

Feed intake could also be inhibited by imbalances in the circulating levels of amino acids (Harper [43]) and perhaps also by a tendency for acidosis resulting from Ac accumulation. Factors that act to reduce feed intake are probably additive. Acetate load, heat load and amino acid imbalance will further reduce appetite already subject to other inhibitory stimuli from, for example, rumen and abdominal distension (McClymont [44], Forbes [45]).

6. METABOLIC INTERRELATIONSHIPS

The metabolic interrelationships between rates of synthesis of glucose and the availability of other nutrients in ruminants are maintained by complex control systems (Bassett [46]). The model described by Gill et al. [36] is probably the most complete representation presently available to enable the various relationships to be quantitatively evaluated. But even in this model, hormonal (e.g. insulin, glucagon, epinephrine, growth hormone) and other controlling factors (e.g. neural) are not fully accounted for, and in particular their effects on feed intake are not predictable. The

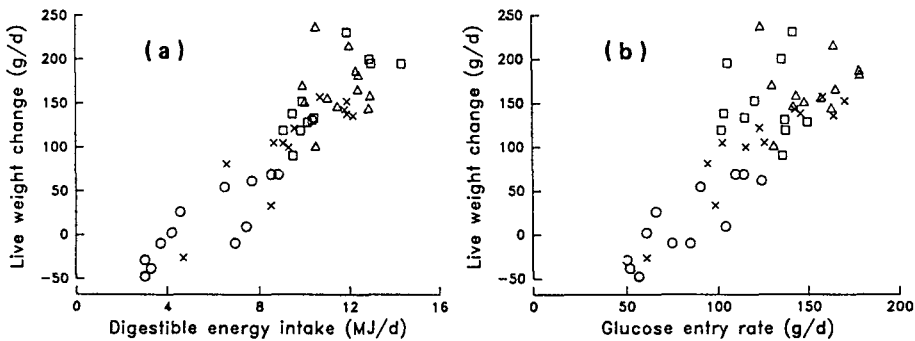


FIG. 1. Relationships between (a) live weight change and digestible energy intake, and (b) live weight change and glucose entry rate in 48 lambs given free access to a basal diet of oats chaff-sugar (1:1) and either 0 (O), 40 (X), 80 (□) or 120 (Δ) g fishmeal/kg basal diet [50].

possibility of modelling these control systems has been discussed by Waghorn and Wolff [47].

The metabolic relationships between nutrients make it difficult to design definitive experiments to identify the role of glucose in metabolism and to calculate the true minimum requirement for glucose. It has been argued (Leng [12]) that the rate of entry of glucose into the glucose pool in the blood, estimated by isotope dilution methods ^3H - or ^{14}C -glucose, may give a good indication of this requirement because synthesis of glucose in excess of its needs for essential purposes would be energetically wasteful. The glucose entry rate (GER) is linearly related to DE intake (DEI) in mature (Judson and Leng [48]) and growing (Kempton and Leng [49]) animals, but the reasons for the close correlation are not fully understood. Appetite and DE intake of ruminants are under complex metabolic control and are dependent on stimulatory and inhibitory signals from the liver, visceral organs and adipose tissue that are integrated by the higher centres in the brain [50]. In growing ruminants, live weight gain is also closely related to GER [49, 50]. These relationships are typified by the results of Bird [50] given in Fig. 1.

The relationship between live weight gain and GER may suggest that glucose availability is a primary determinant of growth rate; however, because live weight gain is also closely correlated with DE and therefore metabolizable energy (ME) intake, it may be argued that the former relationship has little metabolic significance. We therefore decided to re-examine a number of experimental results, including those in Fig. 1, for evidence of an association between glucose availability and growth rate that was not explainable if glucose were considered simply to be a source of ME. The question was as follows. Are the variations in live weight gain between different animals at a given ME intake (i.e. differences in efficiency of ME use for growth) associated with differences in glucose availability?

TABLE II. EFFECTS OF SUPPLEMENTARY BYPASS GLUCOSE OR PROTEIN ON FEED INTAKE, LIVE WEIGHT GAIN AND FEED CONVERSION RATIO (FCR: g FEED/kg LIVE WEIGHT) AND ON GLUCOSE FLUX AND UREA SYNTHESIS RATES IN LAMBS [54, 55]

Treatments*	Feed intake (g/d)	Growth (g/d)	FCR	Glucose flux rate (g C/d)	Urea synthesis rate (g C/d)
B	890 ^a	97 ^a	10.1 ^a	54 ^a	31 ^a
B + G	770 ^a	129 ^b	6.9 ^b	56 ^a	24 ^b
B + P	1139 ^b	202 ^c	6.0 ^c	71 ^b	45 ^c
B + G + P	1074 ^b	257 ^d	4.3 ^d	90 ^c	40 ^d

* The lambs were given a basal diet (B) consisting of a mix of oaten chaff and sucrose (1:1) to which urea (40 g/kg mix), minerals and vitamins were added, or the basal diet plus 80 g glucose/d by abomasal infusion (B + G), or a dietary supplement of 60 g fishmeal/kg basal diet (B + P), or both latter treatments simultaneously (B + G + P).

Values in columns with different superscripts are significantly different at $P < 0.05$.

The results from a number of studies [50–53] with sheep and cattle on diets that resulted in variable rates of growth were examined by multiple regression procedures. The following model was used:

$$\text{Live weight gain} = \text{constant} + \alpha(\text{ME}) + \gamma(\text{GER}) + \text{residual variation}$$

For these purposes ME intake was assumed to be $0.81 \times \text{DE intake}$.

In the case of the study by Bird [50] of lambs (18 to 34 kg) given the diets described in the legend to Fig. 1, the inclusion of GER in the above model did not significantly improve the prediction of live weight gain, which had already been well predicted ($R^2 = 82\%$) by ME alone (Fig. 1). The situation was similar with the results from a study made by Kempton [52] of 16 growing lambs (21 to 27 kg) given oaten chaff plus casein or formaldehyde-treated casein and from a study made by Hennessy et al. [53] with 27 growing cattle (ca 142 kg) given mature, subtropical grass hay alone, or supplemented at two levels with protein meal, or two levels of cracked sorghum grain, or combinations of grain and protein meal. In contrast, when analysing results from a study [53] of 30 growing lambs (17 to 33 kg) given either a basal diet of bagasse (containing also small amounts of sucrose, lucerne and urea) or the basal diet with supplements of rice bran and fishmeal, 49% of the variation was removed by the inclusion of ME in the model, and a further 20% was significantly ($P < 0.01$) removed by GER. Inclusion of GER in addition to ME in the multiple regression model also significantly improved the prediction of live weight gain with results from a study with nine growing lambs (see Table II [54, 55]) and from

a study made by West and Passey [56] with 14 growing cattle (190 to 260 kg) given a basal diet of molasses-urea ad libitum plus 1.5 kg/d of mature cereal hay alone or with fishmeal (8 or 12% of the basal diet).

The clear demonstration that at times GER plays a significant role in the prediction of differences in live weight gain, which is additional to the role played by ME intake, i.e. that availability of glucose is apparently associated with different efficiencies of the use of ME, is of considerable interest.

In seeking to explain the association of GER with higher live weight gain and higher feed conversion efficiency in some of the studies examined and not in others, several points may be relevant. First, live weight gain at times bears little or no relationship to changes in energy storage in the body; its measurement, although convenient, provides an unsatisfactory basis for estimating the efficiency of ME utilization. Second, responses in live weight gain to GER (in addition to MW) would be more likely, on the basis of the discussion above, to be evident when the animal's capacity to synthesize its requirements for glucose (from dietary bypass starch, ruminal propionate, or amino acids from dietary bypass protein or microbial protein) was limited. This hypothesis receives general support from the results obtained using the multiple regression model. In animals consuming the molasses-based or bagasse-based diets, where GER made a significant contribution (in addition to ME intake) to the prediction of LWG in the model, the molar proportions of Pr in the ruminal VFAs were low, and the animals could have been more dependent on amino acids to supply glucose requirements. In contrast, in the lambs given the diets based on oaten chaff [50], the molar proportions of ruminal Pr in the VFAs were abnormally high (0.27 to 0.38) and there would have been a more than adequate capacity for glucose to be synthesized from this source. In the cattle supplemented with sorghum [53] some of the grain probably escaped ruminal fermentation and supplied starch to the intestine, augmenting the potential for glucose supply; again, inclusion of GER did not significantly improve the prediction of live weight gain after ME was included in the model. It was also noted in the studies examined that the effect of GER appeared to be more readily identified in animals growing at moderately fast rates.

7. FEEDING RUMINANTS FOR PRODUCTION

The above discussion supports the recommendation of Leng and Preston [3] that, in practice, when the N content of the basal diet is low, feeding strategies should focus on maximizing fermentative digestion and then on promoting adequate glucose availability for the animal, either by providing additional glucose for intestinal absorption or by providing additional precursors for gluconeogenesis.

Increased rates of absorption of glucose may inhibit gluconeogenesis in fasted ruminants (West and Passey [56]) and reduce gluconeogenesis in mature fed animals (Judson and Leng [57]). In contrast, intraruminal infusions of propionate or intra-

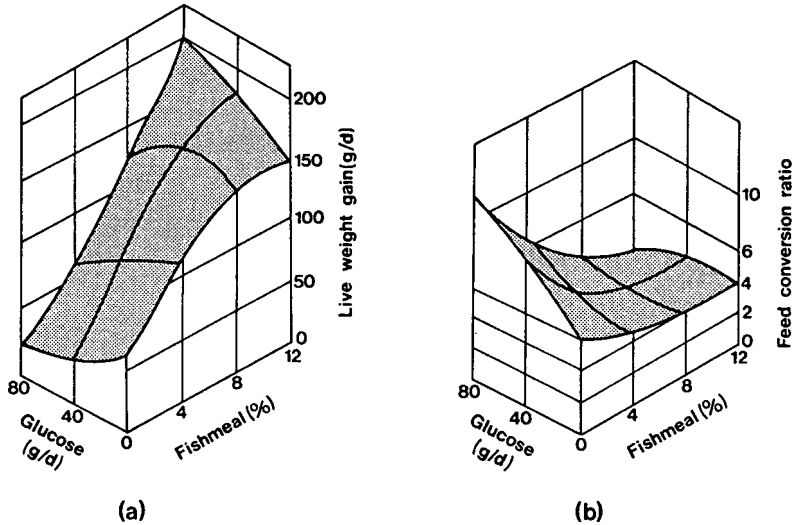


FIG. 2. (a) Growth rate (g/d) and (b) feed conversion efficiency (g feed/g live weight) in lambs given a basal diet of oat chaff-sucrose and supplemented with bypass amino acids or bypass glucose [59].

abomasal infusions of casein hydrolysate stimulate gluconeogenesis in sheep fed roughage diets (Judson and Leng [58]). It appears rational to supplement animals so as to improve Pr production in the rumen, or protein absorption from the small intestine, rather than to provide bypass starch or bypass glucose. This conclusion appears to be borne out in feeding trials. Leng et al. [54] compared the effects on feed intake and growth of supplying glucose directly (by abomasal infusion) or amino acids (by giving dietary bypass protein) to lambs fed a forage-sugar diet containing negligible amounts of true protein (i.e. 0.48 oat chaff, 0.48 sucrose and 0.04 urea, minerals and vitamins) (see Table II [54, 55]). Glucose infusion tended to reduce feed intake but to increase the efficiency of feed conversion and live weight gain. The increased efficiency was accompanied by a reduction in the rate of urea synthesis, which suggests that less protein was degraded (less amino acids were deaminated and the carbon moiety used in synthesis of glucose) when glucose was infused, and therefore more amino acids would have been available for deposition in tissues. In contrast, the bypass protein supplement increased both feed intake and feed conversion efficiency and produced an additional increase in live weight gain. When both supplements were given together, there was a further improvement in feed conversion in live weight gain.

Similar results were obtained in a study (Kempton et al. [59]) in lambs on the same basal diet. Bypass nutrients were provided by different combinations of fishmeal (bypass protein, lipid and starch) and abomasally available glucose (via the suckling reflex) (see Fig. 2 [59]). The growth rate and efficiency of feed conversion

TABLE III. LIVE WEIGHT CHANGE AND OTHER MEASUREMENTS IN CATTLE ON A BASAL DIET OF SUBTROPICAL GRASS HAY, EITHER UNSUPPLEMENTED OR SUPPLEMENTED WITH UREA-MOLASSES OR PROTEIN MEAL IN COMBINATION WITH EITHER CRUSHED MAIZE (CM) OR WHOLE MAIZE (WM) (From Lee et al. [60])

	No Nitrogen			Urea-molasses			Protein meal		
	Nil	CM	WM	Nil	CM	WM	Nil	CM	WM
Live weight change (g/d)	-676	-417	-482	-65	167	9	111	268	361
Total DOMI ^a (kg/d)	0.79	1.63	1.73	1.93	2.01	2.33	2.40	2.36	2.27
ME from hay (MJ/d)	36	44	51	78	72	77	79	0	71
ME from supplements (MJ/d)	0	7	7	2	9	9	7	14	13
N intake (g/d)	8	21	25	70	76	84	69	70	79
Rumen NH ₃ concentration (mg N/L)	10	45	13	115	172	221	92	142	82
Glucose entry rate (g glucose/kg W ^{0.75} per day)	3.6	3.3	4.8	4.2	4.2	4.8	5.6	5.2	6.5
Glucose entry rate ^b (g glucose/kg W ^{0.75} per day)		4.3			4.3			5.5	

^a DOMI = digestible organic matter intake.

^b Corrected by co-variance analysis to same DOMI = 1.9 kg/d.

were increased by fishmeal supplementation, but with glucose positive effects were obtained only in combination with more than 4 to 8% fishmeal in the basal diet.

A revealing study was made by Lee et al. [60] of the effect of supplements containing combinations of urea-molasses (rumen degradable N), protein meal pellets (rumen degradable N + bypass protein), crushed maize (largely ruminally fermentable) and whole maize (a fraction of the maize starch escapes ruminal fermentation) on the intake of mature subtropical grass hay, glucose availability and live weight change (some 'loss') in 27 young cattle. The nine treatments consisted of N supplements (nil, 112 g urea/d, or 800 g protein pellets/d) offered in combination with maize supplements (nil, crushed (570 g/d) or whole (680 g/d)). The urea and protein meal containing supplements were iso-nitrogenous and the maize and protein meal in the supplements were estimated to provide the same amounts of ME. The changes in live weight and other relevant metabolic measurements in response to the various supplements are given in Table III [60]. The intakes of digestible OM (DOM) accounted for 71% of the variance in live weight change, but as with the lamb growth results given in Section 6 other factors, e.g. efficiency of ME utilization, also affected production.

Supplements of maize alone did not significantly reduce weight loss. Supplements containing urea or protein meal had larger positive effects such that, on average, cattle gained 37 g/d with urea and 247 g/d with protein. The additional 210 g/d gain in protein compared with urea-supplemented cattle was associated with an increase in intake of only 110 g digestible OM (DOM)/d, indicating an effect of supplements containing protein pellets, not only on rumen fermentation but also on the efficiency of utilization of absorbed nutrients. Higher efficiencies were associated with higher glucose availabilities, which were obtained with supplements containing protein pellets. After adjusting for the effect of DOM intake, glucose availability was not different between the unsupplemented cattle and cattle supplemented with urea, but glucose availability was higher in cattle given protein pellets alone or in combination with maize.

Urea-molasses supplementation appeared to maximize the intake of grass hay; however, overall the protein meal-maize combinations were the most effective supplements for these growing cattle, providing not only the highest levels of production but also the most efficient use of the basal diet of grass hay.

8. CONCLUSIONS

Optimum levels of production in ruminants can only be achieved if all the nutrients required for tissue synthesis and metabolism are available to the tissues in sufficient amounts and in the correct proportions. Ruminants fed fibrous diets may benefit from supplementary nutrients in two ways, namely through enhanced ruminal fermentation (supplying more VFAs and microbial amino acids and lipids) and, with supplements that resist ruminal degradation, through the greater availability of

dietary nutrients for absorption from the small intestine. The supply of additional glucogenic nutrients and lipids may be particularly helpful in growing and fattening ruminants on fibrous diets; high efficiencies of utilization of absorbed nutrients will be achieved only if amino acids and glucose availability are balanced with the energy availability from the non-glucogenic energy substrates — principally acetate and butyrate. The processed protein meals (meat, fish and oil-seed meals) are the most reliable supplements fulfilling these roles.

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EFFECT OF DIET AND HORMONES ON PROTEIN METABOLISM IN MUSCLE

P.J. BUTTERY, L. BRYAN

Department of Applied Biochemistry
and Food Science,
University of Nottingham School
of Agriculture,
Sutton Bonington, Leicestershire,
United Kingdom

Abstract

EFFECT OF DIET AND HORMONES ON PROTEIN METABOLISM IN MUSCLE.

Muscle protein is in a state of continual flux. Protein is constantly being synthesized and degraded and it is the balance between these two processes which controls the rate of change in the protein content of muscle. The protein degradative rate and its rate of change is as important as the synthetic rate in controlling muscle protein mass. Generally, a change in synthetic rate is accompanied by a change in the same direction in the degradative rate, although the magnitude of the responses may differ. Post-natal muscle growth is by cross-sectional and longitudinal hypertrophy of existing muscle fibres. The ability of an animal to effect the net catabolism of muscle protein enables this tissue to provide amino acids for other tissues, e.g. the foetus and the brain. Individual muscles show different responses to their hormonal environment and this is reflected in their differing response to nutritional stress. The hormones insulin and cortisol have antagonistic effects on muscle protein metabolism; insulin tends to be anabolic, while cortisol tends to be catabolic. The ratio of these two hormones in the plasma can be correlated to responses seen in muscle both during dietary insufficiency and during growth.

1. INTRODUCTION

This review considers aspects of the hormonal control of protein metabolism in muscle and relates them to the changes seen in muscle and whole body protein metabolism when animals receive poor diets or are subjected to complete or partial starvation. Much of the work on malnutrition has been conducted with the response of Man in mind and, as such, has largely used Man and the rat as experimental animals. There are, however, good reasons to believe that the data obtained have direct relevance to domesticated species subjected to harsh environments.

2. GROWTH OF MUSCLE

Post-natal growth of muscle takes place by cross-sectional and longitudinal hypertrophy of existing muscle fibres, but in the embryo there is obviously *de novo*

production of muscle fibres. In the embryo presumptive myoblasts, originating from the mesoderm layer, multiply under the influence of unidentified serum factors and subsequently align themselves and fuse to form myotubes. At this stage the myotube starts to become filled with myofibrils. Secondary myotubes then form around the primary myofibre (a myotube with myofibrils in it) and also become filled with contractile protein. At this stage the connective tissue is laid down and the adult muscle structure emerges. Subsequent growth of the muscle takes place by enlargement of the existing myofibres. The number of nuclei do, however, increase but these originate from satellite cells which are intimately associated with the adult muscle fibres. The satellite cells proliferate and fuse with existing myofibres. Changes in the mass of muscle in the post-natal animal take place largely by changes in the protein content of the muscle fibre; some change in water content is also often noted.

Proteins in muscle are constantly being synthesized and degraded, i.e. they are being turned over, and this balance between these two processes controls whether a muscle increases or decreases in size. The rate of degradation of the protein is as important as the rate of protein synthesis.

3. PROTEIN TURNOVER IN MUSCLE

The rate of total body turnover is related to metabolic body size with approximately 16 g protein being synthesized per kilogram body weight^{0.75} per day [1]. Of this turnover, muscle accounts for a relatively constant proportion in different species (see Table I [2]). In a sheep of 40 kg approximately 200 g of protein are being synthesized per day. The rate of protein deposition is considerably less than this. The ratio of synthesis to accretion in rapidly growing cattle is not likely to be less than 8:1, while in older cattle it would be much higher, 20:1, even when the animal is receiving an adequate diet.

Individual muscles do not behave identically. The turnover rates are quite different and can be related to such things as the ratio of glycolytic to oxidative muscle fibres and the hormonal sensitivity of the muscle. Data available for the ruminant have been discussed elsewhere [3] and are illustrated by the recent observations presented in Table II [4-10]. It will not, therefore, be surprising to find that different muscles respond differently to nutritional stress.

4. HORMONAL CONTROL OF MUSCLE GROWTH

Many hormones have been shown to influence protein synthesis and degradation in muscle; some of the responses are shown in Table III.

As a first approximation whole animal growth is often equated with muscle growth, especially in large domesticated species where it is often difficult to obtain carcass composition data. Since glucocorticoids have a catabolic effect it might be

TABLE I. PERCENTAGE CONTRIBUTION OF MUSCLE PROTEIN SYNTHESIS TO WHOLE BODY PROTEIN TURNOVER

Species	Body weight (kg)	Percentage contribution
Rat	0.1	19
Rabbit	3.8	18—21
Pig	24	24
Pig	30	33
Pig	75	26
Lamb	2.7	2.8
Sheep	35	19
Human	70	20—26
Cattle	236—628	19—22

Data are from table compiled by Lobley et al. [2]; it should be consulted for further information.

expected that their circulating level would be correlated to growth rate, but in domesticated species much of the data is equivocal. In cattle a negative relationship has been reported; however, other studies have failed to confirm this (see Ref. [11]). In sheep this relationship has not been demonstrated. Circulating concentrations of hormone do not, however, always give a true picture of the activity of a hormone. Steroid hormones in particular are found bound to circulating proteins in the blood; transcortin in the case of glucocorticoids. Ninety per cent of the circulating glucocorticoid is bound, while 10% is free. It is commonly suggested that it is the free component that is active. For this component to have an effect in the tissues it must combine with a receptor protein found in the cytosol of the cell and this complex must migrate to the nucleus to interact with the chromatin. Thus, the biological activity of glucocorticoids is related to the amount of transcortin which controls the free concentration of the hormone in the plasma, and the activity and numbers of receptors available in the target tissue. Insulin and glucocorticoids have antagonistic effects on muscle protein metabolism. In a recent experiment Sharpe et al. [11] studied various parameters of cortisol status and growth in sheep whose growth was manipulated by diet or by implantation with either the growth promoter trenbolone acetate or the growth promoter zeranol. The correlations observed are presented in Table IV [11]. Free cortisol was negatively correlated with growth rate ($P < 0.05$), but the most significant correlation was seen between the free cortisol to insulin ratio

TABLE II. VARIATION IN FRACTIONAL SYNTHETIC RATES (FSR) FOR INDIVIDUAL MUSCLES OF THE SHEEP

Muscle	FSR/d ^a	Remarks	Refs
Longissimus dorsi	0.024	Wethers (67 kg), tyrosine infused, fed a hay-concentrate diet	[4]
Gastrocnemius	0.028		
Vastus lateralis	0.031		
Vastus intermedius	0.076		
Semi-tendinosus	0.022		
Diaphragm	0.028		
Longissimus dorsi	0.030 ^b	Ewes (65 kg), tyrosine infused, fed hay-concentrate diet	[5]
	0.032 ^c		
	0.035 ^d		
Semi-tendinosus	0.030 ^b		
	0.021 ^c		
	0.024 ^d		
Longissimus dorsi	0.05	Wether lambs (20 kg), leucine infused, mean of animals fed ryegrass clover with and without abomasal infusions of casein	[6]
Biceps femoris	0.04		
Heart	0.09		
Heart	0.032	Wether (45 kg), lysine infused, fed concentrate diet	[7]
Diaphragm	0.022		
Longissimus dorsi	0.018		
Gastrocnemius	0.017		
Longissimus dorsi	0.060	Ewes (36 kg), leucine infused, fed concentrate diet	[8]
Heart	0.99	Lambs in uterus (2.4 kg)	[9]
Gastrocnemius	0.040	Ewe lambs (30 kg), lysine infused, tyrosine infused	[10]
Semi-tendinosus	0.039		

^a Values for FSR are calculated from intracellular specific activities.

^b In unmated animals.

^c In animals 16–31 d after mating.

^d In animals 56–57 d after mating.

TABLE III. EFFECTS OF HORMONES AND OTHER STIMULI ON MUSCLE PROTEIN SYNTHESIS AND DEGRADATION

	Growth	Synthesis	Degradation
Insulin	↑	↑	↓
Growth hormone	↑	↑	↔
Glucocorticoids	↓	↓	↑
Testosterone	↑	↑	↑
Thyroid	↑	↑	↑
(hyperthyroidism)	↓	↑	↑
Exercise	↑	↑	↑
Starvation ^a	↓	↓	↓
Protein-free diet	↓	↓	↓

Data are assembled from the literature and represent general trends; for example, responses to starvation and protein-free diets^a depend on the severity of treatment. Hormone responses are often diet dependent.

TABLE IV. RELATIONSHIPS BETWEEN VARIOUS PLASMA HORMONE PARAMETERS AND INDIVIDUAL GROWTH RATE (g/d) OF SHEEP WHOSE GROWTH WAS MANIPULATED BY DIETARY RESTRICTION OR ANABOLIC AGENT TREATMENT

Total cortisol (C) (ng/mL)	Growth = $2.84 \times C + 230$; $r = 0.19$; $P > 0.10$
Free cortisol (F) (ng/mL)	Growth = $-39.7 \times F + 418$; $r = -0.35$; $P < 0.05$
Transcortin (TB) binding capacity (ng/mL)	Growth = $4.62 \times TB + 95$; $r = 0.57$; $P < 0.01$
Insulin (I) (i.u./mL)	Growth = $1.6 \times I + 249$; $r = 0.16$; $P > 0.10$
$\frac{\text{Total cortisol}}{\text{insulin}}$ ratio	Growth = $-92.8 \times C/I + 392$; $r = -0.34$; $P < 0.05$
$\frac{\text{Free cortisol}}{\text{insulin}}$ ratio	Growth = $-843.6 \times F/I + 435$; $r = -0.69$; $P < 0.001$
$\frac{\text{Transcortin}}{\text{insulin}}$ ratio	Growth = $25.8 \times TB/I + 244$; $r = 0.18$; $P > 0.10$

The growth rate of each animal was regressed against the various hormone characteristics of that animal (taken from Ref. [11]).

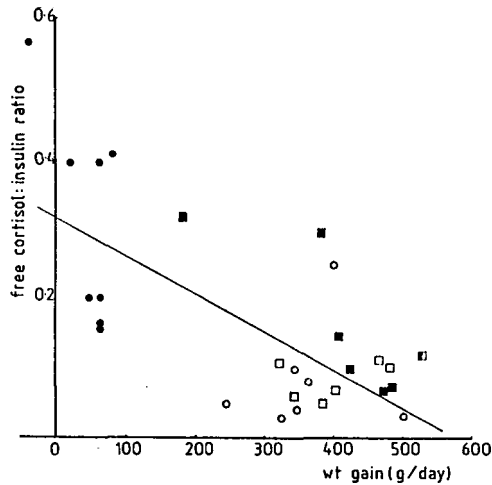


FIG. 1. Free cortisol/insulin ratios and weight gain in individual sheep whose growth was manipulated: ○ control animals; ● restricted diet; □ zeranol implant; ■ trenbolone acetate implant; $y = -5.65 \times 10^{-4}x + 0.32$; $r = -0.69$. (See Sharpe et al. [11] for details.)

($P < 0.001$). Figure 1 also presents these data [11]; the correlation is seen, irrespective of the method used to manipulate the animal. There is, however, the possibility in the trenbolone-treated animals that while the relationship still holds ($r = 0.81$, $P < 0.01$) the numerical value of the ratio is increased. Trenbolone did, however, reduce the numbers of muscle receptors to glucocorticoids, thereby reducing the responsiveness of the tissue to cortisol. These data suggest that the antagonistic action of glucocorticoids and insulin may be of significance in controlling the growth of animals. The glucocorticoid/insulin ratio is also important in regulating muscle protein metabolism during dietary insufficiency (see Section 5).

5. SHORT SUPPLY OF NUTRIENTS

Hammond [12] discussed the proposition that when nutrients were in short supply certain tissues have higher priorities for available nutrients than others. This concept is illustrated in Fig. 2 [12]. The order of priority of the tissues for the available nutrients was suggested to be the brain and the central nervous system, the placenta and foetus, the bone, muscle and fat. Vezu et al. [13] discussed the concept that on a protein-free diet initially the labile proteins are catabolized, then the mobilizable proteins and lastly the fixed proteins before death occurs. The metabolically active tissues lose proportionately greater quantities of protein than less active tissues such as the muscle. Vezu et al. [13] observed that when adult rats were fed

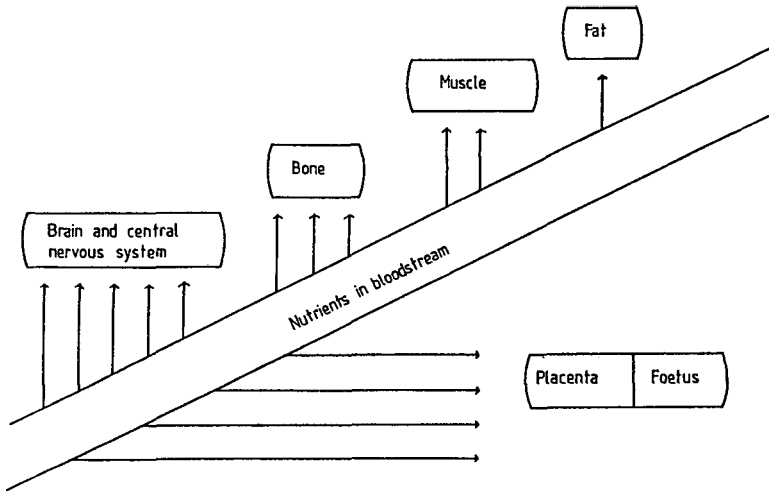


FIG. 2. Diagram showing how the different tissues compete for nutrients in the blood stream. The number of arrows denotes the priority of each tissue for nutrition when there is limitation of supply. When the plane of nutrition is reduced (deduct one arrow from each) fat ceases to grow but the other tissues grow at a reduced weight. At a lower level of nutrition (2 arrows removed) muscle ceases to grow and the arrow for fat is reversed, so that fat enters the blood stream and helps in the reduced growth of brain and bone (see Ref. [12]).

a 10% protein-free diet the contribution of individual tissues to nitrogen excretion was related more to the rate of turnover in the individual tissues than to their size, but when severely depleted of protein (2% in the diet) the size of the tissue had a greater effect. The liver is particularly sensitive to dietary change.

Mobilization of protein from the muscle during dietary insufficiency is manifested by a net output of amino acids by the tissue (Table V [14]). Not surprisingly, the composition of the mixture of amino acids released basically corresponds to that of the protein in the tissue. However, from the muscle in particular, there are some notable differences, which largely result from the catabolism of amino acids by the muscle to serve as an energy source and by the use of certain amino acids, e.g. alanine, glycine and glutamine, to transport nitrogen from the muscle to the liver. This production of amino acids also enables carbon intermediates derived from glycolysis to be transported back to the liver to be converted back to glucose (see Ref. [14]).

The restriction of diet reduces muscle mass and this is brought about by a decrease in mean fibre size with a reduction of the number of myofibrils in the fibre (see Goldspink Ref. [15]). Not all muscles respond in the same way to dietary stress. Spence and Hansen-Smith [16] compared the composition of 13 different muscles following dietary restriction in the rat. They found that the magnitude of the changes

TABLE V. OUTPUT OF INDIVIDUAL AMINO ACIDS AS PERCENTAGE OF TOTAL OUTPUT FROM MUSCLE

Species Muscle Length of fast (h)	Rat Diaphragm 48	Human Forearm 60	Sheep Hindlimb 48—96	Cattle Hindlimb 20
Alanine	11	30	21	24
Glutamine	25	99	10	27
Glycine	9	10	24	10
Serine	6	2	4	—
Proline	5	8	7	—
Glutamic	6	—	0.5	Uptake
Aspartic	0.8	—	Uptake	0
Asparagine	—	—	Uptake	—
Valine	2.3	4.1	7.1	8.1
Leucine	4.5	3.7	5.9	6.4
Isoleucine	3.4	2.9	1.9	6.4
Threonone	5.3	7.4	8.3	—
Lysine	5.6	9.9	5.1	Uptake
Histidine	3.5	3.8	2.2	0.5
Methionine	3.2	1.5	0.9	5.8
Phenylalanine	3.4	2.1	2.4	3.7
Tyrosine	2.7	2.4	1.0	0.5
Arginine	3.9	6.2	4.0	2.7

Adapted from Ref. [14], which should be consulted for details of original studies.

depended to a certain extent on the anatomical position of the muscle, the muscles of the trunk showing a smaller change than those of the limbs.

Whole body protein turnover responds to dietary supply. Generally, both protein synthesis and protein degradation respond positively to increased dietary protein, but as pointed out by Reeds and Fuller [17] the magnitude of the slope varies. The rate of increase of protein synthesis with increasing dietary supply is greater than that of degradation such that below maintenance degradation exceeds synthesis. The changes in muscle protein synthesis and degradation are inherently more sensitive to nutritional change than most other tissues. The response of protein turnover in individual muscles is not uniform; for example, the soleus muscle is much less sensitive to starvation than the gastrocnemius muscle and this can be correlated to the rela-

TABLE VI. RESPONSE OF RATS TO DIETARY PROTEIN QUALITY

	Gluten	Gluten plus lysine and threonine	Lactalbumin
Weight gain (g/d)	0.74	4.80	3.74
Feed efficiency (gain/feed)	0.10	0.43	0.37
Fractional synthetic rate—gastrocnemius muscle (%/d)	5.3	12.0	9.1
Fractional degradative rate—gastrocnemius muscle (%/d)	5.4	9.4	6.2
Net synthesis (%/d)	-0.1	2.6	2.9

Data taken from Ref. [22].

tive sensitivity of the two muscles to glucocorticoids and insulin [18, 19]. In a pregnant animal muscle protein synthesis and degradation adapt to a low protein diet in an attempt to provide some protection to the foetus [20]; such a response would be expected from the tissue priority concept outlined above. In starvation, muscle protein breakdown is reduced to minimize body protein losses (see Ref. [21]), but as death approaches protein degradation would increase.

The changes in protein synthesis and degradation are, not surprisingly, responsive to the quality as well as the quantity of protein fed. This is illustrated by the data presented in Table VI [22], which shows that protein synthesis in the muscle of rats fed a gluten diet can be made to approach that of animals fed lactalbumin, provided the gluten is supplemented with lysine and threonine. Again, these changes are brought about by alterations in both the synthetic and catabolic rates. The relative changes in synthetic and degradative rates may not be constant for all muscles; for example, in the chick feeding a diet limited in lysine causes almost a threefold increase in fractional degradative rate in the breast muscle but only a 30% increase in that of the leg muscle. Restriction of energy or of both energy and protein had similar effects (Table VII [23]).

On refeeding following a period on a protein-free diet, protein synthesis rises rapidly; in a chick fed a protein-free diet from 4 to 6.5 weeks of age the fractional synthetic rate is restored to a value above that of the control animals within 1 day. The protein degradation rates also increased (Table VIII [24]); these data refer to the breast muscle, which is very sensitive to diet. The rapid change in synthetic rate partially accounts for the compensatory increase in weight gain seen on refeeding animals. The compensatory growth in the liver in contrast to that of the muscle is associated with a reduction in protein breakdown.

Insulin/glucocorticoid ratios in the plasma change dramatically on fasting and refeeding. Starvation increases glucocorticoid and dramatically decreases insulin, but

TABLE VII. SYNTHESIS AND DEGRADATION RATES OF CHICKS FED DIETS LIMITED IN ENERGY, LYSINE AND PROTEIN

	Control	Energy restricted	35% lysine	Protein energy-nitrogen restricted
Body weight (g)	103	98	69	77
Growth rate (%/d)	9.4	1.6	0.7	1.3
Leg muscle				
Synthesis (%/d)	22	16	18	20
Degradation (%/d)	12	15	16	21
Difference (%/d)	10	1	1	2
Breast muscle				
Synthesis (%/d)	18	18	18	23
Degradation (%/d)	6	15	16	21
Difference (%/d)	12	3	2	2

See Ref. [23] for details.

TABLE VIII. BODY WEIGHT AND BREAST MUSCLE PROTEIN TURNOVER IN STARVED AND REFED CHICKS

	Fractional body growth rate (%/d)	Fractional protein synthetic rate (%/d)	Fractional protein degradative rate (%/d)
Control (4 weeks old)	4.5	24	18
Protein free			
17 days	-1.6	21	23
+ 1 day refed	5.6	32	21
+ 2 days refed	10.3	41	24
+ 5 days refed	6.4	45	36
Control (6 weeks old)	2.7	17	14
Control (7 weeks old)	3.6	17	13

Data taken from Ref. [24].

TABLE IX. EFFECT OF REFEEDING 4-DAY FASTED RATS

	Protein synthesis (%/d)	Insulin (μ units/mL)	Corticosterone (μ g/100 mL)	Insulin/corticosterone ratio
Well fed	15.7	32.8	26.9	1.22
4-day fasted	4.3	4.3	62.2	0.07
60-min refed	8.0	25.0	7.8	3.21

Data taken from Ref. [25].

on refeeding insulin rises while glucocorticoid decreases to a value often well below that of the control animals. These responses are seen in rats within 60 minutes of refeeding (Table IX [25]). These changes may well be primarily responsible for the changes in the relative magnitude of muscle synthetic and degradative rates seen on refeeding.

Protein metabolism in animals is able to adapt partially to dietary stress. Long-term protein restriction, for example, is associated with a fall in muscle synthetic and degradative rates; however, in children, at least, on recovery whole body protein synthesis remains unresponsive to dietary intake, at least when the dietary intake is greater than 0.6 g N/kg body weight, unlike protein degradation which increased with decreasing dietary intake [14].

The responses reported in the literature of muscle protein metabolism to dietary insufficiency and subsequent refeeding are not consistent. The information above presents the general trends. The differences in the literature presumably reflect differences in experimental design and consequential subtle differences in the response of the endocrine system, which is the main regulator of muscle protein metabolism.

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USE OF MARKERS FOR MEASUREMENT OF FEED DIGESTIBILITY IN RUMINANTS*

L.D. SATTER, D.K. COMBS,
J.M. LOPEZ-GUISA, W.F. NELSON
United States Dairy Forage Research Center,
Agricultural Research Service,
United States Department of Agriculture,
Madison, Wisconsin,
United States of America

Abstract

USE OF MARKERS FOR MEASUREMENT OF FEED DIGESTIBILITY IN RUMINANTS.

Use of markers for estimating digestibility in the whole gastrointestinal tract or in different sections of the gut is discussed. Emphasis is given to use of rare earth marker elements, such as ytterbium, lanthanum, samarium and cerium. The rare earth elements are not absorbed from the gut, and their analysis can be precise and inexpensive. Estimates of digestibility are more variable with the marker technique than with total collection, and 2 to 6 times as many animals may be required to provide the same degree of accuracy as total faecal collection. Measuring digestibility at different sites along the gastrointestinal tract can be done with a digestibility marker, but unrepresentative digesta samples from abomasal or intestinal cannulae pose problems in calculating the nutrient flow at a given location. It appears difficult with the markers available to totally overcome this problem by using dual-phase (liquid and solid) markers to attempt reconstitution or 'correction' of sampled digesta. Effort might better be expended to improve cannula design or to develop improved labelling techniques to minimize problems with unrepresentative digesta samples.

1. INTRODUCTION

Digestibility measurements continue to be important for determining the nutritive value of feedstuffs. The standard digestibility trial involves accurate measurement of feed consumption and faecal output. To obtain the latter, close confinement in digestion crates or use of collection bags is required. This is not possible for all animal experiments due to lack of facilities, inability of the animal to cope with the stress of close confinement, or insufficient labour to properly carry out total collection digestion trials involving large numbers of animals. Therefore, less stressful and more labour-saving techniques involving use of markers have been used for estimating the digestibility coefficients from a limited number of faecal grab samples.

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Markers have also been used to partition digestion within the gastrointestinal tract of ruminants. The characteristics of markers and their behaviour in the gastrointestinal tract become especially important when used to calculate digesta flow at different locations in the gut. The characteristics of the wide variety of markers that have been used in the study of various aspects of digestion have been reviewed [1,2]. The criteria for evaluating markers were listed by these authors and may be summarized as follows. The marker should be: (1) non-absorbable and hence completely recoverable in the faeces; (2) unaffected by and have no influence on the gastrointestinal tract or its microbial population; (3) physically similar to or associated with the dietary material it marks; and (4) capable of sensitive and specific quantitative analysis.

None of the available markers satisfy all of these criteria, and it is important when selecting a marker for a particular purpose that the marker's shortcomings be considered. The objective of this paper is to point out some of the relative strengths and weaknesses of markers, and to discuss their use in digestion studies.

2. USE OF MARKERS TO DETERMINE DIGESTION IN THE TOTAL GASTROINTESTINAL TRACT

Markers commonly used for determining digestibility in the total tract include chromic oxide (Cr_2O_3), lignin, acid insoluble ash and rare earth elements such as ytterbium, samarium, cerium, dysprosium, europium and lanthanum. There is no question that Cr_2O_3 administered via the feed (in a gelatin capsule) or impregnated in paper [3] has been used very successfully to calculate total tract digestibility. It has sometimes been noted, however, that digestibility determined with Cr_2O_3 is slightly lower than digestibility determined by total faecal collection [4, 5]. This is partly due to incomplete recovery (97.5%) of Cr_2O_3 from the gastrointestinal tract [6], and may partly be related to the large diurnal changes in Cr_2O_3 concentration in the faeces, which poses problems for obtaining representative faecal samples [7]. Chromic oxide will continue in use as a fairly reliable digestibility marker. However, future use should depend on its comparison with the rare earth elements, which may offer some advantages in terms of cost, quantitative analyses and total recovery from the gastrointestinal tract.

Lignin has been used extensively as an internal marker in digestion trials, but there are problems with incomplete recovery of lignin in the faeces and analytical techniques that are variable and inadequate. Lignin is often regarded as being indigestible because there are no known anaerobic microbial or mammalian enzymes capable of lignin degradation. However, as pointed out by Fahey and Jung [8] in a review of lignin as a marker in digestion studies, there are numerous reports of lignin being digested or degraded in the ruminant digestive tract. The probable reasons for incomplete lignin recovery in the faeces include: (1) true digestion; (2) apparent

digestion due to formation of soluble lignin-carbohydrate complexes that pass from the rumen and possibly the gut as polymers not recovered in the fibrous residues of faeces; and (3) physical and/or chemical differences between feed and faeces in the nature of materials empirically defined as lignin by the analytical procedure employed. Muntifering [6] reported recoveries in the faeces of permanganate lignin, acid detergent lignin and acetyl bromide soluble lignin to be 78.0, 78.4 and 65.0%, respectively. Such low marker recoveries will obviously result in unacceptable error. Muntifering [6] also found that acid detergent lignin in a cottonseed hull or corn-cob-based diet was extensively digested, but lignin from a fescue-based diet was not. Thus, the diet composition and method of lignin analysis may determine whether lignin can be used as a marker.

Acid insoluble ash has been used successfully as a marker [9, 10], but potentially large errors due to soil contamination of feed reduces the reliability of the method. Also, the passage of accumulated soil and sand particles from the ventral rumen is erratic, perhaps due to occasional but exceptionally strong ruminal or reticular contractions [11]. This can lead to extreme variation in the ash content of faecal samples.

Rare earth elements such as ytterbium, samarium, lanthanum, dysprosium and europium have been used as digesta markers with increasing frequency. Interest in the rare earths and their involvement in biological systems began in the late 1950s and early 1960s as scientists became concerned about the fate of nuclear fission products released into the atmosphere. Although rare earth elements play no known metabolic role in living organisms, Garner et al. [12] were concerned that deposition of rare earth nuclides on plants could potentially lead to accumulation of radioactive fallout in grazing ruminants. They found, however, that ^{144}Ce was only slightly absorbed from the gastrointestinal tract of grazing cows. They noted that the excretion pattern of ^{144}Ce was similar to that of chromic oxide, leading them to suggest that the rare earths may be useful digesta markers.

Ellis [13] and Ellis and Huston [14] were among the first to critically evaluate rare earth elements as markers. They and many others have measured element recovery after feeding, and Table I is a summary of recoveries in faeces of several animal species [12, 13, 15-24]. Rare earth elements have consistent recoveries in the faeces of about 100%.

Rare earth elements can be analysed by several techniques. Colorimetric methods for determination of rare earths are available [25], but these procedures are of limited usefulness because they do not distinguish between individual rare earths. Other elements, particularly calcium and phosphorus, also interfere with the analysis and must be removed, which makes these procedures time consuming and laborious.

Radioisotopes of Co, Cr and the rare earths offer high sensitivity for detection and speed of analysis. Their major disadvantage is that special facilities are required for housing animals and storing animal faeces, urine and used bedding. The animals must also be disposed of after the experiment, which may make certain studies cost prohibitive. Neutron activation analysis can also be employed [26-28], but this

TABLE I. RECOVERY OF ORALLY ADMINISTERED LANTHANONS IN FAECES

Element	Recovery (%)	Animal	Refs
¹⁴⁴ Ce	93-119	Cows	[12]
	104.2	Goats	[15]
	97.0	Cows	[16]
	96-99	Calves	[17]
	107	Cows	[17]
¹⁴¹ Ce	> 95	Chickens	[18]
Ce	101.2	Cows	[19]
Ce	99.6	Cows	[20]
⁹¹ Y ^a	97.9	Rats	[21]
¹⁴⁷ Pm	98.4	Goats	[15]
Dy	99.8	Sheep	[13]
	91.0	Steers	[22]
Sm	106.8	Monkeys	[23]
Sm	101.3	Cows	[19]
Sm	104.6	Cows	[20]
La	96.8	Monkeys	[23]
La	101.7	Cows	[20]
Eu	104	Monkeys	[23]
Tb	99	Rats	[24]
Ir	99	Rats	[24]
Lu	99	Rats	[24]
Yb	99	Rats	[24]

^a Yttrium and iridium are not rare earth elements but have similar properties.

requires more extensive facilities for element analysis. The cost of neutron activation analysis can be very expensive, depending on the availability of facilities.

Plasma emission spectroscopy offers potential as a specific and sensitive method of rare earth analysis. Atomic emission techniques for the rare earths are five to ten times as sensitive as atomic absorption techniques [29] and plasma emission techniques are even more sensitive [30]. Emission intensity in response to element con-

TABLE II. MINIMAL CONCENTRATIONS SUGGESTED FOR ANALYSES OF Co, Cr, La, Sm, Eu and Yb BY NEUTRON ACTIVATION ANALYSIS, ATOMIC ABSORPTION SPECTROPHOTOMETRY, INDUCTIVELY COUPLED PLASMA EMISSION SPECTROSCOPY AND DIRECT CURRENT PLASMA EMISSION SPECTROSCOPY

Element	Neutron activation ^a ($\mu\text{g} \cdot \text{g}^{-1}$)	Atomic absorption spectroscopy ^a ($\mu\text{g} \cdot \text{mL}^{-1}$) ^{c,d}	Inductively coupled plasma emission spectroscopy ^b ($\mu\text{g} \cdot \text{mL}^{-1}$) ^{c,e}	Direct current plasma emission spectroscopy ($\mu\text{g} \cdot \text{g}^{-1}$) ^{c,e}
Cobalt	0.5	1	0.003	0.02
Chromium	1.5	2	0.001	0.01
Lanthanum	0.02	—	0.003	0.04
Samarium	0.004	—	0.02	0.01
Europium	0.02	4	0.001	0.002
Ytterbium	0.07	2	0.0009	0.009

^a From Ref. [28].

^b From Ref. [30].

^c Expressed as μg element $\cdot \text{mL}^{-1}$ solution aspirated.

^d Minimal concentration in aspirated solution to yield an increase in optical density of 0.05 unit.

^e Detection limit expressed as two times standard deviation of background scatter.

centration is usually linear over two to three orders of magnitude, which makes standard curve fitting easier than with atomic absorption procedures. High sensitivity is important, because most feeds have a limited binding capacity for the rare earths [31, 32]. Table II contains a comparison of suggested minimum concentrations of several elements by neutron activation, atomic absorption and plasma emission spectroscopy [28, 30]. The greater sensitivity of inductively coupled plasma emission spectroscopy compared with direct current plasma emission spectroscopy is due to the higher plasma temperatures obtained with the former.

Based on what is essentially 100% recovery of rare earth elements in the faeces and sensitive and precise analytical techniques, rare earth elements are good candidates for digestibility markers. When used in low concentration, i.e. 20 to 30 ppm in the feed, the second and third criteria of a good marker (see earlier discussion) are satisfied by rare earth elements. Hodnett [19] has compared digestibility values obtained with total collection or by the marker technique where acid detergent lignin or cerium or samarium were used as markers. Seventeen non-lactating cows were

TABLE III. COMPARISON OF DRY MATTER (DM) AND ACID DETERGENT FIBRE (ADF) DIGESTIBILITIES USING TOTAL COLLECTION, RARE EARTH ELEMENT MARKER OR ACID DETERGENT LIGNIN MARKER TECHNIQUES [19]

Method	Digestibility (%)			
	DM		ADF	
	Mean	SE	Mean	SE
Total collection	64.4	0.57	52.4	0.88
Rare earth element ^a	64.4	0.79	51.8	1.13
Acid detergent lignin	62.5	0.68	49.6	1.10

^a Either cerium or samarium were used; there was no difference between elements.

used in the study and the results are shown in Table III [19]. There were no differences in digestibilities calculated from the total faecal collection or by the cerium/samarium marker technique. As might be anticipated, digestibility calculated using acid detergent lignin as a marker was slightly lower. The variation in digestibility estimates, as evidenced by a higher standard error of the mean for the marker techniques, suggests more animal replicates will be needed with the marker technique than with the total faecal collection.

Lopez-Guisa [33] has measured the variation in ytterbium concentration in the faeces of Holstein heifers throughout a three-day excretion period. Dry matter digestibilities calculated from each of eight individual faecal samples obtained from 11 heifers are presented in Table IV. The standard deviation of calculated digestibilities within animals averaged 2.6, which is slightly lower than the standard deviation across animals which averaged 3.5. Unpublished studies at the United States Dairy Forage Research Center suggest that the degree of variance in digestibility values calculated from individual faecal samples within lactating cows is a little higher (standard deviation of 4 to 5) than the value of 3.5 observed with heifers. The variation in element concentration from one faecal sample to another underscores the need to obtain several samples for marker analyses. We typically obtain eight faecal samples over a three- or four-day period and composite these before element analyses.

The larger variance associated with digestibility estimates obtained with the marker technique raises the question of how many additional animals are needed in a marker-based digestion trial compared with a complete collection trial. To answer this we conducted two trials. In the first study ytterbium chloride was sprayed onto

TABLE IV. DRY MATTER DIGESTIBILITIES CALCULATED FROM INDIVIDUAL RECTAL SAMPLES

Heifer identification	Sampling time								Mean	SD
	06:00	08:00	11:00	13:00	15:00	17:00	21:00	23:00		
	Dry matter digestibility (%)									
406	68.6	68.5	71.8	70.9	67.7	74.5	68.3	70.1	70.0	2.3
410	68.8	72.5	74.9	67.7	69.9	71.6	75.6	72.8	71.7	2.8
415	67.8	70.2	70.7	71.5	77.5	69.9	70.9	71.8	71.3	2.8
418	69.3	72.2	79.2	67.7	71.7	73.5	71.5	70.5	72.0	3.4
429	70.0	67.2	70.6	65.2	65.2	67.4	67.4	67.0	67.5	2.0
431	76.2	77.8	67.7	76.4	—	—	68.6	73.2	74.8	3.4
432	65.8	70.1	75.4	71.8	69.2	65.8	69.5	69.7	69.7	3.1
435	69.9	69.9	70.5	69.3	71.1	72.2	72.4	75.5	71.4	2.0
438	67.9	69.5	68.3	70.1	76.4	72.4	68.8	75.3	71.1	3.2
447	71.8	72.1	72.1	72.8	77.0	77.0	77.5	76.7	74.6	2.6
456	74.9	76.8	76.2	78.0	75.0	74.7	75.4	78.9	76.2	1.5
Mean	70.1	71.5	73.3	71.0	72.1	71.9	71.4	72.8		
SD	3.1	3.3	3.4	3.8	4.2	3.4	3.4	3.5		

a portion of the diet, which in turn was mixed with all diet ingredients to give a total diet concentration of 20 mg Yb/kg diet dry matter. Forty-four Holstein heifers were divided into four groups of eleven animals each and fed test diets in the form of total mixed rations. A total of eight faecal samples were obtained from the rectum over a period of three days at 06:00, 08:00, 11:00, 13:00, 15:00, 17:00, 21:00 and 23:00 hours. In the second study ytterbium chloride was sprayed onto a portion of the diet, which in turn was mixed with other diet ingredients to give a ytterbium concentration of 23 mg/kg diet dry matter. Fifty-five lactating Holstein cows were divided into three groups of 18, 18 and 19 animals and fed test diets in the form of total mixed rations. A total of seven faecal samples were obtained from the rectum over a period of four days at 01:00, 03:00, 07:00, 10:00, 11:00, 16:00 and 17:00 hours. Samples from both studies were dried, ground and wet ashed. Ytterbium content was determined by emission spectroscopy.

The results from each experiment were considered as a separate population, and variance between animals within sampling times was measured as well as variance

TABLE V. NUMBER OF ANIMALS REQUIRED PER TREATMENT FOR DETECTING A GIVEN DIFFERENCE IN DIGESTIBILITY WITH 90% PROBABILITY [34]

True difference (as per cent of digestibility)	No. of animals ^a		
	Heifers	Cows	Sheep ^b
1.0	65	228	28
1.5	29	101	14
2.0	16	57	8
2.5	10	36	6
5.0	3	9	3

^a Calculated according to Ref. [35].

^b From Ref. [36].

between sampling times within animals. Variance among cows was larger than variance among heifers within times. One might expect greater variance in digestibility estimates determined with cows differing more in feed intake than a group of heifers or sheep consuming feed at near maintenance levels. The number of animals required per treatment to detect a specified difference in digestibility with a given probability was calculated based on the variance in each population. These were compared with the number of sheep required in a total faecal collection trial to give the same degree of precision (Table V) [34–36] in the digestibility estimate. To illustrate the numbers in Table V, if the true dry matter digestibility of a diet is 60%, then 10 heifers or 36 lactating cows using a rare earth marker technique or 6 sheep in a total collection study of complete random design would be required per treatment to claim a real difference between 60.0 and 61.5% digestibility 90% of the time. Although more animals must be used to obtain the same accuracy in measuring digestibility coefficients with a marker technique compared with total faecal collection, the advantages of using a marker technique may offset the increased number of animals required. The labour and animal stress associated with total collection studies with high producing dairy cows, for example, may prevent collection of accurate data at high levels of feed intakes if total collection of faeces is required.

3. USE OF MARKERS TO PARTITION DIGESTION WITHIN THE GASTROINTESTINAL TRACT

Measurement of nutrient digestion within segments of the ruminant gastrointestinal tract is done through use of cannulated animals and indigestible markers.

Cannulation of the proximal duodenum has allowed researchers to measure digesta flow from the abomasum and partition digestion between the stomach and intestines. Digesta samples can be obtained by total collection through duodenal re-entrant cannulae or by spot-sampling of digesta through a simple T-cannula. Simple T-cannulae are preferred, since animals fitted with re-entrant cannulae are more difficult to maintain and collection procedures are labour intensive or require sophisticated collection apparatus [37]. Re-entrant cannulae also disturb intestinal motility, causing some pooling of digesta proximal to the cannula.

The choice of marker can influence estimates of nutrient digestion and flow. Kung et al. [38] observed that organic matter digestion in the forestomach was low when Cr-EDTA or lanthanum was used as a single digesta marker and that lignin appeared to give reasonable estimates of digestion in the rumen. Redman et al. [39] also used lignin as a marker in preference to radiolabelled ruthenium-phenanthroline ($^{103}\text{Ru-P}$) in a dual-phase marker procedure because of very low estimates of digestion in the stomach based on the latter marker. Egan and Doyle [40] suggest, however, that lignin used as a single marker overestimated digestion by about 25% in sheep.

Theurer [41] found that estimates of nitrogen flow to the small intestine in sheep and cattle were influenced by the choice of digesta marker in several experiments that he reviewed. Nitrogen flow to the small intestine appeared to be consistently higher when chromic oxide was the digesta marker than when lignin was used.

