ISOTOPE AND RELATED TECHNIQUES
IN ANIMAL PRODUCTION AND HEALTH

PROCEEDINGS OF A SYMPOSIUM, VIENNA, 15-19 APRIL 1991
JOINTLY ORGANIZED BY IAEA AND FAO

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1991
ISOTOPE
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AND HELD IN VIENNA, 15–19 APRIL 1991

INTERNATIONAL ATOMIC ENERGY AGENCY
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FOREWORD

The papers published in this book comprise the proceedings of an international symposium on Nuclear and Related Techniques in Animal Production and Health, organized jointly by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations and held in Vienna from 15 to 19 April 1991. The symposium was attended by over one hundred participants from about forty countries and included 61 presentations of which 25 were posters. Many of the participants were from developing countries and much of the work described was conducted within developing countries, often with assistance from the joint FAO/IAEA programme. However, without the generous support of a number of bilateral and many national funding organizations, the scope and general success of this symposium would have been substantially reduced. It is therefore with pleasure that the IAEA and FAO acknowledge the assistance provided by: the British Council; the Commonwealth Scientific and Industrial Research Organization of Australia; the Italian Department of Co-operation for Development; the Swedish International Development Authority; and the Department of Technical Assistance of the Dutch Ministry of Foreign Affairs.

The purpose of the symposium was to provide a forum for livestock production and health specialists in developing and developed countries to review advances in the nutrition and reproduction of ruminant and other herbivorous animals, as well as in new approaches to disease diagnosis and control. Within this general framework, consideration was given to those isotope and related techniques currently employed in research, but more importantly to the application of research findings to improving the productivity of livestock reared in tropical and subtropical developing countries. For example, the general theme of the session on animal nutrition was the feeding of ruminant animals whose diets consist mainly of poor quality roughages. Here, consideration was given to tracer techniques based on isotopes which are an integral part of the armoury used by animal nutritionists to establish the value of feeds as well as the protein, energy and mineral requirements of the animals themselves. Particular emphasis was given to how the principles developed from these and other methods could be used in practice to improve animal growth and productivity, e.g. through the feeding of urea–molasses blocks and the provision of sources of rumen non-degradable protein and/or minerals.

During the session on animal reproduction and breeding, advances were considered in the development of highly sensitive radioimmunoassay methods for measuring the concentrations of reproductive and other hormones which control or influence reproduction. Examples were also provided of how these methods can be used to assess the reproductive status of animals, monitor the effectiveness of feed supplementation strategies, devise corrective measures for reproductive disorders, and improve the efficiency of artificial insemination and embryo transfer programmes.
The session on animal health emphasized developments in the use of enzyme linked immunosorbent assays, not only for diagnosis of diseases with major relevance to developing countries, but perhaps more importantly for the future, the applicability of these methods for monitoring the effectiveness of large scale vaccination programmes and conducting epidemiological surveys. Some of the new approaches to diagnosis, vaccine production and increasing animal resistance to disease arising from developments in molecular biology were also discussed. These developments themselves often rely on isotopes to provide the required levels of sensitivity and specificity.

The authorship of this volume is truly international and the information provided comes from a wide variety of experiences, disciplines and animal production systems. It was impossible to cover all existing and new technologies and all of the problems connected with rearing livestock in developing countries. It is nevertheless hoped that by providing an insight into how isotope and related methods are used within the context of research to study and solve specific problems, these proceedings will assist scientists in developing countries in improving livestock production and health.

EDITORIAL NOTE

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A HISTORY OF THE USE OF ISOTOPIC TRACER TECHNOLOGY IN RUMINANT NUTRITION: LESSONS FOR PRESENT RESEARCH

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Abstract

A HISTORY OF THE USE OF ISOTOPIC TRACER TECHNOLOGY IN RUMINANT NUTRITION: LESSONS FOR PRESENT RESEARCH.

Isotopically labelled materials may be used to delineate metabolic pathways, but their major role in animal nutrition has been to provide quantitative data on the availability, utilization and turnover of nutrients in the whole animal and in defined tissues. The development of isotopic dilution procedures in the 1950s and 1960s made it possible to measure the inflows into body pools of the major energy yielding nutrients (glucose, acetate, 3-hydroxybutyrate and plasma non-esterified fatty acids) and amino acids. When 14C labelled substrates are used, rates of release of 14CO2 provide data on oxidative metabolism. The dynamics of nitrogen metabolism in the rumen have been defined in similar studies using 15N-ammonium sulphate, 15N-urea and 14C-urea. Studies in which isotope dilution procedures are combined with classical arteriovenous difference studies have provided comprehensive data on the uptake, oxidation and release of substrates in tissues which include the mammary gland, liver, hind limb skeletal muscle and brain. The same procedures have been used to measure protein turnover in the whole animal and in defined tissues. The application of new technology to the continuous measurement of both blood flow and blood oxygen content has made it possible to combine isotope dilution studies with the simultaneous measurement of energy expenditure in the whole animal and in defined tissues. The power of these combined procedures is such that a range of key parameters in animal nutrition may be monitored. These include inflows of nutrients from the alimentary tract; production rates of the major energy yielding nutrients; the uptake and utilization of nutrients by productive tissues, e.g. skeletal muscle and mammary gland; and the influence of the balance of energy yielding nutrients and essential amino acids on the oxygen consumption (energy expenditure) of both the whole animal and defined tissues. The use of isotope dilution, particularly when combined with other quantitative procedures, has yielded data which have led to improved efficiency of ruminant production, and identified
areas which require more research effort, as indicated in the review. In particular, the use of isotopes has emphasized that nutrition today must be concerned more with matching nutrient availability to tissue requirements than with the metabolizable energy and crude protein content of feedstuffs, if the efficiency of livestock production is to be maximized.

1. INTRODUCTION

The foundations of modern ruminant nutrition were laid in Cambridge, United Kingdom, in the 1940s with the discoveries that both dietary carbohydrate [1] and protein [2] are fermented in the rumen to volatile fatty acids (VFAs) and ammonia respectively. Both end products were shown to be absorbed from the rumen, the VFAs constituting a major energy source for maintenance and production. Much of the absorbed ammonia was shown to be recycled to the rumen as salivary urea, and used for microbial protein synthesis [2]. In the early 1950s it was discovered that dietary lipids are rapidly hydrolysed in the rumen, and that the unsaturated fatty acids which predominate in a herbage undergo extensive biohydrogenation, as outlined by Garton [3]. Although the fermentation of plant materials in the rumen had been recognized a century earlier, the main features of the extraordinarily complex rumen ecosystem did not emerge until the late 1930s and 1940s. These developments were brilliantly reviewed by Hungate [4], the major contributor to this field in those formative years.

The use of isotopes in the investigation of metabolism began in the 1930s, when Schoenheimer and Rittenberg used deuterium to study fat metabolism in mice [5]. A later study with $^{15}$N labelled glycine demonstrated for the first time the turnover of protein in the body [6]. The use of stable isotopes to study nutrition, however, has always been hampered by the complexity and costs of analysis, and the relative insensitivity of stable isotope detection. This has severely limited the use of isotope dilution procedures based on $^{15}$N labelled materials, although the first comprehensive quantitative study of nitrogen metabolism in the rumen was based on the use of $^{15}$NH$_3$ [7].

Nuclear techniques based on radioactive and stable isotopes have been used to determine body composition and to investigate many facets of mineral metabolism. These topics were comprehensively reviewed in 1972 [8]. The fact that isotopes of many trace elements are readily obtainable has encouraged studies on their availability for utilization by tissues, but as stressed by Suttle [9], many factors impinge on the absorption of these elements, and great care is required in the design and interpretation of experiments. The application of nuclear techniques to mineral metabolism will not be discussed further in this review.

The availability of metabolites labelled with the radioactive isotopes $^{14}$C and $^3$H in the 1950s had a dramatic impact on two areas of ruminant nutrition. These were the delineation of the metabolic pathways of key nutrients, and the application
of isotope dilution to measure the availability and rates of oxidation of the major energy yielding nutrients (acetate, glucose, 3-hydroxybutyrate and plasma non-esterified fatty acids (NEFAs)) in the whole animal, and in organs and defined tissues. Early studies on rumen metabolism using $^{14}$C labelled substrates were reviewed by Shaw in 1961 [10].

The utility of the classical arteriovenous (AV) difference method for the study of tissue metabolism was greatly enhanced when combined with isotope dilution [11]. Current knowledge of the role of circulating glucose, acetate, amino acids and lipids in milk biosynthesis, for example, is largely based on these combined procedures [12]. New developments in technology which make it possible to monitor continuously both blood flow and blood oxygen content add another dimension to the AV difference-isotope dilution procedure. In addition to an already impressive list of quantitative metabolic data provided simultaneously on both defined tissues and the whole animal, the oxygen consumption of the tissue and overall oxygen consumption can be continuously monitored [13]. This capacity to monitor energy expenditure continuously will facilitate the study of a key area of animal nutrition, namely the interrelationships of nutrient imbalances and energy cost. Other areas which will profit from the application of this new technology include exercise physiology and the evaluation of drugs and hormones which influence energy metabolism.

The use of isotope dilution, particularly when combined with other quantitative procedures, has yielded data which have led to improved efficiency of ruminant production, and identified areas which require more research effort, as will be indicated in this review. In particular, the use of isotopes has emphasized that nutrition today must be concerned more with matching nutrient availability to tissue requirements than with the metabolizable energy and crude protein content of feedstuffs. In production systems the objective is to optimize livestock production from available sources.

2. DELINEATION OF METABOLIC PATHWAYS IN RUMINANTS

2.1. Mammary gland metabolism

Mammary gland metabolism has been studied using a range of in vitro and in vivo procedures. Tissue slices proved useful in comparisons of the relative importance of glucose and acetate in milk fat synthesis (see below), but current knowledge of milk biosynthesis is largely based on the classical AV difference technique brilliantly adapted by Linzell [14] for use in goats and cows. Application of the AV difference technique to mammary metabolism is facilitated by the ease of access to both the glands, and their venous drainage. An essential feature of the approach is that measurements are made in conscious, unstressed animals.
Isotopically labelled substrates are of obvious value in studies of milk biosynthesis, and Hevesy, a pioneer in the use of isotopes in biology, administered inorganic $^{32}$P to lactating goats in 1938 [15]. This first report of the application of isotopes in lactation showed that milk inorganic P, casein P and phospholipid P are derived from plasma inorganic P. The transfer of other ions ($^{89}$Sr, $^{59}$Fe, $^{22}$Na) into milk was later demonstrated, but as pointed out by Linzell [16], only the proportion of the administered dose of isotope excreted in milk was reported. Information on the rates of transfer of ions, or on mammary permeability, requires time course studies of changes in specific radioactivity (SRA).

The availability of $^{14}$C labelled metabolites in the early 1950s led to numerous studies on milk biosynthesis. In most instances, likely precursors of milk fat, lactose or casein were injected or continuously infused into lactating animals. Milk was collected at intervals after the administration of labelled materials, and the relevant constituent assayed for radioactivity. Studies of this nature were greatly aided by the criteria for the design and interpretation of experiments seeking to elucidate precursor–product relationships outlined by Zilversmit et al. in 1943 [17], which hold good today.

2.1.1. The role of acetate in milk fat synthesis

By the late 1940s, the quantitative importance of acetate as an energy source for ruminants had been established [1], but little was known of the pathways of acetate utilization. At that time, Folley and French [18] were incubating mammary slices from lactating rats, rabbits, sheep and cows with acetate as the sole substrate. Ruminant mammary tissue respired actively, with a respiratory quotient (RQ) above 1, but non-ruminant tissue was much less active, with an RQ below 1. These data suggested that ruminant tissue, but not non-ruminant tissue, could synthesize fat when acetate was the sole substrate. In later studies, mammary slices from lactating rats or sheep were incubated with a mixture of $^{13}$C-acetate and $^{14}$C-glucose. About 38 and 62%, respectively, of the carbon in the fatty acids synthesized by rat mammary tissue were derived from acetate and glucose, but the corresponding values for sheep tissue were 97 and 3% [19]. The small contribution of glucose to fatty acid synthesis in ruminant mammary tissue, unlike non-ruminant tissue, was shown later to occur in adipose tissue [20].

A more dramatic demonstration of the role of acetate in ruminant milk synthesis was achieved in 1951 by Popjak and his colleagues [21], who injected $^{14}$C labelled acetate intravenously into a lactating goat. Blood and milk samples were taken at intervals and the milk fatty acids assayed for SRA. The results showed that short chain fatty acids in milk fat are not derived from the degradation of long chain fatty acids in the mammary gland, as suggested by earlier workers, and that all of the short and medium chain length fatty acids up to palmitic acid are synthesized in mammary tissue from acetate. The $C_{18}$ fatty acids stearate and oleate were
not derived from acetate, and later work has confirmed the absence of the chain elongation system in ruminant mammary tissue.

2.1.2. The role of blood glucose in lactose synthesis

Time course studies of the SRA of circulating glucose and lactose following the injection or continuous infusion of $^{14}$C-glucose have shown that in both the cow and goat, blood glucose accounts for about 80% of lactose carbon [12].

In general, the interpretation of data based on the continuous infusion of labelled substrate to achieve constant SRA of circulating substrate, as in the measurement of entry rates (Section 4), is much simpler than with corresponding data from single injection experiments. Furthermore, the SRA of arterial blood CO$_2$ or of respiratory CO$_2$ provides a rough guide to the contribution of the substrate to total oxidative metabolism [11].

2.1.3. Mechanism of uptake of circulating triacylglycerol

The observation of Linzell and his colleagues that circulating low density lipoproteins and chylomicrons are taken up by the mammary gland and used for milk fat synthesis [16] raised the issue of the mechanism of uptake of relatively large aggregates of lipid from the mammary capillary bed into secretory cells. Indirect evidence of prior hydrolysis was obtained during the infusion of albumin-bound $^{14}$C labelled free palmitate, stearate or oleate. Net uptake from these fatty acids was small and variable, but considerable radioactivity appeared in milk fat, indicating mammary uptake, and of equal significance, the SRA of the labelled fatty acid fell considerably across the mammary gland [12]. The latter observation proved that unlabelled fatty acid was being released during the passage of blood across the gland. Direct evidence that hydrolysis precedes the uptake of triacylglycerol was obtained by infusing into the mammary artery chylomicrons in which the glycerol and fatty acid residues had been labelled with $^3$H and $^{14}$C respectively [12]. Labelled glycerol and fatty acids both appeared in milk fat, but in different proportions to those in the original labelled chylomicrons, and only free labelled glycerol and fatty acids were detected in venous blood and lymph.

2.1.4. Milk protein synthesis

Early studies on amino acid utilization by the ruminant mammary gland based on AV difference measurements were bedevilled by inadequate techniques for the measurement of blood flow. This led to the view that plasma globulins, and not free amino acids, were the precursors of milk protein [22]. Experiments in which
14C labelled amino acids were injected into goats [22] clearly showed that circulating amino acids are the precursors of casein. Plasma proteins were found to be of only minor importance as precursors of components of the whey fraction of milk.

Progress in the area of amino acid metabolism in the 1950s was greatly facilitated by the development of ion exchange chromatography for the separation and identification of mixtures of amino acids [23].

3. NITROGEN METABOLISM IN THE RUMEN

The degradation of dietary protein to ammonia in the rumen [2] and the fact that ammonia is an important source of N for microbial protein synthesis were recognized in the late 1940s. The economic significance of the potential replacement of dietary protein with inexpensive non-protein nitrogen (NPN), and in particular urea, encouraged research in this area which culminated in the demonstration by Virtanen [24] that ruminants could be maintained on diets in which NPN was the sole nitrogen source. By the late 1950s, 15N labelled urea and ammonium salts had been used to demonstrate the synthesis of microbial protein, carcass protein and milk protein from NPN, as discussed by Annison [25].

The most important pathway for amino acid synthesis in the rumen is transamination, mediated through glutamic and aspartic acid, and the relevant enzymes for the synthesis of most amino acids have been identified in cell free extracts of rumen bacteria [25].

Elucidation of the metabolic pathways involved in the use of NPN for microbial protein synthesis was followed by the use of 15N and 14C labelled materials to measure rates of N entry, exit and interconversions, as outlined in Section 4.6.

4. APPLICATION OF ISOTOPE DILUTION TO NUTRITION

An important outcome of the availability of isotopically labelled substrates was that it became possible to measure the rates at which nutrients become available for utilization by tissues. This was achieved by isotope dilution procedures, which may also be used to measure the uptake, release and oxidation of nutrients in defined tissues. Furthermore, the use of 14C labelled substrates in isotope dilution studies provides additional data on the amount of substrate oxidized, and on the contribution of substrate to oxidative metabolism [12].

Isotope dilution procedures are based on the administration of labelled substrates, followed by the withdrawal of blood at intervals of several hours for the assay of the SRA of circulating substrate [12]. An important feature of the technique is the use of labelled substrates of high radioactivity but insufficient mass to provoke
a hormonal response to the injection or infusion of the substrate. Adequate mixing of labelled substrate with the body pool of substrate, and maintenance of relative constancy of pool size, are essential. Utilization by tissues of labelled substrate before complete mixing with body pool must obviously be avoided, but the metabolism of significant amounts of unlabelled substrate entering the body pool before complete mixing with labelled substrate is also unacceptable. The latter situation would preclude measurement of glucose entry rates in fed non-ruminants, for example, in situations where a significant proportion of absorbed glucose is utilized by the liver before equilibration with body pool. Both constant infusion and single injection techniques require steady state conditions in which body pools remain of constant size while undergoing replacement with an input equal to rate of outflow. In practice, blood levels of the substrate should not fluctuate by more than ±10% of the mean concentration during the experiment.

Several reports of the limited use of 14C labelled glucose and acetate in ruminants in vivo had appeared in the early 1960s, but the most concentrated studies on the biokinetics of the major energy yielding nutrients in sheep were carried out at the University of New England (UNE), Armidale, Australia, from 1961. These studies were based on the use of 14C labelled substrates, and measurement of the SRA of expired 14CO2 or of arterial blood 14CO2 provided data on substrate oxidation. This approach to quantitative nutrition culminated in 1967 [26] with comprehensive measurements of the rates of entry and oxidation of glucose, the major VFAs and plasma NEFAs. In this study, for the first time in ruminants, O2 consumption, CO2 production and the SRA of expired 14CO2 were monitored during the continuous infusion of 14C labelled substrates. Somewhat disappointingly at the time, the sum of the values for the contribution of individual substrates to total oxidative metabolism accounted for only 70% (fed sheep) and 58% (starved sheep) of total CO2 production. Similarly, corresponding values for the proportion of substrate entry directly oxidized indicated that the substrates studied accounted for 63 and 43% of total energy expenditure on the basis of oxygen measurement. The failure to account for total CO2 production was largely due to the entry of 14C labelled carbon into pools of low turnover rate. More surprising, however, was the inability to account for more than two thirds of total energy expenditure. New developments in methodology, however, which make it possible to monitor oxygen consumption continuously (Section 7.2) will allow this apparent anomaly to be re-examined.

4.1. Glucose biokinetics

In the 1950s, data on several aspects of glucose metabolism suggested that this substrate was of minor importance in ruminants. Factors contributing to this view included the paucity of alimentary glucose, since on most diets little α-linked glucose
polymer reaches the small intestine, the relative insensitivity of sheep to the hypoglycaemic action of insulin [12], and the negligible role of glucose as a carbon source for milk fatty acid synthesis in ruminants (see above). The data on milk fat synthesis were obtained with $^{14}$C labelled substrates, and fittingly, the use of $^{14}$C labelled glucose in isotope dilution studies led to a re-evaluation of the quantitative importance of glucose in ruminants.

In the first report from UNE in 1961 [27], glucose entry rates in sheep measured by isotope dilution were shown to be similar to values obtained in starved non-ruminants. Entry rates were measured by the continuous infusion of uniformly $^{14}$C labelled glucose, a method developed for use in dogs [28]. The method depends on the achievement of constant SRA of circulating glucose, which usually occurs 4–5 h after the start of the infusion. The constancy of blood glucose SRA at that time is useful when examining precursor–product relationships, particularly the extent of glucose oxidation. Single injection procedures based on $^{14}$C labelled glucose yield data of similar precision [12], but are less useful when assessing precursor–product interrelationships.

The use of tritiated glucose labelled on specific carbons of the glucose molecule which are lost early in metabolism (e.g. 2-$^3$H-glucose) when combined with $^{14}$C-glucose has allowed estimates of total entry rates and irreversible loss rates simultaneously [29].

Glucose entry rates measured by isotope dilution represent the flow of glucose into the circulation from all sources, which includes absorption from the alimentary tract, hepatic gluconeogenesis and glycogenolysis. A key feature of glucose metabolism in ruminants is that gluconeogenesis is a major metabolic activity in both fed and starved states.

The importance of gluconeogenesis in ruminant metabolism led to attempts to measure the relative contribution of potential precursors, including propionate and glucogenic amino acids, by isotope dilution. An obvious approach was based on the continuous intravenous infusion of $^{14}$C labelled substrate for long enough to achieve constancy of SRA in both the precursor and blood glucose. A major problem with this procedure is that most glucogenic precursors pass through, or are in equilibrium with, reactions of the tricarboxylic cycle, which results in the transfer of part of the labelled carbon into CO$_2$ rather than glucose [30]. This crossover of radioactivity, which leads to an underestimate of the contribution of precursor to glucose, can be corrected for from the distribution of labelled carbon in glucose [31].

Glucose supply to ruminant tissues, measured as entry rate by isotope dilution, is well correlated with feed intake in sheep [32] and lactating cows [33]. This relationship is not unexpected since the fermentation of carbohydrate in the rumen gives rise to propionate, an important precursor of glucose, and much of the energy generated during fermentation is utilized for microbial protein synthesis. The latter contributes significantly to the inflow of amino acids from the small intestine, many of which are glucogenic.
4.2. Acetate biokinetics

The role of acetate as the major energy source in ruminants encouraged the use of $^{14}$C labelled acetate in biokinetic studies in the early 1960s. These early studies were based on single injection procedures, and it was quickly appreciated that the small pool size and rapid turnover of acetate led to appreciable loss of labelled acetate before complete mixing with body pool [34]. Continuous infusion procedures proved more effective [35], but the finding that many tissues release acetate (endogenous acetate), even when extracting acetate from blood, implied that acetate entry rate could not be equated with acetate production in the alimentary tract. Endogenous acetate production may be identified by the decline in SRA of acetate across tissues [35].

The use of $^{14}$C labelled acetate in isotope dilution experiments has revealed that this substrate is a major contributor to total $\text{CO}_2$ production in fed goats (21%), sheep (26%) and lactating cows (33%), and not surprisingly, the value falls to about 10% in starved goats [36].

In the early-mid-1960s, labelled VFAs were used by several investigators to measure production rates in the rumen, as reviewed at an earlier symposium organized jointly by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency [37]. Single injection and continuous infusion procedures were used, but the latter procedure introduced by Leng and Leonard [38] is now the method of choice. The precision of the method is inevitably adversely affected by the need to infuse into, and sample from, the rumen. Corrections may be made for the interconversions of acetate and butyrate revealed by the use of labelled substrates [39]. More recent studies [40] have shown that the extent of acetate–butyrate interconversion precludes the measurement of the production rates of all three major rumen VFAs from a knowledge of their molar proportions and data based on the intraruminal infusion of one $^{14}$C labelled VFA, as suggested in earlier reports. A procedure involving the separate infusion of $^{14}$C tracers of all three VFAs into hourly fed animals is recommended [40].

4.3. 3-Hydroxybutyrate biokinetics

Entry rates of 3-hydroxybutyrate in ruminants were first measured by Leng and Annison in 1964 [41] using $^{14}$C labelled 3-hydroxybutyrate prepared by the incubation of $^{14}$C labelled butyrate with sheep liver slices or rumen epithelium. Continuous infusion procedures were used, and comparison of the SRA of circulating 3-hydroxybutyrate and $\text{CO}_2$ during the terminal stages of the infusion suggested that the contribution of 3-hydroxybutyrate to total oxidative metabolism in fed sheep was about 5%. Later studies [26] showed that this value rose to about 10% in starved sheep. More recent work [42] has shown that since there is incomplete interconversion of 3-hydroxybutyrate and acetoacetate, neither entry rate nor oxidation of
ketone bodies can be estimated by assuming that circulating ketone bodies represent a single metabolic component. This difficulty was overcome by the use of a three compartment model. In fasted pregnant ewes, the oxidation of 3-hydroxybutyrate plus acetoacetate accounted for about 30% of total CO$_2$ production [42].

4.4. Plasma non-esterified fatty acid biokinetics

The triacylglycerols which make up adipose tissue, the major source of stored energy in animals, are mobilized only as NEFAs. In fed animals, plasma NEFA levels are low, but when adipose tissue is mobilized, as in starvation, or when tissue lipolysis is stimulated, plasma NEFA concentrations rise. The relatively low concentrations of plasma NEFAs, however, must reflect high turnover, and studies with $^{14}$C labelled fatty acids confirmed their high entry rate and ready oxidation, as discussed by Annison in 1963 [43].

Isotope dilution studies based on the single injection or continuous infusion of $^{14}$C labelled long chain fatty acids confirmed the metabolic importance of plasma NEFAs in man, dogs and rats, but the first biokinetic studies with these labelled substrates in ruminants were made at UNE in 1964 [44]. Sheep were continuously infused with $^{14}$C labelled palmitate bound to albumin, and the assumption was made that palmitate behaved as a typical representative of plasma NEFAs. Rates of entry of palmitate and the contribution of palmitate to total CO$_2$ production were both correlated with plasma concentration. In later studies with pregnant sheep based on the continuous infusion of $^{14}$C labelled palmitate, stearate and oleate, Pethick et al. [45] showed that there is a common linear relationship between plasma NEFA concentration and rates of entry and oxidation. Oxidation of NEFAs accounted for 34 and 58% of respiratory CO$_2$ in fed and fasted (3–4 d) pregnant ewes. In starved non-pregnant sheep the contribution of NEFAs to total CO$_2$ production is about 30%.

4.5. Amino acid and protein biokinetics

Isotope dilution procedures have been used to measure flux rates of amino acids and their contribution to gluconeogenesis and oxidative metabolism, as discussed by Lindsay [31]. In addition, amino acid flux rate has been used to calculate total body protein synthesis. In a typical study Lobley et al. [46] continuously infused $^{14}$C labelled leucine to measure leucine flux rate and the rate of oxidation of leucine from the SRA of expired $^{14}$CO$_2$ in steers. The leucine flux rate was used to calculate total body protein synthesis, using the mean leucine content of the body protein of cattle. A limitation of the method, recognized by the authors, is the assumption that the SRA of circulating amino acid, in this case leucine, is identical with or closely similar to the SRA of the aminocacyl-tRNAs involved in protein synthesis.

Measurements of whole body protein synthesis by isotope dilution are time consuming and expensive, and the differential responses of individual tissues to
changes in nutritional or physiological status severely weaken the usefulness of whole animal data. Studies with defined tissues or organs using combined isotope dilution and AV difference methods are likely to prove more useful (Section 6).

4.6. Nitrogen transactions in the rumen

As discussed earlier (Section 3), qualitative data on nitrogen metabolism in the rumen emerged over the period following the initial recognition by McDonald [2] of the significance of the degradation of dietary protein in the rumen. Quantitative data were sparse, however, until Mathison and Milligan [47] and Nolan and Leng [7] used isotope dilution techniques with $^{15}$N-ammonium sulphate, $^{15}$N-urea and $^{14}$C-urea to measure ammonia and urea exchanges in the rumen of sheep. These comprehensive studies yielded data which were used to construct models of nitrogen metabolism in sheep showing the movement of nitrogen between the various nitrogenous pools in the body. A particularly striking finding was that only about 20% of the urea passing from plasma to the digestive tract entered the rumen, the remainder being degraded in other parts of the tract.

Subsequent studies in this area were reviewed in 1984 by Leng and Nolan [48], who emphasized the significance of the efficiency of microbial synthesis in the rumen for the ratio of protein to energy in the nutrients which become available to the animal.

5. MICROBIAL GROWTH IN THE RUMEN

In the late 1960s, microbial cell growth in the rumen, and therefore the supply of microbial protein, were measured in animals fitted with cannulae in the rumen, abomasum and duodenum using a range of markers to label bacteria, digesta and the liquid phase of gut contents. Flows of microbial and non-microbial protein from the rumen were found to be highly variable [49]. More importantly, later studies have shown that the efficiency of microbial growth varies widely. This inability to predict with acceptable precision microbial protein yield from the amounts of organic matter digested has greatly weakened the utility of systems seeking to predict amino acid supply from the amount and characteristics of the diet [50]. Furthermore, these difficulties have tended to obscure the significance of one of the crucial features of metabolism in the rumen, namely that microbial growth and VFA production are stoichiometrically and inversely related [51]. These and other stoichiometric relationships were developed by Baldwin [52] and Leng [51] from data on the metabolic pathways involved in the fermentation of carbohydrate to VFA, and from the dynamics of nitrogen metabolism in the rumen [48].

The manipulation of rumen metabolism to increase microbial growth, and hence the supply of amino acids to the animal, is a vital research objective, since
in many systems of livestock production essential amino acids are rate limiting nutrients [53]. Indeed, the ratio of protein to energy (P/E ratio) of the nutrients which become available to the animal influences both efficiency of production and voluntary food intake, as discussed by Leng [54]. It is pertinent to stress that when bypass protein (protein largely undegraded in the rumen) is fed to increase directly amino acid supply, the ruminal degradation of even a small proportion of bypass protein generates peptides, amino acids and ammonia, all of which may stimulate microbial growth [55].

The background to systematic attempts to increase the supply of microbial protein emerged from studies of nitrogen flows in the rumen, which revealed an unexpectedly high rate of bacterial N turnover [48]. Factors contributing to this include the predation of bacteria by protozoa [56, 57] and the activities of rumen bacteriophages [58]. The beneficial effects of rumen defaunation are well established [57], and efforts to control rumen bacteriophage will inevitably follow the demonstration of their significance.

6. ISOTOPE DILUTION COMBINED WITH AV DIFFERENCE MEASUREMENTS

The quantitative study of tissue metabolism began with Chauveau in 1887 [59], who measured AV differences of $O_2$, $CO_2$ and glucose across the levator muscle of the upper lip of the horse. This was the first known application of the principle stated by Fick in 1870, and cited by Zierler in 1976 [60]. The Fick principle postulates that net substrate uptake or release by a defined tissue or organ may be measured as the product of AV difference and blood flow. When using this technique, several stringent conditions must be satisfied. These are that blood flow, tissue uptake of substrate and arterial concentration of substrate must all remain constant. Also, the venous blood that is sampled must be representative of total venous drainage [11].

The precision of AV difference measurements is directly related to the accuracy with which blood flow can be measured. In recent studies at the University of Sydney a new system based on ultrasonics (Transonics Systems Inc., Ithaca, New York) has been used to measure blood flow in the pulmonary artery, portal vein and mammary artery [13]. Blood volume flow is sensed independently of blood vessel size, vessel alignment and flow profile. The flow probe is surgically implanted around the blood vessel, and connected to a benchtop meter. Electrical signals from the meter provide inputs to a micropower data logger. The system, which measures blood flow continuously, has proved to be robust and reliable. Most previous AV difference studies have relied on intermittent measurements of blood flow, and the ability to monitor blood flow continuously will greatly increase the precision of AV difference measurements.
Measurement of entry rate based on the continuous infusion of $^{14}$C labelled substrates requires constancy of SRA of circulating substrate for several hours, as discussed earlier (Section 4.1). AV difference measurements across defined tissues are made during this period, and data are generated on the uptake, oxidation and release of the metabolite being studied. The procedure, first used in ruminants to examine the role of glucose and acetate in the oxidative metabolism of the testis and epididymis in 1963 [61], has proved outstandingly successful in studies of nutrient utilization by the mammary gland, hind limb skeletal muscle and brain, as discussed below.

6.1. Mammary gland

Much of current knowledge of milk biosynthesis and mammary gland metabolism in ruminants was obtained by combining AV difference measurements with isotope dilution [12]. A single experiment based on the continuous infusion of $^{14}$C labelled substrate yields an impressive array of data which includes the entry rate of the substrate and its contribution to CO$_2$ production in the whole animal; the uptake of substrate by the mammary gland, the amount oxidized and its contribution to the oxidative metabolism of the gland; and the rate of release of substrate by the gland, detected by a fall in SRA across the gland. Comprehensive data on the utilization of glucose and acetate by the mammary glands of goats, sheep and cows have been reported [12].

Isotope dilution combined with AV difference procedures based on the continuous infusion of $^{14}$C labelled leucine has been used to measure protein synthesis and degradation in the mammary gland of lactating goats [62]. Convincing evidence was obtained of significant turnover of milk proteins, an unexpected finding which should be studied further.

6.2. Hind limb skeletal muscle

The technique developed by Domanski et al. in 1974 [63] for the study of hind limb metabolism in sheep in vivo has been widely used in studies in which AV difference measurements have been combined with isotope dilution. In a modification of the technique, catheterization was modified to sample venous drainage from a defined muscle bed [64]. The modified preparation was used to examine valine metabolism in skeletal muscle by isotope dilution combined with AV difference measurements. Data were obtained on rates of protein synthesis in sheep hind limb muscle [65]. In a more comprehensive study, Oddy and Lindsay [66] used the same procedures with $^{14}$C labelled leucine to measure rates of protein synthesis, gain and degradation in the hind limb muscle of lambs. The finding that rate of protein degradation had more influence on net protein deposition than rate of protein synthesis was an important and controversial finding.
The data discussed above were obtained in resting animals, but the same procedures have been applied to the hind limb muscle of working animals [67]. Glucose and NEFAs were found to be the principal metabolic fuels for contracting muscles in the working ruminants. The uptake of acetate by muscle was influenced more by the level of feed intake than the extent of exercise, but the relationship between circulating level of acetate and utilization by muscle was not established. Future work in this area will be facilitated by the new procedures for the continuous measurement of blood flow and blood oxygen content.

6.3. Brain

The AV difference procedure combined with isotope dilution has been used to study metabolism in the sheep brain in vivo, by sampling venous drainage from the superior sagittal sinus. This approach showed that the sheep brain, unlike the human brain, has no capacity to utilize ketones even in the marked hyperketonaemia of undernourished pregnant animals [68].

7. MEASUREMENT OF ENERGY EXPENDITURE

The basic principles of energy metabolism in animals emerged from the use of simple calorimeters by Lavoisier and Laplace in the eighteenth century, as discussed by Blaxter [69]. Improvements in animal calorimetry, and in particular the widespread adoption of indirect calorimetry [70], have provided data on the energy expenditure of ruminants over a range of nutritional states. Most research groups use automated open circuit respiration chambers, and improved systems are available for gas analysis, measurement of air flows and computerized data acquisition. The precision of these systems is excellent, but equipment costs are high and animals must be confined within respiration chambers.

Alternative approaches to energy metabolism include the measurement of energy expenditure from CO₂ production and the direct measurement of oxygen consumption by AV difference across the lungs.

7.1. Carbon dioxide production

The measurement of energy expenditure from CO₂ production rather than oxygen consumption is somewhat less precise [71], but in many situations the loss of precision is outweighed by practical advantages.
7.1.1. Carbon dioxide entry rate

The widespread use of isotope dilution procedures based on $^{14}$C labelled substrates to measure the entry rate (rate of entry into total body pool) and extent of oxidation of energy yielding nutrients led Leng and his colleagues to use $^{14}$C labelled sodium bicarbonate to measure CO$_2$ entry rates in ruminants. Single injection [72] and continuous infusion [73] procedures are equally effective, but the former require multiple sampling of body fluids. In the continuous infusion procedure, the objective is to achieve constancy of SRA of $^{14}$CO$_2$ in blood, and for this reason the infusion is continued for up to 12 h [74]. The intrinsic problem with the method is that the CO$_2$ pool is not homogeneous, but consists of many interlinked pools of varying turnover rate. Also, CO$_2$ is fixed in a number of metabolic cycles, and the recycling of labelled CO$_2$ is probably the major reason why even after 12 h complete constancy of the SRA of $^{14}$CO$_2$ in expired air, blood, urine and saliva is not achieved. Nevertheless, the method is relatively simple and inexpensive, and has provided useful data on the energy expenditure of grazing animals [73].

In general, the method is only applicable when the metabolic rate of the animal is relatively constant during the sampling period. With resting animals reported values for CO$_2$ output obtained by isotope dilution differed by only 2–4% from values obtained by direct measurement of gas exchange [74]. The procedure is inapplicable to working animals, where major changes in CO$_2$ pool size occur over short periods. Also, the nature of the system precludes its use to monitor responses over short time intervals.

7.1.2. Doubly labelled water

The basis of the method using doubly labelled water is that whereas hydrogen is lost from the body mainly as water, oxygen is lost both in water and as CO$_2$ [75]. This implies that if the animal is given a dose of water enriched with $^2$H$_2$$^{18}$O to label the total H$_2$O pool, the degree of enrichment (specific activity) of $^{18}$O will decline faster than that of deuterium. Further, the difference in the two rates of decrease multiplied by the volume of the total H$_2$O pool is a measure of loss of CO$_2$. Total body water, incidentally, may be estimated from the specific activity of body water shortly after administration of the labelled water.

In practice, the specific activities of both deuterium and $^{18}$O are determined in any body fluid, such as saliva or urine, for up to 14 d following the administration of doubly labelled water. Measurements are made during the first few hours to determine both the initial equilibrium specific activity and total body water.

Application of the doubly labelled water method to ruminants must take into account the large reservoir of water in the rumen, which may hinder the rapid and complete equilibrium of oxygen atoms between CO$_2$ and water. Also, changes in rumen volume during the course of an experiment may cause unacceptably large
changes in total body water. Midwood et al. [76], however, have shown that in sheep, volume of water in the rumen is not a critical factor, and that it is possible to correct for changes in total body water. Loss of methane, which may cause energy consumption to be underestimated by 3%, can be compensated for by estimating methane production from the composition of the feed.

The main advantage of the use of doubly labelled water to measure energy expenditure is that the procedure is non-invasive, and requires only urine samples at infrequent intervals. The disadvantages are that the accurate determination of the isotopes requires expensive analytical techniques based on mass spectrometry, and that the method gives only average data on total CO₂ production over time intervals of days. It is unlikely that changes in metabolic rate of less than 10–12% can be determined by this method.

7.2. Direct measurement of oxygen consumption

Although not involving nuclear technology, the recent development of procedures which continuously measure oxygen consumption in normal, conscious animals [13] could be integrated with isotope dilution procedures to investigate the interrelationships of nutrient balance and the efficiency of energy utilization.

The new system is based on the measurement of cardiac output and the difference in blood oxygen content across the lungs. The concept is not new, but successful application of the principle has been made possible by the availability of new methods for the continuous monitoring of both blood flow and blood oxygen content in the pulmonary artery.

The new ultrasonics procedure was discussed earlier (Section 6). The development of fibre optic catheter technology, largely for use in human medicine, has made it possible to monitor continuously the oxygen content of blood. The method is based on the reflectance and measurement of light of a particular wavelength reflected from a ‘window’ located at the end of the catheter. The Oximetrix catheters currently available [13] have proved to be entirely suitable for use in sheep.

The new procedure could be combined with both isotope dilution and AV difference procedures to study the effects of changes in the balance of nutrients on energy expenditure in the whole animal and in defined tissues. An obvious candidate for study by these procedures is the vexed question of the efficiency of utilization of acetate, particularly in circumstances where acetate supply is high. This controversial subject was reviewed recently by Leng [54].

8. LESSONS FOR PRESENT RESEARCH

Isotopic tracer technology has proved invaluable in most areas of ruminant nutrition, and indispensable in the elucidation of metabolic pathways in the rumen,
and in defined tissues like skeletal muscle and mammary gland. Closer definition of the factors which impinge on the efficiency of utilization of energy yielding nutrients and amino acids in livestock will be facilitated by combining current tracer technology with new methods for the continuous measurement of energy expenditure. The importance of optimizing P/E ratios to ensure maximum efficiency of livestock production cannot be overemphasized. In many instances this may be achieved by increasing amino acid supply to tissues, either by increasing rumen microbial yields or by supplying bypass protein. These approaches, based on the supplementation of high forage diets with either urea-molasses blocks or bypass protein, have proved outstandingly successful in the field [53]. Further efforts to manipulate rumen metabolism to increase microbial supply must be encouraged.

Studies involving nuclear technology are complex and time consuming, and it is often difficult to obtain data on enough animals to satisfy statistical criteria. This problem would be minimized by the availability of groups of identical animals obtained by cloning techniques [77]. Experiments with identical twins have shown that their responses either to changes in nutritional or physiological status or to challenge by drugs or hormones are virtually the same.

Many of the procedures applied to experimental animals discussed in this review are invasive, and it is important to stress that at this time essential data cannot be obtained in any other way. Since even minor discomfort, irrespective of cause, may result in significant physiological changes, minimization of stress in animals is a vital factor in quantitative nutritional studies. A promising non-invasive procedure for the measurement of metabolic parameters, such as gluconeogenesis, which has been applied at this stage only to small laboratory animals, is based on nuclear magnetic resonance [78]. At present, equipment costs are very high, but the approach has great promise for the future.

REFERENCES


ANNISON and LENG


OPTIMIZING THE USE OF POOR QUALITY FORAGE FEED RESOURCES FOR RUMINANT PRODUCTION: SUPPLEMENTATION WITH BYPASS NUTRIENTS

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Abstract

OPTIMIZING THE USE OF POOR QUALITY FORAGE FEED RESOURCES FOR RUMINANT PRODUCTION: SUPPLEMENTATION WITH BYPASS NUTRIENTS.

In many parts of the world poor quality forage crops are the major feed resource available for ruminants for a large part of the year. A number of alternatives are available for attempting to optimize the utilization of these types of feeds. The paper examines the option of supplying deficient nutrients post-ruminally on the utilization of poor quality forages where these forages constitute the bulk of the diet. Methods of supplying nutrients post-ruminally and factors likely to affect that supply are examined and discussed. It is concluded that post-ruminal supply of carbohydrate sources is generally effective in increasing the efficiency of nutrient utilization but also results in reduced basal dry matter intake. Enhanced amino acid supply to the small intestine results in increased production responses and in some instances improves dry matter intakes.

1. INTRODUCTION

In many parts of the world poor quality hays, pastures and cereal crop residues form the major part of the feed resources available for ruminants. The utilization of these poor quality roughages by the animal is limited by nutrient deficiencies (particularly protein), low voluntary intake and low digestibility. Attempts to improve the intake and utilization of poor quality roughages have included physical processing, chemical treatment or a mixture of both [1, 2]. Supplementation of poor quality forage diets with protein or non-protein nitrogen has been shown to increase voluntary intake [3, 4], which has been attributed, at least in part, to improved digestion in and increased passage from the rumen. It has also been reported that post-ruminal protein supply and subsequent metabolic effects enhanced the voluntary intake of low quality forages [5, 6] but it is not clear whether this was through alleviation of an amino acid deficiency, through the utilization of amino acids as energy sources or by increasing urea recycling to the rumen. The present paper will examine the effects of bypass nutrient supply, particularly nitrogen and energy sources, on the intake and utilization of poor quality roughages. Earlier reviews of this subject have been published [6–8].
2. TECHNIQUES AND PROCEDURES

Post-ruminal supply is derived from three sources, namely the rumen microbes, endogenous secretions and dietary components which have survived passage through the rumen. The last group is generally referred to as bypass nutrients, a succinct but erroneous description. The estimation of the relative contributions of these sources to total flow into the small intestines is extremely difficult. In fact only one component, the microbial part, has been measured to any extent and this can only be achieved directly using fistulated animals. A microbial marker such as incorporated $^{35}$S or $^{32}$P or some other internal component is used to establish marker:component ratios which are then used to establish what proportion of total component flow at the duodenum is of microbial origin. Endogenous secretions tend to be ignored but can contribute significantly to the nitrogen flow. An estimated value derived according to the live weight of the animal can be used [9]. Thus surviving feed component contribution to duodenal digesta flow is derived as the total component flow minus the sum of microbial and endogenous flows.

Given the generally severely modifying effect of the rumen on ruminant feeds, techniques such as maintaining the oesophageal groove reflex [10] in ruminating animals or the use of duodenal or abomasal fistulae have been used to some extent to determine the possible influence of bypass nutrients on the intake and utilization of poor quality forage. However, in the latter case experiments using chronically fistulated animals are extremely intensive and difficult to perform. There are also practical limitations on the numbers of animals which can be used in any one experiment and results obtained, particularly production responses, must be treated with caution.

3. POST-RUMINAL NITROGEN SUPPLY

3.1. Post-ruminal infusion

The infusion of casein into the duodenum of sheep receiving diets of oaten chaff resulted in a significant increase in the intake of roughage dry matter [5] (Table I) of the order of about 40%. A similar increase in dry matter intake (DMI) was also observed with sheep receiving ground corncob diets when soybean protein was infused into the abomasum [11] (Table I). The increases were assumed to be, at least in part, a result of increased urea recycling to the rumen and enhanced fibre digestion and dry matter outflow. Infusions of urea solutions into the duodenum of the sheep receiving the oaten chaff diets [5] confirmed the partial effect of urea recycling in that there was a small increase (about 23%) in DMI compared with protein infusion (Table I). In the same experiments inclusion of 4% urea in the diet resulted in only a 10% increase in forage DMI from protein infusions (Table I),
TABLE I. EFFECT OF POST-RUMINAL PROTEIN INFUSION ON FORAGE DRY MATTER INTAKE

<table>
<thead>
<tr>
<th>Animal</th>
<th>Diet</th>
<th>Infusate</th>
<th>Forage DMI (g/d)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>94% oaten chaff</td>
<td>Phosphate</td>
<td>593</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Casein</td>
<td>837</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urea</td>
<td>731</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>94% oaten chaff + 4% urea</td>
<td>Phosphate</td>
<td>667</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Casein</td>
<td>737</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>Ground corncobs</td>
<td>Carbonate</td>
<td>780</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soybean</td>
<td>1 104</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>70% ground corncobs 30% alfalfa hay</td>
<td>Nil</td>
<td>1 182</td>
<td>[11]</td>
</tr>
<tr>
<td>Sheep</td>
<td>Chopped lucerne + wheaten hay</td>
<td>Water</td>
<td>1 103</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Casein</td>
<td>1 075</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>Chopped ryegrass</td>
<td>Water</td>
<td>769</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Casein</td>
<td>831</td>
<td></td>
</tr>
<tr>
<td>Steers</td>
<td>85% prairie hay 15% concentrates</td>
<td>Urea</td>
<td>4 198</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Casein</td>
<td>4 287</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>98% alfalfa silage</td>
<td>Nil</td>
<td>19 900</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soya protein</td>
<td>18 300</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nil</td>
<td>18 500</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soya protein</td>
<td>16 400</td>
<td></td>
</tr>
</tbody>
</table>

Further suggesting that at least part of the intake effect was due to improved microbial fermentation. Further support was found when 30% alfalfa was included in the ground corncob diets given to sheep (Table I), which resulted in a similar increase in DMI compared with that seen with the protein infusions. Differences in effects between protein and urea infusions in sheep were not observed in steers. In both sets of experiments protein infusion post-ruminally increased rumen volumes [5, 11] but apparently decreased cellulose digestion [5], whereas post-ruminal urea infusion enhanced cellulose digestion [11] and the inclusion of alfalfa in the diet [11] increased digesta passage but not rumen fill. All of these effects could be reflected in a more active or efficient microbial fermentation with increased throughput influencing intake.
On the other hand there is also a large body of data showing no significant effects of post-ruminal infusion of protein sources on roughage DMI [12-15] (Table I). There are no immediate obvious differences between the two sets of data to explain the differences in response but importantly in all experiments post-ruminal infusion did not significantly reduce intakes of the basal forage and did result in positive production responses, indicating the value of post-ruminal supply of amino acids in animals on poor quality forages.

3.2. Protected protein supplements

Addition of protected protein (formaldehyde treated casein) to a semisynthetic diet of oat hulls and cellulose powder given to lambs resulted in increased DMIs

<p>| TABLE II. EFFECTS OF PROTECTED PROTEIN SUPPLEMENTS ON FEED INTAKE AND ANIMAL PERFORMANCE |
|---------------------------------|-----------------|-----------------|-------------|</p>
<table>
<thead>
<tr>
<th>Animal</th>
<th>Diet</th>
<th>Dry or organic matter intake (g/d)</th>
<th>Live weight change (g/d)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs</td>
<td>Oaten chaff</td>
<td>493</td>
<td>10</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>+ urea</td>
<td>525</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ urea + casein</td>
<td>463</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ urea + FT(^a) casein</td>
<td>553</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ urea + casein + FT casein</td>
<td>509</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Heifers</td>
<td>Chaffed straw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ urea</td>
<td>2830</td>
<td>-189</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>+ casein</td>
<td>3000</td>
<td>-108</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ FT casein</td>
<td>2650</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>Oat straw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ urea</td>
<td>1316</td>
<td>30</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>+ FT casein</td>
<td>1779</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ rapeseed meal</td>
<td>1750</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ FT rapeseed meal</td>
<td>1899</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ sunflower meal</td>
<td>1909</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ FT sunflower meal</td>
<td>1977</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>Chaffed hay</td>
<td>4020</td>
<td>100</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>+ urea</td>
<td>4370</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ FT casein</td>
<td>4290</td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) FT = formaldehyde treated.
[16]. Other early experiments where protected protein was added to poor quality roughage diets were summarized by Kellaway and Leibholz [17]. These authors concluded that supplements of non-protein nitrogen (NPN) and proteins (free and protected) were equally effective in stimulating forage intake, that is, when rumen degradable nitrogen (RDN) was not limiting, protein supplementation had no effect on intake. From their survey, the authors found production responses in only half of the experiments, which they attributed to increased absorption of amino acids from the small intestines.

Other work (Table II) confirmed the earlier assertion that protected protein supplementation does not significantly increase DMI over NPN supplementation. In these later experiments there were generally positive production responses when protein was the supplement compared with urea, with some further increases with protected protein supplements, although these were not always significant. Protected protein supplementation of long chopped roughages given to cattle had no effect on DMI but did affect partition of nutrients via a positive insulin and negative growth hormone response [22].

In a comparison of the effects of supplements of rumen degradable protein and rumen undegradable protein on the intakes of two low nitrogen containing forages by different breeds of cattle [23], it was found that formaldehyde treated casein had no additional effect on DMI over that of a rumen degradable supplement. Protein requirements for maximum feed intake of the two forages were met by supplements of RDN, although the calculated digestible crude protein (DCP) supplied by one of the forages was less than half that calculated to be required and the DCP supplied by the other forage was almost exactly the theoretical amount. Thus it seems that lack of response was influenced by completely different mechanisms with each forage.

3.3. Dietary protein supplements

In ruminants dietary proteins are subjected to microbial fermentation and proteolytic action in the reticulorumen, yielding peptides, amino acids and ammonia. All of these end products are essential for or enhance microbial growth and efficiency. Different feed proteins are resistant to varying degrees (Table III) [24–26] and thus some sources of protein supplements will provide greater amounts of undegraded dietary protein (UDP) at the duodenum than others. However, many of the values presented in Table III were obtained in animals receiving mixed forage–concentrate diets. In systems with high forage diets, particle residue time in the rumen will be longer and degradabilities of protein supplements could be higher. Responses in intakes and growth by cattle receiving protein supplements have been shown to be greater than with other supplements [27–29]. Similar responses in intakes, digestibilities, nitrogen retention, etc., have been recorded with different proteins supplying different amounts of UDP [30].
### TABLE III. ESTIMATES OF UNDEGRADABLE PROTEIN FRACTIONS FOR SOME COMMON SUPPLEMENTS [24–26]

<table>
<thead>
<tr>
<th>Feed</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnut meal</td>
<td>0.13–0.37</td>
<td>0.28</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>0.19–0.45</td>
<td>0.31</td>
</tr>
<tr>
<td>Rapeseed meal</td>
<td>0.13–0.28</td>
<td>0.16</td>
</tr>
<tr>
<td>Linseed meal</td>
<td>0.22–0.54</td>
<td>0.36</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>0.10–0.61</td>
<td>0.31</td>
</tr>
<tr>
<td>Cottonseed meal (solvent)</td>
<td>0.26–0.61</td>
<td>0.41</td>
</tr>
<tr>
<td>Cottonseed meal (pre-press)</td>
<td>0.35–0.38</td>
<td>0.36</td>
</tr>
<tr>
<td>Cottonseed meal (screw press)</td>
<td>0.43–0.57</td>
<td>0.50</td>
</tr>
<tr>
<td>Corn gluten feed</td>
<td>0.14–0.26</td>
<td>0.20</td>
</tr>
<tr>
<td>Brewer’s dried grains</td>
<td>0.27–0.66</td>
<td>0.53</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>0.46–0.51</td>
<td>0.50</td>
</tr>
<tr>
<td>Distiller’s dried grains</td>
<td>0.47–0.68</td>
<td>0.56</td>
</tr>
<tr>
<td>Blood meal</td>
<td>0.54–0.82</td>
<td>0.68</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>0.48–0.70</td>
<td>0.58</td>
</tr>
<tr>
<td>Fish meal</td>
<td>0.48–1.00</td>
<td>0.73</td>
</tr>
<tr>
<td>Meat meal</td>
<td>0.76</td>
<td>0.76</td>
</tr>
</tbody>
</table>

### TABLE IV. FEED INTAKE AND LIVE WEIGHT CHANGES OF LAMBS RECEIVING VARIOUS SUPPLEMENTS [31]

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Roughage DMI (g/d)</th>
<th>Live weight change (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>397</td>
<td>-76</td>
</tr>
<tr>
<td>Rolled lupins</td>
<td>537</td>
<td>-12</td>
</tr>
<tr>
<td>Pelleted lupins</td>
<td>593</td>
<td>13</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>580</td>
<td>22</td>
</tr>
<tr>
<td>Pelleted cottonseed meal</td>
<td>665</td>
<td>61</td>
</tr>
</tbody>
</table>
TABLE V. INTAKE OF HERBAGE, DRY MATTER DIGESTIBILITY AND ABOMASAL NITROGEN FLOW IN CATTLE [34]

<table>
<thead>
<tr>
<th>Forage</th>
<th>Forage DMI (g/d)</th>
<th>Flow at abomasum (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-ammonia N</td>
<td>Microbial N</td>
</tr>
<tr>
<td>Mature pasture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(17.8 g N/kg DM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>4601</td>
<td>89</td>
</tr>
<tr>
<td>Fish meal (752 g/d)</td>
<td>5554</td>
<td>136</td>
</tr>
<tr>
<td>Barley straw stubble</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5.3 g N/kg DM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>4178</td>
<td>45</td>
</tr>
<tr>
<td>Fish meal (752 g/d)</td>
<td>3170</td>
<td>96</td>
</tr>
</tbody>
</table>

Different proteins have also been shown to elicit different responses (Table IV) [31]. In lambs receiving a basal diet of oaten chaff all protein supplements significantly increased roughage DMI, with pelleted cottonseed meal being significantly better than the other proteins. Differences in live weight change reflected to some extent intakes but there were significant differences between different proteins as well. Differences in intake and live weight change observed for the two lupin supplements are possibly a result of the rolling process making the seeds more readily available to attack in the rumen or more probably a result of protection of the protein by heating during the pelleting process.

Other processing procedures can markedly influence the degradability of a protein supplement. For example, there are three major processes for extracting oil from cottonseed, each subject to different pressures and heats, and so each by-product meal will be of different rumen degradability (Table III) [32]. Other factors will influence the proportion of dietary protein reaching the small intestine, such as fractional outflow rate from the rumen [25] and feeding level [33]. Other components of the supplements may also influence this utilization, such as residual oils in oilseed meals or inorganic components in sources such as fish meal.

The type of forage may also have a marked influence on the response to protein supplementation. When cattle grazing mature pasture or oat stubble received supplements of fish meal, DMIs of the pasture were significantly increased by about 20% whereas those of the stubble were decreased by about 10% (Table V) [34]. Overall dry matter digestibilities of the forages were similar, at about 0.50 g/g intake, with a significantly higher proportion (0.86) of the stubble dry matter disappearing in the
rumen compared with the mature pasture (0.76). Non-ammonia nitrogen and microbial nitrogen flows were significantly higher on the pasture diets, with roughly similar amounts of feed nitrogen escaping degradation in the supplemented diets. Parameters other than rumen degradable protein (RDP) and UDP per se must be responsible for the varied responses. The greater digestion of stubble dry matter in the rumen suggests a reduction in rumen outflow which could result in an inefficient microbial protein synthesis and restricted intake capacity.

Smith and Warren [31] found that increasing supplements of cottonseed cake to animals grazing oat stubble resulted in significantly greater mean weight gains compared with similarly supplemented animals grazing dry matter pasture. The authors suggested that there were probably advantages from spilt grain in the stubble and the taller, denser forage of the stubble compared with the pasture. The potential digestibilities of the forages probably also play an important part. Responses to supplements of potentially rumen undegradable protein are extremely varied, reflecting the wide variety of situations under which they have been tested.

4. POST-RUMINAL SUPPLY OF CARBOHYDRATES

4.1. Post-ruminal infusion

The ruminant small intestine has a high capacity for the hydrolysis of starches from different sources and for the absorption of large amounts of glucose [35]. In the high producing ruminant where the requirement for UDP has been satisfied, an additional production response can be obtained from an extra glucose supply [15, 36]. Effects of post-ruminal supply of glucose on DMI are perhaps as equivocal as those of protein (Table VI). In experiments with grazing cattle [34] the intake and digestion of barley straw stubble (5.3 g N/kg DM) and mature pasture (17.8 g N/kg DM) were studied. The animals received supplements of 752 g of fish meal dry matter directly into the rumen once daily with or without the continuous infusion of 450 g of glucose into the abomasum.

Glucose infusion significantly reduced the intake of mature pasture but had no effect on the barley straw intake (Table VI). Whole tract digestibilities of the two forages were identical at about 0.5 g/g intake but a significantly higher proportion of barley straw dry matter was digested in the rumen compared with the mature pasture. The reduced proportion of dry matter digested in the rumen with both these forages during glucose infusion implies either an increased rumen outflow rate, which would be expected to increase intake, or a reduced rumen volume. Glucose infusion into the abomasum of lactating cattle [15] (Table VI) significantly reduced DMI, the effect of which was mitigated by the infusion of protein with the glucose. The alleviating effect of UDP was also observed when glucose was infused into the abomasum of lambs receiving 50:50 roughage:concentrate diets [36, 37]. There
**TABLE VI. EFFECT OF POST-RUMINAL GLUCOSE INFUSION ON FORAGE DRY MATTER INTAKE**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Diet</th>
<th>Infusate</th>
<th>Forage DMI (g/d)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>85% prairie hay</td>
<td>Urea</td>
<td>4 198</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>15% concentrates</td>
<td>Urea + glucose</td>
<td>4 372</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>Mature pasture</td>
<td>Nil</td>
<td>5 554</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>(+ fish meal into the rumen)</td>
<td>Glucose</td>
<td>4 945*</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>Barley straw stubble</td>
<td>Nil</td>
<td>3 710</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>(+ fish meal into the rumen)</td>
<td>Glucose</td>
<td>4 073</td>
<td></td>
</tr>
<tr>
<td>Lactating cows</td>
<td>98% alfalfa silage</td>
<td>Nil</td>
<td>18 500</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td></td>
<td>15 100*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose + protein</td>
<td></td>
<td>17 200</td>
<td></td>
</tr>
</tbody>
</table>

* P > 0.05.

There were no significant effects on DMIs if sufficient UDP was present in the diet but reduced intakes were observed when glucose infusions were with low UDP containing diets. Other experiments with protein and carbohydrates infused post-ruminally resulted in significant reductions in DMI [34]. Infusions of urea plus glucose into cattle receiving diets of 85% prairie hay had no effect on DMIs compared with animals receiving infusions of urea only [14].

Thus it would appear that post-ruminal glucose infusion with high roughage diets can result in reduced DMI, which may be alleviated by increased post-ruminal protein supply. However, glucose infusions did increase the efficiency of feed conversion and live weight gain.

### 4.2. Protected carbohydrate supplements

Supplementation of forage diets with starchy concentrates is known to reduce the number and activity of cellulolytic bacteria. The changes occur partly as a result of reduced rumen pH from the rapid fermentation of the starch and strong competition for other nutrients by amylolytic bacteria [38]. Treatment of rolled barley with an acidified formaldehyde reagent resulted in cross-linking of barley protein and starch with associated reductions in their rate of digestion [39]. Three experiments with lactating cows were carried out where treated barley was used to supplement ad libitum grass silage diets [39]. In two experiments, treated barley supplements
TABLE VII. EFFECT OF INCREASING CARBOHYDRATE SUPPLEMENTS ON FORAGE DRY MATTER INTAKE AND DIGESTIBILITIES [42]

<table>
<thead>
<tr>
<th>Supplemental ground corn (kg/d)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay (g/d)</td>
<td>8762</td>
<td>8177</td>
<td>6402</td>
<td>5065</td>
</tr>
<tr>
<td>Digestible organic matter (g/d)</td>
<td>3425</td>
<td>3809</td>
<td>3228</td>
<td>3299</td>
</tr>
</tbody>
</table>

were associated with improvements in silage intake and milk production. Other experiments with sheep receiving diets of rolled barley and dried grass (39:61) at restricted intakes showed an increased flow of non-ammonia nitrogen (NAN) and starch at the duodenum of animals receiving the treated barley [40]. The roughage component of the above diets was highly digestible and only contributed 60-75% of total intake. To the author's knowledge, no supplementation of poor quality forages with protected carbohydrate has been attempted.

4.3. Dietary carbohydrate supplements

Virtually no soluble sugars and generally less than 10% of dietary starches from most feed sources escape fermentation in the rumen. Ground maize inclusion in diets resulted in increased rumen outflow rates and about 30% of the maize starch escaped rumen fermentation [41]. Supplementation of low quality native grass with different levels of ground corn resulted in an almost linear reduction in hay DMI (Table VII) [42]. The unequal replacement of hay by supplement meant that although cattle fed 2 and 3 kg of corn daily were consuming a diet of greater overall digestibility than those receiving diets without corn supplements, digestible organic matter intake was not enhanced. Feeding 1 kg/d of corn supplement slightly improved digestible organic matter intake over the control diet. Supplementation of Bermuda grass (BG) or orchard grass (OG) with ground corn resulted in increased microbial nitrogen at the duodenum of animals receiving BG but not for those receiving OG. Conversely, rumen digestibility of neutral detergent fibre (NDF) was significantly increased with corn supplementation of OG but not in the case of BG [43]. Low quality pasture intakes have also been shown to increase when crushed or whole maize was given as supplement [44], and small supplements of starch have improved intakes and digestibilities of straw [45, 46]. However, it is unlikely that any of the reported beneficial effects of corn supplementation are a result of post-ruminal influence. More probably they result either from increasing the readily available fermentable substrate in the rumen, thus improving the efficiency of nitrogen capture.
by the microbes, or from the simple effect of the addition of the supplementary corn nitrogen, or a combination of both.

It seems improbable that sufficient dietary carbohydrate will survive to the duodenum to be of any benefit to the animal under feeding conditions which will not adversely affect the intake of the basal roughage.

5. MICROBIAL ACTIVITY

Enhancing the flow of dietary nutrients, particularly nitrogen, to the small intestines must be done so as to facilitate maximum microbial activity and not reduce it.

Much of the nitrogen requirements of rumen microbes for protein synthesis may be met from the rumen ammonia. Concentrations found necessary to sustain maximum bacterial activity and microbial protein synthesis have been variously reported between 0.4 and 6.0mM [46-49]. Some of these values were determined in vitro, where requirements may be lower than in vivo. Rumen ammonia concentrations required to achieve maximum microbial protein or NAN flow at the duodenum have been reported to range from 6 to 17mM [50-53]. Maximum efficiency of capture of nitrogen by rumen microbes appears to occur below ammonia concentrations of 7mM, and increasing rumen levels above this results in reduced efficiency of nitrogen capture [54]. Rumen ammonia levels in animals receiving low nitrogen diets may frequently fall very low, in which case supplementation with urea is recommended. This will result in high levels for some time, and although high rumen ammonia concentrations (>0.20mM) have not been found to be detrimental with respect to the efficiency of microbial protein synthesis [55] they may adversely affect fibrolytic bacterial activity [56].

In contrast, Perdok and Leng [57] found that rice straw intake in cattle was maximized at rumen ammonia concentrations above 14mM whereas the straw digestibility was maximum at 4-5mM. Protein supplements, on the other hand, not only release ammonia more slowly than urea but also release amino acids and peptides. Peptide uptake by bacteria is proportional to peptide size: the larger the peptide the higher the uptake, up to a maximum of 45 amino acid residues [58]. What happens above that size is not known and it may be that hydrophobicity is more important than chain length [59]. Different proteins have different ratios of hydrophobic to hydrophilic amino acids. In animals receiving diets of 50:50 roughage:concentrates, different protein supplements elicited different responses in fibre digestion and efficiencies of microbial protein synthesis [60], and in animals receiving low quality roughage diets the source of nitrogen supplementation affected the degradation of dry matter, organic matter and cell wall carbohydrates [61, 62].

Protein degradability and structure are important in supporting protozoal growth [63] and protozoa may be important in some aspects of fibre digestion [64],
but little precise information on their importance is available. The action of fungi in
the rumen with respect to fibre digestion is also an unknown area although a role
has been ascribed to them in the reduction of particle size in the rumen [65].
However, little is known of their contribution to the overall fermentation in the
rumen or their contribution to nutrient supply at the small intestines [65].

Conditions required in the rumen to maximize one particular function are not
necessarily the same as those required to maximize a different function. So this raises
the question whether it is more important to enhance fibre digestion in the rumen
with low quality roughage, increasing intake but not necessarily resulting in a
production response, or whether it is better to maximize microbial protein synthesis
and flow to the small intestines, which may also result in increased intake but will
lead to improved performance.

6. GENERAL CONSIDERATIONS

It is scarcely surprising that there is such a range of equivocal data concerning
responses to rumen bypass nutrient supply, considering the large number of variables
between experiments. For example, data from sheep and cattle are compared
although it is known that there are differences between sheep and cattle in intakes
and digestibilities of the same roughage when offered at the same level [66, 67]. In
addition the length of time food is retained in the rumen is higher for cattle than for
sheep [68]. Feed intake also increases with fine grinding of roughages and the degree
of distension and fill of the reticulorumen can be one of the most important factors
influencing the intake of bulky diets. Between-animal differences can be very great.
Rumen volumes of dairy cows vary widely even when they are receiving identical
diets [69]. In situations where animals are consuming the same amounts of identical
rations, differences in rumen volumes must influence rumen retention times and
hence the digestibility of the feed. In other circumstances differences in rumen
volumes could be reflected in different DMIs of the same feed. Data from sheep
receiving straw diets showed that intakes could vary two- to threefold between
animals [5, 19]. Perhaps a long term solution to improving the utilization of poor
quality roughages would be animal selection on the basis of rumen fill and turnover.
Associative effects with other dietary supplements may also influence responses to
bypass nutrient supplementation, as indeed could substitutive effects. Cattle receiv­
ing daily supplements of 2.18 kg of barley and 0.43 kg of protected soybean meal
consumed 7.34 kg/d of barley straw. When the barley was isoenergetically replaced
by molassed sugar beet pulp, straw intake was significantly reduced to 5.65 kg/d
[70].