Faichney and Griffiths [42] reported that $^{103}\text{Ru-P}$ moved from the originally labelled feed particles in the acid conditions of the abomasum and tended to label smaller feed particles. They concluded that if digesta samples contained more large particles than were present in true digesta, flow of fibre from the abomasum would be underestimated. Allen [32] concluded that unless rare earth elements are applied to feeds by a soaking procedure followed by an acid rinse, substantial quantities of the marker element will disassociate from originally labelled feeds and reattach to fine feed particles and bacterial residues. It is then likely that these markers would actually flow with the more fluid phase of digesta than with larger feed particles that were originally labelled. Chromium-mordanted fibre is known to be stable to acid conditions that would be encountered in the abomasum [43]. However, Ehle [44] demonstrated that flow of chromium-mordanted feed particles is dependent on the density of the mordanted particle.

From the foregoing discussion it is apparent that problems exist with the current techniques for measuring digestibility along the gastrointestinal tract. Digestibility determinations based on spot-sampling of digesta from T-cannulae in the abomasum or intestine are subject to basically three sources of error. The first concerns the changing or erratic composition of digesta leaving the abomasum. Digesta leaving the abomasum is not homogeneous, as particulate matter separates from the fluid phase of digesta and settles into the fundic region of the abomasum in sheep [45]. Spot-samples, whether obtained from a T-cannula or a re-entrant cannula, will be subject to this erratic flow. Obtaining a large number of samples will minimize the problem,

but not necessarily eliminate it. Opening of the cannula, causing changes in intraluminal pressure and possibly on gut motility, may cause deviations in the composition of digesta leaving the abomasum. Leek and Harding [46] have noted that manipulation of the duodenal cannula at sampling excites mechano-receptors of the abomasal mucosa, enhancing propulsive contractions. The only solution to this problem of erratic discharge of abomasal contents, if indeed it is a problem, would be to have continuous sampling of digesta from a re-entrant cannula.

The second possible source of error has to do with the unrepresentative sampling of digesta at the T-cannula. It has long been recognized that bias in digesta composition can occur through collection of digesta with a T-cannula [47, 48]. It is possible that the 'gush' of digesta approaching a T-cannula may be representative of digesta flow, but the fraction of digesta which actually exits the T-cannula may not be representative. We have looked at this problem by occluding the intestine distal to the T-cannula by inserting and inflating a Foley catheter at the time of sampling, thus diverting all digesta to the outside. Estimates of dry matter digestion based on the occlusion of the intestine were 5.15% higher than when the intestine was not occluded (Table VI) [49]. Analysis of samples collected by the two methods indicates that the samples collected by the occlusion technique contained higher levels of fibre than samples collected by the conventional sampling procedure. Interestingly, the ratios of the so-called solid-phase markers (Cr, La, Yb, Ru and lignin) to the liquid-phase marker (Co-EDTA) were not affected by occlusion. The solution to the problem of an unrepresentative digesta sample leaving the T-cannula is to have a cannula design that forces all digesta to the outside [50, 51] without interrupting digesta flow in the intestine, or again, to use a re-entrant cannula.

The third possible source of error involves the marker. If the sample obtained is not truly representative of digesta, then different digestibility values will be obtained with different markers. This is illustrated in Table VI. Lower digestibilities were obtained with the liquid-phase marker (Co-EDTA) than with solid-phase markers. A small part of this difference may be due to the fact that 2 to 5% of the chromium or cobalt complexed with EDTA can be absorbed and excreted in the urine [31, 52]. A more likely and obvious explanation is that the digesta exiting the T-cannula had an altered liquid/solids ratio and thus an altered marker content. This certainly does happen, and it is the reason that Faichney [52] suggested using a liquid-phase and solid-phase marker in combination to allow reconstitution of a 'true digesta' sample. With the reconstitution method digesta is physically separated by straining or centrifugation. Concentrations of both markers in the intact sample and in one of the separated fractions are analysed. Neither marker is required to exclusively label the liquid or solid phase of digesta, but it is critical that each marker uniformly label its respective phase of digesta if this technique is to be valid. True digesta can then be mathematically calculated or physically synthesized by adding (or subtracting) the separated phase of digesta back to the original sample, until the ratio of liquid-phase and solid-phase marker equals the ratio of the two markers in the diet consumed. The problem with the dual-phase marker system is that there is no pair of markers that

TABLE VI. EFFECT OF CONVENTIONAL OR OCCLUSION SAMPLING TECHNIQUES ON CALCULATION OF APPARENT DRY MATTER DIGESTION COEFFICIENTS AT THE DUODENUM [49]

Sampling method	Marker					
	Cr	La	Co	Yb	Ru	Lignin
Conventional ^a	38.2	35.6 ^c	26.4 ^c	38.6 ^c	40.5	30.4 ^e
Occlusion ^b	40.8	40.0 ^d	29.4 ^d	43.5 ^d	42.4	34.9 ^f
SE	1.6	0.8	0.8	0.8	0.9	1.2

^a Digesta obtained by opening the cannula and collecting the effluent.

^b Digesta obtained by occluding the duodenum distal to the cannula with an inflated Foley catheter.

^{c,d} Means in same column with different superscripts are different ($P < 0.01$).

^{e,f} Means in same column with different superscripts are different ($P < 0.05$).

can accurately represent a liquid and solid phase. Moreover, digesta does not flow as two identifiable phases, namely liquid and solid. Attempting to correct digesta flows by using dual-phase markers can be helpful, but it does not solve the problem. The reason is that the available markers do not distribute themselves equally amongst the liquid or solid fractions that we may prepare. The fact that there is no logical dividing point between the so-called liquid and solid phases is part of the problem.

This problem can be illustrated further with results from the study cited earlier where the intestine was occluded distal to the T-cannula at the time of sampling. Table VII [49] contains the ratios of solid to liquid markers in the duodenal digesta and faeces divided by the ratio of the two markers in the feed. A value of unity (1) means that the two markers are present in digesta or faeces in the same proportions as they appear in the feed, implying that a representative digesta sample was obtained. The values in Table VII are all greater than those for digesta samples, suggesting that the digesta sample obtained from the T-cannula had a disproportionate amount of solids, since the concentration of marker representing solids was present in relative excess to the marker representing the liquid phase. As expected, marker ratios approached unity for the faecal samples, because uniform faecal samples can be obtained. Conventional wisdom would say that digestibility calculations based on a single marker would not be appropriate because of what appears to be an unrepresentative sample. This is borne out by the large differences in dry matter flow to the duodenum, as calculated by use of a single marker (Table VIII) [49]. Reconstitution, however, does not solve the problem, as shown in Table VIII [52].

TABLE VII. MARKER RATIOS IN DUODENAL DIGESTA AND FAECES [49]

Sampling method	Marker ratio ^a					
	Cr:Co	La:Co	Yb:Co	Ru:Co	Lignin:Co	% DM
Conventional ^b	1.30	1.14 ^a	1.20	1.22	1.06	6.47
Occlusion ^c	1.30	1.18 ^b	1.24	1.21	1.11	6.26
Standard error	0.05	0.01	0.03	0.03	0.02	
Faeces	1.00	1.05	1.04	0.98	0.93	
Standard error	0.15	0.10	0.08	0.08	0.12	

^a Ratio of solid to liquid marker in the duodenal digesta or faeces divided by the ratio of the two markers in the feed.

^b Digesta obtained by opening the cannula and collecting the effluent.

^c Digesta obtained by occluding the duodenum distal to the cannula with an inflated Foley catheter.

The calculated flow of dry matter varies with the marker combination used as well as the method employed to separate liquid and solid phases.

As pointed out earlier, the problem with the dual-phase marker system is that we do not have markers that distribute themselves in a repeatable manner between the liquid and solid phases. Rare earth elements, for example, can travel largely in the liquid phase because of their affiliation with predominantly small particles, which in turn are suspended in the liquid phase. Figure 1 [49] shows the distribution of particle size as well as the markers in duodenal digesta obtained from cows fed 35% alfalfa silage, 35% maize silage and 30% of a maize-based grain mix (dry basis). Less than 10% of the dry matter in duodenal digesta was retained on a 2 mm screen during wet sieving. A considerably smaller proportion of total marker was retained with these larger particles. The opposite was true for dry matter which passed a 1 mm screen but which moved to the pellet with a 1500g centrifugation. Dry matter of this size constituted about 30% of total digesta dry matter, but contained 60 to 80% of the particulate markers. Interestingly, about 40% of the digesta dry matter was actually in solution in the 35 000g supernatant. From Fig. 1 it is evident that the majority of so-called solids or particulate-phase markers are actually travelling with very small particles suspended in the liquid phase. In essence, the rare earth element is behaving as a liquid-phase marker.

This leads to the situation where the marker ratio in duodenal digesta may be very acceptable, but calculated dry matter digestibilities can be very erroneous. For example, diets containing large amounts of maize silage and/or maize grain will

TABLE VIII. DUODENAL DRY MATTER FLOW USING SINGLE MARKERS VERSUS RECONSTITUTION^a

Sample preparation	Marker	Cow identification			
		489	503	2488	2591
<i>Dry matter intake (kg·d⁻¹)</i>		16.2	15.9	16.6	16.8
		<i>Dry matter flow (kg·d⁻¹)</i>			
<i>Digested sample not reconstituted</i>	Co	4.5	11.6	13.0	9.1
	La	8.1	13.9	17.6 ^b	11.0
	Cr	9.5	13.4	13.7	12.5
	Lignin	17.3 ^b	19.0 ^b	16.8 ^b	14.0
<i>Reconstituted sample</i>					
Liquid phase—dry matter and fluid not precipitated by 25 000g centrifugation for 30 min	Co:La	6.2	12.1	20.6 ^b	9.9
	Co:Cr	7.0	11.7	13.4	10.7
	Co:lignin	10.2	10.6	15.1	11.0
<i>Reconstituted sample</i>					
Liquid phase—dry matter and fluid not precipitated by 1500g centrifugation for 5 min	Co:La	6.7	12.4	18.1 ^b	10.1
	Co:Cr	7.1	11.8	13.4	10.7
	Co:lignin	9.4	12.4	14.5	10.7
<i>Reconstituted sample</i>					
Liquid phase—dry matter and fluid not retained on 1 mm screen after wet sieving	Co:La	27.3 ^b	14.8	38.0 ^b	24.2 ^b
	Co:Cr	14.2	12.1	14.4	16.0
	Co:lignin	10.0	14.6	16.5	17.4

^a Samples reconstituted mathematically by the procedure used in Ref. [52].^b Dry matter flow at duodenum exceeded dry matter consumed.

result in significant amounts of maize kernels in the duodenal digesta. These will carry little marker, and their presence in a duodenal digesta sample may have almost no influence on the ratio of liquid/solid marker. From our own experience, high maize silage or maize grain diets are difficult to work with in terms of obtaining accurate digestibility measurements at the duodenum. We feel this relates to the presence of maize kernels which have uneven flow patterns from the stomach. Diets consisting of all grass or legume forage probably result in digesta that is more uniform in particle size and possibly in marker distribution. Errors in calculating digestibilities at the duodenum are possibly smaller with these diets.

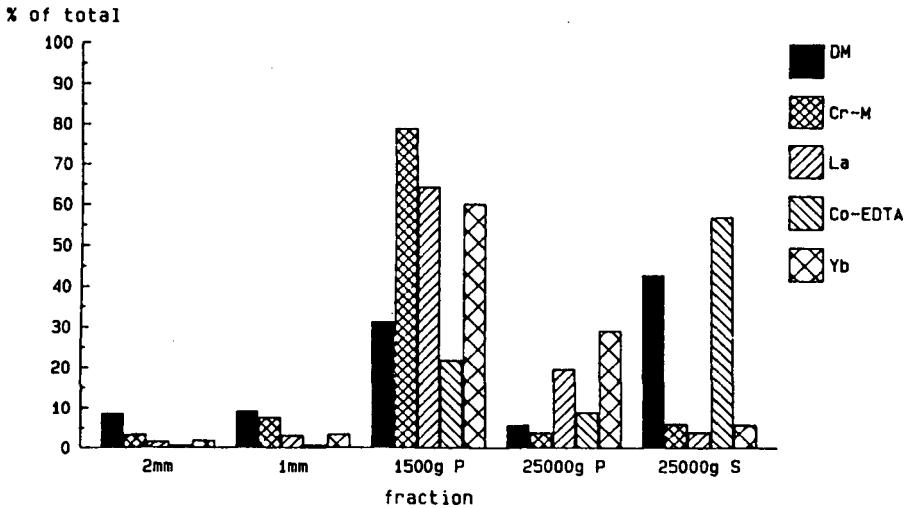


FIG. 1. Summary of dry matter and marker distribution in duodenal digesta [49] (P = pellet; S = supernatant).

It may be possible to improve upon the two-phase system by using more phases and a comparable number of markers; theoretically this is possible [53]. Research is needed to determine if digesta can be reliably divided into three or more phases, and if markers can be identified that will uniformly label each phase.

Nothing has yet been mentioned about calculating digestibilities at the terminal ileum site. It is our experience that ileal samples are much more homogeneous than duodenal samples, and that marker ratios as well as calculated digestibility values seldom deviate from expected ranges. This is in contrast to the duodenal site, where deviant digestibility values are common.

Space does not permit full discussion of all the markers that have been used in digestibility studies, nor of the detailed methodology involved. Details on the use of rare earth elements as markers, including methods of marker application, sample preparation and analyses, are available in the literature [27, 31, 32, 49, 54, 55].

4. CONCLUSIONS

Markers have enjoyed moderate use in studies where digestibility in the total tract is measured. Traditional markers for these studies, such as lignin and chromic oxide, have some limitations, including incomplete recovery from the gut, and in the case of lignin, imprecise analytical procedures. Rare earth elements have some advantages, for they are not absorbed from the gastrointestinal tract and can be precisely and economically analysed for. The concentration of element in the faeces does

vary from one faecal grab sample to another, so replicate faecal samples to make a composite sample for marker analyses are necessary to minimize error. A minimum of eight replicates is suggested. There is greater variation in digestibility estimates obtained by a marker technique, so more animals are required to obtain the same precision as with total faecal collection.

Measurements of digestion along the gastrointestinal tract are still subject to considerable error, particularly at the duodenum. There appear to be no markers that distribute themselves uniformly in the digesta or in fractions of digesta that can be readily obtained. Thus, it is very difficult to 'correct' digesta samples that are not representative of digesta flow past the cannula site. Given the variable composition of digesta, it is hard to obtain representative samples short of using re-entrant cannulae, which are notoriously difficult to maintain and still have the animals functioning normally. T-cannulae are much easier to work with, but more effort is needed to obtain a T-cannula design that enables representative sampling of digesta. In the meantime, the search must continue for markers that minimize the errors which originate with unrepresentative sampling of digesta from abomasal or intestinal cannulae.

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A SIMPLE MODEL TO DESCRIBE THE FLOW OF LARGE AND SMALL PARTICLES IN THE RUMEN

J.W. CZERKAWSKI
Hannah Research Institute,
Ayr, United Kingdom

Abstract

A SIMPLE MODEL TO DESCRIBE THE FLOW OF LARGE AND SMALL PARTICLES IN THE RUMEN.

A brief outline of the structure of the reticulo-rumen is given and some of the important aspects of rumen function (movement, rumination, microbial activity, throughput of fibrous materials) are discussed in the light of published information. A model is proposed in which the fibrous digesta (large particle pool) is subject to a slow cyclic flow and in which the liquid and small particle pool is subject to reciprocating flows. The two pools interact during rumination and during cross-flows through the raft of digesta which acts as a renewable double-sided filter. The validity of important parts of the model was tested in a series of experiments *in vitro*, using the rumen simulation technique (Rusitec). These experiments showed that neither the linear velocity of flows of liquid through the fibrous mass, nor the physical action of rumination had any marked effect on the efficiency of microbial degradation of feeds. On the other hand, the increased removal of microbial matter by washing the solid digesta and removing the washings resulted in a marked redistribution of microbial matter within the system and in a steady increase in its efficiency. At constant 'gut fill', the digestibility of feed dry matter was a simple function of the residence time of feed, but the outputs of end-products of fermentation per unit dry matter digested and the efficiency of the microbial system increased with residence time and then decreased. The results are discussed in relation to the proposed model, which not only stresses the importance of particle size reduction but also underlines the controlling role of rumen movement and rumination.

1. INTRODUCTION

1.1. Structure and function of the rumen

Fibrous feeds contain large proportions of refractory cell wall material (cellulose, hemicellulose), and the rumen makes it possible for the ruminant animals to use these low quality feeds in their own nutrition. Digestion of these materials is possible because of the intervention of the rich microbial population that inhabits the rumen. Microorganisms degrade the plant cell walls, convert the carbohydrate to the end-products of fermentation and use the energy so released for their own growth and maintenance. The host animal digests the microbial mass and uses it together with the end-products of fermentation for growth and fattening. Enormous advances in this field were possible due to the use of isotopic tracers.

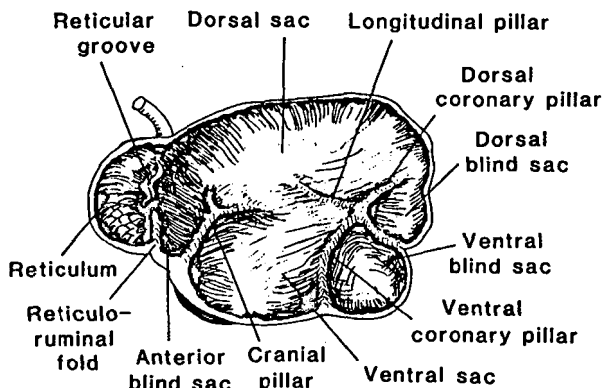


FIG. 1. Schematic representation of the inner structure of the reticulo-rumen (right side).

The rumen is a large organ and with its contents can account for 15 to 20% of the live weight of the animal. Occasionally the microbial system of the rumen shows some of the characteristics of a classical continuous culture, but in general and particularly when the animals consume predominantly roughage diets the system is very different from the ordinary laboratory fermentor.

The reticulo-rumen is a multilobe structure with the entry point (oesophageal orifice) and the exit point (omasal orifice) situated relatively close to each other. The reticulum is separated from the rumen proper by a fold and the rumen itself is divided into clearly defined sacs separated from each other by thick muscular projections, the pillars (see Fig. 1). The dorsal rumen is separated from the ventral rumen by the longitudinal pillar and the two main chambers are also provided with pillars which define the dorsal and ventral blind sacs. The cranial sac is between the reticulum and the main chamber of the rumen. The contents of the rumen are heterogeneous. The region between the dorsal and ventral chambers is occupied by a firm mass of fibrous digesta ('the raft'). The raft is bathed in rumen liquor and the upper parts of the dorsal and ventral rumen contain variable amounts of gas, the gas cap.

It is clear that the internal structure of the reticulo-rumen is not designed to stir the solid and liquid contents in the way that one would stir the contents of a round bottom flask. On the contrary, its structure appears to favour minimal movement of the solid mass of digesta. This must be advantageous, because it is unlikely to disturb the balance of the animal in which the rumen contents could account for up to one-fifth of its weight and whose survival frequently depends on its agility. Furthermore, as we shall see later, immobilization of the raft of fibrous digesta ensures its controlled and predictable throughput in this organ.

The walls and pillars of the reticulo-rumen are provided with strong muscles whose controlled rapid contractions result in rumen movement. Two main cycles have been identified (see Fig. 2). The A sequence (the primary or mixing cycle)

commences with a double contraction of the reticulum and is followed by a caudally moving wave which results in a marked reduction in the volume of the dorsal rumen, while the ventral rumen is relaxed (about 15 s). The cycle continues with contraction of the ventral rumen, while the dorsal rumen is relaxed (completed after about 30 s). These cycles are repeated at approximately 1-min intervals. The B sequence (secondary or eructation cycle) consists of cranially moving contractions which do not involve contractions of the reticulum. The B sequences are associated with removal of fermentation gases that collect in the gas caps. The rumination cycle starts with an additional contraction of the reticulum followed by the normal double contraction. The coarse material is forced from the upper part of the reticulum into the cranial sac and hence to the cardia region by aspiration to the oesophagus and mouth. During the first jaw movement, the regurgitated material is squeezed and the fluid is immediately swallowed. The solid material is mixed with saliva, masticated and swallowed before the next rumination cycle.

During eating the swallowed bolus is deposited in the upper part of the reticulum. When two or three boli have accumulated, the reticular contraction moves them into the cranial sac and the contraction of the latter forces the coarse material over the cranial pillar into the dorsal rumen, often as far back as the caudo-ventral blind sac.

The structure and function of the rumen have been discussed in papers and review articles (see, for example, Refs [1-7]). The papers on the size and distribution of particles leaving the rumen are much more numerous, but there have been virtually no attempts to put forward a controlling mechanism.

It has been established that the particulate material in the rumen must be reduced in size to about 1 mm before it can pass to the omasum [8] and yet the diameter of the omasal orifice is considerably greater than 1 mm (2 cm in the cow). The density of particles has been implicated [3] and although there is evidence of well controlled selection of particles, a clear description of the mechanism is lacking.

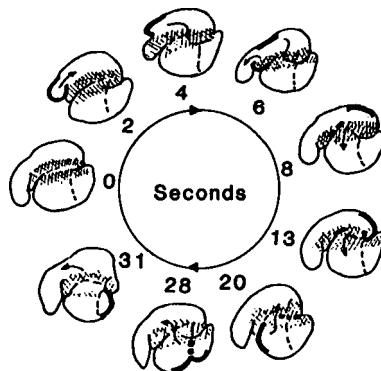


FIG. 2. Schematic representation of the contractions of reticulo-rumen during the mixing sequence. The cross-hatched region indicates the raft of fibrous digesta.

Waghorn and Reid [1] described cyclic clockwise and anti-clockwise flows of digesta in the ventral and dorsal rumen, respectively. They found no evidence of circulation in a lateral plane and the movement of radio-opaque markers suggested a linear flow of 0.5 to 1.0 cm/min. Ulyatt [4] calculated that the pool of fibrous digesta in the rumen of a typical sheep (about 580 g DM) would be ruminated three times per day. If the spent boli are thrown to the caudal part of the rumen and then moved slowly forward to the reticulum, the linear flow of the solid digesta would be very slow (about 0.06 cm/min). Deswysen and Ehrlein [2] observed that BaSO_4 was forced from the dorsal to the caudo-ventral blind sac.

A patient half an hour with one's arm in the cow's rumen shows that there is no tumbling movement of fibrous mass of digesta. On the contrary, the raft of digesta appears to be immobilized in the constricted part between the dorsal and ventral sacs of the rumen. Contraction of the dorsal rumen (complete in about 15 s) may force at least 0.5 L of fluid from the dorsal to the ventral rumen in sheep. This liquid would pass through the fibrous mass, which is constricted to an area of about 80 cm², and a transfer of 0.5 L of liquid would give a linear flow in excess of 25 cm/min. This is much faster than the progression of digesta due to rumination (0.06 cm/min) or the spread of radio-opaque material (0.5 to 1.0 cm/min). These apparently conflicting observations could be reconciled if we accept that the flow of fluid through the raft of digesta is reciprocating [3].

1.2. The model and its implications

A schematic representation of the proposed model is given in Fig. 3. Basically, the model represents two flows, the cyclic flow of fibrous digesta (cranial sac → mouth → caudal dorsal rumen → cranial sac) and a reciprocating flow of fluid digesta (between the mouth and ventral rumen, with branching in the reticulum region). In this model, the circulating pool of large particles does not leave the rumen. The size of this pool is diminished by digestion (due to microbial activity) and by conversion to small particles; it is increased by the input of food. The flows of the liquid and small particle pool are complex. The reciprocating flows through the raft of solid digesta are regular and are governed by the A sequence of ruminal contractions; the amplitude of these flows is unlikely to be large (3 to 4 cm in sheep). During regurgitation of the bolus, the small and large particle pools move together, but the two pools are separated during chewing. The small particles that eventually flow into omasum can come directly from the swallowed liquid portion of the bolus or from the dorsal rumen during the contraction of the ventral rumen. Since the cranial sac contracts rapidly when the reticulum is relaxed, there is a reciprocating flow of digesta between these two sacs. The cranial sac and the reticulum constitute an effective mixing mechanism, which ensures that outflows to omasum are not biased in favour of particles coming from the rumen or squeezed out of the bolus during rumination.

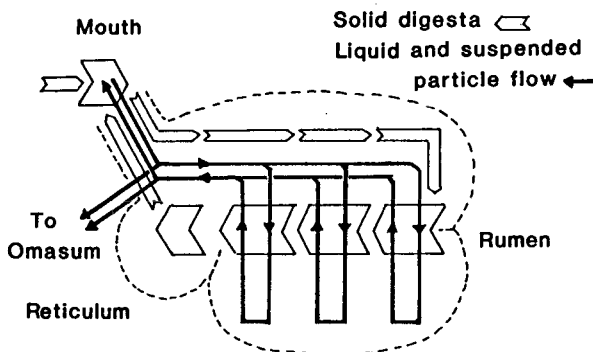


FIG. 3. A model of flows of solid digesta and suspension of small particles in the reticulo-rumen.

The other sites, where the large and small particle pools interact, are the mouth and the solid raft of digesta. The first is clearly concerned with particle size reduction and with their initial separation from the fibrous mass. The interactions at the other site explain why only small particles leave the rumen; the fibrous mass acts as a continuously renewable double-sided filter. Some of the particles could be trapped and then released during one of the reverse flows of liquid. Particles which intertwine would become part of the solid matrix and thus move slowly towards the site of rumination. The particles that are least likely to be trapped will be those that are as long as they are thick which, with hay, means about 1 mm.

Although ruminal contractions are rapid, giving rise to sharp changes in pressure, the flows of liquid are probably slower because of the presence of the gas caps in both the dorsal and ventral sacs of the rumen. These are analogous to gas in car shock-absorbers, resulting in smooth, sinusoidal flows of liquid. The linear velocity of these flows would range from zero to a maximum within a short period of time and this suggests that the microbial activity within the matrix of digesta should be independent of the velocity of such flows. The other factors that may affect the rate of microbial degradation of fibrous feeds could be the rate of removal of small particles, the colonization of new feed, the number of rumination cycles per unit weight of fibrous feed and the residence time of this material in the rumen.

2. TESTING THE VALIDITY OF THE MODEL

2.1. Work with animals

The model is consistent with most of the published observations. It implies that the throughput of fibrous feed is not a random process, but is carried out in a controlled and predictable manner. It reconciles the apparently fixed nature of the fibrous

mass of digesta with its controlled removal, and it explains the excellent mixing of the small particle pool and the selection of particles that are allowed to leave the rumen. It is essentially a deterministic model, whereas a great deal of work on particle flows relies on stochastic procedures. Therefore, it seemed that one would obtain more information at this stage by studying constituent parts of the system using controllable techniques *in vitro*.

2.2. Rumen simulation technique (Rusitec)

Rusitec was developed some 10 years ago [9] and was used in many long-term experiments at this Institute and in other experiments elsewhere. The basic feature of this technique is that the actual residence time of the solid feed inside the reaction vessels can be chosen. Provision is made for a reciprocating flow of liquid through the fibrous mass of digesta and correct physiological conditions are achieved by maintaining the pH, temperature and redox potential within the range of values found in the rumen. A steady microbial population is maintained by daily provision of solid feed and by regular removal of part of the undigested residue. The process of rumination is simulated *in part* by washing and squeezing the solid digesta and returning the washings to the reaction vessel.

The technique simulates the rumen closely in terms of the microbial population that can be maintained and in terms of microbial function (digestibility of dry matter, fermentation patterns, maintenance of protozoal population, etc.). However, Rusitec is only a model system and differs in many aspects from the rumen. For instance, whereas in the rumen small proportions of solid digesta are ruminated (about 8 h/d in sheep), in Rusitec about half of the solid digesta is 'ruminated' for a short time. There is no chewing in Rusitec and there is no rumen wall. The fact that the digestibilities obtained without chewing are not very different from those obtained in the rumen supports our ideas about microbial activity from within [10]. Clearly it does not matter whether the end-products leave the system by absorption or by dilution as long as they are removed.

Work with Rusitec led to important concepts on compartmentation of microbial matter and its function in the rumen [10]. Three microbial compartments were identified in Rusitec: a free suspension of microorganisms (compartment 1), a loosely adherent population (compartment 2) and a population that is closely associated with the solid, and from which it cannot be washed out (compartment 3). It was possible using Rusitec to study these populations in some detail, to define their properties and the interrelations between compartments [11]. Microbial sequestration could be demonstrated directly and it was possible by a modification of the procedure to maintain a healthy population of protozoa without any of these microorganisms leaving the system [12].

Although the concepts of compartmentation are of great value in explaining the mechanisms of microbial degradation of lignocellulosic feeds in the rumen, there is no simple correspondence of the microbial compartments and the two main particle

pools postulated in the present model. The population of compartment 1 is clearly confined to the liquid and small particle pool, while the population of compartment 3 is largely confined to the large particle pool. However, there is no doubt that small particles will also contain strongly adherent microorganisms (compartment 3). The 'shuttle' compartment 2 is associated with the solid mass of digesta, but can be partly separated from it by rumen movement and more completely by rumination.

3. INVESTIGATION OF RUMEN FUNCTION USING RUSITEC

3.1. Reciprocating flows of liquid through the fibrous mass

Two experiments were made to test the hypothesis that the velocity of linear flow of liquid would have no marked effect on fermentation. In the first experiment the angular velocity of the reciprocating movement was kept constant (8 cycles/min), but amplitude of the stroke was varied from 2.8 to 6.0 cm. This changed the mean linear velocity of liquid passing through the solid digesta in the nylon gauze bag from 46 to 96 cm/min. The actual linear velocities ranged from 0 to 150 cm/min. Since the cross-sectional area of each reaction vessel was 50 cm², the flows of liquid through solid digesta ranged from 0 to 7.5 L/min.

The food, good quality hay (15.6 g DM), was supplemented with bruised barley and soya bean meal (0.86 g DM each) and with urea (about 100 mg/d infused with artificial saliva). The experimental and analytical procedures were as described earlier [9]. The results given in Table I are the means for three experimental periods

TABLE I. EFFECT OF AMPLITUDE OF THE STIRRING MOVEMENT IN RUSITEC ON DIGESTION OF DM AND ON THE OUTPUT OF END-PRODUCTS OF FERMENTATION. THE RESULTS ARE MEANS FOR THREE PERIODS OF 4 DAYS (EXPERIMENT 2a)

Vessel	1	2	3	SE
Amplitude (cm)	2.8	4.4	6.0	—
DM digested (g/d)	9.24	9.54	9.72	0.14
End-products per unit DM digested				
CH ₄ (mol/kg)	1.68	1.77	1.87	0.085
VFA (mol/kg)	8.15	8.29	8.73	0.339
Microbial DM (g/kg)	183	187	220	10
Y _{ATP} (g/mol)	9.09	9.13	10.24	—

TABLE II. EFFECT OF THE RATE OF STIRRING IN RUSITEC AT CONSTANT AMPLITUDE (4.6 cm) ON DIGESTION OF DM AND ON THE OUTPUT OF END-PRODUCTS OF FERMENTATION. THE RESULTS ARE MEANS FOR TWO PERIODS OF 4 DAYS IN EACH VESSEL (EXPERIMENT 1a)

Vessel	1	2	3	4	SE
Rate (cycles/min)	2.24	4.63	9.27	17.9	—
DM digested (g/d)	9.41	9.50	10.49	10.89	0.25
End-products per unit DM digested					
CH ₄ (mol/kg)	1.41	1.52	1.73	1.83	0.15
VFA (mol/kg)	7.34	7.34	7.45	7.30	0.31
Microbial DM (g/kg)	164	171	162	164	8
Y _{ATP} (g/mol)	9.02	9.28	8.69	8.90	—

of 4 days. There was a slight increase in the digestion of DM and a somewhat greater increase in the output of end-products of fermentation with the amplitude of the stroke. This resulted in a slight and possibly not significant improvement in the efficiency of the system (Y_{ATP}). The differences were probably due to the detrimental effect of small amplitude, rather than different velocities of flow of liquid. This was confirmed by the results of another experiment in this series (Table II).

In the second experiment, the amplitude of the stroke was the same in all four vessels (4.6 cm), but the rates of stirring ranged from about 2 to 18 cycles/min. The quantities of food were the same as those used in the first experiment. There was no difference in the output of volatile fatty acids or microbial matter per unit DM digested, although the digestibility increased slightly with the rate of stirring. The apparent increase in methane production was due to a more efficient removal of the gas trapped in the solid digesta and hence a better recovery. This conclusion was confirmed by measurements of the volumes of the liquid reaction mixture in the vessels after removal of the solid digesta. The values were 445, 470, 505 and 590 mL in vessels 1, 2, 3 and 4, respectively.

It was concluded that the mean rates of linear flow of perfusing liquid, ranging from 21 to 165 cm/min, had no effect on microbial degradation of feed in Rusitec.

3.2. Partial removal of small particle pool

In routine use, the nylon gauze bag containing food that had been incubated for 48 h is removed from a Rusitec reaction vessel, is placed in a larger polythene bag and the undigested residue is washed with 2×40 mL of artificial saliva by

TABLE III. EFFECT OF REMOVAL OF PROGRESSIVELY LARGER PROPORTIONS OF WASHINGS FROM THE REACTION VESSELS (EXPERIMENTS 1b, 2b, 3). A LINEAR RELATION IS ASSUMED WITH COLUMNS 2, 3 AND 4 GIVING THE INTERCEPT (NO REMOVAL), THE CHANGE DUE TO COMPLETE (100%) REMOVAL AND THE ASSESSMENT OF FIT, RESPECTIVELY

	Diet	Control (no removal)	Change (due to complete removal)	r
DM digested (g/d)	Mixed	10.05	1.22	+ 0.80
	Hay	8.53	0.74	+ 0.99
End-products (mol/kg digested)				
CH ₄	Both	1.94	- 0.11	- 0.44
VFA	Both	7.17	- 0.53	- 0.66
Microbial output (g/kg DM digested)	Both	149	+ 61	+ 0.89

squeezing repeatedly for a few minutes. The residue is then squeezed out and the pooled washings are returned to the reaction vessel together with a new bag of food. The residues are dried and weighed to determine the amounts of DM digested.

In the rumen, the bolus of regurgitated digesta is squeezed and the liquid is swallowed at once, but it is not known how much of this material and similar material produced during the remainder of the rumination cycle flows to the omasum and how much is returned to the rumen. To investigate this aspect of rumination, experiments 1 and 2 were extended (experiments 1b, 2b). During this part of both experiments different proportions of washings were deliberately not returned to the reaction vessels. The material removed from the reacting system in this manner contained some microbial matter and volatile fatty acids and these were added to the outputs in the effluent to determine the total daily outputs.

The removal of some 7 to 50% of the total washable material (i.e. in both the 24- and 48-h bags) had no effect on DM digestibility or the output of methane and volatile fatty acids per unit DM digested. The output of microbial matter in the effluent and in the washed undigested residues decreased with increasing proportions of washings removed, but this was more than compensated for by the microbial matter removed in the washings. The efficiency of microbial synthesis was therefore increased.

In a separate experiment (experiment 3), when the vessels were opened both the 48- and 24-h bags were washed and increasing proportions of the washings were permanently removed from the reaction vessels. As before, the 48-h undigested

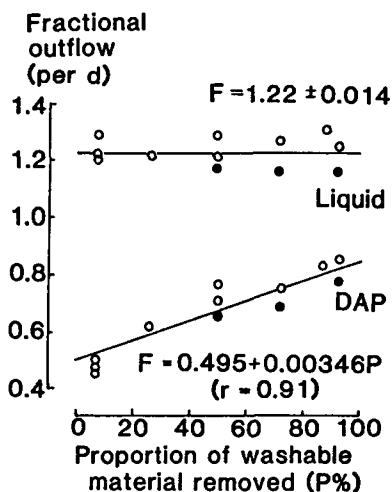


FIG. 4. Fractional removal of water and diaminopimelic acid (DAP) in Rusitec as a function of the proportions of washable material removed from the reaction vessels. (Experiment 3, (○) periods I, II, III, hay with small amounts of concentrate; (●) period IV, hay only.)

residues were removed and replaced by new bags of food. In this experiment, the proportions of washings removed from the reaction vessels ranged from 26 to 96% (Table III).

There was a consistent increase in the digestibility of DM with the increasing removal of washable material from the reaction vessels. There was a small and possibly not significant decrease in the output of methane and volatile fatty acids per unit DM digested, but in agreement with experiments 1b and 2b there was a marked increase in the output of microbial matter.

Even though the amounts of liquid removed from the reaction vessels increased slightly with the removal of washings, the amounts of liquid in the reaction vessels increased too and therefore there was no significant change in the fractional outflow of liquid (Fig. 4). On the other hand, as can be seen in the same figure, the fractional outflow of bacterial matter, measured as diaminopimelic acid (DAP), increased. There was a marked redistribution of DAP between the liquid (effluent + washings) and the solid (washed residue) phases leaving the reaction vessels. This is summarized in the following equations, where Y is the DAP output (mg/d) and X is the proportion of total washable material removed (%)

$$\text{Effluent + washings: } Y = 2.05 + 0.0146X \quad (r = +0.900)$$

$$\text{Residue: } Y = 1.67 - 0.0056X \quad (r = -0.944)$$

Thus, the extreme change from the standard procedure (complete removal of washings) would result in a change from about 45% DAP leaving the vessels in the

washed residue to about 24%. If this signified a change in microbial colonization of fibrous feeds, it obviously had no effect on the digestibility of DM, which increased.

The increase in the fractional removal of bacterial matter (but not of liquid, Fig. 4), probably decreased microbial lysis and made the system more efficient. This was confirmed by the results of measurements of the recovery of the α -NH₂ group (a measure of true protein). This recovery (Y%) increased with the proportion of total washings removed (X%) according to the equation

$$Y = 45.1 + 0.33X \quad (r = 0.988)$$

Since the output of end-products of fermentation declined slightly with the proportions of washings removed and since the microbial output increased, the biochemical efficiency of the system (Y_{ATP}) increased. This can be seen clearly in Fig. 5, where the values obtained in all three experiments are given.

3.3. Effect of 'rumination'

In the fourth experiment in the series, attempts were made to increase rumination. The experiment started with a control period when only the 48-h bags were

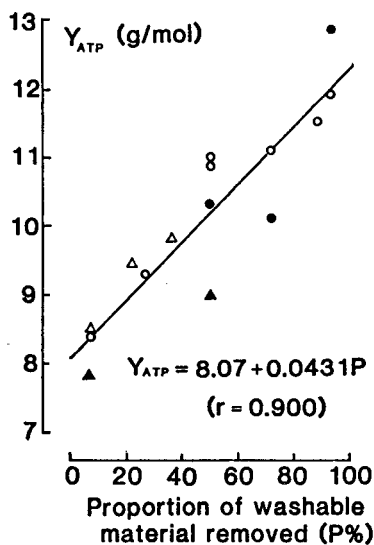


FIG. 5. Biochemical efficiency of microbial synthesis (Y_{ATP}) as a function of the proportions of washable material removed in experiments 1b (Δ), 2b ($\blacktriangle$), experiment 3 mixed diet ($\circ$) and experiment 3 hay only ($\bullet$).

washed and the washings were returned to the reaction vessels (routine use). During the treatment periods, the washings from the 48-h bags were not returned to the vessels. In agreement with results of experiments 1b, 2b and 3, compared with the control, there was an increased digestibility of DM, decreased output of methane and volatile fatty acids and an increased output of microbial matter.

During the treatment periods, the 24-h bags were washed as well and the washings were returned to the reaction vessels. Some vessels were opened twice a day (6 h after the normal feeding time) and one or both bags were washed. In this way a series of treatments was obtained in which the solid digesta were not ruminated at all, were ruminated once (after 6 h), twice (after 6 and 24 h) or three times (after 6, 24 and 30 h).

The 'rumination' had no effect on digestion of DM, even though the wet solid became softer and was much easier to squeeze when the number of ruminations increased. The outputs of methane and volatile fatty acids decreased slightly, but there was no change in the output of microbial matter. The efficiency of the system increased by about 1% per rumination. We must conclude that, at least in Rusitec, rumination has little or no effect on the microbial breakdown and utilization of fibrous feeds. There is no doubt that in the rumen the process of rumination helps in the physical breakdown of large particles and in the reduction of the mean particle size. Rumination also helps in the separation of small and large particles and in the rapid despatch of the former to the reticulum and hence to the omasum. Thus, the beneficial effect of rumination may be an indirect one.

3.4. Residence time of fibrous feeds

In this experiment (experiment 5) the time of incubation was varied by placing more than 2 bags of food inside the reaction vessels. The 'gut fill' was kept constant at 30 g hay. Vessel 1 contained 3 bags (10 g each) and every day two bags were removed (one 24-h bag and the 48-h bag) and replaced by two new bags of food. Vessel 2 contained two bags with 15 g hay in each, while vessels 3 and 4 contained 3 bags with 10 g hay and 4 bags with 7.5 g hay, respectively. In vessels 2, 3 and 4 only the oldest bag was removed for washing and analysis. This gave residence times of 24, 48, 72 and 96 h, with inversely decreasing intakes of feeds (Table IV).

The digestibility of DM increased with time of incubation in the reaction vessel. The results were consistent with an equation $D = At/(B+t)$, where D is digestibility, t is time of incubation, A is the potential digestibility and B is the time to reach half the potential digestibility. The values of A and B obtained were 65.3% and 25.8 h, respectively. At the end of the experiment all the bags in each vessel were washed and the digestibilities of dry matter were determined; these again depended on the time of incubation and for any given time were the same in all vessels even though the input of food varied widely. The outputs of end-products of fermentation and microbial matter per unit DM digested increased with the time of incubation and then

TABLE IV. EFFECT OF DIFFERENT RESIDENCE TIMES OF SOLID DIGESTA ON THE DIGESTIBILITY AND THE OUTPUTS OF END-PRODUCTS OF FERMENTATION IN RUSITEC WITH A CONSTANT 'GUT FILL' OF 25.9 g DM

Vessel	1	2	3	4
Residence time (h)	24 and 48	48	72	96
DM input (g/d)	17.26	12.94	8.63	6.47
Digestibility of DM (%)	30.0 $\pm$ 0.31	44.3 $\pm$ 0.38	48.2 $\pm$ 0.26	51.1 $\pm$ 0.24
and	42.7 $\pm$ 0.42			
End-products (per unit DM digested)				
CH ₄ (mol/kg)	1.59	1.88	1.84	1.59
VFA (mol/kg)	6.26	6.67	7.29	7.02
Microbial matter (g/kg)	166	197	141	100
Y _{ATP} (g/mol)	10.5	11.5	7.6	5.7

declined. The efficiency of synthesis of microbial matter (Y_{ATP}) appeared to have a maximum value after about 2.2 days.

An interesting property of this constant 'gut fill' system was that the inputs of food and the times of incubation of material removed from the vessels were inversely related. Thus, with this arrangement the higher the input of food the shorter was the time of incubation and the lower was the digestibility.

In Rusitec, which is a model system with no provision for chewing or proper rumination, the small particle pool is confined to the nylon bags (the particulate matter in the effluent is almost entirely microbial). Part of the mixed pool (large and some small particles) in the bags is removed every day. For instance, in vessel 4 when the vessel was opened, only the bag that had been incubated for 96 h was removed; the bags incubated for 24, 48 and 72 h were left in the vessel.

In the rumen the situation is different. We could imagine that the large particle pool consists of many packets of fibrous digesta in various stages of digestion, ranging from new food to residues from which all the potentially digestible material had been removed. Since no large particles leave the rumen, one could argue that the estimate of potential digestibility in Rusitec (the plateau value in the hyperbolic or other equation) would predict the digestibility in the rumen. However, not all the potentially digestible material is removed from small particles of feed. The large particles that had been well digested without change in size are more likely to be broken down physically to small particles and this could be an important part of the particle selection mechanism in the rumen.

4. DISCUSSION

Although there are many differences between the rumen and the simulation technique (Rusitec), use of the latter gave much insight on what is going on in the former.

We have considered a model in which the essential features were the slow circulating movement of fibrous mass and the reciprocating flow of liquid and small particles, with part of the large particle pool acting as a renewable double-sided filter. We have shown that the model is consistent with observations *in vivo* and that it allows a controlled throughput of fibrous feed in the rumen.

Experiments with Rusitec showed that the digestion of fibrous material is largely independent of the linear speed of liquid perfusing the solid digesta. Therefore, the main function of such a perfusion is to filter the larger particles and to retain them for a longer time in the rumen. This finding also underlines the importance of the strongly adherent microbial population in the rumen.

The process that appears to have the greatest effect on the efficiency of the microbial system is the increased removal of microbial matter. It can be shown that the microbial matter in free suspension in the rumen accounts for less than 10% of the total microbial matter [11]. Therefore, with a liquid dilution rate of about 1.5/d, the removal of microbial matter as a free suspension would be only 0.15 of the total/d. This means that at steady state some 85% of microbial matter would be lysed. However, rumination offers an effective mechanism for increasing the removal of microbial matter from the rumen. The squeezing of the bolus will remove large proportions of compartment 2, where the microbial concentrations are at least 10 times higher than in free suspension [10]; subsequent chewing will release small undigested feed particles which contain the strongly adherent population of compartment 3 (about 10% of the DM).

Using the data of Ulyatt [4], it can be shown that the rumen of sheep fed 1 kg lucerne hay daily would contain 13 g of microbial matter in compartment 1 and 116 g in compartments 2 and 3. Of this material, about 56 g/d would leave the rumen with undigested residue and 20 g/d would leave as free suspension. Thus, of the total microbial matter leaving the rumen (100 g/d) about 24 g would be removed via the squeezed boli. If the fibrous material is ruminated 3.4 times/d, rumination itself could remove 196 g of microbial matter. The total potential output of microbial matter (free suspension, ruminated and adherent material) could be as high as 272 g/d, giving an efficiency of 60 g microbial N/kg DM digested. According to stoichiometric and energetic considerations [13], such a high output of microbial matter is unlikely. A large proportion of the separated compartment 2 population is mixed with digesta in the reticulum region and returned to the rumen. This may be necessary for colonization of new feed and chewed fibrous residues in which new surfaces had been exposed during rumination.

The extent of digestion of fibrous feeds depends on their residence time in the rumen. As we have seen, a very long residence time tends to lower the microbial effi-

ciency and with a given rumen fill would reduce intake; this would be counterproductive.

It is inconceivable that a worthwhile control mechanism would be possible in a simple reaction vessel filled with a randomly stirred mixture of large and small particles in a liquid medium. The proposed model underlines the importance of particle size reduction, their segregation and removal. It also provides a clear rationale for the controlling role of rumination and rumen movement in these processes.

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EFFECTS OF CARBOHYDRATE AND NITROGEN SOURCE ON THE UTILIZATION OF SUGAR-CANE DIETS

J.T.K. MUNTHALI*, F.M. PATE

Department of Animal Science,
University of Florida,
Gainesville, Florida,
United States of America

Abstract

EFFECTS OF CARBOHYDRATE AND NITROGEN SOURCE ON THE UTILIZATION OF SUGAR-CANE DIETS.

A 133-day feedlot trial and a digestibility and nitrogen (N) balance experiment were conducted to compare sugar-cane-based diets supplemented with either molasses/cotton seed meal (MCS), molasses/urea (MU) or corn meal/urea (CoU). Sugar-cane comprised 70% of the diets' dry matter (DM), while urea supplied 50% of the nitrogen in the diets containing urea. The diets contained 10.4, 10.6 and 11.2% crude protein (CP) on a DM basis for MCS, MU and CoU, respectively. DM intakes on the feedlot trial were similar, but steers fed either MCS or CoU showed, on average, a higher ($P > 0.05$) daily gain than those given MU. The DM intake of steers offered the MU diet in the digestibility and N balance experiment was lower ($P < 0.02$) than that of steers fed MCS but similar to that of steers given CoU. Digestibility coefficients for DM were highest for steers fed MU ($P < 0.05$). The digestibility coefficients for crude fibre, neutral detergent fibre, acid detergent fibre, cellulose and lignin were similar for all dietary treatments. The digestibility of N was lower ($P < 0.03$) for the MCS diet than for the MU and CoU diets. Nitrogen retention was similar for the MCS and CoU diets, but both groups retained more N than the MU diet. There were no differences in any rate of passage parameters, rumen fluid ammonia levels and plasma glucose levels. Propionic acid was higher ($P < 0.05$) in the rumen fluid of steers fed MU than for those fed either the MCS or CoU diets. The studies showed that urea was inferior to cotton seed meal as a CP source in sugar-cane diets when fed with molasses as an energy supplement.

1. INTRODUCTION

Low animal productivity is usually synonymous with the tropical and subtropical regions of the world, most of which are developing countries. Inadequate nutrition, which is either quantitative (semi-arid and arid areas), qualitative (wet hot regions), or both (wet dry tropics), is the major constraint to improved levels of animal production. Quality is decreased by rapid maturation of forages, whereas quantity is lowered by reduced forage growth caused by lack of precipitation for several months each year.

* Present address: Chitedze Research Station, Lilongwe, Malawi.

Sugar-cane offers an attractive alternative for feeding animals during periods of feed scarcity because it is generally unlimited by both quantitative and qualitative factors. Dry matter (DM) yields from sugar-cane are often in excess of 40 short tons per hectare [1] and its level of soluble carbohydrate concentration increases with maturity. The main constraint to feeding sugar-cane to cattle is its low crude protein (CP) content, averaging 2% of the DM in mature cane [2]. When sugar-cane is fed without supplemental protein, animal growth response is normally negative, changing to slight gains when supplemented with urea [3]. Cattle growth responses to protein nitrogen supplements and concentrates have been variable and usually less per unit of added protein [4–6] when sugar-cane or molasses are fed. Widespread cultivation of sugar-cane and production of its by-product, molasses, combined with the availability of non-protein nitrogen (NPN) sources encourage investigation into their utilization for animal production. Use of cereal grains and protein nitrogen supplements is limited by the high prices and scarcity of these feed ingredients in most developing countries.

The objectives of this study were to determine the effects of different dietary energy and nitrogen (N) sources on the nutrient digestibility, rate of passage, N metabolism and performance of steers fed a basal diet of sugar-cane. Comparisons were made between molasses and corn meal as energy sources in a diet containing a high level of urea, and then urea and cotton seed meal as CP sources.

2. EXPERIMENTAL PROCEDURES

A feedlot trial and a digestibility–N balance experiment were conducted. Thirty-six *B. taurus* × *B. indicus* steers averaging 12 months old and 239 kg live weight were used in the feedlot trial. The steers were divided into six groups of six steers each and then allocated at random to three dietary treatments in a completely randomized design. The dietary treatments were molasses/cotton seed meal (MCS), molasses/urea (MU) and corn meal/urea (CoU), and these were fed as concentrate supplements to a basal diet of sugar-cane. The concentrate supplements were formulated to give 11% CP on a DM basis in the total diet. Total diets comprised 68% sugar-cane and 32% concentrate supplements (Table I).

Steers were housed in a concrete floored, covered shed which was divided into six pens measuring 9.8 × 9.8 m each. Two groups of steers were fed one of the three diets. Sugar-cane was harvested and chopped every morning with a forage harvester. Weighed sugar-cane was placed in the feed troughs and known quantities of molasses or premixed dry concentrate supplements were poured over it and then mixed thoroughly by hand. All diets were fed ad libitum once daily. Feed refusals were removed and weighed before the following feeding.

The digestibility–N balance experiment used similar diets to those used in the feedlot trial (Table I). Urea supplied 50% of the total dietary N in the two diets containing urea. The complete rations were prepared immediately before

TABLE I. COMPOSITION OF SUGAR-CANE DIETS USED IN FEEDLOT AND METABOLISM EXPERIMENTS

Ingredient ^a	Feedlot trial diets			Metabolism trial diets		
	MCS ^b	MU ^c	CoU ^d	MCS	MU	CoU
	% on DM basis					
Sugar-cane (IFN 2-04-687)	68.86	68.20	68.70	70.00	70.00	70.00
Molasses (IFN 4-04-696)	11.28	24.32		12.00	23.31	
Corn meal (IFN 4-02-849)			26.35			25.68
Cotton seed meal (IFN 5-01-621)	18.81	3.83	1.32	16.95	3.00	0.65
Urea		2.07	2.05		2.11	2.11
Minerals ^e	0.85	0.85	0.85	0.85	0.85	0.83
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Monobasis phosphate (IFN 6-01-082)		0.53	0.53		0.53	0.53

^a Vitamin A and D added to each diet to supply 3000 and 300 i.u./kg of DM, respectively.

^b Molasses/cotton seed meal.

^c Molasses/urea.

^d Corn meal/urea.

^e Contained 11.5% P, 18% Ca, 0.3% Fe, 0.3% Cu, 0.2% Co, 0.2% Mn, 0.5% Mg, 2% Zn and 12.5% NaCl.

each feeding by thoroughly mixing all feed ingredients in plastic tubs and transferring the mixed feed into feed-bunks attached to the metabolism crates. Steers were fed twice daily. A complete cross-over design [7] was used in which a total of 12 *B. taurus* × *B. indicus* yearling steers were used. By the end of the experiment each steer had been tested on all three dietary treatments.

Steers were housed in elevated metabolism crates for a 14-day adaptation period followed by a 7-day collection period. During the adaptation period steers were offered ad libitum feed to establish their voluntary intake. After establishing voluntary intake, the amount of feed offered to each steer was kept constant, allowing for 5 to 10% feed refusals.

During the collection period feed offered, feed refused and faeces and urine excreted were measured. Daily aliquots of faeces and urine (acidified with H₂SO₄) were obtained. Samples of sugar-cane, molasses, mixed feeds and orts were collected at each feeding. All feed samples and faeces were oven-dried for 48 h at 60°C, equilibrated to ambient temperature and then ground to pass through a 1 mm screen.

The rate of passage was measured using chromic oxide (Cr₂O₃) as a feed marker. On the first day of each collection, 100 g Cr₂O₃ powder was mixed with

1 kg of feed and then offered to each steer. Thirty minutes were allowed for steers to finish their Cr_2O_3 feed followed by the main diet. Total faecal collections were made at 6-h intervals for the first three days and at 12-h intervals for the remaining four days [8, 9]. Rumen fluid samples were obtained with a stomach tube at the end of each trial, 3 to 3½ h after the morning feeding. Blood samples were collected from the jugular vein of each steer at the end of each period using heparinized vacutainer tubes immediately before the collection of the rumen fluid.

Analyses included DM and total N [10]; neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and lignin were determined [11]. Urine samples were analysed for total N [10]. Chromic oxide was analysed according to Kimura and Miller [12] and modified by digesting the organic matter in the faecal samples with nitric acid; the sample was then boiled to dryness before oxidizing further with 70% perchloric acid. Rumen fluid samples were analysed for pH and acetic, propionic, butyric, isovaleric and valeric acids using gas liquid chromatography. Rumen NH_3 was determined by a Technicon autoanalyser and plasma glucose was analysed by using a glucose assay kit [13].

Statistical analysis for the feedlot trial was carried out using the analysis of variance procedure of a completely randomized design; the metabolism experiment was analysed as a complete cross-over design [7].

TABLE II. PERFORMANCE OF STEERS FED SUGAR-CANE DIETS WITH DIFFERENT NITROGEN AND ENERGY SOURCES

Item	Dietary treatment			SE ^a
	Molasses/ cotton seed meal	Molasses/ urea	Corn meal/ urea	
Number of steers	12	12	12	
Initial weight (kg) ^b	239	239	241	4.10
Final weight (kg) ^c	339 ^d	315 ^e	335 ^d	6.60
Average daily gain (kg)	0.75 ^d	57 ^e	71 ^d	0.04
DM intake (kg/d)	6.66 ^d	6.37 ^d	6.29 ^d	0.22
NEg intake (Mcal/d) ^f	5.33	4.33	5.34	
DM intake to gain ratio	8.88 ^d	11.38 ^d	8.86 ^d	0.45
Hot carcass weight (kg)	186 ^d	171 ^e	184 ^d	4.10

^a Standard error of mean.

^b Taken after cattle were held 16 h without feed and water.

^c Calculated from hot carcass weight and a 55% dressing percentage as follows: final weight = hot carcass weight/0.55.

^{d,e} Values in the same row with different superscripts differ ($P < 0.05$).

^f Net energy for gain (NEg).

TABLE III. AVERAGE CHEMICAL COMPOSITION OF SUGAR-CANE DIETS FED WITH DIFFERENT SOURCES OF NITROGEN AND ENERGY IN THE METABOLISM EXPERIMENT

	Dietary treatment		
	Molasses/ cotton seed meal	Molasses/ urea	Corn meal/ urea
	% dry matter basis		
Dry matter	32.4	32.3	31.6
Organic matter	94.5	93.9	96.4
Crude protein	10.4	10.6	11.6
Ether extract	0.9	0.8	1.3
Crude fibre	18.3	17.0	18.4
NDF ^a	39.0	35.8	41.0
ADF ^b	26.2	23.1	25.5
Cellulose	19.9	17.6	19.5
Lignin	5.4	4.7	5.3

^a Neutral detergent fibre.