The physiological state of the animal will be a factor in the response to post­
rumen nutrient supply, as will previous dietary history [71]. Poor quality roughages
are all considered together but differences in chemical composition, physical proper­
ties and morphology will influence responses. There seems to be some considerable way to go before accurate identification can be made of situations when bypass nutrients should be used and even further before accurate prediction of responses is possible.

7. CONCLUSIONS

Although carbohydrates supplied post-ruminally had beneficial effects, such as increasing the efficiency of nutrient utilization, they also had adverse effects on intake. This, as well as economic considerations, would make their use with low quality forages improbable.

Increasing amino acid supply at the duodenum of animals receiving low quality diets generally resulted in improved production responses and occasionally improved intake. Economic considerations would probably rule out supplying amino acids post-ruminally by means of protected proteins. However, amino acid supply to the duodenum can be increased by dietary protein supplementation. This increased supply results from both enhanced microbial protein synthesis and flow and undegraded feed protein. The relative contributions of the two sources will probably vary quite widely depending on a number of factors. It is not known whether the amino acids supplied to the metabolic processes are used as nitrogen or energy sources, but their relationship with production responses needs to be defined.

REFERENCES


OPTIMIZING THE USE OF POOR QUALITY FORAGE FEED RESOURCES FOR RUMINANT PRODUCTION: MANIPULATION OF THE RUMEN MICROBIAL ECOSYSTEM

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Abstract

OPTIMIZING THE USE OF POOR QUALITY FORAGE FEED RESOURCES FOR RUMINANT PRODUCTION: MANIPULATION OF THE RUMEN MICROBIAL ECOSYSTEM.

Poor quality forages are characterized by their high content of lignocellulose and low level of nitrogen. Consequently these forages are generally poorly digested and are unable to support a maintenance level of nutrition for ruminant animals. Low intakes and poor utilization of these feeds can be partly attributed to an inefficient rumen ecosystem and an imbalance in the products of rumen fermentation. Successful strategies which have been used to increase the efficiency of utilization of low quality forages include: pretreatment of the forage, dietary supplements and manipulation of the rumen ecosystem. Manipulation of the rumen aims to increase the rate and extent of digestion of fibre and/or optimize the supply of specific nutrients to the host animal. The elimination of protozoa from the rumen (defaunation) is one form of rumen manipulation which offers considerable promise as a viable method of increasing ruminant production. Ciliate protozoa are normally present in the rumen but research results indicate that ciliate free animals may be more productive whenever dietary protein availability is low. It is now generally accepted that defaunation increases the post-rumen supply of protein (microbial and dietary) and increases the total protein available relative to volatile fatty acids (P:E ratio). Increasing the P:E ratio increases the efficiency of utilization of nutrients by the host and may reduce metabolic heat.

1. INTRODUCTION

Productivity of ruminants is a function of digestible feed intake and the efficiency with which absorbed nutrients are used for productive functions [1]. Therefore, the important characteristics of a diet for ruminants are its capacity to supply nutrients for an efficient microbial ecosystem in the rumen and its capacity to provide 'rumen bypass' nutrients to balance the products of rumen fermentation. It is
generally accepted that the major limitations to the utilization of low quality pasture and crop residues by ruminant animals are associated with low content of essential nutrients (i.e. nitrogen and sulphur), low digestibility and low intake. As a consequence, the efficiency of microbial growth (\( \gamma_{\text{ATP}} \)) in the rumen of animals receiving high fibre diets is low, resulting in a low availability of microbial protein relative to volatile fatty acid (VFA) energy (P:E ratio) to the host animal. In this situation the animal has to either wastefully oxidize the excess VFA energy (mainly acetate), which will increase metabolic heat, or reduce feed intake. Leng [2] has suggested that a low P:E ratio is a major constraint in most ruminant production systems.

Successful strategies which have been used to increase the efficiency of utilization of low quality forages include: pretreatment of the forage, dietary supplements and manipulation of the rumen ecosystem. Fermentation efficiency and nutrient outflow from the rumen can be adjusted favourably by manipulating the microbes and their activities with chemical agents [3]. Maintaining the rumen free of ciliate protozoa has also shown considerable promise as a means of increasing the outflow of nutrients from the rumen (notably amino acids) and increasing animal production (see Ref. [4] for review). Removal of the protozoa (defaunation) from the rumen can be achieved by dietary manipulation (i.e. high starch diets) or chemical drenching. In a closed ruminant production system where animal movements are controlled, the ciliate free condition can be maintained indefinitely, which makes this form of manipulation a more attractive proposition than chemical manipulators, which require regular administration. To understand why the ciliate free animal is more productive it is necessary to consider the role of protozoa in rumen function and their effects on nutrient availability to the host animal.

2. THE RUMEN PROTOZOA

Protozoa are normally present in domestic ruminants. Most of the rumen protozoa are ciliates although several species of flagellates may also be present. The number of flagellates in the rumen is generally low [5] and their mass small [6] and they are not considered in this discussion. The rumen ciliates are classified into two orders: Trichostomatina (family Isotrichidae, Butschi), commonly referred to as holotrichs, and Entodiniomorphida (family Ophryoscolecidae, Stein), commonly referred to as entodiniomorphs. The holotrichs and entodiniomorphs are able to degrade and metabolize the principal protein, carbohydrate and lipid components of the feed material ingested by the host animal [7]. The ciliates are also responsible for the engulfment and degradation of large numbers of rumen bacteria [8]. Estimates of the biomass of protozoa in the rumen indicate that the contribution of the ciliate protozoa to the total microbial biomass may vary from 40 to 80% [9] and as a result their influence on rumen function must be at least comparable to that of the rumen bacteria.
3. THE MICROBIAL ECOSYSTEM IN THE RUMEN

The three major groups of microorganisms that are responsible for the fermentation of ingested plant material in the rumen are: bacteria, protozoa and fungi. These organisms coexist in a dynamic equilibrium, which means that one group cannot be influenced independently from the other two. Protozoa and bacteria compete for the same substrates in the rumen and protozoa engulf and kill bacteria [8]. In the absence of protozoa there is an increase in the bacterial population [10]. A similar relationship apparently exists between protozoa and fungi in animals fed high fibre diets. The concentration of viable zoospores (motile stage in the life cycle of fungi) in rumen fluid collected from ciliate free sheep was two to five times higher than in rumen fluid collected from faunated sheep (Table I).

The changes in rumen function associated with a ciliate free state are therefore a balance between the protozoal activity which is lost and the bacterial and fungal activity which is gained.

4. DEGRADATION OF INGESTED FEED IN THE RUMEN

Microbial degradation of feed material entering the rumen begins with the rapid colonization of feed particles by large populations of bacteria, protozoa and fungi. In vitro studies indicate that the colonization of plant material by the protozoa is rapid and maximal between 5 and 35 min after exposure to the plant particles [14]. The vast array of enzymes capable of digesting proteins and complex carbohydrates that have been isolated from the protozoa [15, 16] is further evidence that protozoa actively participate in the digestion of feed material in the rumen.

<p>| TABLE I. CONCENTRATIONS OF VIVABLE ZOO­SPORES CULTURED FROM RUMEN FLUID COLLECTED FROM FAUNATED ( + F ) AND DEFAUNATED ( - F ) SHEEP |
|---------------------------------------------------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Diet</th>
<th>Viable zoospores (10³/mL)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ F</td>
<td>- F</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>7</td>
<td>16 [11]</td>
</tr>
<tr>
<td>Ammoniated wheat straw</td>
<td>4</td>
<td>12 [12]</td>
</tr>
<tr>
<td>Native pasture</td>
<td>7</td>
<td>30 [13]</td>
</tr>
</tbody>
</table>
4.1. Carbohydrate digestion

There is apparently very little competition between the two groups of protozoa for energy substrates as the holotrichs use soluble sugars and the entodiniomorphs utilize starch and more complex carbohydrates [17]. The holotrichs are attracted to sources of soluble carbohydrates by chemotaxis [18] and attach to damaged ends of plant material entering the rumen [19]. The holotrichs rapidly assimilate soluble sugars, which may be converted to storage polysaccharide reserves within the cell [20]. All the entodiniomorphs with the exception of the smallest *Entodinium* spp. can ingest starch [7] and it has been shown that the highest starch degrading activity is found in the protozoa population [21]. Results from microscopic, in vitro and in vivo studies indicate that protozoa have a significant role in the degradation of plant cell wall structural polysaccharides. Enzymes capable of degrading cellulose, hemicellulose and pectin have been isolated from the entodiniomorphid protozoa [15, 22] and it has been demonstrated that protozoa may account for 5–90% of cellulolytic activity in the rumen [23].

Although it is apparent that protozoa have a significant role in the digestion of fibre in the rumen, it is not possible to measure their contribution directly. From a survey of the early in vitro and nylon bag (in sacco) studies, Demeyer [24] calculated that the protozoa were responsible for 34% of the total microbial digestion of fibre. Of course, in the absence of the protozoa the digestion of fibre in the rumen will not be depressed by this amount because of increases in the sizes of the bacterial population [10, 25] and fungal population [11, 12] and changes in the enzyme activity of the bacterial population [26, 27]. In a more recent review, Veira [28] noted that the apparent digestibility of organic matter in the rumen of ciliate free sheep was on average only 85% of that measured in faunated sheep given concentrate and roughage diets. However, this situation may not be true for animals receiving high roughage diets. For example, the absence of protozoa had no effect on the digestibility of timothy grass (*Phleum pratense*) in sheep given roughage diets [22] and the in sacco digestibility of cereal straw was increased in ciliate free sheep [12]. In this latter study the numbers of sporangia and zoospores were increased in the absence of protozoa, indicating that fungal growth may have been greater in these animals (Table II).

4.2. Protein digestion

The bacteria, protozoa and fungi found in the rumen all have proteolytic activity. There are contrasting views on the relative importance of these groups. Blackburn and Hobson [29] concluded that more than half the proteolytic activity was present in the protozoal population, while Brock et al. [30] and Wallace [31] suggest that bacteria are responsible for most of the proteolytic activity in the rumen. It is apparent that the characteristics of the dietary protein are important. Bacteria
TABLE II. IN SACCO DIGESTIBILITY (24 h INCUBATION) OF WHEAT STRAW (WS) AND AMMONIATED WHEAT STRAW (NH₃-WS) IN FAUNATED (+F) AND DEFAUNATED (−F) SHEEP [12]

<table>
<thead>
<tr>
<th>Diet</th>
<th>Feed material in nylon bag</th>
<th>In sacco DM digestibility (% DM disappearance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+F</td>
</tr>
<tr>
<td>WS + urea</td>
<td>WS</td>
<td>26</td>
</tr>
<tr>
<td>WS + urea + lucerne (150 g/d)</td>
<td>WS</td>
<td>31</td>
</tr>
<tr>
<td>NH₃-WS</td>
<td>NH₃-WS</td>
<td>39</td>
</tr>
<tr>
<td>NH₃-WS + lucerne (150 g/d)</td>
<td>NH₃-WS</td>
<td>44</td>
</tr>
</tbody>
</table>

appear to have a major role in the degradation of soluble proteins [32] while the entodiniomorphs only utilize insoluble particulate protein sources, including bacteria and chloroplasts [8]. The holotrichs are able to use both soluble and particulate sources of protein [33]. Only a small proportion of the peptides and amino acids formed by the digestive activities of the protozoa are used for protein synthesis in the cell [8]. The remaining amino acids and peptides are either excreted directly into the rumen liquor or catabolized within the cell, giving rise to ammonia which is also secreted into the medium [34].

4.3. The rumen environment

In addition to their direct contribution to the enzymic degradation of feed materials in the rumen, the presence of the ciliates has been shown to influence other components of the rumen ecosystem which may influence digestion in the rumen.

(a) **Digesta outflow from the rumen.** Defaunation is often associated with changes in rumen volume and digesta outflow but the results of defaunation studies are not consistent [22, 27]. There is a positive correlation between DM digestion and DM retention in the rumen. There is no proven explanation for the existence of a relationship between digesta kinetics and the presence of ciliate protozoa.

(b) **pH of rumen fluid.** Compared with faunated animals the pH of rumen fluid in defaunated animals is often lower [35], and the difference is exacerbated when the diet is rich in starch. This effect may be due to an increase in amylolytic activity of the bacteria in the absence of the ciliates [36] or to differences
between the bacteria and protozoa with respect to VFA production rates. Some protozoal species also metabolize lactic acid [37]. Cellulolytic activity is inhibited at low pH [38] and by lactic acid accumulation [39].

(c) *Ammonia concentration in rumen fluid.* Ammonia concentrations in the rumen are consistently higher in faunated than in ciliate free animals [40, 41]. The lower ammonia concentration in the defaunated rumen may be the result of: a low production rate (i.e. lower degradation of dietary and bacterial protein), an increased rate of utilization, an increased rate of absorption across the rumen wall, or an increase in rumen volume. Low ammonia concentration in the rumen of ciliate free animals may limit carbohydrate digestion [25, 42]. However, varying levels of urea supplementation had no effect on the productivity of defaunated lambs [43].

5. **END PRODUCTS OF RUMEN FERMENTATION**

5.1. **Volatile fatty acids**

The fermentation products of the rumen protozoa cultured in vitro are H₂, CO₂, formate, acetate, propionate, butyrate and lactate [44, 45]. The proportions of the major metabolites produced in vitro are influenced by the nature and concentration of the substrate and by environmental conditions [46]. Therefore, it is not surprising to find that defaunation of the rumen is not associated with consistent changes in VFA proportions [47].

5.2. **Methane**

The production of methane during fermentation represents a loss of energy. Methanogenic bacteria have been observed to be attached to protozoa [48] and the protozoa have been reported to be the major methane producing fraction in the rumen [49]. Compared with faunated steers the production of methane was lower in the rumen of ciliate free steers fed a high barley ration [50], which is consistent with an increased microbial cell yield and lower VFA production in the ciliate free animals.

5.3. **Microbial protein**

Microbial protein synthesized in the rumen is a major source of protein for the ruminant. The quantity of microbial protein that is available to the host animal is a function of: the efficiency of microbial cell synthesis (\(Y_{\text{ATP}}\)), the amount of organic matter degraded in the rumen, and the proportion of microbial cells which leave the rumen. Microorganisms use ATP for two purposes: to provide energy for cell
growth and to provide energy for cell maintenance (Me) [51], and the Me for a particular microorganism is positively correlated to generation interval [52]. The ciliate protozoa have a longer generation interval than the bacteria [53] and therefore a smaller proportion of the available ATP will be used for growth of protozoal cells. Unfortunately for the host animal only a small proportion of the protozoal cells in the rumen flow to the lower digestive tract [54]. This retention of protozoa in the rumen results ultimately in the lysis of large numbers of protozoa [55–57] and, compared with ciliate free animals, a higher recycling of N within the rumen [58]. In addition the predatory activity of the protozoa [8] will significantly reduce the availability of bacterial cells for intestinal digestion.

Results from both in vitro and in vivo studies indicate that the efficiency of microbial protein synthesis is increased in the absence of the protozoa. The synthesis of microbial N in rumen fluid incubated in vitro (collected from faunated and defaunated goats) was 15% higher in the protozoa free incubation [59], and Demeyer and Van Nevel [60] reported a 33% increase in the net synthesis of microbial protein in a protozoa free incubation (rumen fluid collected from faunated and defaunated sheep). Comparative studies with faunated and defaunated sheep have demonstrated that on average the post-rumen supply of microbial protein is 20% higher in the defaunated animals (for a review see Ref. [61]).

6. NUTRIENT AVAILABILITY FROM THE CILIATE FREE RUMEN

The size and composition of the ciliate population in the rumen are variable and controlled by a complex set of factors [17]. Accordingly the contribution of the ciliates to rumen function and supply of nutrients to the host animal is not constant and not easily predicted. It is obvious that there are both costs and benefits for the host animal associated with the presence of a ciliate population in the rumen. With the exception of high roughage diets, the digestion of carbohydrate in the rumen is often reduced when the ciliates are removed. Therefore the amount of energy (VFA) available to the animal will also be reduced. However, this loss will be partially compensated for in the ciliate free animal by an increased digestion of carbohydrate in the hindgut [28] and an increase in the outflow of starch (when present in the diet) from the rumen [62]. In contrast, more microbial protein and dietary protein will be available to the host animal when protozoa are absent from the rumen. Therefore while it is not possible to predict the quantities of nutrients available from the rumen, with or without protozoa, the absence of protozoa will result in an increase in the total protein available relative to VFAs absorbed from the rumen (P:E ratio). An increase in the P:E ratio should increase the efficiency of energy utilization by the animal [1] and can therefore be expected to increase ruminant production under most conditions.
7. CILIATE FREE ANIMALS AND PRODUCTION

7.1. Background

Interpretation of results from the first comparative study of faunated and defaunated animals was that rumen protozoa were not essential to the host [63]. In this study the growth rates of faunated and protozoa free lambs were 134 and 151 g/d respectively, which represented a 12% improvement in growth rate of the ciliate free lambs (difference statistically non-significant). Further comparative studies with calves were conducted as a result of an observation that under dairy farm conditions protozoa and some characteristic rumen flora (normally seen in mature animals) were not established in calves until they were several weeks old [64]. In these studies calves were isolated from their dams at three days of age (calves were therefore ciliate free) and reared in isolation from other ruminants. Rumen inoculations (freshly obtained cuds from mature cows) were given to some of the calves and successfully established populations of protozoa and some of the characteristic rumen bacteria in these animals. Growth rates of the inoculated and non-inoculated calves over a six week period were the same [65]. Attention was again focused on the role of protozoa in ruminant nutrition when it was demonstrated that the protozoa could metabolize carbohydrate to produce VFAs [44, 65]. As a result of the studies of Pounden and Hibbs [65] isolation of newborn animals was used as the method for obtaining protozoa free animals in contrast to the chemical drenching method (CuSO₄) used by Becker and Everett [63]. Experiments with lambs [40, 66], buffalo calves [67], Bos indicus calves [68] and B. taurus calves [69] demonstrated that inoculation of the isolated animal with rumen fluid increased live weight gain. These positive responses were attributed to the establishment of protozoa in the inoculated animals and have had a considerable impact on the perceived role of protozoa in the nutrition of the ruminant. However, as was noted by Pounden and Hibbs [65], the isolated animal would not only be lacking ciliate protozoa but also some of the microflora normally found in the rumen, some of which would have been introduced with the rumen fluid inoculation. The only conclusion that can be drawn from these studies is that the inoculated animals had faster growth rates than the non-inoculated animals in the early months following inoculation. That this benefit can be maintained or that it can be attributed to the presence of protozoa is not substantiated by these studies.

7.2. Body weight gain

With the exception of the studies in which the ciliate free animals were obtained by isolation of newborn animals, most comparative studies with faunated and defaunated animals have demonstrated that ciliate free animals grow faster than their faunated controls (see Ref. [4] for review). Body weight gain responses to
TABLE III. GROWTH RATES OF FAUNATED (+F) AND DEFAUNATED (−F) RUMINANTS GRAZING PASTURE

<table>
<thead>
<tr>
<th>Production system</th>
<th>Live weight gain (g/d)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+F</td>
<td>−F</td>
</tr>
<tr>
<td>1984</td>
<td>89</td>
<td>109</td>
</tr>
<tr>
<td>Green oats — hoggets</td>
<td>78</td>
<td>104</td>
</tr>
<tr>
<td>Native pasture — calves</td>
<td>210</td>
<td>340</td>
</tr>
</tbody>
</table>

defaunation have been obtained with lambs [43, 70–73], steers [74] and buffalo heifers [75]. In addition to these pen feeding studies positive growth responses have also been obtained under grazing conditions with ciliate free lambs [13], hoggets [76] and calves (see Table III). In contrast to these results, the growth rates of lambs given diets rich in starch were depressed in ciliate free animals [41, 71], indicating that protozoa may have an important role in rumen function when diets contain high levels of starch.

The weight gain responses of the ciliate free animals are associated with a significant improvement in feed conversion efficiency (Table IV). Therefore, compared with faunated animals the use of metabolizable energy for growth must be more efficient in ciliate free animals. This finding supports the concept that it is often the availability of total protein (amino acids) relative to oxidizable substrates (mainly VFAs) that is the primary limitation to the efficient use of absorbed nutrients [1]. The higher feed intakes of the ciliate free animals which occurred in some studies (Table IV) may be due to an increased rate of fibre digestion in the rumen [12], more amino acids available for intestinal digestion [79] or, in hot climates, an increase in the efficiency of utilization of absorbed nutrients in reducing heat stress (i.e. less heat production) [2].

7.3. Wool production

Wool growth is highly correlated to the intestinal absorption of sulphur amino acids [80]. Therefore the increased availability of protein from the ciliate free rumen could be expected to increase wool production. With the exception of the studies of Cottle [81, 82], comparative studies with faunated and defaunated sheep have demonstrated that wool production over a wide range of dietary conditions and production levels was significantly higher in ciliate free animals (see Table V). These
TABLE IV. FEED INTAKE, BODY WEIGHT CHANGE AND FEED CONVERSION EFFICIENCY (FCE) OF FAUNATED (+F) AND DEFAUNATED (−F) RUMINANTS GIVEN STRAW BASED DIETS

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total DM intake (kg/d)</th>
<th>Growth rate (g/d)</th>
<th>FCE (DMI/gain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+F</td>
<td>−F</td>
<td>+F</td>
</tr>
<tr>
<td>Lambs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat straw&lt;sup&gt;a&lt;/sup&gt; (WS) + urea (14 g/d)</td>
<td>455</td>
<td>510</td>
<td>−69</td>
</tr>
<tr>
<td>WS&lt;sup&gt;a&lt;/sup&gt; + urea (14 g/d) + lucerne (150 g/d)</td>
<td>535</td>
<td>710</td>
<td>−20</td>
</tr>
<tr>
<td>WS&lt;sup&gt;a&lt;/sup&gt; + urea (10 g/d) + cottonseed meal (CSM) (80 g/d)</td>
<td>606</td>
<td>650</td>
<td>9</td>
</tr>
<tr>
<td>WS&lt;sup&gt;a&lt;/sup&gt; + urea (10 g/d) + maize (M) (160 g/d)</td>
<td>644</td>
<td>684</td>
<td>22</td>
</tr>
<tr>
<td>WS&lt;sup&gt;a&lt;/sup&gt; + urea (10 g/d) + CSM (30 g/d) + M (160 g/d)</td>
<td>674</td>
<td>734</td>
<td>43</td>
</tr>
<tr>
<td>Buffalo heifers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensiled WS + burseed (3 kg/d) (basal diet, B)</td>
<td>3.13</td>
<td>2.57</td>
<td>220</td>
</tr>
<tr>
<td>B + groundnut cake (GNC) (250 g/d)</td>
<td>3.16</td>
<td>3.08</td>
<td>277</td>
</tr>
<tr>
<td>B + GNC (500 g/d)</td>
<td>3.50</td>
<td>3.24</td>
<td>360</td>
</tr>
<tr>
<td>B + GNC (750 g/d)</td>
<td>3.48</td>
<td>3.14</td>
<td>400</td>
</tr>
</tbody>
</table>

<sup>a</sup> Wheat straw was offered ad libitum.
TABLE V. WOOL GROWTH OF FAUNATED (+F) AND DEFAUNATED (−F) SHEEP GIVEN A VARIETY OF DIETS

<table>
<thead>
<tr>
<th>Diet</th>
<th>Clean wool growth (g/d)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+F</td>
<td>−F</td>
</tr>
<tr>
<td>Wheat straw (WS) + urea (14 g/d)</td>
<td>1.6</td>
<td>2.3</td>
</tr>
<tr>
<td>WS + urea (14 g/d) + lucerne (150 g/d)</td>
<td>2.2</td>
<td>3.2</td>
</tr>
<tr>
<td>WS + urea (10 g/d) + cottonseed meal (CSM) (80 g/d)</td>
<td>3.7</td>
<td>4.7</td>
</tr>
<tr>
<td>WS + urea (10 g/d) + maize (M) (80 g/d)</td>
<td>4.1</td>
<td>4.9</td>
</tr>
<tr>
<td>WS + CSM (160 g/d) + M (80 g/d)</td>
<td>5.6</td>
<td>6.2</td>
</tr>
<tr>
<td>Oaten chaff + sugar + fish meal (4%) + urea (3%)</td>
<td>8.0</td>
<td>10.8</td>
</tr>
<tr>
<td>Oaten chaff + sugar + fish meal (4%) + urea (5%)</td>
<td>7.9</td>
<td>11.0</td>
</tr>
<tr>
<td>Green oats (grazing)</td>
<td>10.0</td>
<td>11.9</td>
</tr>
<tr>
<td>WS (10%) + oats (89%)</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td>WS (10%) + oats (80%) + crushed lupins (10%)</td>
<td>6.8</td>
<td>7.6</td>
</tr>
<tr>
<td>WS (10%) + oats (80%) + whole lupins (10%)</td>
<td>8.2</td>
<td>7.9</td>
</tr>
</tbody>
</table>

production responses were achieved without a change in N intake (pen feeding studies), indicating that there is a more efficient conversion of dietary N into wool growth in the ciliate free animal. The absence of a wool growth response in the studies of Cottle [81, 82] may have been due to the high level of oats fed to the sheep in these studies.

Other positive production responses that have been reported to be associated with defaunation include increased milk production from dairy cows [83] and increased birth weights of lambs [13]. In the study of Moate [83] the daily production of both milk fat and milk protein was increased following defaunation treatment.

8. CONCLUSION

The ciliate protozoa in the rumen are quantitatively important in the digestion of the major carbohydrate, protein and lipid components of feed material ingested by the ruminant. Removal of the ciliates may result in a reduction in the digestion
of carbohydrate and protein in the rumen. However, with the exception of high starch diets, their presence is apparently not essential to the host animal and in many situations their presence represents a constraint to production. Ciliate protozoa actively degrade dietary and bacterial protein in the rumen and only a small proportion of protozoal cells are available for intestinal digestion. In many ruminant production systems this behaviour of the rumen ciliates results in a critical loss of protein to the host animal. Consequently the ciliate free animal can be expected to achieve higher levels of production whenever the availability of amino acids to the host animal is the primary limitation to production, i.e. whenever a relatively low P:E ratio results in an inefficient use of absorbed nutrients. The P:E ratio, and its effects on efficiency of utilization of nutrients, assume greater importance in animal production systems in hot climates because of the reduced ability of the animal to dissipate metabolic heat.

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MANIPULATION OF NUTRIENT PARTITIONING: IMPLICATIONS FOR RUMINANTS FED LOW QUALITY FORAGES

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Abstract

MANIPULATION OF NUTRIENT PARTITIONING: IMPLICATIONS FOR RUMINANTS FED LOW QUALITY FORAGES.

Ruminants can produce two sources of food, milk and meat. To increase the efficiency of production it is necessary that the nutrients absorbed from the gut are partitioned towards the desired product. Low quality forages are usually deficient in protein and any attempt to increase the extent of lean deposition in the animal by manipulation of its endocrine status will be limited unless the intake of protein and energy is increased. Lean deposition is restricted not only by protein supply per se but also by the regulation of the endocrine axis, in particular the growth hormone axis, which is also sensitive to nutritional status. Although the use of exogenously administered hormones and hormone analogues is an accepted practice in many countries, in some it is now forbidden. Currently much research is being conducted on the use of the immune response to manipulate endocrine status and hence nutrient partition. The potential use of transgenesis to improve productivity is the subject of much speculation but unless methods can be devised to significantly increase the efficiency of gain, this technology is likely to be of little use in manipulating nutrient partition except where the animal receives an adequate supply of nutrients. The use of metabolism modifiers to reduce basal metabolic rate during times of nutrient restriction thus warrants exploration.

1. INTRODUCTION

Low quality forages have been defined by Leng [1] as those forages which are less than 55% digestible and are deficient in true protein (less than 80 g/kg crude protein) as well as being low in soluble sugars and starches (usually less than 100 g/kg). These diets are therefore quite different from the high protein:high energy diets usually used in studies investigating the control and manipulation of fat and lean deposition in animal tissues and milk production by the mammary gland. Most of the recent studies on nutrient partition in developing countries have concentrated on
investigating ways of manipulating the animal to enhance lean deposition at the expense of fat. Since the development of technology to produce large quantities of growth hormone, numerous studies have also been conducted on methods of enhancing milk production by high producing dairy cows. Unfortunately there are relatively few studies on nutrient partition in ruminants fed low quality diets. It is, however, clear that the potential to manipulate nutrient partitioning with significant effect is acutely dependent upon nutrient intake.

2. NUTRITIONAL INFLUENCES ON MUSCLE AND FAT DEPOSITION

At a given level of energy intake, protein deposition increases with protein intake up to a value determined by that level of energy intake [2]. An increase in total nutrient intake above that required for maintenance generally results in a co-ordinated increase in protein synthesis and protein degradation, in both the whole body and skeletal muscle [3, 4], the increase in synthesis exceeding the increase in degradation, resulting in net protein accretion. Changes in the levels of protein or energy intake or in protein quality may increase protein deposition through differential effects on protein synthesis and degradation rates [5]. An inadequate intake of protein and/or energy, however, can severely constrain protein deposition. When protein synthesis is constrained by protein intake, extra energy is available for lipogenesis, resulting in high body fat content and slow, inefficient growth.

This can be illustrated by the situation with cattle fed grass silage where poor productivity is often associated with high fat:protein ratios in the carcass. Supplementation of silage with fish meal has been shown to increase live weight gain

TABLE I. GROWTH AND PROTEIN AND FAT DEPOSITION (g/d) IN SILAGE FED STEERS OFFERED SILAGE ALONE OR IN COMBINATION WITH FISH MEAL<sup>a</sup> AND/OR A GROWTH PROMOTER<sup>b</sup> [6]

<table>
<thead>
<tr>
<th>Gain</th>
<th>Control</th>
<th>+ Fish meal</th>
<th>+ Oestradiol</th>
<th>+ Oestradiol fish meal</th>
<th>Standard error</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty body weight</td>
<td>569</td>
<td>787</td>
<td>568</td>
<td>891</td>
<td>30.0</td>
<td>19</td>
</tr>
<tr>
<td>Protein</td>
<td>94.6</td>
<td>145.2</td>
<td>93.7</td>
<td>167.8</td>
<td>8.06</td>
<td>19</td>
</tr>
<tr>
<td>Fat</td>
<td>92.1</td>
<td>97.5</td>
<td>101.6</td>
<td>100.4</td>
<td>9.80</td>
<td>19</td>
</tr>
</tbody>
</table>

<sup>a</sup> 150 g fish meal/kg silage DM.
<sup>b</sup> Compudose 365 implant.
TABLE II. GROWTH RATES AND GROWTH HORMONE AND IGF-1 CONCENTRATIONS IN YOUNG STEERS FED SILAGE OR A FORAGE-CONCENTRATE DIET OF DRIED GRASS–BARLEY (70:30)  
(J.M. Dawson, unpublished observations)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Silage (n = 6)</th>
<th>Forage-concentrate (n = 6)</th>
<th>Pooled SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight gain (kg/d)</td>
<td>0.42</td>
<td>0.86</td>
<td>0.100</td>
</tr>
<tr>
<td>GH (ng/mL)</td>
<td>11.7</td>
<td>5.9</td>
<td>0.88</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>118</td>
<td>374</td>
<td>42.4</td>
</tr>
</tbody>
</table>

and the protein content of the carcass and empty body, without affecting fat content (Table I) [6]. Non-ammonia nitrogen flow to the duodenum and amino acid uptake were significantly increased by fish meal but there was no evidence for an increase in efficiency of utilization of the absorbed nutrients, which was surprisingly low on both diets [7]. Increasing the efficiency of utilization of absorbed nutrients would be a way of enhancing the performance of these animals.

In our studies, an attempt was made to further enhance protein deposition by implanting some animals with oestradiol [6]. Implanted animals showed no increase in growth or change in carcass composition when fed silage alone, but when protein supply was increased by fish meal supplementation there was a significant interaction between the growth promoter and fish meal. Of particular note was the increase in lean deposition, suggesting that the increased growth potential cannot be achieved in the absence of an adequate protein supply (Table I).

Nutritional status influences endocrinological status, which in turn affects growth. The growth hormone–IGF-1 axis is often implicated in this process. Basal concentrations of growth hormone (GH) are often elevated and those of IGF-1 are reduced when protein and/or energy intake is low [8, 9] (see also Table II). A possible explanation for this and for the lack of response to oestradiol in silage fed animals [6] may be inferred from the data of Breier et al. [10–12], who demonstrated that animals on a low plane of nutrition have hepatic GH binding sites of reduced affinity, presumably resulting in an uncoupling of the growth hormone–IGF-1 axis. Indeed, the presence of a high affinity GH binding site was only detectable in well fed animals. The capacity of the high affinity binding site was shown to correlate with weight gain and with plasma IGF-1 concentrations. Oestradiol increases plasma GH in animals on both low and high planes of nutrition. Although the number of high affinity hepatic GH binding sites in the steers on high nutritional intakes was shown to be increased by oestradiol, this did not occur in the implanted
animals on the low plane of nutrition. Thus the IGF-1 response to GH was elevated
to a greater extent in the well fed than in the poorly fed animals.

Leng [1] examined the response to protein supplementation of cattle fed low
quality forage, with particular reference to food intake, in tropical and subtropical
environments and suggested that the observed response was related to the location
of the experiment. It was concluded that the increase in intake of forage as a result
of supplementation of a diet with protein was considerably greater in the tropics
and subtropics than in the temperate zones. Furthermore, Leng concluded that supple­
ments which increase the protein to energy ratio of the nutrients absorbed by cattle
fed low quality forages result in a reduction in basal metabolic heat production. This
in turn was suggested to reduce the suppression of feed intake normally associated
with increased body temperature. Acetate, the major volatile fatty acid produced dur­
ing fermentation of forage diets, has been shown to be metabolized less efficiently
than propionate, hence giving increased heat production. It is possible that the
 gluconeogenic precursors in dietary protein improve the efficiency of acetate
metabolism. Furthermore, in hot climates, as the need to generate metabolic heat to
 maintain body temperature is reduced, this may allow more of the rumen fermenta­
tion products to be partitioned into synthetic reactions, e.g. animal growth [1],
 provided that there are sufficient gluconeogenic precursors present to facilitate effi­
cient acetate metabolism.

3. GENERAL COMMENTS ON MANIPULATION BY ALTERATION OF
ENDOCRINE STATUS

In recent years many experiments have been conducted in attempts to find a
safe and efficacious method of manipulating fat and lean deposition in the carcass
of farm animals; the vast majority involve some form of manipulation of the animal’s
endocrine status. Most of these studies have concentrated upon well fed animals and
relatively few experiments have been conducted which have investigated the efficacy
of these agents over a range of dietary intakes. The data that are available, however,
would indicate that endocrinological manipulation of lean deposition or of milk
production will only show significant effects when the animals receive a good diet.
Although dramatic changes in lean accretion can be achieved, efficiency of protein
deposition and production is rarely markedly enhanced by the currently available
endocrinological manipulation systems.

4. ANABOLIC STEROIDS

Although the use of anabolic steroids is banned in some countries, notably
members of the European Economic Community, their use in many other countries,
including the United States of America, is widespread. The response of treated animals has been observed to show a marked interaction with diet as demonstrated above [6]. A possible explanation for the lack of response to oestradiol in the animals fed the non-supplemented silage diet was the reduced capacity of the liver to produce IGF-1 in response to GH (see above). Other data have also suggested an interaction between diet and response to anabolic agents [13, 14].

The anabolic agent Trenbolone acetate enhances muscle deposition by reducing the extent of muscle protein breakdown and hence muscle protein turnover [15, 16]. This prompted Hunter and Vercoe [17], working in Rockhampton, Australia, to investigate the possibility of using hormone-like implants to reduce the weight loss often seen in grazing ruminants during the dry season in the tropics and subtropics. Trenbolone implanted cattle showed similar weight losses to their controls albeit with a reduced feed intake. However, when the treated animals were given a urea plus sulphur supplement the implant not only prevented weight loss but actually enhanced animal growth. Despite this interesting application of anabolic agents, these studies again indicate that a positive growth response cannot be achieved unless there are sufficient nutrients in the diet to allow this greater potential to be expressed.

The possibility of using other methods of endocrinological manipulation to reduce basal metabolic rate and hence reduce the loss of body condition during periods of dietary insufficiency is currently being investigated by the Rockhampton group (see Ref. [18]).

5. EXOGENOUS GROWTH HORMONE APPLICATION

Studies of the effects of injections of GH in growing ruminants have been reviewed by Enright [19]. Cattle generally show an improvement in daily live weight gain of 10–15% with lean content being increased by 5–10% and carcass fat content reduced. These studies were largely conducted with animals fed high quality diets. Unfortunately there appear to be little data for ruminants where the response of the animals has been titrated against the quality and intake of nutrients. Examination of data obtained with pigs, however, provides evidence that in some experiments the amino acid availability has restricted the response to the hormone treatment [20].

The effects of GH on fat are thought to be mediated at least in part via adipose tissue GH receptors. IGF-1 receptors are not thought to occur on adipocytes [21]. In muscle, however, the effects are more likely to involve the IGF-1 receptor, probably on the myotube itself. It therefore follows that nutritional regulation of the GH–IGF-1 axis will play an important role in regulating the extent of lean deposition in ruminants fed poor quality diets. This will also apply when the animal is receiving exogenous supplies of GH [12]. Surprisingly, however, Pell [22] reported that exogenous GH increased lean even when lambs were given a 12% protein diet. The increase in lean content is brought about by an increase in the rate of
protein synthesis with a smaller increase in degradation rate [23]. There is evidence that the response of adipose tissue to GH will also depend on nutritional state. In well fed ruminants plasma non-esterified fatty acids are not increased following GH administration, but in feed restricted animals or in animals in negative energy balance, non-esterified fatty acids are increased [24]. This suggests that in well fed animals the main action of GH in reducing fat accretion may be via a reduction in lipogenesis. Furthermore, Pell [22] found that adipose tissue was only reduced by GH in ad lib fed animals but not when feed intake was restricted [22]. Since the ad lib fed lambs had almost 2.5 times more carcass fat than restricted lambs this supports the suggestion that the effects of exogenous GH are more pronounced in animals with a tendency to deposit fat.

GH injections have been demonstrated to increase milk production by lactating cows in numerous trials conducted in many countries. With high producing animals fed good quality diets, dramatic increases in milk production have been reported. This increase in milk production requires additional nutrients as hormone treated animals will attempt to increase their feed intake. On poor quality roughages this may not be possible and the response seen will be constrained by the nutrients available to the mammary gland. The nutritional needs of a cow with a genetic potential for 9000 kg of milk per year producing 10 000 kg following hormone treatment will be identical to those of a genetically superior cow producing 10 000 kg without hormone treatment [24]. If cows are fed rations with inadequate nutrient balances or which provide insufficient nutrients to the tissue the response seen to GH will be small or even non-existent.

6. IMMUNE MANIPULATION OF GROWTH HORMONE AXIS

Alternative methods of increasing the activity of the GH axis in ruminants have been explored. Of particular significance for extensive agriculture is the use of the immune response. Animals are often immunized against disease and it might be possible to immunize animals to alter endocrine status and hence nutrient partition at the same time.

For example, somatostatin inhibits GH secretion in all species tested. The inhibitory effects of somatostatin are not confined to GH; thyrotrphin secretion from the pituitary gland and insulin and glucagon release from the pancreas are also inhibited. The concept of autoimmunizing farm animals against somatostatin to enhance GH release was initially explored in sheep by Spencer et al. [25]. These initial studies reported an increase in growth in the immunized animals, associated with increased plasma concentrations of GH and IGF-1. Many subsequent studies have produced variable responses, some showing increased growth rates of about 17% without effects on plasma GH [26] or IGF-1 [27] concentrations, others showing increased GH or IFG-1 concentrations without effect on growth [28]. Varner
et al. [29] immunized mature wether lambs against linear somatostatin and found an increase in basal GH secretion which correlated positively with antibody titre, but the antibody titre correlated negatively with weight gain. Bass et al. [28], using groups of lambs (which included animals autoimmunized against somatostatin) fed either a good quality pelleted diet of lucerne and barley or cut pasture, concluded that nutrient intake may influence the growth response to immunization with somatostatin. Although immunization increased plasma IGF-1 concentrations in sheep on both diets, mean levels were higher in pellet fed animals than those fed cut pasture and only the pellet fed animals showed an increase in weight gain in response to immunization. Presumably the forage fed animals, being on a low plane of nutrition, had tissue GH receptors which were partially refractory to GH and, consequently, IGF-1 release (see above). Little evidence for an effect on increased lean acceleration has been obtained in any experiment; in fact limited evidence suggests that carcass fat content may be slightly increased in well fed animals [28, 30, 31]. The reason for this is unclear. The possibility that the effects observed on immunization against somatostatin are not mediated through GH but are due to alterations in the rate of digesta flow through the gastrointestinal tract [32] needs to be considered.

Other immunological methods enhancing the GH axis, e.g. by the use of anti-idiotypes to GH [33] or the use of specific monoclonal antibodies [34], are likely to show the same interaction with diet as is seen with exogenous GH administration.

7. BETA AGONISTS

Beta agonists, which are substituted phenylethanolamines, are structural analogues of the naturally occurring catecholamines adrenalin and noradrenalin and as such are most likely to act via specific membrane bound beta adrenoreceptors.

Numerous studies have demonstrated the profound effects that oral administration of this group of compounds has on fat and lean deposition in most livestock (for a summary of the results obtained, see Table III). All mammals tested appear to respond, with the effects in ruminants being particularly noteworthy. Responses in poultry are less dramatic than those seen in mammals; this is probably associated with differences in the sites of lipid metabolism in birds and mammals and with differences between the roles of adrenalin and glucagon in these two classes of animals. Although there is some evidence that young animals are not very responsive to beta agonist treatment — for example, Mersmann et al. [36] were unable to demonstrate an effect in young pigs fed Cimaterol — Williams et al. [37] demonstrated a clear repartitioning effect in veal calves fed milk replacer containing Clenbuterol, indicating that a functional rumen is not necessary.

As indicated above, these materials probably act via the beta receptor and it therefore follows that their action within the target tissues is mediated via cyclic AMP. Most evidence suggests that the enhancement of lean is due to a reduction in
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Poultry</th>
<th>Ruminants</th>
<th>Swine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight gain</td>
<td>+2</td>
<td>+5 to +15</td>
<td>+5</td>
</tr>
<tr>
<td>Feed intake</td>
<td>−1.5</td>
<td>−2 to −9</td>
<td>−3</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>+2</td>
<td>+15</td>
<td>+6</td>
</tr>
<tr>
<td>Dressing percentage</td>
<td>+1</td>
<td>+6</td>
<td>+1.5</td>
</tr>
<tr>
<td>Carcass lean</td>
<td>+2</td>
<td>+10 to +15</td>
<td>+7</td>
</tr>
<tr>
<td>Carcass fat</td>
<td>−7</td>
<td>−25 to −30</td>
<td>−25</td>
</tr>
</tbody>
</table>

the rate of muscle protein degradation although there is some evidence that there is an increase in protein synthesis [38]. Although this reduction in the rate of protein turnover might be expected to reduce the energy costs of protein deposition, whether this is of significance can only be a matter of debate as beta agonist treatment also increases basal metabolic rate [39]. It is generally accepted that beta agonists act directly on adipose tissues via the beta receptor and this results in an increase in lipolysis, the main supporting evidence for this being the increase in circulating plasma free fatty acids in treated animals (see, for example, Ref. [40]). Some doubt on this being the method by which beta agonists reduce adipose tissue, however, comes from in vitro studies where no effect on lipolysis has been observed but lipogenesis has been decreased [41]. A detailed review of the literature does give indications that the response by in vitro adipose tissue preparations may be dependent upon the species, the incubation medium and the type of beta agonist used [42]. One of the major difficulties in coming to an exact conclusion as to the mode of action of beta agonists on adipose tissue is that it is very difficult to measure the rates of lipolysis and lipogenesis in vivo, especially in large domesticated livestock.

There have been relatively few reports in the literature where the interaction between diet and response of ruminants to beta agonists has been fully explored. Data from the pig would indicate that ractopamine becomes more effective at improving growth performance and carcass composition as protein in the diet increases [43]. Kim et al., however, observed enhanced protein accretion in lambs fed a maintenance diet, with no effect on dry matter digestibility [39]. The effects of these agents on animals fed low quality forages have not apparently been reported. Beta agonists are likely to reduce fat in the carcass irrespective of the diet; of course, the quantity of fat lost will depend upon that present pre-treatment.
The metabolic stimulation associated with beta agonist treatment will increase basal heat production [39] and this could make animals more vulnerable in hot environments. The loss of insulating adipose tissue would, in contrast, make the animals more susceptible to the cold [44].

8. TRANSGENIC LIVESTOCK

Since the early reports of the significantly increased growth rate of mice harbouring GH transgenes, the possibility of producing domesticated livestock with enhanced production characteristics using similar technologies has been the subject of much speculation. Reports of the successful production of transgenic livestock appeared in 1985 (e.g. Ref. [45]). The majority of studies have concentrated upon the introduction of extra copies of the GH gene. A number of groups have produced transgenic animals (pigs — US Department of Agriculture, Beltsville, Maryland, USA, and sheep — Commonwealth Scientific and Industrial Research Organization, Prospect, Australia). Unfortunately the majority of these animals suffer from a variety of abnormalities. This is presumably due to the failure to regulate effectively the GH transgene expression (that is, the introduced genes are constitutively expressed). Also, in the initial studies with transgenic animals, the transgenes were expressed in many tissues of the animal. Techniques are now available, however, to ensure tissue specific expression of transgenes by using specific promoters which, under normal conditions, are primarily expressed in the desired tissue; for example, using lactose synthesis promoter would give site specific expression in the mammary gland. Furthermore, the efficiency in producing transgenic animals using the conventional technique of injecting the gene construct into fertilized ova and then implanting the ova into a surrogate mother is very low (approximately 1% of all eggs injected [46]). Despite the difficulties, the GH enhanced animals often show enhanced growth rate and repartitioning. The efficiency of production of transgenic ruminants is likely to improve dramatically when it becomes possible to culture ruminant embryonic stem cells [47].

The major problem associated with the production of transgenic animals with altered nutrient partition is our lack of understanding of the control of nutrient partition in normal animals. It will be necessary to identify a single gene trait and then to be able to precisely regulate its activity in the manipulated animal. The interaction between diet and the response of transgenic animals with enhanced GH or beta receptor activity is likely to be similar to that seen with animals given GH or beta agonists exogenously. A much more exciting prospect would be to be able to manipulate the efficiency of animal metabolism, for example by increasing the efficiency of ATP production in the mitochondria. Such animals may also be less susceptible to heat stress. To be able to do this we would clearly need to know much more about animal metabolism than we do now.
While it is exciting to speculate upon the potential ways of manipulating the extent of tissue growth in domestic livestock by transgenes it could be argued that it would be far better as far as animals fed low quality roughages are concerned to concentrate effort on reducing susceptibility to disease and increasing efficiency of digestion or food intake. In addition a detailed evaluation of the consequences of releasing transgenic animals into the environment and also into the human food chain would be desirable. It would take a brave person to speculate when, if ever, a commercially viable transgenic ruminant will be produced with improved productivity when fed low quality roughages.

9. CONCLUSIONS

Attempts to manipulate nutrient partition using some of the techniques being developed for animals fed high quality diets are unlikely to succeed in animals fed low quality roughages. The only possible way of achieving a significant response to these techniques is to supplement the diet with protein. The alternative use of metabolic modifiers to reduce basal metabolic rate during periods of food deprivation is worthy of further examination, especially in situations where economics and the scale of animal production warrant it. Enhanced lean deposition or milk production will only occur when the diet contains sufficient protein and energy.

REFERENCES

FEED TECHNOLOGY AND OTHER METHODS FOR INCREASING THE BYPASS PROTEIN FOR RUMINANTS FED LOW QUALITY FORAGES

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Abstract

FEED TECHNOLOGY AND OTHER METHODS FOR INCREASING THE BYPASS PROTEIN FOR RUMINANTS FED LOW QUALITY FORAGES.

In order to make use of crop residues, it is necessary to meet the protein needs of the ruminant as well as the energy needs. Inexpensive sources of rumen ammonia (urea or ammonia from ammoniation) can meet the needs of the rumen microorganisms. The cost of protein supplementation can be reduced by using sources of high bypass protein with the sources of ammonia. Accurate evaluation of protein is necessary. Estimates of the bypass values for protein sources can be made from laboratory analyses or by directly measuring the bypass of a protein source with intestinally fistulated animals. These values are useful as supporting evidence; however, measurement of animal growth or production is the best way to obtain these bypass values. It is essential that proteins be compared below the animal's protein requirement. Otherwise, protein is not the first limiting nutrient and valid comparisons cannot be made. The protein in grain, especially corn and milo, is bypassed to a great extent and, therefore, the grain by-products are high in bypass protein. Heating reduces rumen degradation of proteins and, therefore, the drying of dehydrated alfalfa, blood meal and meat meal causes them to be high bypass protein sources. Hydrolysed feather meal and non-enzymatically browned protein are also good sources of bypass protein.

1. INTRODUCTION

Low quality roughages, including crop residues, have tremendous potential as feed resources worldwide. These roughages have two primary deficiencies: low energy digestibility and low protein. There are alternative methods by which we can enhance the energy digestibility of these roughages. However, the protein deficiency often precludes improved animal performance. Urea can be used as an inexpensive source of rumen ammonia but that may only partially solve the deficiency problem. Supplemental protein is often expensive.
Concepts of protein metabolism in the rumen have changed appreciably in the past few years. Some of these changes increase the possibility for reducing supplemental protein costs and/or increasing animal performance. The greatest potential for savings, especially in situations where producers want to use natural protein, is with the use of high bypass proteins. Bypass protein is that protein which escapes (or bypasses) digestion in the rumen. This protein is then digested in the lower tract of the animal and absorbed as amino acids to be used for productive functions. The animal has two sources of protein to use for these functions: bypass protein and microbial protein. We must always be aware of the significant role that microbial protein plays in meeting the animal’s needs. In some cases, the microbial protein is sufficient to meet these needs. When the microbial protein is inadequate, the only way to supply additional protein to the animal is with bypass protein. Therefore, the value of a protein source for ruminants is highly dependent upon its bypass value. Protein broken down in the rumen supplies ammonia which can be supplied more cheaply by urea. Growing calves and lactating cows have high protein requirements and usually require some bypass protein to achieve maximum performance.

2. EVALUATION SYSTEMS

Accurate evaluation of protein is necessary. Estimates of the bypass values for protein sources can be made from laboratory analyses or by directly measuring the bypass of a protein source with intestinally fistulated animals. The disadvantages of this technique are that two markers are needed (flow and microbial), the animal is surgically altered, intakes are seldom ‘normal’ and the metabolizability of the protein is not measured. These values are useful as supporting evidence; however, animal growth or production is the best way to obtain these bypass values.

The best means of estimating bypass is through the use of animal performance. The disadvantages of this technique are that protein sources may supply rumen degradable protein or other nutrients which affect animal performance, and animal variation makes measurement difficult. The techniques used in conducting animal performance experiments are critically important and, therefore, will be discussed in some detail [1].

The most important procedural consideration in growth studies is to demonstrate that protein is the first limiting nutrient. This technique is a generally accepted means of determining animal requirements or availability of nutrients in feedstuffs, especially with non-ruminants. Unfortunately, workers have not reliably demonstrated protein deficiencies in previous ruminant growth studies. In a review of studies involving the treatment of soybean meal, we found protein to be limiting in only 6 of 28 animal production studies [2].

A second point that is often overlooked is that rumen ammonia is limiting. This raises an interesting question. Should crude protein or protein level be reduced
to make protein limiting? In other words should non-protein nitrogen (NPN) be supplied to make sure the needs of the microorganisms are met? Basically the researcher wants to determine the ability of a protein source to supply bypass protein to the small intestine. If that is the case, all rations should be isocaloric and should supply sufficient ammonia and rumen degradable protein to meet microbial needs. It should be noted, however, that high bypass proteins supply less ammonia and rumen protein for microbial synthesis.

These requirements present two procedural problems which can be difficult to overcome. First, a basal ration must be designed which is low in protein yet relatively high in energy and has 'typical' feedstuffs. Also, in many cases animals fed the control NPN ration consume less feed than those fed protein supplements. This raises an important procedural question. Should intakes be equalized so that energy intake does not confound the animal gain response to protein?

We have developed a system in an attempt to meet the considerations mentioned above. Young growing calves are individually fed a high forage, low protein diet, generally one half sorghum silage and one half corncobs. The control animals are supplemented with urea and all rations are fed at an equal percentage of body weight based on the intake of the urea controls. Test proteins are fed at increasing levels replacing the urea. This is called the slope ratio technique and has been used in non-ruminant studies of amino acid, mineral or vitamin availabilities. The procedure is similar to that for a dose response curve in a drug study.

The increase in live weight gain from increasing levels of protein is a direct measure of the value of the protein. At some point the protein requirement of the animals is met, and no further response to protein is obtained. It is essential that proteins be compared at levels below the animal's requirement. Otherwise, protein is not the first limiting nutrient and valid comparisons cannot be made.

3. BYPASS VALUES

Protein efficiency is defined as the gain obtained per unit of test protein fed. This value is equivalent to the slope of the regression of gain on protein intake (Fig. 1). Generally, the higher the protein bypass, the higher the protein efficiency value. Blood meal, a high bypass protein compared with soybean meal, meets the animal's protein requirement for maximum gain with about 40% as much supplemental protein (Fig. 1). The protein efficiency as measured by the slope of the regression line was over 2.5 times greater for the blood meal than the soybean meal. This indicates that soybean meal could be improved by at least 2.5 times, leading to reduced rumen protein degradation, assuming that a specific amino acid does not become limiting.
**FIG. 1.** Daily gain above that of urea control versus natural protein fed daily, plotted using a non-linear program [3].

**TABLE I. BYPASS ESTIMATES OF PROTEIN SOURCES [4]**

<table>
<thead>
<tr>
<th>Protein source</th>
<th>% protein escape (bypass)</th>
<th>Duodenal collection</th>
<th>Animal growth</th>
<th>Soybean meal equivalent value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal</td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Blood meal, ring dried</td>
<td></td>
<td>24.6</td>
<td>30</td>
<td>1.0</td>
</tr>
<tr>
<td>Meat meal</td>
<td></td>
<td>82.4</td>
<td>84.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td></td>
<td>63.9</td>
<td>61.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Dehydrated alfalfa, 20% crude protein</td>
<td></td>
<td>60.3</td>
<td>57.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Distiller's grains</td>
<td></td>
<td>50.8</td>
<td>54.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Distiller's grains plus solubles</td>
<td></td>
<td>54.3</td>
<td>60.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Brewer's dried grains</td>
<td></td>
<td>48.6</td>
<td>43.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Feather meal</td>
<td></td>
<td>55.0</td>
<td>56.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Zn treated soybean meal</td>
<td></td>
<td>65</td>
<td>60</td>
<td>2.0</td>
</tr>
<tr>
<td>Non-enzymatic browned soybean meal</td>
<td></td>
<td>75</td>
<td>70</td>
<td>2.5</td>
</tr>
</tbody>
</table>

$^a$ Value relative to soybean meal.
### TABLE II. RUMINAL BYPASS PROTEIN (STEERS) AND DIGESTIBILITY (LAMBS) OF VARIOUS PROTEIN SOURCES [5]

<table>
<thead>
<tr>
<th></th>
<th>Urea</th>
<th>Soybean meal</th>
<th>Blood meal</th>
<th>Feather meal</th>
<th>Blood and feathers⁶</th>
<th>Blood and feathers³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escape protein (٪)</td>
<td></td>
<td>25.6d</td>
<td>90.0e</td>
<td>73.2f</td>
<td>76.1f</td>
<td>81.8g</td>
</tr>
<tr>
<td>Daily intake (g/kg body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mass</td>
<td>18.5</td>
<td>18.5</td>
<td>17.8</td>
<td>18.5</td>
<td>18.3</td>
<td>18.5</td>
</tr>
<tr>
<td>N</td>
<td>0.35</td>
<td>0.37</td>
<td>0.33</td>
<td>0.35</td>
<td>0.33</td>
<td>0.35</td>
</tr>
<tr>
<td>Digestibility (٪)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mass</td>
<td>55.02d</td>
<td>58.3e</td>
<td>58.0e</td>
<td>59.3e</td>
<td>54.0d</td>
<td>58.2e</td>
</tr>
<tr>
<td>N</td>
<td>66.7de</td>
<td>67.5de</td>
<td>67.7d</td>
<td>65.2e</td>
<td>62.7f</td>
<td>65.5de</td>
</tr>
<tr>
<td>Protein source</td>
<td>102.3d</td>
<td>103.0d</td>
<td>95.0e</td>
<td>85.2f</td>
<td>95.9de</td>
<td></td>
</tr>
<tr>
<td>Metabolizable protein (٪)</td>
<td></td>
<td>25.6</td>
<td>90.0</td>
<td>69.0</td>
<td>63.0</td>
<td>78.0</td>
</tr>
</tbody>
</table>

a Raw blood added to feathers, then hydrolysed and dried.
b Raw blood added to hydrolysed feathers and dried.
c Protein remaining after 12 h of in situ incubation in steers.
defg Means in the same row with different letters differ significantly (P < 0.10).
h Calculated as 100 — (N digestibility of urea control diet — N digestibility of test protein diet)/(protein source N as ٪ of total diet N).
i ٪ escape protein minus indigestibility.

![FIG. 2. Regression of gain on protein intake from growth study. The resulting slopes (numbers on graph) are the protein efficiencies from the growth study. Standard errors of treatment means were 0.228 for soybean meal, 0.374 for blood meal, 0.227 for feather meal and 0.296 for blood meal + feather meal [5].](image-url)
FIG. 3. Regression of gain on protein level from growth study. The resulting slopes (numbers on graph) are the protein efficiencies from the growth study. Standard errors of treatment means were 0.188 for 100% feather meal (FTH), 0.191 for 87.5:12.5 feather meal and blood meal, 0.195 for 75:25 feather meal and blood meal, 0.197 for 50:50 feather meal and blood meal and 0.086 for 100% blood meal (BM). Protein efficiencies with different letters differ significantly ($P < 0.05$) [6].

The data collected with animal growth trials are consistent with those from intestinally fistulated animal trials (Table I). This gives credibility to both techniques.

Feathers are a keratinous protein source of low nutritional value in their native state. Steam and pressure processing increase their protein availability. Even though the protein in processed feather meal is highly digestible, its use in non-ruminant diets is limited owing to amino acid deficiencies. As a result, feather meal is priced about the same as soybean meal but it contains twice the protein. When used as a source of supplemental protein for cattle, feather meal is high in bypass protein and is digestible.

When ammonia was not limited, results from a digestion study (Table II) indicated no differences in dry matter or total tract nitrogen digestion as affected by urea, soybean meal, blood meal or feather meal supplementation. These data show that feather meal protein is as digestible as the other protein sources and the laboratory estimates support the high protein escape value.

The utilization of feather meal protein may be increased when fed in combination with high quality (high lysine) protein sources. An experiment was conducted where bypass protein was shown to be limiting. Performance of steers in this growth trial (Fig. 2) indicates that calves consuming blood meal, feather meal or a 50:50 combination of blood meal and feather meal gained faster than steers fed soybean
meal. The improved protein efficiency for blood meal plus feather meal compared with the average of the two fed alone may be due to the sulphur amino acids supplied by the feather meal and the lysine and/or other amino acids supplied by blood meal. A second experiment compared several levels of blood meal and feather meal (Fig. 3). There was a positive associative effect of the mixtures (Fig. 4) which was maximized at 10–15% blood meal.

Corn protein is insoluble in water and relatively resistant to ruminal degradation. Therefore, by-products containing corn protein are generally good sources of bypass protein. Titgemeyer et al. [7] have recently estimated the bypass of corn gluten meal protein to be 86% using intestinally fistulated cattle compared with 21% for soybean meal. Somewhat lower bypass values were reported by Stern et al. [8] and Zinn et al. [9]. Distiller’s grains are also good sources of corn protein.

The amino acid profile of bypass protein is an important criterion in the evaluation of sources for practical diets [10, 7]. Combinations of slowly degraded protein sources to provide an optimum amino acid pattern for the lower tract are desirable. Combinations of two slowly degraded protein sources (blood meal and corn gluten meal) improved performance of steers above the weighted average of the two individual sources [11]. This may be due to an improved amino acid pattern reaching the lower tract. Methionine and lysine have been shown to be limiting in ruminant rations [12]. Blood meal contains high levels of lysine [13], with corn gluten meal being somewhat deficient. The higher level of methionine in corn gluten meal could offset any deficiency of that amino acid. Combinations of slowly degraded proteins

![FIG. 4. Complementary effect of feather meal–blood meal combinations on protein efficiency of steers (quadratic, effect P < 0.01) [6].](image)
and rapidly degraded proteins would not increase the response, because only the bypass proteins would reach the lower tract, and little would be gained by the combination [14]. It would probably be advisable to use combinations of feather meal or grain by-products with higher quality, high bypass proteins.

4. PROTEIN PROTECTION

Soybean meal has good palatability, good amino acid balance and good availability, but it is highly degraded in the rumen (70% or more). Various attempts have been made to increase the bypass of soybean meal, including formaldehyde treatment and heating.

Heating has been shown to improve the protein bypass of soybean meal, but not enough and not consistently. We therefore undertook a research project to study the effects of heating on soybean meal protein bypass [15]. It has generally been accepted that the non-enzymatic browning process, which occurs when hay heats and turns brown or black or when foods are cooked and browned, negatively affects proteins. Obviously some browning is not bad because most cooked foods have some degree of browning. In fact, normal soybean meal is toasted during processing, darkening its colour as well as destroying enzymes and inhibitors.

In the chemistry of non-enzymatic browning, there are two distinct phases. The initial phase is a condensation of amino groups from proteins with sugars. The second is a polymerization that makes the protein indigestible. We decided to try to apply the first phase to protect protein from rumen degradation while minimizing the second phase, polymerization.

![Graph of Ammonia release from soybean meal treated in the laboratory](image)

**FIG. 5.** Ammonia release from soybean meal treated in the laboratory (bars with different letters differ significantly ($P < 0.05$)) [15].
In the laboratory, soybean meal was heated under various conditions. Moisture, pH and sugar sources were varied. We found that xylose was the most reactive sugar source available. Heating temperature, heating time and sugar level all interacted so that several combinations were able to produce the desired results.

Most xylose does not naturally occur as a free sugar but is normally found as a component of hemicellulose, part of the fibre in forages. Corn cobs, for example, contain high levels of xylose as a part of the fibre. In the wood pulping process, hemicellulose is hydrolysed to the component sugars as the lignin is removed. The resulting fibre is primarily cellulose. The hydrolysed xylose is a component of sulphite liquor, the by-product of the wood pulping industry. Sulphite liquor is also commonly used as a pellet binder. Not all sulphite liquor contains xylose in economical quantities. Good sources contain about 20% on a dry matter basis.

The possibility of using sulphite liquor as a commercial source of xylose was studied in a laboratory experiment. Protein bypass was estimated by determining ammonia release in the artificial rumen. The more ammonia released, the less the bypass of the protein.

Heat alone had only a small effect on ammonia release (Fig. 5). Xylose and sulphite liquor both reduced ammonia release to less than one third that of the control. To test whether xylose was the active ingredient in sulphite liquor, a treatment was also included with 90% of the xylose removed by ultrafiltration. This treatment was relatively ineffective and shows that the xylose is the active ingredient in the sulphite liquor.

Two growth trials were conducted with growing beef calves using the slope ratio technique to determine the bypass of the sulphite liquor treated soybean meal
The calves were fed increasing levels of the protein sources and their gain increased until their requirement was met. The slopes of the lines are measures of the efficiency with which the proteins were used. Even though two different base rations were used that had different energy levels, the slopes of the lines are similar. The heated soybean meal had about two and a half times the bypass value of the control [15].

The advantages of using bypass proteins are as follows: (1) the amount of preformed protein fed is reduced; (2) the use of non-protein nitrogen is increased (to supply rumen ammonia); (3) the cost of supplementation is reduced compared with conventional protein; and (4) animal performance is maintained compared with conventional protein supplements or increased compared with non-protein nitrogen.

An example of combining all of these concepts is shown in Fig. 7. Young growing beef calves were fed ammoniated corncobs with or without bypass protein (blood meal and corn gluten meal) and with various levels of alfalfa hay. The residual ammonia in the treated cobs supplied rumen ammonia for the microorganisms. Alfalfa apparently supplied rumen degradable protein which stimulated microbial growth. Finally, bypass protein increased the efficiency of growth of the calves. A similar response was obtained with untreated cobs in another experiment. Inexpensive bypass protein is an important key to the efficient utilization of crop residues.
REFERENCES


MANIPULATION OF FERMENTATION AND DIGESTION TO OPTIMIZE THE USE OF FORAGE RESOURCES FOR RUMINANT PRODUCTION

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South Perth, Western Australia,
Australia

Abstract

MANIPULATION OF FERMENTATION AND DIGESTION TO OPTIMIZE THE USE OF FORAGE RESOURCES FOR RUMINANT PRODUCTION.

Chemical manipulation of digestive fermentation in the ruminant is discussed in relation to: improving the utilization of the basal diet of grazing animals for meat, fibre and milk production; facilitating the use of starch based supplements; and reducing methane production. A range of ionophore compounds and other antibiotic rumen modifiers may significantly increase body weight in grazing animals. Some of these rumen modifiers have been found to increase wool and milk production under some dietary conditions. These compounds may be administered to grazing animals via mineral licks and blocks, drinking water, protein supplements or intraruminal slow release devices. A number of rumen modifiers, and virginiamycin in particular, may have a role in overcoming problems associated with feeding starch rich supplements such as cereal grains. These compounds control some of the negative effects which result from the rapid fermentation of starch and reduce the risk of lactic acid accumulation. Compounds such as virginiamycin allow grain supplements to be fed at weekly or fortnightly intervals and, in addition, the supplementary nutrients appear to be utilized more efficiently. A number of chemical agents are known which inhibit the production of methane gas during fermentation in the rumen. Reducing methane production in this way does not consistently improve the efficiency of feed conversion to the same extent as the ionophore compounds and for this reason the use of these agents has not been promoted commercially. In view of the rapid rise of atmospheric methane concentrations and the important role of this gas in the ‘greenhouse effect’ the potential for inhibiting methane production in ruminants is re-evaluated.

1. INTRODUCTION

While it is common to use chemical feed additives which modify rumen fermentation and digestion in the intensive animal production industries, the potential of these compounds in grazing ruminants has not been fully explored or well utilized. This review focuses on two ways in which these chemical agents may be applied to improve the efficiency with which roughage based diets are used by grazing animals and also discusses the potential for reducing methane gas production from domestic
The use of rumen modifiers to increase the efficiency of meat and fibre production in grazing animals is a logical extension to their use in intensive feeding systems. The diet available to grazing animals is far more variable in quality than in the case of intensively fed animals and efficient digestion of fibrous roughage is more important than in the case of feed lot diets. The benefits of using rumen modifiers under grazing conditions can therefore not be predicted from pen feeding studies.

In addition to the potential benefits of more efficient production the rumen modifiers may have a specific role in overcoming problems associated with feeding carbohydrate rich supplements to ruminants. There are a range of compounds, including the ionophores and virginiamycin, which control the buildup of lactic acid during the rapid fermentation of α linked carbohydrate. These compounds have the potential to make the feeding of cereal based supplements and molasses safer and more effective.