^b Acid detergent fibre.

3. RESULTS AND DISCUSSION

In the feedlot trial, the average daily gain of steers consuming the MU diet was lower ($P < 0.05$) than that of steers fed either MCS or CoU diets, which were similar (Table II). Since DM intakes were similar, the lower performance by steers fed the MU diet is a reflection of the poorer utilization of that diet, as shown by a high DM intake to gain ratio of 11.4 compared with 8.9 for the other two diets. Such poor performance by animals fed sugar-cane-based diets supplemented with either molasses/urea or urea alone has been reported [3, 14, 15]. Low performance on such diets is frequently attributed to a reduced supply of metabolizable protein and glucose precursors. A low total supply of metabolizable protein rather than amino acid composition [16] might have been responsible for the decreased performance by steers fed the MU diet. Lack of glucose precursors might have been negligible because it was shown that equivalent amounts of energy in the form of acetic or propionic acid produced similar growth rates to glucose in lambs [17].

Good performance by steers fed CoU suggested that more urea N was converted to microbial protein caused by starch in corn meal [18-20] than sugar in molasses.

TABLE IV. DRY MATTER INTAKE, APPARENT DIGESTIBILITY AND NITROGEN METABOLISM BY STEERS FED SUGAR-CANE DIETS WITH DIFFERENT NITROGEN AND ENERGY SOURCES

Item	Dietary treatment			SE ^a
	Molasses/ cotton seed meal	Molasses/ urea	Corn meal/ urea	
Number of observations	12	12	12	
Initial weight (kg)	263	265	260	4.0
Dry matter intake (kg/d)	4.7	4.1	4.3	0.1
Digestibility (%)				
Dry matter	64.9 ^b	67.0 ^c	65.7 ^{bc}	0.6
Organic matter	65.7 ^b	78.0 ^c	67.0 ^{bc}	0.5
Crude protein	56.7 ^b	61.7 ^c	65.0 ^c	1.2
Neutral detergent fibre	37.0 ^b	38.2 ^b	40.4 ^b	1.6
Acid detergent fibre	39.9 ^b	40.5 ^b	42.8 ^b	1.5
Cellulose	46.0 ^b	45.1 ^b	48.0 ^b	1.0
Nitrogen N metabolism				
N intake (g/d)	78.4 ^b	68.1 ^c	78.2 ^b	3.1
Faecal N (g/d)	33.9 ^b	26.0 ^c	27.3 ^c	1.2
Urinary N (g/d)	25.6 ^d	30.0 ^b	29.3 ^b	0.6
N balance (g/d) ^e	18.9 ^b	12.1 ^b	21.6 ^b	2.8
Digested N retained (%) ^f	40.6 ^b	20.7 ^b	40.3 ^b	6.6

^a Standard error of mean.

^{b,c,d} Values in the same row that do not have a common superscript differ ($P < 0.05$).

^e N balance = N intake - (faecal N + urinary N).

^f Digested N retained = (N intake - (faecal N + urinary N)) $\times$ 100/(N intake - faecal N).

On the other hand, better performance by steers fed MCS than by those fed MU, both of which contained molasses as supplemental energy sources, also suggests that the supply of metabolizable protein was inadequate with the MU diet. In summary, it appears that starch (as in corn meal) fed with NPN (urea) can be used to substitute protein nitrogen in sugar-cane-based diets. It also appears that poor performance by steers fed readily fermentable sugars with NPN is caused more by a shortage of metabolizable protein than energy.

In the digestibility and nitrogen balance experiment, steers consuming the MU diet showed sporadic signs of restlessness, aimless kicking, inappetance, excessive salivation and scouring. Uneasiness and excessive salivation in steers are early signs of urea toxicity caused by high levels of NH_3 in the blood [21, 22]. Lack of the above clinical signs by steers fed the CoU diet indicated that most of the NH_3

TABLE V. MEAN RETENTION TIME, 5%, 80% AND 5 TO 80% EXCRETION TIME OF CHROMIC OXIDE BY STEERS FED SUGAR-CANE DIETS WITH DIFFERENT SOURCES OF NITROGEN AND ENERGY

Variable ^a	Dietary treatment ^b		
	Molasses/ cotton seed meal	Molasses/ urea	Corn meal/ urea
Retention (h)	38.6	40.1	37.4
5% excretion (h)	18.5	18.5	17.4
80% excretion (h)	63.6	64.0	62.4
5 to 80% excretion (h)	45.1	45.5	45.0

^a Retention time from Castle [30]; 5%, 80% and 5 to 80% excretion from Balch [31].

^b Dietary treatments were not different ($P > 0.05$).

TABLE VI. VOLATILE FATTY ACIDS IN THE RUMEN CONTENTS OF STEERS FED SUGAR-CANE DIETS WITH DIFFERENT NITROGEN AND ENERGY SOURCES

Variable	Dietary treatment			SE ^a
	Molasses/ cotton seed meal	Molasses/ urea	Corn meal/ urea	
Acid				
Acetic ^b	59.6 ^d	59.1 ^d	62.5 ^d	1.1
Propionic ^b	21.2 ^e	26.0 ^d	20.5 ^e	0.7
Butyric ^b	17.9 ^d	13.6 ^d	16.1 ^d	1.2
Isovaleric ^b	0.2 ^d	0.1 ^d	0.0 ^d	0.1
Valeric ^b	1.2 ^d	11.2 ^d	0.9 ^d	0.2
Total ^c	75.4 ^d	70.3 ^d	75.9 ^d	2.3
pH	7.0 ^d	7.0 ^d	6.8 ^e	0.1

^a Standard error of mean.

^b Expressed as molar percentage of the total volatile fatty acids.

^c Expressed as molar concentration per litre.

^{d,e} Values in the same row with different superscripts differ ($P < 0.05$).

evolved from the hydrolysis of urea was captured by the rumen microorganisms when corn meal was fed than when molasses was fed. This observation further supports the better performance obtained by steers fed CoU over those fed the MU diet. Lack of urea toxicity signs in steers fed MU in the feedlot trial suggests that stress caused by confinement in metabolism crates predisposed steers to urea toxicity.

The average chemical composition of the experimental diets is given in Table III. The Mu diet had higher ($P < 0.05$) DM and organic matter digestibility (Table IV), probably due to the low level of fibre in the MU diet (Table III). The higher intake of DM by steers fed MCS might have further decreased DM digestibility in this diet. There were no differences ($P > 0.05$) in the digestibility of the fibre components (Table IV) and this was ascribed mainly to the presence of high levels of fermentable carbohydrates (45%), of which 40% came from sugar-cane alone in all the diets. Similar results have been reported elsewhere [23–26]. Sugars from sugar-cane alone might have caused a general decline in fibre digestibility, enough to eliminate the dietary treatment effects.

The apparent N digestibility was higher ($P < 0.01$) for the diets containing urea than for MCS (Table IV), mainly due to the complete hydrolysis of urea to NH_3 in the rumen. There was little difference in the digested N retained and N balance ($P > 0.08$ and $P > 0.07$, respectively) over all the diets, although both parameters were much lower for the MU diet than for the other two diets (Table IV). The results indicated that molasses was inferior to corn as an energy supplement to diets containing urea. The N balance data generally supported the growth data of steers in the feedlot trial discussed earlier.

The rumen NH_3 concentration for individual steers ranged from 0 to 20.1 mg/100 mL of rumen fluid compared with 0 to 130 mg/100 mL reported in the literature [23, 27]. The mean rumen fluid NH_3 levels were 1.63 ± 0.59 , 4.39 ± 1.01 and 5.94 ± 1.76 mg/100 mL for MCS, MU and CoU, respectively, but the differences were not significant. Diets containing urea tended to give higher NH_3 levels in the rumen fluid of steers, indicating high degradation of urea in the rumen. The suggested approximate upper level for maximum dietary N utilization is 5 mg of $\text{NH}_3\text{-N}$ per 100 mL rumen fluid [28]; 23.5 mg/100 mL rumen fluid has also been reported to be the optimum for microbial fermentation [29]. Although the preceding discussion suggested a deficiency of $\text{NH}_3\text{-N}$ with the diets used, the lower N balance along with high urinary N losses for the MU diets imply that the optimum $\text{NH}_3\text{-N}$ level for maximum urea utilization is below the suggested 5 mg $\text{NH}_3\text{-N}$ per 100 mL of rumen fluid.

Using Cr_2O_3 as a feed marker, there were no differences ($P > 0.05$) among the dietary treatments for any of the rate of passage parameters (Table V [30, 31]). Lack of differences among the treatments indicated that rate of passage did not influence utilization of the three diets.

Acetic acid tended to be lower in the rumen fluid of steers fed molasses diets than for those fed corn meal, whereas propionic acid was higher ($P < 0.05$) for the MU fed steers (Table VI). Since propionic acid, a glucose precursor, was within the

normal range (and even higher for the MU diet ($P < 0.05$)) lack of glucose or its precursors was not responsible for the poorer N retention or the growth of steers in the metabolism and feedlot trials, respectively. Above all, plasma glucose levels were similar for all the diets ($P > 0.05$), and the plasma levels were 78.5, 81.1 and 84.9 mg/100 mL for MCs, MU and CoU, respectively, indicating further that glucose or its precursors did not cause poor performance by steers fed the sugar-cane-based diets.

4. CONCLUSIONS

These experiments showed that molasses was inferior to corn meal as a source of energy, and that urea was inferior to cotton seed meal as the N source in diets containing high levels of sugar-cane. The moderate performance obtained with sugar-cane diets containing high levels of urea when molasses was fed showed that use of molasses and urea should be encouraged in areas where their availability is high as these might be the most economical means of supplementing sugar-cane diets for growing and fattening cattle.

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STUDIES ON CELLULOLYTIC BACTERIA ISOLATED FROM A STEER AND A BUFFALO*

F. FOONG**, H. KUDO***,
K.-J. CHENG***, S. JALALUDIN**

**Faculty of Veterinary Medicine and
Animal Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia

***Research Station,
Department of Agriculture,
Lethbridge, Alberta, Canada

Abstract

STUDIES ON CELLULOLYTIC BACTERIA ISOLATED FROM A STEER AND A BUFFALO.

A simple method using medium enriched with Whatman No. 1 filter paper as the sole selective substrate proved to be very effective in the isolation of cellulolytic bacteria from the rumen of a steer and a buffalo. Both animals were fed a high fibre ration. Most of the cellulolytic bacteria isolated were identified as *Bacteriodes succinogenes*; *Ruminococcus flavefaciens* was detected less frequently. However, no *R. albus* was isolated. The colonies were contaminated with *Butyrivibrio* sp. but not with *Borrelia* sp. The method described is effective, requires less time than the conventional cellulose agar method of isolation and is superior to the latter because pure colonies of *B. succinogenes*, which usually escape detection because of their failure to produce clear zones, can be detected. In clear zone experiments with cellulose (Whatman No. 1 filter paper) agar (1.3% wt/vol.) roll tubes, *B. succinogenes* and *Butyrivibrio* sp. were unable to produce clear zones, whereas mixed cultures were. However, *R. flavefaciens* in its pure form was able to produce clearings which had centre colonies.

1. INTRODUCTION

The rumen is an extremely complex community of many organisms and includes species which are capable of digesting cellulose. These microorganisms are very important in the initial attack on cellulosic materials and provide degraded products to other microorganisms which otherwise cannot utilize undegraded cellulose. The cellulolytic bacteria that inhabit the rumen of animals are largely responsible for the digestion and conversion of substrate which is otherwise useless. These microbes are therefore an indispensable part of the ruminant digestive system and warrant more intensified research. Their manipulation for the benefit of man can only begin with a greater understanding of their basic characteristics. In view of their

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importance to nutrition, experiments were carried out on the isolation and cellulolytic characteristics of anaerobic cellulolytic bacteria isolated from the rumen of a steer and a buffalo to determine if there are any species differences.

2. MATERIALS AND METHODS

2.1. Rumen content inoculum

The solid rumen contents used were collected from a fistulated water buffalo and a fistulated steer 2 h after morning feedings. The solid contents were strained through two layers of cheese cloth and the filtrate used as an inoculum. The steer was fed daily amounts of cubed hay equal to 0.5% of the body weight, with chopped straw offered *ad libitum*. The water buffalo was fed a daily ration of molasses equal to 0.1% of its body weight, together with mineral supplements and palm press fibre *ad libitum*.

2.2. Culture technique and media

All media were prepared under anaerobic conditions with a CO₂ gas phase using the technique of Hungate [1] as modified by Bryant and Burkey [2]. The liquid enrichment medium (10 mL) used for the initial enrichment and maintenance of bacteria was modified by Scott and Dehority [3] (MOD-SD) with 1.5 cm² (about 20 mg) Whatman No. 1 filter paper as the sole source of carbohydrate. The same medium, with a reduced amount (3 mm²) of cellulose paper, was used to detect cellulolytic bacteria. The dilution fluid was the MOD-SD medium without rumen fluid and carbohydrate. To prepare RGCSA (rumen fluid-glucose cellobiose-starch-agar) roll tubes for bacterial isolation, the MOD-SD medium was supplemented with 0.025% (wt/vol.) of cellobiose and glucose, 0.05% (wt/vol.) of starch and 1.8% (wt/vol.) of agar, as described by Grubb and Dehority [4]. This medium was placed in 10 mL quantities in 22 × 150 mm roller culture tubes (Bellco Glass Inc., Vineland, USA) with the appropriate No. 4 butyl rubber stoppers.

2.3. Isolation and characterization of cellulolytic bacteria

One millilitre of inoculum was incubated in 10 mL of the enrichment medium at 39°C for 36 h. At the end of incubation, the tubes were vortexed until the filter paper had completely disintegrated, then cooled to 0°C by placing them in ice for 30 min with occasional vortexing. This cooling procedure is effective in releasing cellulolytic bacteria from the cellulosic fibres [5]. One millilitre of this cooled culture was then diluted serially with dilution fluid to 10⁻⁹. Another 1 mL of each of the 10⁻⁷, 10⁻⁸ and 10⁻⁹ dilutions was introduced into the RGCSA roll tubes, spun mechanically (Tube spinner, Bellco Glass Inc., Vineland, USA), then incubated at

39°C for 72 to 96 h. Colonies were picked at random from the roll tubes which contained about 10 to 20 colonies, were introduced into the detection medium and incubated at 39°C. Cultures from the detection medium that showed any visible signs of filter paper disintegration were transferred to an enrichment medium. Culture purity and cellular morphology were examined by phase contrast microscopy, culture homogeneity and electron microscopy, and strains were characterized by the fermentation products obtained using gas chromatography. The samples used for this determination were deproteinized overnight with 24% (wt/vol.) metaphosphoric acid in 5N sulphuric acid and then centrifuged at 7700g for 30 min. For volatile fatty acid (VFA) analyses, the supernatant was mixed with an equal amount of 30 mmol crotonic acid as an internal standard. For lactic and succinic acids analyses, 0.5 mL of supernatant was mixed with 0.5 mL of 20 mmol malonic acid as an internal standard, methylated overnight at 39°C with 1 mL of 14% (wt/vol.) boron trifluoride and extracted with 0.5 mL of chloroform. Samples were treated by these procedures and analysed for VFAs (column: 10% polyethylene adipate + 3% phosphoric acid, chromosorb W 80/100) and other organic acids (column: 10% polyethylene adipate + 3% phosphoric acid, chromosorb 60/80) with a Varian Model 3700 gas chromatograph equipped with a Shimadzu Chromatopac C-RIB data processor. For clear zone formation tests, 1.3% (wt/vol.) of enzymatically digested Whatman No. 1 cellulose filter paper or untreated ball-milled Whatman No. 1 filter paper was substituted for the carbohydrates in the Scott and Dehority medium and 1.3% (wt/vol.) of agar was added. Filter paper cellulose was enzymatically prepared by placing 12 g of shredded Whatman No. 1 filter paper in 1 L of culture waste from a cellulolytic bacterium for 96 h, with stirring and by washing and autoclaving the treated fibre.

For colony homogeneity tests the inoculum was serially diluted, then cultured in RGCSA roll tubes and incubated for 72 h at 39°C. The tubes were then checked to determine whether the colonies were homogeneous. To confirm the morphological homogeneity of the cells, the purified isolates were transferred into the MOD-SD medium containing glucose and cellobiose (each at 0.17% (wt/vol.)) and examined under the phase contrast microscope after 12 h of incubation at 39°C.

2.4. Electron microscopy

Partially digested cellulose filter paper from the 24-h enrichment cultures and bacterial cells in pure cultures were prefixed by the addition of 1 mL of fixation solution to 10 mL of sample. The cellulose fibres were allowed to settle, while cells in pure cultures were centrifuged at 20 000g for 10 min and the samples were then fixed for 2 h at room temperature in a fixative solution of 5% glutaraldehyde in 10 mmol cacodylate buffer (pH 7.0) containing 0.15% ruthenium red. For transmission electron microscopy the samples were then washed five times in cacodylate buffer, post-fixed in 2% OsO₄ in cacodylate buffer, dehydrated in a water-acetone series [6] and examined using a Hitachi 500 series electron microscope. For scanning electron

TABLE I. FERMENTATION PRODUCTS BY *B. succinogenes* sp. ISOLATED FROM A STEER^a

Strain	Fermentation products (mmol)				
	Acetic acid	Succinic acid	Strain	Acetic acid	Succinic acid
A	5.0	7.9	U	4.6	7.4
B	4.6	6.6	V	4.2	7.9
C	5.5	7.7	W	4.2	6.3
D	5.2	7.5	Y	3.5	7.6
E	4.0	6.7	Z	5.2	8.7
F	4.5	7.2	a	5.0	9.2
G	3.8	6.9	b	4.9	8.8
H	4.6	7.5	d	4.6	7.6
I	3.5	7.1	e	3.7	7.8
J	3.2	7.1	f	2.5	7.2
K	4.1	8.0	g	2.6	7.1
L	4.4	7.5	h	2.6	7.6
M	5.5	7.9	i	2.8	8.4
N	4.6	7.5	j	2.5	7.7
O	4.8	7.5	m	3.1	8.2
P	4.2	7.8	n	3.1	8.7
Q	3.5	6.9	q	3.9	8.7
R	3.7	7.7			
S	3.6	7.6			
T	4.2	7.7			

^a Cultures were incubated in 10 mL of Scott and Dehority's medium with 30 mg of Whatman No. 1 cellulose filter paper.

microscopy the samples were internally metallized by the thiocarbonylhydrazine technique [7], dehydrated by a critical point drying method [8] and examined without metal evaporation using a Hitachi 500 series scanning electron microscope.

3. RESULTS

Direct scanning electron microscopy (SEM) examination of filter paper fibres from the 24-h enrichment cultures of samples from the rumen of a steer showed that

TABLE III. FERMENTATION PRODUCTS BY
R. flavefaciens sp. ISOLATED
FROM THE RUMEN OF A STEER^a

Strain	Fermentation products		
	Acetic acid (mmol)	Lactic acid (mmol)	Succinate acid (mmol)
1	2.9	2.2	4.5
2	3.4	2.2	4.4
3	2.8	2.0	4.4
4	3.3	2.3	4.1
5	3.5	1.7	4.2
6	2.7	0.9	4.3
7	4.9		5.3

^a Cultures were incubated for 5 days in 10 mL of Scott and Dehority's medium with 30 mg of Whatman No. 1 cellulose filter paper at 39°C.

TABLE II. FERMENTATION PRODUCTS BY
Butyrivibrio sp. ISOLATED
FROM THE RUMEN OF A STEER^a

Strain	Fermentation products	
	Butyric acid (mmol)	Lactic acid (mmol)
B Bf	11.8	1
E Bf	11.5	2
X Bf	11.2	1.8
e Bf	10.5	2.1
f Bf	4.3	20

^a Cultures were incubated for 5 days in 10 mL of Scott and Dehority's medium with 30 mg of cellobiose.

TABLE IV. FERMENTATION PRODUCTS BY *B. succinogenes* sp. ISOLATED FROM THE RUMEN OF A WATER BUFFALO^a

Strain	Fermentation products								
	Acetate (mmol)	Succinate (mmol)	Lactate (mmol)	Butyrate (mmol)	Strain	Acetate (mmol)	Succinate (mmol)	Lactate (mmol)	Butyrate (mmol)
101	6.9	11.3			119	4.5	9.7	1.0	2.1
103	5.7	11.8			120	4.4	12.3	1.8	
104	8.1	11.5			121	7.4	10.1	0.8	1.2
105	6.3	10.5			122	3.9	10.5	0.7	
107	7.0	11.5			123	6.1	12.6	1.1	1.2
108	6.4	9.4			126	5.6	12.7	1.7	
110	2.4	8.7	0.7		127	7.1	11.9	1.2	0.6
112	10.1	11.5		1.3	128	4.8	11.1	2.8	
113	3.6	9.8	1.8		129	6.0	9.4	2.1	
114	8.0	10.2	1.7		130	3.4	9.0	1.7	
116	7.3	12.7	1.2		135	5.6	9.3	0.5	

^a Cultures were incubated for 5 days in 10 mL of Scott and Dehority's medium with 30 mg of Whatman No. 1 cellulose filter paper at 39°C.

TABLE V. FERMENTATION PRODUCTS BY *R. flavefaciens* sp. ISOLATED FROM THE RUMEN OF A WATER BUFFALO^a

Strain	Fermentation products		
	Acetic acid (mmol)	Lactic acid (mmol)	Succinate acid (mmol)
102	6.5		8.5
106	6.9		6.8
111	2.5	0.9	0.1
115	6.2	2.2	7.8
132	8.7	0.5	0.2
136	8.7	0.6	9.4

^a Cultures were incubated for 5 days in 10 mL of Scott and Dehority's medium with 30 mg of Whatman No. 1 cellulose filter paper at 39°C.

the surfaces of these cellulosic materials were very heavily colonized by bacteria. Transmission electron microscopy (TEM) of sections of these partially digested cellulose fibres from the 24-h enrichment cultures also showed that areas of their surface were colonized by various combinations of bacterial species. Over most of the surface of the fibres one bacterial species formed a primary colonizing population, while cells of another bacterial species were found in lesser numbers in association with these primary colonizers. Spiral cells resembling *Borrelia* sp. and curved rod-shaped cells resembling *Butyrivibrio* sp. were seen in association with these surfaces; they were heavily colonized by polymorphic rod-shaped cells and slightly elongated coccoid cells that resembled those of *Ruminococcus* sp. Culture material from the enrichment cultures derived from the rumen contents of the steer was used to inoculate the roll tubes, and 120 colonies were randomly picked from the agar surface of the culture material. Of these 120 colonies, 45 were found to be cellulolytic. Thirteen were contaminated, of which six were cellulolytic rods resembling *B. succinogenes* accompanied by curved rods resembling *Butyrivibrio* sp.; five were cells resembling *B. succinogenes* and an unidentified small rod, and two were cells resembling *R. flavefaciens* and an unidentified rod-shaped bacterium. None of the colonies picked from the roll tubes contained cells resembling *Borrelia* sp., even though direct observation of cellulose fibres from the origin enrichment cultures revealed large numbers of spiral organisms.

After purification, the isolates were transferred into the MOD-SD medium containing glucose and cellobiose (each at 0.17% (wt/vol.)) and were examined (after 12 h incubation for morphological homogeneity) by SEM and TEM to confirm the

purity and cell structure. All the cultures that were found to be pure had colonies of similar appearance and their morphology under the phase contrast microscope was homogeneous within the same strain. The cellulolytic rods were Gram-negative and produced large amounts of acetate and succinate (Table I) and we concluded that these are cells of *B. succinogenes*. When pure strains of cellulolytic rods were tested for their ability to produce 'clear zones' in cellulose (Whatman No. 1 filter paper) agar (1.3% (wt/vol.)) roll tubes, none could produce this reaction. After similar purification, the curved rod-shaped cell resembling *Butyrivibrio* sp. also showed no sign of cellulolytic activity in the cellulose roll tubes. However, co-cultures of the curved rod-shaped cells resembling *Butyrivibrio* sp. with cells of *B. succinogenes* were able to produce clear zones. Fermentation products produced from cellobiose showed that all but one strain of *Butyrivibrio* sp. produced high levels of butyrate (Table II).

Examination of 12-h cultures of the cellulolytic cocci revealed Gram-positive cells in pairs or in chains of 5 to 14 cells. The cells in older cultures (18 h) were seen to be spherical or slightly elongated and they occurred predominantly as diplococci and were found to be Gram-negative or Gram-variable. The major fermentation products from cellulose were acetate, lactate and succinate (Table III), but one strain did not produce lactate. All pure strains of this species could produce clear zones accompanied by yellow pigment production on the cellulose (Whatman No. 1 filter paper) agar (1.3% (wt/vol.)) roll tubes. Yellow pigment production was also observed in the Scott and Dehority liquid medium with cellulose filter paper as the sole source of carbohydrate. On the basis of fermentation products and the production of the characteristic yellow pigment, these cocci were characterized as *R. flaveflaciens*.

In a manner similar to that of the steer, a total of 90 colonies were picked from inoculated roll tubes of the buffalo rumen contents. Thirty-six of these 90 colonies showed cellulolytic activity when transferred to the detection medium. Of the 36 isolates, 22 were identified as *B. succinogenes*, six were identified as *R. flaveflaciens*, five were cultures of *B. succinogenes* with *Butyrivibrio* sp. and three were mixed cultures of unidentifiable species. There was a general similarity in morphology, fermentation products (Table IV) and cellulolytic activity between the strains of *B. succinogenes* isolated from the buffalo and the steer. The six strains of *R. flaveflaciens* isolated from the buffalo demonstrated a higher production of acetate and succinate from cellulose than those from the cattle, except for one strain which had very low values of fermentation products (Table V).

4. DISCUSSION

A high proportion (45 out of 120 colonies from a steer and 36 out of 90 colonies from a water buffalo) of cellulolytic bacterial strains was obtained by using this simple enrichment method with Whatman No. 1 filter paper substrate. Scanning and

transmission electron micrographs of these rumen populations in the enrichment medium revealed that members of the important consortia are accounted for among the isolates. Both the morphological and biochemical data generated in this study attest to a general similarity between the cellulolytic bacterial populations of the steer and the buffalo. *Borrelia* sp. was seen by SEM and TEM in both animals, and *B. succinogenes*, *R. flavefaciens* and *Butyrivibrio* sp. were all seen, isolated and characterized in both animals. Fermentation products were produced in higher concentrations from *R. flavefaciens* from the buffalo rumen, and both *B. succinogenes* and *R. flavefaciens* from the buffalo rumen showed a greater dependence on rumen fluid. These minor microbiological differences may result from the well known influence of diet (straw versus palm press fibre) on the bacterial rumen population [9-11].

Most of our isolates were found to be pure, but some were mixed cultures of organisms; all produced disintegration of filter paper fibres in the liquid detection media. However, while all isolates of *R. flavefaciens* produced a cellulolytic effect in both this liquid detection media and on cellulose agar, isolates containing *B. succinogenes* failed to produce the clearing reaction on cellulose agar except when mixed with other organisms such as *Butyrivibrio* sp. This suggests that isolates of *B. succinogenes* could not be detected as being cellulolytic by the conventional method of direct plating on cellulose agar unless they were contaminated by other organisms. This may account for the generally reduced recovery of this species as a cellulolytic organism, in relation to *Ruminococcus* sp. which clears cellulose agar even as pure isolates, and for the finding that *B. succinogenes* which are isolated as cellulose-clearing colonies are always contaminated by *Borrelia*, *Butyrivibrio* or other bacterial species [12, 13]. This infers that some sort of commensal relationship may occur between *B. succinogenes* and its contaminant. Electron microscopic examination of mixed rumen population has revealed that *B. succinogenes* is the main primary colonizer of cellulose fibres, while *Butyrivibrio* or *Borrelia* sp. can often be seen to be associated with *B. succinogenes* in close juxtaposition.

The method used for the isolation of cellulolytic rumen bacteria in this study yields a high recovery of these organisms, and the results of the isolation agree well with those generated by direct morphological examination. The results from clear zone experiments suggest that direct plating on cellulose agar fails to detect pure colonies of *B. succinogenes*; thus, its presence may be underestimated and it is difficult to isolate without contamination by *Borrelia* and/or *Butyrivibrio* sp. Our isolation medium allows the recovery of pure cultures of *B. succinogenes*, and the cellulolytic activity of all species, or a combination of species, is accurately assessed in our liquid detection medium. Co-ordinated use of electron microscopy and this isolation technique also assists in a better understanding of the microbial ecology of cellulose digestion in the rumen.

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MINERAL IMBALANCES AND THEIR DIAGNOSIS IN RUMINANTS

L.R. McDOWELL, J.H. CONRAD, J.K. LOOSLI

Department of Animal Science,
Institute of Food and Agricultural Sciences,
University of Florida,
Gainesville, Florida,
United States of America

Abstract

MINERAL IMBALANCES AND THEIR DIAGNOSIS IN RUMINANTS.

The most reliable method of confirming mineral deficiencies is response derived from specific mineral supplementation. However, such studies are costly in time and resources if conducted with adequate control and assessment. Clinical signs of mineral deficiencies, pathological and biochemical examinations, along with soil, water, plant and animal tissue and fluid mineral analyses have all been used with varying degrees of success to establish mineral deficiencies and excesses. However, the majority of mineral imbalances, particularly borderline conditions, do not result in pathological observations or clinical signs specific to a single mineral. Therefore, in order to determine mineral insufficiencies, chemical analyses and biological assays are often required. Analyses to determine the available forms of soil minerals can sometimes provide clues to livestock mineral deficiencies, but more often they are unreliable and difficult to interpret. Data from Brazil, Bolivia, Florida (USA) and Guatemala have indicated that mineral correlations among soil, plant and animal tissue concentrations were highly variable among locations and often low or non-existent. Relying on a forage mineral analysis to establish mineral status assumes that the sample is representative of what animals consume. Nevertheless, forage mineral analyses are preferable to soil analyses, while appropriate animal tissue and fluid analyses most accurately portray the contribution of the total dietary environment (forage, soil, water, etc.) in meeting livestock mineral requirements. Since mineral analyses are complicated and expensive, it is important to select and analyse the minimum number of plant and animal tissues, which are more indicative of the mineral status. Tissues used to evaluate the status of specific minerals include liver, blood, bone, milk, hair and saliva. Other non-chemical techniques for evaluating the mineral status of bone minerals include bone breaking strengths, ultrasound procedures and photon absorption. In vitro techniques involving addition of radioisotopes to collected blood samples have been used to evaluate the mineral status of a number of minerals (e.g. ^{75}Se and ^{65}Zn).

1. INTRODUCTION

Even when there is an abundant feed supply for grazing livestock, investigations have shown that ruminant livestock may deteriorate and fail to achieve a reasonable level of productivity because of deficiencies or excesses of minerals in soils and plants. Furthermore, such deficiencies or excesses may not only result in low production but may give rise to problems of reproduction and to clinical signs indicating mineral deficiencies that are encountered worldwide, such as wasting diseases, loss of hair, depigmented hair, skin disorders, non-infectious abortion, diarrhoea, anaemia, loss of appetite, bone abnormalities, tetany, low fertility and pica.

Mineral deficiencies and imbalances for herbivores are reported from almost all tropical regions of the world [1, 2]. These reports include both confirmed and highly suspected geographical areas of mineral deficiencies and toxicities for ruminants. The extent of affected areas is not generally appreciated and it is inevitable that reports of mineral inadequacies will greatly increase as more tropical countries undertake mineral research and thereby improve their methods of detection. Except occasionally for common salt, grazing ruminants in tropical and subtropical regions rarely receive mineral supplementation and have to depend for their requirements entirely on grazing and browsing, which can hardly ever satisfy all the mineral requirements of the animal. Research from tropical regions has shown mineral supplementation to increase calving percentages by 20% to more than 100%, to increase growth rates from 10 to 25% and to reduce mortality significantly [1]. Mineral nutrition disorders range from acute mineral deficiency or toxicity diseases, characterized by well marked clinical signs and pathological changes, to mild and transient conditions difficult to diagnose and expressed as a vague unthriftiness or unsatisfactory growth and production [3]. The latter assume great importance because they occur over large areas and affect a large number of animals, in addition to the fact that they can be confused with energy and/or protein deficiencies or parasitism [3].

Detection of mineral element deficiencies or excesses involves clinical, pathological and analytical criteria as well as response from specific element supplementation. Clinical signs of mineral deficiencies along with soil, water, plant and animal tissue analyses have all been used with varying degrees of success to establish mineral deficiencies and toxicities. The most reliable method to confirm mineral deficiencies is response derived from specific mineral supplementation. However, supplementation studies are costly in time and resources if conducted with adequate control and assessment.

For several decades, a major goal in mineral research has been to discover and/or develop simple and accurate biochemical measurements of the status of animals for the minerals in which there are important practical problems [4]. Like soils and plants, animal tissue-mineral concentrations are influenced by many factors. Nevertheless, when appropriate interpretation is made animal tissue concentrations are often better indicators of the mineral status of livestock than either plant or soil concentrations [5]. When the evidence obtained from clinical, pathological and biochemical examinations of the animal and from chemical analysis of the diet and its components is combined and assessed, it is usually possible to detect and define any nutritional abnormality of mineral origin, even when it is mild [3].

2. CLINICAL AND PATHOLOGICAL EVALUATION

Changes in animal appearance or level of production can often be an early indication of diet inadequacy. Where the nutritional abnormalities are acute or severe, well marked clinical and pathological stigmata appear, making detection and correc-

tion relatively easy. As an example, severe or acute deficiencies of I, Mg and Cu and toxicities of Se and F are often characterized by specific clinical signs, but nutritional disorders are often mild or marginal and expressed only as a vague unthriftiness (e.g. hair losing its sheen) or suboptimal growth, fertility or productivity. Unfortunately, these changes are often non-specific and indistinguishable from those resulting from inadequate energy-protein or vitamins, or from parasitism or toxic plants. Therefore, it often becomes necessary to resort to chemical analyses in order to adequately determine mineral insufficiencies.

Clinical and pathological observations of animals have become essential diagnostic tools in investigations of all mineral element deficiencies and toxicities. Since sudden death in the absence of clinical signs may also be a consequence of acute infectious disease, it is important to conduct a post-mortem examination for purposes of differential diagnosis. When evidence of gross pathology is supported by histological findings, it is often possible to present a tentative diagnosis of mineral element deficiency [6].

It is important to recognize the limitations of clinical and pathological findings. For instance, anaemia can be caused by deficiencies of Fe, Cu and Co as well as protein and certain vitamins and by toxicities of Zn, Se and Mo. Similarly, abnormalities in the size, shape, strength and chemical composition of the bones can occur in Ca, P, Cu, Mn, Zn, Si and vitamin A and D deficiencies as well as in toxicities of F and Mo. Since early diagnosis is the key to preventive treatment, and preventive treatment is clearly superior to curative treatment given after productivity losses or mortality have occurred, it is important to develop detection techniques capable of giving an early and secure diagnosis [3].

3. ANALYSIS OF WATER, SOIL AND FORAGE

Mineral deficiencies and excesses have been established by soil, water and plant analyses. Although highly variable, all mineral elements essential as dietary nutrients occur to some extent in water. Nevertheless, grazing livestock obtain the majority of their mineral requirements from forages that, under some conditions, are contaminated with soil.

Plants withdraw essential elements from the soil solution in quantities to satisfy their own requirements as well as satisfying many of the requirements of grazing livestock. Besides essential plant elements, plants also withdraw Se, Co and I, which are essential for the grazing ruminant. The soil-plant relationship is direct in that the plant must obtain all the mineral nutrients from the specific soil with which it has contact.

In some instances, a soil survey can provide clues to potential livestock deficiencies. Soil concentrations of Co, Mo and I reflect the plant's concentrations of these elements to a certain degree. However, numerous factors affect forage mineral uptake from soils, including yield of plant, stage of maturity, species and strain

TABLE I. DIETARY REQUIREMENTS AND TOXICITIES OF MINERAL ELEMENTS FOR DAIRY, BEEF CATTLE AND SHEEP^{a,b}

Element	Dairy cow ^{a,c}	Beef cattle ^{a,d}	Sheep
<i>Deficiency</i>			
Calcium (%)	0.54	0.18—0.53	0.21—0.52
Magnesium (%)	0.20	0.05—0.25	0.04—0.08
Phosphorus (%)	0.38	0.18—0.37	0.16—0.37
Potassium (%)	0.80	0.65	0.50
Sodium (%)	0.18	0.08	0.04—0.10
Sulphur (%)	0.20	0.10	0.14—0.26
Cobalt (ppm)	0.10	0.10	0.1
Copper (ppm)	10	8	5.0
Iodine (ppm)	0.50	0.5	0.1—0.8
Iron (ppm)	50	50	30—50
Manganese (ppm)	40	40	20—40
Selenium (ppm)	0.1	0.20	0.1
Zinc (ppm)	40	30	35—50
<i>Toxic concentration</i>			
Copper (ppm)	80	115	8—25
Fluorine (ppm)	30	20—100	60—200
Manganese (ppm)	1000	1000	
Molybdenum (ppm)	6	6	5—20
Selenium (ppm)	5	5	> 2.0
Zinc (ppm)	500	500	1000

^a Requirements below which a deficiency occurs [2].

^b For grazing livestock soil concentrations suggesting deficiencies are as follows: calcium (0.35 meq/100 g), potassium (0.15 meq/100 g), magnesium (0.07 meq/100 g), phosphorus (10 ppm), cobalt (0.1 ppm), copper (0.6 ppm), manganese (19 ppm) and zinc (2 ppm).

^c Recommendations for lactating dairy cows (500 kg) giving 17 to 23 kg of milk.

^d Recommendations for growing and fattening steers and heifers.

differences, climatic and seasonal conditions, chemical forms of minerals and factors of the soil, including pH and degree of aeration of waterlogging.

The concentration of a mineral in a soil is an uncertain guide to its concentration in the forage. Soil analysis, though useful for pasture fertilization, has been eliminated in some investigations because of lack of a direct relationship to the mineral content of herbage growing on the soil [7]. For instance, plants growing on Co-deficient soil may not necessarily be deficient in Co, nor would a soil rich in Co

necessarily yield plants with high levels of Co [8]. However, in the Netherlands soil analysis is preferred to that of forage analysis to establish a Co deficiency [9].

A more satisfactory soil analysis to relate mineral concentrations for livestock is the use of soil extracts (i.e. 0.1 N HCl or 2.5% acetic acid), which contain the more available forms of soil minerals. Analyses to determine the available forms of soil minerals can sometimes provide evidence of livestock mineral deficiencies but, unfortunately, more often they are unreliable and difficult to interpret. Hartmans [10] reports that the available Cu of the soil does not show any positive relationship with the Cu status of the animal. Data from Brazil, Bolivia, Guatemala, Malawi and Florida (USA) have indicated that mineral correlations among soil, plant and animal tissue concentrations were highly variable among locations, and are often low or non-existent [2]. Typical forage-soil correlations reported in Brazil were Fe ($r = 0.12$), Mn ($r = -0.12$), and Zn ($r = 0.30$). In Malawi, few correlation coefficients in soil, forage and animal tissues were greater or equal to ± 0.5 .

Mineral analysis of the forage consumed by the grazing animal is basic to mineral status diagnosis. If mineral concentrations are below minimum requirements or above the minimum tolerance level, there is an immediate suggestion of a nutritional problem (Table I [2]). However, relying on a forage mineral analysis to establish mineral status assumes that the sample is representative of what animals consume. Fick et al. [11] have summarized procedures to obtain a representative sample as follows: (1) Carefully observe livestock grazing patterns and hand pluck a sample to represent the animals' diet; samples should not be taken from areas where excessive excretion has accumulated or from large clumps of grass that have obviously been rejected by animals; (2) Separate samples from each of the major species should be taken, with estimates made as to the percentage each particular species represents of the total consumption; (3) The aerial parts should be clipped using stainless steel scissors, with the cutting representing the height being grazed.

Additional disadvantages of forage element analyses to assess mineral adequacy are the difficulties in estimating forage intake and digestibility. The majority of mineral requirements is given in percentage or ppm (mg/kg), which assumes the expected consumption as estimated by dietary standards (i.e. National Research Council (NRC) or Agricultural Research Council (ARC)). Unfortunately, commonly used dietary standards are based on temperate forage consumption data and, therefore, would overestimate the intake of minerals. It is generally accepted that tropical forages are less digestible than temperate species and, therefore, daily consumption by grazing ruminants is lower. For more accuracy, total grams of specific minerals consumed per day, and not forage concentrations, determine the true adequacy of a mineral. Likewise, relative adequacy based on forage mineral concentrations is dependent on interactions with other nutrient fractions, such as proteins, lipids or other elements that can greatly affect the availability of the respective elements for digestion, absorption and retention [12].

Forage analysis for certain trace minerals will be erroneously high due to the inherent problem of sampling forages free from contaminating soil. Mineral elements

such as Ca, K, P and Mo would not be greatly affected by soil contamination, since soil levels would be approximately equal to or less than plant mineral concentrations. In contrast, soil mineral levels of Co, Fe, I, Na, Mn and Se and, to a lesser extent, Zn and Cu would be higher than forages and even slight contamination caused by splashing rain could give an erroneously high impression of the concentration of these elements. Mitchell [13] indicated that soil contains 20 to 1000 times the Co and Fe content found in pastures grown on a particular soil.

The disadvantages of forage element analyses to assess mineral adequacy for grazing livestock can be summarized to include: (1) uncertainty of samples representing what livestock consume; (2) difficulty of estimating forage intake; (3) variation in the availability of forage elements; and (4) the possibility of soil-contaminated forage samples.

4. EXAMINATION OF TISSUES AND FLUIDS

Without question, forage analysis is a much better indicator of mineral status for ruminants than soil analysis. Likewise, animal tissue-mineral concentrations are better indicators of the availability of minerals than forage mineral analyses. Grazing livestock obtain part of their mineral supply from the consumption of water, soil, leaves, tree bark, etc. rather than entirely from forages. Livestock tissue-mineral concentrations, therefore, more accurately portray the contribution of the total environment in meeting the mineral requirements of grazing animals. As an illustration, neither the available Cu content of the soil nor the Cu content in the herbage show any positive relationship with the Cu status of the animal [10]. However, liver Cu concentrations of less than 25 ppm coincide with Cu deficiency signs in cattle [2].

Animal tissue and fluid levels of minerals, in addition to concentrations of particular enzymes, metabolites or organic compounds with which the mineral in question is associated functionally, are important indicators of mineral status. The diagnostic significance of tissue and fluid analysis is based upon evidence that mineral element deficiencies are ultimately reflected in subnormal concentrations of the element, in altered concentrations of related metabolites or in changed activities of affected enzymes. The concentrations of minerals in the tissues, or of their functional forms such as thyroxine (I) and vitamin B₁₂ (Co), must be maintained within narrow limits if the growth, health and productivity of the animal are to be sustained. Departures from these normal limits, which are now well defined for most elements, therefore constitute useful diagnostic indicators. A further valuable aspect of such tissue composition changes is that they frequently arise before the appearance of adverse clinical signs [3].

Ideally, animal scientists would like to determine the mineral status of an animal by measuring the mineral content of one tissue that is readily available from a live animal. Unfortunately, no mineral concentration of any one tissue or fluid will portray the status of all minerals. Blood, urine, saliva, milk, faeces and hair may

easily be sampled, and even liver and bone may routinely be biopsied with a minimum of time and danger to the animal. Surgical techniques for the acquisition of biopsy samples of liver [14] and rib bone [15] from cattle and sheep have been described. Liver taken either by biopsy or from sacrificed animals is an excellent indicator of the status of certain trace elements, but bone is the preferred tissue for evaluating bone-forming minerals, particularly Ca and P. Other non-chemical techniques for evaluating the mineral status of bone minerals include bone breaking strengths, ultrasound procedures and photon absorption [16].

The organ, tissue or fluid chosen for analysis varies with the element, but estimations of whole blood, plasma or serum trace element, or enzyme concentrations have wide applicability and do not, of course, require sacrifice of the animal. The levels of certain mineral elements in hair or wool, urine and even in milk are also of value in the detection of deficiency or toxicity status, although individual variability can be very high and external contamination provides problems for trace element status evaluation.

Whole blood or blood serum or plasma is widely used for studies in mineral nutrition. Values significantly and consistently above or below 'normal' concentrations or ranges provide suggestive but not conclusive evidence of a dietary excess or deficiency of particular minerals [3]. Precautions must be taken in interpreting blood mineral data collected or prepared under less than optimum conditions. The factors responsible for elevations of serum or plasma minerals include stress, exercise, haemolysis, temperature and serum separation time [11]. These factors have often been difficult to control in studies in Latin America and Africa and have resulted in high serum P concentrations compared with extremely low levels of forage P [17].

Because of the many factors that cause variation in the mineral content of hair, hair analyses are not likely to be precise indicators of the mineral status of animals. While concentrations of Ca, P and Cu in hair are not affected by dietary intake, the Zn and Se contents of hair may reflect dietary intake as well as toxic consumptions of Pb, As and possibly Cd [18].

Enzymes have been used to detect mineral deficiencies. Because of the essential role of many minerals in enzymes (i.e. serum, ceruloplasmin for Cu and glutathione peroxidase for Se), enzyme activity has been developed into a useful measure in certain mineral deficiencies [4]. Perhaps this approach will have far wider application in the future.

5. RADIOISOTOPE DETECTION OF MINERAL DEFICIENCIES

Various methods for utilization of radioisotopes have been well described in an IAEA publication [19]. In determining mineral deficiencies or excesses, element analyses are indispensable. Neutron activation is a desirable method of analysis for a large number of mineral analyses. The method is non-destructive, results in extremely low detection limits and allows for a high number of simultaneously detec-

table elements. This method does not require any operation except radiation by neutrons in the reactor and measuring activity on a suitable detector. An example illustrated for Se [20] would be for samples and standards to be activated with neutrons for 7 to 21 hours in a Triga III type reactor with a neutron exposure of $1 \times 10^{13} \text{ N}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$. Eight to 20 weeks were allowed for radiation decay and ^{75}Se , which has a half-life of 120 days, was counted on a multichannel analyser using the 0.265 MeV peak.

Isotopic methods have been used to examine the nutritive value of locally available feedstuffs and to determine the dietary intake of specific minerals. A technique for measurement of dietary P content has been used successfully to also measure the dietary intake of P [21]. In this technique, P of salivary origin is distinguished from forage samples extruded by oesophageally fistulated animals by prior labelling of salivary P with ^{32}P .

True absorption of an element, a fundamental parameter in nutritional studies, requires use of a radioisotope to measure endogenous faecal excretion, which can be independent of mineral intake or vary with it. Constant endogenous faecal excretion cannot be assumed, but must be measured on each occasion for some elements such as P [22].

The extent of absorption, retention and excretion of an element is dependent upon the status of the animal. Thus, deficiency of a mineral can be established on the basis of absorption, retention and excretion of an isotope of a particular element. Kirchgessner and Schwarz [23] discussed three models for the use of radioisotopes in the diagnosis of mineral element deficiency: (1) determination of intestinal absorption of an orally administered radioisotope by measuring the activity of blood; (2) determination of the retention of a parenterally administered dose in blood or plasma; and (3) determination of the endogenous faecal excretion of a parenterally administered dose. With an oral dose of the labelled element, absorption of this dose can be measured and, with a parenteral injection of the label, retention and endogenous excretion of this dose can be assessed. Determination of the endogenous secretion of the trace element after intravenous injection of its radioisotope may be considered as the simplest and most informative method. Assessment of the endogenous secretion appears to be feasible without major difficulties, even under practical farm conditions [23]. For on-farm studies employment of isotopes of short half-life is preferable.

Employment of radioisotopes makes it possible to demonstrate the dynamics of mineral elements and to arrive at quantitative assessments on pathways of homeostatic regulation. For rats, Kirchgessner and Schwarz [23] reported higher *in vitro* absorption for deficiency versus adequacy (in nanograms) for Cu (28.5 versus 8.9), Zn (68.1 versus 17.0) and Fe (17.9 versus 6.7). *In vitro* techniques involving addition of radioisotopes to collected blood samples have been used to evaluate the mineral status of a number of minerals (e.g. ^{75}Se and ^{65}Zn). As an example, the erythrocyte uptake of ^{75}Se has proved to be a promising technique to evaluate Se status. The erythrocytes of a Se-deficient animal will have a much higher uptake of

^{75}Se than animals with a normal Se status [24]. This procedure is of value, since blood samples could be collected even under nomadic conditions as animals are at times assembled at stations for vaccination or other reasons.

Mineral interrelationships and antagonism of some minerals towards others, which result in deficiencies, have been better explained by the use of radioisotope studies. As an example, doubling the dietary Ca did not affect ^{54}Mn absorption, but endogenous faecal loss of ^{54}Mn was increased by approximately 28% in both dry and lactating cows [25]. Pope et al. [26] studied the level of dietary S on Se (^{75}Se) absorption and retention. Urinary excretion of ^{75}Se accounted for between 12% (0.05% S) and 22% (0.24% S) of the dose after 7 days. The levels of radioactivity in blood were significantly higher in sheep fed the 0.05% S diet than those fed higher levels.

Copper deficiency is not only influenced by low forage Cu but also excessive intakes of Mo and S. Attention is paid to Cu–Mo–S interactions in ruminants, mainly regarding the mechanisms by which body Cu stores are depleted by dietary Mo and S. In work with sheep and cattle using radioisotopes, the mechanisms by which Cu metabolism is affected under such dietary conditions, together with the sites affected, have been reported [27]. It was demonstrated that the ability of the liver to store ^{64}Cu and the utilization of the tracer in ceruloplasmin production was impaired by high dietary Mo and S. Failure in the synthesis of cuproproteins by the liver was postulated to explain the dysfunction.

6. ANALYSES MOST INDICATIVE OF MINERAL STATUS

Since mineral analyses are complicated and expensive, it is important to select and analyse the minimum number of those plant and animal tissues (or fluids) that are more indicative of the mineral status of ruminants. Tables I and II [2, 5, 17] illustrate analyses of considerable value in assessing specific mineral deficiencies and toxicities. The word 'critical' is used in Table II to note a concentration in forages below (or above with excesses) what is considered the requirement. This assumes the expected consumption of dry matter. Critical animal tissue concentrations (Table II) are levels below or above values associated with specific clinical signs as reported in the literature.

7. MAPPING TECHNIQUE FOR DETERMINING MINERAL DEFICIENCIES AND TOXICITIES

Mineral deficiencies or toxicities in grazing livestock can be predicted by use of a systematic mapping survey technique or regional reconnaissance. Analysed Se and Co levels of US forages have been related to Se- and Co-responsive diseases [28]. Similar mapping techniques based on forage analyses have been undertaken for

TABLE II. CRITICAL LEVELS FOR DIAGNOSIS OF SPECIFIC MINERAL DEFICIENCIES OR TOXICITIES IN CATTLE USING TISSUE AND FLUID MINERAL CONCENTRATIONS^{a,b}

Element	
<i>Deficient</i>	
Calcium	Fat-free bone (24.5%), bone ash (37.6%), plasma (8 mg/100 mL)
Magnesium	Serum (1–2 mg/100 mL), urine (2–10 mg/100 mL)
Phosphorus	Fat-free bone (11.5%), bone ash (17.6%), plasma (4.5 mg/100 mL)
Sodium	Saliva (100–200 mg/mL)
Cobalt	Liver (0.05–0.07 ppm, dry basis)
Copper	Liver (25–75 ppm, dry basis), serum (0.65 µg/mL)
Iodine	Milk (300 µg/d)
Iron	Haemoglobin (10 g/100 mL), transferrin (13–15% saturation)
Manganese	Liver (6 ppm, dry basis)
Selenium	Liver (0.25 ppm, dry basis), serum (0.03 µg/mL), hair (0.25 ppm dry basis)
Zinc	Serum (0.06–0.8 µg/mL)
<i>Toxic</i>	
Copper	Liver (700 ppm, dry basis)
Fluorine	Bone (4500–5500 ppm, dry basis)
Manganese	Hair (70 ppm, dry basis)
Molybdenum	Liver (4 ppm, dry basis)
Selenium	Liver (5–15 ppm, dry basis), hair (10 ppm, dry basis)

^a References for critical levels are found in reviews [5, 17].

^b Non-mineral assays for the following elements are sensitive diagnostic techniques: cobalt (vitamin B₁₂), iodine (free thyroxine), copper (ceruloplasmin), and selenium (glutathione peroxidase); in vitro techniques using radioisotopes (e.g. ⁷⁵Se and ⁶⁵Zn) are also useful tools.

Ca and P in Brazil and Se in Venezuela [2]. Egan [12] reported that sampling and analysis of stream bed sediments have revealed areas of hitherto unsuspected Mo-induced Cu deficiency in sheep and cattle, Mn deficiency in cattle and Co deficiency in sheep. Cobalt and/or Cu deficiencies of grazing ruminants have also been established in specific Brazilian regions as a result of low liver concentrations of these elements [29]. Deficiencies of P were likewise established in Venezuela and Panama [30] on the basis of low serum P levels.

In Uganda, the mineral status of dairy farms was established from analyses of local pastures [31]. Boyazoglu [32] analysed liver samples for trace elements and

identified mineral deficiencies in ten regions of South Africa and adjoining territories. Liver Cu concentrations from the Sudan [33] and South Africa [34] have been used to detect areas of Cu deficiency.

Since 1974, the University of Florida, with the support of the Agency for International Development, has been engaged in co-operative mineral research with institutions in Latin America, Africa and South-East Asia. The purpose of this

TABLE III. LATIN AMERICAN, AFRICAN
AND ASIAN STUDIES ON EFFECTS OF
MINERAL SUPPLEMENTATION ON
INCREASED CALVING PERCENTAGES

Country	Control ^a	Control + mineral supplement
Bolivia	67.5	80.8 ^b
Bolivia	73.8	86.4 ^c
Brazil	55.0	77.0 ^d
Brazil	49.0	72.0 ^b
Brazil	25.6	47.3 ^b
Colombia	50.0	84.0 ^d
Panama	62.2	68.8 ^e
Panama	42.0	80.0 ^b
Peru	25.0	75.0 ^f
Philippines	57.0	79.0 ^d
Philippines	76.0	80—82 ^d
South Africa	51.0	80.0 ^b
Thailand	49.0	67.0 ^b
Uruguay	48.0	64.0 ^b
Uruguay	86.9	96.4 ^b
Uruguay	50.0	75.0 ^d
Uruguay	27.0	70.0 ^c

Complete references cited by McDowell [2].

^a Control animals received only common salt (NaCl).

^b Bone meal.

^c Bone phosphate.

^d Complete mineral mixture.

^e Dicalcium phosphate + triple superphosphate.

^f Dicalcium phosphate + copper sulphate.

TABLE IV. FOUR-YEAR COLOMBIAN STUDY EVALUATING SUPPLEMENTAL MINERALS^a

Item	Salt only	Complete minerals ^b
Abortions (%)	9.3	0.75
Death losses, birth weaning (%)	22.6	10.5
Calves weaned (%)	38.4	60.4
Weaning weight (9 months) (kg)	117	147
Gain of growing cattle (572 days) (kg)	86	141
Average daily gain (g)	150	247
Kilogram per year per cow ^c	44.8	88.7

^a Miles and McDowell [35].

^b Evaluation of the complete mineral supplement indicated adequate concentrations of most minerals but suboptimum levels of Zn and Cu, with no added Se and S.

^c Weaned calf per cent multiplied by weaning weight.

research has been to locate mineral deficiencies or excesses for grazing livestock by use of a systematic mapping technique that analyses plant and animal tissues and by observing the biological response from mineral supplements. A systematic mapping technique using forage and animal tissue analyses has been employed in large areas of Colombia, Costa Rica and Venezuela [17].

8. RESPONSE TO SUPPLEMENTATION

Animals that show deficiency signs and that improve upon supplementation when unsupplemented animals do not provide confirmation of other diagnostic procedures. Supplementation studies, however, are costly in time and resources if conducted with adequate control and assessment. Since each investigation of a suspected subclinical or marginal deficiency is experimental in nature, control groups of animals must be run in common with treated groups but denied access to the supplement. Where separate uniform paddocks are unavailable for experimental treatments, injections or methods of drenching can be used.

Many reports from tropical regions of the world dating back to the early part of the century have revealed the beneficial effects of P supplementation on overall performance. Increased reproductive performance due to mineral supplementation is illustrated in Table III for 17 locations in Latin America, Africa and Asia [2].

Averaging the 17 reports together resulted in a mean calving percentage of 52.6% for animals receiving salt only versus 75.5% for those receiving additional supplemental minerals. Reports of improved weight gains by mineral supplemented cattle have been summarized for various world regions. The importance of mineral supplementation on overall production of cattle in the Llanos regions of Colombia is presented in Table IV [35]. Supplemental minerals dramatically increased all parameters of production. Multiplication of the weaning percentage by the weaning weight gave 88.7 kg of calf produced per cow with complete minerals compared with 44.8 kg with salt alone.

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URINARY TRACT OBSTRUCTION IN CALVES INDUCED BY DIETARY MAGNESIUM

Incidence and pathogenesis

F.A. KALLFELZ, C. CROSETTI*, I. TUKENMEZ**

Department of Physiology,
New York State College of Veterinary Medicine,
Cornell University,
Ithaca, New York,
United States of America

Abstract

URINARY TRACT OBSTRUCTION IN CALVES INDUCED BY DIETARY MAGNESIUM: INCIDENCE AND PATHOGENESIS.

Twenty-three Holstein calves were divided into five groups and fed a basal ration to which Mg was added as follows: Group 1 (control): 0.2%; Group 2: 0.5%; Group 3: 0.8%; Group 4: 1.1%; Group 5: 1.4%. For 9 weeks, the calves were weighed weekly and blood samples were taken for Ca, Po_4 , Mg, BUN and creatinine assays. Calves developing urinary obstruction were killed and a necropsy performed. Recovered uroliths were analysed for chemical composition. In a second experiment in rats a dose of 0.1 μCi ^{47}Ca was given via a saphenous vein catheter. Principals were then given intravenous Mg (3 mg/mL) as the chloride in saline at 0.026 mL/min. Controls were infused with saline. Animals were killed at varying times from 10 to 160 min; blood samples were collected at these times from a jugular catheter. Blood, urine, femur, liver and skeletal muscle were analysed for stable and radioactive Ca and for Mg. Increased dietary Mg caused increases in BUN and serum creatinine. At 9 weeks Group 5 calves had BUN and creatinine levels of 19 and 1.8 mg/dL, respectively, compared with 5 and 1.1, respectively, in Group 1. Increased Mg decreased blood Ca and increased blood Mg and PO_4 levels. Growth was inversely correlated to dietary Mg. Five calves in Groups 3 to 5 developed obstructive uroliths composed of calcium apatite. One calf in Group 2 had cystic crystals. In rats, infusion of Mg caused a rapid decrease in blood Ca specific activity. Bone, liver and muscle ^{47}Ca reflected the blood level, while urinary ^{47}Ca was dramatically increased by Mg. After 10 min, urinary ^{47}Ca was 20%/dL in Mg-infused rats but only 3%/dL in controls. This increased to 160%/dL compared with 6%/dL at 65 min. The total urinary Ca was increased by Mg infusion, reaching 1.32 mg compared with 0.05 mg in controls at 2 hours. Dietary magnesium levels above 0.2% caused urinary tract disease in calves; levels of 0.8% and higher caused obstruction. Increased blood Mg dramatically increased urinary Ca, which may explain the formation of apatite uroliths in calves fed high Mg diets.

* Present address: Molinos, Concepción, Buenos Aires, Argentina.

** Present address: Lalahan Nuclear Research Institute of Animal Health, Ankara, Turkey.

1. INTRODUCTION

Urinary tract obstruction is a common cause of illness and death in feedlot cattle [1]. Death losses have been estimated to be as high as 3% in weaned steer populations in the United States of America [2]. Phosphate calculi have been the most common type reported in cases of obstruction of feedlot cattle and a high phosphorus intake has been implicated as being of etiological significance in both feedlot calves and lambs [3, 4]. Also, most of the research related to this disease has been concentrated on the level of phosphorus in rations for these animals and on methods of prevention using alterations of dietary mineral levels [5, 6]. Little attention has been focused on the possible role of Mg in urolithiasis in calves, even though high levels of dietary Mg have been shown to cause an increased incidence of urolithiasis in cats [7, 8].

Only a few reports of the effects of elevated dietary Mg in calves are available. In one study bull calves 3 to 6 months of age were fed a ration containing 4% Mg for 14 days [9]. Decreased feed consumption and diarrhoea were noted. In the same study, no abnormalities were noted in older calves fed 1% Mg for 6 weeks. In another study bull calves 4 months of age were fed rations containing 0.7 or 1.15% Mg for 28 days [10]. The 0.7% level appeared to be safe, while a level of 1.15% caused decreased food intake and growth rates.

In a recently completed study we evaluated the effects of high dietary Mg (1.4%), Mg and P (1.4, 1.6%) or Mg and Ca (1.4, 2.2%) on the incidence of urolithiasis in growing bull calves [11]. Within two weeks of initiation of feeding the experimental diets the BUN and plasma creatinine levels of calves fed high Mg or Mg and P became significantly elevated. The serum Mg level became significantly elevated as well in these two groups, while in the group fed high Mg and Ca the serum Mg level became elevated with respect to the controls but did not reach levels comparable to those occurring in calves fed high Mg or Mg and P.

In association with elevated serum Mg, there was a significant decrease in serum Ca, a phenomenon of unknown cause which has been previously observed in man [12, 13].

During this study, five of twenty calves developed urolithiasis and were killed. Analysis of the recovered calculi showed them to be composed of calcium apatite.

The experiments reported here were undertaken to determine the maximum safe level of dietary Mg for growing calves and to evaluate the pathophysiology of reduced serum Ca and urolithiasis resulting from elevated dietary and serum Mg levels.

2. MATERIALS AND METHODS

2.1. Calf study

A group of 23 newborn male Holstein calves were raised to weaning using a standard milk replacer diet. At weaning the animals were randomly assigned to a con-

trol or to one of four treatment groups. They were fed a growing calf ration that met all nutrient requirements for growing calves with the exception of the Mg level [14]. The control group (Group 1) was fed Mg at a level of 0.2% on a dry matter basis, while the treatment groups, Groups 2 to 5, were fed rations containing magnesium levels of 0.5, 0.8, 1.1 and 1.4% on a dry matter basis, respectively. Groups 1 and 2 consisted of four calves each; Groups 3 to 5 had five calves each. The calves were fed the respective diets *ad libitum* for 9 weeks after weaning. Feed intake was measured daily, and water was made available free choice. The calves were weighed and blood samples were taken weekly by jugular vein puncture. The blood samples were centrifuged and serum was collected for the evaluation of Ca and Mg by atomic absorption spectrophotometry, phosphorus using an automated procedure [15] and BUN and plasma creatinine using colorimetric procedures [16, 17].

Calves developing clinical signs of urinary tract obstruction such as anorexia, depression and anuria were killed and a complete necropsy examination was performed. Additional calves were killed at the end of the experiment so that necropsy reports were available on at least two calves from each group. Uroliths recovered during necropsy procedures were submitted for optical and X-ray crystallographic analysis of chemical composition.

Group differences in feed consumption, weight gain and blood chemical parameters were evaluated by analysis of variance for the effects of diet and time.

2.2. Rat experiment

To investigate the cause of the depressive effect of elevated serum Mg on the serum Ca level, a second experiment was performed in rats. Male rats weighing 300 to 400 g were used. General anaesthesia was initiated and maintained by intraperitoneal injections of sodium pentobarbital at an initial dose of 5 mg/100 g of body weight. Intravenous catheters were then placed in the saphenous vein for infusion of Mg-containing or control solutions and in the jugular vein for blood collection.

Initially a bolus dose of 0.1 μCi ^{47}Ca was given by intravenous injection to all animals.¹ Thereafter, intravenous infusion of Mg was begun in the principals using a concentration of 3 mg/mL Mg as the chloride in physiological saline solution at a rate of 0.026 mL/min. Control rats were infused at the same rate with physiological saline solution only.

Groups of four to six animals in each of the principal and control groups were killed at 10, 25, 45, 65, 85, 105, 125 or 160 minutes. Blood samples (0.5 mL) were collected at each of these time periods in all groups through the jugular catheter. The blood samples were centrifuged and the serum collected for subsequent analysis. At termination, total urine produced as well as skeletal muscle, liver and bone were collected. Blood, urine and tissues were analysed for stable Ca and Mg content using

¹ 1 Ci = 3.70×10^{10} Bq.

TABLE I. NUTRIENT LEVELS IN EXPERIMENTAL CALF RATIONS (DRY MATTER BASIS)

Ingredient	Calculated Mg content				
	0.2%	0.5%	0.8%	1.1%	1.4%
Analysed nutrient content					
Protein (%)	17.8	18.8	16.2	17.6	16.7
ADF (%)	12.6	12.4	13.2	12.9	10.4
ENE (mcal/kg)	1.74	1.72	1.73	1.73	1.78
Ca (%)	0.60	0.74	0.54	0.65	0.54
P (%)	0.61	0.67	0.55	0.60	0.57
Mg (%)	0.19	0.58	0.83	1.18	1.42
K (%)	1.11	1.16	1.09	1.07	1.01
FE (ppm)	258	329	281	331	336

TABLE II. BUN AND CREATININE LEVELS OF CALVES FED ELEVATED LEVELS OF DIETARY Mg

Group	Dietary Mg (%)	BUN (mg/dL)			Creatinine (mg/dL)	
		Week			Week	
		1	5	9	5	9
1	0.2	6.7 ± 1.7 ^a	5.7 ± 1.6	6.2 ± 1.5	0.95 ± 0.1	1.15 ± 0.1
2	0.5	7.5 ± 0.5	7.1 ± 0.9	7.5 ± 1.2	0.95 ± 0.1	1.15 ± 0.1
3	0.8	8.5 ± 1.6	8.1 ± 0.9	13.2 ± 6.1	0.92 ± 0.1	1.40 ± 0.1
4	1.1	9.6 ± 1.8	11.3 ± 7.0	16.4 ± 3.7	1.32 ± 0.5	1.53 ± 0.1
5	1.4	9.7 ± 1.4	16.1 ± 4.4	18.1 ± 0.9	1.44 ± 0.1	1.66 ± 0.1

^a Mean ± SD.

TABLE III. SERUM Ca, PO₄ AND Mg LEVELS OF CALVES FED ELEVATED LEVELS OF DIETARY Mg

Dietary Mg Group	(%)	Ca (mg/dL)		PO ₄ (mg/dL)		Mg (mg/dL)	
		Week		Week		Week	
		8	9	8	9	8	9
1	0.2	9.9 ± 0.3 ^a	10.1 ± 0.2	7.7 ± 1.2	7.8 ± 0.5	1.8 ± 0.2	2.0 ± 0.1
2	0.5	9.8 ± 0.1	9.9 ± 0.3	8.7 ± 1.0	8.4 ± 0.6	2.5 ± 0.4	2.4 ± 0.2
3	0.8	9.2 ± 0.5	9.5 ± 0.4	8.3 ± 1.4	8.9 ± 0.7	3.1 ± 0.1	3.0 ± 0.4
4	1.1	8.9 ± 0.6	9.4 ± 0.3	9.9 ± 1.5	9.3 ± 0.2	4.2 ± 2.0	3.5 ± 0.6
5	1.4	8.6 ± 0.6	8.1 ± 0.5	9.3 ± 1.1	10.2 ± 2.2	5.6 ± 0.7	6.2 ± 0.6

^a Mean ± SD.

TABLE IV. FEED INTAKE AND WEIGHT GAIN DURING WEEK 9 IN CALVES FED ELEVATED LEVELS OF DIETARY Mg

Group	Dietary Mg (%)	Weight (kg)	Feed intake (kg/d)	Weight gain (kg/d)
1	0.2	127.5 $\pm$ 1.8 ^a	5.3	1.4
2	0.5	123.8 $\pm$ 3.6	4.7	1.0
3	0.8	121.8 $\pm$ 7.7	4.0	1.2
4	1.1	119.6 $\pm$ 2.6	4.2	0.9
5	1.4	106.9 $\pm$ 3.0	3.2	0.8

^a Mean $\pm$ SD.TABLE V. PATHOLOGICAL CHANGES OBSERVED IN CALVES FED ELEVATED LEVELS OF DIETARY Mg^a

Group	Dietary Mg (%)	No significant findings	Bladder sand	Ureter stones	Bladder stones	Urethra rupture	Bladder rupture
1	0.2	X X					
2	0.5		X				
3	0.8			X X	X	X	
4	1.1			X	X X	X	X
5	1.4			X	X X		X

^a Results from post-mortem examinations on two calves from each group.

atomic absorption spectrophotometry and for ⁴⁷Ca content using a well-type solid scintillation detector. Results were plotted as a function of time and analysed by regression analysis.

3. RESULTS

The actual composition of the rations fed to the calves is shown in Table I. Magnesium levels in the diets as analysed closely approximated the calculated values.

Within one week of the initiation of feeding of the experimental rations increases in BUN were noted, these increases being directly correlated with the level

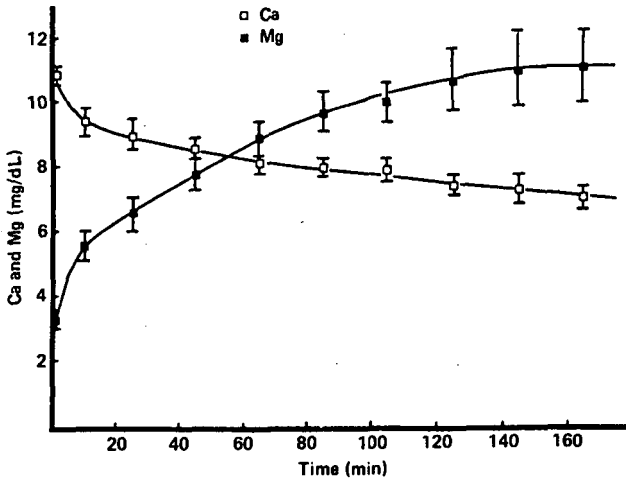


FIG. 1. Effect of intravenous Mg infusion on serum Ca and Mg levels in rats (linear plot).

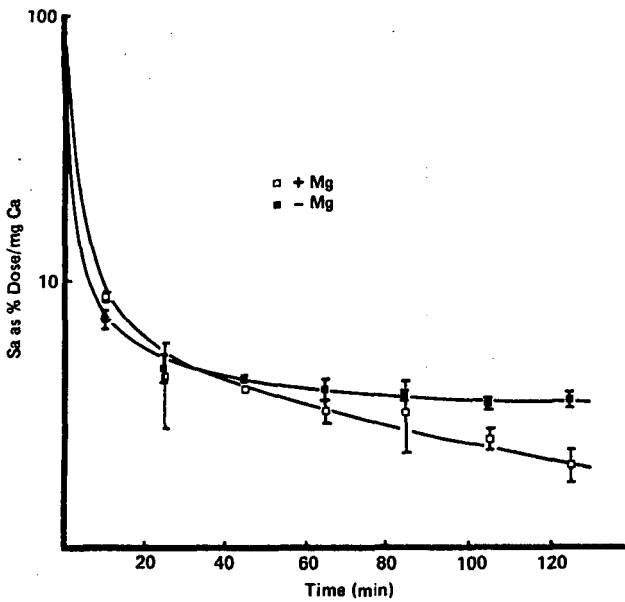


FIG. 2. Effect of intravenous Mg infusion on serum ^{47}Ca -specific activity in rats (semi-logarithmic plot).

TABLE VI. CALCIUM-47 CONTENT OF SERUM AND TISSUES IN Mg-INFUSED AND CONTROL RATS

Time (min)	Control				Mg infused			
	Serum (% dose/dL)	Muscle	Liver (% dose/g)	Bone	Serum (% dose/dL)	Muscle	Liver (% dose/g)	Bone
10	67.3 $\pm$ 4.3 ^a	0.13 $\pm$ 0.01	0.27 $\pm$ 0.02	0.78 $\pm$ 0.03	84.3 $\pm$ 4.7	0.10 $\pm$ 0.02	0.19 $\pm$ 0.02	0.90 $\pm$ 0.08
45	39.8 $\pm$ 0.8	0.07 $\pm$ 0.01	0.15 $\pm$ 0.02	1.30 $\pm$ 0.15	35.0 $\pm$ 2.2	0.07 $\pm$ 0.01	0.10 $\pm$ 0.01	1.13 $\pm$ 0.14
85	35.2 $\pm$ 0.5	0.07 $\pm$ 0.01	0.07 $\pm$ 0.01	1.36 $\pm$ 1.08	26.9 $\pm$ 7.8	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	1.21 $\pm$ 0.24
125	33.1 $\pm$ 0.9	0.07 $\pm$ 0.01	0.07 $\pm$ 0.01	1.42 $\pm$ 0.10	15.6 $\pm$ 2.3	0.06 $\pm$ 0.01	0.05 $\pm$ 0.01	1.09 $\pm$ 0.13

^a Mean $\pm$ SD.

TABLE VII. URINARY LOSS OF LABELLED AND STABLE Ca IN Mg-INFUSED AND CONTROL RATS

Time (min)	Control		Mg infused	
	Urinary ^{47}Ca (% dose/dL)	Urinary Ca (mg)	Urinary ^{47}Ca (% dose/dL)	Urinary Ca (mg)
10	3.16 ± 0.36^a	0.05 ± 0.01	19.56 ± 3.08	0.03 ± 0.00
25	3.60 ± 2.00	0.05 ± 0.01	111.37 ± 10.93	0.19 ± 0.00
45	4.49 ± 1.37	0.05 ± 0.01	110.65 ± 22.90	0.17 ± 0.01
85	7.53 ± 1.24	0.09 ± 0.00	100.49 ± 42.3	0.53 ± 0.05
125	11.41 ± 1.20	0.05 ± 0.00	75.28 ± 35.0	1.31 ± 0.18

^a Mean $\pm$ SD.

of dietary Mg (Table II). By the end of the 9-week feeding trial, Group 5 calves (fed 1.4% Mg) had BUN and serum creatinine values of 19 and 1.8 mg/dL, respectively, compared with 5 and 1.1 mg/dL, respectively, in the controls. These were highly significant differences. Groups 2 to 4 had corresponding intermediate values (Table II).

Increased dietary Mg resulted in increases in serum Mg and PO_4 levels and decreases in serum Ca values which were evident by two weeks after initiation of feeding the experimental rations, but which were most pronounced during the last two weeks of the experiment (Table III).

Feed intake and weight gain were both adversely affected by dietary Mg level, these effects being most pronounced at the end of the experiment (Table IV).

Post-mortem evaluations revealed lesions relating only to the urinary system. The severity of these lesions was again directly related to the dietary Mg level (Table V). No abnormalities were found in the two calves examined from the control group fed 0.2% Mg. Sand-like crystals were found in the bladder of one of the calves in Group 2 fed 0.5% Mg. A total of five calves developed urinary tract obstruction, one from Group 3 fed 0.8% Mg and two each in Groups 4 and 5 fed 1.1 and 1.4% Mg, respectively. Analysis of the recovered uroliths from calves with urinary tract obstruction showed that they were composed of calcium apatite. Histopathological evaluation of the kidneys revealed microcrystalline obstruction of renal tubules, the severity of which was proportional to the dietary Mg level.

Intravenous infusion of Mg in rats resulted in a rapid increase in serum Mg level and a fall in serum Ca level (Fig. 1). Serum levels of Mg and Ca were 10.5 and 7.3 mg/dL, respectively, after 125 minutes of Mg infusion compared with 3.0 and 10.8 mg/dL, respectively, before initiation of infusion. In addition to the fall in serum

Ca, Mg infusion also resulted in a rapid fall in blood ^{47}Ca -specific activity as compared with animals not infused with Mg (Fig. 2). The ^{47}Ca content of muscle, bone and liver reflected the blood level of labelled calcium in both Mg-infused and control rats (Table VI). However, the urinary elimination of ^{47}Ca was greatly increased by intravenous administration of Mg (Table VII). After 125 minutes, 3.9% of the dose of ^{47}Ca was present in the urine of rats infused with Mg compared with 0.08% of the dose in controls. Total urinary calcium loss was similarly increased by Mg infusion. During 125 minutes a total of 1.32 mg of Ca was lost to the urine in Mg-infused rats compared with only 0.05 mg in controls.

4. DISCUSSION

The results of this study confirm previous reports that elevated dietary Mg is associated with an increased incidence of urolithiasis in male calves [11]. Furthermore, the results of the study show that urinary tract obstruction can occur at levels of dietary Mg as low as 0.8% and potentially at levels in the range of 0.5%.

As noted previously, increases in BUN and serum creatinine occurred within a few weeks of the feeding of elevated dietary Mg levels [11]. In the current study it was further shown that the elevations in BUN and creatinine were correlated to the Mg level in the diet (Table II). These early increases in BUN and serum creatinine levels strongly suggest that kidney damage had been taking place for some time before the formation of uroliths large enough to cause urinary tract obstruction since, in fact, the cases of urinary tract obstruction did not occur until the weeks 5 to 9 of the experiment. This hypothesis was also supported by the finding of microcrystalline obstruction of renal tubules on histopathological examination of the kidneys of calves receiving elevated levels of dietary Mg.

These results also confirm previous reports that increased dietary Mg is associated with decreased feed intake and weight gain [9–11]. Further, the data reported here show that the decrease in weight gain and feed efficiency is inversely correlated to the Mg level in the diet (Table IV).

Previously observed decreases in serum Ca as a result of increased dietary and serum Mg [11, 12] were confirmed by this study. This could be due to decreased intestinal absorption of calcium as a result of elevated dietary Mg [18, 19], or to decreased levels of circulating parathyroid hormone [20, 21] resulting in insufficient bone Ca resorption to maintain serum calcium levels.

A more likely explanation of the decreased serum Ca level is, however, strongly suggested by the results of the second experiment reported here. The calcium kinetic study in rats showed that elevated serum Mg was associated with a more than 20-fold increase in urinary Ca during a 125-minute study (1.32 versus 0.05 mg). In the Mg-infused rats, this is equivalent to a loss of almost 100% of the blood calcium into the urine during the two hours of Mg infusion. Such a significant loss of blood calcium might easily explain a 30% decrease in the blood calcium level. The finding

that muscle, liver and bone levels of ^{47}Ca were correlated with blood radiocalcium levels (Table VI) tends to negate previous suggestions that hypermagnesaemia may result in shifts of Ca into bone or intracellular spaces [22]. The significant urinary calcium loss might also explain the predominance of calcium apatite uroliths in obstructed calves.

Urinary tract disease appeared to be closely correlated with dietary Mg level (Table V). The fact that no abnormalities were noted in the control group indicates that a dietary level of 0.2% Mg is not harmful. Calves fed 0.5% Mg did demonstrate some crystalline concretions on preputial hairs and one calf was found to have sand-like crystals in the bladder on post-mortem examination. Calves in this group also had somewhat elevated BUN levels (Table II). These findings indicate that dietary Mg levels as low as 0.5% can result in urinary tract disease. Dietary levels of 0.8% Mg and higher were associated with obstructive urolithiasis (Table V).

In this study, as was the case in a previous report [11], the uroliths formed in response to elevated levels of dietary Mg were found to be composed of calcium apatite. The results of the study done in rats show that elevated serum Mg causes a significant increase in urinary calcium loss. A similar effect of hypermagnesaemia has been reported in man [12]. This increase in urinary calcium, perhaps occurring as a result of competition by Mg for renal tubular reabsorption of Ca [23], may explain the formation of calcium-containing uroliths in response to elevated levels of dietary Mg.

The results reported here have proved that dietary Mg levels in the range of 0.5% and above can result in urinary tract disease and obstruction in male calves. The urinary tract obstruction occurring in calves fed elevated levels of dietary Mg appears to be due to the formation of calcium-containing uroliths formed in response to enormously increased urinary calcium losses induced by elevated dietary and serum Mg. Dietary Mg levels should therefore be carefully monitored in steer and bull raising enterprises.

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MINERAL REQUIREMENTS OF DAIRY SHEEP*

S. ECONOMIDES

Agricultural Research Institute,
Ministry of Agriculture and Natural Resources,
Nicosia, Cyprus

Abstract

MINERAL REQUIREMENTS OF DAIRY SHEEP.

The mineral requirements of dairy sheep under semi-intensive and intensive management systems are reviewed. Basically, mineral requirements obtained with mutton breeds are supplemented with specific information obtained with dairy sheep. The daily requirements of sheep in major elements have been estimated using the factorial method, first to calculate the net requirements during maintenance, growth, reproduction and lactation, and second to assess the efficiency of absorption for converting net requirements into dietary allowance. The requirements in trace elements were estimated from the results of feeding trials. The choice of values for endogenous losses and particularly the precision with which efficiency of absorption can be predicted are the main sources of error in estimates of dietary allowances, especially when efficiency of absorption is low. The net requirements in major elements are given for maintenance, pregnancy and lactation, together with the recommended coefficients of absorption. Dietary requirements in trace elements are expressed as milligram per kilogram dry matter. The requirements in all nutrients and particularly in Ca and P increase during late pregnancy, especially during lactation. The requirements during lactation mainly reflect milk composition, the level of milk yield and lactation length, but also the feed intake level. The factors related to the confinement, feeding and management of dairy breeds, which differ from those of mutton breeds, may affect their mineral requirements. Changes in husbandry practices resulting in increased production may increase the stress on the animal and therefore alter its metabolic equilibrium. Direct supplementation of the diet of dairy sheep is the cheapest and most convenient method of mineral supplementation. The cost is outweighed by the enhanced performance of sheep. Determination of the mineral composition of feedstuffs available for feeding dairy sheep indicates the type and level of mineral supplementation needed.

1. INTRODUCTION

In southern Europe countries from the Atlantic to the Black Sea and in the Middle East [1] sheep are used as dual purpose animals, i.e. for milk and meat production.

In certain areas milk production is given higher priority than meat production, whereas in others milk and meat are of equal importance. Within the dual purpose system of production there are different management systems [2], such as extensive (free range, migratory), tethering (small size flocks, family size), semi-intensive (pasture grazing with supplementary feeding of crop residues and concentrates in confined areas) and intensive (confined all the year round to about 2 h grazing daily

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on improved pastures, zero grazing and supplementary feeding with increased use of concentrates). In the latter two systems there is an integration of animal and crop production. Moving from extensive to intensive management systems improves the performance of animals and higher inputs (feeds and management) are necessary to obtain higher output.

In dairy sheep systems the lactation length is generally 2 or 3 months longer than in suckling systems, where weaning takes place from 1 to 4 months after birth. Also, in dairy sheep the period of milking is always preceded by a suckling period, and in some areas (Cyprus) ewes are also milked during the suckling period to increase commercial milk yields. Studies carried out on the mineral requirements of suckling ewes could be applied to dairy ewes [3] when suitable adjustments are made for the level of milk yield, the lactation length and the time required during the dry period to replenish loss of nutrients during the previous lactation.

Therefore, in this paper the mineral requirements of mutton breeds of sheep will be reviewed as well as the information obtained for dairy sheep under semi-intensive and particularly intensive management systems.

2. NET REQUIREMENTS

The major minerals required are Ca, P, Na, Cl, Mg, K and S and the trace elements required are I, Fe, Cu, Mo, Co, Mn, Zn and Se [4]. Severe deficiencies or toxicities result in characteristic diseases causing severe production losses, but marginal deficiencies also impair production.

The mineral requirement is that amount of mineral that must be supplied in the diet to meet the needs of a normal healthy sheep when it consumes an otherwise completely adequate diet. The daily requirements in major elements have been estimated using the factorial method, first to calculate the net requirements during maintenance, growth, reproduction and lactation, and second to assess the efficiency of absorption for converting net requirements into dietary allowance. Finally, these allowances were compared with the results of practical feeding trials in which the element was given in two or more different amounts [5, 6]. For trace elements the requirements were estimated largely from the results of practical feeding trials [4]. The choice of values for endogenous losses and particularly the precision with which efficiency of absorption can be predicted are the main sources of error in the estimates of dietary allowances, especially when efficiency of absorption is low.

2.1. Maintenance requirements

Maintenance requirements are defined as the inevitable losses in faeces and urine which occur when the element is absent from the diet. The recommended estimates [6] of endogenous excretion (mg/kg live weight (LWT) per day), and hence of the maintenance requirements, for the major elements are the following: Ca: 16;

P: 14; Mg: 3; K: 1 g/kg dry matter intake for faecal losses plus 37 mg/kg LWT per day for urinary losses; Na: 6 plus 2 g/d; Cl: 8; S: 1.1 to 1.6 g/kg dry matter intake (the degradable nitrogen multiplied by 0.07).

The above values change whenever new information from research work becomes available. For example, the endogenous losses for Ca were reduced from 40 [5] to 16 mg/kg LWT per day [6]. However, more recent results with mutton sheep [7] and dairy sheep [8] suggest that endogenous faecal Ca loss is closely related to dry matter intake and therefore a variable factor based on some measure of level of feeding should be introduced when estimating the Ca requirement. Endogenous faecal Ca loss increased by about 0.64 [7] and 0.78 [8] mg/kg LWT per day for each 1 g/kg LWT per day increase in dry matter intake. With dairy sheep it has been reported [8] that when the dry matter intake of dry, pregnant and lactating sheep was 11.2, 15.5 and 32.9 g/kg LWT per day, respectively, the corresponding endogenous faecal Ca loss was 11.7, 13.3 and 29.3 mg/kg LWT per day, respectively. The pooled regression equation for dry, pregnant and lactating sheep describing endogenous faecal Ca loss (mg/kg LWT per day, $\hat{Y}$) and dry matter intake (g/kg LWT per day, X) was $\hat{Y} = 2.87 + 0.78 X$ ($R^2 = 0.84$).

No information is available on the net requirements for trace elements.

2.2. Pregnancy

The requirements for essential nutrients including trace elements are greatly enhanced after the third month of pregnancy. The transfer rate of Ca from the mother to the foetus was related to the needs of the foetus [9], whereas failure of individual quadruplets to grow as fast as a single lamb [10] was associated with a limitation in the capacity of the placenta to transfer minerals. The same authors suggested that the maximum transfer rate of Ca and P was 2.8 and 1.4 g/d, respectively, irrespective of the number of foetuses. The rate of daily accretion of macro- and microminerals increases during the last six weeks of pregnancy [11]. Daily accretion per kilogram lamb birth weight at 102 and 144 days of pregnancy are as follows:

Stage of gestation (d)	102	144
Ca (mg)	111	237
P (mg)	69	115
Mg (mg)	3.4	6.8
Na (mg)	24	33
K (mg)	22	29
Cu (μ g)	28	59
Zn (μ g)	161	501
Mn (μ g)	13	13
Se (μ g)	0.34	0.43

2.3. Lactation

The amount of minerals in 1 kg of ewe's milk is as follows [5, 6]: Ca: 1.9 g; P: 1.5 g; Mg: 178 mg; Na: 0.5 g; K: 1.25 g; Cu: 0.32 mg; Zn: 7.2 mg; Mn: 0.03 mg; Se: 37 μ g. The contents of other trace elements vary greatly with the dietary intake. For instance, the values of Mo can vary between 10 and 980 μ g/kg and of I from 30 to 1000 μ g/kg.

3. EFFICIENCY OF ABSORPTION

The efficiency of absorption is affected by many factors such as the chemical form of the mineral, dietary levels of other minerals and organic substances, interaction between animals and diets, and inherited differences between individuals of the same breed or between breeds of sheep [12-14]. Assessment of the availability of minerals in feeds or feed supplements presents difficulties. Development of procedures with radioactive isotopes to assess the endogenous faecal mineral has greatly facilitated mineral utilization studies in sheep [15].

Availability of Ca for absorption ranges from 0.6 to 0.7 [6], although efficiency of absorption is inversely related to calcium intake [8, 16].

Phosphorus is absorbed more efficiently in young (0.73) than older animals (0.6); the efficiency of absorption of magnesium ranges from 0.1 to 0.3, but 0.17 is the preferred value; Na: 0.91; Cl: 0.85; S: 0.6; Mo: 0.3 to 0.8 (with high or low S intakes, respectively). Efficiency of absorption of most trace elements is less than 0.1.

4. REQUIREMENTS FOR MAJOR ELEMENTS

4.1. Calcium and phosphorus

There is no change in the Ca transfer to foetuses in late pregnancy (4.3 to 5.4 g/d) and Ca excretion in milk in early lactation (4.3 g/d) for mutton breeds of sheep [17]. However, Ca excretion in milk for dairy breeds [8] is higher than Ca deposited in late pregnancy (Fig.1). Demands for all nutrients and particularly Ca and P increase in late pregnancy and early lactation. Since the lactating mature ruminant is dependent on a continuous intake of Ca from its digestive tract, an increase in feed intake and Ca intake after parturition is essential for the maintenance of an adequate Ca supply for peak lactation [18].

Feeding low Ca diets during pregnancy and then increasing the Ca intake at parturition prevents hypocalcaemia in dairy cows [19] by increasing bone resorption and

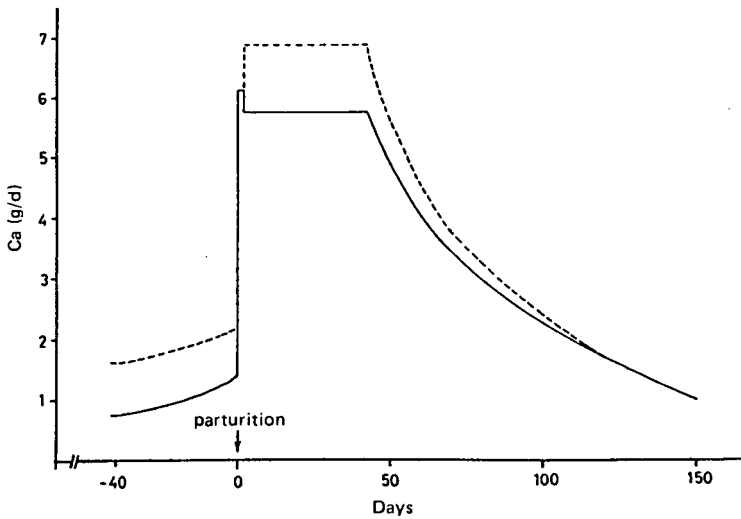


FIG. 1. Calcium transfer to fetuses and calcium excretion in milk. (—), ewes with singles; (---), ewes with twins.

the active absorption of Ca from the intestine. Similarly, ewes that received a Ca-deficient diet in pregnancy absorbed Ca at a greater rate, and with a greater efficiency, and were in a more positive Ca balance in early lactation than ewes that received normal Ca diets [20]. However, with high producing dairy sheep no such differences were found between ewes which received prepartum Ca-deficient or Ca-normal diets [8]. Ca intake was high in early lactation and ewes were in a positive Ca balance (Tables I and II). Although ruminants can tolerate a wide range of Ca:P ratios in their diets when vitamin D, Ca and P intakes are adequate [21], low P in the diet may enhance Ca excretion in faeces and may result in lower efficiencies of Ca absorption. Similar results have been obtained with dairy sheep [8].

Sheep diets based on low quality roughage or diets high in concentrates require Ca supplementation [4]. Intensive feeding of sheep with concentrates necessitates high Ca supplementation, and Ca deficiency can be a problem in animals that are normally housed during the greater part of their lives. In the case of phosphorus, forage legumes and cereal grains and their by-products and oil meals are good sources of P, but grass forage and poor quality roughage are poor sources. Excessive P may promote the development of urinary calculi, particularly in young lambs.

4.2. Magnesium

The requirements in Mg increase from 0.7 g for maintenance to about 2 g daily during late pregnancy; the magnesium requirements per kilogram milk is 1 g. The net endogenous faecal loss of Mg may be reduced in animals receiving a diet deficient

TABLE I. ENDOGENOUS FAECAL Ca LOSS, RATE OF Ca ABSORPTION AND Ca BALANCE (mg/kg LWT per day) OF PREGNANT EWES

	Diets		SE
	Normal Ca	Ca-deficient	
Phosphorus intake (g/d)	4.9	5.2	0.24
Ca intake (g/d)	8.2	3.0	0.09
Total faecal Ca loss	101.7	26.4	1.54
Urinary Ca loss	1.2	0.5	0.35
Endogenous faecal Ca loss	13.3	8.8	1.13
Rate of Ca absorption	36.3	24.3	2.23
Efficiency of Ca absorption (%)	29.1	57.6	2.14
Ca balance	21.8	14.9	1.74

TABLE II. ENDOGENOUS FAECAL Ca LOSS, RATE OF Ca ABSORPTION AND Ca BALANCE (mg/kg LWT per day) OF LACTATING EWES

	Prepartum treatment		SE
	Normal Ca	Ca-deficient	
Phosphorus intake (g/d)	10.3	10.3	0.20
Ca intake (g/d)	27.2	27.4	0.45
Milk yield (kg/d)	1.87	1.88	0.33
Total faecal Co loss	369	409	26.4
Urinary Ca loss	2.0	2.0	1.01
Ca loss in milk	75.2	79.7	10.63
Endogenous faecal Ca loss	30.9	33.2	3.26
Rate of Ca absorption	162	175	13.0
Efficiency of Ca absorption (%)	32.4	31.8	0.68
Ca balance	54	61	11.7

in Mg [22]. Lower endogenous faecal losses for Ca were also reported with Ca-deficient diets [8, 23]. Excessive amounts of magnesium salts in cereal-based diets have been associated with the formation of magnesium containing urinary calculi in lambs.

4.3. Potassium

With the exception of all-concentrate diets, all other diets are rich in potassium. Requirements for sheep are about 3 g K per kilogram dry matter.

4.4. Sodium and chlorine

Sodium and chlorine excreted in milk are a substantial part of the total requirement of lactating animals. Usually 0.5% of the complete diet is adequate. In many areas water may contain enough salt to meet the requirements and supplemental salt need not be offered [4]. Most feeds contain some sodium and chlorine but the main source is sodium chloride. Sheep are able to tolerate dietary intakes of Na in excess of requirement, especially when there is free access to water, but a high dietary concentration of Na may depress feed intake and the efficiency of feed utilization.

4.5. Sulphur

The sulphur need is calculated by multiplying the degradable nitrogen requirement by 0.07, i.e. the N:S ratio approximately equals 14:1 [6]. Sulphur in the diet ranging from 1.1 to 1.6 g per kilogram dry matter is adequate, depending on protein concentration. Sulphur deficiency may arise where the diet is also deficient in protein or contains a significant proportion of non-protein nitrogen. When diets are low in sulphur, or when urea is fed, performance can be improved by feeding a sulphur supplement.

5. REQUIREMENTS FOR TRACE ELEMENTS

The dietary allowances of trace elements can be met with diets containing the following concentrations of trace elements per kilogram dry matter [4, 6]:

Zinc: 30 mg, but diets high in Ca adversely affect Zn utilization. This is important for dairy sheep because their diets are supplemented with high levels of Ca.

Manganese: 20 to 25 mg.

Selenium: 0.1 mg Se and 10 to 15 mg of vitamin E. Selenium and vitamin E are usually studied together because of similar functions. Supplementing diets with Se is not recommended because of the narrow margin between the normal and the toxic level [24].

Iodine: 0.5 mg, increasing to 2.0 mg when the presence of goitrogens is suspected. The concentration in milk is related to dietary iodine intake. Iodine supplementation of the diets is usually needed.

Cobalt: 0.1 mg. Cobalt is an essential component of vitamin B12. The synthesis of B12 by rumen microorganisms is reduced with low Co intakes and milk yield is reduced by a moderate or severe Co deficiency [6]. Requirements in Co are higher when diets low in roughage and high in concentrates are fed [24]. The ingestion of soil improves the vitamin B12 status of sheep.

Molybdenum: The concentration of Mo in milk varies considerably (10 to 980 $\mu\text{g/L}$), depending on the Mo content of the diet. Sulphate limits Mo retention, both by reducing intestinal absorption and by increasing urinary excretion.

Copper: The dietary concentrations of Cu for sheep are 5, 6 to 7.5 and 6 to 9 mg/kg dry matter for maintenance, pregnancy and lactation provided the Mo and S levels are normal. Sheep are sensitive to excess Cu intake, and chronic copper toxicity is usually a problem with intensive sheep rearing. Copper toxicity may arise with roughage diets containing normal levels of copper (8 to 11 mg Cu/kg dry matter) if Mo levels are less than 1 mg/kg dry matter. Concentrate feeds usually contain high levels of copper. Haemolysis and jaundice due to copper toxicity can be precipitated by stress, change in the diet and other factors. It has been reported [24] that the addition of 4 mg Mo and 2 g sulphate/kg diet provides adequate protection for intensively reared lambs.

6. FACTORS AFFECTING THE MINERAL REQUIREMENTS OF DAIRY SHEEP

As stressed in Section 1, studies on the mineral requirements of suckling ewes could be applied to dairy ewes when suitable adjustments are made for the level of milk yield and lactation length. However, other factors related to the confinement, feeding and management of dairy sheep, which differ from those of mutton sheep, may affect their mineral requirements. Intensification of production imposes restrictions on movements until some animals become dependent on a single soil type or a narrower range of soil types which may be incapable, without appropriate treatment, of sustaining the health, fertility and productivity of livestock [24]. Furthermore, intensification of ruminant production with larger numbers of animals confined in small areas and fed large amounts of concentrates results in increasing metabolic disorders [25]. The demand for high animal production results in changes in diet which may result in deficiencies of organic or mineral requirements. Changes in husbandry practices may increase the stress on the animal and therefore alter its metabolic equilibrium [25, 26]. Marginal trace element deficiencies are likely to occur with intensified use of confined management systems and industrially produced feed-stuffs, often of a non-conventional character [27]. Feeds of higher digestibility are fed to dairy sheep. Hence, less feed is needed to meet the energy and protein require-

ments, and consequently mineral intake is also low. Limited grazing reduces soil ingestion and thereby limits mineral intake. In addition, with increasing feeding of concentrates the proportion of roughage in the total diet becomes low, altering the pattern of rumen fermentation and thereby the absorption of minerals.

7. SIGNIFICANCE OF MINERAL SUPPLEMENTATION

It is evident that it is difficult to estimate an exact mineral allowance because many factors are involved in assessing a requirement. For example, a high Ca efficiency of absorption (0.68) was accepted [6], which was obtained with diets low in calcium. However, in dairy sheep dry matter intake is high [28, 29] and mineral intake is also high, and since Ca absorption is inversely related to calcium intake the efficiency of absorption of Ca in lactating sheep is not expected to be higher than 0.30 [8].

Intensification of production imposes restrictions on movements and sheep become dependent on certain soil types only. For grazing animals it is not possible to measure or even estimate mineral intake with an acceptable degree of precision. With stall-fed dairy sheep the actual feed intakes are more or less known. It is unlikely that P or Mg deficiencies will arise in dairy sheep under an intensive system of management because cereal grains or forage legumes comprise a large proportion of their diets. Supplementation to meet the additional energy and protein requirements during lactation will also increase the P and Mg intakes. However, high Ca supplementation is necessary.

Determination of the mineral composition of feedstuffs will indicate the type and level of supplementation needed. The mineral composition of feedstuffs will also help to avoid preparation of supplements with imbalanced composition. Direct supplementation of the diet of dairy sheep is the cheapest and most convenient method of mineral supplementation. With stall-fed dairy sheep minerals are usually incorporated in the concentrate mixture. The contribution to the total cost of the concentrate mixture from the vitamin and mineral supplement is less than 1% with dairy sheep on high concentrate diets. This extra cost is always insignificant compared with the high productivity and health afforded the dairy sheep.

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POSTER SESSION

IAEA-SM-292/1P

**SHORT-TERM CALF REMOVAL TO INCREASE
CONCEPTION RATES IN ANGONI COWS***

J.A.S. CHIPEPA, M.A. OMAR,
B. SAKALA, R.C. CHANGA
Livestock and Pest Research Centre,
National Council for Scientific Research,
Chilanga, Lusaka,
Zambia

1. INTRODUCTION

Long intervals from calving to first postpartum oestrus and consequently opportunity for conception have long been recognized as a major factor reducing the annual calf crop. Research done on the use of short-term calf removal on its own or in combination with hormone treatments has shown potential as a means of shortening the postpartum interval in beef cows [1—3]. A pilot study was initiated to investigate the use of short-term calf removal on its own (without additional treatments) in Angoni cows (Zambian Shorthorn Zebu) to stimulate early postpartum cyclicity.

The results obtained in this study prompted the initiation of a study to closely investigate ovarian activity after short-term calf removal by monitoring the progesterone hormone levels [4].

2. MATERIALS AND METHODS

A total of 80 mature Angoni cows in late stages of gestation were utilized in the study, with calf separation at 20 and 40 days postpartum for either 24 or 48 hours. In the control group the calves were left with the cows for either 24 or 48 hours. Bulls fitted with chin-ball markers ran with the herd throughout the duration of the period of experimentation. The cows and calves were weighed and condition scored 1 day post-calving. Blood samples were collected every other day after calf removal until calving. From the collected blood, serum is separated and frozen at -20°C until assayed for plasma progesterone using radioimmunoassay techniques. The date

(Text cont. on p. 568)

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TABLE I. CONDITION SCORES, WEIGHTS, INTERVALS FROM PROGESTERONE PROFILES IN COWS SEPARATED FROM OR LEFT WITH THEIR CALVES FOR 24 HOURS, 20 DAYS POSTPARTUM (PP)

Control						
Cow No.	Condition score	Weight of cow (kg)	Postpartum interval to first detectable P4 levels (d)	Length of interval from calf removal to first detectable P4 levels (d)	Length of postpartum interval to conception from P4 levels (d)	
1	6	233.5	344	323	345	
4	7	243	127	107	127	
26	7	256	ND	ND	ND	
(Up to 214 days PP)						
Total	20	20	732.5			
Mean	6.7	244.2				
Test						
6	8	272	120	100	120	
10	7	231	103	83	103	
15	7	270	ND	ND	ND	
(Up to 151 days PP)						
18	6.5	234	ND	ND	ND	
(Up to 240 days PP)						
Total	28.5	1007				
Mean	7.1	251.8				

ND = non-detectable P4 levels.

TABLE II. CONDITION SCORES, WEIGHTS, INTERVALS FROM PROGESTERONE PROFILES IN COWS SEPARATED FROM OR LEFT WITH THEIR CALVES FOR 48 HOURS, 20 DAYS POSTPARTUM (PP)

Control					
Cow No.	Condition score	Weight of cow (kg)	Postpartum interval to first detectable P4 levels (d)	Length of interval from calf removal to first detectable P4 levels (d)	Length of postpartum interval to conception from P4 levels (d)
16	7	248	ND	ND (Up to 189 days PP)	ND
19	8	285	ND	ND (Up to 206 days PP)	ND
24	7	245	ND	ND (Up to 164 days PP)	ND
Total	22	778			
Mean	7.3	259.3			
Test					
7	6	200	366	346	OPEN
12	8	273	368	348	368
22	6	219	ND	ND (Up to 200 days PP)	ND
28	7	250	ND	ND (Up to 148 days PP)	ND
Total	27	942			
Mean	6.8	235.5			

ND = non-detectable P4 levels.

TABLE III. CONDITION SCORES, WEIGHTS, INTERVALS FROM PROGESTERONE PROFILES IN COWS SEPARATED FROM OR LEFT WITH THEIR CALVES FOR 24 HOURS, 40 DAYS POSTPARTUM (PP)

Control						
Cow No.	Condition score	Weight of cow (kg)	Postpartum interval to first detectable P4 levels (d)	Length of interval from calf removal to first detectable P4 levels (d)	Length of postpartum interval to conception from P4 levels (d)	
2	8	310	ND	ND (Up to 365 days PP)	ND	
17	8	300	ND	ND (Up to 166 days PP)	ND	
23	7	250	ND	ND (Up to 130 days PP)	ND	
Total	23	23	860			
Mean		7.7	286.7			
Test						
13	6	219	376	336	376	
3	6	231	263	223	263	
9	6	204	284	244	284	
27	6	236	ND	ND (Up to 240 days PP)	ND	
Total	24	890				
Mean	6	222.5				

ND = non-detectable P4 levels.

TABLE IV. CONDITION SCORES, WEIGHTS, INTERVALS FROM PROGESTERONE PROFILES IN COWS SEPARATED FROM OR LEFT WITH THEIR CALVES FOR 48 HOURS, 40 DAYS POSTPARTUM (PP)

Control						
Cow No.	Condition score	Weight of cow (kg)	Postpartum interval to first detectable P4 levels (d)	Length of interval from calf removal to first detectable P4 levels (d)	Length of postpartum interval to conception from P4 levels (d)	
11	7	248	249	209	249	
14	7	250	ND	ND (Up to 194 days PP)	ND	
21	7	254	ND	ND (Up to 186 days PP)	ND	
25	8	270	ND	ND (Up to 224 days PP)	ND	
Total	29	1022				
Mean	7.25	255.5				
Test						
8	7	250	346	306	346	
5	6	200	ND	ND (Up to 242 days PP)	ND	
20	7	260	ND	ND (Up to 226 days PP)	ND	
29	7	244	ND	ND (Up to 140 days PP)	ND	
Total	27	954				
Mean	6.75	238.5				

ND = non-detectable P4 levels.

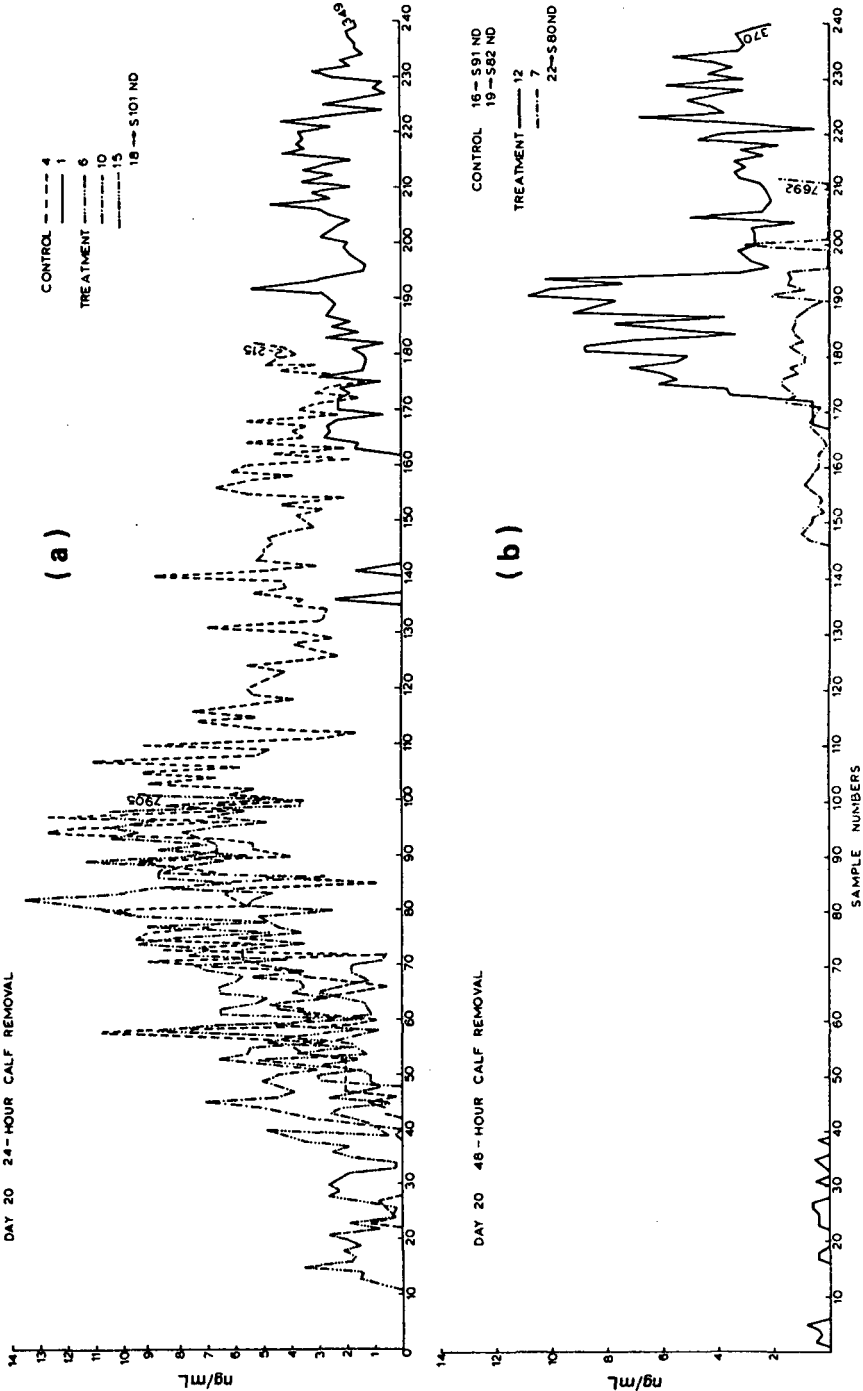


FIG. 1. Calf removal: (a) day 20, 24 hours; (b) day 20, 48 hours.

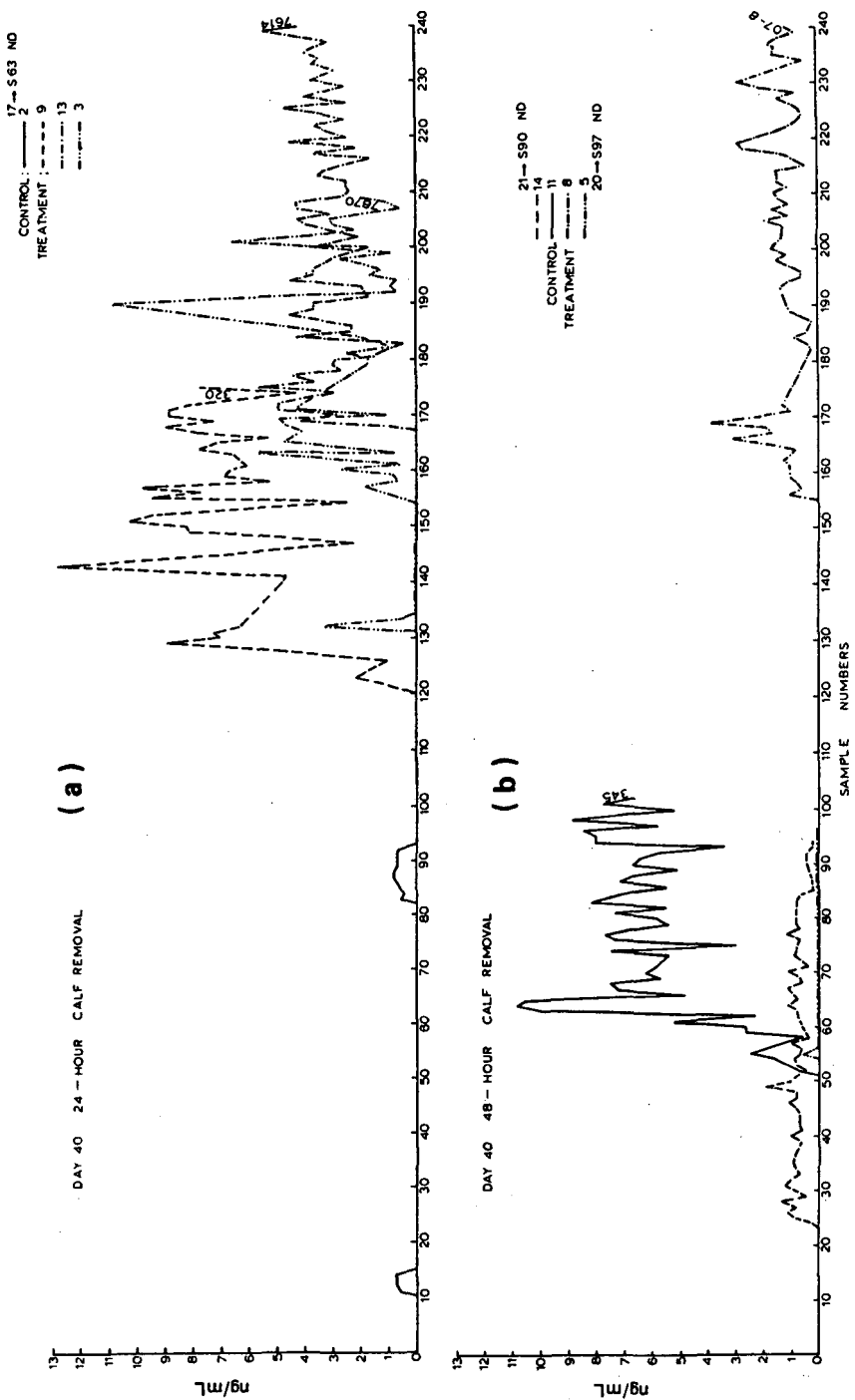


FIG. 2. Calf removal: (a) day 40, 24 hours; (b) day 40, 48 hours.

of calving and approximate date of conception after calf removal are recorded. The following information has been obtained from the progesterone (P4) profiles: interval from parturition to first ovulation; interval from parturition to conception; number of cows conceiving within an 80-day period. The number of cows pregnant by 130 days was obtained by rectal palpations.