Prior to the clearance of the first ionophore as a feed additive for cattle in 1975, the control of methane production was regarded as the most promising way to improve the efficiency of fermentative digestion in ruminants. Methane production and loss may represent around 8% of the gross energy intake and the potential to improve efficiency through blocking of this ‘wasteful’ loss was a clear objective of several groups [1]. While a number of effective products are identified in the patent literature the subsequent production trials in the scientific literature suggest that this approach to manipulating rumen fermentation does not have the same commercial potential shown by the ionophore compounds. For this reason no methane inhibitors have been developed to the point of clearance by the United States Food and Drug Administration or any other regulatory authority. In view of our more recent understanding of the importance of methane gas in the ‘greenhouse effect’ it is relevant to reassess the potential for controlling its production in ruminants.

2. MODE OF ACTION OF SOME TYPES OF RUMEN MODIFIERS

The mode of action of the rumen modifiers has been the subject of many review papers [2–4]. No attempt is made here to provide a complete summary of all compounds and their unique attributes. The aim of this section is to illustrate the usefulness of isotope techniques in quantifying some of the biological effects of feed additives and to provide a broad summary of some aspects of the activity of these compounds for subsequent discussion. It needs to be emphasized that only some of the many effects of compounds used as ‘rumen modifiers’ and ‘feed additives’ are understood. Most studies have concentrated on measuring effects in the rumen where the modifiers have been shown to alter bacterial, protozoal and fungal populations. In addition, there are many changes in the overall pattern of end product formation. There is also evidence for an increased flow of lipid from the forestomachs when
monensin is included in the diet [5]. However, it is clear from work in monogastric animals that many of the compounds have effects in the small intestine and hindgut. Furthermore, there is evidence for the direct and indirect action of some compounds on the gastrointestinal tract itself. Since we do not fully understand the mode of action of these compounds it is impossible to extrapolate from results under one set of conditions to predict what will happen under other conditions. There are a wide range of chemical agents available and even compounds from the same chemical groups appear to behave differently. There is, however, significant scope for improving productive efficiency under many practical conditions, and with a better understanding of the modes of action more effective use of these compounds should be possible.

2.1. Availability of energy to the animal

The amount of metabolizable energy available to ruminant animals can be affected by changing the feed intake, the amount of fibre digestion, the pattern of volatile fatty acid (VFA) production and the methane and hydrogen gas loss. Most of the rumen modifiers increase the molar proportion of propionate relative to the other VFAs. This pattern of fermentation results in a more efficient capture of energy in the form of VFAs and is thought to be one of the major ways in which the animal may obtain more metabolizable energy [4, 5]. As a result of increased propionate production and more lipid synthesis [6] there is less hydrogen available for methane production and there is less energy lost through this pathway. The results of a study, based on isotope dilution techniques, to measure the production of fermentation end products in sheep fed diets with or without the ionophore monensin are summarized in Table I. These results indicate that approximately 10% more metabolizable energy may be available to the animal when the pattern of fermentation is changed towards propionate production by ionophores. However, this simple explanation of the mode of action of monensin and other propionate enhancers does not provide a ‘general rule’. There are numerous studies where significant increases in the molar proportion of propionate were measured without any improvement in feed conversion efficiency, and there are also studies in which the increase in propionate was far too small to explain the magnitude of the improvement observed.

Most of the rumen modifiers cause some decrease in feed intake and the improvement in feed conversion efficiency observed in feed lots often results from a combination of reduced feed intake and a marginal improvement in live weight gain. There is considerable variation between different compounds in the extent of their effect on feed intake. The ionophore monensin can have a marked effect on feed intake and this aspect of its activity has even been investigated as a means of controlling the amount eaten when animals have free access to feed [7, 8]. Most of the rumen modifiers are antibiotics and as such often reduce the overall capacity of the rumen
TABLE I. QUANTITATIVE CHANGES IN THE PATTERN OF RUMEN FERMENTATION AS A RESULT OF INCLUDING THE IONOPHORE COMPOUND MONENSIN IN A DIET FED TO SHEEP [6]

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Monensin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g C/d</td>
<td>MJ/d</td>
<td>g C/d</td>
<td>MJ/d</td>
</tr>
<tr>
<td><strong>Net VFA production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>110</td>
<td>4.01</td>
<td>102</td>
<td>3.72</td>
</tr>
<tr>
<td>Propionate</td>
<td>12</td>
<td>0.51</td>
<td>49</td>
<td>2.09</td>
</tr>
<tr>
<td>Butyrate</td>
<td>25</td>
<td>1.14</td>
<td>22</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Total VFA energy</strong></td>
<td>—</td>
<td>5.66</td>
<td>—</td>
<td>6.81</td>
</tr>
<tr>
<td><strong>Wasted energy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane</td>
<td>4.4</td>
<td>0.323</td>
<td>2.7</td>
<td>0.198</td>
</tr>
<tr>
<td>Acetate–butyrate interconversion</td>
<td>72</td>
<td>0.052</td>
<td>50</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>Total wasted energy</strong></td>
<td>—</td>
<td>0.375</td>
<td>—</td>
<td>0.228</td>
</tr>
<tr>
<td><strong>Net useful energy</strong></td>
<td>—</td>
<td>5.285</td>
<td>—</td>
<td>6.582</td>
</tr>
</tbody>
</table>

*a Available after accounting for interconversion of carbon between VFAs.

microbes to ferment fibre. There is some evidence of reduced digestibility [9] and while it does not appear to be a major feature [10] it is an aspect that should be considered in identifying reasons for variable responses in different trials. Under grazing conditions the intake of active compound can vary from animal to animal. This can have a negative effect on performance as when too much compound is consumed total feed intake may be adversely affected and in animals not eating enough there may be no response. The net result would be reduced performance for the group. Where reduced feed intake and decreased digestibility do occur these factors may override the benefits of a more efficient pattern of fermentation and digestion and thus contribute to the variability between trials discussed below.

2.2. Availability of protein to the animal

Availability of protein to the animal can be altered in a number of ways by the antibiotic feed additives and ionophore compounds. The main effect of the ionophore compounds is to reduce the amount of dietary protein degraded in the rumen. There may also be some reduction in microbial protein synthesis. The overall effect appears
to be a slight increase in protein flow to the small intestines [3, 9, 11], as summarized in Fig. 1. On the other hand the glycopeptid antibiotic avoparcin has been shown to increase absorption of amino acids from the small intestine [12] and the glycolipid flavomycin may reduce the reactivity of mucosa of the intestinal wall and thereby make more protein available for protein accretion elsewhere [13]. This range of activities suggests some scope for combinations of compounds but this has not been investigated — mainly because of the cost of gaining approval of regulatory authorities for this type of product.

**FIG. 1.** Nitrogen digestion measured using $^{15}$N in sheep fed an unmedicated diet or one containing monensin (30 mg/kg). All flows of N are in g/d. (From Ref. [11].)
2.3. Reduction in lactic acid accumulation in the rumen

There appear to be two ways in which feed additives act to reduce the accumulation of lactic acid during the rapid fermentation of starch and soluble carbohydrate. Lactate is a key intermediate in the production of propionate from pyruvate and on this basis it would be expected that the feed additives which increase the production of propionate may actually increase the likelihood of lactate accumulation during rapid fermentation of starch and sugars. There are two pathways by which propionate is synthesized in the rumen: through lactate-acrylate and through the dicarboxylic acid, or succinate, pathway. In the case of the ionophore compounds there is evidence that propionate production is stimulated specifically through the succinate pathway and therefore away from lactate production. When monensin is fed there is an increase in the concentration of succinate dehydrogenase [14]. There is also an increase in the proportion of $^{14}$C labelled carbon from the CO$_2$–bicarbonate pool incorporated into propionate [15] and this indicates a greater degree of equilibration through the carboxylation–decarboxylation process which occurs as part of the dicarboxylic acid pathway, but not the lactate–acrylate pathway.

A second way in which feed additives may affect lactate accumulation is through their antibiotic activity. The most important lactate producers in the rumen are Gram positive organisms (*Streptococcus bovis*, *Lactobacillus* spp.) while the lactate utilizers tend to be Gram negative. The ionophore compounds as well as additives such as virginiamycin, flavomycin and avoparcin are specifically active against Gram positive organisms and selectively control the proliferation of the lactate producers. In vitro screening of antibiotic feed additives [16] indicates a number of compounds to be effective in controlling lactate accumulation. On the basis of this screening, virginiamycin has been tested as a means to facilitate rapid introduction of high grain diets under feed lot conditions and it appears that animals can safely be given direct access to 90% wheat diets (with virginiamycin). The introduction of grazing animals to diets containing grain or soluble carbohydrate could be improved by use of these compounds and this is discussed in Section 4.

3. IMPROVING PRODUCTIVITY OF GRAZING ANIMALS

3.1. Body weight

Under intensive feed lot conditions the advantages associated with the use of chemical feed additives are mainly due to more efficient feed conversion. In grazing animals the subtle improvement in feed conversion efficiency is not measurable and has no real economic value. Responses in body weight change are therefore of the greatest interest and there are several examples showing that major improvements are possible. The feed additives which have been most widely tested under grazing
FIG. 2. Relationship between the live weight change of control (unmedicated) groups of grazing cattle and the response to rumen modifiers monensin, lasalocid and avoparcin in 'treated' animals. The data summarized are from Refs [17-23].

conditions are monensin, avoparcin and lasalocid. These additives have been compared in several experiments [17, 18] and there is no evidence that responses are significantly different between them. In order to gain an overview of the effect of these compounds on live weight gain the results from a number of trials are summarized in Fig. 2, which shows the relationship between the performance of the unmedicated control group and the response in the treated group. This provides a means of investigating the effect of the quality of the basal diet on the growth response. It appears that in animals grazing low quality dry pastures, maintaining or losing weight, there is less chance of a positive response to the use of rumen manipulators than in animals grazing better quality pastures and gaining weight. It is also clear that there is a lot of variation in the response to these feed additives whatever the quality of the pasture and the growth rate of the unmedicated animals. One possible explanation could be unsatisfactory compound dosing techniques. However, in the case of monensin, which has been widely evaluated using a rumen release device to administer the compound, there is still considerable variation in response.

Under experimental conditions compounds are normally administered with a small amount of concentrate supplement on a daily basis. This is costly and labour intensive in situations where the supplement itself is not essential or cost effective. The use of lick blocks and mineral supplements is a cheaper means of administration but there is even more variation between animals using this technique and it is likely to be unsatisfactory particularly when compounds have a narrow effective dose range. Water medication can be used for compounds which are water soluble [18]. The rumen release device developed for monensin is the only product which has been
widely tested under grazing conditions [22]. This technology is still relatively expensive but indicates that the approach is feasible and with more effort in development may become cost effective for more compounds.

As with all systems of supplementary feeding the economics must be evaluated in the light of the potential for compensatory gain once supplementation is no longer necessary. This is particularly important when animals grow to their market weight and fatness over two or more growing seasons. This is illustrated in the results of an excellent study in Queensland [20] where the benefits of protein supplements, hormone growth promoters and feed additives were evaluated. The conclusion reached was that even with a good response in growth rate of around 80 g/d when feeding avoparcin during the dry season (143 d) the subsequent growth of the cattle during the wet season resulted in no economic advantage. There is, however, good evidence that in the year in which animals are being prepared for market or for mating there may be considerable economic advantages in achieving a faster growth rate by using feed additives.

3.2. Manipulation of fermentation and digestion to improve wool growth

As would be expected from the range of ways in which protein digestion may be affected by various feed additives it is possible to increase wool growth using a number of different compounds. Although increased wool growth has been observed in response to ionophores [24, 25] the best response in our studies has been to the compound flavomycin, which MacRae and Lobley [13] suggest acts to reduce the amount of protein required to maintain the integrity of the intestine. Even with this compound the responses in wool growth have been variable and appear to be influenced by the quality of the basal diet as well as the age and previous nutritional history of the animals. Our results suggest that good responses may be expected in mature animals which have unrestricted access to a relatively good basal diet and which have not undergone severe nutritional deprivation during a seasonal drought [26]. In these animals wool growth may be increased by 15–20% or by 1.5–2 g of clean wool per day.

None of the feed additives which we have studied has given any increase in wool growth in animals fed poor quality roughage based diets. On the other hand there is some evidence that by adding bentonite clays to the drinking water of sheep grazing low quality roughage wool growth could be increased by 10–20% [27]. Despite these initial results subsequent experiments have shown that responses to bentonite may also be variable [27–30] and there are practical problems in incorporating the clay into the drinking water. Although it is often claimed that the variability of the wool growth response to feeding bentonite is due to differences in the source and type of the bentonite this does not appear to be the case. There have been a number of studies conducted using exactly the same source of bentonite where the
<table>
<thead>
<tr>
<th>Additive</th>
<th>Diet</th>
<th>Level of intake</th>
<th>Control wool growth (g·m⁻²·d⁻¹)</th>
<th>Response above control (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ionophores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lasalocid</td>
<td>Pellet</td>
<td>Maintenance</td>
<td>6.4</td>
<td>0</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Maintenance</td>
<td>5.1</td>
<td>0</td>
<td>[31]</td>
</tr>
<tr>
<td>Tetronasin</td>
<td>Pellet</td>
<td>Ad libitum</td>
<td>11.5</td>
<td>9.6</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Ad libitum</td>
<td>5.9</td>
<td>0</td>
<td>[25]</td>
</tr>
<tr>
<td><strong>Glycopeptides and glycolipids</strong></td>
<td></td>
<td></td>
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<tr>
<td>Avoparcin</td>
<td>Pellet</td>
<td>Maintenance</td>
<td>6.4</td>
<td>0</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Maintenance</td>
<td>5.1</td>
<td>0</td>
<td>[31]</td>
</tr>
<tr>
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<td>Chaff</td>
<td>Maintenance</td>
<td>6.2</td>
<td>0</td>
<td>[33]</td>
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<tr>
<td></td>
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<td>Ad libitum</td>
<td>11.9</td>
<td>0</td>
<td>[33]</td>
</tr>
<tr>
<td>Flavomycin</td>
<td>Pellet</td>
<td>Maintenance</td>
<td>6.4</td>
<td>6.6</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Maintenance</td>
<td>5.1</td>
<td>8</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>Pellet</td>
<td>Ad libitum</td>
<td>11.5</td>
<td>18.6</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Ad libitum</td>
<td>5.9</td>
<td>7.7</td>
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<tr>
<td></td>
<td>Pellet</td>
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<td>13.4</td>
<td>3</td>
<td>[32]</td>
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<tr>
<td></td>
<td>Pellet</td>
<td>3.5% of live weight</td>
<td>13.2</td>
<td>17.4</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Pellet</td>
<td>3.5% of live weight</td>
<td>13.7</td>
<td>13.9</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Pellet</td>
<td>3.5% of live weight</td>
<td>14.6</td>
<td>0</td>
<td>[26]</td>
</tr>
<tr>
<td><strong>Bentonite clays</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bentonite 1</td>
<td>Pellet</td>
<td>3.5% of live weight</td>
<td>13.4</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Maintenance</td>
<td>4.8</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Ad libitum</td>
<td>7.6</td>
<td>15.8</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>Grazing</td>
<td>Green pasture</td>
<td>6.5</td>
<td>16.8</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>Roughage</td>
<td>2.5% of live weight</td>
<td>3.2</td>
<td>18.8</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Roughage</td>
<td>2.5% of live weight</td>
<td>8.2</td>
<td>19.5</td>
<td>[30]</td>
</tr>
<tr>
<td>Bentonite 2</td>
<td>Pellet</td>
<td>3.5% of live weight</td>
<td>13.4</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Maintenance</td>
<td>4.8</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td>Bentonite 3</td>
<td>Pellet</td>
<td>3.5% of live weight</td>
<td>13.4</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Chaff</td>
<td>Maintenance</td>
<td>4.8</td>
<td>0</td>
<td>[28]</td>
</tr>
</tbody>
</table>
responses have been quite different [27-29] and, as in the case of other classes of feed additives, our understanding of how these compounds act is incomplete.

The results of a number of trials investigating the use of feed additives to improve wool growth are summarized in Table II. These results demonstrate the variability in the level of response from compound to compound and for any single compound with varying conditions.

3.3. Milk production

Milk production is influenced by metabolizable energy and the amount and quality of protein available to the animal. Since feed additives alter both protein and energy availability it is not surprising that they can also affect milk production. Because milk forms an important part of the diet of young children possible contamination of the milk with feed additives, or any other artificial substance, continues to be an emotional issue. In many countries there is also surplus milk production and this is another reason why the potential use of feed additives to increase production has not been a primary commercial target. However, avoparcin has recently been cleared for use in dairy cattle in Europe and it appears that an increase in milk production of between 4 and 7% may be achieved under conditions prevailing in the United Kingdom [34]. Slight increases in milk production have also been reported for the ionophore compound lasalocid [35] and there are unpublished reports of increased milk production in response to flavomycin. All of the data available at this stage are for dairy cattle on good temperate pasture or fed well balanced concentrate based diets. It is not possible to use these data to predict the potential advantages for cattle grazing low quality pastures or crop residues. This question is important not only for commercial milk production but also for cow-calf operations where the performance of the calf is directly affected by the milk production of the cow.

4. IMPROVING THE UTILIZATION OF STARCH BASED SUPPLEMENTS

One of the major problems in providing energy rich supplements to grazing animals is the adverse effect of the starch, or soluble carbohydrate in the case of molasses, on the utilization of the fibrous basal diet. These adverse effects are further confounded by the interval of feeding [36]. It is not clear whether the inefficiency occurs in the digestion and absorption of the nutrients or in the utilization of those nutrients at the tissue level. However, the difference in the response of grazing animals to cereal grain or legume/oilseed supplements can be quite dramatic, as shown in Fig. 3. There is considerable debate as to whether or not the animal has a specific requirement for protein (or protected protein) or whether the difference in performance results from the negative effects of starch/soluble carbohydrate fermentation in the rumen. The review by McAllan [37] concludes that there is no
FIG. 3. Change in live weight of sheep given no supplement (●) or fed twice a week with either lupins (○) or barley grain (Δ) to provide the equivalent of 200 g/d.

TABLE III. USE OF VIRGINIAMYCIN WITH BARLEY GRAIN TO ALLOW A GREATER INTERVAL OF FEEDING AND IMPROVED UTILIZATION OF SUPPLEMENT [38]

<table>
<thead>
<tr>
<th>Supplementation rate</th>
<th>Lupins</th>
<th>Barley + virginiamycin</th>
<th>Barley</th>
<th>Nil</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight gain (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>91</td>
<td>85</td>
<td>86</td>
<td>48</td>
<td>8</td>
</tr>
<tr>
<td>Twice-weekly</td>
<td>93</td>
<td>81</td>
<td>68</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Weekly</td>
<td>72</td>
<td>70</td>
<td>43</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Fortnightly</td>
<td>62</td>
<td>56</td>
<td></td>
<td></td>
<td>—</td>
</tr>
</tbody>
</table>

Chaff intake (g/d)

| Daily                | 1077   | 1026                   | 1084   | 1232 | 47  |
| Twice-weekly         | 1086   | 1038                   | 939    |     | —   |
| Weekly               | 934    | 974                    | 926    |     | —   |
| Fortnightly          | 948    | 950                    |        |     | —   |
evidence for increased feed intake in response to protected protein. This suggests that the difference in performance between animals fed these two types of supplements may be due to the depression in nutrient utilization associated with the starch rather than to an improvement due to the additional protein.

Protein supplements, in addition to providing amino acids and metabolizable energy, also provide significant quantities of substrates for rumen microbes. Therefore, in experiments comparing protein and energy supplements under pen conditions protein is often indicated as being beneficial, not because amino acids are required, but because of insufficient nitrogen and sulphur for microbial activity provided by the basal diet. It is therefore possible that cereal grain may result in improvements in animal production that are similar to those seen in response to legume grains and oilseed meals when the cereal supplements are supplied with adequate nitrogen and sulphur for the microbes and also with some means of preventing the adverse effects of uncontrolled starch fermentation. This approach was the subject of an experiment in which adequate nitrogen and sulphur were provided in the roughage fraction of the diet and animals were then fed supplements of lupin, barley or barley coated with virginiamycin, supplying 40 mg/kg grain [38]. The results are summarized in Table III. The supplements, equivalent to 200 g/d, were fed daily, twice a week, weekly or once a fortnight. There were similar responses in feed intake and live weight gain in animals fed barley with virginiamycin or lupin grain even when the supplements were fed at fortnightly intervals. On the other hand animals fed a supplement of barley on its own showed a reduced feed intake even with twice-weekly feeding and there was no live weight response at all when the grain was fed weekly. With the substantially higher prices of oilseed meals and legume grains compared with cereal grain and molasses, further work is justified to explore more fully the use of feed additives.

5. INHIBITION OF METHANE PRODUCTION

Of all aspects of the greenhouse gas problem the control of methane production from ruminants holds most promise as one which could be achieved without detrimental effect to the economy. Methane contributes 20% of the total radiative forcing (greenhouse) effect, compared with approximately 50% for carbon dioxide. The rate at which the concentration of methane is increasing is nearly twice that of carbon dioxide and each molecule of methane has 36 times more capacity for radiative forcing than a carbon dioxide molecule. It is estimated that 50 million tonnes more methane enter the atmosphere each year than break down [39]. To put this imbalance in context, domestic ruminants are estimated to produce around 80 million tonnes per year [40]. Therefore, if methane production could be significantly reduced in a high proportion of the world's domestic ruminants then the current
increase in atmospheric methane concentration could potentially be controlled. This is technically feasible.

The ionophores and the antibiotic feed additives which increase propionate production also decrease methane production. Ionophore antibiotics may reduce methane production by 20–30% [41–43] but are only effective for short periods, with methane production returning to normal within a week or two [41]. The real effect on methane production is through compounds which have been identified specifically as methane inhibitors. These compounds are all polyhalogenated molecules which inhibit the enzyme system involved in the final methyl transfer reaction leading to methane. While the simple halomethanes appear to be the most active methane inhibitors [44, 45] these compounds are too volatile to be used as feed additives and can be toxic to the animal. The groups of compounds which have been most seriously considered include hemiacetal of chloral and starch [46, 47] and the chlorosubstituted benzol-1,3-dioxins [48]. These compounds achieve almost total control of methane production during the first few days and thereafter there is variable persistence of antimethanogenic activity. For example, in an experiment with hemiacetal of chloral and starch methane production was reduced to around 36% of the level in the control animals after 2 d but returned to 88% of control values by the end of 30 d of feeding [49]. On the other hand methane production was reduced by the chlorosubstituted benzol-1,3-dioxin, ICI 13409, to 8% of the control output over the first 21 d of feeding and even over the full 196 d feeding period the production of methane by treated animals was only 37% of that measured in the control group [48]. Over the 28 week period of this feeding trial the animals treated with ICI 13409 also grew 8% faster and had a feed conversion which was 12.5% more efficient than that of the control animals [48].

Although there are examples of experiments where the growth rate and feed conversion efficiency have improved in response to using methane inhibitors, the overall picture shows variability. Summarizing the results of ten sheep and cattle experiments which examined halogen containing chemicals, Chalupa showed that there were improvements in live weight gain in 48% of cases and improved feed conversion in 90% of cases [50]. These experiments involved a number of halogenated compounds known to have a short period of effectiveness and others with a negative effect on feed intake. Although it is probable that variations in animal production responses resulting from the use of methane inhibitors are similar to those seen with ionophore and other antibiotic feed additives, in view of the problems relating to the greenhouse effect there appears to be a good case for additional effort to understand the situations in which positive responses are likely with the second generation compounds such as the chlorosubstituted benzol-1,3-dioxins. It would also be desirable to examine combinations of antibiotic feed additives and methane inhibitors with the objective of achieving more economically attractive responses in conjunction with effective methane inhibition.
6. CONCLUSIONS

Chemical manipulation offers a cost effective means of improving live weight gain under grazing conditions. Although responses are variable at present, there is scope for understanding better the mechanisms by which improved efficiency is achieved and for predicting more accurately the conditions under which benefits will be realized. There are also new possibilities for more efficient use of low cost energy supplements if feed additives can be used successfully to overcome the detrimental effects associated with the rapid fermentation of starch and soluble carbohydrate. Chemical methane inhibitors provide a realistic approach for tackling some of the greenhouse problems. More work is needed on the development of low cost and effective methods for the administration of additives to grazing animals. This is particularly important in the case of methane inhibitors where the economic value of the production response may be marginal.

REFERENCES


MINERAL SUPPLEMENTATION OF LOW QUALITY ROUGHAGES

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Edinburgh,
United Kingdom

Abstract

MINERAL SUPPLEMENTATION OF LOW QUALITY ROUGHAGES.
Mineral concentrations in low quality roughages (LQRs) are often low and availability for absorption has often been presumed to be low. However, radioisotope dilution studies have shown that the fraction of P in rice bran, wheat bran and barley straw which is available is surprisingly high (0.57–0.72). Chemical processing ought to improve the release of minerals from LQR in the rumen but possible effects of the chemicals on absorption and retention of the released mineral make it impossible to predict net effects on the mineral status of animals. Effects of physical processing have received little attention but are probably small and not worth pursuing. Supplementation of LQRs with energy and protein may indirectly enhance Mg and Zn absorption respectively and improve intakes of minerals by increasing food intake. However, feeding LQRs will increase the faecal endogenous losses and hence maintenance requirements for P, Ca and possibly other elements. Improving the quality of LQRs by improving their digestibility may further increase both maintenance needs and negative balances by increasing food intake. Because of uncertainties in the stated mineral requirements of ruminants, in the ability of LQRs to meet those needs and in the capacity of animals to adapt to deficient diets, production responses cannot be predicted: responses to mineral supplementation in long term feeding trials are the best guide to the need to supplement. The ideal method is conservative supplementation of complete diets based on LQRs with all essential minerals at the time of treatment with energy and protein. The need to supplement may be greater in the field than in experiments because parasitic infections increase mineral requirements.

1. INTRODUCTION

Each year some one million white-bearded wildebeest (Connochaetes taurinus Burchell) migrate great distances south and east from Tanzania to Kenya across the Serengeti–Mara ecosystem to spend the wet season on the short grasslands of the Serengeti plains. Many driving forces have been advanced for such migrations, including the increased need for water, digestible energy and protein in lactation: none is more convincing than the need for phosphorus, since the wildebeest becomes severely hypophosphataemic during the dry season (plasma P 0.6 mmol/L) and
moves to wet season pastures much richer in P [1]. In farmed ruminants there are other natural drives to conserve minerals which can afford protection from short term deficiencies in the diet: these adaptive powers help to explain the inconsistency of responses to mineral supplementation of low quality roughages (LQRs) in the literature and why some researchers still try to improve nutritional quality without adding minerals. The objectives of the present paper are: firstly, to confirm that the health and performance of domesticated livestock given LQRs will often be impaired by mineral deficiencies unless they are corrected; secondly, to show that appropriate use of radioisotopes can improve the characterization of LQRs as sources of minerals; thirdly, to indicate how supplementation should be practised to overcome inherent mineral deficiencies.

2. MINERAL CONCENTRATIONS IN LOW QUALITY ROUGHAGES

The mineral composition of LQRs is not well documented but there are reasons for expecting such materials to be of low mineral content. ‘Low quality’ is synonymous with ‘low protein’ [2] and, since many essential minerals are associated primarily with the protein component of the plant (e.g. Cu, P and S), ‘low quality’ will also mean ‘low in some essential minerals’. A few examples of mineral deficiencies in LQRs can be given. Table I presents data for the macromineral composition of large numbers of wheat and barley straws and a few oat straws in the United Kingdom [3]. The average wheat straw was deficient in P, Mg and Na and the

<table>
<thead>
<tr>
<th>Element</th>
<th>Wheat $(n = 62)$</th>
<th>Barley $(n = 51)$</th>
<th>Oats $(n = 5)$</th>
<th>Effectsa</th>
</tr>
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<tbody>
<tr>
<td>P</td>
<td>0.8 (0.36)</td>
<td>1.0 (0.55)</td>
<td>1.4 (0.86)</td>
<td>S</td>
</tr>
<tr>
<td>Mg</td>
<td>0.9 (1.06)</td>
<td>0.7 (0.56)</td>
<td>1.1 (0.24)</td>
<td>—</td>
</tr>
<tr>
<td>Na</td>
<td>0.5 (0.87)</td>
<td>1.4 (1.60)</td>
<td>4.3 (1.61)</td>
<td>Y, S, C</td>
</tr>
<tr>
<td>S</td>
<td>1.5 (0.82)</td>
<td>1.8 (1.06)</td>
<td>4.0 (3.46)</td>
<td>Y, S, C</td>
</tr>
</tbody>
</table>

a Significant effects of year (Y), species (S) and county (C).
average barley straw lacked P and Mg: neither was particularly low in S and the oat straws were only marginally low in P and Mg. Data for rice straw [4] show similar macroelement deficiencies and also lower values for S (1.1 g/kg DM): microelement concentrations were highly variable with some rice straws deficient in Cu (range 2–10 mg/kg DM) or Zn (range 15–60 mg/kg DM). Leaves of leguminous trees are generally poor sources of P and S though adequate in Ca according to chemical analysis [5].

Stage of maturity is one of many factors which will contribute to variation in mineral concentrations in LQRs. Loneragan [6] has shown that Cu concentrations in cereal leaves decline markedly as maturity approaches. Similar trends with maturity have been reported in four tropical forages, with Cu concentrations falling from 5.5–9.8 mg/kg DM in 6 week regrowths to deficient values of 3.1–5.8 in 12 week regrowths [7].

Minson [8] has recently published extensive data on mineral concentrations in forages: the most striking feature for most species was the wide range of reported values, some of which will be due to interlaboratory error. There is a need to establish a comparable database for the mineral composition of LQRs but new data should be collated from laboratories using internationally accepted quality control standards and organized through an agency such as the International Network of Feed Information Centres.

3. AVAILABILITY OF MINERALS IN LOW QUALITY ROUGHAGES

3.1. Untreated roughages

It has been suggested that the problem of mineral deficiencies in LQRs is often compounded by low availabilities, particularly for S [9], but there is little evidence to support that claim. The database for mineral availabilities is even more restricted than that for mineral concentrations. The reasons for the lack of meaningful data on the intrinsic nutritive value of minerals in even the expensive, staple foods have been reviewed elsewhere and the importance of appropriate radioisotope dilution techniques to differentiate the effects of food and animal attributes on absorption has been stressed [10]. The only data for LQRs are for P and show little evidence of low availability. The mean availability coefficient for P in rice bran given to sheep was 0.64, only 7% below the value obtained for soybean meal (0.69, Table II). Sareen et al. [12] reported a slightly lower value (0.57) for P in wheat bran given to buffalo (Bubalus bubalis) but the average availability of P in mixed diets for cattle is only 0.58 [12].

There have been claims that some roughages contain Ca of low availability but a Technical Committee on Responses to Nutrients (TCORN) Working Party [13]
argued that valid assessments have yet to be made. Because animals absorb Ca according to need, only roughages providing no more than the Ca requirement can be assessed for their full potential (i.e. availability) as Ca sources. The use of animals at peak lactation to increase need does not help because there is probably obligatory mobilization of Ca from the skeleton and the mobilized Ca lessens the ‘felt need’ [13]. Measurements using Ca isotopes in the manner employed by Braithwaite [14] of Ca absorption in early weaned animals or those in late lactation given untreated roughages would provide a rigorous test of the inference [13] that Ca availability will not be low in LQRs.

The availability of minerals in LQRs may be reduced by the formation of complexes with unusual constituents such as oxalates and tannins [15]. There is physical evidence of low Ca availability in certain oxalate rich feeds in that crystals of calcium oxalate from such feeds (e.g. alfalfa) can be found unchanged in the faeces. However, Ca can be well absorbed from oxalate rich tropical grasses by cattle and Ca supplements only marginally improved the growth of cattle given oxalate rich *Acacia aneura* leaves [16]. The high tannin content of the leaves of leguminous shrubs and trees may reduce Fe availability because tannins form complexes with Fe which are stable at normal abomasal pH [17]. Although complexes of tannin and Co should dissociate below pH4.0, i.e. upon arrival at the abomasum, that is too late for Co to be incorporated into vitamin B12 by rumen microbes. In animals with parasitic infections of the abomasum, pH increases to 4–5 and complexes of tannins with Cu (stable up to pH3.7) may not be dissociated. Although Mg–tannin complexes dissociate below pH6.95, Mg absorption occurs predominantly from the rumen and mixtures of tannin rich leaves with alkali treated straw, itself low in Mg (cf. Table I),
could create a seriously Mg deficient diet. The availability of Mg in other LQRs can be expected to be at least as low as that in hay and lower than that in cereals [18]. Large reductions in the intake of *A. aneura* by cattle without S supplementation have been attributed to the formation of indigestible tannin–protein (i.e. S amino acid) complexes in the rumen [16].

Field [19] suggested that the rate of release of minerals from roughages in the rumen may be a major determinant of mineral availability. Wide ranges of release rates have been noted for macroelements in tropical hays [20], uniformly high rates for silages [21] and low rates for trace elements in tropical forages [7] but there are as yet no data relating mineral absorption to ruminal release rate.

### 3.2. ‘Chemically improved’ roughages

If slow release is a problem one would expect it to be greatest in fibrous, lignified LQRs and to be improved by chemical treatments which increased the rumen degradability of cell wall matrices. Improved release could be particularly significant for Mg, which is absorbed extensively from the rumen, and Co, which is incorporated there into vitamin B12. There are, however, no data to support this speculation. The net effect of chemical treatment on Mg absorption is particularly hard to predict because absorption from the rumen is enhanced by Na and inhibited by NH₃ and decreases with pH [18]: with alkali treatment, the type of alkali (e.g. NaOH or NH₄OH) may therefore be important. Reductions in plasma Mg have been reported in heifers given NaOH treated straw [22] but the treatment was confounded with a halving of the concentrate supplement used. Neutralization of NaOH treated straw did not change Mg balance in a small experiment with calves [23].