3. RADIOIMMUNOASSAYS

Plasma progesterone levels were measured using a milk progesterone assay kit from Farnos Diagnostica (Finland). To correct for the non-specific effects of using unextracted plasma samples from cows, 0.025 mL of steer plasma was added to each of the standards [5]. The rest of the assay was performed as described in the kit.

4. RESULTS AND DISCUSSION

The average per cent binding of the assays was 38% from 42 assays, while the within- and between-assay variations were 5.7 and 15.9, respectively. The sensitivity of the assay was 0.4 ng/mL.

Out of a total of 44 cows that were subjected to calf separation, 29 have had their sera assayed in part or completely for progesterone hormone levels. As the study has not yet been completed the preliminary data have not been subjected to statistical analysis. In the group confined with their calves for 24 hours, 20 days postpartum, 1 out of 3 cows in the control group was able to exhibit detectable P4 values, which showed that it was pregnant. This occurred 127 days postpartum and 107 days after calf removal. In the group separated from their calves for 24 hours, 20 days postpartum, 2 out of 4 cows exhibited detectable P4 values at 120 and 103 days postpartum. The length of the interval from calf separation to conception was 100 and 83 days, respectively (Table I and Fig. 1(a)). Of the rest of the animals that were assayed none showed detectable P4 levels earlier than 130 days postpartum (Tables II—IV, Figs 1(b), 2(a) and (b)). There was a range of 130 to 368 days without any detectable levels. Although the effects of short-term calf removal in combination with or without hormone treatments have been fully documented [6—8], in these preliminary findings they were not readily apparent. However, only 29 of 80 cows in the study have been subjected to partial or complete plasma progesterone analysis, therefore no final conclusions can be deduced from these data.

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IAEA-SM-292/2P

**POSTPARTUM REPRODUCTIVE STATUS OF DAIRY COWS
IN TWO AREAS OF ICELAND (1983-1984)***

J. ELDON, T. ÓLAFSSON

Institute of Experimental Pathology,
University of Iceland,
Reykjavik, Iceland

This presentation describes the postpartum reproductive performance of dairy cows in two areas of Iceland: in the north (Eyjafjörður) and in the south (Skeid) (Fig. 1). The first postpartum ovulation occurred earlier in cows in the south ($\bar{x} = 38$; $SD = 22.27$; $N = 100$) than in the north ($\bar{x} = 43$; $SD = 21.26$; $N = 113$). This difference becomes statistically significant ($P < 0.05$) if cows that ovulated later than 99 days postpartum ($n = 5$) are omitted from the calculations ($\bar{x} = 35$; $SD = 17.29$, and $\bar{x} = 42$; $SD = 13.03$ d, respectively).

The average time of first postpartum AI was 72 days in both areas. The conception rate to first AI in these cows was 58% in the north and 53% in the south, and the number of AIs per conception was 1.55 and 1.78, respectively. In the north 29 cows were inseminated at the time of first postpartum ovulation; 18 or 62% of these cows conceived. In the south 20 cows were inseminated at the time of first postpartum ovulation; 9 or 45% of these cows conceived. The time of conception was 92 days postpartum (average number of days) in the north and 100 days postpartum in the south (see Refs [1, 2] for relevant comparisons with these data).

Comparison of the results from individual farms showed an increase in the number of conceptions to first postpartum AI (35 to 72%) and a decrease in the number of services per conception (2.0 to 1.3), with an increased mean number of days from calving to first AI ($\bar{x} = 62$ to 82 d).

On one farm in the south, Arakot, the cows were set loose from their tie stalls twice a day during milking. The first postpartum ovulation on this farm occurred, on average, after 29 days, which is statistically significant from other farms in the south ($\bar{x} = 41$ d; $P < 0.05$). The conception rate to first service in 25 cows was 75% and the number of AIs per conception was 1.36. Claus et al.[3] reported that acycilia was longer in cows housed in stanchion barns compared with loose housing.

Our data showed differences in the time of first postpartum ovulation between primiparous and multiparous cows and we found a correlation between the onset of cyclicity and the calving month.

* Research carried out with the support of the Icelandic Science Foundation and the IAEA under Research Contract No. 3340/RB.

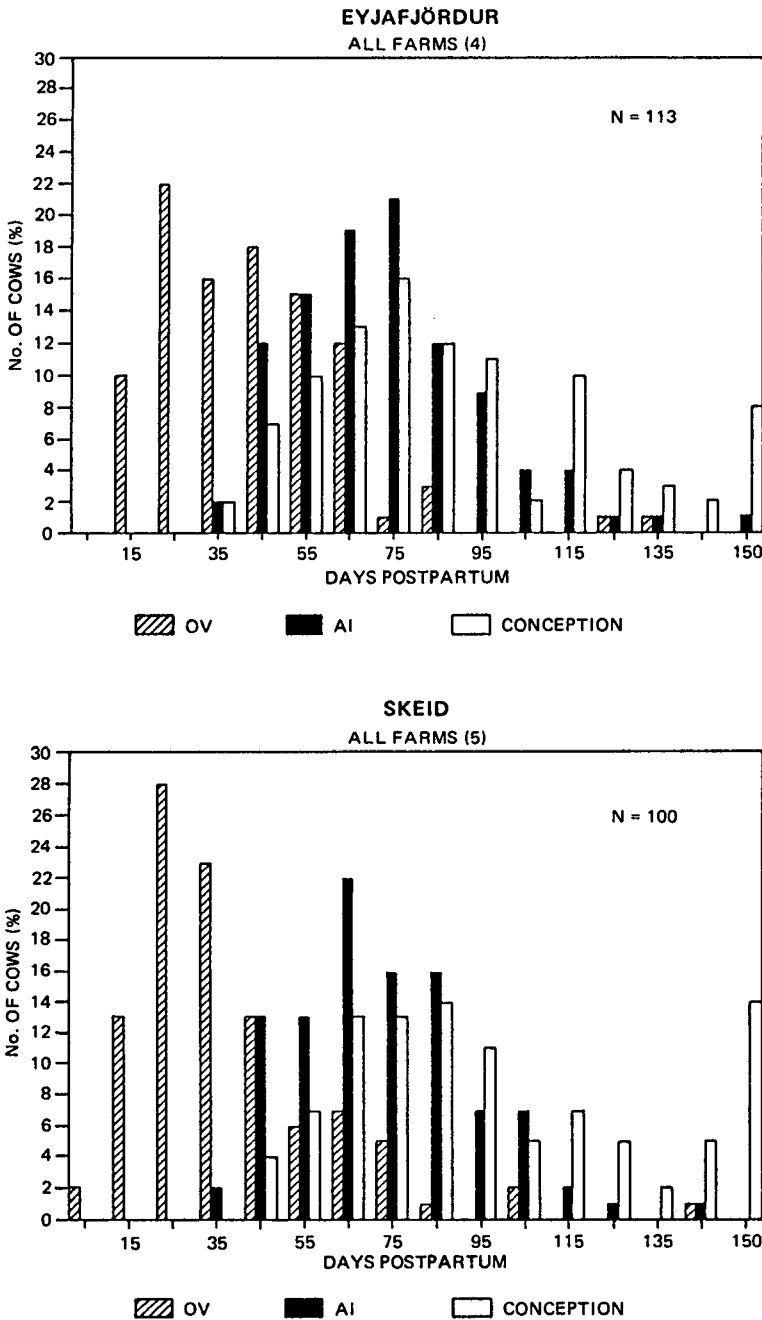


FIG. 1. Time of first postpartum ovulation, first postpartum artificial insemination and conception in dairy cows in the north (Eyjafjörður) and south (Skeid) of Iceland.

The results indicate that:

- (1) First postpartum ovulation occurred earlier in 'normal' cows in the south than in the north and earlier in cows set loose from their tie stalls for part of the day.
- (2) Conception to first AI increased and the number of AIs per conception decreased with the increasing number of days from calving to first AI.
- (3) Different insemination practices (first postpartum AI early/late) can give similar results to time of conception.

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IAEA-SM-292/3P

MILK PROGESTERONE PROFILES DURING THE POSTPARTUM PERIOD IN DAIRY COWS AT THE THAI-DANISH DAIRY FARM*

Y. INTRARAKSA**, K. NITICHAI***,
P. SUKHAMPA**

**Faculty of Veterinary Medicine,
Kasetsart University, Bangkok

***Thai-Danish Dairy Farm,
Saraburi Province
Thailand

The reproductive efficiency of dairy herds is low [1, 2], especially in tropical countries. Reproductive performance is one of the most important factors determining the profitability of dairy herds. Ideally, the calving interval should be, on average, 1 year but this can only be achieved if the rates of conception and detection of oestrus are high and the interval between parturition and first service is less than 90 days. Some animals have a longer postpartum interval and may still be acyclic

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TABLE I. SUMMARY OF BREEDING MONITORED BY MILK PROGESTERONE AT 21 DAYS AND RECTAL PALPATION AT 60 AND 100 DAYS AFTER INSEMINATION

Total cows	Conception at 21 days post AI	Successful pregnancy	Embryonic mortality	No conception	Inactive ovaries
97	30	9	21	60	7
%	30.93	9.28	21.65	61.86	7.22

TABLE II. COMPARISON OF POSTPARTUM ACYCLIC PERIODS OF PRIMIPAROUS AND MULTIPAROUS COWS BY OESTRUS OBSERVATION AND MILK PROGESTERONE ASSAY

	Total No. of cows	Postpartum acyclicity by oestrus observation ($\bar{x} \pm SD$) (d)	Total No. of cows	Postpartum acyclicity by milk progesterone assay ($\bar{x} \pm SD$) (d)
Primiparous	37	60.35 $\pm$ 14.53	26	34.96 $\pm$ 16.36
Multiparous	53	62.43 $\pm$ 14.87	33	34.82 $\pm$ 14.49

during the period when they should be inseminated [3]. While studying reproductive performance in a large herd using only artificial insemination, milk progesterone testing was instituted as an aid to monitoring oestrous cycle activity. Because of the ease with which it is obtained repeatedly without disturbing the animal, it offers the possibility of monitoring certain physiological or pathological conditions of postpartum ovarian activity, confirming oestrus, timing insemination and diagnosing early pregnancy [4].

The objective of our study was to evaluate reproductive efficiency and to determine the postpartum acyclic period under routine practical herd management conditions using milk progesterone profile assays.

The reproductive performance of dairy cows at the Thai-Danish dairy farm was evaluated. Following 97 parturitions, no cow was inseminated until 45 or more days postpartum. Thirty cows had conception confirmed by milk progesterone analysis 21 days after insemination. Pregnancy was confirmed by rectal palpation at 60 and

100 days in 15 and 9 cows, respectively; in a total of 21 cows embryonic mortality occurred. Sixty dairy cows did not conceive after first insemination and returned to heat within 21 days. Only seven dairy cows had inactive ovaries and the levels of milk progesterone were very low, without fluctuation. No signs of oestrous behaviour were observed (see Table I).

Milk samples were collected every 3 days from 66 cross-bred dairy cows, commencing at day 15 of parturition and continuing until each animal was inseminated at first oestrus, which occurred after 45 days postpartum. The milk progesterone profiles of each cow were collated with the records of oestrus and insemination. The average postpartum anoestrous periods of primiparous and multiparous cows were 34.96 ± 16.86 and 34.82 ± 14.39 days, respectively. In 68% of the primiparous cows and 64.71% of the multiparous cows oestrus occurred between days 20 and 40 of the postpartum period (Table II).

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IAEA-SM-292/4P

FERTILITY OF CROSS-BRED DAIRY COWS IN MALAYSIA*

W. SHARIFUDDIN, M.R. JAINUDEEN, K. AZIZUDDIN

Department of Veterinary Clinical Studies,
Faculty of Veterinary Medicine and Animal Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia

1. INTRODUCTION

In an attempt to achieve 20% self-sufficiency in milk production by 1990, the Veterinary Division of the Ministry of Agriculture, Malaysia, embarked on a Small-holder Dairy Farming Programme using imported F₁ Sahiwal × Friesian cows. On

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TABLE I. BODY SCORE OF SAHIWAL $\times$ FRIESIAN COWS IN RELATION TO REPRODUCTIVE PERFORMANCE

Body score	Farm ^a	No. of cows	Calving interval (d)	Calving to conception (d)	Calving to oestrus (d)
1	IF SF	—	—	—	—
2	IF SF	5 4	461.5 $\pm$ 124.0 542.3 $\pm$ 43.1	217.4 $\pm$ 125.5 297.2 $\pm$ 96.5	165.8 $\pm$ 69.1 211.2 $\pm$ 43.5
3	IF SF	7 8	459.8 $\pm$ 85.2 513.5 $\pm$ 139.2	210.1 $\pm$ 105.5 281.4 $\pm$ 124.7	161.2 $\pm$ 72.8 198.4 $\pm$ 82.0
4	IF SF	10 7	453.9 $\pm$ 120.2 493.0 $\pm$ 139.3	190.2 $\pm$ 93.2 273.6 $\pm$ 109.2	158.8 $\pm$ 58.3 187.2 $\pm$ 73.6
5	IF SF	13 3	420.6 $\pm$ 117.4 435.4 $\pm$ 121.5	177.0 $\pm$ 86.2 259.2 $\pm$ 97.5	142.4 $\pm$ 67.3 179.0 $\pm$ 59.7
6	IF SF	7 2	425.2 $\pm$ 96.7 429.5 $\pm$ 84.2	164.9 $\pm$ 59.3 251.8 $\pm$ 101.3	133.8 $\pm$ 45.2 172.7 $\pm$ 67.4
7	SF SF	—	—	—	—
Overall mean	IF SF	42 24	447.3 $\pm$ 103.6 ^b 536.3 $\pm$ 88.6 ^b	179.5 $\pm$ 84.0 ^b 213.4 $\pm$ 83.2 ^b	149.6 $\pm$ 74.3 ^b 186.0 $\pm$ 40.5 ^b

^a IF = institutional farm; SF = smallholder farms.

^b Significant at the 5% level.

a typical smallholder dairy farm 4 to 6 dairy cows and their calves are maintained in sheds located close to the farmer's house. Cows are hand-milked following stimulation of milk let-down by suckling; 6 to 10 kg of milk are produced daily. However, serious infertility problems are reflected in the long calving to conception intervals. The reproductive performance of these cross-breds under smallholder farming conditions was therefore compared with similar animals maintained in an institutional herd.

2. MATERIALS AND METHODS

The reproductive records of 24 F₁ Sahiwal $\times$ Friesian cross-bred cows on six smallholder farms and 42 cows on an institutional farm were analysed for calving

intervals, body condition scores during the first 150 days postpartum and the resumption of ovarian cyclicity. Three milk samples were collected at 8-day intervals from each cow between 60 and 100 days postpartum and were analysed for progesterone in defatted milk by a solid-phase radioimmunoassay technique [1, 2].

3. RESULTS

The calving interval for cows on the institutional farm was significantly shorter ($P < 0.05$) than that on the smallholder farms (Table I). Milk progesterone profiles of postpartum cows revealed that ovarian activity was initiated by day 100 postpartum in 36% of the institutional cows and 12% of the smallholder cows. On smallholder farms, the mean body score for all cows was 3.75 ± 1.14 with fertile cows having a significantly higher ($P < 0.05$) body score than infertile cows.

4. DISCUSSION

Several factors may contribute to the long calving interval in F_1 Sahiwal $\times$ Friesian dairy cows under the smallholder farming system. A significant feature was the lower body score of cows maintained on smallholder farms; these cows also suckled their calves. These factors, either alone or in combination, could account for the failure of cows to resume ovarian cycles during the first 100 days postpartum. Reproductive failure associated with the AI technique can be another contributing factor to the long calving interval [3].

Postpartum anoestrus must be regarded as the major obstacle confronting the Sahiwal $\times$ Friesian cross-bred cows on smallholder farms in Malaysia. Most farmers are reluctant to provide adequate nutrition for their cows and, as a result, ovarian dysfunction is further aggravated. This study revealed that under both management systems an increase in body score corresponded to a shorter calving interval, calving to conception and calving to oestrus. This suggests that greater attention should be focused on reproduction and its interaction with nutrition.

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IAEA-SM-292/5P

POSTPARTUM ANOESTRUS IN BEEF COWS

*Comparison of the plasma progesterone
profiles of two groups of animals
calving at different times of the year**

J. ROBALO SILVA, A. HORTA,
I. RIOS VASQUES, R.M. LEITÃO,
D. CLÁUDIO

Instituto Nacional de Investigação Agrária,
Estação Zootécnica Nacional,
Fonte Boa, Santarém, Portugal

A study was undertaken to determine the seasonal variation in ovarian activity in postpartum beef cows of the Alentejano breed. Observations performed on cows calving in January to March (group A) and August to October (group B) over a 2-year period showed a mean interval from parturition to the onset of cyclic ovarian activity of 53.9 ± 28.8 and 92.1 ± 38.9 days for cows from group A that calved in 1984 and 1985, respectively, and 26 ± 10.6 and 30.3 ± 13.6 days for cows from group B that calved in 1984 and 1985, respectively. Cows that calved in late summer/autumn resumed postpartum ovarian activity significantly sooner than cows that calved in winter/early spring ($P < 0.01$).

Reproductive performance was assessed during the period from mating to pregnancy diagnosis by rectal palpation at 45 to 60 days after service (mating seasons were April to May and November to December for groups A and B, respectively). During a 60-day mating period the percentage of served cows was 91.5 and 87.5 in groups A and B, respectively. The percentage of pregnant cows in group A was 91.8 and 80.0 at 24 and 45 to 60 days, respectively, and in group B 92.3 and 87.7 at 24 and 45 to 60 days, respectively.

It seems that spring may not be a very convenient season for reproduction in this breed of cattle. This is discussed in relation to the usual system of reproductive management followed for this breed in Portugal.

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**DEFATTED MILK PROGESTERONE RADIOIMMUNOASSAY
AS A TOOL TO CONFIRM OESTRUS, EARLY PREGNANCY
AND EARLY EMBRYONIC DEATH IN DAIRY CATTLE***

L. MAHAPUTRA**, S. HARDJOPRANJOTO**,
M.R. INDRA***, M. SOERJOATMODJO**

**Department of Reproduction and Obstetrics,
Faculty of Veterinary Medicine,
Airlangga University

***Faculty of Medicine,
Airlangga University
Surabaya, Indonesia

Confirmation of true oestrus at the time of artificial insemination could be achieved in order to increase the female reproductive efficiency of dairy cattle. Of the factors which influence the female reproductive efficiency, early embryonic mortality and undetected oestrus are the most prominent.

In the United Kingdom and the Federal Republic of Germany 20 to 22% of cows were inseminated during the luteal phase [1, 2]. By analysing progesterone to confirm the time of true oestrus before insemination Oltner and Edqvist [3] found that in Sweden only 4% were inseminated during the luteal phase. In the United States of America the pregnancy rate achieved in cows that were inseminated during the luteal phase was 8% compared with 60 to 62% when inseminated during the follicular phase [4, 5].

To determine whether the reproductive efficiency of dairy cattle in Indonesia is similar to that in temperate climates, we carried out our study to monitor the percentage of dairy cows in Surabaya inseminated during the luteal phase and the occurrence of early pregnancy and early embryonic mortality by analysing the milk progesterone. Early pregnancy and early embryonic death were detected by a progesterone concentration of more than 0.5 ng/mL and were confirmed by rectal palpation on day 60.

The milk samples of 108 cows were collected (stripping) from the healthy quarter of the udder on the day of insemination (day 0), day 22 and day 29 after service; this was followed by rectal palpation on day 60. The defatted milk samples were analysed with solid phase radioimmunoassay, with ^{125}I -P₄ as a tracer [6, 7].

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TABLE I. ACCURACY OF OESTRUS DETECTION AND PREGNANCY RATE AFTER ARTIFICIAL INSEMINATION

Parameter	Cows inseminated		Cows pregnant		Cows non-pregnant		Early embryonic mortality			
							(23—29 d)		(30—60 d)	
	No. of cows	%	No. of cows	%	No. of cows	%	No. of cows	%	No. of cows	%
Follicular phase	94	87	60	55.5	18	16.6	9	8.3	7	6.5
Luteal phase	14	13	4	3.7	7	6.5	3	2.8	0	0
Total	108	100	64	59.2	25	23.2	12	11.1	7	6.5

TABLE II. ACCURACY OF PREGNANCY DIAGNOSIS BASED ON PROGESTERONE CONCENTRATION CONFIRMED BY RECTAL PALPATION ON DAY 60 POST-AI

Parameter	Progesterone concentration (> 0.5 ng/mL)	
	Day 22 post-AI	Day 29 post-AI
Pregnant (%)	82.4 (n = 83)	93.5 (n = 71)
Non-pregnant (%)	100 (n = 25)	100 (n = 37)

The results show that only 13% of the cows were inseminated during the luteal phase, with a pregnancy rate of 3.7% compared with 55.5% when insemination was carried out during the follicular phase (Table I). The accuracy of early pregnancy and non-pregnant diagnosis on day 22 post-insemination was 82.4 and 100%, respectively; 93.5 and 100% were found on day 29 (Table II).

The conclusions reached are as follows:

- (1) Although the farmers in Surabaya have observed their cows for many generations, visual detection of oestrus has proved to be insufficiently accurate.
- (2) Confirmation of oestrus by analysing the progesterone concentration before insemination could result in better oestrus detection.
- (3) The further the cow is into the gestation period, the lower the embryonic mortality.

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IAEA-SM-292/7P

**STUDIES ON REPRODUCTIVE EFFICIENCY
OF NGANDA CATTLE UNDER
SMALL-SCALE MANAGEMENT CONDITIONS***

L. MIYINGO-KEZIMBIRA

Faculty of Veterinary Medicine,
Makerere University,
Kampala, Uganda

1. INTRODUCTION

Most of the indigenous breeds of cattle in the tropics and some in the subtropics have been categorized as animals of poor fertility, i.e. they have late sexual maturity, a high percentage of acyclicity, poor conception rates, long calving intervals and poor weaning rates. Thus, there is a need to study each breed in its prevailing environment in order to identify the limiting factors and to develop a practical scheme of improvement.

The only documented studies on the reproductive functions of the indigenous cattle of Uganda are those of El Wishy [1] and Miyingo [2]. In abattoir studies these authors identified the abnormalities that could cause infertility; however, clinical field investigations and radioimmunoassays were not carried out. Therefore, the present study was designed to investigate, clinically and using RIA, the reproductive efficiency of the Nganda cattle of Uganda.

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TABLE I. RESULTS OF RECTAL PALPATION FINDINGS IN THE 60 3-YEAR OLD HEIFERS

Cycle status	Active heifers	Pregnant heifers	Inactive heifers	Total
No. of heifers	22	31	7	60
Age of group (%)	36.7	51.7	11.7	100

TABLE II. PROGESTERONE LEVELS IN ACTIVE HEIFERS (nmol/L)

Follicular phase				Luteal phase			
Heifer No.	Week 1 sample	Week 4 sample	Week 5 sample	Heifer No.	Week 1 sample	Week 4 sample	Week 5 sample
V 1	1.58	1.17	14.24	V 9	14.24	14.09	10.23
V 2	1.98	2.25	12.28	V51	17.06	15.61	10.64
V 3	9.06	9.56	14.59	V55	6.33	17.23	5.86
V28	7.82	6.06	12.28	V39	15.12	15.92	19.48
V40	2.00	4.01	11.09	V13	16.19	19.18	4.57
V50	2.28	2.39	13.27	V83	15.70	19.36	4.31
V35	4.57	5.76	14.29	V34	18.77	19.19	2.34
V49	1.18	1.08	14.70	V72	11.63	12.27	21.59
V50	8.66	5.49	13.93	V57	21.01	21.59	4.75
V99	4.65	5.55	17.48	V20	1.66	1.05	9.98
V53	6.85	8.66	16.54				
V23	1.62	1.13	11.18				

2. MATERIALS AND METHODS

2.1. Animals

The main animals used in this study were Nganda cattle which are small Short-horn Zebus with a hump and are found mainly in central and southern Uganda.

Sixty heifers about 3 years old were used in the study. Age was determined by eruption and wear of the incisor teeth, as described by Williamson and Payne [3]. The reproductive status of each heifer was obtained by rectal palpation and body score was done following the method developed by Mulvany [4].

2.2. Progesterone assay

For RIA, blood was collected by jugular puncture into heparinized tubes. Plasma was collected and stored at -20°C until assaying was carried out by the Department of Medical Physiology, University of Nairobi, Chiriromo, following the methodology provided by WHO Progesterone Assay Protocol No. 6.

3. RESULTS

According to rectal palpation, the heifers were categorized into three groups, namely active, inactive and pregnant heifers (see Table I). Classification into luteal or follicular phases was first done according to rectal palpation findings and then compared with the progesterone levels (see Table II).

4. CONCLUSIONS

Both the rectal palpation findings and the progesterone assay results show that nearly 90% of the Nganda cattle of Uganda and/or its crosses with other local types will have started cycling by 3 years of age; in fact, about 50% have started cycling by 2.5 years of age. It is concluded that the low parasitic load, the good climatic conditions and supplementation, although often incidental, contribute to early attainment of sexual maturity in Nganda cattle. Since 18 out of the 22 active heifers conceived, fertility is good in cycling females. Pregnancy loss was low, as only one heifer aborted. Neonatal survival will require more research work before any conclusions can be drawn.

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IAEA-SM-292/9P

**USE OF RADIOIMMUNOASSAY TO MONITOR
THE REPRODUCTIVE STATUS OF
CHEJU NATIVE CATTLE AND THE
EFFECT OF SUPPLEMENTARY FEEDING
ON REPRODUCTION**

*Body weight changes, breeding performances and
progesterone levels from weaning to first calving**

D.C. CHOUNG, J.K. KIM, D.C. KIM

Applied Radioisotope Research Institute,
Cheju National University,
Cheju City, Cheju Do,
Republic of Korea

Cheju Island is situated in the Korean Strait, south of the Korean mainland. The island is dominated by Mount Halla, which rises to 1950 m, and has a subtropical climate. It has 110 000 ha of undeveloped grassland, which comprises 61% of the total land mass. This area of grassland provides tremendous potential for the grazing of cattle and horses.

During the 1970s there were more than 70 000 head of cattle on the island, but by 1983 this had been reduced to 45 000, the main reason being the poor breeding performance of the native cattle.

Kim and Kim [1] reported that Cheju native cattle were, on average, 27.9 months old at first oestrus and that the average age at first calving was 38.0 months (see Table I). When compared with mainland cattle and exotic beef breeds, Cheju native cattle were, on average, 10 months later in reaching maturity. On average, 174 days elapsed from calving to first oestrus when bi-annual calving cows were included. Kim et al. [2] stated that the average rate of bi-annual calvers was 15.4%. This poor breeding performance is undoubtedly the main limiting factor to increasing the cattle numbers and is reflected in a loss of farmers' income. Poor breeding performance has always been thought to be due to undernutrition and lack of essential mineral supplementation. Although this conjecture is probably correct, it is nevertheless necessary to clarify the true reasons for poor fertility. Development of a satisfactory livestock industry depends on solving these problems.

Studies were conducted to investigate the relationship between the hormone and nutritional levels for improving the breeding performance of Cheju native cattle (Fig. 1). In June 1984 a trial was initiated using eight Cheju calves (after weaning)

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TABLE I. REPRODUCTIVE STATUS AND BODY WEIGHT ON SUPPLEMENTARY FEEDING LEVELS

Nutritional levels	Cow No.	First oestrus		First pregnancy		First calving	
		Age (month)	Body weight (kg)	Age (month)	Body weight (kg)	Age (month)	Body weight (kg)
NRC 100%	1	13.3	200	13.9	207	23.3	360
	2	13.0	234	15.4	234	25.0	370
	3	12.3	291	26.0	303	36.1	555
	4	19.8	335	21.3	253	30.5	459
	Mean	14.6	265	19.1	274.1	28.9	436.0
NRC 70%	1	20.6	197	25.3	285	34.9	379
	2	26.0	257	36.0	336	45.1	360
	3	25.0	297	27.1	318	44.6	425
	4	20.2	250	21.2	256	30.8	340
	Mean	23.0	250.1	27.4	298.8	38.9 $\pm$ 7.16	376 $\pm$ 36.3

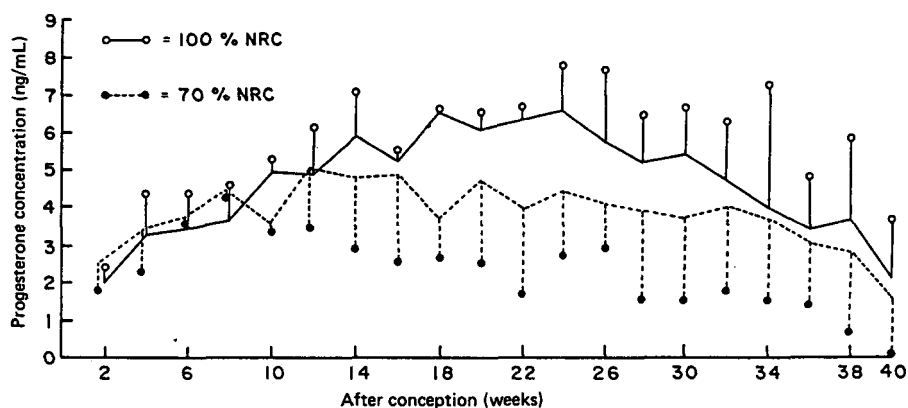


FIG. 1. Changes in serum progesterone concentration during pregnancy in cows receiving different feeding levels.

fed at two supplementary feeding levels (100 or 70% NRC). The body weight, breeding performance and change in progesterone level during pregnancy were evaluated.

The mean body weight of heifers at 6 months of age was 155 kg when fed the 100% NRC ration, but it was only 137 kg when they received the 70% NRC ration. At 10 months the difference in body weight was 66 kg, at 15 months 70 kg, at 20 months 160 kg and at 25 months 115 kg. This showed that heifers fed the standard ration gained weight rapidly.

The average size of the left ovary in the standard group was 2.1×1.6 cm and the right ovary was 2.6×1.8 cm. However, in the restricted feeding group the ovaries were found to be smaller (left 1.5×1.2 cm, right 2.0×1.4 cm). The diameter of Graffian follicles showed a similar tendency in the two groups. The Graffian follicles in three heifers were over 10 mm in diameter in the standard feeding group, but only one heifer in the restricted group had follicles in excess of 10 mm.

First oestrus in the standard feeding group appeared at 14.6 months when body weight was 265 kg. Age at first pregnancy was, on average, 19.1 months when the average weight was 249.2 kg. Age at first calving was, on average, 29.1 months at a body weight of 436 kg. On the other hand, when heifers were fed the restricted ration first oestrus appeared at 23.0 months at a body weight of 250 kg. Average age at first calving was 38.9 months, which was 10 months later than the average in the standard feeding group.

No calving difficulties were experienced in the standard group and all cows gave birth to healthy calves, but in the restricted group one cow had a retained placenta.

In the standard feeding group the progesterone level was 1.95 ng/mL two weeks after pregnancy and gradually increased up to 10 weeks. Thereafter, the level declined slightly for about two weeks and then rose and peaked at 24 weeks. This peak (5.6 to 6.0 ng/mL) was maintained up to 38 weeks, when the progesterone level decreased until it reached 0.87 ng/mL at the end of pregnancy. In the restricted group the progesterone level up to 16 weeks followed a similar pattern to that of the standard group, but there was a tendency in the restricted group to have lower progesterone levels.

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IAEA-SM-292/12P

**IMMUNIZATION AGAINST LHRH AS AN AID
IN MANAGING REPRODUCTION
IN GRAZING FEMALE BEEF CATTLE***

T.B. POST

Tropical Cattle Research Centre,
Division of Tropical Animal Science,
Commonwealth Scientific and Industrial
Research Organization,
Rockhampton, Queensland, Australia

1. INTRODUCTION

Immunization against LHRH (luteinizing hormone-releasing hormone) in female beef cattle is being investigated as a simple non-traumatic method for preventing unwanted pregnancies in female beef cattle where the control of bulls is difficult. This procedure has potential usefulness in females being fattened for slaughter, in heifers that are too young for breeding, and possibly for preventing out-of-season conceptions.

2. METHODS

LHRH (Carlsberg Biotechnology Ltd) was conjugated to human serum albumin (HSA) by B. Moss (CSIRO, Division of Molecular Biology, Sydney) using the method of Goodfriend et al. [1]. Twelve Africander-cross heifers 2½ years of age were immunized, four with 5 mg LHRH conjugate, four with 10 mg LHRH conjugate, and four controls with HSA using Freund's complete adjuvant and multiple subcutaneous and intramuscular injection sites. Primary immunization was followed by a booster 12 weeks later.

The reproduction activity of the heifers was monitored before and after immunization by twice weekly measurements of serum progesterone (by radioimmunoassay) and by observations of oestrus (using an androgenized steer fitted with a chin-ball marker). Antibody titres were determined weekly by measuring the per cent binding of ¹²⁵I-labelled LHRH (0.24 pg) to 300 µL of a 1:500 dilution of serum.

* Research carried out in association with the IAEA under Research Agreement No. 3405/CF.

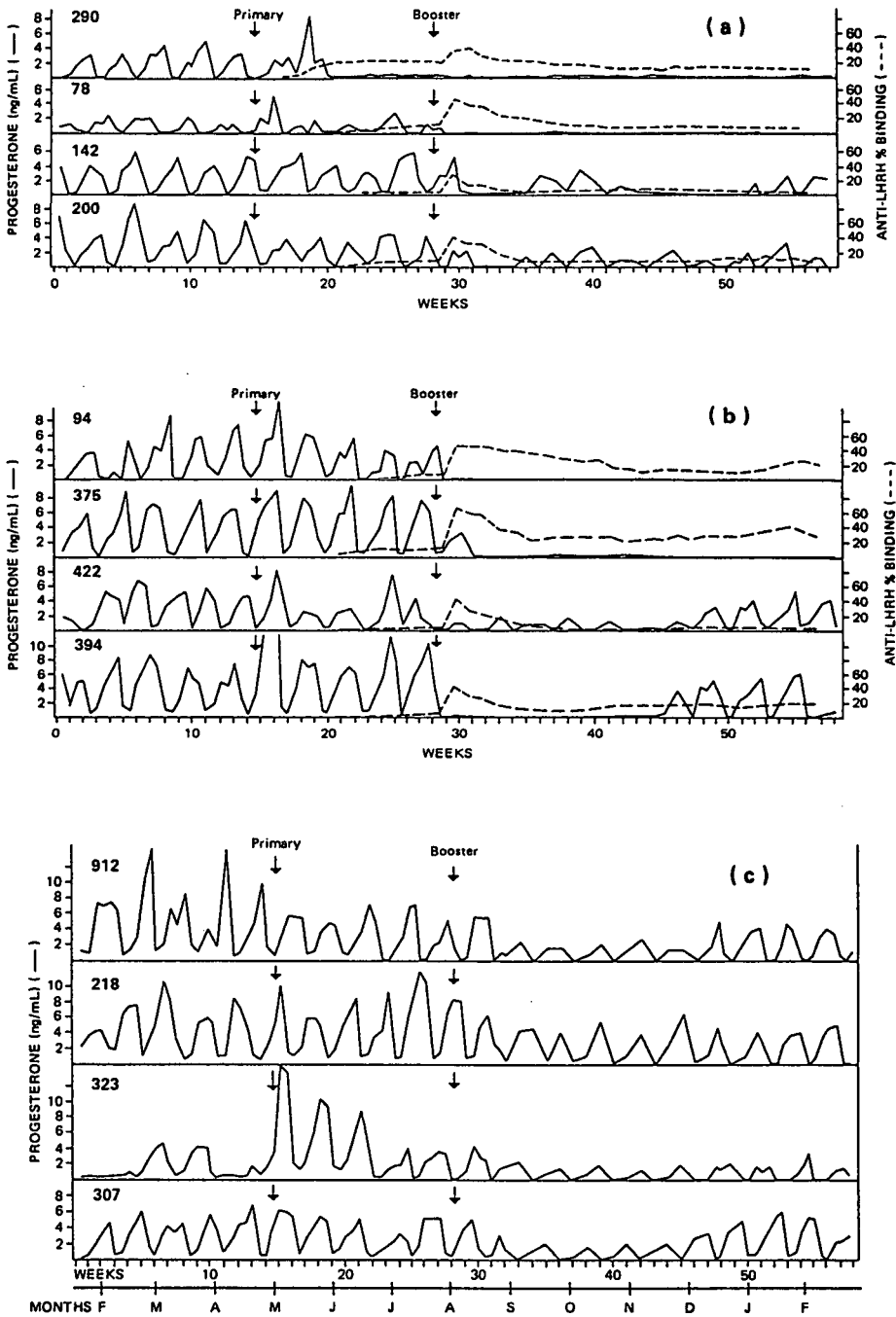


Fig. 1. Progesterone profiles and anti-LHRH titres for twelve heifers: (a) immunized, 5 mg LHRH-HSA; (b) immunized, 10 mg LHRH-HSA; (c) controls, HSA.

3. RESULTS AND DISCUSSION

The progesterone profiles and LHRH antibody titres are shown in Fig. 1. During the 12 weeks following the primary immunization LHRH antibody titres rose gradually to a mean of 10.2%. In heifer 290, with the most rapid rise in antibody titre, ovarian cyclicity ceased when the binding of labelled LHRH reached 20%. The remaining heifers with lower antibody titres continued to cycle.

Booster immunization was followed by a rapid rise in LHRH antibody titres to a mean of $45.2 \pm 4.0\%$ at 12 days post-injection in all animals immunized against LHRH. Progesterone measurements and oestrus observations indicated that ovarian cyclicity ceased in these animals soon after booster immunization. All the controls continued to demonstrate normal ovarian cyclicity. At the end of the study (8 months after the booster) half (2 low dose and 2 high dose) of the eight immunized heifers remained anoestrous. Antibody titres of heifers that remained anoestrous reached a mean peak of $51.7 \pm 4.0\%$ after the booster and declined to $18.2 \pm 3.8\%$ at the end of the study. Heifers in which cycling resumed reached an initial peak of $40.0 \pm 2.8\%$ and declined to $9.1 \pm 2.5\%$. Antibody titres of the 5 and 10 mg dose groups reached peaks of $39.8 \pm 4.7\%$ and $51.9 \pm 5.4\%$, respectively. The final titres were $7.8 \pm 1.8\%$ and $19.4 \pm 3.5\%$, respectively. There was no difference between controls and animals immunized against LHRH in live weight gains.

A major objective of this study was to develop a procedure that is as simple as possible to implement with a minimum requirement for booster immunizations. Although antibody titres determined in this study were continuing to rise in all animals at 12 weeks following primary immunization, at least one booster was necessary in most animals for a rapid rise in titres to a level that would stop ovarian cyclicity.

So far, these preliminary results indicate that immunization against LHRH is an effective means of preventing undesired reproduction in female beef cattle, but development of this technique into a practical management tool will require continued work to determine acceptable adjuvants, routes of administration and methods of manipulating the duration of response.

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COMPARISON OF OESTROGEN CONCENTRATIONS IN FAECES AND BLOOD PLASMA OF CYCLIC AND PREGNANT SOWS

E. BAMBERG, H.S. CHOI, J. HOIS

Institut für Biochemie,
Veterinärmedizinische Universität Wien

and

Ludwig Boltzmann-Institut für
Veterinärmedizinische Endokrinologie

Vienna, Austria

Concentrations of oestrone sulphate in blood plasma and oestrogens in faeces were measured from sows throughout the normal oestrous cycles and during early pregnancy to determine the possibility of utilizing oestrogens in faeces as an indicator of pregnancy. Blood and faeces samples were collected every two days from six non-pregnant sows during the oestrous cycle and from 30 pregnant animals between days 16 and 35 of gestation.

The validity of the assay was checked according to the method of Cekan [1]. After extraction of 1 g faeces with sodium hydroxide (2 mol/L) and chloroform (3:1, vol./vol.), the alkaline phase was acidified (pH 3 to 4) with acetic acid and re-extracted using a mixture of petroleum ether and diethyl ether (9:1, vol./vol.). The extract was subjected to high performance liquid chromatography (FZ Seibersdorf Si60) using chloroform and n-hexane (6:4, vol./vol.) as the eluant, and showed two immunoreactive substances which appeared in the same fractions as oestrone and oestradiol-17 β .

Concentrations of oestrone sulphate in plasma and those of oestrogens in faeces were measured by enzymeimmunoassay on microtitre plates as well as by radioimmunoassay.

The concentration of oestrone sulphate in plasma was low in non-pregnant animals and increased to a peak between days 25 and 30 after mating. Similarly, the oestrogen concentration in faeces was low in non-pregnant sows and high between days 25 and 30 after mating. The results show that oestrogen concentrations in faeces reflect oestrone sulphate levels in plasma and can be used for pregnancy confirmation from days 25 to 30 of gestation in sows.

Our previous studies on cattle and horses have demonstrated a marked increase of oestrogen concentrations in faeces during pregnancy [2-5]. Measurement of oestrogens in faeces offers a useful method for pregnancy confirmation with an easy, non-invasive procedure of sample collection.

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IAEA-SM-292/15P

**EARLY PREGNANCY DIAGNOSIS IN
DAIRY COWS BASED ON MILK
AND HAIR PROGESTERONE DETERMINATION***

Dazhi GUO**, Xianyi LUI**, Faju CHEN**,
Yongle ZHANG***, Xuehua SON**, Xingyu LIAO**

**Isotope Research Laboratory,
Sichuan Agriculture University

***Yaan Dairy Farm

Yaan, Sichuan, China

1. METHOD

A comparative protein-binding assay (CPBA) for progesterone using chicken plasma as binding protein was developed. The sensitivity of the assay was 50 pg/tube. The assay range from the standard curve was 50 to 8000 pg. Recovery for the standard and labelled progesterone was 84.8% (n = 6) and 94% (n = 6), respectively. The intra- and interassay coefficients of variation were 8.8% (n = 6) and 14.5% (n = 8), respectively. The relative activities of cross-reacting steroids in the assay were: progesterone 100%, corticosterone 12%, oestrone 3%, pregnenolone 3%, oestradiol 9% and cortisol 14%. Although the specificity of chicken plasma is

* Research carried out with the support of the IAEA under Research Contract No. 3474/RB.

TABLE I. ACCURACY OF PREGNANCY DIAGNOSIS IN DAIRY COWS BASED ON HAIR PROGESTERONE DETERMINATION (%)

Days	Head		Back		Udder		
	21	24	21	24	21	24	
Pregnant	81	87	85	92	81	86	n = 44
Non-pregnant	92	86	80	79	85	100	n = 15

not as good as that of specific antisera, it is easy to obtain from various sources. It can be stored for more than 6 months at 0°C. One chicken's plasma is enough to perform the assays for 2000 to 3000 tubes. In routine practice, each assay can be conducted within 4 hours. Thus, the cost of the assay is relatively low and analysis is easy to prepare and perform.

1.1. Milk progesterone determination

First, drops of milk were collected from 250 black and white dairy cows on the afternoon of day 24 after artificial insemination. Progesterone was extracted from 0.1 mL of milk with petroleum ether and assayed by CPBA.

1.2. Hair progesterone determination

The hair samples taken from the head, back and udder of 59 black and white dairy cattle on days 21 and 24 after artificial insemination were clipped with scissors and cut into small pieces. Five milligrams of the hair fragments from different parts of the body were distributed in each glass tube. After extracting the hairs with 1 mL of petroleum ether, the progesterone contents were assayed using CPBA.

2. RESULTS

2.1. Pregnancy diagnosis by milk progesterone determination

The criterion for discriminating pregnancy and non-pregnancy was 5 ng of progesterone per millilitre of milk, i.e. a level higher than 5 ng/mL indicated pregnant animals; otherwise, non-pregnant. The accuracy of positive and negative diagnoses compared with rectal palpation at three months after artificial insemination was 86.4% (186 out of 216) and 94.2% (32 out of 34), respectively.

TABLE II. PROGESTERONE CONTENTS OF THE HAIR OF COWS

Days	Head		Back		Udder	
	21	24	21	24	21	24
Pregnant	13.18 ± 6.36	14.23 ± 10.39	14.58 ± 8.76	13.90 ± 7.33	16.21 ± 12.48	14.88 ± 10.89
Non-pregnant	3.14 ± 0.94	3.50 ± 0.86	3.71 ± 0.78	3.22 ± 1.43	2.72 ± 0.81	3.00 ± 0.80

2.2. Pregnancy diagnosis by hair progesterone determination

The criterion for classifying pregnancy and non-pregnancy was 5.5 ng progesterone per 50 mg of hair. The accuracy of positive and negative diagnoses compared with rectal palpation is shown in Table I.

The progesterone contents of the hair taken from different parts of the cows' body are shown in Table II.

3. CONCLUSIONS

Early pregnancy diagnosis in dairy cows by determination of milk progesterone using CPBA has been successfully achieved. It appears that pregnancy could also be detected by determination of the progesterone contents of cows' hair.

ACKNOWLEDGEMENTS

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IAEA-SM-292/20P

**GLUCOSE, PROTEIN AND ENERGY METABOLISM
OF LACTATING GOATS FED A GRASS DIET
AND STEAM-COOKED CASSAVA-UREA SUPPLEMENT**

I.G. MANIK, D. SASTRADIPRADJA

Department of Physiology and Pharmacology,
Faculty of Veterinary Medicine,
Bogor Agricultural University,
Bogor, Indonesia

Tropical countries with inadequate sources of vegetable protein could utilize concentrates made of tubers plus urea for ruminant feeding. In addition, it is known that heat-moisture treatment of cereals, cassava and sweet potato intensifies starch susceptibility to α -amylase; hence, it can improve urea utilization by rumen microflora [1, 2]. Addition of fermentable carbohydrate to the diet results in the rumen fluid containing relatively less acetate than that of a fibre-rich fed ruminant. Since the proportions of volatile fatty acids can be manipulated by such dietary means, it is important to assess their utilization in meeting the energy and nutrient requirements for the various physiological functions of the animal. In vivo kinetic techniques to obtain data on the metabolism of glucose and other nutrients in ruminants are widely applied. Tracer labelling of the bicarbonate pool may enable estimation of gluconeogenesis from carbon transfer involving CO_2 fixation into the glucose pool [3, 4] and measurement of the energy expenditure indirectly from the CO_2 production rates [5].

The purpose of the experiment described below was to estimate quantitatively in the ruminant the effects of steam-cooked cassava in the carbohydrate-urea feed supplement on the body metabolism of glucose and protein. The study used 18 lactating goats (16 to 18 kg body weight) housed in individual metabolism cages which were arranged in a complete randomized block design in a feeding-digestion trial. The animals were fed ad libitum napier grass (*Pennisetum purpureum*) plus a concentrate supplement consisting of urea and dried steam-cooked cassava (diet C) or dried raw cassava (diet B); the control goats did not receive any concentrate (diet A). The level of cassava given was 1% of the metabolic body weight ($W^{0.75}$) of the animal, while the content of urea was 4% of the cassava weight. A mineral mix and ad libitum water were given. On the last day of the total collection period of the digestion trial,

TABLE I. PERCENTAGE COMPOSITION OF DIETS (DRY MATTER BASIS) AND THEIR RESPECTIVE APPARENT DIGESTIBILITIES (n = 6) USED IN THE FEEDING-DIGESTION TRIAL WITH LACTATING GOATS*

Composition	Diet A	Diet B	Diet C
Ash	10.99	8.65	8.33
Protein	11.41 (62.92 ± 4.72) ^A	17.33 (66.94 ± 6.77) ^B	15.96 (72.94 ± 4.10) ^B
Crude fibre	46.00 (60.87 ± 8.29) ^a	3.97 (57.94 ± 4.82) ^{a,b}	3.01 (68.67 ± 9.57) ^{a,c}
Fat	1.24 (11.12 ± 5.31) ^a	0.66 (15.08 ± 4.35) ^b	0.53 (25.84 ± 9.07) ^c
N-free extract	29.69 (44.27 ± 7.91) ^A	66.45 (55.89 ± 4.86) ^B	69.37 (66.93 ± 4.27) ^C
Ca	0.28	1.88	1.77
P	0.39	1.06	1.01

* All values in parentheses are percentage apparent digestibilities of the particular nutrients ± SE of test diets A, B and C. The figures for compositions of diets B and C are for the concentrate component only (excluding grass).

A-C; a-c Means in the same row not sharing a common letter differ (A-C: P < 0.01; a-c: P < 0.05).

glucose-2-³H (55.2 µCi)¹ was injected as a single pulse to assess glucose flux and pool size from the time course change of plasma ³H-glucose specific activities [6]. The primed continuous infusion of NaH-¹⁴CO₃ (0.254 µCi·min⁻¹) into the jugular vein was used to label the body bicarbonate pool and CO₂ production was measured by dilution [5]. The transfer quotient (TQ) from CO₂ into glucose as an index of gluconeogenesis was calculated as the ratio between the plateau specific activities of plasma ¹⁴C-glucose and blood ¹⁴C-bicarbonate expressed in percentage. The rate of gluconeogenesis which involves CO₂ fixation was calculated by multiplying the value of the glucose flux by the TQ × 6. All data were subjected to analysis of variance.

The composition of diets and digestibility by lactating goats are presented in Table I. Data on the various metabolic parameters measured are shown in Table II. Steam-cooked processing significantly increased the fermentability of N-free extract. Urea utilization was not significantly improved, however (diet C versus B). Addition of the carbohydrate source did not improve crude fibre utilization compared with the control, but fat digestibility was significantly improved. These increases in digestibility of the various components of the diet correlate with the increase in dry matter intake. Effective utilization of nutrients in the rumen results in an increased supply of substrates (propionate) to support endogenous glucose production, as

¹ 1 Ci = 3.70 × 10¹⁰ Bq.

TABLE II. EFFECTS OF DIET ON THE METABOLIC PARAMETERS IN LACTATING GOATS (n = 6; mean $\pm$ SE)

Metabolic parameter	Diet A	Diet B	Diet C
Dry matter intake (g/d)	308.85 $\pm$ 8.79 ^A	406.22 $\pm$ 38.56 ^B	411.70 $\pm$ 48.72 ^C
Plasma glucose concentration (mg/100 mL)	70.0 $\pm$ 1.5 ^a	70.5 $\pm$ 4.2 ^a	73.1 $\pm$ 1.9 ^a
TQ CO ₂ -glucose (%)	9.12 $\pm$ 1.75 ^a	6.16 $\pm$ 1.42 ^{a,B}	13.22 $\pm$ 2.09 ^{a,C}
Gluconeogenesis (CO ₂ fixation) (mg/min)	9.70 $\pm$ 2.99 ^a	5.94 $\pm$ 2.77 ^{b,B}	24.89 $\pm$ 8.05 ^{c,C}
Glucose flux (mg/min)	17.72 $\pm$ 9.24 ^a	16.08 $\pm$ 6.81 ^{a,B}	31.38 $\pm$ 9.03 ^{a,C}
Protein catabolism* (g/d)	16.59 $\pm$ 4.33 ^a	16.80 $\pm$ 3.97 ^b	8.34 $\pm$ 1.78 ^c
Protein retention** (g/d)	6.06 $\pm$ 1.69 ^A	16.00 $\pm$ 4.38 ^B	23.94 $\pm$ 5.31 ^C
Energy expenditure*** (kcal/d)	726.83 $\pm$ 169.10 ^a	659.48 $\pm$ 124.35 ^{a,B}	1110.94 $\pm$ 187.19 ^{a,C}

* Calculated from urine-N production rate $\times$ 6.25.** N retention $\times$ 6.25; values include protein secreted in milk.*** Calculated from CO₂ production rate, assuming a caloric equivalent of 5.44 kcal/L CO₂ produced.

A-C:a-c Means in the same row not sharing a common letter differ (A-C: P < 0.01; a-c: P < 0.05).

evidenced by the significant increase in gluconeogenesis due to feeding the steam-cooked cassava. With this dietary treatment, gluconeogenesis involving CO₂ fixation provided 79% of the total glucose flux (Table II). It also caused a significant reduction in protein catabolism favouring retention of protein, which increased almost 50% above the value obtained with the unheated cassava supplement. Energy metabolism was also enhanced, and considering all these data it can be concluded that providing steam-cooked cassava in the urea supplement diet causes modification in the ruminal fermentation pattern favouring the formation of glucogenic precursors (propionate), which in turn improves post-ruinally anabolic processes — sparing protein from catabolism and for the production of endogenous glucose — supported by the active energy supply of the lactating goats.

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SERUM PROGESTERONE LEVELS IN MONITORING OVARIAN ACTIVITY IN WHITE FULANI (ZEBU) DAIRY CATTLE AFTER PARTURITION

O. ADEYEMO

Department of Veterinary Anatomy,
University of Ibadan,
Ibadan, Nigeria

The interval between calving and resumption of ovarian cycle is important in determining the postpartum interval. This study was designed to determine the time of occurrence of first oestrus and conception after calving, and the postpartum interval in White Fulani (Zebu) cattle in a seasonal equatorial climatic zone of southern Nigeria [1].

TABLE I. POSTPARTUM CONDITIONS OF WHITE FULANI (ZEBU) DAIRY CATTLE

Animal identification	First observed oestrus (d) ^a	Second oestrus (d) ^a	Third oestrus (d) ^a	Oestrus preceding conception	Parturition ^b to conception interval
637	27	49	Nil	2nd	50
534	25	ND	68	3rd	69
808	30	Nil	Nil	1st	31
700	ND	Nil	Nil	1st	44 ^c
726	ND	Nil	Nil	1st	26 ^c
648	42	Nil	Nil	1st	43

^a Days postpartum.^b Conception was assumed to have occurred 1 day after oestrus.^c Oestrus was assumed to have occurred 4 to 5 days before the first observed elevation of serum progesterone levels.

ND = Not observed by herdsmen.

TABLE II. SERUM PROGESTERONE CONCENTRATION IN WHITE FULANI DAIRY CATTLE

Weeks postpartum	3	4	5	6	7	8	9	10	11	12	13
Animal identification	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)	P (ng/mL)
637	0.06	0.06	1.8	2.58	1.03	2.16	6.41	5.2	3.92	5.68	5.88
534	0.08	0.15	3.3	3.90	1.27	2.86	4.74	0.81	3.82	3.5	4.26
808	0.16	0.09	2.6	3.12	4.65	3.82	5.64	6.4	6.5	4.72	6.29
700	0.06	0.12	0.08	0.16	2.95	3.16	3.41	2.87	5.90	4.55	5.29
726	0.87	2.07	2.73	4.20	4.83	3.66	4.29	6.51	6.99	5.88	4.97
648	0.07	0.06	0.06	0.11	2.46	2.03	3.92	2.59	5.20	6.41	6.69

P: progesterone.

Six cows in a herd of White Fulani dairy cows on the University of Ibadan Teaching and Research Farm were selected for blood sampling by jugular venipuncture once or twice weekly from 2 weeks after calving for 90 days. The cows were kept within the herd, which was run with fertile bulls. Herdsmen recorded oestrus.

The first oestrus was observed in three of the cows within 30 days of calving and on day 42 in cow 648. Oestrus was not recorded in cows 700 and 726 before pregnancy, but was diagnosed by the serum progesterone (P) levels. By calculating backwards from the day elevated levels of P were determined, oestrus was estimated to have occurred on days 43 and 25 after parturition in cows 700 and 726, respectively. The mean interval between parturition and first oestrus in the six cows was 32 ± 8.3 SD days (Table I). Herdsmen overlooked oestrus three times (27%), but each oestrus missed was indicated by low serum P levels (Table II). The mean interval between parturition and conception was 43.8 ± 15.2 SD days. One cow (637) conceived at the second service (second oestrus), another (534) at the third service, while the remaining four cows conceived at the first service (first oestrus).

Serum P levels were determined by the radioimmunoassay technique [2], with some modifications [3]. Serum P levels ranged between 0.06 and 1.18 ng/mL during the interval between calving and dioestrous following the first oestrus. The values ranged between 2 and 7 ng/mL during the dioestrous phase of oestrous cycles and pregnancy. The mean interval between parturition and the first elevation of serum P was 37.0 ± 8.7 SD days.

The herd record showed that 34, 54 and 19% of cows conceived within the first 60 and 90 and after 200 days following parturition, respectively. The mean postpartum interval of the herd was 398.3 (± 74 SD) days. Since ideally the average calving interval should be 1 year [4], the performance of the herd is considered to be fair. However, the mean interval between parturition and first oestrus observed in the six Zebu cows was much longer than that reported in *Bos taurus* cattle (24 days) in a temperate climate [5].

The factors responsible for the relatively prolonged interval between parturition and first oestrus in cow 648 and repeat breeding in cow 534 are not known. However, further studies are required on the effects of season and feed availability on the postpartum ovarian activity and fertility of Zebu cattle. The assay of milk P should facilitate monitoring ovarian activity after parturition and investigating repeat breeders, since sample collection would be less tedious and a large number of animals can be involved in the study.

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HEALTHY ANIMALS IN A SOUND ENVIRONMENT

Perspectives of a study with copper isotopes

W.T. BINNERTS

Department of Animal Physiology,
Agricultural University,
Wageningen

P. POLAK, J. GERADTS,

R. v.d. VLIST, L. LINDNER

National Institute for Nuclear and
High Energy Physics (NIKHEF-K),
Amsterdam

Netherlands

Copper deficiency is a widespread phenomenon which adversely affects animal production and health in many countries. It is coupled with low copper absorption in ruminants, more so in cows than in sheep. In high producing areas with heavy fertilizer applications the already low copper absorption is further reduced and over 96%, sometimes nearly 99%, of the administered copper is excreted (unused) into the environment. The continuing accumulation of copper in the environment due to cattle rearing is the second largest problem after that of intensive pig farming, which will probably cease to be a problem in near future. It is conceivable that considerable effort will then be given to increase the efficiency of copper use by the dairy cow. This presentation describes the methods used for the production and application of copper isotopes to check the copper metabolism, as well as some recent results.

Copper-64, with a half-life of 12.9 h, has a penetrating 0.5 MeV positron annihilation gamma radiation, and is thus suitable for in vivo measurements with large animals. The amount of copper absorption has been determined by comparing liver counts after feeding and injecting ^{64}Cu . In reverse experiments with four milk cows a total of 10 mCi was needed each time, with 4 to 5 mCi for orally dosed cows and

0.2 mCi for injected cows.¹ Our experience is that milk cows had nearly completely emptied their intestines of radio-copper within 3 to 4 days, so that parallel descending lines are obtained in the count-time plot due to liver copper secretion.

Copper-64 is produced by neutron activation. Use of electrolytically pure copper and a short irradiation time result in an excellent preparation, so that no purification is needed; the isotope can be used for periods up to 120 h. For administration nearly neutral solutions of an amino acid complex, usually glycine, are prepared in order to prevent precipitations.

Copper-67, with a half-life of 62 h, enables full consideration of the metabolism during a period of 3 weeks. The 93 and 185 keV gamma rays used for measurement are not sufficiently penetrating for simple external *in vivo* counting. However, it is possible to count large 3 L volumes of milk, urine, faeces, intestinal fluids, and eventually blood from Marinelli beakers, using known standard solutions of identical volume. Endogenous excretion can then be routinely computed by isotope dilution methods (faeces, urine, milk).

Production of ⁶⁷Cu has previously been carried out in a cyclotron by exposing a zinc target to a beam of 50 MeV protons [1]. Currently, ⁶⁷Cu is being produced in the Medium Energy Electron Accelerator facility (MEA of NIKHEF-K) by exposing a zinc oxide target to bremsstrahlung of electrons with energies higher than 100 MeV through the reaction ⁶⁸Zn (γ , p) ⁶⁷Cu. The main problem in the recovery of ⁶⁷Cu is the large amount of zinc oxide needed to overcome the high penetrating power of the photons and the low cross-section of photonuclear reactions. Therefore, a chromatographic separation method has been developed using a Chelex 100 column which is capable of binding copper strongly and selectively, even in the presence of excessive amounts of zinc ions (column saturation). After elution of the copper fraction, further purification is achieved using an anionic exchanger. Thus, very pure solutions of a few millilitres volume were prepared with up to 4 mCi of ⁶⁷Cu and with specific activities of at least 40 mCi/mg, the carrier copper stemming from impurities in the target material.

To complete the picture of available isotopes mention should be made of the availability of enriched ⁶⁵Cu. This stable isotope has been used in calculations of the copper absorption in dairy cows with isotope dilution after liver biopsies [2]. A short-cut method has been described for which periods of 3 to 6 weeks are mentioned, but much longer periods of observation have been used.

¹ 1 Ci = 3.70×10^{10} Bq.

In summary, a number of methods are available for determination of absorption and endogenous excretion of ruminants. As an example, some recent results with radioisotope methods are presented.

Cow	Absorbed copper (mg/d)	Endogenous loss (mg/d)
A	13.2	16-18
B	12.0	8.3
D	14.6	12.6-14
Average/ range	12.0 (n = 12)	1.6-18

The percentage absorption could be directly measured in two weeks. It was usually below 4% in cows (and below 10% in sheep). Grass feeding resulted in a lower absorption than hay + concentrate feeding; the younger the grass the lower the absorption. Animals differed in their absorptive capacity. The endogenous losses of cows were also sensitive to the composition of the feed, and very large individual variation has been found.

Although it must be admitted that more fundamental knowledge has yet to be gathered, the time seems to have come for using these methods in closely following the absorptive capacities with different feed formulations and for spotting cows with excessive copper losses. This may help considerably in reducing animal production losses due to deficiency and in protecting the environment.

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MEDIATION OF PANCREATIC AND THYROID HORMONES IN THE UTILIZATION OF NUTRIENTS IN DAIRY COWS

R. KRZEMINSKI, G. KULASEK, W. BAREJ

Department of Animal Physiology,

Warsaw Agricultural University,

Warsaw, Poland

The relationship of food composition, rumen fermentation processes and body utilization of nutrients in ruminants has been proved for many years. The rumen fermentation rate plays a key role in such dependence. It influences microbial synthesis in the rumen, volatile fatty acid composition as well as the concentration of plasma hormones and many physiological processes.

The aim of the present study was to estimate milk production and the concentration of plasma pancreatic and thyroid hormones in dairy cows fed a low protein diet with different energy contents.

Two groups of four cows each, with a similar milk production, were fed a diet consisting of corn silage, hay and grain concentrate for a 90-day period. The daily ration contained 12.5 kg dry matter with 11% crude protein. In addition, the cows of one group were fed 2 kg sugar beet molasses per cow daily. This increased the daily intake of metabolizable energy from 112 to 136 MJ.

The average daily milk production for the entire experimental period was 12.4 kg fat corrected milk (FCM) per cow in the control group and 14.7 kg FCM in the group given molasses. The results of radioimmunoassay estimation of insulin, glucagon, thyroxine and triiodothyronine are presented in Table I. The average concentration of all estimated hormones (measured during different periods of the experiment and several times during the day) was higher in the group of cows given molasses than in the control; however, only the differences of thyroxine and glucagon were significant statistically.

Several authors [1, 2] have reported an increase in milk production in cows fed molasses. Ostaszewski and Barej [3], in an experiment carried out on sheep, observed the rapid increase of insulin and glucagon secretion after intraruminal administration of light fermenting carbohydrates. High producing dairy cows are characterized by a lowering of the plasma insulin and thyroxine concentration and an increase in the growth hormone [4]. All these hormones are responsible for supporting the metabolic homeostasis in the body. The performance and productivity of animals as well as the food composition regulate the plasma hormonal profile and then the utilization of nutrients. Addition of molasses to the low protein diet stimulated secretion of both

TABLE I. PLASMA CONCENTRATION OF INSULIN, GLUCAGON, THYROXINE (T₄) AND TRIIODOTHYRONINE (T₃) IN DAIRY COWS FED THE CONTROL AND EXPERIMENTAL (MOLASSES) DIET

Group	Insulin milli-unit/L	Glucagon (ng/L)	T ₄ (nmol/L)	T ₃ (nmol/L)
Control	12.6	90	68.3	2.3
Experimental	14.5	113 ^a	82.4 ^a	2.5

^a Statistically significant difference.

pancreatic and thyroid hormones, which improved the protein metabolism and utilization (probably oxidation) of energy compounds in the body, resulting in higher milk production. It appears that similar changes in the hormonal profile will act as a protection against metabolic disorders.

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IAEA-SM-292/25P

**ESTIMATION OF THE KINETIC PARAMETERS
OF INSULIN, GLUCAGON AND GROWTH HORMONE
IN SHEEP FED SUPPLEMENTAL ENERGY**

P. OSTASZEWSKI*, S. NISSEN, A. TRENKLE

Iowa State University,
Ames, Iowa,
United States of America

Several studies have documented the changes in plasma insulin, glucagon and growth hormone (GH) in ruminants fed additional energy [1-3]. The underlying mechanism responsible for changes in plasma hormone concentrations with changes in energy intake is not completely known, that is, are these changes in hormone concentrations due to alterations in removal or secretion?

The purpose of the present studies was to measure the effect of supplemental energy in the diet on basal plasma concentration, secretion rate, half-life and volume of distribution of insulin, glucagon and GH.

Six mature, mixed-breed wethers were used in the experiment after an overnight fast. Two dietary intakes, identical in protein but different in energy content, were used. The kinetic parameters were determined by measuring the rate of disappearance of immunoreactive hormone [4, 5] following an intravenous injection of all three hormones. The concentrations of plasma insulin, GH and glucagon were determined by radioimmunoassay. Bovine insulin, ovine GH and bovine-pork glucagon were used for iodination with ^{125}I by the procedure of Greenwood et al. [6]. The same hormones were used as standards.

Before hormone injection, the plasma growth hormone and glucagon concentrations in the low energy group were significantly ($P < 0.05$) higher than the high energy fed sheep (see Table I). In spite of elevated GH in the low energy fed sheep, no statistically significant differences in other kinetic parameters of GH were observed. Higher glucagon concentrations were, however, associated with increased secretion. Feeding the high energy diet decreased ($P < 0.01$) the half-life of injected insulin from 15.5 to 11.6 min, while concentrations were not significantly altered.

* Fellow of the International Atomic Energy Agency Fellowship Programme. Present address: Department of Animal Physiology, Warsaw Agricultural University, Warsaw, Poland.

TABLE I. PLASMA INSULIN (A), GLUCAGON (B) AND GROWTH HORMONE (C) CONCENTRATION, SECRETION RATE, HALF-LIFE AND VOLUME OF DISTRIBUTION OF SHEEP FED HIGH AND LOW ENERGY DIETS

Diet	High energy	Low energy	P
(A) Basal plasma insulin (ng/mL)	1.25	1.09	NS
Secretion rate ($\mu\text{g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$)	0.36	0.25	NS
Half-life (min)	11.61	15.45	0.01
Volume of distribution (% body weight)	7.82	8.55	NS
(B) Basal plasma glucagon (pg/mL)	111	137	0.05
Secretion rate ($\text{ng}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$)	73	103	0.05
Half-life (min)	5.89	6.46	NS
Volume of distribution (% body weight)	9.50	11.93	NS
(C) Basal plasma GH (ng/mL)	6.01	9.25	0.01
Secretion rate ($\mu\text{g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$)	1.27	1.40	NS
Half-life (min)	11.70	12.95	NS
Volume of distribution (% body weight)	6.53	5.37	NS

NS = not significant.

In conclusion, the results of the experiment suggest that:

- (1) Differences in the plasma concentration of GH and glucagon in the high energy fed sheep are most likely due to changes in the rates of hormone secretion
- (2) Insulin concentration in the plasma seems to be influenced less by secretion and more by clearance from plasma.

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IAEA-SM-292/26P

**OXYTOCIN THAT MAY BE RELEASED FROM
THE CORPUS LUTEUM DURING
THE CAPRINE OESTROUS CYCLE**

A.M. HOMEIDA

Department of Medicine, Pharmacology
and Toxicology,
Faculty of Veterinary Science,
University of Khartoum,
Khartoum, Sudan

Plasma concentrations of oxytocin (OT) and progesterone are raised during the luteal phase of the caprine oestrous cycle [1, 2], suggesting that the corpus luteum may be the source. To test this, Cl was removed from three goats between days 6 and 9 of the oestrous cycle, and acetic acid extracts prepared. The mean ($\pm$ SEM) OT concentrations were 1445 ± 16 ng/g wet weight, using a specific radioimmunoassay (RIA), and 1204 ± 19 ng/g wet weight using the superfused rat uterus bioassay. The extracts stimulated release of $\text{PGF}_{2\alpha}$ from the postpartum rat uterus in a quantitatively similar manner to standard OT. The oxytocic activity was not affected by either atropine or cyproheptidine, but was abolished by pretreatment of the extracts with either thioglycollate or a specific OT antibody. Column and paper chromatography of the extracts demonstrated that the fraction with marked biological activity behaved in a similar manner to the OT standard.

In a further three goats jugular blood samples were obtained at frequent intervals between 12 and 18 days after oestrus, and plasma-assayed for oxytocin and oxytocin-associated neurophysin [3]. Oxytocin (10 to 76 pg/mL) was released in a pulsatile pattern and in all three goats was accompanied by a simultaneous release of neurophysin (1.7 to 8.5 ng/mL).

The correlation coefficient for plasma oxytocin and neurophysin concentrations was +0.80 ($n = 135$, $P < 0.001$).

The simultaneous release of neurophysin and ovarian oxytocin suggests that oxytocin synthesis may occur within the ovary.

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IAEA-SM-292/27P

CHANGES IN THE RATE OF LIQUID FLOW FROM THE RUMEN AND TURNOVER OF BODY WATER OF THE ACUTE HEAT-STRESSED BUFFALO*

N. CHAIYABUTR, C. BURANAKARL,
V. MUANGCHAROEN, P. LOYPETJRA,
A. PICHACHARNARONG

Department of Physiology,
Faculty of Veterinary Science,
Chulalongkorn University,
Bangkok, Thailand

Buffaloes are known to differ from other domestic ruminants both in heat tolerance and water requirement [1]. In hot environments maintenance of the buffalo's thermal balance depends mainly on cooling derived from the evaporation of water from respiration and salivation. Body water is known to play a central role in heat dissipation mechanisms. However, there is a lack of information regarding the effects of short, extreme heat exposures on the balance of body fluid in buffaloes. The aim of the present work was to investigate whether (over a period of 5 h) exposure to heat affects the rate of liquid flow from the rumen or the turnover of body water of buffaloes. A better understanding of such changes may throw some light on the incidence of omasal impaction that might occur in heat-stressed buffaloes.

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TABLE I. EFFECTS OF ACUTE HEAT EXPOSURE ON THE RATE OF LIQUID FLOW FROM THE RUMEN, TURNOVER OF BODY WATER AND PLASMA VOLUME OF SIX NON-PREGNANT BUFFALOES (mean $\pm$ SE)

Parameters	Control	Heat exposure (5 h)
Ambient temperature ($^{\circ}\text{C}$)	30 $\pm$ 0.4	41 $\pm$ 0.4
Humidity (%)	46 $\pm$ 1.3	44 $\pm$ 0.9
Rumen fluid volume (L)	27.5 $\pm$ 3.7	35.5 $\pm$ 4.3 ^{NS}
Rumen fluid flow rate (L/h)	1.82 $\pm$ 0.42	3.12 $\pm$ 0.61*
Biological $T_{1/2}$ ^{51}Cr -EDTA (h)	12.9 $\pm$ 2.5	9.3 $\pm$ 2.0 ^{NS}
Water turnover (L/d)	43.80 $\pm$ 12.0	82.53 $\pm$ 14.1*
Biological $T_{1/2}$ tritiated water (h)	87.7 $\pm$ 6.8	49.2 $\pm$ 5.7**
Total body water (L/100 kg)	63.0 $\pm$ 7.2	62.6 $\pm$ 4.1 ^{NS}
Plasma volume (mL/kg) (using T-1824)	47.5 $\pm$ 2.9	50.8 $\pm$ 2.8*

By paired T-test: *P < 0.05; **P < 0.01 with respect to control state of the same animals; NS = not significant.

Two series of experiments were carried out on six non-pregnant buffaloes, weighing 320 to 368 kg. In the first series the animal was kept during the control period in a room at normal ambient temperature and relative humidity in the range of 29 to 31 $^{\circ}\text{C}$ (dry bulb) and 45 to 58%, respectively. Over a period of about 4 weeks after the first series each animal was exposed in a hot room at an ambient temperature of 41 $\pm$ 1 $^{\circ}\text{C}$ (db) and a humidity range of 42 to 48% for 5 h. In both series of experiments, measurement of the flow rate of liquid from the rumen was carried out by transferring 250 μCi ^{51}Cr -EDTA as a marker through the rumen cannula (OD: 0.5 cm).¹ After 1 hour, and then every hour for 7 hours, 20 mL of rumen liquor were taken manually for analysis. The total body water and water turnover were measured in each animal in each period using the ^3H -radioisotope dilution technique [2]. A single dose of 3000 μCi /animal of carrier-free tritiated water was injected intravenously. Blood samples for water turnover measurements were collected at intervals of up to 50 h after dosing.

It was found that the heart rate, respiratory rate, rectal temperature and packed cell volume remained constant throughout the control period. When animals were

¹1 Ci = 3.70 $\times 10^{10}$ Bq.

exposed to heat, the rectal temperature rose (from the beginning to the end of exposure) from 38.6 ± 0.2 to $39.7 \pm 0.4^\circ\text{C}$. The respiratory rate significantly increased from 26 ± 10 to 86 ± 38 (SD) breaths/min. The mean initial heart rate from the first to the fifth hour of heat exposure significantly increased from 43 ± 6 to 51 ± 3 beats/min. During the 5 h of heat exposure, the plasma volume significantly increased, while the packed cell volume decreased slightly from 25.9 ± 2.8 to $25.2 \pm 3.5\%$. The plasma solids consisted mainly of protein and glucose which increased 6.5% (9.21 to 9.81 g per 100 mL plasma) and 73% (53.9 to 93.4 mg per 100 mL plasma), respectively, by the fifth hour of heat exposure. The results in Table I show that the flow rate of liquid from the rumen and the body water turnover were significantly higher during exposure to heat, whereas the biological half-life of tritiated water and ^{51}Cr -EDTA were decreased by approximately 44% and 28%, respectively.

These results conclude that buffaloes require more water to change the physiological state during 5 h of exposure to heat. An increase in water turnover simply reflects part of a process of adaptation for evaporative cooling to maintain a normal body temperature during a rise in heat from exogenous sources. An increase in plasma water during heat stress could be due to an increase in colloidal osmotic pressure that is caused by an increase in the plasma protein concentrations. This might augment the passage of water from the extravascular tissue space to the intravascular compartment. Another possibility for heat-stressed buffaloes to balance an excessive evaporative water loss might be by water replacement from the digestive tract [3] during an increase in the rate of liquid flow from the rumen.

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IAEA-SM-292/28P

**HORMONE DETERMINATION AS A TOOL
FOR IMPROVING REPRODUCTIVE EFFICIENCY
IN SWAMP BUFFALOES*****M. KAMONPATANA**

Biochemistry Unit,
Faculty of Veterinary Science,
Chulalongkorn University,
Bangkok, Thailand

Hormonal measurement in buffaloes has become important for monitoring genetic progress and productivity since the introduction of artificial insemination and embryo transfer in the field of buffalo reproduction. The study of endocrine patterns and their regulation employing hormonal measurement is becoming a well used tool for improving reproductive efficiency in association with reproductive management in buffaloes.

Concerning reproductive efficiency in swamp buffaloes, evaluation may be based on the number of surviving calves per breeding female, which is commonly low. This low reproductive rate is due to a high percentage of calf mortality, abortion and embryonic loss. Environmental and health factors must be taken into account. Thus, there is a need to assess the reproductive efficiency of swamp buffaloes in terms of: age at first calving, pregnancy rate, conception rate, calving rate, weaning rate and percentages of embryonic mortality, abortion and calf mortality. These are the requirements needed in order to keep accurate records, which can only be produced from repeated breeding in confinement. It is difficult to make such evaluations in the smallholder system, since there are so many confusing factors, e.g. migration of animals due to the season, inaccurate control of feeding and management, and inadequate health services to prevent losses, particularly of young calves.

It is our intention to evaluate reproductive efficiency in swamp buffaloes resulting from improved feeding and management. Since the intensive breeding programme of swamp buffaloes was established at the Surin Buffalo Breeding Centre in 1976, records of all reproductive data and details of improved feeding and management have been collected. These records will provide details of reproductive efficiency in terms of the aspects mentioned above from the data collected during 1976-1983.

The Surin Buffalo Breeding Centre is responsible for producing high-grade weaners to be used in the performance testing programme at the Lumpayaklang

* Research carried out with the support of the IAEA under Research Contract No. 3873/RB.

Breeding Station. After the performance test was completed, top quality animals were sent back to Surin to provide a replacement herd. During 1976–1983 the buffalo breeding programme was divided into two and three breeding periods of 90 days and 70 days, respectively. Pregnancy diagnosis was performed by rectal palpation at 2 to 3 months after removal of the bulls, and also by using three blood samples at 15, 21 and 27 days after removal of the bulls for progesterone tests employing the radioimmunoassay and microtitre plate enzymeimmunoassay techniques used by Kamonpatana et al. [1]. Analysis of reproductive efficiency was based on a combination of both methods of pregnancy detection.

Various criteria of the efficiency of reproduction are set in the *average age of first calving* (54.96 ± 10.60 months), *pregnancy rate* (71.23% in heifers and 63.20% in cows), *conception rate* (12.95% during 100 to 150 days postpartum and 15.40% during 151 to 200 days after parturition) and *calving interval*. The latter decreased as parity increased. Of 409 repeat-breeding buffaloes, an average first calving interval of 652 ± 117 days was decreased to a fourth calving interval of 487 ± 89 days. Reproductive disorder was found in 44% of the animals, with an irregular oestrous cycle ranging from 3 to 55 days; regular oestrous cycles ranged from 17 to 24 days. The breeding cows included 14.23% subfertile animals, which contributed a rather wide range of average calving intervals. Other reproductive losses due to embryonic mortality, abortion and calf mortality were 14.93, 9.33 and 4.63%, respectively. The results of this study indicate that more factors concerning endocrinology as related to feeding and breeding management through genetic selection require in-depth research in order to provide new technology to rear swamp buffaloes for beef consumption.

Concerning buffalo reproduction, particularly in the female, one major area of endocrinology besides nutrition and management requires in-depth study, namely endocrine regulation of ovarian activity by synchronization, which is practised in swamp buffaloes both in confinement and in smallholder systems. Almost 50% of the animals showed no ovarian activity at the time of synchronization and insemination. It is suggested that regulation of the endocrine status in these animals is needed to remedy the subfertility. Steroidal immunization seems to be the promising area of research as it may increase fecundity. Also, an efficient AI service will no doubt increase fertility. Thus, if the ovulation rate can be significantly increased, one of the most important limitations to increasing reproductive efficiency will be overcome.

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IAEA-SM-292/29P

**POSTPARTUM OVARIAN CYCLICITY OF
CROSS-BRED COWS IN TROPICAL ADAPTATION**

S. SOPHON, K. SRISAKWATTANA,
M. KAMONPATANA

Biochemistry Unit,
Faculty of Veterinary Science,
Chulalongkorn University,
Bangkok, Thailand

According to the Dairy Promotion Organization, Thailand, three major exotic breeds of Red Dane, Red Sindhi and Holstein Friesian were introduced to Native $\times$ Sahiwal cross-breeds by artificial insemination. Twenty milking cows were selected for this study; they were divided into five groups according to the percentage of exotic blood and breeds. Fourteen cows in groups 1, 2 and 3 were Red Dane cross-breeds with percentages of 50% ($n = 3$), 60% ($n = 8$) and 71 to 81% ($n = 3$), respectively. Three cows in group 4 were 75% Holstein Friesian crossbreeds and three cows in group 5 were 75% Red Sindhi. The animals were raised in the same state of environment, feeding and health care. The animals were let loose to graze in well developed pasture, and during milking they were fed green fodder and concentrate. Milk samples were collected at 3-day intervals from 20 days postpartum until the animals were inseminated, the limit being around 100 days after parturition. A daily milk yield was collected. Milk progesterone was determined by the radioimmunoassay and microtitre enzymeimmunoassay techniques.

As shown in Table I, most of the cows were first inseminated 50 to 70 days postpartum. First ovarian cyclicity occurred before 40 days postpartum, except for three cows in groups 1, 3 and 5, which showed first cycling at days 44, 50 and 68, respectively. The first calving interval of nine cows ranged between 332 and 637 days, while the second calving interval of five animals ranged between 322 and 532 days. Thus, parity may affect the resumption of cyclicity in postpartum cows.

The milk yield of seven cows in group 2, two cows in group 3 and one cow each in groups 4 and 5 started to decrease around day 60 postpartum (Figs 1-3). The decrease in milk yield may be a consequence of postpartum sexual function as suggested by the progesterone profiles in a full luteal function shown in 11 cows.

TABLE I. REPRODUCTIVE EFFICIENCY OF TWENTY MILKING COWS ASSESSED IN TERMS OF NUMBER OF CALVINGS (No. CV), CALVING INTERVAL (CV), LAST GESTATION PERIOD (LG), FIRST AI DAYS POSTPARTUM (1st AI DAYS pp) AND DAY AT FIRST CYCLICITY POSTPARTUM

Blood line	Cow No.	No. CV	CV (days)		LG (days)	1 st AI days pp	First cyclicity day pp (cycle duration in days)
			1	2			
<i>Group 1</i>	14 302	3	359	464	288	50	20 (9)
Red Dane 33-50%	51 144	1	—	—	277	64	27 (15)
	5 221	10	637	365	291	89	44 (15)
<i>Group 2</i>	14 608	1	—	—	289	66	26 (18)
Red Dane 62-68%	51 151	1	—	—	281	47 LAI	29 (25)
Red Sindhi 18-31%	51 098	1	—	—	286	65	27 (18)
	14 282	3	339	402	286	72	32 (19)
	14 495	2	470	—	289	57	39 (18)
	51 085	1	—	—	277	75	35 (24)
	14 582	2	336	—	—	87	29 (10)
	14 129	3	332	532	—	103	32 (9)
<i>Group 3</i>	51 118	1	—	—	289	46	26 (18)
Red Dane 71-81%	51 125	1	—	—	284	55	38 (14)
	51 079	1	—	—	275	No AI	50 (18)
<i>Group 4</i>	9 239	3	371	322	287	78	32 (21)
HF 75%	51 014	—	—	—	—	74	20 (16)
HF 50%	9 354	1	—	—	283	NE	20 (9)
<i>Group 5</i>	14 204	2	338	—	—	53	34 (23)
Red Sindhi 75%	14 593	2	—	—	—	51	33 (17)
	14 091	3	434	—	281	40 HF	68 (15)

LAI = wrongly inseminated; No AI = not inseminated; NE = no oestrus;
HF = Holstein Friesian.

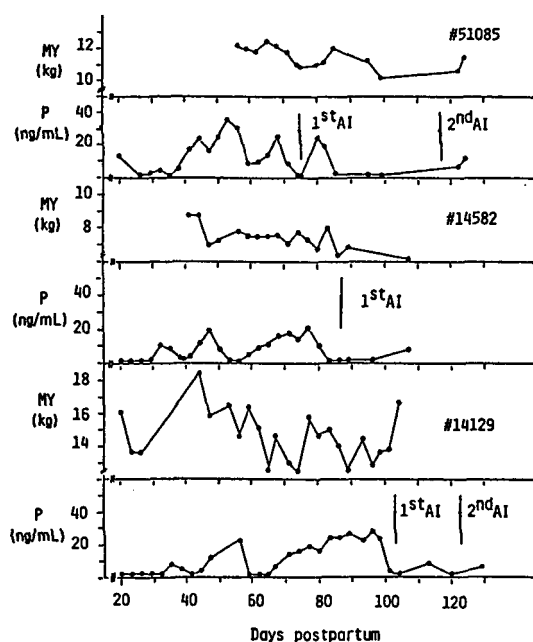


FIG. 1. Relation between decreasing milk yield (MY) and ovarian cyclicity determined by progesterone (P) profiles during postpartum in three cross-bred cows (62–68% Red Dane and 18–31% Red Sindhi).

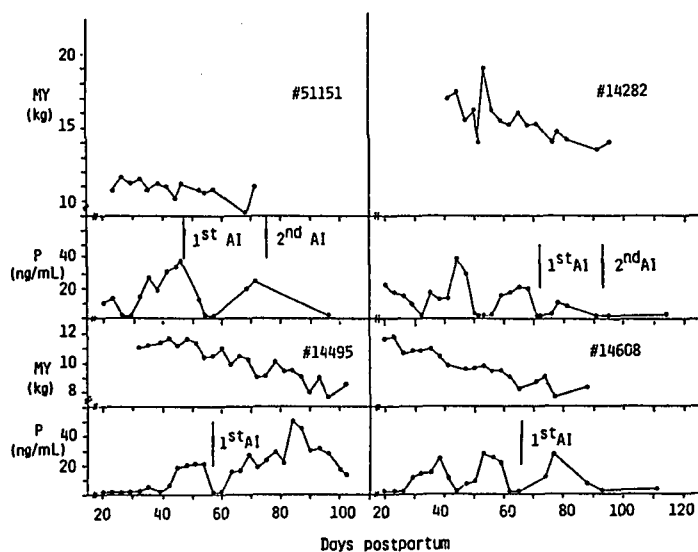


FIG. 2. Relation between decreasing milk yield (MY) and ovarian cyclicity determined by progesterone (P) profiles during postpartum in four cross-bred cows (62–68% Red Dane and 18–31% Red Sindhi).

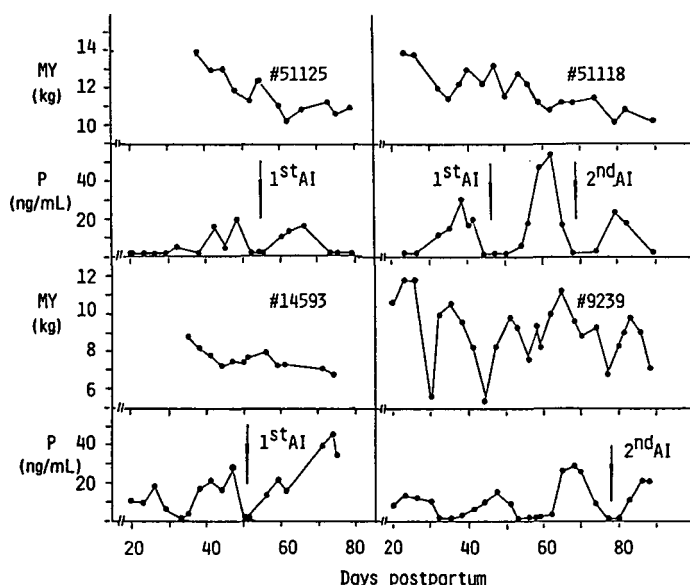


FIG. 3. Relation between decreasing milk yield (MY) and ovarian cyclicity determined by progesterone (P) profiles during postpartum in four cross-bred cows (71-81% Red Dane, Nos 51125, 51118; 75% Red Sindhi, No. 14593; 75% Holstein Friesian, No. 9239).

Thus, we arrive at the following conclusions:

- (1) The blood level of exotic breeds may be an important factor in the postpartum resumption of the oestrous cycle.
- (2) The decrease in milk yield may be a result of postpartum sexual function as suggested by the progesterone profiles in a full luteal function. It is not known if the sex of the foetus can regulate the milk yield during early postpartum.
- (3) The parity may affect the resumption of cyclicity in postpartum cows.

Further studies on a suitable blood level of certain breeds should be carried out in order to obtain the shortest interval to conception postpartum.

IAEA-SM-292/30P

**RESPONSE OF LAMBS VACCINATED WITH IRRADIATED
Trichostrongylus colubriformis LARVAE
TO IMPULSE AND SEQUENTIAL CHALLENGES
WITH NORMAL LARVAE**

M. ALABAY, H. ÇERÇİ, F. AYKOL

Department of Parasitology,
Lalahan Nuclear Research Institute of Animal Health,
Turkish Atomic Energy Authority,
Lalahan, Ankara, Turkey

Previous studies have shown that sheep aged between about 6 and 10 months can be protected against homologous challenge by vaccination with irradiated larvae of *Trichostrongylus colubriformis* [1-3]. Gregg et al. [4] have reported that vaccination was less effective in lambs aged 3 months and while some lambs developed partial immunity, others did not respond.

In the present study lambs were vaccinated with two and three doses of 10 000 irradiated *T. colubriformis* larvae at fortnightly intervals and the response of lambs to impulse and sequential challenges with normal larvae was compared.

Infective larvae were obtained from the Moredun Research Institute in Edinburgh, UK. Larvae were cultured from the faeces of a sheep with a monospecific infection of *T. colubriformis* [5, 6]. Vaccine larvae were attenuated by exposure to 60 krad from a ^{137}Ce source.¹ Larvae were administered to the lambs by means of plastic tubes.

Eighteen male merino lambs aged between 2 and 3 months were used. They were allotted randomly to three groups of six. Lambs in Group A were vaccinated with two doses of 10 000 irradiated larvae on days 0 and 14. Animals in Group B were vaccinated with three doses of 10 000 irradiated larvae on days 0, 14 and 28. Group C was not vaccinated and served as the non-immune control.

Groups A and B were given tetramisole hydrochloride (Poliverm-Vetaş) at 15 mg/kg body weight on day 48 to remove worms developed from the irradiated larvae. Group C was also treated with the anthelmintic on day 48. On day 62, three animals in each group were dosed by mouth with 20 000 normal larvae (impulse challenge). The other halves of the all groups were given four doses of 5000 larvae, i.e. a total of 20 000 larvae (sequential challenge) on days 62, 69, 76 and 81.

Faecal egg counts were made once a week on all lambs from day 76. The group mean faecal egg counts in vaccinated and control lambs after challenge infections are depicted in Fig. 1.

¹ 1 rad = 1.00×10^{-2} Gy.

TABLE I. EXPERIMENTAL DESIGN AND GROUP MEAN FAECAL EGG COUNTS IN VACCINATED AND CONTROL LAMBS AFTER IMPULSE AND SEQUENTIAL CHALLENGES WITH 20 000 *T. colubriformis* LARVAE (epg = eggs per gram)

Groups	No. of animals	Double vaccination	Triple vaccination	Anthelmintic	Type of challenge	Mean of each animal (epg)	Group geometric mean (epg)	% protection
A	1					513.3		
	2	+	-	+	4 × 5000	290.0	558.8	
	3					963.3		
	4					465.6		
	5	+	-	+	20 000	1046.8	1071.8	
	6					1700.0		64.5
B	7					471.8		
	8	-	+	+	4 × 5000	553.1	669.4	
	9					983.3		
	10					550.0		
	11	-	+	+	20 000	440.6	596.8	
	12					800.0		72.9
C	13					1796.8		
	14	-	-	+	4 × 5000	3183.3	2433.9	
	15					2321.8		
	16					2418.7		
	17	-	-	+	20 000	1765.6	2248.9	
	18					2562.5	2341.4	-

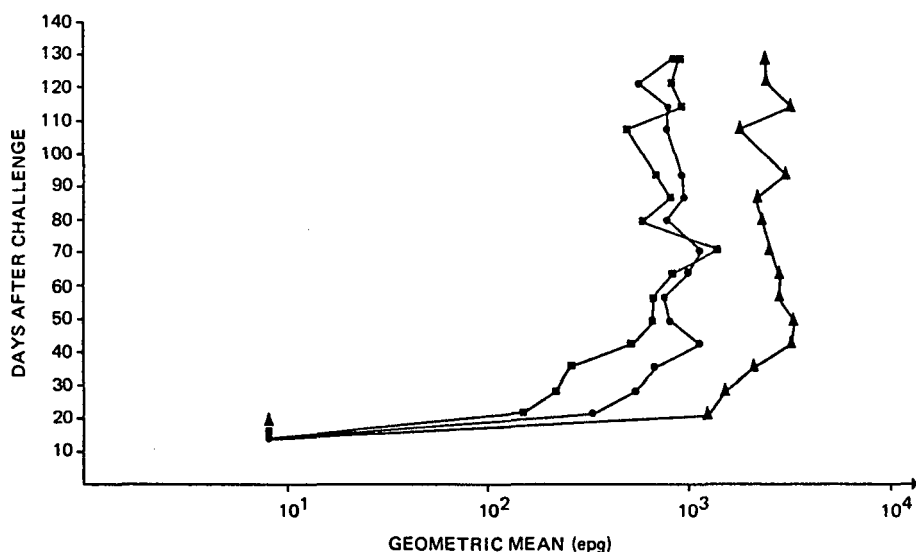


FIG. 1. Mean faecal egg counts (epg = eggs per gram) on three groups of lambs subjected to impulse and sequential challenge infections with *T. colubriformis* (●—● Group A; ■—■ Group B; ▲—▲ Group C).

As judged by the faecal egg count parameter, there were significant differences between the means of the vaccinated and control groups ($P < 0.01$). The efficacy of vaccination in Groups A and B was 64.5 and 72.9%, respectively. The degree of resistance acquired by vaccination was not affected by impulse or sequential challenge infections. The results of our experiment are shown in Table I.

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IAEA-SM-292/31P

**SOME MANAGERIAL FACTORS AFFECTING
THE REPRODUCTIVE PERFORMANCE OF
DAIRY COWS IN TURKEY***

S. ÖZSAR**, B. GÜVEN**, H. IZGÜR***,
Z. EMRE**, S. COŞAR⁺

**Lalahan Nuclear Research Institute
of Animal Health,
Turkish Atomic Energy Authority,
Lalahan

***Clinic for Obstetrics and Gynaecology,
Faculty of Veterinary Medicine,
University of Ankara

⁺Lalahan Research Institute of Zootechnics,
Ministry of Agriculture,
Lalahan

Ankara, Turkey

An extended calving interval in dairy cows, largely due to managerial problems such as inefficient detection of oestrus (timing of AI, silent or unobserved heat) and also failure of early non-pregnancy diagnosis between parturition and conception, is one of the major causes of losses to dairy production in Turkey.

This study was aimed at providing a more manageable rate of increase in production potential; therefore, skimmed milk progesterone determination (as compared with regular examinations of the ovarian structures by rectal palpation) was used to demonstrate management influences on suboptimal reproductive performance.

The studies were carried out on a government experimental farm and involved 85 dairy cows (Holstein, Jersey, Swiss Brown × local breed) which were maintained at a good nutrition level and under stanchion conditions in a barn. At the beginning of the study all the cows were separated into those that were pregnant and those that were not according to the farm records (41.7 and 58.3%, respectively). Forty lactating non-pregnant cows selected at random were studied. The ages of the cows averaged (mean ± SD) 4.25 ± 1.73 years and the interval between the last calving and

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the beginning of the study was 105.33 ± 75.30 days. First, the cows were examined by rectal palpation and 17.5% were found to be pregnant (approximately 1.5 to 2 months), 12.5% had endometritis problems (without any ovarian structures) and 7.5% were still in anoestrus (mean $\pm$ SD, 72.5 ± 24.2 days postpartum). The remaining cows, whose progesterone concentrations showed that they were on a normal cycle, were confirmed as having silent or unobserved heat (repeat breeders). In spite of the fact that after calving these animals had been inseminated several times they were still open, suggesting that they had been inseminated at an unsuitable time (improper timing of AI), which means incorrect oestrus observations had been made during the luteal phase or AI had been carried out too early to have any chance of conception.

Milk samples were collected at morning milking on every other day. The progesterone concentrations measured by RIA in skimmed milk were carried out according to the method described by Meyer et al. [1], and all the animals were controlled twice daily by herdsmen for visible oestrus symptoms; rectal palpation tests were made periodically (twice a week). During the experiment 25 cows were inseminated after confirmation of heat (as judged by skimmed milk progesterone levels and rectal palpation). The results of non-pregnancy diagnosis by progesterone concentrations at 21 days after AI showed that 88% of the cows were pregnant. The skimmed milk progesterone levels were significantly higher in pregnant cows (mean $\pm$ SD, 3.87 ± 2.11 ng/mL) compared with those in non-pregnant cows (0.22 ± 0.09 ng/mL). Cows with a progesterone concentration in skimmed milk <0.4 ng/mL were considered to be non-pregnant and those with a concentration of 0.9 ng/mL or more were regarded as pregnant. The accuracy of the test with RIA for judgement of pregnancy confirmed by rectal palpation about 50 days after AI was high, about 87%. However, evaluations of the data from a larger number of cows are now under way. On the other hand, to demonstrate the time from parturition to commencement of ovarian activity, ten cows were examined regularly (beginning from parturition) and it was found that seven had commenced ovarian activity (as indicated by a rise in the skimmed milk progesterone level) by 43 days postpartum (mean $\pm$ SD, 30.28 ± 9.56 days).

In conclusion, in terms of productivity and economic performance the most important factor in lowering bovine fertility was a failure to detect oestrus in normal females. The results demonstrated that the oestrus detection rate affects the interval from calving to first service. Thus, use of skimmed milk progesterone concentrations will help in reducing losses due to infertility, selecting therapeutic methods and increasing the profitability of dairy farming.

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IAEA-SM-292/33P

**INVESTIGATION OF PROGESTERONE PROFILES
IN POSTPARTUM DAIRY COWS***

R. KOVAČEVIĆ, L. KRSMANOVIĆ,
D. MARIĆ, S. VESELINOVIĆ, R. PERKUČIN
Institute of Biology and Veterinary Medicine,
University of Novi Sad,
Novi Sad, Yugoslavia

Investigations were carried out on 245 Holstein-Friesian dairy cows from nine herds located on one large commercial farm. The cows were housed and from November to April were fed a diet of alfalfa hay, corn silage, noodle beet with supplemental protein and minerals according to the levels of milk production; from May to October the animals were fed a diet of different herbage and supplemental protein and minerals. They were milked twice daily.

Analysis of the distribution of 245 cows according to the intervals between calving and first artificial insemination (AI) and calving and conception showed that the greatest proportion of cows were first inseminated during the third and fourth months. They became pregnant during the fourth and fifth months, or even later, which means that the intervals between calving and conception were extended (i.e. extended calving intervals). On the other hand, almost 50% of the cows became pregnant after the first or second AI (Fig. 1).

To explain such extended calving intervals, milk progesterone profiles were measured in 20 cows from parturition up to 80 days postpartum. Samples of first milk were collected twice a week and the levels of progesterone were determined by means of radioimmunoassay, using tritiated progesterone and antiserum No. 377. According to the progesterone levels determined, 12 out of 20 cows (60%) showed no ovarian activity until 60 days postpartum, while half of them were in anoestrus even after 75 to 80 days postpartum. Of these cows, four were after first calving, which according to the results of Hansen et al. [1] could partly explain such a long anoestrus. Another group consisted of eight cows which started to cycle during the first or second month postpartum. Two of them were inseminated at the wrong time. Three cows were not inseminated during the period under observation, even though ovarian activity was initiated at the end of the first or beginning of the second month postpartum. It is known, however, that fertility improves if cows are mated at the second

* Research carried out with the support of the Voivodina Research Association and the IAEA under Research Contract No. 3187/IG.

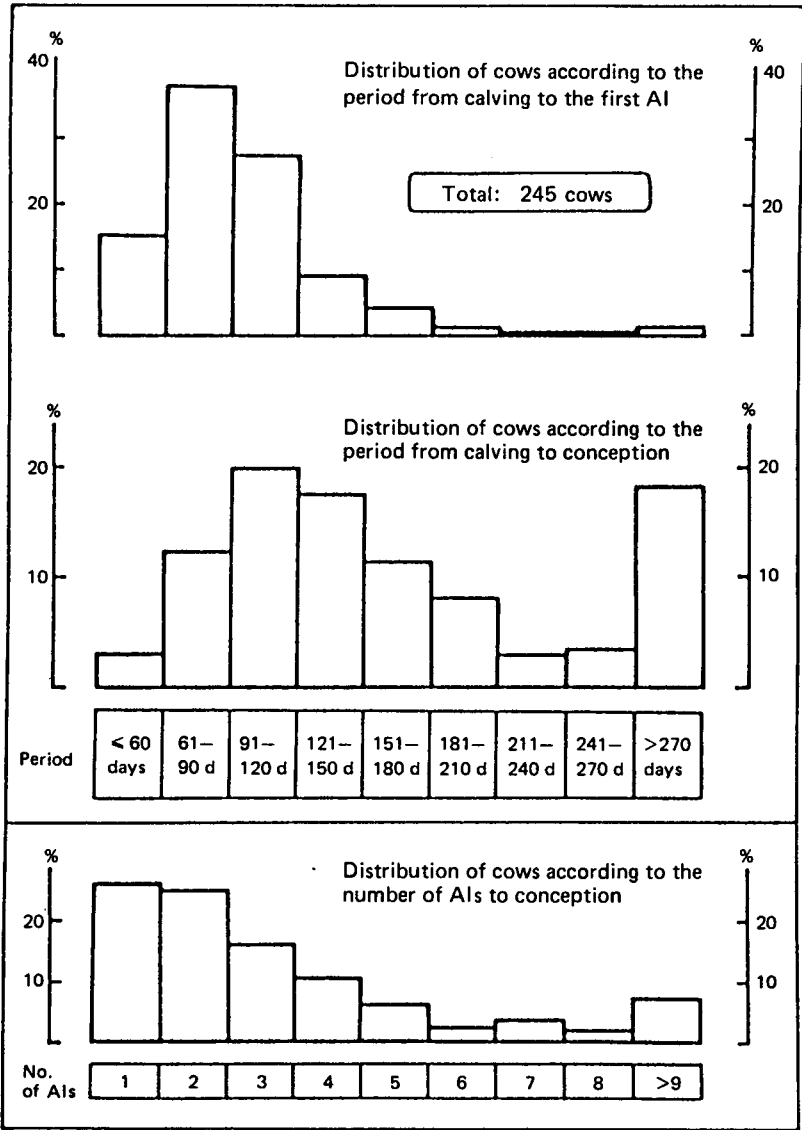


FIG. 1. Distribution of dairy cows according to the intervals between calving and first insemination, calving and conception, and the number of AIs to conception.

or third ovulation compared with the first [2] and that breeding early in the postpartum period is associated with lower fertility. On the other hand, it has also been suggested that cows that exhibited oestrus earlier conceived earlier, even though their fertility was lower [1]. Some cows in both groups had retention of foetal membrane and/or endometritis. These disturbances were more frequent in the first group.

The results suggested that extended calving intervals could partly be explained by the occurrence of anoestrus in 60% of the cows under investigation, and that 'fertility prophylaxis' is necessary in these herds to achieve restoration of ovarian activity earlier in the postpartum period. On the other hand, it is obvious that some problems exist concerning heat detection (occurrence of silent heat or inadequate detection of heat by personnel), which could also account for the prolonged calving intervals.

ACKNOWLEDGEMENT

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IAEA-SM-292/34P

POSSIBILITY OF EARLY PREGNANCY DIAGNOSIS IN SOWS BY RIA OF SERUM PROGESTERONE

Dj. DJURDJEVIĆ, P. NIKOLIĆ, V. STOJIĆ
Faculty of Veterinary Medicine,
University of Belgrade,
Belgrade, Yugoslavia

Early pregnancy diagnosis in sows is of great importance for the successful breeding of pigs, particularly on large farms where artificial insemination (AI) is applied on a wide scale. Different methods were used for pregnancy diagnosis in sows. Many are very complicated and difficult to perform, need special equipment (X-ray machine, ultrasonic generator) and are not sufficiently sensitive.

Having had good experience in the application of progesterone radioimmunoassay in early pregnancy diagnosis in cows [1-3], we decided to evaluate the possibility of its application in sows, since the oestrous cycle in this species is 21 days, on average, with the difference that more corpus lutea are formed on the ovaries.

In 1983 and 1984, 20 and 23 healthy cross-bred sows of the Swedish Landrace and large Yorkshire breeds were examined, respectively. The blood samples for progesterone RIA were taken by puncture of the vena cava cranialis before AI (day 0) and 7, 14 and 21 days after AI. Highly specific progesterone antiserum produced in rabbits and progesterone labelled with ^{125}I were used in RIA of progesterone. All measurements of radioactivity were done in a well-type scintillation counter (Nuclear-Chicago Corporation, USA).

To check pregnancy diagnosis, the number of healthy and dead piglets was determined at parturition.

The results of the first experiment showed that serum progesterone levels varied from 0 to 1.65 nmol/L, with an average of 0.95 ± 0.41 nmol/L on day 0 (before AI). These low values demonstrated that detection of oestrus was accurate in all sows. The serum progesterone levels then increased to reach average values of 21.0 ± 6.0 , 43.2 ± 15.6 and 61.3 ± 15.9 nmol/L on days 7, 14 and 21, respectively.

On the basis of the high progesterone levels on day 21 after AI, all sows were diagnosed as pregnant. During pregnancy one sow died on day 85, and one sow returned to oestrus on day 95. The remaining 18 sows farrowed at term, producing 179 healthy piglets and four dead piglets. Thus, diagnosis on day 21 after AI on the basis of high progesterone concentration was confirmed in 90% of the sows.

Similar results were obtained in the second experiment, in which the serum progesterone concentrations were, on average, 2.38, 52.73, 76.3 and 74.41 nmol/L on days 0, 7, 14 and 21, respectively. On day 21 one sow had a low progesterone level (2.6 nmol/L) and therefore was designated as non-pregnant. This diagnosis was confirmed, since the sow returned to oestrus two weeks later, showing that negative diagnosis of pregnancy was 100% accurate.

There was no direct relationship between the serum progesterone levels in pregnant sows on day 21 after AI and the number of piglets farrowed, probably due to the death of different numbers of embryos and their mummification and resorption.

On the basis of these results it can be concluded that RIA of serum progesterone on day 21 after insemination is a very suitable and precise method for early pregnancy diagnosis in sows. There is no direct relationship between serum progesterone concentration on day 21 after insemination and the number of piglets farrowed.

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IAEA-SM-292/37P

YIELD AND FLOW OF MICROBIAL NITROGEN IN P-DEPLETED SHEEP AS DETERMINED BY USE OF ^{15}N *

H. HÖLLER, G. BREVES

Physiologisches Institut der Tierärztlichen
Hochschule Hannover,
Hannover, Federal Republic of Germany

1. INTRODUCTION

Phosphorus depletion of sheep is known to have effects on various body tissues and functions, including phosphorus concentrations and turnover in the gastrointestinal tract. Since microbial protein synthesis in the rumen requires certain concentrations of electrolytes, e.g. phosphate, it was of interest to study gastrointestinal nitrogen turnover in P-depleted sheep. Nitrogen-15 was used as a marker of microbial N, and two different experimental approaches were compared.

2. METHODS

Castrated male sheep (8 to 9 months, 45 to 50 kg) were supplied with rumen cannulae, re-entrant duodenal and T-shaped ileal cannulae. They were fed a semi-synthetic diet of chopped straw (320 g/d) and pellets (660 g/d) consisting of corn starch (72%), sucrose (6.5%), soybean meal (13.8%), urea (2.3%), minerals and trace minerals (5.2%) and vitamins (0.2%). This diet was deficient in P and provided, on average, 1.05 g P per day during depletion periods. For P repletion the sheep received additionally a twice daily intraruminal or intraduodenal infusion of 350 mL isotonic phosphate solution to adjust the total daily P intake to approximately 4.2 g.

Gastrointestinal N balances in six sheep were obtained from N analyses in lyophilized samples from food, duodenal and ileal contents and from faeces, ammonia-N was determined after samples had been incubated with urease, and non-ammonia-N (NAN) was calculated by difference. Digesta flow into the proximal duodenum and from the terminal ileum was obtained by continuous intraruminal infusion of Cr-EDTA as a fluid marker and repeated dry matter determination in duodenal and ileal contents.

In experimental series 1, five depletion and five repletion experiments were carried out on three sheep to estimate the daily flow of microbial N into the duodenum

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and out of the ileum. $^{15}\text{NH}_4\text{Cl}$ and Cr-EDTA were infused intraruminally for 134 hours, and samples were repeatedly taken from the rumen, duodenum and ileum during the last 48 hours. A bacterial reference fraction from the rumen was prepared by differential centrifugation; ^{15}N analyses were carried out by emission spectrometry. The intestinal flow of microbial N was obtained by multiplying the daily flow of NAN by the ratio between ^{15}N in intestinal N and rumen bacterial N.

The daily flow of microbial N was:

$$\frac{\text{at. \% } ^{15}\text{N in intestinal N}}{\text{at. \% } ^{15}\text{N in rumen bacterial N}} \times \text{daily flow of NAN}$$

In experimental series 2, 11 depletion and 11 repletion experiments were performed in six sheep to calculate the daily net yield of microbial N in the rumen. $^{15}\text{NH}_4\text{Cl}$ and Cr-EDTA were infused intraruminally for 105 to 110 hours. Bacterial reference fractions from the rumen were prepared as before, and rumen fluid samples for ammonia, ^{15}N and Cr analyses were taken during the last 2 days of infusion.

The daily net yield of microbial N was then:

$$\frac{^{15}\text{N infusion rate} - (\text{NH}_3\text{-}^{15}\text{N net absorption} + \text{total ammonia-}^{15}\text{N outflow})}{^{15}\text{N in rumen bacterial N}}$$

$\text{NH}_3\text{-}^{15}\text{N}$ net absorption and total ammonia- ^{15}N outflow from the rumen with the liquid phase were calculated on the basis of ^{15}N enrichment in the total ammonia-N fraction, average ammonia concentrations and fluid turnover.

3. RESULTS

Phosphorus depletion did not affect the flow of total N and NAN into the duodenum and from the ileum, i.e. the apparent digestibility of total N in the small intestines remained unchanged (50 to 55%). In the large intestines the total N digestibility was 41% in P-repleted and 29% in P-depleted sheep, constituting a digestibility reduction of 29%. Microbial N made up 94% of the NAN flowing into the duodenum and 88% of that leaving the ileum in P-repleted sheep compared with 72 and 64% in P-depleted sheep. This resulted in a reduction of microbial N flow into the duodenum by 26% and from the ileum by 33% (experimental series 1).

In experimental series 2 the daily yield of rumen microbial N was found to be reduced by 27% when the sheep were brought from the repleted into the depleted state (9.5 versus 13.1 g N/d).

4. CONCLUSIONS

Dietary phosphorus depletion with resultant reduction of concentrations of inorganic P in the rumen fluid led to a significant decrease of the daily net yield of microbial N in the rumen. Since dry matter digestibility in the gastrointestinal tract before the duodenum was not changed by the depletion procedure, the efficiency of microbial growth was lower in the P-depleted state. Almost identical results were obtained irrespective of whether ^{15}N was used to calculate the net yield of microbial N in the rumen or to measure the flow of microbial N into the proximal duodenum.

IAEA-SM-292/38P

EFFECT OF DIETARY N ON FIBRE DIGESTION*

R.H. SMITH, A.B. McALLAN, R.J. MERRY

National Institute for Research in Dairying**,
University of Reading,
Shinfield, Reading,
United Kingdom

1. INTRODUCTION

Earlier experiments with young steers have shown that supplementing diets deficient in available nitrogen (rumen degradable nitrogen (RDN)) with urea or proteins led to increases in fibre digestion and the efficiency of microbial protein synthesis in the rumen [1], and that the former was greater when the N supplement was fish meal [2].

These studies were extended to compare other protein sources in vivo and to examine further the fish meal, urea comparison in vitro.

2. IN VIVO STUDIES

Four young Friesian steers (approximately 135 kg body weight) were given four diets supplying metabolizable energy intakes sufficient for a growth rate of 0.5 kg/d and apparently adequate RDN intakes [3] in a Latin square design. The main dietary components (proportions of total diet dry matter in parentheses) were alkali-treated straw pellets (0.43) and rolled barley (0.39) with: (A) tapioca (0.13), casein (0.02),

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** Now Animal and Grassland Research Institute.