In using alkali to improve feed quality, the mineral contribution from the alkali must not be ignored. In studies comparing Ca(OH)₂ with other alkalis, up to 25 g Ca/kg DM has been added to straws. While ruminants can tolerate very wide Ca:P ratios in diets adequate in P, they are less tolerant when the diet is marginal in P. Wan Zahari et al. [24] found that lamb growth and plasma P were depressed when the Ca:P ratio of a high quality, semi-purified diet containing 2.0 g P/kg DM was raised to 3.6:1. A corresponding reduction in the inorganic phosphate concentration in rumen liquor from 20 to 7 mmol/L was attributed in part to a Ca induced decrease in P solubility in the rumen. Ternouth and Sevilla [25, 26] have recently performed similar studies with a diet consisting mainly of LQR (barley straw) but found no reduction in P availability or plasma P when the Ca:P ratio was increased from 2.1 to 8.1. The basal diet was, however, so low in P (0.75 g/kg) that it is doubtful if the Ca × P antagonism could have been expressed.

The solubilized ash of crop wastes such as cocoa pods [27] is a cheap and convenient source of alkali for the treatment of LQRs: ash will contain a mixture of cations, chiefly K, but P and S may not be present in sufficient quantity to avoid the
need for further supplementation. If the feeding of alkali treated straw changed the acid–base balance of the recipient, the degree of mineralization of the skeleton might increase (cf. Ref. [28]) but it is not clear whether this would represent an obligatory increase in growth requirements for Ca, P and Mg and restrict mobilization in times of dietary deficiency.

3.3. Physically processed roughages

Superficially, one might expect the reduction in particle size of LQRs to improve mineral release and availability by increasing the surface area exposed to digestive processes. As far as P is concerned, milling barley straw had no effect on P availability [29]. The lack of effect may be partly explained by a decrease in dwell time and hence cellulose breakdown in the rumen, since grinding can reduce the digestibility of straw. Effects on other elements will depend partly on whether the rumen is a favourable or unfavourable environment in terms of the sustained absorbability of the released element. In the case of Cu, for example, a decreased dwell time might enhance availability because Cu can form irreversible complexes with sulphide and thiomolybdates which enter the solid phase in the rumen, rendering the Cu unavailable [30].

3.4. Energy- and nitrogen-supplemented roughages

The addition of fermentable carbohydrate and protein to an LQR diet may influence mineral availability in and beyond the rumen. Fermentable carbohydrate would be expected to enhance Mg availability by lowering rumen pH [18]. Addition of protein to a diet based on low quality hay has increased the availability of dietary Zn for sheep [31]. Ternouth [29] recorded high availabilities of 0.69 and 0.72 for P in two barley straws which were fed to sheep with molasses and urea but the low digestibility coefficients of the mixed diets (0.54 and 0.51) suggest that the availability of P in straw was intrinsically high rather than improved by the molasses and urea.

4. MAINTENANCE REQUIREMENTS

The adequacy of LQR as a source of minerals is determined not only by its content of available minerals but also by the effect which the feed has on animal needs for maintenance and production. Faecal endogenous losses of Ca (FE_{Ca}) increase with dry matter intake (DMI) and at times when DMI is high relative to body weight (i.e. in lactation) the maintenance need for Ca is much higher than hitherto realized [13]. Maintenance needs for P similarly increase with DMI but, in addition, roughage diets stimulate higher faecal endogenous losses (FE_{P}) by
Table III. Increased dry matter intake (DMI) of a low quality roughage can improve or diminish P balance in sheep depending on the P concentration present because faecal endogenous losses increase with DMI.

<table>
<thead>
<tr>
<th>P in barley straw (g/kg DM)</th>
<th>1.47</th>
<th>0.47</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI (g/kg LW/d)</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Absorbed P (A) (mg/kg LW)</td>
<td>10.4</td>
<td>20.1</td>
</tr>
<tr>
<td>Faecal endogenous P (E) (mg/kg LW)</td>
<td>16.7</td>
<td>22.7</td>
</tr>
<tr>
<td>A - E (mg/kg LW)</td>
<td>-6.3</td>
<td>-2.6</td>
</tr>
</tbody>
</table>

Note: A and E are predicted from equations describing their relationships with DMI in Ref. [30].

drawing more P, via saliva, past an absorptive surface which is not 100% efficient. To allow for this effect, TCORN [13] proposed the use of a roughage factor whereby the maintenance need for P was increased by 1.6 for diets of low digestibility; this would include all those based predominantly on even nutritionally improved roughages.

Putting precise figures on the influence of DMI and roughage intake on $FEP$ is difficult because of the correlated effect of P intake and adaptive changes which can occur in the animal [13]. Net effects of DMI and P intake on P balance are more tangible and one is shown in Table III. With a high P straw, P absorption increased more than $FEP$ as DMI increased and P status improved as more roughage was consumed. By contrast, the more low P straw that was consumed, the greater was the negative P balance because the effect of DMI on $FEP$ outweighed the additional P absorbed. The improvement of LQR will (if allowed to) increase DMI of the roughage and thus maintenance needs: some diets will therefore become more deficient in P and Ca unless there are associated improvements in mineral availability.

The effect of DMI on maintenance requirements may well be a general phenomenon applying to all elements, not just Ca and P. If the LQR is improved sufficiently in digestibility and as a source of microbial protein to sustain production then all mineral needs will be further increased.
5. VARIATIONS IN TOTAL MINERAL REQUIREMENTS

A common approach to the assessment of mineral status in tropical pastures has been to compare herbage composition with standards of animal requirement (e.g. Ref. [32]) but two sources of variation weaken this approach.

5.1. Human sources

Widely different estimates of mineral requirements by different national authorities (for Ca and P see Ref. [13]) indicate that the real need to supplement any diet with minerals cannot be judged simply by referring to tables of requirement [30]. The different estimates reflect a lack of data and differences of interpretation: both sources of divergence will be magnified when dealing with a heterogeneous and understudied commodity such as LQR. The latest recommendations [13] allow for the skeleton to be fully mineralized at all times; this provision may be a wise precaution but the need is not obligatory for lifetime health or production.

5.2. Animal sources

Variations in mineral requirements within and between species have been recorded for P: wider differences may exist and extend to other minerals, influencing the need to supplement LQRs. Read et al. [33] found that sheep maintained viability and almost maintained production on pasture containing far less P than the latest tabulated requirements (3–15 vs. 12–43 g P/kg DM [13]) over four seasons whereas calf mortality quickly and greatly increased on the same pasture. It was suggested that sheep may be the more tolerant species because they have greater time to replenish lactation losses before the next pregnancy begins and they also have a higher DMI/kg LW than cattle [33]: the latter could, however, be a disadvantage (see Section 4). The TCORN review [13] of P availabilities suggests another reason, sheep having a higher efficiency of absorption than cattle on mixed diets (0.68 vs. 0.58).

Wildebeest appear to have a very low blood P concentration (1.0 mmol/L) when kept in a zoo and presumably supplied with adequate P [1]. Since faecal endogenous losses are related to salivary and blood P [29], it is possible that wildebeest have developed low maintenance requirements for P as an adaptation to P deficiency associated with low quality, natural grazings. It is also possible that Bos taurus and B. indicus differ in their requirements for P.

Variation in P metabolism within and between breeds of sheep has been reported by Field and his co-workers (e.g. Ref. [11]). In Table II it can be seen that genetic variation was as important as feed variation in determining P availability. Since dietary requirement is inversely related to availability, it follows that the need to supplement with P will be influenced by variation within sheep breeds. Genetic variation provides opportunities as well as problems: it should be possible to select...
genotypes which can thrive on P supplies which would impair performance in others. Addition of minerals to LQR is not the only solution to anticipated deficiency problems.

6. RESPONSES TO MINERAL SUPPLEMENTATION

From what has been written so far, it is clear that there is not sufficient information on mineral content, mineral availability or animal need to be able to predict benefits from mineral supplementation of LQR. The best index of a need to supplement is a positive response in a feeding trial [30]. While many instances of responses to minerals under grazing conditions can be quoted (e.g. Ref. [32]), those for supplemented LQRs have often been disappointing or equivocal. Minerals have usually been applied with urea and/or energy with the result that the specific contribution from minerals could not be determined. There are probably as many examples of lack of responses to such mixtures (e.g.Refs [34-36]) as there are of significant improvements in performance (e.g. Refs [37-39]).

Two examples of specific responses to mineral supplements have recently appeared. Morrison et al. [40] confirmed earlier observations that S supplementation of spear grass (Heteropogon contortus), which is very low in S (0.5 g/kg DM), can greatly increase intake of LQR by cattle. In addition, they found that S deficiency deleted anaerobic fungi from the rumen population and suggested that improved digestibility in the rumen was an important component in the response to S. Ternouth and Sevilla [25, 26] have shown repeatedly that addition of P to a diet of barley straw supplemented with sugar and gluten will improve appetite in sheep. They suggested that P may have been beneficial by increasing the rate of DM flow from the rumen, the signal coming from systemic sources rather than a rumen distended because of a failure of microbial digestion [26]. The P content of the straw diet which was improved by supplementation was similar to that found in United Kingdom wheat straw (Table I) and many Australian tropical forages.

Ternouth and Sevilla's [25, 26] experiments contained important features which maximized the likelihood of revealing the undoubted dietary P deficit. Firstly, large sucrose and urea supplements were given in equal measure to all individuals so that neither energy nor nitrogen deficiencies could have limited digestion. Van Niekerk and Jacobs [35] could not significantly improve upon the food intake response to nitrogen by also including energy (10% of estimated requirement, ER) and P (50% of ER) in a mineral lick for cattle (180 kg LW) given sugar cane tops low in P (0.8 g/kg DM) for 70 d. Others have attributed such failures to low average intakes of lick [36] but variations within the group may have been just as important [41], leading to inadequate energy provision for some animals. Secondly, the weaned sheep were depleted of Ca and P for one month before the supplements were fed to minimize the recycling and redistribution of skeletal reserves. There is a large
increase in bone mineralization after weaning because solid diets have a better mineral:metabolizable energy ratio than milk [13]. Tolerance of Ca and P deficient diets will increase with time after weaning owing to the combined effects of skeletal growth and improved skeletal mineralization. Variations in responses to mineral supplementation of LQR may be due to variations in initial mineral reserves in the skeleton at the start of experiments, which have been as short as 21 d [34].

In seeking to show the benefits or otherwise of mineral supplementation of LQR, trials should be conducted with neither energy nor protein in short supply and with the onset and duration of feeding similar to those likely to be adopted in local husbandry systems. Until such trials have been carried out, the many reports of no responses to mineral supplementation should not tempt development workers to dispense with minerals instead of dispensing them. More prominence should be given to the fact that (a) responses to N can be found when minerals are mixed thoroughly with an LQR (e.g. Ref. [42]) and (b) they have not been found when minerals were omitted from an improvement regimen [43].

Another reason for not writing off the need for mineral supplementation too readily is that research workers usually take deliberate and effective steps to remove gastrointestinal parasites from their experimental animals: in doing so, they remove an important factor which often reduces mineral status in the field, where the norm is a state of chronic parasitic infection. With intestinal parasitism, there is impaired P absorption [30] and the resultant adverse effects on P balance are likely to be increased on roughage diets which carry more P past the damaged mucosal surface. In nematodiasis there are also unexplained systemic effects which cause extensive bone demineralization (cf. Ref. [44]). Current research at the Moredun Research Institute is revealing disturbances of Na and/or K metabolism in lambs with mixed nematode infections, such that salivary Na:K ratios decline to levels indicative of Na deficiency (5 vs. 15) on pastures adequate in Na (1.5 g/kg DM; N.F. Suttle, unpublished data). The need to supplement LQRs with minerals may therefore be partly dependent on the degree and persistency of nematode infections and be greater than indicated by trials with worm free stock.

7. SUPPLEMENTATION STRATEGIES

7.1. How to supplement

While the wildebeest may be able to sort out its own P deficiency problem [1], there is no evidence that animals instinctively and invariably recognize and satisfy a need for minerals when given the opportunity. In addition to the considerable variation in uptake of mineral blocks already referred to, variation also occurs in the intake of liquids [45] and of loose, dry mineral–vitamin mixtures between individuals, breeds and seasons in a grazing flock [46]. Mineral intakes may,
however, be less variable when such mixtures or blocks are offered separately to housed groups on LQR [45]. Nematodiasis [46] and previous experience of minerals (N.F. Suttle, unpublished data) can also drastically affect uptake of mineral mixtures given in separate troughs. Wherever possible, minerals should be distributed evenly over the roughage when it is treated with other supplements (e.g. at baling) or at feeding. With silages, it may be convenient to supplement with minerals at the time of ensiling. The incorporation of by-products such as poultry wastes into silages will greatly enhance the Ca and P value of the silage.

7.2. What to supplement

The most common need is likely to be for S closely followed by P, Mg and Na. The provision of urea to increase microbial protein synthesis on LQR must be accompanied by S (preferably elemental and of slow degradability) in a 1:10 ratio with added N. Only where the drinking water is naturally rich in sulphate should none be added. Calcium is usually found in considerable excess of P in mineral blocks and mixes because it is a cheap and convenient diluent or setting agent: in supplementing LQRs directly, P should be given in excess of Ca. Trace element deficiencies may arise and the cost of adding them to complete mineral mixtures is so small that in most situations all essential minerals should be added. Mineral excesses can, however, be as harmful as deficiencies and caution should be exercised. With individual intakes guaranteed by mixing mineral and LQR, ‘safety factors’ can be reduced: a target of supplementation to meet 75% of the lowest textbook requirements is suggested. The fact that S supplementation can reduce Cu availability while increasing the DMI of straw by sheep [39] confirms the importance of using minerals conservatively: provoking a need to supplement with Cu could create a potentially more damaging imbalance, that of Cu excess. The other principal overdose risks are Se toxicity in seleniferous areas and phosphaturic urinary calculi; the latter indicates an overfeeding of P and a scandalous waste of a precious resource.

REFERENCES


THE IMPORTANCE OF UREA-MOLASSES BLOCKS AND BYPASS PROTEIN IN ANIMAL PRODUCTION: THE SITUATION IN TROPICAL LATIN AMERICA

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Abstract

THE IMPORTANCE OF UREA-MOLASSES BLOCKS AND BYPASS PROTEIN IN ANIMAL PRODUCTION: THE SITUATION IN TROPICAL LATIN AMERICA.

The grasslands of Latin America sustain 25% of the world cattle population. Most of the cattle are located in tropical Latin America. There are two predominant systems of production, dual purpose and beef, with diets based primarily on cultivated or native pastures and to a minor extent on conserved feeds and fibrous agricultural by-products. Urea-molasses blocks and bypass protein supplements are recently introduced technologies that could contribute to solution of some of the feeding constraints of the systems. The use of bypass protein has been mainly studied using fish meal as a supplement to medium quality basal feeds. Large live weight gain responses have been obtained with growing animals, but the influence on milk yield of dual purpose animals is small or non-existent. Supplements with fish meal offered once a day did not increase the utilization of basal fibrous diets in the rumen and were not able to sustain adequate levels of ammonia nitrogen throughout the day. Lower responses have been obtained with other bypass protein sources. The use of urea-molasses blocks has been investigated less. The major impact of this technology should be in extensive grazing systems with diets based on native pastures low in nitrogen. The scarce evidence available indicates that the sporadic consumption of blocks in these conditions prevents them from fulfilling the main objective, which is to provide a constant source of degradable nitrogen throughout the day. But there is still a positive effect on live weight change and reproduction during the dry season. The blocks should be adapted to this situation by incorporation of sources of slow nitrogen release.

1. INTRODUCTION

Latin America contains large areas of grassland advantageous for cattle production. There are approximately 380 million ha of permanent pastures supporting about 347 million cattle, or 25% of the world population [1]. Two regions must be differentiated: temperate South America (Argentina, Chile and Uruguay) and tropical Latin America. In the tropical regions lower levels of production have been achieved, partly because it has not been possible to use technology transferred from
developed countries. However, much of the livestock population is located in this area and there is a vast potential to increase production through the improvement of existing production systems via solving its main constraint.

2. CATTLE PRODUCTION SYSTEMS IN TROPICAL LATIN AMERICA

Two major groups of production systems can be identified in tropical Latin America: dual purpose and beef. In the general description that follows those parameters were selected which have a large potential for improvement through better nutrition.

The characteristics of the dual purpose systems in this region were summarized by Sere and Vaccaro [2] and Vaccaro [3] from a large sample of studies and farms, and some parameters are shown in Table I. The authors stressed the importance of these systems, pointing out that between 45 and 85% of milk production in different tropical Latin American countries is obtained from dual purpose herds.

A large proportion of the farms use cultivated pastures, some of them introduced from Africa: *Panicum maximum*, *Brachiaria* spp., *Cynodon* spp., *Hyparrhenia rufa*, *Echinochloa polystachya*, *Digitaria* spp., *Andropogon* spp., etc. [4]. The nutritive value of these and other tropical forages has been evaluated in numerous digestibility trials reviewed by Minson [5]. The concentration of crude protein was most frequently found to be between 6 and 9% and the dry matter digestibility between 55 and 60%. However, most farms are in areas with a dry season of three to six months, when the availability of pasture is limited and its nutritive value is severely depressed. The use of fertilizers is not common, and the recent elimination of subsidies in order to reduce the huge external debts of most countries in the region has probably reduced their use even further. The use of concentrate and other energy and protein supplements is restricted to a small number of farms and these supplements are fed mainly to lactating cows. Growing animals are usually not supplemented, so growth rates remain below maximum during the dry season when herbage availability and nutritive value are low. The use of minerals is more frequent, but approximately half of the farms do not supplement these nutrients and frequently inadequate mineral mixtures are used [6].

Feeding and other factors determine the productivity parameters summarized in Table I. Milk yields refer to saleable milk because restricted suckling by calves is the usual practice. Average values were 4.0 kg/d and 1180 kg/lactation. Live weight gain and age at first calving are negatively influenced by feeding deficiencies during the dry season. Low calving rates affect milk and beef production per hectare.

There is no similar study on beef systems in this region, but some productive parameters reviewed by Plasse [7, 8] are indicative of the improvements that could be achieved by better feeding (Table II). The herds studied are located on poorer soils than the dual purpose herds and are sustained on native pastures of lower yield
TABLE I. DESCRIPTION OF COMMERCIAL DUAL PURPOSE FARMS IN TROPICAL LATIN AMERICA (1984) [3]

<table>
<thead>
<tr>
<th>Pastures and supplements</th>
<th>n</th>
<th>Range&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated pastures (% of total)</td>
<td>7</td>
<td>794</td>
<td>33–87</td>
</tr>
<tr>
<td>% of farms which:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fertilize pasture</td>
<td>7</td>
<td>929</td>
<td>8–50</td>
</tr>
<tr>
<td>use concentrate</td>
<td>9</td>
<td>1039</td>
<td>6–73</td>
</tr>
<tr>
<td>use any supplement</td>
<td>4</td>
<td>362</td>
<td>10–73</td>
</tr>
<tr>
<td>use minerals</td>
<td>8</td>
<td>848</td>
<td>13–83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal productivity</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield (kg):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cow/d</td>
<td>10</td>
<td>852</td>
<td>2.8–6.5</td>
</tr>
<tr>
<td>cow/lactation</td>
<td>4</td>
<td>130</td>
<td>749–1584</td>
</tr>
<tr>
<td>cow/year</td>
<td>3</td>
<td>267</td>
<td>186–1156</td>
</tr>
<tr>
<td>Lactation length (d)</td>
<td>5</td>
<td>157</td>
<td>244–311</td>
</tr>
<tr>
<td>Calving (%)</td>
<td>16</td>
<td>1298</td>
<td>39–81</td>
</tr>
<tr>
<td>Age at first calving (months)</td>
<td>8</td>
<td>994</td>
<td>32–43</td>
</tr>
<tr>
<td>Weight gain of calves (kg/d)</td>
<td>3</td>
<td>33</td>
<td>0.29–0.48</td>
</tr>
<tr>
<td>Stocking rate (AU/ha)</td>
<td>12</td>
<td>1096</td>
<td>0.72–1.90</td>
</tr>
<tr>
<td>Beef production (kg/ha/year)</td>
<td>4</td>
<td>744</td>
<td>45–192</td>
</tr>
<tr>
<td>Milk production (kg/ha/year)</td>
<td>10</td>
<td>990</td>
<td>182–749</td>
</tr>
</tbody>
</table>

<sup>a</sup> Range: mean values per publication.
<sup>b</sup> Mean: non-weighted average of published means.
TABLE II. ESTIMATED AVERAGE PRODUCTION LEVELS OF BEEF CATTLE IN TROPICAL LATIN AMERICA [7, 8]

<table>
<thead>
<tr>
<th>Trait</th>
<th>Production level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calving rate</td>
<td>35-60%</td>
</tr>
<tr>
<td>Birth weight</td>
<td>22-25 kg</td>
</tr>
<tr>
<td>Weaning weight of calf/cow</td>
<td>35-80 kg</td>
</tr>
<tr>
<td>Age at first calving</td>
<td>3-4 years</td>
</tr>
<tr>
<td>Slaughter age (males)</td>
<td>3.5-5 years</td>
</tr>
<tr>
<td>Slaughter weight</td>
<td>350-450 kg</td>
</tr>
<tr>
<td>Extraction rate</td>
<td>8-15%</td>
</tr>
</tbody>
</table>

and quality than the introduced species. All animals lose weight during the dry season and this is reflected in the low calving rates and advanced ages at first calving of females and slaughter ages of males.

3. FEEDING CONSTRAINTS

The characteristics of the systems described indicate that there is ample scope for improving production through better feeding. The main feeding constraints are the low herbage availability during the dry season, physical limitations to intake of tropical forages, nutrient deficiencies limiting rumen digestion of fibrous feeds, and an imbalance of the nutrients required by the animal.

To improve the availability of feeds during the dry season several alternatives have been proposed, including fibrous agricultural by-products, conserved forages, and legumes. But the probability of economic success depends on the particular conditions of each farm. Conservation technologies are being applied in mixed systems of crops and dual purpose animals, where machinery and experience with the crop are available. Grazing of maize and sorghum stubbles is used in farms located in cereal areas. But in most farms the lack of adequate forage in the dry season is probably the main feeding constraint to production. Finding a profitable solution is even more difficult in extensive beef systems. An alternative being evaluated by the Centro Internacional de Agricultura Tropical (CIAT) in Colombia is the use of improved grass-legume pastures [9].
Tropical forages have a large proportion of lignified cell walls with low digestibility and fermentation rates, leading to a low rate of disappearance through digestion or passage to the lower tract and limiting intake. There is a positive relationship in low quality feeds between digestibility and intake [10] and a linear relationship was observed with grazing animals in tropical pastures [11]. However, manipulation of this factor is only possible to a limited extent, either by increasing digestibility through pasture management or improved varieties, or by optimizing fermentation conditions in the rumen.

The supplementation of limiting nutrients to microorganisms to enhance their growth and increase the utilization of fibrous feeds has received much attention in recent years as a way to improve feeding in the tropics. Two groups of nutrients could be deficient: minerals (sulphur, phosphorus, magnesium, ammonia) and peptides/amino acids [12]. The cellulolytic microbes have apparently lower requirements of peptides/amino acids than those utilizing sugars or starches, according to evidence reviewed by the author. And, as pointed out in the review by Vaccaro [3] (Table I), the use of mineral supplements with limiting macro- and microelements is one of the few practices followed already by more than half of the farms.

Emphasis has been given in recent studies to the timing and supply of degradable nitrogen to meet the continuous requirements of microorganisms. The rumen ammonia concentrations required to achieve maximum intake and digestibility in low quality forages are far greater than those suggested earlier [13] to maximize microbial growth [14]. Information on ammonia concentrations in the rumen of grazing animals in Latin America is non-existent. But there is evidence indicating that when crude protein falls below 6-8% the appetite of the animal is depressed, and a large proportion of tropical grasses, particularly from native pastures, have values below this range [5]. Two approaches are being investigated in Latin America to find a practical solution to the problem of continuous nitrogen supply to grazing animals: tree legumes and urea–molasses blocks.

The addition of bypass nutrients to complement the products of rumen fermentation and balance the nutrients required by the animal has been another of the main objectives of new approaches to improve the utilization of low quality fibrous feeds [15, 16]. The needs for dietary amino acids, lipids and starch that escape rumen fermentation vary according to many factors, including climate, basal diet and physiological function, and it is difficult in practice to identify the primary limiting nutrient and determine a strategy to correct any imbalance [15].

4. USE OF BYPASS PROTEIN

The use of bypass protein in cattle has been a topic of research in this region in recent years. Unfortunately the information on grazing systems, by far the most
### TABLE III. EFFECT OF FISH MEAL (FM) ON INTAKE AND LIVE WEIGHT GAIN (LWG) OF GROWING CATTLE

<table>
<thead>
<tr>
<th>Diet</th>
<th>LW (kg)</th>
<th>DM intake (kg/d)</th>
<th>LWG (kg/d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay 2 kg/d + urea-molasses</td>
<td>234</td>
<td>6.22</td>
<td>0.54</td>
<td>Veitia [23]</td>
</tr>
<tr>
<td>100 g/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 g/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 g/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 g/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize silage + concentrate</td>
<td>125</td>
<td></td>
<td></td>
<td>Vasquez et al. [24]</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;: 2 kg/d with canavalia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;: T&lt;sub&gt;1&lt;/sub&gt; + 0.3 kg/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;: 2 kg/d with soybean meal (SBM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;: T&lt;sub&gt;3&lt;/sub&gt; + 0.3 kg/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh forage + concentrate</td>
<td>175</td>
<td></td>
<td></td>
<td>Silva et al. [25]</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;: 1.9 kg/d with canavalia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;: T&lt;sub&gt;1&lt;/sub&gt; + 0.3 kg/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh forage + 2.5 kg/d concentrate</td>
<td>180</td>
<td></td>
<td></td>
<td>Godoy et al. [26]</td>
</tr>
<tr>
<td>Treatments without FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatments with FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh forage + 4 kg/d concentrate</td>
<td>300</td>
<td></td>
<td></td>
<td>Chicco et al. [27]</td>
</tr>
<tr>
<td>Treatments without FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatments with FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize silage</td>
<td>143</td>
<td></td>
<td></td>
<td>Calzadilla and Combellas [28]</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;: 2 kg/d maize meal (MM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;: T&lt;sub&gt;1&lt;/sub&gt; + 0.3 kg/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;: 1 kg/d MM + SBM + urea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;: T&lt;sub&gt;3&lt;/sub&gt; + 0.3 kg/d FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize silage + 1.3 kg/d concentrate</td>
<td>203</td>
<td></td>
<td></td>
<td>Letta and Combellas [29]</td>
</tr>
<tr>
<td>MM + urea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM + SBM</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MM + FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize silage + 1.5 kg/d concentrate</td>
<td>270</td>
<td></td>
<td></td>
<td>Rattia and Combellas [30]</td>
</tr>
<tr>
<td>MM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM with 10% FM</td>
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<td></td>
</tr>
<tr>
<td>MM with 20% FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE III. (cont.)

<table>
<thead>
<tr>
<th>Diet</th>
<th>LW (kg)</th>
<th>DM intake (kg/d)</th>
<th>LWG (kg/d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh forage + 2 kg/d concentrate</td>
<td>129</td>
<td>4.41</td>
<td>0.65</td>
<td>Melendez and</td>
</tr>
<tr>
<td>MM + urea</td>
<td></td>
<td></td>
<td></td>
<td>Combelllas</td>
</tr>
<tr>
<td>MM + FM</td>
<td></td>
<td>4.42</td>
<td>0.81</td>
<td>(unpublished)</td>
</tr>
<tr>
<td>MM + urea + FM</td>
<td></td>
<td>4.61</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Fresh forage + 1.5 kg/d concentrate</td>
<td>155</td>
<td>4.94</td>
<td>0.62</td>
<td>Reaño and</td>
</tr>
<tr>
<td>$T_1$: MM + SBM + urea</td>
<td></td>
<td></td>
<td></td>
<td>Combelllas</td>
</tr>
<tr>
<td>$T_2$: FM + brewer’s grains</td>
<td></td>
<td>5.05</td>
<td>0.67</td>
<td>(unpublished)</td>
</tr>
<tr>
<td>$T_3$: 50% $T_1$ + 50% $T_2$</td>
<td></td>
<td>4.90</td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

extensive systems used, and on reproduction is less abundant than for stall fed systems. The experiments reviewed have been grouped together as those involving growing animals and those involving lactating cows.

4.1. Bypass protein in the diets of growing cattle

Experiments with bypass protein to supplement cattle consuming a basal diet of forage can be divided into early work using sugar cane and more recent studies with harvested grasses, silages and pastures.

Sugar cane is a peculiar tropical grass that accumulates large amounts of sucrose in its stem, and its composition is different from others in that it is high in soluble sugars and low quality fibre and very low in nitrogen. However, the molar proportion of volatile fatty acids (VFAs) in the rumen of animals given sugar cane is similar to that for other grasses [17]. A series of experiments was carried out by Preston and co-workers in the Dominican Republic and Mexico in the 1970s to evaluate the effect of different supplements on live weight gain and rumen digestion [16, 18]. Large responses were obtained when derinded or chopped sugar cane and urea–molasses were supplemented with rice polishing [19, 20]. This feed leaves the rumen quickly and supplies bypass nutrients to the animal as protein, starch and lipids [21, 22]. The non-ammonia N reaching the duodenum also increased, indicating that microbial protein synthesis was stimulated.

However, Preston [18] pointed out that responses were not as high when bypass protein sources such as meat meal, meat and bone meal and fish meal were used alone as supplements compared with rice polishing or ground maize and a
<table>
<thead>
<tr>
<th>Diet</th>
<th>$T_{1/2}$ (h)</th>
<th>Silage or forage</th>
<th>FM</th>
<th>Ammonia N (mmol/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize silage + 30% concentrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize meal (MM) + urea</td>
<td>57</td>
<td>69</td>
<td></td>
<td>104</td>
<td>315</td>
</tr>
<tr>
<td>MM + soybean meal</td>
<td>64</td>
<td>75</td>
<td></td>
<td>163</td>
<td>200</td>
</tr>
<tr>
<td>MM + FM</td>
<td>61</td>
<td>82</td>
<td></td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Fresh forage + 25% concentrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM + urea</td>
<td>85</td>
<td>112</td>
<td></td>
<td>140</td>
<td>300</td>
</tr>
<tr>
<td>MM + FM</td>
<td>84</td>
<td>113</td>
<td></td>
<td>150</td>
<td>142</td>
</tr>
<tr>
<td>MM + urea + FM</td>
<td>84</td>
<td>113</td>
<td></td>
<td>172</td>
<td>240</td>
</tr>
</tbody>
</table>

Cabeza and Combells [31]

Marquez and Combells (unpublished)
source of protein. The author concluded from these results that gluconeogenic precursors are the most important supplements to these diets, followed by bypass protein. The short supply of gluconeogenic precursors was explained by the absence of starch, the low protein levels, the moderate molar proportions of propionic acid in the rumen and the low levels of this acid because of low turnover rates.

Most experiments with other grasses have been carried out with stall fed animals using fish meal (FM) as the bypass protein source (Table III). The use of FM in supplements had a large influence on intake and live weight gain (LWG). The effect was greater when the control diet was deficient in nitrogen [28, 30] because of the supply of this element to rumen microorganisms. However, with low nitrogen basal diets the offer of supplements containing FM once a day does not prevent the fall of ammonia nitrogen to 100 mg/L or less after a few hours (Table IV), levels which are below those required to optimize intake and digestibility. The observed effect of slow nitrogen release of FM in nylon bags probably quickly disappears because of the rapid transit of FM out of the rumen. The addition of urea to a supplement based on a maize meal by-product and FM did not increase animal LWG (Melendez and Combellas, Table III).

The FM also increased the intake of the basal diet in most trials (Table III) but without influencing the rate of dry matter (DM) digestion (Table IV). This could be explained by an improvement in the balance of nutrients to the animal through bypass protein or other nutrients, or by an increase in microbial growth, but these parameters were not evaluated in the trials.

The level of bypass protein required to optimize responses in LWG seems to be low. Veitia [23] did not observe increments over levels of 200 g/d of FM in animals of approximately 230 kg. In another trial with fresh forage as the basal diet (Reaño and Combellas, unpublished) the animal response to a protein concentrate based on FM and dehydrated brewer’s grains compared with a conventional concentrate based on degradable sources of protein was similar to that in other experiments where the bypass protein source was a minor part of the supplement (Table III).

Responses with other bypass sources have been smaller. Alvarez et al. [32] obtained no effect from substituting cottonseed meal for a maize meal by-product in the supplement of animals consuming fresh forage, and Godoy et al. [26] and Chicco et al. [27] found lower LWG with formaldehyde treated sesame meal than with FM. Limitations to the extensive use of FM in the region are its low availability and the competition from other species. Only Peru and Ecuador in tropical Latin America produce large amounts of FM [33]. Other sources produced in larger quantities in the area, such as oil meals, should receive more attention.