TABLE I. DIGESTIBILITIES OF FIBRE-
BOUND SUGARS AND EFFICIENCIES OF
MICROBIAL PROTEIN SYNTHESIS
BETWEEN MOUTH AND ABOMASUM

Diet	Estimated fractional true digestibility mouth to abomasum			Efficiency of microbial protein synthesis (g N/kg TDOM)
	Cellulose glucose	Xylose	Arabinose	
A	0.61	0.63	0.67	20.6
B	0.68	0.67	0.71	17.7
C	0.75	0.77	0.79	16.4
D	0.75	0.79	0.82	18.0
Significance of effects (P < or not significant (NS))				
A/B	NS	0.05	0.01	NS
A/C	0.01	0.02	0.01	NS
A/D	0.01	0.01	0.01	NS
B/C	0.05	0.05	NS	NS
B/D	0.05	0.05	0.05	NS
C/D	NS	NS	NS	NS

urea (0.02); (B) tapioca (0.05), single cell protein (0.11), urea (0.01); (C) tapioca (0.03), maize gluten meal (0.12), urea (0.01); (D) rapeseed meal (0.19), urea (0.01). The experimental and analytical procedures were essentially as described before, with estimations of N, carbohydrate and organic matter (OM) flows at the abomasum using Cr_2O_3 and polyethylene glycol in a dual marker technique [4] and ^{35}S infusion to distinguish microbial N flows [5]. The efficiencies of microbial protein synthesis were calculated in terms of truly digested organic matter (TDOM). The results are shown in Table I.

Although no diets were deficient in RDN according to Agricultural Research Council recommendations [3], the presence of maize gluten or rapeseed protein led to greater fibre digestion than did that of the other protein sources.

TABLE II. DIGESTIBILITIES OF FIBRE-
BOUND SUGARS AND EFFICIENCIES OF
MICROBIAL PROTEIN SYNTHESIS IN
IN VITRO CONTINUOUS CULTURE

Diet	Fractional true digestibility in apparatus			Efficiency of microbial protein synthesis (g N/kg TDOM)
	Cellulose glucose	Xylose	Arabinose	
1	0.43	0.56	0.54	31.0
2	0.53	0.58	0.58	30.3

3. IN VITRO STUDIES

The apparatus and technique employed and the composition of the urea-based diet (Diet 1) and the fish meal + urea-based diet (Diet 2) have been described elsewhere [6].

The results for digestibilities of structural sugars and efficiencies of microbial protein synthesis estimated from ^{32}P incorporation rates are shown in Table II.

In the presence of fish meal, digestion of fibre components was enhanced, as has been shown earlier in in vivo experiments. Differences were significant ($P < 0.05$) for cellulose glucose and arabinose.

4. GENERAL CONCLUSIONS

The findings provide support for the view that the kind, as well as the amount, of RDN provided in ruminant diets may be important in ensuring optimum digestion of dietary fibre even though the efficiency of microbial protein synthesis (g N incorporated/kg TDOM) may be unaffected. It appears likely that the most effective fibre digestion requires the presence of adequate preformed amino acids and/or peptides in a relatively undegradable form.

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IAEA-SM-292/39P

**MANIPULATION OF RUMEN FERMENTATION
TO ENHANCE MICROBIAL ACTIVITY
BY USING MOLASSES-UREA BLOCKS***

H.A. EL FOULY

Department of Radiobiology,
Nuclear Research Centre,
Abou Zaabal, Egypt

R.A. LENG

Department of Biochemistry, Microbiology
and Nutrition,
University of New England,
Armidale, New South Wales,
Australia

Various ways of supplementing non-protein nitrogen (NPN) to ruminant animals are used to raise the rumen ammonia levels and thereby increase the microbial activity and digestibility of straw [1]. The success of any of these systems depends mainly on considering the nutritional principles that lead to optimized rumen function and the overall animal requirements, along with the existing socio-economic conditions of the users. This presentation deals with the research findings of specific formulations of multnutritional urea-molasses solidified licks (blocks). In these formulas the following set of scientific principles were considered:

- (1) Maintaining sufficient and uninterrupted release of ammonia in the rumen by continuous licking of the block.
- (2) Providing a limited quantity of fermentable energy (molasses and corn) to minimize the inverse interaction between fermentable carbohydrates and fibre digestion.

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TABLE I. DIETARY INTAKES AND LIVE WEIGHT CHANGE OF LAMBS FED EITHER PELLETTED FEED, BLOCKS OR MOLASSES-UREA-STRAW FEED

	Block	Rate of consumption		Live weight change (g/head per day)
		Rice straw	Urea	
A (pelleted rations)	—	510	—	10
B Block	146	450	22	17
C Molasses-straw feed	—	550	25	40

A = 420 pelleted rations which cover the maintenance requirements of the animals.

B = Urea-molasses block (15% urea).

C = 300 g molasses containing all the ingredients equivalent to 170 g of the block, except corn. These were well mixed with 300 g of rice straw.

(3) Adding sulphur to maintain an optimum N/S ratio in the rumen required for the de novo biosynthesis of thioaminoacids. Intraruminal infusion of a solution containing both ^{35}S and ^{15}N reveals that the ratios of these two isotopes present in bacterial cells ranged between 6.8 and 13.1, with an overall average of 11.1. In the blocks the N/S ratio was near 10.

(4) Adding potassium ions (3 to 4% in the block). K^+ was recently found to enhance $\text{NH}_3\text{-N}$ utilization by rumen microorganisms, probably by regulating the rumen osmotic balance and acid-base-equilibrium and through its catalytic function on many of the enzymes [2]. Besides, it compensates for urinary K^+ losses due to the diuretic action of the absorbed ammonia.

(5) Adding the recommended levels of vitamins and macro- and micro-elements.

Many research trials connected with the preparation of the block, the response of sheep to consuming the block and how to stimulate further the animal's productivity are discussed.

Heating and the levels of urea and molasses are related factors that determine the degree of solidification of the block. Excellent blocks were obtained by pre-heating the molasses to 80 to 100°C before adding and mixing the other ingredients. In blocks containing a high urea level (20%), the molasses percentage should not exceed 40 to 45% of the ingredients. The molasses percentage can be increased up to 55% of the components of the block if a low urea content (10%) is required. The blocking agents used in these trials were limestone, dolomite and either crushed corn, rice bran, casein or ground rice hulls. However, all the in vivo trials were made using the crushed corn blocks.

In a 3-week trial, three groups of lambs (5 to 6 animals each) were offered three types of urea-molasses licks plus ad libitum rice straw. The licks were almost similar in composition, but contained 10, 15 or 20% urea. The rate of consumption from the blocks and rice straw were progressively decreased by increasing the urea level in the block. On average, the lambs consumed 136, 112 and 18 g/head per day of the blocks and 441, 550 and 326 g/head per day of rice straw, relevant to lamb's given the 10, 15 and 20% urea, respectively. Individual animals within each group varied in their intakes of the blocks; there were high, moderate and poor consumers. With the 20% urea blocks, four out of five lambs did not lick any of their blocks.

To evaluate the nutritional potential of the molasses blocks in view of the present system of ruminant nutrition in Egypt and the licking behaviour of lambs, three groups of lambs (five each) were given either diet A, B or C and ad libitum rice straw (see Table I). The data reveal that the blocks (diet B) have the same if not better nutritional potential as the pelleted ration (diet A) when both were provided to cover the maintenance requirements of the lambs. Providing a fixed amount of the nutrients present in the block (170 g) in the molasses-urea-straw feed (diet C) improved both the plain rice straw consumption and live weight gain of the lambs compared with diets A and B (Table I). These results indicate that the overall performance of the animals can be further improved by developing molasses-urea-rice straw blocks.

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RESPONSES OF SHEEP FED PERUVIAN FISH MEAL AS A SUPPLEMENT TO AGRO-INDUSTRIAL BY-PRODUCTS AND NATIVE PASTURES OF THE HIGH ANDES*

V. TALAVERA

Departamento de Nutrición,
Universidad Nacional Agraria,
La Molina, Lima, Peru

Previous experiments have shown that when the nitrogen content of a basal diet is low, feeding strategies should focus on maximizing fermentative digestion and then on promoting adequate availability of bypass protein and glucose for ruminants [1]. Also, addition of fish meal as a source of protein that escapes ruminal fermentation (bypass protein) is an effective way of promoting efficient growth and production in ruminants consuming mature roughages [2]. Based on these findings, experiments were conducted to observe the effects of the addition of Peruvian fish meal (PFM) to the diet of sheep maintained on native pastures or fattened with agro-industrial by-products.

Rumen protein degradability on nylon bags (Tables I, II [3]) was lowest for PFM dried by a thermal process (flame dried). By supplementing PFM, corn grain or urea to a basal diet of sugar-cane molasses, corn-cobs, corn stover and wheat (27 to 31, 41 to 46, 9 to 11 and 8 to 10%, respectively) showed that PFM and PFM + urea increased the feed intake, live weight gain and feed conversion ratio (Table III [4]); a combination of PFM, urea and corn grain supplement to the basal diet proved to be better than the other treatments.

On native pastures of the Peruvian High Andes (3800 m) castrated sheep were supplemented with 36 g of PFM per day during the rainy season; this was not enough to show a significant difference to a diet with mineral supplementation only (Table IV [5]). The same experiment carried out in the dry season showed poor animal growth rates for all the treatments.

Use of PFM as a supplement (50 g/d per animal) during the breeding and gestation periods of ewes clearly showed an increase in the weight gain during gestation, the number of lambs born alive, the weaning rate and the wool weight (Table V [6]).

The results of these experiments demonstrated that PFM is the highest source of non-fermentable protein in Peru and that it increases the feed intake, weight gain and feed conversion ratio of sheep fed agro-industrial by-products; it also improves the reproduction performance of ewes maintained on native pastures.

* Research carried out with the support of the IAEA under Research Contract No. 2879/RB and Technical Co-operation Project No. PER/5/014.

TABLE I. RUMEN PROTEIN DEGRADABILITY OF DIFFERENT SOURCES (%)

Incubation time	Corn grain	Feather meal poultry	Alfalfa meal sun-cured	Cotton seed meal	Soybean meal	Rice polishings
4	18.7	19.5	31.6	30.4	21.0	35.5
8	27.5	23.8	36.21	34.1	21.0	42.0
12	29.3	27.7	44.9	38.0	27.7	46.5
24	38.6	30.4	49.5	41.7	32.3	56.0
48	37.3	37.3	59.6	63.4	66.8	68.0

Talavera and Tinoco [3].

TABLE II. EFFECTS OF PROCESSING AND STORAGE TIMES ON THE DEGREE OF RUMINAL PROTEIN DEGRADABILITY OF FISH MEAL

Incubation time (h)	Per cent of degradable protein		
	Sun dried	Sun dried, stored for 5 months	Thermal process stored for 5 months
4	26.7	14.9	19.9
8	27.0	17.9	21.1
12	28.1	20.1	24.5
24	31.3 ^a	23.2 ^b	26.6 ^c
48	4.4	31.7	35.6

^{a,b,c,d} Values on the same line with different superscripts differ ($P < 0.05$).
Talavera and Tinoco [3].

TABLE III. EFFECTS OF UREA, CORN GRAIN AND FISH MEAL SUPPLEMENTATION ON PERFORMANCE OF SHEEP*

	Basal	Basal + urea	Basal + urea + fish meal	Basal + fish meal	Basal + corn	Basal + corn + fish meal	Basal + urea + corn	Basal + urea + corn + fish meal
Initial weight (kg)	18.8	18.5	18.4	19.1	19.0	19.1	18.9	18.7
Final weight (kg)	18.1	19.5	24.8	25.3	16.4	24.5	17.5	26.3
Daily gain (g)	-28	42	108 ^{a,b}	101 ^b	-59	89 ^b	-20 ^c	125 ^a
Days fed	24	24	60	60	45	60	60	60
Daily intake (kg)	0.79	0.81	1.05	1.07	0.72	1.03	0.76	1.00
Feed/gain	—	19.13	9.80	10.6	—	11.7	—	8.0
mg/N-NH ₃ per 100 mL	4.7	9.8	11.6	9.1	3.6	7.6	3.6	12.0
Wool length (cm)	—	—	1.88 ^a	1.46 ^b	0.73 ^d	1.55 ^b	1.0 ^c	1.88 ^a
Economic merit (%)	—	—	8.5	7.6	—	1.1	-21.1	8.0

* Fifteen castrated sheep/treatment; 6 months of age.

^{a,b,c,d} Values on the same line with different superscripts differ ($P < 0.05$).

Talavera and Carrión [4].

TABLE IV. EFFECTS OF MINERALS AND FISH MEAL SUPPLEMENTATION TO SHEEP ON NATIVE PASTURE DURING THE RAINY SEASON IN THE HIGH ANDES (3800 m)*

	Treatments			
	Salt + vitamin A	Salt + vitamin A + minerals	Salt + vitamin A + fish meal	Salt + vitamin A + minerals + fish meal
Initial weight (kg)	30.2	30.4	29.4	29.8
Final weight (kg)	35.5	37.3	37.2	38.2
Daily gain (g)	58.1 ^a	76.6 ^b	86.1 ^b	93.7 ^b
Carcass yield (%)	39.2 ^a	40.7 ^b	39.8 ^{a,b}	41.1 ^b

* Twenty-four castrated sheep/treatment; 15 months of age; 90 days on native pasture.

^{a,b} Values on the same line with different superscripts differ ($P < 0.05$).

Talavera and Gómez [5].

TABLE V. EFFECTS OF FISH MEAL AND MINERAL SUPPLEMENTATION ON THE BREEDING PERFORMANCE OF EWES FOLLOWING FIRST MATING*

Measurements	Treatments				
	Season Breeding	Fish meal + minerals**	Minerals	Fish meal + minerals	Minerals
	Gestation	Fish meal + minerals	Fish meal + minerals	Minerals	Minerals
	Lambing	Fish meal + minerals	Fish meal + minerals	Minerals	Minerals
Ewes initial weight (kg)	31.3	30.9	31.0	31.1	
Ewes final weight (kg) (135 d)	40.6 ^a	39.7 ^a	37.8 ^b	37.58 ^b	
No. of lambs born alive	242	238	231	221	
Weight of lambs born (kg)	3.80 ^a	3.75 ^a	3.58 ^b	3.54 ^b	
Weaning number	229	223	207	193	
Weaning rate (%)	91.6	89.2	82.8	77.2	
Ewes wool weight (kg)	3.14				

* Two hundred and fifty ewes/treatment; native pasture, High Andes (3800 m).

** Minerals (iodine, copper, cobalt and zinc).

^{a,b} Values on the same line with different superscripts differ ($P < 0.05$).

Talavera and Ríos [6].

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FORAGE PARTICLE BREAKDOWN AND MOVEMENT IN THE RETICULO-RUMEN OF CATTLE*

P.M. KENNEDY**, A. LIRETTE,
K. CHAI, L.P. MILLIGAN
Department of Animal Science,
University of Alberta,
Edmonton, Alberta, Canada

Two steers fitted with cannulas in the oesophagus and rumen were given hay diets of alfalfa, red clover, reed canary grass or brome grass in either a chopped or ground and pelleted form. Collections from the oesophageal fistula were made of boluses regurgitated during rumination of the chopped diets. Diets were given at 95 % of voluntary feed consumption at intervals of 2 h.

Rumination of the chopped diets required 28 to 36 000 chews/d, compared with values of 3 to 10 000 chews/d for the pelleted forms. The weight of material regurgitated during rumination was 786 g, of which 493 g were swallowed before chewing commenced. Fractionation of particulate DM by wet sieving showed that the material

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** Present address: Commonwealth Scientific and Industrial Research Organization, Aitkenvale, Queensland, Australia.

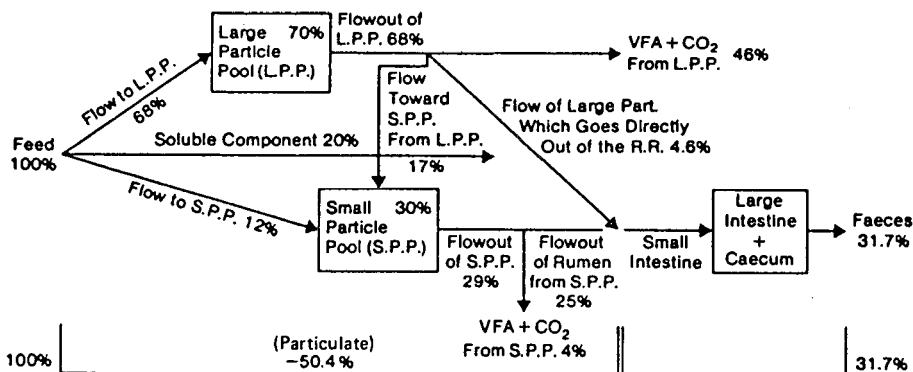


FIG. 1. Quantitative kinetic model of ruminant digestive tract with ^{51}Cr -mordanted feed particles at a low concentration (0.01% Cr).

retained in the mouth for chewing contained 41% more large particulate matter than did regurgitated material. Approximately 70% of the large particles retained in the mouth were comminuted to small particles during each cycle of rumination. Comminution per chew was positively related to the amount of large particulate matter retained in the mouth for chewing and negatively to the number of chews/cycle. The specific fragility (specific effectiveness of breakdown) of the acid detergent fibre of the legumes was 35% greater than that of the grasses. Rumination could account for comminution of 80 to 93% of the large particles which entered the rumen. Measurements of digestion of dietary materials from nylon bags and rates of outflow of small particles (Cr-mordanted) and water (Co-EDTA) from the rumen were used in conjunction with intake and chewing measurements to predict the rumen content of large particles.

Subsequently, a quantitative kinetic reticulo-rumen model was developed for forage particles using grass stems mordanted with ^{51}Cr at very low concentrations. This technique maintains a high digestibility of the feedstuff. Four fistulated steers fed brome grass hay were used in a Latin square design. Two different Cr concentrations (0.01 and 0.5%) were used to mordant two different lengths (0.1 to 0.2 cm and 1 cm) of neutral detergent fibre extracted brome stems. Observations made from the model (Fig. 1) representing the results are:

- (1) The ratio of the large particle pool (> 3.35 mm) to the small particle pool (< 3.35 mm) is 2:1
- (2) Volatile fatty acids and CO₂ are produced mainly from the large particle pool
- (3) Most of the digestion of the hay takes place in the reticulo-rumen
- (4) The soluble portion of the hay is a major fraction
- (5) Exit from the reticulo-rumen is composed of 40% soluble components, 50% small particles and 10% large particles
- (6) The size of the particles influences their turnover time in the caecum.

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ESTIMATION OF NITROGEN DIGESTIBILITY IN UNDEGRADED DIETARY PROTEIN BY THE IN SACCO PROCEDURE

T. HVELPLUND

Department of Research in Cattle and Sheep,
National Institute of Animal Science,
Ørum Sønderlyng, Denmark

Digestibility of undegraded dietary protein is an important factor in the new protein evaluation systems proposed [1-3], but only few estimates are available on the digestibility of this fraction in the small intestine of ruminants [4]. This presentation describes further experiments with an in sacco procedure which enables easy estimation of the digestibility of this protein fraction [4].

Nylon bags measuring 6×6 cm and made from polyamid cloth with a pore size of $9 \mu\text{m}$ were used. One gram of undegraded dietary protein, which was isolated from nylon bags after incubation in the rumen of a hay-fed cow for 16 hours, was placed in each bag which was then closed using a heat-sealing machine.

The nylon bags were incubated in a pepsin-HCl solution adjusted to a pH of 2.4. The nylon bags were incubated at 37°C for 2 hours with constant stirring. During incubation the pH was kept constant at 2.4 by titration with HCl.

Cows with a T-piece cannula in the duodenum and also a cannula in the distal ileum were used. The bags were introduced to the intestine with a spatula via the duodenal cannula, and as soon as the digesta passing the duodenum at the insertion point had washed the bag away the next bag was introduced. Bags were recovered from both the ileum and faeces. The bags were collected from the ileum by means of a magnet placed in the ileum cannula. They were caught at this point as they also contained three small iron balls. The bags were recovered from the faeces by washing the faeces in a sieve basket. After recovery the bags were washed thoroughly under running tap water (approximately 40°C) for at least 2 hours to remove all water-soluble nitrogen. Disappearance of nitrogen from the bags was calculated and taken as an estimate of the true digestibility of undegraded dietary nitrogen.

The influence of recovery of the bags at either the ileum or in the faeces on the N-disappearance from the bags is shown in Table I for undegraded residues from eight different feedstuffs. The table shows that there are only small differences in the observed N-disappearance from the bags recovered from either the ileum or the faeces and that the influence of place of recovery was found to have no significant effect on N-disappearance from the bags. This finding increases the potential of this method, as recovery of bags from the faeces is much easier than from the ileum.

TABLE I. DISAPPEARANCE OF NITROGEN FROM NYLON BAGS EITHER RECOVERED FROM THE ILEUM CANNULA OR THE FAECES

Undegraded residue from	Degradability of nitrogen	N-disappearance from bags			
		Ileum		Faeces	
		Mean	SEM	Mean	SEM
Cotton seed cakes	0.63	0.84	0.020	0.85	0.017
Fish meal (heat damaged)	0.21	0.05	0.008	0.03	0.009
Grass pellets	0.62	0.54	0.026	0.56	0.039
Coconut cakes	0.58	0.90	0.004	0.87	0.003
Palm kernel cakes	0.40	0.74	0.005	0.74	0.005
Rapeseed meal	0.50	0.77	0.014	0.79	0.020
Soybean meal	0.82	0.97	0.002	0.97	0.003
Soybean meal (heat-treated)	0.32	0.74	0.001	0.78	0.015

TABLE II. PASSAGE TIME OF THE BAGS BETWEEN THE DUODENUM AND THE ILEUM AND BETWEEN THE DUODENUM AND UNTIL EXCRETION WITH THE FAECES

Place of recovery	No. of bags	Passage time (h)		
		Mean	Minimum	Maximum
Ileum	46	5.6	4.0	7.4
Faeces	80	14.2	6.3	34.0

The time the bags spent in the intestine was recorded for 46 bags collected at the ileum and for 80 bags recovered from the faeces; the results are given in Table II.

The average passage time between the duodenum and the ileum was found to be 5.6 hours and the average time between the duodenum and until excretion with the faeces was found to be 14.2 hours. The table also shows a marked variation in passage time, but this variation had no significant effect on N-disappearance from the bags. This indicates that release of nitrogen from the feed inside the bags was not limited by the time the bags stayed in the intestine.

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ENZYMEIMMUNOASSAY OF OESTRONE CONJUGATE FOR A PREGNANCY DIAGNOSIS TEST IN SOWS AND GOATS

G. GALEATI, S. LODI,
F. CONTE, A. PRANDI
Istituto di Fisiologia Veterinaria,
Università di Bologna,
Bologna, Italy

1. INTRODUCTION

Seren et al. [1] set up an early pregnancy diagnosis test by measuring the levels of oestrone conjugate (E1C) in the urine of sows 25 days after insemination. Similarly, Heap et al. [2] and Tamanini et al. [3] found that the plasma levels of oestrone sulphate can be used as an index of pregnancy in goats. These authors measured the levels of the oestrone conjugate by using radioimmunoassay techniques. Bearing in mind the disadvantages of using radioisotopes, we performed a reliable enzyme-immunoassay suitable for measuring plasma and urine E1C concentrations.

2. MATERIALS AND METHODS

2.1. Animals and collection of samples

Three hundred large white sows and three hundred alpine goats were used. Samples of urine were collected from the sows 25 days after insemination according to the method of Seren et al. [1]. Blood samples were taken from the goats 55 to 80 days after mating. The samples were stored at -20°C until assayed.

TABLE I. CROSS-REACTIONS OF THE ANTISERUM ANTI-OESTRONE-3HS-BSA (%)

Oestrone	100
Oestrone glucuronate	62
Oestrone sulphate	48
Oestradiol-17 α	1
Oestradiol-17 β	1
Oestriol	0.01

2.2. Enzymeimmunoassay of E1C

2.2.1. Preparation of the enzymatic tracer

Oestrone-3-hemisuccinate (2.5 μ mol) was conjugated with horseradish-peroxidase (HPR) (0.25 μ mol) according to the method of Erlanger et al. [4] modified by Rajakowski et al. [5]. Conjugated oestrone was purified by gel chromatography on a column (50 $\times$ 1.5 cm) of Sephadex G100 according to the method of Osterman et al. [6], then it was dialysed at 4°C against phosphate buffer, 0.1M, pH 7, according to the method of Rajakowski et al. [5].

2.2.2. Antibody production and its adsorption on solid phase

The antibody anti-oestrone was produced by immunizing rabbits with oestrone-3-hemisuccinate-BSA according to the experimental protocol described by Motta et al. [7] and Tamanini et al. [8]. The antibody was purified as described by Axen et al. [9] and then dialysed against phosphate buffer 0.1M, pH 7. The cross-reactions of the antibody are shown in Table I.

To prepare the solid phase adsorbed antibody, polystyrene balls (6.5 mm in diameter) were incubated with the antiserum diluted to 1:2000 for 4 h at 4°C under continuous agitation. The balls were washed with phosphate buffer, 0.1M, pH 8, and then incubated for 2 h at 4°C in phosphate buffer, 0.1M, pH 8, containing 0.3% BSA and 0.01% thimerosal to prevent any successive non-specific absorption.

2.3. Enzymeimmunoassay procedure

To 0.1 mL of swine urine diluted to 1:25 or 0.02 mL of goat plasma, 0.1 mL of oestrone HRP (1:50 000) was added. The final volume was raised to 0.4 mL with phosphate buffer; then an antibody-coated ball was added to any assay tube, which

TABLE II. ACCURACY AND PRECISION OF E1C ENZYMEIMMUNOASSAY

	Urine	Plasma
Recovery (% + SE)	97.1 + 3.1	103.7 + 5.3
Sensitivity (pg)	4.2	4.2
Inter-assay precision (% CV)	6.9	5.3
Intra-assay precision (% CV)	9.1	9.2

TABLE III. ACCURACY OF PREGNANCY DIAGNOSIS TEST

	Sows		Goats	
	No.	(%)	No.	(%)
Animals	300		300	
Diagnosis (E1C test)				
Pregnant	259		261	
Not pregnant	41		39	
Accuracy				
Positive diagnosis	238	91.9	246	94.3
Negative diagnosis	39	9.51	39	100.0

was then incubated overnight at 4°C. To remove the free steroid the antibody-coated balls were washed twice with distilled water. O-phenylenediamine dihydrochloride (5.5 mmol) and hydrogen peroxide (4.4 mmol) in PBS, 0.1M, pH 6, were added to any assay tube, which was then incubated in the dark for 30 min at room temperature. To stop the reaction, 200 µL of H₂SO₄ (4N) were added and the enzyme activity was measured by reading the absorbance at 492 nm.

3. RESULTS AND DISCUSSION

The characteristics of E1C enzymeimmunoassay are shown in Table II and the accuracy of the pregnancy diagnosis test in Table III. These results are similar to those of Seren et al. [1], Heap et al. [2] and Tamanini et al. [3], who measured E1C levels by RIA.

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**UTILIZATION OF SUGAR-CANE BAGASSE
AS BASAL ROUGHAGE FOR GROWTH
OF NATIVE BULLS***

A.M.M. TAREQUE, S.A. CHOUDHURY

Department of Animal Nutrition,
Bangladesh Agricultural University,
Mymensingh, Bangladesh

A feeding trial was conducted with 16 bulls aged about 2½ years with a mean body weight of 108 kg. They were divided in a completely randomized manner into four groups of four each for a period of 135 days to study the effects of urea-treated or untreated bagasse on the chemical composition of ration ingredient, feed intake, body weight gain, feed efficiency and digestibility of proximate components.

Sugar-cane bagasse obtained from the sugar industry was treated with 5% urea and stacked for 15 days before feeding. Four groups of animals were designated as A, B, C and D. Animals of all four groups were supplied with ad libitum untreated rice straw, untreated bagasse, urea-treated bagasse and urea-supplemented bagasse, respectively. All the animals received about 1.0 kg wheat bran, 200 g molasses, 50 g green german grass, 100 g bone meal and 50 g common salt in order to satisfy

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the nutrient requirements. The urea-supplemented group (D) also received 20 g of Na_2SO_4 daily. Animals were fed individually in separate stalls and the parameters studied were recorded individually. A conventional digestion trial was carried out at the mid-experimental period. The proximate components of feed and faeces were determined according to the Association of Official Analytical Chemists [1]. The acid detergent fibre and neutral detergent fibre contents of feed and faeces were determined according to Van Soest and Wine [2]. In vitro determination of digestible organic matter (DOM), digestible energy (DE) and metabolizable energy (ME) were determined after Menke [3]. Data were analysed statistically for any significance.

It is evident from the results that the contents of crude protein and nitrogen-free extract of bagasse were found to be lower and that the crude fibre and ash contents were higher (Table I) than those reported by Paliwal et al. [4]. The ash content of bagasse was found to be lower than that of rice straw. Treatment of bagasse with 5% urea showed a $\pm$ threefold increase in crude protein content. The highest intake was shown by animals of group A supplied with rice straw (0.6 to 2.4 kg), followed by groups C and D with treated bagasse and urea-supplemented bagasse, respectively; the lowest dry matter intake was observed in group B with untreated bagasse. Dry matter intake per kilogram metabolic body weight and per 100 kg body weight showed a similar trend. Dry matter intake from roughage ($\text{g/kg W}^{0.75}$) was significantly ($P < 0.005$) higher in the rice-straw-based ration than that from bagasse-based rations. Of the bagasse-based rations, significantly ($P < 0.005$) more dry matter intake from roughage ($\text{g/kg W}^{0.75}$) was observed in group C which was supplied with urea-treated bagasse (Table II), indicating that urea treatment of bagasse improved the dry matter consumption, which is in agreement with the observations of Horton [5], Oji and Mowat [6] and Saadullah et al. [7]. Significantly ($P < 0.005$) more body weight gain was observed in rice straw and urea-treated bagasse rations than untreated bagasse and urea-supplemented bagasse rations. No significant difference in body weight gain was observed between untreated bagasse and urea-supplemented bagasse groups, which may be due to the lower intake of energy and proteins by the animals of group D. Significantly more body weight gain in the urea-treated bagasse group proved the superiority of urea treatment, but this was similar to the results shown in the rice straw ration.

The digestibility of dry matter, crude fibre and nitrogen-free extract was significantly ($P < 0.005$) higher in the urea-treated bagasse group C than in groups A and D. The lowest digestibility of dry matter and crude fibre was observed in group D. There was no significant difference in the digestibility of crude protein among the groups. The improvement in the digestibility of dry matter, crude fibre and nitrogen-free extract in group C may be attributed to the urea treatment of bagasse, which is in agreement with the observations of Horton [5], Horton et al. [8] and Lawlor and O'Shea [9]. The lowest digestibilities of all proximate components, total digestible nutrients and ME intakes in the animals of group D indicated that lower protein and energy intakes as well as a lower supply of concentrate might have affected the protein and energy concentration.

TABLE I. PROXIMATE COMPONENTS OF RATION INGREDIENTS (AS FED)

Ingredients	% dry matter (DM)	% crude protein (CP)	% crude fibre (CF)	% ether extract (EE)	% ash	% nitrogen-free extract (NFE)	% organic matter (OM)
Rice straw	87.3	3.5	29.3	3.3	18.2	33.0	69.0
Bagasse	62.2	1.1	27.9	0.4	5.1	27.7	57.2
5% urea-treated bagasse	37.0	2.3	19.1	0.2	0.8	14.6	36.2
Molasses	73.0	3.1	—	0.1	7.9	61.9	65.1
German grass	21.9	1.7	8.5	0.5	1.9	9.4	20.0
Wheat bran	85.5	12.6	10.0	3.1	6.2	53.5	79.3

TABLE II. PERFORMANCE OF ANIMALS ON DIFFERENT DIETS

Subject	Group A (straw + concentrate)	Group B (bagasse + concentrate)	Group C (treated bagasse + concentrate)	Group D (bagasse + urea + concentrate)
Initial weight (kg)	112.2	105.6	109.6	103.9
Final weight (kg)	154.8	120.8	151.3	128.9
Live weight gain (g/d)	374.5	179.4	308.3	184.7
DM intake from:				
Straw-bagasse (kg/d)	2.4 ± 0.0 ^a	0.6 ± 0.2 ^b	1.5 ± 0.0 ^c	0.9 ± 0.0 ^d
Concentrate (kg/d)	1.1	1.2	1.1	0.9
Total DM intake (kg/d)	3.7 ± 0.0	1.9 ± 0.2	2.7 ± 0.0	1.9 ± 0.0
DM intake (g/kg W ^{0.75})	102.7 ± 4.9 ^a	59.5 ± 3.9 ^c	77.9 ± 2.0 ^b	60.1 ± 5.0 ^c
DM intake				
(kg/100 kg body weight)	2.7 ± 0.2	1.6 ± 0.1	2.1 ± 0.0	1.7 ± 0.2
CP intake (g/d)	254.7 ± 12.8 ^a	181.6 ± 4.0 ^b	255.5 ± 6.1 ^a	178.1 ± 8.7 ^b
Feed conversion				
(kg/kg feed DM)	1:9.89	1:10.79	1:8.84	1:10.44
TDN intake (kg/d)	2.1 ± 0.1	1.2 ± 0.1	2.0 ± 0.1	0.7 ± 0.0
ME intake (MJ/kg)	23.4 ± 1.0	11.9 ± 0.4	14.4 ± 0.3	9.9 ± 0.2

Values with similar superscripts do not differ significantly ($P < 0.005$).

Significantly more body weight gain in the urea-treated bagasse group (C) proved the superiority of urea treatment, but this gain was similar to that shown by animals fed straw plus concentrate (group A).

These investigations indicated that urea treatment of low quality sugar-cane bagasse improved its nutritive values, making it perhaps as good as rice straw. Urea-treated bagasse enriched with molasses and 1 kg wheat bran can effectively be used as a ration for growing animals, for example during periods of scarcity such as when cyclones and droughts occur.

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IAEA-SM-292/46P

ENVIRONMENT CONDITIONING*

P.H. VUISTER

Division of Life Sciences,
International Atomic Energy Agency,
Vienna

1. INTRODUCTION

Maintenance of equipment poses serious problems for many nuclear laboratories in developing countries [1-3]. Instruments suffer from a hot and humid environment and poor quality AC power supply. In many cases they cannot be maintained

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in a properly functioning condition owing to a lack of trained staff and factory trained representatives and the regular loss of trained personnel to better rewarding jobs. Spare parts are difficult to obtain due to bureaucratic restrictions. Directors of nuclear laboratories do not always appreciate the value of preventive maintenance and quick repair. Therefore, in 1975 the International Atomic Energy Agency established a programme to assist the staff of such laboratories in finding solutions to these problems [4]. Two solutions are described.

2. POWER-CONDITIONING

A conditioned AC power supply is necessary for the reliable functioning of instruments. Electric mains power is produced for industry, workshops, lighting and households. In principle, its quality is adjusted to these uses. This means that inevitable disturbances consequential upon laws of physics, such as those of Ohm and Lenz, are intended to be kept within limits acceptable for these uses. In areas and countries with a fast growing demand for electric power, even these rather undemanding requirements are far from being met. Electronic instruments and computers, especially in developing areas, need protection against disturbances of the mains supply [4].

Mains disturbances can be categorized as voltage fluctuations and power failures with durations between a fraction of a second and several hours, frequency fluctuations, harmonics, transients and electric noise. In the framework of the programme the quality of the mains supply has been evaluated with the help of voltage recordings in all the participating laboratories and continues to be checked regularly. To prevent instrument damage by power failures and transients, and malfunction by any of the disturbances, an effective and economical power-conditioning system was developed. It consists of a dedicated power line (i.e. a separate shielded power line from the last step-down transformer) which reduces interference from other power users, a drop-out relay with varistors which prevents damage by power failures and transients, a ferroresonant constant-voltage transformer which filters out transients and noise and minimizes voltage fluctuations, and a dedicated earth line which prevents interference from currents to earth from other laboratories and workshops. More than 300 locally produced drop-out relays are now in use and in each of the 50 participating laboratories one or more complete conditioning systems have been installed. The use of these conditioners has led to an appreciable decrease in the number of failures of electronic instruments in several pilot laboratories. New equipment requested for the Agency's technical co-operation projects will, in the near future, be supplied with power-conditioners.

3. AIR-CONDITIONING

A clean and low humidity environment is needed for the reliable functioning and long life of instruments. High humidity, especially at higher temperatures, changes the electrical characteristics of electronic components. Moreover, under these conditions fungal growth causes leakage currents and corrosion causes poor contacts. The presence of dust enhances these effects. They give rise to the malfunction of instruments, particularly of high voltage equipment. Furthermore, very high or very low air temperature and humidity are uncomfortable for the staff and other persons present in the laboratory [4].

In the framework of the programme, guidelines for an economical air-conditioning system to be used in tropical areas were worked out which aim at the preservation of the proper conditions of instruments and at the comfort of persons present. Economical air-conditioning requires minimizing heat, dust and humidity intake. Therefore, walls and ceilings must be good heat insulators and double-window and double-door systems must be installed. Windows, doors and other openings should be kept closed and well sealed as much as possible and direct sunlight should be screened off. To dehumidify most effectively and to cool most economically, two air-conditioners, each of half of the needed capacity, should be installed. One should run continuously and one be controlled by its thermostat. Persons feel uncomfortable when exposed to low temperatures and cold draughts, detectors can break easily when they change temperature quickly or unevenly, and water condenses readily on cold instruments. Therefore the temperature in the laboratory should usually not be more than 8°C lower than the outside temperature and the airflow of air-conditioners should not be directed at persons or instruments. The best compromise for the value of the relative humidity between preservation of instrument conditions, comfort of persons and economy is 50%.

Similar systems are being worked out for other types of climates.

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IAEA-SM-292/47P

ACTIVITIES OF THE FAO/IAEA ANIMAL PRODUCTION UNIT OF THE IAEA SEIBERSDORF LABORATORY

FAO/IAEA Animal Production Unit,
IAEA Seibersdorf Laboratory,
Vienna

Part 1

Solid-phase radioimmunoassay (RIA) appropriate for use in developing countries

R.F. Nachreiner, S.J. Oschmann,
L.-E. Edqvist, J.I. Richards

The main task of the FAO/IAEA Animal Production Unit is research and development of suitable techniques for use by similar laboratories in developing countries. In animal reproduction, progesterone is a key hormone which can be used to assess reproductive performance in most domestic species. The following data are given on the development of a RIA procedure to measure this hormone in blood or milk.

At the outset it was decided to develop a direct RIA (avoiding extraction) of high capacity; to use an iodinated tracer (low cost counting system); to employ a minimum of pipetting steps (to improve precision); and to avoid a centrifugation step. All these requirements are fulfilled by the 'coated tube technique' in which the antibody is bound to (or coated on) the wall of a polystyrene test-tube and the separation of antibody bound and free hormone is achieved by simple decanting. A solid-phase RIA (using coated tubes) that fulfils these criteria was subsequently developed; an anti-serum was raised in rabbits against progesterone-3-[O-carboxymethyl]oxime-BSA (obtained from E. Seren, Bologna, Italy) and polystyrene Star tubes (Nunc Laboratories, Denmark) were coated with it. The whole assay requires six pipetting steps (two for coating, two for washing and two for the assay), centrifugation is avoided and several hundred assay units can be processed in one day. An antibody dilution of 1:8000 is used for coating and the process is complete after 90 minutes at 20°C (Fig. 1-1). However, after 72 hours of coating, binding becomes progressively less (Fig. 1-1). This coating procedure yields a maximum binding of approximately 45 to 55% for 10 µL skim milk and 30 to 45% for 20 µL blood serum or plasma when about 20 000 counts/min of ¹²⁵I-11α-hydroxyprogesterone-hemisuccinate (Amersham International, UK) is used as tracer.

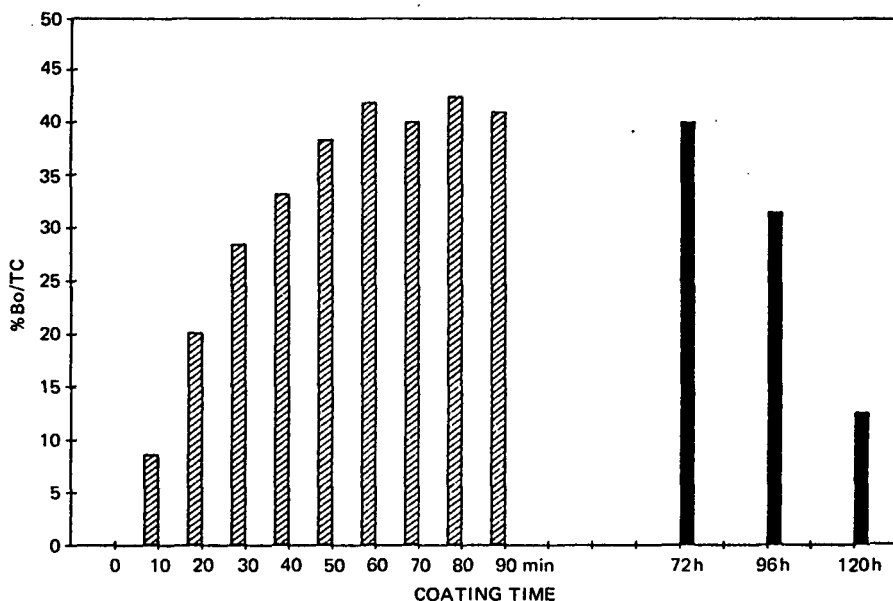


FIG. 1-1. Effect of tube coating time on maximum binding (Bo) at 20°C (%Bo/TC = percentage bound per tube coating).

To minimize variability within the assay the maximum antibody binding (%Bo/TC) should be stable for at least a time equal to that needed to complete pipetting. This feature is temperature dependent: thus, %Bo/TC reaches a plateau within 2 hours at 37°C, 3 hours at 0°C and 5 hours at 18°C, indicating that antibody affinity is lowest at 37°C and highest at 18°C (Fig. 1-2). The speed of the reaction, however, is most rapid at 37°C. The results for blood plasma are similar to those obtained with skim milk. Decantation of solid-phase tubes eliminates the need for assay centrifugation. However, non-specific binding (NSB) is relatively high (~ 8%) without tube washing (Fig. 1-3). Two washings with a dilute detergent solution reduced NSB to ~ 0.2%, with a minimal effect on specific binding.

Under optimum conditions the assay shows the following characteristics: the *calibration curve* is linear in a logit-log plot in the 2.5 to 75.0 nmol/L range for skim milk and in the 1.0 to 40.0 nmol/L range for blood plasma. *Precision*: intra-assay coefficients of variation (CV) of 7.2 and 11.2% and inter-assay CV of 8.3 and 9.9% for plasma and skim milk, respectively. *Sensitivity*: defined as 100% bound - 2 SD is 0.6 and 1.5 nmol/L, and defined as 95% B/Bo is 0.4 and 0.4 nmol/L for blood plasma and skim milk, respectively. *Specificity*: expressed as reactivity of related steroids at 50% displacement of B/Bo is 14% for 11 α -hydroxy-progesterone, 12.7% for 17 α -hydroxyprogesterone, 5.3% for corticosterone, 3.5% for

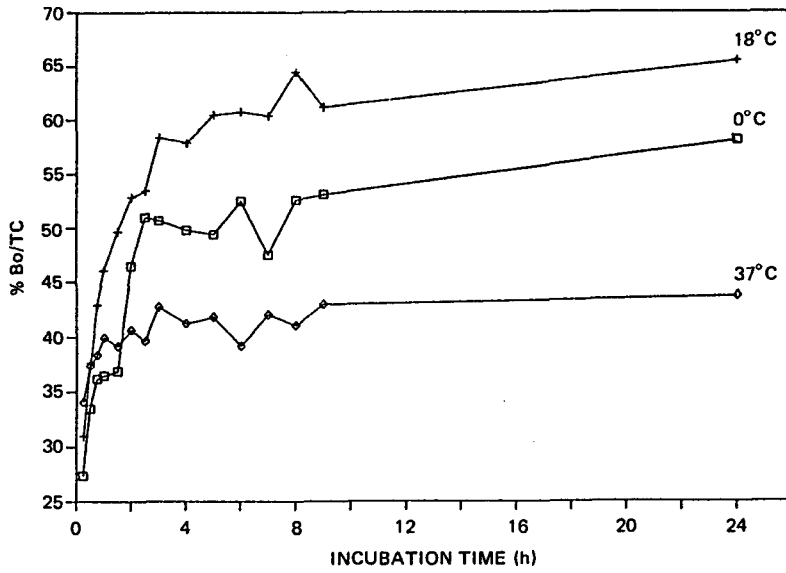


FIG. 1-2. Effect of incubation time and temperature on maximum binding (Bo) with skim milk.

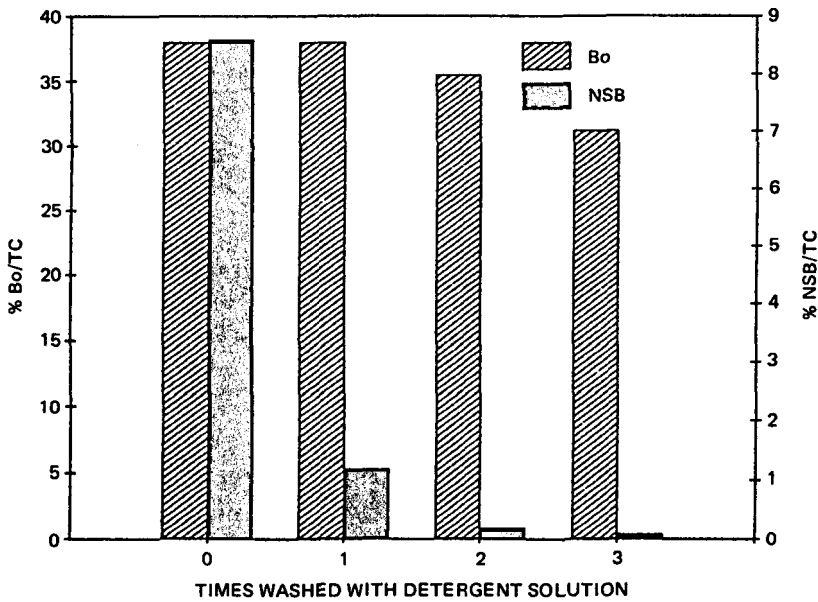


FIG. 1-3. Influence of tube washing on maximum binding (Bo) and non-specific binding (NSB).

desoxycorticosterone and 0.4% for 20 α -dihydroxyprogesterone. Cross-reactivity is < 1% for testosterone, androstenedione, cortisol, pregnenolone, oestradiol 17 β , oestrone and cholesterol. *Recovery*: as assessed by addition of progesterone to blood plasma samples ranges from 86.5 to 117.7%; for skim milk it ranges from 79 to 101%. Thus, in general, the assay meets the commonly accepted criteria for assay validation for the direct measurement of progesterone in blood plasma and skim milk; it offers significant advantages over tritiated and liquid-phase systems while maintaining a combination of maximum simplicity with high reliability and accuracy.

The FAO/IAEA kits for measurement of progesterone in skim milk or blood plasma/serum are available with 100 tubes or 1000 tubes. They consist of the requisite number of polystyrene tubes, iodinated progesterone (1 μ Ci/100 tube kit)¹, progesterone antibody, buffer and BSA, freeze-dried progesterone standards (seven for the milk kit, six for the blood kit), and two quality control samples (one low and one intermediate-high progesterone).

A single-well, crystal gamma counter is appropriate for the number of samples likely to be handled. If a larger throughput of samples is required, several single-well counters or one multi-well counter can be used. The other pieces of equipment required to complete a viable RIA laboratory are a multipipette, two three-volume pipettes (10, 20 and 25 μ L and 50, 75 and 100 μ L), a Vortex mixer and semi-logarithmic paper. Currently, the cost of these items, including a single-well counter, is less than US \$2000.

Other factors which affect the true progesterone concentration of biological samples include sample collection, processing and storage. It is well established that the red blood cells of ruminant species have the capacity to metabolize progesterone to other steroids not recognized by highly specific antibodies to progesterone. Thus, serum and plasma should be separated from the red blood cells as soon as possible after collection. If this cannot be done within 30 minutes, the samples should be put on ice to retard the enzymatic processes. Adhering to these criteria results in insignificant changes in progesterone obtained from blood samples using EDTA or heparin as anti-coagulants or using serum progesterone obtained from blood collected in polystyrene (PS) tubes, with or without polystyrene beads (PS-beads), or glass tubes (Fig. 1-4). This figure also illustrates that the addition of one, two or three tablets of sodium azide or potassium dichromate to 20 mL whole milk does not alter the progesterone concentration of the resulting skim milk, which indicates that these preservatives do not damage the progesterone and do not interfere with the assay reagents.

The progesterone content in skim milk depends on the temperature at which the whole milk is centrifuged (Fig. 1-5). When using a ³H-progesterone tracer to follow progesterone movement, warm butterfat (centrifuged at 21°C) seems to have a higher affinity for progesterone than cold butterfat (centrifuged at 4°C). In addition, the time of sample collection during milking can be significant. It has previously

¹ 1 Ci = 3.70 $\times$ 10¹⁰ Bq.

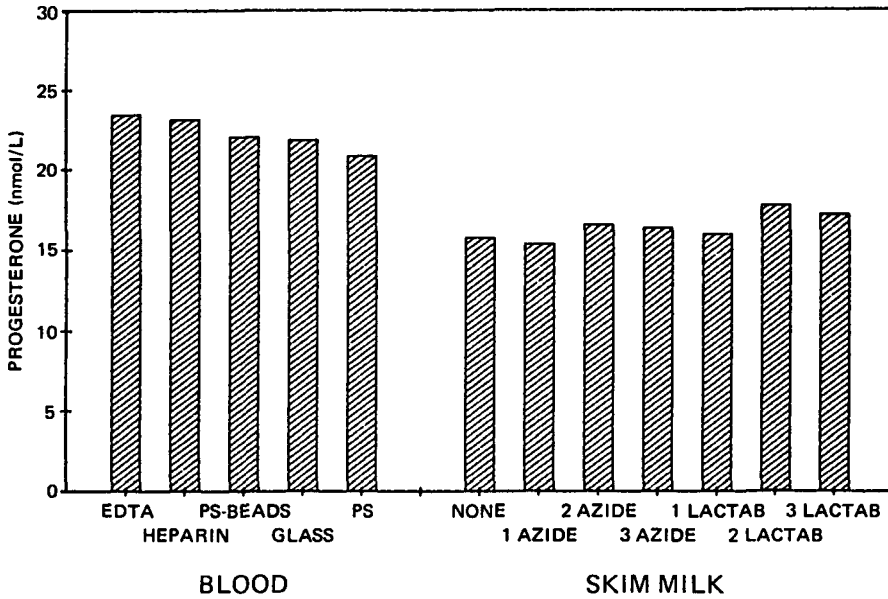


FIG. 1-4. Effect of sample processing on progesterone concentrations in blood and skim milk.

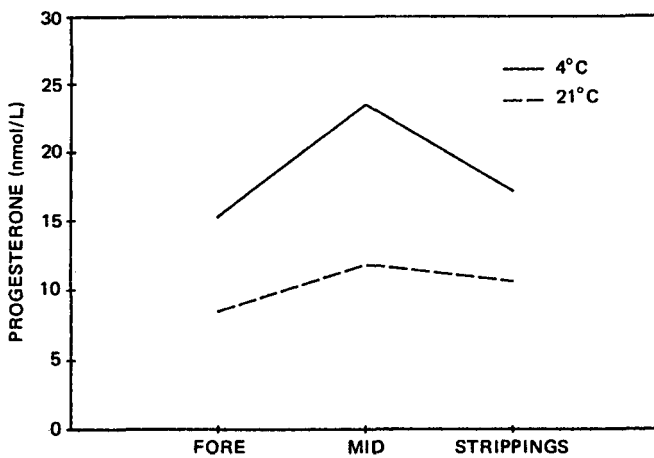


FIG. 1-5. Concentration of progesterone in skim milk when whole milk is centrifuged at 4°C or 21°C.

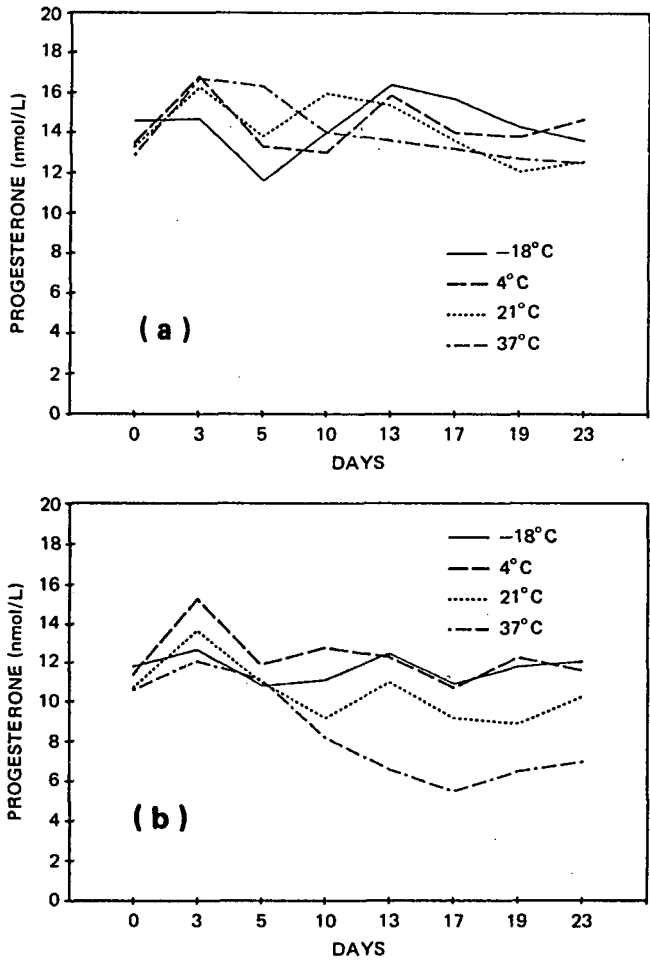


FIG. 1-6. Effect of storage temperatures on concentration of progesterone in skim milk preserved with: (a) sodium azide, and (b) potassium dichromate.

been reported that there is a difference between fore milk, composite milk and strippings [1-3]. This difference is less obvious in the resulting skim milk, but our assay system still detects a significant difference. Since the time of sample collection during milking and the temperature of the milk at centrifugation are significant variables, consistency in sample handling is considered imperative. Sedimentation of whole milk to remove butterfat can be used in place of centrifugation; however, it takes 2 days of sedimentation to achieve approximately the same values obtained with centrifugation and the milk obtained from the bottom of the tube has less progesterone than that near the top — presumably because of better fat separation.

Sample storage is a problem in many countries because of frequent power failures. In one experiment, skim milk samples preserved with sodium azide (1 tablet/20 mL) or potassium dichromate (1 tablet/20 mL) were stored at -18°C , 4°C , 21°C or 37°C for 23 days (Fig. 1-6). Potassium dichromate began to be ineffective for preservation after 10 days at 37°C ; sodium azide remained effective over the whole experimental period. In a long-term study, skim milk samples preserved with sodium azide (1 tablet/10 mL) or Thimerosal (100 mg/100 mL) and stored at 37°C and 21°C began to coagulate within 30 days; however, samples stored at 4°C and -18°C showed no significant deterioration for at least 7 weeks. Use of preservatives should help to alleviate concern in the event of short power failures.

ACKNOWLEDGEMENTS

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Part 2

Characterization of some fibrous agricultural residues using an artificial rumen

M.C.N. Jayasuriya, R. Hamilton

Fibrous agricultural residues have been the subject of considerable research during the past decade both in developed and developing countries. In an attempt to assist developing Member States in this particular area of research, the IAEA Seibersdorf Laboratory established a Fibrous Residue Bank (FRB) in 1985 with a view to characterizing and establishing the feeding value of some of the potential but low quality fibrous feed materials. To date, more than 15 countries have contributed

TABLE 2-I. FERMENTATION CHARACTERISTICS OF SOME FIBROUS RESIDUES IN THE RUSITEC

Type of residue	Country of origin	Dry matter digestibility (%)	pH of effluent	Ammonia-N in effluent (mg/L)	End-products of fermentation (mmol/d)			Particulate dry matter (g/d)
					VFA	Methane		
Wheat straw	Pakistan	45.6	6.83	N/A	44.9	9.6		1.134
Wheat straw	Czechoslovakia	34.7	6.99	108.7	33.1	3.5		0.423
Wheat straw	Egypt	45.2	6.98	106.8	47.9	7.9		0.973
Wheat straw	Yugoslavia	42.3	6.93	N/A	39.1	4.2		0.405
Wheat straw	Portugal	39.4	6.91	N/A	39.9	6.6		0.571
Wheat straw	Portugal	53.6	6.80	N/A	54.4	9.8		0.830
(5% urea ammonia treated)								
Rice straw	Bangladesh	39.6	6.99	90.8	35.4	4.1		0.334
Rice straw	Pakistan	43.8	6.92	N/A	37.2	6.4		0.843
Rice straw	Thailand	44.9	6.90	72.3	34.7	4.5		0.471
Rice straw	Malaysia	37.5	7.05	102.0	29.9	6.1		0.443
Rice straw	Sri Lanka	31.2	7.22	332.9	25.2	N/A		0.741
Rice straw	Sri Lanka	53.4	6.97	251.7	47.8	N/A		1.263
(4% urea ammonia treated)								
Rice straw	Egypt	37.2	7.06	107.6	36.9	6.1		0.410

Barley straw	Cyprus	54.9	6.80	85.0	63.3	12.6	1.166
Barley straw	Austria	36.2	6.99	60.5	42.3	5.4	1.008
Grass hay (medium quality)	Austria	51.3	7.09	151.7	49.7	N/A	0.760
Grass hay (good quality)	Austria	71.6	6.79	197.4	67.1	11.5	1.418
Hay	Portugal	49.1	6.94	109.8	53.3	8.0	0.825
Hay (5% urea ammonia treated)	Portugal	53.5	6.95	202.5	52.0	5.9	1.000
Clover hay	Egypt	61.6	7.01	219.8	61.5	8.4	1.612
Sugar-cane bagasse	Bangladesh	24.6	7.08	106.2	29.6	4.0	0.280

N/A = not available.

as many as 30 feed samples to the FRB. This presentation reports the characterization of 21 fibrous residues from 11 countries using the artificial rumen (rumen simulation technique) developed by Czerkawski and Breckenridge [1]. Apart from the dry matter digestibility of a feed, the rumen simulation technique (Rusitec) can also measure the production rates of end-products of fermentative digestion in the rumen such as volatile fatty acids (VFAs), fermentation gases and microbial biomass. These values, when expressed on a 'per unit dry matter digested' basis, provide valuable information on the fermentation characteristics of the feed material.

The design, incubation procedure and preparation of samples for analyses were essentially as described by Czerkawski and Breckenridge [1]. The roughage and a concentrate at a ratio of 80:20 were enclosed in nylon mesh bags of approximately 200 μm pore size. The infusion medium consisted of artificial saliva prepared according to McDougall [2], i.e. containing 50 mg urea/L as a source of fermentable nitrogen. The infusion rate of saliva was similar for all 21 diets. Adequate trace mineral nutrients were supplied by infusing 3 mL/d of a mineral mixture containing 0.64 g zinc sulphate, 0.34 g manganese sulphate and 0.02 g cobalt sulphate/L into the reaction vessel immediately after feeding.

All roughages were chopped into 1 to 2 cm lengths before being enclosed in nylon bags. The input of dry matter was approximately 15 g/d for all diets. Ground wheat bran containing 2.5% nitrogen was used as the source of concentrate. Rumen fluid for inoculation was collected from a fistulated cow maintained on a standard diet of hay and concentrate. Each roughage characterization was carried out either in duplicate vessels or over two periods. An adjustment period of 7 to 10 days was allowed for the vessels to reach 'steady state' prior to taking samples for measurements.

The concentration of VFAs in the effluent was determined by the gas-liquid chromatography procedure of Czerkawski [3] using pivalic acid as the internal standard. The volumes of gas produced were measured with a wet meter, and gases were analysed chromatographically for methane and carbon dioxide [4]. To determine particulate dry matter (PDM) in effluent, 75 to 100 mL of preserved suspension were centrifuged at 15 000g for 30 minutes and the residue washed twice with water and dried to constant weight at 105°C. The ammonia nitrogen in the effluent was determined by steam distillation of a 2 mL aliquot into 2% boric acid solution containing a mixed indicator and titration of the distillate with 0.01N sulphuric acid. After washing twice with 40 mL saliva the undigested residues were dried at 105°C to constant weight to determine the dry matter loss.

The fermentation characteristics of the fibrous residues are given in Table 2-I. The dry matter digestibility of cereal straws varied from 31.2 to 54.9, indicating the vast variability in digestibility among cereal straws. In general, rice straws showed a lower digestibility (range: 31.2–44.9%; mean: 39.0% $\pm$ 4.9) than wheat straws (range: 34.7–45.6%; mean: 41.4% $\pm$ 4.5). Urea ammonia treatment increased the dry matter digestibility of both rice (Sri Lanka) and wheat (Portugal) straws so that it was equivalent in quality to that of a medium quality hay. While clover hay from

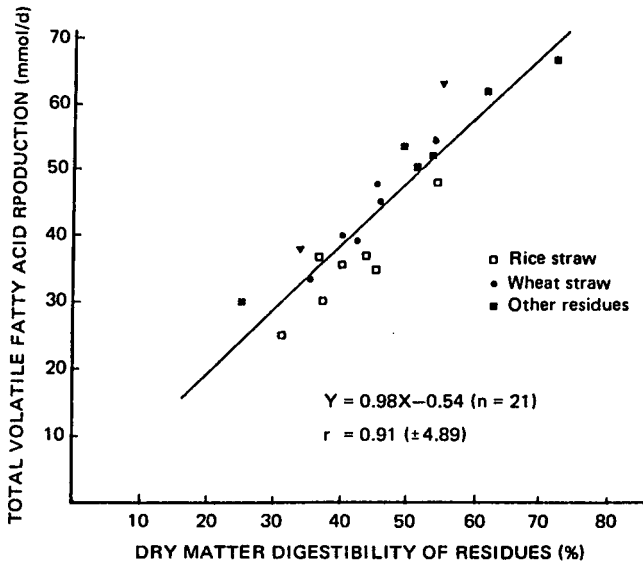


FIG. 2-1. Relationship of DM digestibility of fibrous residues with VFA production in the Rusitec.

Egypt showed the highest digestibility (61.6%), sugar-cane bagasse from Bangladesh showed the lowest (24.6%).

The addition of 50 mg urea/L of saliva provided sufficient fermentable nitrogen to raise the rumen ammonia concentration to acceptable levels of over 50 mg ammonia nitrogen/L, but the production of microbial matter had no significant correlation with the ammonia concentration. However, contrary to this observation, the production of VFAs was highly dependent ($r = 0.91$; $P < 0.001$) on the digestibility of the feed material (Fig. 2-1). Similarly, the output of microbial matter appeared to be significantly ($P < 0.001$) related to the digestibility of dry matter ($r = 0.83$) and total VFA production ($r = 0.80$).

An interesting observation was that the output of microbial matter was significantly ($P < 0.001$) correlated ($r = 0.91$) to the combined effects of total VFA production and ammonia concentration, substantiating the view that both fermentable nitrogen and readily available energy in adequate quantities are essential for optimum rumen function.

Table 2-II shows the molar percentage of VFAs and some derived quantities from the fermentative digestion of the residues. Acetate was the predominant VFA in the effluent ranging from 57 to 72%. The propionate component ranged from 18.5 to 28.5%, while the butyrate component ranged from 4.9 to 16.4%.

The output of VFA and methane/kg of dry matter digested was in general agreement with values reported for similar diets in in vivo experiments [1]. Similarly, the

TABLE 2-II. SOME DERIVED QUANTITIES OF FERMENTATION OF FIBROUS RESIDUES IN THE RUSITEC

Type of residue	Country of origin	Molar percentage of VFA			C ₂ :C ₃	Output/unit DM digested		
		Acetic	Propionic	Butyric		VFA (mol/kg)	Methane (mol/kg)	Particulate DM (g/kg)
Wheat straw	Pakistan	72.2	18.6	7.3	3.9	7.2	1.6	181.4
Wheat straw	Czechoslovakia	70.9	21.7	5.2	2.1	6.9	0.8	88.5
Wheat straw	Egypt	60.9	19.7	12.6	5.3	7.6	1.3	154.3
Wheat straw	Yugoslavia	64.6	28.5	5.4	1.6	6.6	0.8	68.2
Wheat straw	Portugal	67.5	23.9	6.9	1.9	7.6	1.3	108.3
Wheat straw	Portugal	67.3	25.0	5.7	1.9	7.4	1.4	113.1
(5% urea ammonia treated)								
Rice straw	Bangladesh	67.0	26.3	4.7	1.9	6.5	0.7	60.9
Rice straw	Pakistan	66.9	24.2	6.6	2.3	6.2	1.1	140.3
Rice straw	Thailand	70.3	22.1	5.4	2.1	5.6	0.7	75.9
Rice straw	Malaysia	70.9	21.9	4.9	2.2	5.8	1.2	85.7
Rice straw	Sri Lanka	65.8	18.9	10.6	4.7	6.0	N/A	177.3
Rice straw	Sri Lanka	65.4	21.6	9.7	3.3	6.4	N/A	168.3
(4% urea ammonia treated)								
Rice straw	Egypt	60.1	21.2	13.3	5.5	7.3	1.2	81.2

Barley straw	Cyprus	64.5	22.6	9.5	3.6	2.9	8.4	1.6	154.6
Barley straw	Austria	62.5	22.4	12.7	2.4	2.8	8.6	1.4	206.1
Grass hay (medium quality)	Austria	62.5	24.4	8.8	4.3	2.6	7.3	N/A	110.8
Grass hay (good quality)	Austria	62.9	18.5	15.6	3.7	3.1	7.1	1.2	148.6
Hay	Portugal	57.1	19.8	16.4	6.6	2.9	8.0	1.2	124.1
Hay (5 % urea ammonia treated)	Portugal	58.3	20.4	14.6	6.7	2.9	7.5	0.8	138.7
Clover hay	Egypt	60.3	22.1	11.1	6.7	2.8	7.4	1.0	193.5
Sugar-cane bagasse	Bangladesh	60.2	22.3	13.0	4.5	2.7	8.7	1.2	82.6

N/A = not available.

production of microbial matter/kg of dry matter digested was within the expected values for high roughage diets.

It is concluded that the Rusitec is a versatile in vitro system for measuring the fermentation characteristics of fibrous residue-based diets. As expected, all cereal straws under consideration were found to be potential basal feed resources for ruminant production in developing countries. Their manipulation, either by treatment and/or supplementation, could bring about changes in rumen fermentation leading to an increased supply of energy and protein for the host animal. The future role of the FRB could be twofold: (1) to characterize and evaluate non-conventional and new feed resources which may have a potential in the future as a basal diet or as a supplement for existing basal diets, and (2) to formulate and test new diets for ruminant production systems in developing countries using locally available feed resources.

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IAEA-SM-292/48P

INTERNATIONAL INFORMATION SYSTEM FOR THE AGRICULTURAL SCIENCES AND TECHNOLOGY (AGRIS)

R. PORTEGIES ZWART

AGRIS Processing Unit,

Food and Agriculture Organization of the United Nations,
Vienna

AGRIS is a co-operative system for collecting and disseminating current bibliographical information on the world's agricultural literature, in which at present 132 national and multinational centres take part. The world production of literature of agricultural interest is vast, being proportionate to the importance of food production: the AGRIS database had accumulated about 1 200 000 references by the beginning of 1986 and is increasing at a monthly rate of some 10 000 references.

AGRIS was created to serve the agricultural information needs of developed and developing countries alike, on a co-operative basis. It enables information published in one country to be made available to all other countries in the system. Each AGRIS participating country brings into the system information published within the country itself, and receives from it the information it needs on materials published elsewhere. Each participating country has designated an organization or centre to act as the national AGRIS centre, while the Food and Agriculture Organization of the United Nations acts as the co-ordinating agency for the entire system. The AGRIS Co-ordinating Centre, located at FAO Headquarters in Rome, has overall responsibility for management and development of the system. Processing of the data, maintenance of the database and the generation of output are carried out by the AGRIS Processing Unit (APU), which is housed with the IAEA in Vienna.

Since its start in 1975 all the data fed into AGRIS have been incorporated in the AGRIS database, and each month new data are published in the monthly issue of the printed bibliography Agrindex and stored in one consolidated computer on-line database held on the IAEA computer in Vienna.

Agrindex can be consulted at any participating centre and in many libraries throughout the world; generally, these are the libraries of ministries of agriculture and of agricultural universities. Information on subscription rates can be obtained from the Distribution and Sales Section, FAO, Rome, Italy.

AGRIS output tapes are sent to any participating centre that wishes to receive them. Subject to the availability of the necessary computer facilities, AGRIS can provide services based on the tapes, either at the centres themselves or at other institutions.

With the rapid progress made in telecommunications in recent years, it is becoming faster, cheaper and more efficient to consult the central database. On-line access to the database (on the IAEA computer in Vienna, on DIMDI in Cologne, on DIALOG in California or via ESA in Frascati) can be obtained via ordinary public telephone, telex or various telecommunication networks.

Full information on any of the AGRIS products can be obtained from national AGRIS centres, from the AGRIS Co-ordinating Centre, FAO, Rome, or from the AGRIS Processing Unit, c/o IAEA, Vienna.

IAEA-SM-292/49P

**EFFECTS OF ACUTE NUTRITIONAL DEPRIVATION
ON LOCAL IMMUNE RESPONSES TO
GASTROINTESTINAL HELMINTHS IN
RABBITS AND SHEEP***

E. SIŃSKI, H. WĘDRYCHOWICZ,

I.B. HELAL, B. BEZUBIK

Department of Parasitology,

Institute of Zoology,

University of Warsaw,

Warsaw, Poland

An attempt was made to investigate the effects of a low protein (LP) and a high protein (HP) diet, local immunization, immunomodulation by cyclophosphamide (CY) and *Corynebacterium parvum* on the inhibition phenomenon, resistance and immune response during primary and challenge infections of rabbits with *Obeliscoides cuniculi*.

O. cuniculi-infected rabbits were used as a model system to study the immune response to gastrointestinal *Trichostrongylidae*.

1. DESIGN OF EXPERIMENTS

Experiment I. Twenty-four baby rabbits were weaned 4 weeks after birth; 12 were kept on a LP diet (8% protein) and 12 were fed a HP diet (21% protein). All the animals were exposed to 10 000 larvae when they were 16 weeks old.

Experiment II. Fifteen adult rabbits were used; five were exposed to three doses of 10 000 larvae given on weeks 0, 4 and 8. Each infection was cut-off with 200 µg/kg of Ivermectin (MSD) 2 weeks later in order to stimulate the hosts with larval antigens in the histotropic phase only. The animals were challenged with 10 000 larvae on week 12 of the experiment. Post-mortems were performed 6 weeks after challenge infection (WACH). Five rabbits were exposed to a single dose of 10 000 larvae; this group served as the challenge infection control. Also, a group of five rabbits was included as the non-treated and non-infected control.

Experiment III. Five animals were infected with 10 000 larvae and treated with 10 mg/kg of CY on days 0, 2 and 4 of the experiment. Also, two groups of five rabbits were included as the non-treated and infected controls and non-infected controls, respectively.

* Research carried out with the support of the IAEA under Research Contract No. 3572/RB.

Experiment IV. Five animals were infected with 10 000 larvae and treated three times with 4.2 mg of dry weight *C. parvum* on days 0, 2 and 4 of the experiment. As in experiment III, two groups of control animals were included.

The effectiveness of infection and percentage of inhibited larvae were examined by worm and larvae counts. Live weight gain, egg output and haematological and serological changes were examined weekly. The level of total antibodies in serum and mucosal antibody isotypes against somatic antigens of adult forms of *O. cuniculi* were measured by haemagglutination (HA) and radioimmunoprecipitation (RIP) tests. Labelling of somatic antigens with ^{125}I was performed according to the method of Greenwood et al. [1]. Also, the level of total proteins in scraped mucosa samples of the stomach and small intestine and in the bile, and coproproteins were examined. The relationships between live weight gain and immunological responsiveness were examined by means of analysis of variance.

The protein-deficient diet (LP) did not significantly alter the percentage of inhibition and effectiveness of infection as measured at the sixth WACH, compared with the CY- and *C. parvum*-treated groups. The percentage of inhibited larvae of both dietary groups was found to be statistically non-significant. However, the highest percentage (45%) of inhibition in larval development was recorded in *C. parvum*-treated animals. Also, local immunological stimulation by premature termination of infection only showed a clear-cut effect on the inhibition phenomenon. There was no inhibition of larvae after challenge infection; 87% of immunological protection was found at the sixth WACH. These results suggest that L_3 and L_4 larvae may be efficiently immunogenic in the induction of resistance to challenge infection.

There was a negative correlation of live weight gain in the LP and HP diet groups. However, within each dietary group the mean daily egg output increased substantially during the time of infection. The LP diet did not affect the egg output and there was no significant difference in the dynamics of egg output between the two groups.

A comparison of the changes in haematocrit levels in both groups of animals showed significant anaemia before and after infection in the LP group of animals. Within each dietary group, the faecal protein loss increased significantly after infection only in the HP group. No correlation was observed between the average values of coproprotein loss and changes in the total serum proteins both before and after infection in the LP group. Anti-parasite antibodies in sera, mucosae, bile and faeces, as detected by the HA and RIP tests, were extremely low. However, relatively higher HA antibody titres and specific secretory IgA from stomach mucosa to the ^{125}I -labelled somatic antigen of adult worms were detected in the LP group.

2. CONCLUSIONS

(1) *O. cuniculi* appears to be relatively non-discriminated against by the host's nutritional condition

- (2) The *O. cuniculi* rabbit system can only be used with a limited scope as a model for experimental studies of the host's response to ruminant haemonchosis
- (3) Lack of a clear-cut correlation between dietary groups and responses within the local immune system indicates that perhaps this response does not compete with the physiological processes of malnourished and infected animals.

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IAEA-SM-292/51P

STUDIES ON THE INVOLVEMENT OF PSEUDORABIES VIRUS (AUJESZKY'S DISEASE VIRUS) ENVELOPE PROTEINS IN VIRULENCE AND PROTECTION USING RECOMBINANT DNA TECHNOLOGY*

T.C. METTENLEITER, H.-J. RZIHA, N. LUKÁCS,
H.-J. THIEL, C. SCHREURS, A. SIMON
Bundesforschungsanstalt für
Viruskrankheiten der Tiere,
Tübingen, Federal Republic of Germany

Aujeszky's disease caused by Pseudorabies virus (PRV) infection is of great economical importance in animal husbandry and there is a need for evaluation of more effective and safer vaccines, although vaccination is carried out using live attenuated or inactivated vaccines. It is well known that herpesviral envelope proteins play a major role in eliciting the host's immune response. Thus, the objective of this study was: (1) to determine the involvement of PRV glycoproteins (gp) in virus neutralization and protection; (2) to investigate their possible role in expression of virulence; and (3) to examine the potential of certain gp as vaccines after expression in foreign cells. In the present study we focused on two major previously characterized gp of PRV [1-3].

* Research carried out with the support of the Commission of the European Communities (Biomolecular Engineering Programme) under Contract No. GBI-2-056-D, and the Deutsche Forschungsgemeinschaft under Grant No. RZ 2/1-4.

Recently we demonstrated [4] that the glycoprotein gI, together with another 40 kilodalton¹ protein, is not expressed in several avirulent PRV vaccine strains, which correlates with a known deletion of DNA sequences comprising most of the gI structural gene. Here we report on in vivo and in vitro isolation of PRV variants displaying significant changes in gI expression. Although no obvious alteration in the genomic region coding for this gp and also no changed transcriptional programme could be detected as compared with the wild-type virus, all the mutated viruses exhibited a truncated gI. After combined hybrid selection and in vitro translation experiments one considerably smaller precursor protein of gI (pgI) was found (64 kilodalton in size), whereas wild-type viruses generally express two pgI, about 80 kilodalton in size. Furthermore, we demonstrated that gI processing was also strikingly altered in these isolates. In contrast to wild-type PRV, these isolates were no longer neutralized with monoclonal antibodies directed against gI. The significance of an altered gI expression for the virulence of PRV is discussed.

With respect to the size pattern of pgI analysed in more than 80 PRV strains and isolates obtained from different parts of Europe, we present data which show a striking correlation between distinct pgI patterns and the regional occurrence of certain virus strains. This finding might provide a valuable, additional diagnostic criterion for defining the origin of virus strains isolated from PRV outbreaks.

Results of investigation on the role of the PRV gp in virus neutralization and protection are presented. A panel of monoclonal antibodies directed against the major gp was used: (1) for in vitro neutralization tests in the presence and in the absence of complement; and (2) for mice protection assays. These results indicated that also the major gpgII representing a disulphide-linked gp complex in PRV [1, 2] is involved in both functions. After mapping the structural gene of gII on the viral genome [5], experiments for the expression of this protein in foreign cells are performed.

ACKNOWLEDGEMENTS

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¹ 1 dalton = 1 u (unified atomic mass unit)
 $\approx 1.66 \times 10^{-27}$ kg.

IAEA-SM-292/52P

VALORES DE PROGESTERONA DURANTE EL CICLO ESTRAL POSTPARTO EN VACAS HOLSTEIN EN PERIODO SECO Y DE LLUVIAS

O. FERNANDEZ-LIMIA, R. FAURE, L. GARCIA

Departamento de Reproducción,
Centro Nacional de Sanidad Agropecuaria,
La Habana, Cuba

La baja fertilidad de las vacas Holstein observada en los países de las zonas tropical y subtropical ha sido atribuida al stress de calor en los meses de verano [1].

En vacas secas y en novillas, Castellanos [2] y Solano [3] han observado siempre niveles elevados de progesterona en el verano; sin embargo estos animales son más fértiles que las vacas en lactación [4].

El objetivo de este trabajo fue corroborar el aumento de la tasa de secreción de progesterona durante el ciclo estral en vacas en lactación durante los meses más cálidos.

Se tomaron muestras de sangre de la vena yugular a vacas Holstein de 2 rebaños de la provincia de La Habana durante los meses de enero a mayo y de julio a septiembre; de uno se tomaron los lunes, miércoles y viernes, y del otro 4 a 5 días después de presentado el celo, y se agruparon a partir del día de presentación de celo, promediándose todos los valores por día. El número de muestras fluctuó entre 3 y 38 valores (vaca).

Se tomaron todos los valores correspondientes a ciclos estrales, eliminando las vacas que quedaban gestantes.

Las muestras fueron centrifugadas y el suero conservado a -20°C , hasta su procesamiento por RIA directo de progesterona.

La comparación de los niveles de progesterona entre los diferentes días del ciclo estral en ambas épocas del año mostró diferencias los días 8 ($P < 0,01$), 9 ($P < 0,001$) y 19 ($P < 0,01$); aunque en el resto de los días las diferencias no fueron significativas, los días 2, 10 y 15 mostraron una $P < 0,1$. Estas diferencias se caracterizaron por ser los valores de progesterona más bajos en verano los días 8, 9, 10 y 15 del ciclo estral, mientras que nuestros resultados anteriores siempre mostraron lo contrario. El aumento relativo encontrado el día 2, en el verano, tiene un valor muy relativo ya que la diferencia es de 0,73 ng/mL. Sin embargo se aprecia en general una tendencia a un desarrollo precoz del cuerpo lúteo en esta época del año, como se podrá ver posteriormente. Algo muy llamativo es la diferencia encontrada el día 19, no relacionada con ningún otro día, y pensamos que es producto de una disminución de rebote de la progesterona en verano, debido al aumento tan apreciable que se observa los días 17 y 18, y que si bien no mostraron diferencias estadísticas,

producto de la gran variabilidad, este mismo efecto fue señalado por Castellanos [2] y Solano [3].

Por último, el análisis de series cronológicas da una mayor secreción de progesterona al principio del ciclo en la época de verano, debida quizá a un desarrollo más rápido del cuerpo lúteo. Esto posiblemente esté relacionado con la observación hecha anteriormente de un pico preovulatorio de hormona luteizante más temprano en el verano. Sin embargo, se hace evidente la disminución a partir del día 5 del ciclo hasta el día 9. Esto, junto con los datos sobre las diferencias encontradas en los días 8, 9 y 10, da a entender una afectación del embrión en el momento de la transformación de morula en blastocitos, que en el bovino ocurre el día 7, día que ha sido planteado como crucial en la mortalidad embrionaria [5, 6]. Esto quizá esté causado por los tenores relativamente bajos de hormona luteizante encontrados por Fernández-Limia [7] y Madan y Johnson [8].

Otro aspecto interesante de este análisis es el aumento de la tasa de secreción de progesterona que se observa en el gráfico en los días 17 y 18, lo cual es realmente contraproducente para el próximo celo y el desarrollo folicular que se produce en ese momento.

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EFFECTS OF SHEARING AND NON-SHEARING ON THE WATER TURNOVER RATES OF LOCAL, EXOTIC AND CROSS-BRED SHEEP UNDER OPEN GRAZING*

M.A. DOLLAH, Y. ZAKARIA

Animal Research Division,
Malaysian Agricultural Research and
Development Institute,
Kuala Lumpur, Malaysia

A comparative study of heat balance was carried out on local (indigenous sheep of Malaysia, ISM), exotic (Dorset Horn, DH) and their 50:50 cross-breed (ISM $\times$ DH) by determining the water turnover rates under open grazing and stall-fed conditions (shade). The effects on heat balance of shearing the wool were also studied. The results are shown in Table I.

TABLE I. WATER TURNOVER OF SHEEP UNDER DIFFERENT MANAGEMENT SYSTEMS

Breed	Treatment	Body weight (kg)	Turnover rate (mL/d per kg W ^{0.82})	Half-life (d)
ISM (n = 5)	Shade + unsheared	24.0 $\pm$ 3.0 ^{A,a}	174.1 $\pm$ 10.2 ^{A,a}	5.7 $\pm$ 1.2 ^{A,a}
	Grazing + unsheared	25.6 $\pm$ 4.0 ^{A,a}	272.6 $\pm$ 11.2 ^{B,a,b}	3.6 $\pm$ 0.6 ^{B,a}
	Shade + sheared	25.8 $\pm$ 2.6 ^{A,a}	238.5 $\pm$ 15.2 ^{C,a}	4.3 $\pm$ 1.4 ^{C,a}
	Grazing + sheared	25.1 $\pm$ 4.0 ^{A,a}	232.6 $\pm$ 20.0 ^{C,a}	4.2 $\pm$ 0.9 ^{C,a}
DH (n = 5)	Shade + unsheared	66.5 $\pm$ 10.2 ^{A,c}	189.3 $\pm$ 10.2 ^{A,a}	6.0 $\pm$ 0.7 ^{A,c}
	Grazing + unsheared	62.5 $\pm$ 5.6 ^{A,c}	268.1 $\pm$ 12.6 ^{B,b}	3.4 $\pm$ 0.5 ^{B,a}
	Shade + sheared	65.5 $\pm$ 5.0 ^{A,c}	294.7 $\pm$ 9.2 ^{C,b}	3.6 $\pm$ 0.9 ^{B,C,b}
	Grazing + sheared	64.8 $\pm$ 10.1 ^{A,c}	300.8 $\pm$ 20.1 ^{C,b}	3.7 $\pm$ 0.6 ^{C,b}
ISM $\times$ DH (n = 5)	Shade + unsheared	28.9 $\pm$ 4.2 ^{A,b}	141.3 $\pm$ 15.2 ^{A,b}	6.9 $\pm$ 0.6 ^{A,b}
	Grazing + unsheared	29.6 $\pm$ 6.1 ^{A,B,b}	293.5 $\pm$ 10.9 ^{B,a}	3.4 $\pm$ 0.8 ^{B,a}
	Shade + sheared	29.0 $\pm$ 3.0 ^{A,B,b}	234.4 $\pm$ 13.2 ^{C,a}	4.2 $\pm$ 0.5 ^{C,a}
	Grazing + sheared	30.5 $\pm$ 5.6 ^{B,b}	244.1 $\pm$ 11.2 ^{C,a}	4.4 $\pm$ 0.5 ^{C,a}

Mean $\pm$ SD.

A,B,C = comparison of mean within breeds at $P < 0.05$.

a,b,c = comparison of mean between breeds with the same treatment at $P < 0.05$.

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The ISM is small (mean body weight of 25.0 kg) compared with DH (average weight of 64.9 kg), but it is highly adaptable to a hot, humid climate. Crossing these two breeds has improved the body size (28.5 kg) of the offspring over ISM.

The water turnover rate of unsheared sheep of all breeds under shade conditions was lowest in all the treatments. During grazing, while the wool was still intact, the water turnover rate increased to 56, 40 and 100% for ISM, DH and ISM $\times$ DH, respectively. The water turnover rate of animals after shearing of the wool but maintained under shade conditions was higher than that of unsheared animals under the same conditions; the increment was 36, 55 and 65% for ISM, DH and ISM $\times$ DH, respectively. When the sheep were released for grazing (sheared), the water turnover rate did not significantly change compared with conditions in the shade.

An overall comparison of water turnover rates indicated that DH has a higher water turnover rate, while both ISM and its cross-breed were similar.

Changes in the half-life of water were inversely correlated with changes in the water turnover rate.

The study concluded that:

- (1) DH has a higher heat load; crossing DH with ISM produced sheep with an improved body size and a lower heat load than ISM and DH, respectively
- (2) Shearing the wool reduces the heat load, even under shade conditions; shearing possibly increased the sweating rate.

IAEA-SM-292/54P

**ELECTRON MICROSCOPICAL DIAGNOSIS
OF PARAPOX (*Ecthyma contagiosum*)
IN CAMELS (*Camelus dromedarius*)**

E. MUNZ*, D. SCHILLINGER**,
M. REIMANN*, H. MAHNEL ***

*Institut für vergleichende Tropenmedizin
und Parasitologie,
Tierärztliche Fakultät,
Universität München,
Munich, Federal Republic of Germany

**Veterinary Research Laboratory,
Kabete, Kenya

***Institut für Medizinische Mikrobiologie,
Infektions- und Seuchenmedizin.
Universität München,
Munich, Federal Republic of Germany

Camel contagious ecthyma (CCE, Auzdyk) is caused by a parapox virus which is different from the orthopox virus cameli (genus: orthopox virus, family: *Poxviridae*). Although the parapox virus has been isolated from camels in the USSR, Mongolia and Somalia, CCE is apparently a sparsely studied disease.

CCE is usually characterized by localized lesions, but generalized lesions resembling true camelpox have been observed.

In July 1984 a camelpox outbreak was reported on a farm in Kenya's Laikipia district, where about 450 camels (of the Somalia and Turkana type) are kept together with other livestock. In the affected animals, proliferous lesions developed on the lips, with an occasional extension to the mucosa of the mouth and nostrils. In calves and immature animals (1 to 4 years old) there was a marked tendency to generalization. Lesions occurred all over the body, with a prevalence on the distal parts of the leg, inner parts of the thighs and, in females, in the vaginal area; sometimes the entire head swelled. The disease resembled true camelpox. The morbidity amounted to almost 100% in young animals compared with 10 to 20% in adult camels. The mortality rate in all age groups was zero. The camels lost condition rapidly and became weak and emaciated. Specimens from the mouth region and from the body of the animals were investigated in a Zeiss EMC/R transmission electron microscope with formvar or pioloform-coated grids contrasted with phosphotungstic acid.

In 20 out of 21 samples typical parapox viruses were readily seen. Their morphology resembled the ovine *Ecthyma contagiosum* virus. The ovoid particles measured 230 to 285 nm $\times$ 157 to 171 nm (average 250 $\times$ 160 nm) in size. Their axis ratio was 1:1.56 and their surface showed a regular criss-cross pattern.

The purpose of this study was twofold: (1) To report for the first time on parapox infections in camels in Kenya, and (2) to underline the useful application of electron microscopical investigations for the rapid differential diagnosis of pox infections.

IAEA-SM-292/55P

IFS ACTIVITIES IN DEVELOPING COUNTRIES

C. AROSENIUS

International Foundation for Science,
Stockholm, Sweden

The International Foundation for Science (IFS) is a non-governmental organization which is active in development aid and is multilaterally financed by nine donor countries. Grants are given to scientists working at universities and research institutes in developing countries who should be at the beginning of their scientific careers and possess a good academic education and some research experience. It is a well-known fact that many developing countries suffer from a brain drain of researchers caused by a lack of funds, particularly foreign currency, and thus difficult research conditions. The aim of the IFS is to diminish this trend and to create in these countries a better working environment for the scientists. This is also why the work of IFS is focused on individuals rather than on projects. Nevertheless, the proposed research must fall into one of the seven priority areas of IFS, which are Aquaculture, Animal Production, Food Crops, Afforestation and Mycorrhiza, Microbiology and Food Technology, Chemistry of Natural Products and Rural Technology.

The maximum amount of the grants is US \$10 000 per research period. If the work progresses successfully, three more grants can be awarded. Grantees may leave all or part of the grant money with the IFS Secretariat; the IFS Purchasing Department can then handle the purchasing of equipment, expendable supplies and the literature needed for the project. Special prices can often be negotiated, packing and shipping is arranged and thus the grantee can get the most benefit out of the funds available. More and more grantees are choosing this service; at present the Foundation administers 70% of the funds granted.

Another important IFS activity is the organizing and financing of workshops, symposia and training courses for the grantees. Scientists working in specific research areas are brought together to discuss their work and to form personal contacts. The formation of regional networks and collaboration is strongly promoted by the Foundation. IFS has organized a total of 19 workshops, four of which were also training courses. The proceedings of all these workshops have been published by the Secretariat and six have also been commercially published. Three of the meetings have been held in Latin America in Spanish only. Most of the workshops in Africa have been bilingual (English/French) with simultaneous translation.

Another way of promoting scientific contacts is by awarding travel grants to grantees. Every year a large number of scientists are able to attend international congresses, symposia and meetings where they can present and discuss their research and thus enter into the international scientific community.

Over the years the Foundation has built up a worldwide network of contacts with scientists and science administrators. A total of 81 research councils, scientific academies and universities in 67 countries make up the IFS Member Organizations. Two-thirds of these are located in developing countries and are able to advise the Foundation on the national scientific priorities and needs.

IFS was founded in 1972, and as of 1985 some 950 researchers have been awarded grants. Most of them have also been awarded one or more renewal grants. The geographical distribution of grants for the period 1982–1985 is 36% for Asia, 26% for Latin America and 38% for Africa.

The number of applications received in the last few years has been 400 to 500 per year. This means that a rather strict selection has to be made. The first screening is made in the Secretariat, where applications which do not fill the formal criteria are rejected. A scientific evaluation is then done with the help of a large group of scientific advisers, persons who play a very important role in the activities of the Foundation. These advisers are experts within the IFS research areas. They are also well acquainted with the situation in developing countries through extensive travelling. The number of advisers helping IFS with evaluation of applications and advice to grantees is steadily increasing and at the moment is close to 450. This work is done free of charge for IFS — out of a general interest in research and as a result of a commitment to helping developing countries.

This network of experts also gives IFS the possibility of offering many grantees a personal visit by an international expert. For financial reasons, IFS cannot afford to send out experts for the sole purpose of visiting grantees; it therefore takes advantage of scientists who travel extensively for other organizations, by paying for a few extra days' visit to one or more grantees in the country the expert is visiting. These visits are much appreciated by the grantees, who get good advice and help in their laboratories. In turn, the expert can form a sound opinion on the existing problems and perhaps guide his younger colleagues towards the solutions. Many advisers and grantees also establish contact by correspondence at the very beginning of the granting period, i.e. when the adviser evaluates the application.

Within the research area of Animal Production, most IFS projects focused on animal nutrition during the first years of the Foundation's activities. The main interest among the researchers was to study the potential of alternative and inexpensive feed for different animals. Many industrial wastes, agricultural by-products and non-utilized plants were analysed. Over the last five years, applications have increased within the areas animal breeding and genetics, exploitation of new animal species, animal diseases, toxicology and reproduction. Nutrition projects, however, still make up some 50% of the total number of grants awarded in the animal production area.

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PURIFICATION OF IODINATED TRACERS FOR HOMOLOGOUS RADIOIMMUNOASSAYS OF OVINE LH AND FSH

B.M. STANKOV*, A.A. USCHEVA**, L.N. KANCHEV*

*Institute of Biology and Immunology of
Reproduction and Development of Organisms

**Institute of Molecular Biology,
Bulgarian Academy of Sciences

Sofia, Bulgaria

Several purification techniques were employed with the aim of obtaining high quality iodine-labelled tracers of ovine luteinizing hormone (LH-LER-1056-C₂) and follicle stimulating hormone (NIAMDD-oFSH-I-1). Binding capacity in the presence of excess specific antibodies, cross-reactivity (single point calculation, CR_n = 200 ng) [1] with other glucoproteins and the electrophoretic behaviour of the samples subjected to SDS electrophoresis [2] were estimated for the collected fractions.

After iodination through modification of the chloramine-T method [3], using 8 to 9 µg chloramine-T per microgram of the hormones, moderate specific activities of 2 to 3 MBq/µg hormone were obtained.

Nevertheless, simple gel filtration (Sephadex G-25 medium, PD-10 column) yielded in every case products with considerable heterogeneity. The small amounts of contaminants with a higher molecular weight, visible in either preparation before iodination only after silver staining of the samples, subjected to electrophoresis [4] were found following iodination and appeared as bands with different intensities at autoradiography.

Additional purification of the preparations on hydroxyapatite [5], ultrogel-AcA-54 [6], cellulose CF-11 [7], DEAE Sepharose Cl-6B or Blue Sepharose Cl-6B [8] (the latter two used for oFSH only), resulted in heterologous profiles in radio- and immunoreactivity. Recovery of the intact hormones varied from 9 to 14% up to 45%, depending on the method and the hormone.

CONCLUSIONS

- (1) A single purification technique applied to the ^{125}I -labelled oLH and oFSH, such as gel filtration on Sephadex G-25 to separate the free iodine, is not sufficient to obtain a high quality tracer, and so guarantee valid assays. Aggregates and free subunits may be formed following iodination; this is especially true for oFSH, even when low concentrations of chloramine-T are used.
- (2) A freely chosen technique for additional purification may result in a tracer with a lowered quality, as was the case with oFSH following cellulose CF-11 adsorption chromatography.
- (3) High resolution gel filtration in these experiments yielded tracers with considerably improved qualities. The recovery and reproducibility of the results were consistently high.

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LIST OF PARTICIPANTS

- Abu Zinada, A. Ministry of Agriculture and Water,
P.O. Box 17285, Riyadh, Saudi Arabia
- Adeyemo, O. Department of Veterinary Anatomy,
University of Ibadan, Ibadan, Nigeria
- Al-Bekairi, S.I. Regional Agriculture and Water Research Centre,
Ministry of Agriculture and Water,
P.O. Box 17285, Riyadh, Saudi Arabia
- Amaral-Mendes, J.J. General Direction of Veterinary Services,
Ministry of Agriculture, Lisbon, Portugal
- Arosenius, C.
(IFS) International Foundation for Science,
Grevturegatan 19, S-114 38 Stockholm, Sweden
- Auriol, P.
(FAO) Animal Production and Health Division,
Food and Agriculture Organization of the United Nations,
Via delle Terme di Caracalla,
I-00100 Rome, Italy
- Aykol, F. Department of Parasitology,
Lalahan Nuclear Research Institute of Animal Health,
Turkish Atomic Energy Authority,
Lalahan, Ankara, Turkey
- Bamberg, E. Institut für Biochemie,
Veterinärmedizinische Universität Wien,
Linke Bahngasse 11, A-1030 Vienna, Austria
- Barej, W. Department of Animal Physiology,
Warsaw Agricultural University,
ul. Nowoursynowska 166, 02-766 Warsaw, Poland
- Barthakur, N.N. Department of Food Science and Agricultural Chemistry,
21, Lakeshore Road, St. Anne de Bellevue,
Quebec H9X 1C0, Canada
- Benlamlih, S. Département de physiologie et thérapeutique,
Institut agronomique et vétérinaire Hassan II,
B.P. 6202 Rabat-Instituts, Rabat, Morocco

- Bezubik, B. Department of Parasitology,
Institute of Zoology, University of Warsaw,
00-927 Warsaw, Poland
- Binnerts, W.T. Department of Animal Physiology,
Agricultural University,
Haarweg 10, NL-6709 PJ Wageningen, Netherlands
- Bono, G. Facoltà di Agraria, Università di Udine,
Via S. Mauro 2, I-33010 Pagnacco, Italy
- Bosticco, A. Consiglio Nazionale delle Ricerche,
Comitato di Consulenza per le Scienze Agrarie,
Piazzale Aldo Moro 7, I-00100 Rome, Italy
- Buttery, P.J. Department of Applied Biochemistry and Food Science,
University of Nottingham School of Agriculture,
Sutton Bonington, Nr. Loughborough,
Leicestershire LE12 5RD, United Kingdom
- Castelino, J.B.
(IAEA) Division of Life Sciences,
International Atomic Energy Agency,
Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
- Chaiyabutr, N. Department of Physiology,
Faculty of Veterinary Science, Chulalongkorn University,
Henri Dunant Street, Bangkok 10500, Thailand
- Chipepa, J.A.S. Livestock and Pest Research Centre,
National Council for Scientific Research,
P.O. Box 49, Chilanga, Lusaka, Zambia
- Choshniak, I. Department of Zoology,
George S. Wise Faculty of Life Sciences,
Tel Aviv University, Ramat Aviv 69978, Israel
- Chukeir Bassam
(LAS) League of Arab States,
Grimmelshausengasse 12, A-1030 Vienna, Austria
- Chuong, D.C. Applied Radioisotope Research Institute,
Cheju National University,
1 Ara-dong, Cheju City, Cheju Do, Republic of Korea
- Czerkawski, J.W. Hannah Research Institute,
Ayr KA6 5HL, United Kingdom
- Dakkak, A. Département de parasitologie et des maladies parasitaires,
Institut agronomique et vétérinaire Hassan II,
B.P. 6202 Rabat-Instituts, Rabat, Morocco

- Das, H.A. Netherlands Energy Research Centre,
P.O. Box 1, NL-1755 ZG Petten, Netherlands
- Djurdjević, Dj. Faculty of Veterinary Medicine,
University of Belgrade,
18, Bul. JNA, YU-110070 Belgrade, Yugoslavia
- Dollah, M.A. Animal Research Division,
Malaysian Agricultural Research and Development Institute,
P.O. Box 12301, 50774 Kuala Lumpur, Malaysia
- Economides, S. Agricultural Research Institute,
Ministry of Agriculture and Natural Resources,
Nicosia, Cyprus
- Edqvist, L.-E.
(FAO) Joint FAO/IAEA Division of Isotope and Radiation
Applications of Atomic Energy for Food and
Agricultural Development,
International Atomic Energy Agency,
Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
- El Fouly, H.A. Department of Radiobiology,
Nuclear Research Centre,
Abou Zaabal, Egypt
- Eldon, J. Institute of Experimental Pathology,
University of Iceland,
P.O. Box 8540, 128 Reykjavik, Iceland
- Farelo Cruz, M.C.S. Laboratório Nacional de Investigação Veterinária,
Estrada de Benfica 701, 1500 Lisbon, Portugal
- Fernández Limia, O. Departamento de Reproducción,
Centro Nacional de Sanidad Agropecuaria (CENSA),
Carretera de Jamaica-Tapaste,
Apartado 10, San José de las Lajas, Havana, Cuba
- Folman, Y. Institute of Animal Sciences,
Volcani Centre, P.O. Box 6, Bet Dagan 50250, Israel
- Foong, F. Faculty of Veterinary Medicine and Animal Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia
- Galina, C.S. Departamento de Reproducción,
Facultad de Medicina Veterinaria y Zootecnia,
Universidad Nacional Autónoma de México,
Mexico 20, D.F. 04510, Mexico
- Giacintov, P. State Veterinary Institute,
Mercova 54, 612 38 Brno, Czechoslovakia

- Gomaa, I.A. Animal Production Research Institute,
Nadi Elsid Street, El Dokki, Cairo, Egypt
- Gombe, S. Reproductive Biology Unit,
College of Biological and Physical Sciences, University of Nairobi,
P.O. Box 30197, Nairobi, Kenya
- Gruber, L. Bundesanstalt für Alpenländische Landwirtschaft Gumpenstein,
A-8952 Irdning, Austria
- Guo, Dazhi Isotope Research Laboratory,
Sichuan Agricultural University,
Yaan, Sichuan, China
- Gustafsson, K.G.B. Institut für Zuchthygiene, Universität Zürich,
Winterthurerstrasse 260, CH-8057 Zurich, Switzerland
- Gyawu, D.
(FAO) Station de physiologie de la reproduction,
Institut national de recherches agronomiques,
F-37380 Nouzilly, France
- Hamdi, A.
(LAS) League of Arab States,
Grimmelshausengasse 12, A-1030 Vienna, Austria
- Hamilton, R.
(IAEA) FAO/IAEA Animal Production Unit,
IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Hampl, J. Veterinary Research Institute,
Hudcova 70, 621 32 Brno, Czechoslovakia
- Höller, H. Physiologisches Institut der Tierärztlichen Hochschule Hannover,
Bischofsholer Damm 50, D-3000 Hannover 1,
Federal Republic of Germany
- Holmes, P.H. Department of Veterinary Physiology,
University of Glasgow Veterinary School,
Bearsdon Road, Glasgow G61 1QH, United Kingdom
- Homeida, A.M. Department of Medicine, Pharmacology and Toxicology,
Faculty of Veterinary Science, University of Khartoum,
P.O. Box 32, Khartoum North, Sudan
- Hvelplund, T. Department of Research in Cattle and Sheep,
National Institute of Animal Science,
P.O. Box 39, DK-8833 Ørum Sønderlyng, Denmark
- Intraraksa, Y. Faculty of Veterinary Medicine,
Kasetsart University,
Bangkok 10900, Thailand

- Jacobson, R.H. New York State College of Veterinary Medicine,
Cornell University,
Ithaca, NY 14853, United States of America
- Jalaludin, S. Faculty of Veterinary Medicine and Animal Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia
- Jasiorowski, H.A. Animal Production and Health Division,
(FAO) Food and Agriculture Organization of the United Nations,
Via delle Terme di Caracalla, I-00100 Rome, Italy
- Jayasuriya, M.C.N. FAO/IAEA Animal Production Unit,
(IAEA) IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Jeggo, M.H. Animal Virus Research Institute,
Pirbright, Woking, Surrey, United Kingdom
- Kallfelz, F.A. Department of Physiology,
New York State College of Veterinary Medicine,
Cornell University, Ithaca, NY 14853, United States of America
- Kamonpatana, M. Biochemistry Unit,
Faculty of Veterinary Science, Chulalongkorn University,
Henri Dunant Street, Bangkok 10500, Thailand
- Karg, H. Lehrstuhl für Physiologie der Fortpflanzung und Laktation,
Technische Universität München,
D-8050 Freising-Weihenstephan, Federal Republic of Germany
- Khanum, S.A. Nuclear Institute for Agriculture and Biology,
P.O. Box 128, Jhang Road, Faisalabad, Pakistan
- Khayal, W. League of Arab States,
(LAS) Grimmelshausengasse 12, A-1030 Vienna, Austria
- Kindahl, H. Department of Obstetrics and Gynaecology,
College of Veterinary Medicine,
Swedish University of Agricultural Sciences,
S-750 07 Uppsala, Sweden
- King, G.J. Department of Animal and Poultry Science,
Ontario Agricultural College, University of Guelph,
Guelph, Ontario N1G 2W1, Canada
- Kovacević, R. Institute of Biology and Veterinary Medicine,
University of Novi Sad,
6, Ilije Djuricica Street, YU-2100 Novi Sad, Yugoslavia

- Lamm, C.G.
(IAEA) Joint FAO/IAEA Division of Isotope and Radiation
Applications of Atomic Energy for Food and
Agricultural Development,
International Atomic Energy Agency,
Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
- Leitão, R.M. Estação Zootécnica Nacional,
Vale de Santarém, 2000 Santarém, Portugal
- Luckins, A.G. Centre for Tropical Veterinary Medicine,
University of Edinburgh,
Easter Bush, Roslin,
Midlothian EH25 9RG, United Kingdom
- Mahaputra, L. Department of Reproduction and Obstetrics,
Faculty of Veterinary Medicine,
Airlangga University, Surabaya, Indonesia
- McDowell, L.R. Department of Animal Science,
Institute of Food and Agricultural Sciences,
University of Florida,
Gainesville, FL 32611, United States of America
- Meyer, H.H.D. Institut für Physiologie,
Süddeutsche Versuchs- und Forschungsanstalt für Milchwirtschaft,
Technische Universität München,
D-8050 Freising-Weihenstephan, Federal Republic of Germany
- Milligan, L.P. Department of Animal Science, University of Alberta,
Edmonton, Alberta, Canada
- Miyingo-Kezimbira, L. Faculty of Veterinary Medicine,
Makerere University,
Kampala, Uganda
- Mohamed, A.R. Veterinary Research Institute,
Gannoruwa, Peradeniya, Sri Lanka
- Moreno-López, J. Section of Virology,
Department of Veterinary Microbiology,
Faculty of Veterinary Medicine,
Swedish University of Agricultural Sciences,
P.O. Box 585, S-751 23 Uppsala, Sweden
- Mrazek, N. Bundesanstalt für Fortpflanzung und Besamung von Haustieren,
Postfach 121, Thalheim, Austrasse 10,
A-4601 Wels, Austria
- Munthali, J.T.K. Chitedze Research Station,
P.O. Box 158, Lilongwe, Malawi

- Munz, E. Institut für Vergleichende Tropenmedizin und Parasitologie,
Tierärztliche Fakultät, Universität München,
Leopoldstrasse 5, D-8000 Munich 40, Federal Republic of Germany
- Nachreiner, R. F. FAO/IAEA Animal Production Unit,
(IAEA) IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Nolan, J.V. Department of Biochemistry, Microbiology and Nutrition,
University of New England,
Armidale, New South Wales 2351, Australia
- Oh, Tae-Kwang Korea Advanced Institute of Science and Technology,
P.O. Box 131, Dongdaemum, Seoul, Republic of Korea
- Ørskov, E.R. Rowett Research Institute,
(FAO) Bucksburn, Aberdeen AB2 9SB, United Kingdom
- Oschmann, S.J. FAO/IAEA Animal Production Unit,
(FAO) IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Ostaszewski, P. Department of Animal Physiology,
Warsaw Agricultural University,
ul. Nowoursynowska 166, 02-766 Warsaw, Poland
- Ozawa, Y. Animal Health Service,
(FAO) Animal Production and Health Division,
Food and Agriculture Organization of the United Nations,
Via delle Terme di Caracalla, I-00100 Rome, Italy
- Özsar, S. Lalahan Nuclear Research Institute of Animal Health,
Turkish Atomic Energy Authority,
Lalahan, Ankara, Turkey
- Perera, B.M.A.O. Department of Veterinary Clinical Studies,
Faculty of Veterinary Medicine and Animal Science,
University of Peradeniya,
Peradeniya, Sri Lanka
- Pfahler, W. Institut für Vergleichende Tropenmedizin und Parasitologie,
Tierärztliche Fakultät, Universität München,
Leopoldstrasse 5, D-8000 Munich 40, Federal Republic of Germany
- Poppi, D.P. Department of Animal Sciences,
Lincoln College, University College of Agriculture,
Canterbury, New Zealand
- Portegies Zwart, R. AGRIS Processing Unit,
(FAO) Food and Agriculture Organization of the United Nations,
P.O. Box 100, A-1400 Vienna, Austria

- Post, T.B. Tropical Cattle Research Centre,
Division of Tropical Animal Science,
Commonwealth Scientific and Industrial Research Organization,
P.O. Box 5545, Rockhampton Mail Centre,
Queensland 4701, Australia
- Prandi, A. Istituto di Fisiologie Veterinaria, Università di Bologna,
Via Belmeloro 8/2, Bologna, Italy
- Rahman, M. Department of Physiology, Nuclear Research Laboratory,
College of Veterinary Science, Assam Agriculture University,
Khanapara, P.O. Guwahati 781022, Assam, India
- Ramirez, A.L.V. (IAEA) FAO/IAEA Animal Production Unit,
IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Richards, J.I. (IAEA) FAO/IAEA Animal Production Unit,
IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Robertshaw, D. Department of Physiology and Biophysics,
College of Veterinary Medicine and Biomedical Sciences,
Colorado State University,
Fort Collins, CO 80523, United States of America
- Rogavić, B. (IAEA) FAO/IAEA Animal Production Unit,
IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Rothauer, D. Facoltà di Medicina Veterinaria,
Istituto di Microbiologia e Immunologia,
Via Celoria 10, Milan, Italy
- Rziha, H.-J. Bundesforschungsanstalt für Viruskrankheiten der Tiere,
Paul Ehrlich Strasse 28, D-7400 Tübingen,
Federal Republic of Germany
- Sastradipradja, D. Department of Physiology and Pharmacology,
Faculty of Veterinary Medicine, Bogor Agricultural University,
Bogor, Indonesia
- Satar Herrmann, C. Veterinärmedizinische Universität Wien,
Linke Bahngasse 11, A-1030 Vienna, Austria
- Satter, L.D. United States Dairy Forage Research Center,
Agricultural Research Service,
United States Department of Agriculture,
Madison, Wisconsin, United States of America

- Schuller, W. Veterinärmedizinische Universität Wien,
Linke Bahngasse 11, A-1030 Vienna, Austria
- Sharifuddin, W. Faculty of Veterinary Medicine and Animal Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia
- Sigurbjörnsson, B.
(IAEA) Joint FAO/IAEA Division of Isotope and Radiation
Applications of Atomic Energy for Food and
Agricultural Development,
International Atomic Energy Agency,
Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
- Siński, E. Department of Parasitology,
Institute of Zoology, University of Warsaw,
00-927 Warsaw, Poland
- Smith, R.H. Animal and Grassland Research Institute,
University of Reading,
Church Lane, Shinfield,
Reading RG2 9AQ, United Kingdom
- Smith, W. Nuclear Energy Board,
Dublin, Ireland
- Sophon, S. Biochemistry Unit,
Faculty of Veterinary Science, Chulalongkorn University,
Henri Dunant Street, Bangkok 10500, Thailand
- Stankov, B.M. Institute of Biology and Immunology of Reproduction and
Development of Organisms,
73, Lenin Avenue, 1113 Sofia, Bulgaria
- Sumar, J. Instituto de Investigaciones Tropicales y de Altura (IVITA),
Universidad Nacional Mayor de San Marcos,
Apartado 4270, Lima 1, Peru
- Susmel, P. Facoltà di Agraria,
Istituto di Produzione Animal, Università di Udine,
Via S. Mauro 2, I-33010 Pagnacco, Italy
- Tabish, A.
(IAEA) FAO/IAEA Animal Production Unit,
IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- Talavera, V. Departamento de Nutrición, Universidad Nacional Agraria,
La Molina, Lima, Peru

- Tan, H.S. Department of Animal Sciences,
Faculty of Veterinary Medicine and Animal Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia
- Tareque, A.M.M. Bangladesh Agricultural University,
Mymensingh, Bangladesh
- Thatcher, W.W. Department of Dairy Science,
Institute of Food and Agricultural Sciences,
University of Florida,
Shealy Drive at Ritchey Road,
Gainesville, FL 32611, United States of America
- Thimonier, J. Station de physiologie de la reproduction,
Institut national de recherches agronomiques,
F-37380 Nouzilly, France
- Thong, Tran-The Agricultural Research Institute,
121 Nguyen Point Khiem,
Ho Chi Minh City, Viet Nam
- Uriyapongson, S. (IAEA) FAO/IAEA Animal Production Unit,
IAEA Seibersdorf Laboratory,
P.O. Box 100, A-1400 Vienna, Austria
- van de Wiel, D.F.M. Research Institute for Animal Production Schoonoord,
Driebergseweg 10d, P.O. Box 501,
NL-3700 AM Zeist, Netherlands
- Vercoe, J.E. Tropical Cattle Research Centre,
Division of Tropical Animal Science,
Commonwealth Scientific and Industrial Research Organization,
P.O. Box 5545, Rockhampton Mail Centre,
Queensland 4701, Australia
- Vuister, P.H. (IAEA) Division of Life Sciences,
International Atomic Energy Agency,
Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
- Wilkins, J.V. Overseas Development Administration/
British Tropical Agricultural Mission,
Casilla 359, Santa Cruz, Bolivia
- Wright, I.G. Long Pocket Laboratories,
Division of Tropical Animal Science,
Commonwealth Scientific and Industrial Research Organization,
Private Mail Bag 3, Indooroopilly, Queensland, Australia
- Zerobin, K. Institut für Zuchthygiene, Universität Zürich,
Winterthurerstrasse 260, CH-8057 Zurich, Switzerland

AUTHOR INDEX

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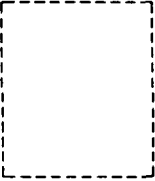
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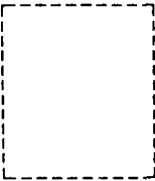
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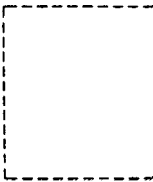
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