4.2. Bypass protein in the diets of lactating cows

Reviewed experiences with bypass protein in lactating cows are summarized in Table V. With the exception of the experiment by García et al. [40] with high
### TABLE V. EFFECT OF BYPASS PROTEIN ON MILK YIELD AND LIVE WEIGHT CHANGE (LWC) OF COWS

<table>
<thead>
<tr>
<th>Diet</th>
<th>Milk yield (kg/d)</th>
<th>LWC (kg/d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low yielding cows</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silage + grazing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 g/d fish meal (FM)</td>
<td>8.7</td>
<td>0.00</td>
<td>Salinas and Figueroa [34]</td>
</tr>
<tr>
<td>225 g/d FM</td>
<td>10.0</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>450 g/d FM</td>
<td>9.8</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Silage + grazing</td>
<td></td>
<td></td>
<td>Ugarte et al. [35]</td>
</tr>
<tr>
<td>Concentrate</td>
<td>9.1</td>
<td>-0.08</td>
<td>Delgado and Combellas [36]</td>
</tr>
<tr>
<td>Concentrate + FM</td>
<td>8.3</td>
<td>-0.38</td>
<td>García et al. [37]</td>
</tr>
<tr>
<td><strong>Maize silage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low concentrate + urea</td>
<td>7.0</td>
<td>-0.16</td>
<td></td>
</tr>
<tr>
<td>Low concentrate + FM</td>
<td>7.4</td>
<td>-0.11</td>
<td></td>
</tr>
<tr>
<td>High concentrate + urea</td>
<td>8.6</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>High concentrate + FM</td>
<td>9.7</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td><strong>Fresh forage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low concentrate</td>
<td>6.5</td>
<td>-0.28</td>
<td></td>
</tr>
<tr>
<td>Low concentrate + FM</td>
<td>6.8</td>
<td>-0.26</td>
<td></td>
</tr>
<tr>
<td>High concentrate</td>
<td>8.7</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>High concentrate + FM</td>
<td>8.8</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td><strong>Grazing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₁: Concentrate + canavalia</td>
<td>8.6</td>
<td></td>
<td>Castillo et al. [38]</td>
</tr>
<tr>
<td>T₂: T₁ + urea + formaldehyde</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₃: T₁ + FM</td>
<td>8.4</td>
<td></td>
<td>Pérez and Romero (unpublished)</td>
</tr>
<tr>
<td><strong>Fresh forage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate</td>
<td>9.4</td>
<td>-0.34</td>
<td></td>
</tr>
<tr>
<td>Concentrate + FM</td>
<td>10.1</td>
<td>-0.50</td>
<td></td>
</tr>
<tr>
<td><strong>High yielding cows</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay + silage + stubble</td>
<td></td>
<td></td>
<td>Salinas et al. [39]</td>
</tr>
<tr>
<td>T₁: 8 kg/d concentrate</td>
<td>20.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₂: 6 kg/d concentrate + soybean meal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₃: T₂ + formaldehyde + SBM</td>
<td>21.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**TABLE V. (cont.)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Milk yield (kg/d)</th>
<th>LWC (kg/d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grazing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_1$: Concentrate + 16% SBM</td>
<td>4245 kg/lactation</td>
<td></td>
<td>García et al. [40]</td>
</tr>
<tr>
<td>$T_2$: $T_1$ + 16% formaldehyde + SBM</td>
<td>4974 kg/lactation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_3$: $T_1$ + 8% formaldehyde + SBM</td>
<td>4609 kg/lactation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fibre concentrate (LFC)</td>
<td>16.0</td>
<td>0.36</td>
<td>Rodríguez and Randel [41]</td>
</tr>
<tr>
<td>LFC + protected protein (PP)</td>
<td>15.8</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>High fibre concentrate + PP</td>
<td>15.0</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE VI. EFFECT OF FISH MEAL (FM) ON MILK YIELD AND REPRODUCTION OF DUAL PURPOSE CATTLE DURING THE DRY SEASON**

<table>
<thead>
<tr>
<th>FM (kg/d)</th>
<th>Farm A</th>
<th>Farm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td></td>
<td>0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet</th>
<th>Farm A</th>
<th>Farm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet</td>
<td>Grazing corn and sorghum stubble</td>
<td>Grazing para and star grass</td>
</tr>
<tr>
<td>Concentrate (kg/d)</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Number of females</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Milk yield during first 90 d of lactation (kg/d)</td>
<td>5.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Intervals (d):</td>
<td>Parturition to first oestrus 68 72</td>
<td>Parturition to conception 84 92</td>
</tr>
<tr>
<td></td>
<td>Parturition to conception 84 90</td>
<td></td>
</tr>
</tbody>
</table>

Note: No significant differences were observed between any measurements ($P > 0.05$) (Martínez, unpublished).
yielding dairy cows, the effect of bypass protein on milk yield was very small or non-existent. These results contrast with the high responses obtained in other tropical regions when agricultural fibrous by-products were used [16], and could be explained by differences in quality between the fibrous diets concerned. Cultivated pastures or conserved forages are used in dual purpose systems and their quality in terms of digestibility and crude protein content is higher than that of native pastures or fibrous by-products. But tropical grasses have a low content of non-structural carbohydrates and their contribution to ruminant nutrient supply is of lesser significance than in the case of temperate grasses [42]. The supply of this fraction could also be low in tropical silages because of the low grain:fodder ratio and the low quality of the vegetative parts [43].

Milk production has a high requirement of glucose for lactose synthesis and for oxidation to synthesize body and milk fat, and there is usually an imbalance in the ratio of gluconeogenic to microbial protein in these animals [16]. This is particularly true when medium quality tropical grasses are used with the characteristics, indicated above, of low non-structural carbohydrates and a small proportion of propionate in the VFAs. A great proportion of protein requirements could be satisfied from microbial protein synthesis in low yielding milking cows [44]. The gluconeogenic precursors and not the amino acids are probably the limiting nutrients in these conditions, explaining the low responses observed in Table V.

The effect of bypass protein on reproduction has been less well studied and the two experiments reviewed (Table VI) show, as with milk yield, no response to the addition of these nutrients.

5. USE OF UREA–MOLASSES BLOCKS

The primary purpose of urea–molasses blocks is to provide ammonia for rumen microorganisms in small amounts throughout the day. This simple technique has been extended with success in other regions, but was introduced fairly recently at the farm level in tropical Latin America. There is, however, great interest in blocks and the Food and Agriculture Organization of the United Nations has received assistance requests in this field from 13 countries in the region [45]. The technique has not been properly evaluated in the region and may require some adaptation to our systems. During the last two meetings of the Latin American Association for Animal Production (1988, 1990) about 10% of the papers in ruminant nutrition were related to bypass protein, but no work was presented on blocks.

The use of blocks should be of major significance during the dry season when the quality of pastures decreases, and particularly in beef systems based on native pastures of low nitrogen content. Preliminary results of two trials carried out in Venezuela are presented in Table VII. The first one was carried out under conditions similar to the systems based on the native grasslands of Brazil, Colombia and
TABLE VII. EFFECT OF UREA-MOLASSES BLOCKS ON LIVE WEIGHT CHANGE (LWC) AND REPRODUCTION OF GRAZING BEEF CATTLE DURING THE DRY SEASON

<table>
<thead>
<tr>
<th>Blocks with:</th>
<th>Without blocks</th>
<th>Sorghum</th>
<th>Fish meal</th>
<th>Cottonseed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mata and Combellas, unpublished data)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>LWC (kg/d):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season (Feb.–May)</td>
<td>-0.26</td>
<td>-0.25</td>
<td>-0.14</td>
<td>-0.10</td>
</tr>
<tr>
<td>Start of rains (Jun.)</td>
<td>0.73</td>
<td>0.76</td>
<td>0.86</td>
<td>0.92</td>
</tr>
<tr>
<td>Block consumption (g/d)</td>
<td>—</td>
<td>296</td>
<td>301</td>
<td>183</td>
</tr>
<tr>
<td>Pregnant + non-pregnant suckling animals (%):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan. 1990</td>
<td>52</td>
<td>52</td>
<td>52</td>
<td>44</td>
</tr>
<tr>
<td>Jan. 1991</td>
<td>47.8</td>
<td>47.8</td>
<td>43.5</td>
<td>73.9</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lupi, López and Martínez, unpublished data)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td>18</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LWC, Feb.–Jun. (kg/d)</td>
<td>0.14</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals pregnant during breeding season, Apr.–Jul. (%):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows</td>
<td>75.0</td>
<td>90.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heifers</td>
<td>25.0</td>
<td>83.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Venezuela, with a low stocking rate (8 ha/animal). The herbage on offer was 740 kg DM/ha with 5.4% crude protein in the dry matter of the green material. The main difference from published evidence obtained with stalled animals [45] is the low consumption of the blocks, and this was related to the sporadic visits, mainly in the evenings, to the feeding troughs. The infrequent intake of blocks may lead to large fluctuations in rumen ammonia [16], and so a source of slow degradable nitrogen release should be sought.

The best response in this trial was obtained with cottonseed in spite of the low consumption of these blocks. This feed degrades slowly in the rumen of cattle, continuously supplying nitrogen to the microbes, and also has a high content of lipids. The use of a protein source of low degradability such as FM fed once a day did not contribute to an increase in the rumen ammonia levels throughout the day (Table IV), probably because of the speed with which FM leaves the rumen.

In the second trial the offer of blocks per animal was restricted to 300 g/d for a group of Brahman females grazing molasses grass at a higher stocking rate (3 ha/animal) in a hill area. There was also a positive effect of blocks on reproduction but no influence on live weight change was observed.

More research is required to adapt urea–molasses blocks to extensive grazing systems and fulfil the main objective of providing degradable nitrogen continuously to the rumen microbes, even where consumption of the supplement is sporadic.

REFERENCES


[31] CABEZA, G., COMBELLAS, J., “Influencia de la urea, la harina de soya y la harina de pescado sobre las características de la fermentación ruminal de bovinos consumiendo ensilaje de maíz”, ibid., pp. 69–70.


THE IMPORTANCE OF UREA-MOLASSES BLOCKS AND BYPASS PROTEIN IN ANIMAL PRODUCTION: THE SITUATION IN PAKISTAN

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* NWFP Agricultural University, Peshawar

** Buner Development Project, Daggar Pakistan

Abstract

THE IMPORTANCE OF UREA-MOLASSES BLOCKS AND BYPASS PROTEIN IN ANIMAL PRODUCTION: THE SITUATION IN PAKISTAN.

The potential of introducing urea-molasses blocks as a dry season supplementary strategy for domestic ruminants was investigated in several on-farm and on-station experiments. These studies demonstrated the obvious advantage of using the blocks as a farm package to increase milk production in dairy cows and buffaloes and growth rates in calves and lambs under village conditions in Pakistan. Positive responses in productivity to block feeding were attributed to improved rumen efficiency caused by a sustained supply of critical microbial nutrients from continuous licking of the blocks. The studies also confirmed the economic benefits of using the blocks as a complement to conventional concentrates in lactating and growing cows and buffaloes. The need for feeding bypass protein for further increasing animal productivity was also emphasized. The block package was readily accepted by local farmers and was found easy to introduce into the existing on-farm feeding practices in Pakistan.

1. INTRODUCTION

Crop residues and grazing constitute more than 70% of the feed resources available for ruminant livestock in Pakistan. The major crop residues are cereal straws and stovers which serve as the main feed for cattle, buffaloes and sheep during dry periods. Unimproved ranges in Pakistan have a low carrying capacity and their vegetative growth is stopped for a period of two to three months during both winter and summer seasons, consisting typically of overgrazed range stubble.
Losses in animal productivity during the dry seasons are commonly observed and are mainly due to inadequacy of quality feeds. The major limitation of crop residues and mature grasses as animal feed is that they are deficient and imbalanced in essential nutrients and thus unable to provide for an efficient rumen, leading to inefficient utilization of the nutrients absorbed [1]. Therefore, the primary aim in evolving a ruminant feeding system based on low quality roughages should be to create optimum conditions in the rumen for maximizing fibre digestion through the supply of deficient nutrients such as fermentable nitrogen, minerals and other microbial nutrients. While the required amounts and sources of such nutrients are well known, the importance of presenting these in a package that can be readily adopted by small farmers in developing countries is not equally recognized. For example, there is a natural reluctance among uneducated small farmers to feed urea to their animals because of the risk of poisoning. Similarly, feeding of liquid molasses, although cheap, has not become popular among the small farmers because of difficulties in transportation, storage and feeding.

Experience in other countries [2, 3] has shown that a solidified urea–molasses block lick which provides critical microbial nutrients, namely nitrogen as urea, readily available energy as molasses and minerals, appears practicable and can be easily introduced into the existing on-farm practices. It has been widely advocated that, after correcting the rumen environment to maximize nutrient output, an additional feed of bypass protein be given to ruminants to further increase the productivity [1].

The aim of the work presented in this paper was to investigate the potential of feeding urea–molasses blocks as a dry season supplementary strategy under the existing feeding system of smallholdings in Pakistan.

2. PREPARATION OF UREA–MOLASSES BLOCKS

The blocks were prepared with the hot method and contained 46% molasses, 10% urea, 1% MgO, 10% Ca(OH)₂, 5% NaCl, 5% mineral mixture and 23% wheat bran. Molasses was heated at 90°C for 20 min, cooled to 60°C and then the urea and other ingredients were added. After thorough mixing, the contents were poured into moulds and solidified in 24 h. Addition of 10% cement or bentonite in place of 1% MgO did not help in the hardening of the blocks.

3. CONSUMPTION OF BLOCKS BY ANIMALS

Large variations in block intake were found among the animals. Adult cattle, buffaloes and sheep on average consumed 400, 500 and 100 g of block per day respectively and generally required an adaptation period of two to three weeks. The highest block intake of 1200 g/d was recorded in buffalo steers weighing 450 kg.
Soft blocks encourage chewing by the animals and may increase the risk of urea poisoning, whereas blocks which are too hard decrease the rate of intake by the animals. It was often found that the urea level in the block was negatively related to block intake. In sheep on a straw based diet, block intake decreased from 133 g/d with 2% urea in the blocks to 127, 118 and 75 g/d when blocks contained 10, 15 and 20% urea respectively. However, in adult buffaloes and cattle, block intake decreased only with urea contents of 15% and higher. Supplementary feeding of cottonseed meal (100 g/d) in sheep did not affect block intake up to a content of 15% urea in the blocks but the intake decreased by 16 g/d when the urea content increased to 20%. When goats, grazing range stubble during the dry season, were offered the blocks for the first time, they tended to consume large quantities of the blocks in a short time, presumably owing to pica resulting from mineral deficiency. After controlled feeding for two weeks, the block intake settled to 100 g/d.

4. EFFECTS OF BLOCK FEEDING ON ROUGHAGE INTAKE

A significantly higher intake of cereal straw in response to block feeding was consistently observed in all domestic ruminants. The increase in straw intake varied from 30% in adult buffaloes to 23% in growing calves. In calves given a basal diet of wheat straw ad libitum plus 300 g/d of wheat bran, the positive effect of block feeding on straw intake was more pronounced after four weeks of feeding (Table I).

**TABLE I. EFFECT OF UREA-MOLASSES BLOCKS ON DAILY INTAKE OF WHEAT STRAW IN CATTLE AND BUFFALOES**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Body weight (kg)</th>
<th>Wheat straw intake (kg/d)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>- Block</td>
<td>+ Block</td>
</tr>
<tr>
<td>Buffalo steers</td>
<td>280</td>
<td>5.33</td>
<td>6.93</td>
</tr>
<tr>
<td>Buffalo calves:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 1 to 4</td>
<td>167</td>
<td>2.57</td>
<td>2.83</td>
</tr>
<tr>
<td>Weeks 5 to 10</td>
<td></td>
<td>2.60</td>
<td>3.01</td>
</tr>
<tr>
<td>Cow calves:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 1 to 4</td>
<td>130</td>
<td>2.52</td>
<td>2.53</td>
</tr>
<tr>
<td>Weeks 5 to 10</td>
<td></td>
<td>2.55</td>
<td>3.00</td>
</tr>
</tbody>
</table>
TABLE II. EFFECT OF UREA CONTENT ON INTAKE OF BLOCK AND WHEAT STRAW IN SHEEP FED A BASAL DIET OF WHEAT STRAW AD LIBUTUM WITH OR WITHOUT A 100 g/d SUPPLEMENT OF COTTONSEED MEAL

<table>
<thead>
<tr>
<th>Urea content in block (%)</th>
<th>2</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block intake (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CSM</td>
<td>131</td>
<td>123</td>
<td>114</td>
<td>82</td>
</tr>
<tr>
<td>+ CSM</td>
<td>135</td>
<td>131</td>
<td>122</td>
<td>66</td>
</tr>
<tr>
<td>Wheat straw intake (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CSM</td>
<td>649</td>
<td>777</td>
<td>725</td>
<td>681</td>
</tr>
<tr>
<td>+ CSM</td>
<td>729</td>
<td>703</td>
<td>648</td>
<td>567</td>
</tr>
</tbody>
</table>

High urea content in blocks (20%) appeared to depress the daily intake of wheat straw in sheep. This was more marked when the animals received an additional 100 g/d of protein rich concentrate (Table II). Apart from the bitter taste of urea, the resulting high concentrations of ammonia N in the rumen fluid, which exceeded 250 mg/L, may have evoked a negative feedback on feed intake.

The effect of block feeding on the intake of basal diet was also studied in dairy buffaloes of five smallholdings under village conditions. With a basal diet of rice straw plus a small quantity of oilcake (0.7-1 kg/d), block lick increased the straw intake by 26%. Generally, protein in oilcake is less degradable, and therefore when given in small amounts may not satisfy the ammonia requirements of rumen microbes. Under these conditions, block lick may prove instrumental in stimulating fermentative digestion by increasing ammonia concentrations in the rumen.

5. EFFECTS OF BLOCK FEEDING ON RUMEN PARAMETERS

The in sacco dry matter (DM) digestibility of wheat straw in buffalo steers increased from 22.05 to 28.20% after 12 h and from 34.95 to 39.20% after 24 h of feeding/incubation in response to block feeding (Table III). This was apparently caused by improved ammonia concentrations in the rumen fluid of the steers.

Ammonia concentrations in the rumen fluid of control steers were below the optimum level of 50 mg N/L [4], which presumably impared microbial digestion in the rumen. Frequent licking of blocks by the animals not only increased ammonia
### TABLE III. EFFECTS OF UREA–MOLASSES BLOCKS ON IN SACCO DIGESTIBILITY OF WHEAT STRAW AND AMMONIA CONCENTRATIONS IN THE RUMEN FLUID OF BUFFALO STEERS FED A BASAL DIET OF WHEAT STRAW AND MINERAL SUPPLEMENT

<table>
<thead>
<tr>
<th>Diet</th>
<th>Time after feeding/incubation (h)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>In sacco DM digestibility of wheat straw (%)</td>
<td>Control</td>
<td>22.05</td>
<td>34.95</td>
</tr>
<tr>
<td></td>
<td>+ Block</td>
<td>28.20</td>
<td>39.24</td>
</tr>
<tr>
<td>Ammonia concentration (mg N/L rumen fluid)</td>
<td>Control</td>
<td>15.5</td>
<td>24.20</td>
</tr>
<tr>
<td></td>
<td>+ Block</td>
<td>50.00</td>
<td>57.00</td>
</tr>
</tbody>
</table>

**Note:** The differences between the control and “+ block” diets for each measurement are significant ($P < 0.05$).

### TABLE IV. MEAN AMMONIA CONCENTRATIONS AND IN SACCO DIGESTIBILITY OF WHEAT STRAW AT 24 h IN THE RUMEN OF SHEEP FED UREA–MOLASSES BLOCKS CONTAINING DIFFERENT LEVELS OF UREA WITH OR WITHOUT ADDITIONAL PROTEIN SUPPLEMENT (COTTONSEED MEAL)

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Urea content in block (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>In sacco DM digestibility of wheat straw (%)</td>
<td>Nil</td>
<td>31.25</td>
<td>35.88</td>
<td>36.10</td>
<td>34.83</td>
</tr>
<tr>
<td></td>
<td>+ CSM</td>
<td>35.08</td>
<td>35.82</td>
<td>34.73</td>
<td>35.61</td>
</tr>
<tr>
<td>Ammonia concentration^a (mg N/L rumen fluid)</td>
<td>Nil</td>
<td>53.45</td>
<td>162.50</td>
<td>206.31</td>
<td>226.38</td>
</tr>
<tr>
<td></td>
<td>+ CSM</td>
<td>101.29</td>
<td>176.79</td>
<td>301.37</td>
<td>225.83</td>
</tr>
</tbody>
</table>

^a Mean of ammonia concentrations at 2, 4, 8, 12 and 24 h after feeding.
concentrations in the rumen but also ensured optimum concentrations throughout the 24 h period. Synchronizing ammonia availability with a slow rate of straw digestion in the rumen is important for maximizing microbial digestion of fibre [5].

The effect of including different levels of urea in the block, with or without additional feeding of protein supplements, on wheat straw digestion and ammonia concentration in the rumen of sheep is given in Table IV. The results suggest that additional feeding of protein meal with blocks containing 10% urea or more may not be effective in stimulating straw digestion. The data also suggest that a rumen ammonia concentration above 100 mg N/L may be required to maximize straw digestion in the case of straw based diets, and show that ammonia levels higher than 162 mg N/L failed to increase the digestibility further. However, it is interesting to note that while straw digestibility levelled off with rumen ammonia levels higher than 162 mg N/L, the daily intake of straw decreased with the same high level of ammonia in the rumen fluid (Table II). These observations suggest that 10% urea in the blocks may be the optimum level for maximizing both digestibility and intake of straw in sheep fed a basal diet of cereal straw or other low quality roughage.

The population density of protozoa and anaerobic fungi in the rumen of sheep tended to decline with increasing urea content in the blocks but the difference was not statistically significant. The average numbers of protozoa 4 h after feeding and fungal sporangia counted on straw blades incubated in the rumen for 24 h were $(15.8 \pm 0.80) \times 10^4$/mL rumen fluid and $(7.48 \pm 1.4) \times 10^3$/cm$^2$ respectively.

6. MILK YIELD RESPONSE TO BLOCK FEEDING

The effect of introducing urea–molasses blocks on milk yield in dairy buffaloes and cows was evaluated under village conditions during the season of fodder scarcity. Sixteen small farms in different villages, each having two buffaloes or cows at about the same stage of lactation, were selected. The conventional feeding of the animals at all the holdings was almost the same and consisted of a basal diet of wheat straw and/or maize stovers given ad libitum plus an additional small amount (0.2–0.5 kg/d) of concentrate (oilcake and wheat bran in a 50:50 ratio). To one of the two buffaloes or cows at each farm, a urea–molasses block (10% urea) was made available in the feed trough all the time. Daily milk yield was recorded for 90 d and the data recordings were checked for accuracy by visiting the farms twice a week during milking time.

All the animals got used to licking the blocks after an initial period of one to two weeks. Cows appeared to have a better taste for the blocks than buffaloes, consuming on average 450 g of block per day compared with 320 g for buffaloes. Block feeding increased ($P < 0.001$) the daily milk yield in both species consistently over
FIG. 1. Effect of urea–molasses block lick on milk yield in dairy buffaloes and cows fed a basal diet of low quality roughage and restricted amounts of concentrate.

the 90 d period (Fig. 1). However, the effect was greater in cows than buffaloes (42% vs. 22%). The average daily milk yield increased from 3.8 to 5.4 L in cows and from 3.7 to 4.5 L in buffaloes.

The difference in the productive responses of the two species may, in part, be attributed to the higher maintenance requirements of buffaloes. The partitioning of absorbed nutrients towards maintenance appears to be of prime importance when the animals are not adequately fed, and under conditions of limited feed availability dairy animals of smaller body size appear to be more suitable.

The low amount of block consumed by the buffaloes may have also affected the milk response in comparison with the cows. However, when consumed together with a small allowance of concentrates, the low intake of urea from the block may not have seriously limited digestibility through lack of ammonia in the rumen because buffaloes have a highly efficient system of urea recycling to the rumen [6].

In another study at a different location (rice growing area), milk yield response to block feeding in 12 buffaloes was investigated in five smallholdings. Three successive periods of 7 d pre-treatment, 28 d during treatment and 14 d post-treatment were used for recording daily milk production in each buffalo. Urea–molasses blocks (10% urea) were provided to the animals as a free choice lick during the treatment period. All the buffaloes received a basal diet of rice straw ad libitum, 3–5 kg/d of
TABLE V. EFFECTS OF UREA-MOLASSES BLOCK LICK ON PERFORMANCE OF GROWING ANIMALS FED LOW QUALITY BASAL DIETS

<table>
<thead>
<tr>
<th>Animals</th>
<th>Diet</th>
<th>Block intake (g/d)</th>
<th>Change in body weight (g/d)</th>
<th>Feed conversion efficiency (gain/feed) (%)</th>
<th>Feed cost per kg gain (PRs)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Block</td>
<td>+ Block</td>
<td>- Block</td>
</tr>
<tr>
<td>Buffalo calves</td>
<td>Wheat straw + 300 g/d</td>
<td>292</td>
<td>+32</td>
<td>+187</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>wheat bran</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow calves</td>
<td>Wheat straw + 300 g/d</td>
<td>196</td>
<td>+137</td>
<td>+307</td>
<td>4.74</td>
</tr>
<tr>
<td></td>
<td>wheat bran</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambs</td>
<td>Dry range grazing</td>
<td>38</td>
<td>-29</td>
<td>+133</td>
<td>-</td>
</tr>
<tr>
<td>Lambs</td>
<td>Dry range grazing + block</td>
<td>200</td>
<td>-11</td>
<td>+38</td>
<td>-</td>
</tr>
<tr>
<td>Lambs</td>
<td>Dry range grazing + concentrate</td>
<td>-</td>
<td>+66</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> 1 US $ = 21 PRs.

<sup>b</sup> Additional cost of block feeding for 1 kg gain.

<sup>c</sup> Additional cost of concentrate feeding for 1 kg gain.
maize stovers and 0.7–1 kg/d of conventional concentrates. The average intake of
block was 320 g/d, which was higher than recorded in the previous study. Block
feeding increased ($P < 0.01$) the average daily milk production from 5.3 to 6.5 L
(+22.6%). Interestingly, the response in milk yield to block feeding noted here was
the same as recorded in the first experiment. Similarly, an increase of 15% in con-
sumption of rice straw ($P < 0.05$) was observed in response to block feeding. The
positive effect of blocks on milk production in buffaloes and cows confirms the find-
ings obtained from a study carried out in Indian villages [3].

With the additional daily cost of 1.35 Pakistan rupees (PRs)$^1$ incurred by the
feeding of 450 g of block per cow, the net return from sale of milk amounted to
11.2 PRs. Similarly, in buffaloes the mean calculated return amounted to 7.0 PRs,
compared with 0.83 PRs spent on daily feeding of blocks. Moreover, the additional
amount of milk that the suckling calves would receive from the block fed dams also
indirectly contributes to the overall economic benefits of block feeding.

7. GROWTH RESPONSE TO BLOCK FEEDING

During an 80 d feeding trial, each of six buffalo calves and six cow calves (one
year of age) was fed a basal diet of wheat straw ad libitum plus 300 g/d of wheat
bran. The calves were divided into two equal groups on the basis of live weight and
sex and one of the groups was given free access to urea–molasses block lick. All the
animals were fed individually.

The daily intake of block averaged 292 and 196 g in buffalo calves and cow
calves respectively. The block fed animals consumed more straw (Table I) and
gained in body weight faster than the respective control groups (Table V).

The control buffalo calves gained 32 g/d and this was increased to 187 g/d
($P < 0.001$) when the animals received urea–molasses blocks. Similarly, block feed-
ing increased ($P < 0.001$) body weight gain in cow calves from 137 to 307 g/d.
Although the net daily gain, due to block feeding in the two species of calves, was
roughly the same, i.e. 155 and 170 g in buffalo and cow calves respectively, the feed
conversion efficiency (gain/feed consumed) was significantly higher ($P < 0.05$) in
cow calves than buffalo calves (9.48% vs. 5.42%). Correspondingly, the cost of feed
to produce a 1 kg body weight gain in the calves was markedly reduced by block
feeding (Table V).

Positive responses of growth rate to block lick for the straw based diets
can be logically attributed to efficient rumen fermentation providing a better balance
of nutrients to the animals for absorption. Samples of rumen fluid, collected 3 h
after feeding from the same calves, showed that the ammonia concentration of

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$^1$ 1 US $ = 21$ PRs.
FIG. 2. Mean protein degradability rate of cottonseed cake (CSC), maize oil cake (MOC), corn gluten feed (CGF), mustard seed cake (MSC) and wheat bran (WB) in the rumen of buffalo steers, cattle steers and sheep measured with the nylon bag technique.

39.23 mg N/L in the rumen fluid of control calves was suboptimal compared with 93.31 mg N/L in the rumen fluid of block fed calves. Wheat bran (2.1% N) is highly degradable in the rumen (Fig. 2). Therefore, ammonia released from digestion of wheat bran in the rumen would peak soon after feeding, leaving little ammonia for microbial activity during the later part of the day. Provision of the blocks, which were always available to the animals and which they licked frequently, supplied small doses of urea for hydrolysis in the rumen throughout the day. This helped in complementing the beneficial effects of wheat bran on microbial growth.

Studies with sheep grazing range stubble during the winter dry season and not given any supplement demonstrated that block feeding was highly effective in improving the growth rate of the yearlings. Two neighbouring flocks each having 70–90 sheep and grazing the same range were used for comparison. All the sheep in one flock were provided with urea–molasses block (10% urea) during the night time. From each flock, six yearlings were randomly selected, ear-tagged and weighed regularly at fortnightly intervals over a period of 40 d. The average intake of block per animal was 38 g/d. Lambs in the control flock lost body weight at the rate of 29 g/d while the block fed lambs gained 113 g/d. This increase incurred a marginal cost of 0.80 PRs/kg body weight gain. Early findings [7] with lambs
grazing identical range in the same geographic zone showed similar positive responses to blocks, though less pronounced than in the study described here (Table V), probably owing to concentrate feeding of the lambs at the Government farm before the experiment. In the same study, comparison of conventional concentrates with block feeding showed the economic benefits of using blocks over concentrates on dry range (7.99 vs. 10.85 PRs/kg gain).

These results clearly demonstrate that block feeding is a useful economic strategy in overcoming dry season losses in grazing lambs. This appears to have a large impact on the lifelong performance of the animals. The lambs showing faster growth on a block lick supplement will reach maturity earlier, and with healthy breeding the overall life productivity of the animals should be greatly enhanced.

8. NEED FOR SUPPLEMENTARY FEEDING OF BYPASS PROTEIN WITH BLOCKS

Proteins that are extensively fermented in the rumen may be of little value when fed along with urea–molasses blocks. Instead, bypass protein is required with blocks to complement the nutrients available from the end products of rumen digestion. Evidence for the positive effects of such a balanced approach on animal performance was provided earlier by Australian workers [8], who found that the growth rate in lambs receiving a block lick supplement to a basal diet of wheat straw further increased from 10 to 90 g/d with additional feeding of 100 g of cottonseed meal, while lambs receiving the same supplement without the blocks were growing at only 38 g/d. Similarly, body weight loss of 320 g/d was changed into a gain of 207 g/d when an additional 500 g/d of bypass protein was fed along with urea supplemented low quality pasture hay to growing cattle [9].

The bypass quality of concentrate feeds available in Pakistan was not known until recently. Rumen degradability studies in this laboratory ranked some of the most common protein concentrates as shown in Fig. 2. Not only were there great differences among the feeds ($P < 0.001$), but cattle appeared to degrade feed protein to a greater extent than buffaloes and sheep on the same diets ($P < 0.05$). The comparison suggests that cottonseed cake, maize oil cake and corn gluten feed, owing to their low protein degradability, would be the most appropriate protein supplements for feeding along with urea–molasses blocks to lactating and growing ruminants in Pakistan.

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THE IMPORTANCE OF UREA-MOLASSES BLOCKS AND BYPASS PROTEIN IN ANIMAL PRODUCTION: THE SITUATION IN BANGLADESH

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Abstract

THE IMPORTANCE OF UREA-MOLASSES BLOCKS AND BYPASS PROTEIN IN ANIMAL PRODUCTION: THE SITUATION IN BANGLADESH.

Molasses remains underutilized as an animal feed in Bangladesh. The total production of molasses in 1980 was about $69.8 \times 10^3$ t. Molasses is mainly utilized by distilleries, in tobacco smoking and for export. Very little is used as livestock feed. Urea and molasses are used for supplementation of fibrous feeds in different ways in many countries. Used in sprayed form on straw or in liquid form, urea–molasses was found to cause practical problems in village farming conditions owing to urea toxicity arising from higher intake by livestock, as well as difficulties in handling, storage and transport to remote villages. To overcome these problems and to produce a supply of easily available energy and nitrogen to ruminants, a simple method has been developed for making urea–molasses blocks with locally available ingredients and equipment at the farm level in the villages. The procedure is being extended to the on-farm level and appears to be easy for the farmers to follow. To evaluate the nutritional potential of urea–molasses blocks fed to livestock on a straw based diet a study has been conducted on the performance of livestock under on-farm conditions. Results from experiments on the nutritional value of the block are reported in the paper. It appears that daily live weight gain, milk yield and draught output of cattle increased significantly. A similar positive response in the performance of sheep and goats was also observed. Once the supply of rumen fermentable nitrogen and energy is ensured to optimize the rate of degradation of fibrous substrate with supplementation of urea–molasses block lick, the next limitation to production is considered to be the availability of amino acids absorbed from the intestine. To overcome this limitation protein with a low rate of degradation, such as fish meal, could be used as bypass protein. Large amounts of fish trash or non-consumable fish species (25 000 t/year) and fish meal from fish plants (650 t/year) are available in Bangladesh. A small quantity is used for poultry feeding. Several experiments were conducted on the effect of supplementation of bypass protein (such as fish meal) on the performance of cattle, sheep and goats in both on-station and on-farm conditions. Results indicated a positive response in daily live weight gain, milk yield and draught power output.
1. INTRODUCTION

Livestock are an essential component of farming systems in Bangladesh, supplying the major part of the draught power requirement for land preparation, transport, threshing and crushing of sugar cane, oil seeds, etc. In addition, they provide meat and milk for human consumption and hide, skin, bone and horn as raw materials for industry and export. Animal manure is used for domestic fuel as well as fertilizer in crop production. Almost all animals are kept on small farms (less than 0.8 ha, with 50% having less than 0.4 ha [1]). The farmers are involved in crop production, with a pair of bullocks or cows as draught animals for cultivation needs. The animals may also be hired out to neighbours. There may also be one or two milking cows, goats or sheep for milk and eventually for meat. A few chickens and ducks are kept for meat and eggs. The livestock population was estimated in 1984 to be about 22 million cattle, 0.5 million buffaloes, 10.5 million goats and sheep and 90 million poultry [1]. Annually, these animals provide $2.3 \times 10^6$ hp (~1.7 GW) of draught power, $0.29 \times 10^6$ t of meat, $1 \times 10^6$ t of milk and $10 \times 10^6$ hides and skins [2].

2. FARMING SYSTEMS

2.1. Types of farm

The types of farm and their surplus, self-sufficiency or deficit in terms of cereal grain produced are shown in Table I. The table shows that 82.5% of all farms (households) are livestock–poultry–crop farms and only 3.5% are involved solely with crop farming. Moreover, there are farms with only livestock and poultry (4%), i.e. without crop enterprises [3].

2.2. Distribution of land

It is reported that 33% of the population own no land, although most of them own some type of domestic animal. The distribution of land ownership is as follows: 29% of households own 10% and 28% own 40% of the total cultivable land and the remaining 10% of the households own 50% of the land [4]. The total cultivated area, including fallow land, is about 22.39 million acres ($9.05 \times 10^6$ ha), corresponding to 147% cropping intensity.

2.3. Feed resources

As in many countries of the world, pressure on available land in Bangladesh is increasing day by day. It has been reported that 77% of the cultivable land is used
TABLE I. TYPES OF FARM AND THEIR SURPLUS, SELF-SUFFICIENCY OR DEFICIT IN TERMS OF CEREAL GRAIN PRODUCED

<table>
<thead>
<tr>
<th>% of households</th>
<th>Surplus</th>
<th>Self-sufficiency</th>
<th>Deficit</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livestock-poultry-crop farms</td>
<td>17.0</td>
<td>15.5</td>
<td>50.0</td>
<td>82.5</td>
</tr>
<tr>
<td>Crop farms</td>
<td>1.0</td>
<td>—</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Fish farms</td>
<td>0.5</td>
<td>2.5</td>
<td>4.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Livestock and poultry farms</td>
<td>—</td>
<td>0.5</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>No enterprise or farm</td>
<td>—</td>
<td>—</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Total</td>
<td>18.5</td>
<td>18.5</td>
<td>63.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

For rice production and the rest for jute, sugar cane, pulses and tea. Nothing is available exclusively for the production of animal feeds [5]. Feed resources for ruminants are mainly derived from crop residues and a small quantity of cereal by-products. Straw is by far the most important crop residue, contributing more than 90% of the total dry matter available to ruminants.

2.4. Limitations on available feed for animal production

Considering the inherent low nutritional value of straw and its availability during the year for animal feeding, it is very difficult to formulate a ration using conventional feeding standards. The strategy, therefore, is to properly manage the limited amounts of straw, supplements from cereal by-products and agroindustrial by-products (i.e. molasses, urea and marine by-products such as fish meal) to ensure the most efficient utilization of the basic feed (crop residues, green grasses, tree leaves, etc.).

Given these constraints it would be possible to increase the availability of feeds and fodder by:

- Increasing crop residue production by increasing cropping intensity;
- Utilizing non-conventional feed resources, e.g. tree leaves, molasses and urea;
- Increasing the availability of arable or non-arable land for feed and fodder production;
- Processing basic feedstuffs, i.e. crop residues (physical, chemical or biological treatment);
- Supplementing the basic feedstuffs.
3. SCIENTIFIC PRINCIPLES FOR DEVELOPING FEEDING SYSTEMS WITH CROP RESIDUES

3.1. Imbalance of nutrients

The primary limitation to ruminant productivity is the imbalance of nutrients in the available feeds, which are of low digestibility and low availability. The principles for developing feeding systems using local feed resources are many; however, one should give emphasis to the following points in any developing country [6]:

— Identify the most abundant and inexpensive carbohydrate;
— Create the optimum conditions for fermentation of this carbohydrate in the rumen (fermentable nitrogen and other nutrients);
— Supplement with bypass nutrients in order to balance the products of digestion according to the productive state of the animal (milk yield, draught output, production of wool, meat, etc.).

3.2. Animal requirements

Microbial fermentation allows the digestion of cellulose and hemicellulose from crop residues, thereby releasing energy substrates and building microbial protein. In order to promote a vigorous microbial population, sufficient energy, nitrogen, sulphur, etc., must be available, especially in the case of crop residues. Lack of protein is often the most important limiting factor in many different types of straw. For the efficient breakdown of lignocellulose by microbes, a nitrogen content of 1–1.5% is considered to be essential [7]. An inadequate supply of nitrogen will retard microbial growth and thereby impede the degradation of the cell wall components. It is an established fact that utilization of ammonia by microorganisms in the rumen depends on the level of nitrogen and type of carbohydrate/energy available in the diet [8]. Furthermore, it is wasteful to ferment high quality protein and convert it into microbial protein. Additional animal production can be obtained from protein supplement which reaches the abomasum intact. This can be achieved by feeding protein with a low rate of fermentation to the rumen or by activating the oesophageal groove reflex so that the protein bypasses the rumen and goes directly into the lower gut for absorption [9].

4. UREA-MOLASSES AS FEED FOR RUMINANTS

Supplementation with non-protein compounds like urea would offset the shortage of protein and increase the efficiency of utilization of crop residues. When urea is incorporated in ruminant rations, the supply of readily available energy substrate in the rumen becomes imperative. Therefore molasses, a low cost energy source,
becomes a necessary component of feeds containing urea. Molasses remains under-utilized as an animal feed in Bangladesh. In 1980, molasses production totalled about \(69.8 \times 10^3\) t. Molasses is mainly utilized by distilleries, in tobacco smoking and for export. Very little is used as livestock feed [10]. The advantages of strategic supplementation of poor quality roughages with urea and molasses to increase energy and nitrogen content have long been realized. Urea and molasses have been used for supplementation of poor quality roughages in many different ways, e.g. by spraying them on the feed or providing them in liquid form [11–13]. Using urea–molasses mixtures, either by spraying or as a liquid lick, has been found to have several drawbacks in on-farm conditions in a developing country like Bangladesh because of the possible high intake by the animals, resulting in urea toxicity, as well as problems of handling, storage and transport to remote villages. To overcome these problems, manufacture of solid urea–molasses blocks has been described by a number of research workers [14–16]. In the making of blocks, different ingredients have been used, depending on their availability under local conditions [16–18]. Differences have been observed mostly in the use of binding agents, such as lime powder, cement, kaolin and bentonite, and in whether or not heat is applied.

4.1. The technique of making urea–molasses blocks in Bangladesh

The composition of the urea–molasses block is shown in Table II [17]. The process of manufacture involves heating of molasses to 70°C for 15–30 min,
depending on the moisture content (thickness), in a metal pot or half of an oil drum. The other ingredients (urea, lime powder, wheat bran and a mineral and vitamin mix) are thoroughly mixed and added to the heated molasses in the metal pot; the contents of the pot are then mixed thoroughly without any more heating. The thick urea-molasses mixture is poured into a mould (usually 22 cm × 11 cm × 10 cm). The cover is placed over the mould and pressure applied. This gives a brick shaped block weighing approximately 2 kg. After pressing, the soft block is removed from the mould and left for about one hour to harden. If the block is not going to be fed to livestock immediately it may be wrapped with polyethylene sheet for storage or transport. The chemical composition of this type of block is shown in Table III.

4.2. Production responses to feeding urea–molasses blocks

Feeding experiments with urea–molasses blocks as a supplement to straw based diets for cattle, goats and sheep were conducted on-station as well as on-farm to investigate the nutritional potential of the blocks. It was observed that supplementation of the urea–molasses block could increase feed intake, daily milk yield and live weight gain, as well as draught output in draught animals, and lengthen the lactation period [15, 16, 19–21]. It was concluded from on-station experiments with lactating draught cows that urea–molasses blocks can effectively be used with a basal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated straw</th>
<th>Ammoniated straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- UMB + UMB</td>
<td>- UMB + UMB</td>
</tr>
<tr>
<td>Dry matter intake (% of live weight)</td>
<td>2.8 3.0</td>
<td>3.4 3.6</td>
</tr>
<tr>
<td>Daily milk yield (g)</td>
<td>452 460</td>
<td>515 570</td>
</tr>
<tr>
<td>Lactation period (d)</td>
<td>220 235</td>
<td>246 255</td>
</tr>
<tr>
<td>Time of sign of first heat after calving (d)</td>
<td>210 210</td>
<td>205 195</td>
</tr>
<tr>
<td>Daily live weight gain from calving to</td>
<td>20 40</td>
<td>87 150</td>
</tr>
<tr>
<td>pregnancy diagnosis (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time for which cows used for draught power (h/d)</td>
<td>3 3</td>
<td>3 3</td>
</tr>
</tbody>
</table>
FIG. 1. Change in live weight of calves fed with or without urea-molasses block (UMB) supplement.

FIG. 2. Change in live weight of sheep fed with or without urea-molasses block (UMB) supplement.

FIG. 3. Daily dry matter intake of sheep fed with or without urea-molasses block (UMB) supplement.

FIG. 4. Daily live weight gain in sheep and feed conversion ratio.

diet of straw, either untreated or treated, to increase daily milk yield, lengthen the lactation period and improve reproductive performance, as shown in Table IV [20]. Calves supplemented with blocks gained 224 g/d whereas calves without blocks gained 80 g/d ($P < 0.01$) [19]. The fortnightly change in live weight of the calves fed with or without blocks under existing feeding systems in the villages is shown in Fig. 1 [21]. Supplementation of straw based diets with urea-molasses blocks in sheep significantly increased the daily dry matter intake ($P < 0.05$) and live weight gain ($P < 0.1$) compared with a group without blocks. The weekly change in live weight of the sheep is shown in Fig. 2 [22]. Their voluntary dry matter intake and feed conversion ratio are shown in Figs 3 and 4.
5. BYPASS PROTEIN IN THE ANIMAL DIET

5.1. Importance of bypass protein

Once the supply of rumen fermentable nitrogen is ensured (rumen ammonia concentrations above 150 mg/L of rumen fluid) to optimize the rate of degradation of fibrous substrate, the next limitation to animal productivity is considered to be the availability of amino acids absorbed from the intestine [23]. In tropical countries where crop residues and by-products are often used in animal feeding, the potential value of protein lies mostly in its ability to stimulate voluntary feed intake. Additional animal production can be obtained from protein supplementation, which reaches the abomasum intact. This can be achieved by feeding protein with a low rate of fermentation in the rumen so that it goes directly into the abomasum. The rate of degradation of various proteins in the rumen needs to be considered. It is likely that differences in degradability exist among protein sources from green roughages, especially tree leaves, which may be important for ruminant feeding in Bangladesh [24]. The protein in oilcakes, which are mostly used to supplement straw based diets for livestock, was found to be extensively degraded compared with that of fish meal and tree leaves [24, 25].

5.2. Availability of fish meal

Fish meal is the most important protein supplement made from fish trash or other marine aquatic organisms that are not used for human consumption. Depending

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Fish meal (150 g/d)</th>
<th>Oilcake (300 g/d)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rice bran (g/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 300 600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily live weight gain (LWG) (g)</td>
<td>143</td>
<td>357 354 335</td>
<td>188 252 235</td>
<td>0.038</td>
</tr>
<tr>
<td>Dry matter intake (DMI) (kg/d)</td>
<td>3.0</td>
<td>3.2 3.6 3.8</td>
<td>3.8 3.6 3.6</td>
<td>0.069</td>
</tr>
<tr>
<td>Feed conversion ratio (kg DMI/kg LWG)</td>
<td>21</td>
<td>9 10 11</td>
<td>18 14 16</td>
<td></td>
</tr>
</tbody>
</table>
on the quality, type and protein content of the raw materials, these are classified into A₁ (55–60% crude protein), A₂ (50–55% crude protein) and B (45–50% crude protein). The quantity of fish meal available from the Bangladesh Fisheries Development Corporation's plants is about 650 t/year. The amount of fish trash available as raw materials is about 25 000 t/year [26]. Data on fish meal availability from different fish processing plants are not available. It has also been reported that 4000–5000 t of raw shrimp wastes are available in the country. The quantity of crab meal available has not yet been measured [26]. Most fish meal is fed to poultry. If more fish meal became available it could be a source of bypass protein supplement with straw based diets for livestock to increase animal productivity.

5.3. Production responses to feeding fish meal as bypass protein

Earlier trials [27–29] strongly suggested that a small quantity of fish meal supplement of not more than 50 g/d with a basal diet of straw significantly improved both feed intake and daily live weight gain in calves [28]. Calves on a basal diet of urea treated straw were fed for 105 d with either 150 g of fish meal or the crude protein equivalent of 300 g of sesame oil cake, together with increasing levels (0, 300, 600 g/d) of rice bran. The results are shown in Table V. The best growth rate was achieved in the group receiving no other supplement than 150 g of fish meal. The effect on feed conversion was particularly marked as the dry matter intake (DMI) requirement per kilogram of live weight gain (LWG) decreased from 21 to 9 kg. Considering the small, nondescript Bangladeshi local calves, these results are remarkable in terms of conversion efficiency, with the given diet.

In view of the clear response to preformed protein, which presumably avoids large scale degradation in the rumen, the next question of interest is to define the
TABLE VI. EFFECT OF INCREASING LEVEL OF FISH MEAL SUPPLEMENT ON PERFORMANCE OF CALVES AFTER WEANING ON A STRAW BASED DIET

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fish meal (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Mean live weight (LW) (kg)</td>
<td>31.8</td>
</tr>
<tr>
<td>Dry matter intake (DMI) (g/kg LW^{0.75})</td>
<td>79.2</td>
</tr>
<tr>
<td>Daily live weight gain (LWG) (g)</td>
<td>160^a</td>
</tr>
<tr>
<td>Feed conversion ratio (kg DMI/kg LWG)</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Note: Significance levels: a < b, P < 0.01; b < c, P < 0.05.

optimum quantity needed to increase animal productivity (i.e. to develop a response curve). It has been reported that a daily supplement of 50 g of fish meal could increase the daily LWG (196 g/d), with little further effect being observed on increasing the level of fish meal (Fig. 5) [30]. On the basis of this result it may be assumed that the provision of 20–25 g of preformed protein effectively escaping degradation in the rumen will have a dramatic effect on animal performance, when the basal diet is ammoniated rice straw. To develop the response curve, the fish meal allocation to each group of calves in another experiment was reduced to 0, 15, 25, 50 and 150 g/d. Results from this experiment revealed that the difference in LWG between animals given 50 and 150 g of fish meal was insignificant but that the LWGs in these two groups were found to be highly significant (P < 0.01) when compared with those of the groups receiving 15 and 25 g/d.

The effects of supplementation of fish meal on weaned calves receiving a straw based diet have been demonstrated and the results are shown in Table VI [31]. The highest LWG of 319 g/d was observed in calves receiving 30 g/d of fish meal in the diet, compared with 309 g/d for a supplement of 20 g/d. Furthermore, the feed conversion ratio was much lower (3.9 kg DMI/kg LWG) in the supplemented group than in the unsupplemented group (6.6 kg DMI/kg LWG).

Figure 6 illustrates the results of trials with native Zebu cattle in which responses to different levels of fish meal (protein) supplement were measured in milking cows. There was a linear increase in milk yield when fish meal was given as a supplement to a basal diet of ammoniated rice straw [30]. Ammoniation of straw increased intake and clearly reduced the need for bypass nutrients since the amount of milk produced per unit of concentrate fed was increased [31].
REFERENCES


THE IMPORTANCE OF UREA–MOLASSES MULTINUTRIENT BLOCKS FOR RUMINANT PRODUCTION IN INDONESIA

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Australia

Abstract
THE IMPORTANCE OF UREA–MOLASSES MULTINUTRIENT BLOCKS FOR RUMINANT PRODUCTION IN INDONESIA.

Early work demonstrated that the growth of rumen microbes in ruminants fed cut and carry grass was low and inefficient but that with supplementation of a mixture of nutrients this improved threefold. Feeding urea–molasses multinutrient blocks (UMMBs) to buffaloes fed cut and carry grass improved rumen ammonia levels and microbial growth yield. Supplementation with UMMBs markedly stimulated milk yield and growth rate of cattle under village management conditions where other nitrogenous supplements were not used but had no effect when a large amount of concentrate or soybean curd waste was included in the diets. When supplemented with UMMBs, cattle or sheep fed cut and carry grass showed a wide variation in growth rate improvement. The best response in sheep was a 126% increase in the live weight gain of Garut fighting sheep, for which the weight gain improved from 140 g/d in the controls to 316 g/d in the supplemented lambs. Reproductive rate, specifically birth weight and survival of goats, was increased by feeding UMMBs in the traditional system of raising goats. In 12 out of 14 trials highly significant and economic increases in production resulting from feeding UMMBs were observed. The potential of UMMBs to increase both the level and efficiency of livestock production and so profitability in Indonesia is substantial and represents a major technology breakthrough for all meat/milk production systems.

1. INTRODUCTION

To attain maximum benefits from agricultural/agroindustrial by-products as feeds for ruminants it is necessary to apply strategic supplements to (a) promote the efficiency of rumen microbial growth and (b) supply the animals with sufficient by-
pass nutrients to balance nutrient availability and nutrient demand [1, 2]. Animals that only have poor quality forages grow slowly unless these concepts are applied. Earlier studies have suggested that ammonia concentration in rumen fluid is the most important constraint governing microbial growth [3, 4], digestibility and intake [5, 6]. Previous research has shown that other microbial growth factors may also be involved in maximizing microbial growth efficiencies. For example, amino acids, peptide [7] and minerals [8] may all have a role in optimizing the growth of rumen microbes [9].

It has thus been established that microbial growth efficiency in the rumen is markedly affected by the availability of a number of nutrients, including minerals, ammonia, amino acids and vitamins, which must be supplied in the diet or must be produced by the interaction of different microbes to their mutual benefit. The efficiency of microbial growth is a major factor that affects the ratio of protein (from microbe digesta in the intestines) to energy (volatile fatty acids absorbed from the rumen), which in turn is the major factor that appears to affect the efficiency and level of growth of ruminants on forage based diets [10].

Earlier studies at the Centre for the Application of Isotopes and Radiation (PAIR), Jakarta, undertaken under a co-ordinated research programme jointly organized by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency, examined the efficiency of microbial growth in the rumen using a radioisotope technique and found highly conflicting results from buffaloes given cut and carry grass and various isonitrogenous supplements [11]. However, mixing together a number of feedstuffs to arbitrarily correct nutritional deficiencies for the microbes in the rumen resulted in remarkable increases in microbial growth (approximately threefold). When the same nutritional practices were applied at the village level the growth rate and feed conversion efficiency of buffaloes were increased markedly [12]. The studies presented here represent some ten years' research to effectively and practically achieve the same production increases more economically.

2. RESEARCH OBJECTIVES

The problem that Indonesian scientists face is how to apply such technology widely and achieve significant increases in cattle, sheep and goat production in the cut and carry grass systems which are typical of the traditional production systems. In these systems growth rates, reproductive rates and milk yields of all species are low, yet the systems effectively use a resource that would otherwise be wasted. It was suspected from the laboratory studies that the low productivity was largely the result of an inefficient rumen system. This results in a lowered digestibility, which in turn limits intake and also leads to a low protein to energy ratio in the nutrients absorbed which reduces efficiency of live weight gain and may further reduce
intake through an added heat load [10]. The provision of multinutrients to feeding systems for ruminants based on crop residues and poor quality pasture has been remarkably successful in other countries [13–15]. The studies reported here were developed simultaneously with the aforementioned work [13–15]. Indonesia, however, has a particular advantage in that it has an abundant and inexpensive supply of molasses.

3. RESEARCH TO ESTABLISH THE USEFULNESS OF UREA-MOLASSES MULTINUTRIENT BLOCKS

3.1. Multinutrient mixtures and microbial growth efficiency in the rumen

The initial research examined the effects of feeding increasing amounts of urea–molasses multinutrient block (UMMB) to buffaloes fed cut and carry grass from which rumen fluids were collected and microbial growth measured in vitro. The method used was based on the incorporation of the radioisotope $^{32}$P into microbes in the incubated rumen fluid [16, 17]. As mentioned in the introduction, the results were spectacular [18].

3.2. Responses to feeding UMMBs to buffaloes and goats

Buffaloes and goats were fed cut and carry pasture with or without UMMBs. In both animals the rumen ammonia levels were increased substantially by supplementation. Microbial growth efficiency measured in vitro in rumen fluid taken from buffaloes [18] and goats [19] indicated that the supplement improved the nutritional status of the rumen microbes considerably. The basic hypothesis, that the microbial growth efficiency in the rumen could be improved markedly in ruminants fed cut and carry grass as used by the farmers, appeared to be proven. The next step

FIG. 1. Composition of feedstuffs in the urea–molasses multinutrient block.
was to determine how this translated into increased productivity of cattle, sheep and goats and milk production from dairy cows in the village. Rather than test these in the research institute and in order to obtain immediately applicable results it was decided to take the research to the village farmers.

Our experience indicated that village farmers prefer to be given a readily usable package of technologies rather than component parts that they have to mix themselves. For this reason research was undertaken to develop an appropriate block composition for use in the field that could be manufactured in the village. The final formulation that appears to give the best results is shown in Fig. 1. Blocks were selected as the best mode for supplementation because of the convenience in transportation, storage and particularly ease of application by the farmers.

3.3. Research organization for field testing

The group of investigators assembled to deliver and test the technology in the field were extension specialists from the livestock services at provincial and district levels who co-operate closely with the first author and her group at the PAIR and with the second and third authors (United Nations Development Programme/IAEA experts). The rest of this paper deals with the practical results of introducing UMMBs into traditional ruminant production systems without other disturbance to the routine. These trials were carried out in a number of sites throughout Java with different species of ruminants [20, 21].

3.4. Results of introducing UMMBs into traditional livestock feeding

3.4.1. Dairy cattle — Central and West Java

Friesian dairy cows given UMMB supplementation in the Magelang district of Central Java gave a total milk yield of 1119 L, as compared with 871 L for the no-UMMB treatment, over a period of 18 weeks (Fig. 2). Results from Lembang in West Java indicated no response in milk yield to UMMB supplementation (1019 vs. 1008 L in 18 weeks), as shown in Table I.

3.4.2. Sheep

Supplementation of UMMBs to sheep gave different responses, apparently depending largely on the particular diet. Animals maintained on cut and carry cultivated forage such as *Panicum maximum* showed a lower but still significant response as compared with sheep receiving mixed cut and carry grass, tree foliage, cultivated forage and agricultural by-products (growth rate increased by 86% from 36 to
FIG. 2. Effect of UMMB supplementation on milk yield in dairy cattle in the Magelang district of Central Java (based on data reported by Suharto and S. Kamarudin from the Central Java Livestock Services, 1990).

TABLE I. EFFECT OF UMMB SUPPLEMENTS ON MILK YIELDS OF FRIESIAN–HOLSTEIN DAIRY CATTLE AND ETAWA CROSSBRED GOATSa

<table>
<thead>
<tr>
<th>N (g/d)</th>
<th>Milk yield (18 weeks) (L)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- UMMB</td>
<td>+ UMMB</td>
</tr>
</tbody>
</table>

Friesian–Holstein dairy cattle

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Milk yield</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lembang, West Java</td>
<td>297</td>
<td>1008</td>
<td>1019</td>
</tr>
<tr>
<td>Garut, West Java</td>
<td>197</td>
<td>900</td>
<td>1107</td>
</tr>
<tr>
<td>Magelang, Central Java</td>
<td>270</td>
<td>871</td>
<td>1119</td>
</tr>
</tbody>
</table>

Goats

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Milk yield</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Java</td>
<td>47</td>
<td>99</td>
<td>121</td>
</tr>
</tbody>
</table>

a Based on data collected in collaboration with West Java [20] and Central Java [21] Livestock Services.
FIG. 3. Effect of UMMB supplementation on live weight increase in Garut sheep (600 m a.s.l.) (based on data reported by A. Nuriati and D. Sartika from the West Java Livestock Services, 1990).

67 g/d), whereas on the mixed materials sheep growth was increased from 140 to 316 g/d (126% increase). Live weight increases are illustrated in Table II for two locations at different sea levels (see also Fig. 3).

3.4.3. Beef cattle

Results in Table II show that beef cattle responded to supplementation with UMMBs when no other nutrient components were added to the diet of cut and carry grass. A remarkable growth rate response to UMMBs was observed in Friesian-Holstein steers, with supplementation increasing growth rates from 400 to 810 g/d. Young Ongole bulls that were supplemented with soybean curd waste did not respond to additional UMMB. However, a significant response to UMMB in Ongole bulls maintained on cut and carry grass plus pineapple residues is evident in Fig. 4, which shows the growth rate over a 16 week observation period.

3.4.4. Goats — Reproduction studies

A trial to examine the effect of UMMB supplements on the reproductive capacity of goats was carried out in Central Java. The data in Table II indicate a major effect of such supplements on the birth weight of offspring and their subsequent production. UMMB supplementation reduced gestation period and also decreased the time between pregnancies (Table III).
TABLE II. EFFECT OF UMMB SUPPLEMENTS ON GROWTH RATE OF FRIESIAN-HOLSTEIN STEERS, ONGOLE STEERS, SHEEP AND GOATS

<table>
<thead>
<tr>
<th>Ration</th>
<th>Amount (fresh weight basis) (kg/d)</th>
<th>N (g/d)</th>
<th>Growth rate (g/d)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>- UMMB</td>
<td>+ UMMB</td>
<td></td>
</tr>
<tr>
<td><strong>Friesian-Holstein steers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cut and carry grass</td>
<td>15.0</td>
<td>156.3</td>
<td>210</td>
<td>560</td>
</tr>
<tr>
<td>Rice bran</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice straw</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cut and carry grass</td>
<td>15.0</td>
<td>171.3</td>
<td>400</td>
<td>810</td>
</tr>
<tr>
<td>Rice bran</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice straw</td>
<td>5.0</td>
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<td></td>
</tr>
<tr>
<td><strong>Ongole steers</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Mixed cut and carry grass</td>
<td>30.0</td>
<td>161.0</td>
<td>333</td>
<td>526</td>
</tr>
<tr>
<td>Cassava tubers</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cut and carry grass</td>
<td>30.0</td>
<td>204.0</td>
<td>478</td>
<td>465</td>
</tr>
<tr>
<td>Rice bran</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean curd waste</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut and carry elephant grass</td>
<td>35.0</td>
<td>291.0</td>
<td>388</td>
<td>822</td>
</tr>
<tr>
<td>Rice bran</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cut and carry grass</td>
<td>25.0</td>
<td>110.0</td>
<td>183</td>
<td>403</td>
</tr>
<tr>
<td>Pineapple waste</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sheep</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Majalengka (100 m a.s.l.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut and carry elephant grass</td>
<td>3.0</td>
<td>30.4</td>
<td>36</td>
<td>67</td>
</tr>
<tr>
<td>(b) Garut (600 m a.s.l.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cut and carry grass + tree foliage</td>
<td>4.0</td>
<td>31.5</td>
<td>140</td>
<td>316</td>
</tr>
<tr>
<td>Young papaya chopped</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Etawa crossbred goats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Does (&gt;6 months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut and carry grass + foliage</td>
<td>3.0</td>
<td>31.7</td>
<td>40</td>
<td>88</td>
</tr>
<tr>
<td>Concentrate</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Lambs (1–90 d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut and carry grass + foliage</td>
<td>3.0</td>
<td>51.7</td>
<td>91</td>
<td>105</td>
</tr>
<tr>
<td>Concentrate</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk from does</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on data collected by West Java [20] and Central Java [21] Livestock Services.
FIG. 4. Effect of UMMB supplementation on live weight increase in Ongole bulls, Subang district, West Java (based on data reported by A. Nuriati and D. Sartika from the West Java Livestock Services, 1990).

### TABLE III. EFFECT OF GIVING UMMB SUPPLEMENTS TO ETAWA CROSSBRED GOATS FED CUT AND CARRY GRASS AND TREE FOLIAGE (AT TIMES Leucaena) ON REPRODUCTIVE PERFORMANCE [22]

<table>
<thead>
<tr>
<th></th>
<th>− UMMB</th>
<th>+ UMMB</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.3</td>
<td>3.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Live weight gain (g/d)</td>
<td>91.6</td>
<td>105.2</td>
<td>14.9</td>
</tr>
<tr>
<td><strong>Dams</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk yield (L/d):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>1.1</td>
<td>1.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Mean</td>
<td>0.8</td>
<td>1.0</td>
<td>20.3</td>
</tr>
<tr>
<td>Kidding interval (d)</td>
<td>152</td>
<td>144</td>
<td>−5.3</td>
</tr>
<tr>
<td>Gestation period (d)</td>
<td>201</td>
<td>192</td>
<td>−4.5</td>
</tr>
</tbody>
</table>
The changes in rumen ammonia concentration and microbial growth in those animals receiving UMMBs with a diet of cut and carry grass suggested that the supplement had a stimulating effect on the rumen ecosystem, particularly in supplying extra ammonia and probably also minerals. Ammonia is probably not the only deficient nutrient supplied and it is highly likely that other microbial growth factors in the blocks are also promoting rumen microbial growth. The most likely of these are sulphur and trace elements. These findings confirm and extend the previous results which indicated that in order to promote microbial growth other nutrients are needed in addition to ammonia. The particular deficiencies corrected almost certainly vary with diet and with the location from where the cut and carry grass is obtained [23, 24]. The conclusion reached from the present studies is that there is an important relationship between rumen microbial growth efficiency and animal growth rate. This implies that microbial growth in the rumen is a sensitive measure that could be used to evaluate feed resources. The N content in the diets of cut and carry grass when supplemented with UMMBs is close to the values suggested by Leng and Kunju [25] to meet the needs of a particular production level.

The results of field experiments on dairy cattle have clearly shown that UMMB supplementation increases milk production in animals on forage based diets including agricultural by-products. Where concentrates are fed in large amounts there are no particular deficiencies in the rumen and the UMMB is not needed. In this situation, presumably the rumen intake is balanced and the animal receives bypass protein from the concentrate that is sufficient to meet its needs for the level of production determined by the specific farming conditions.

UMMB supplementation improved the appearance of the animal’s coat, which provides one way of assessing quickly whether there is likely to be a long term response.

The fat content of milk was increased by 0.1–0.2%. This is presumably the result of a better balanced nutrition with a greater availability of substrates for direct fat synthesis (acetate) and for stimulation of fat synthesis (glucose). The effects of UMMB supplementation during 18 weeks in late lactation are illustrated in Fig. 2. The pattern of milk production indicates that the responses are induced over a long period. UMMB supplements during the late lactation period have been observed by farmers to contribute to successful parturition, quick post-partum recovery, healthy neonatal calves and higher calf growth rate, in addition to increasing milk yield. The observations are supported by results from studies of UMMB supplementation of goats [22].

Growth rate response of Garut fighting sheep to UMMB supplementation is shown to be dependent on the quality and quantity of the basal diet given. An amount of 150 g/d of UMMB was sufficient to increase the growth rate of animals in two locations at different altitudes and with dissimilar climatic conditions and the diets
given were different (Table II). UMMB supplementation of these sheep at the first location (100 m a.s.l.) increased growth rate by 86% as compared with 126% (Fig. 3) for the Garut sheep in the second location at a higher altitude (600 m a.s.l.). The growth rates of sheep on the relatively ‘poor quality’ location at the higher altitude are of significance as they approach those of sheep fed concentrates and high quality proteins [10]. The difference between the results of these two studies emphasizes the importance of basal diets, climate and their interaction in determining animal response to UMMB supplementation [10].

Ongole bulls fed cut and carry grass supplemented with soybean curd waste did not respond to UMMB supplementation. Soybean curd waste contains 20-24% protein [26] but is probably very soluble, providing both ammonia and sulphur in the rumen from amino acid degradation, and therefore it is reasonable not to expect an additional response to urea or sulphur from the blocks. However, the result does tend to indicate that in the other experiments N and/or S are the primary factors which improve feed utilization and growth of ruminants on cut and carry grass. In the absence of supplements of soybean curd waste, however, responses to supplementation of Ongole cattle fed cut and carry grass were extremely high (Fig. 4).

Reproduction studies with goats indicated that UMMB supplementation enhanced reproduction considerably. As reproduction rates are low in all domestic livestock in Indonesia this could be the most important aspect of UMMB technology (Table III).

5. OVERALL CONCLUSION

The results from all the studies conducted during 1987–1990 in many districts of Java are summarized in Tables I–III. The demonstration trials have convinced a large population of the livestock owners of the value of the UMMB, which is now gradually spreading throughout West and Central Java. In the Garut district alone some 2000 cattle are receiving UMMBs prepared locally by the village co-operative units.

Future research needs to examine the potential for manipulation of the rumen to remove protozoa in animals on these diets to increase further the protein to energy ratio, and an antiprotozoal forage is presently being treated for this purpose. The manipulation of the rumen to enhance protein availability to ruminants is particularly important since bypass protein sources are not available generally and they are expensive. A further approach will be to find ways of developing bypass proteins for supplementation from the available protein sources in Indonesia, in particular from tree leaves. In the mean time the UMMB constitutes an innovative feeding strategy for use with ruminants in Indonesia.
ACKNOWLEDGEMENTS

The experiments and field trials described in this paper were parts of activities of the UNDP/IAEA Country Project No. INS/88/013 implemented by the National Atomic Energy Agency (BATAN), Indonesia. The authors appreciate the enthusiastic technical co-ordination from the Animal Production and Health Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (particularly J.D. Dargie, B.M.A.O. Perera and M.C.N. Jayasuriya) and from the National Project Co-ordinator. Special thanks go to E. Suharya, Head of the West Java Livestock Services, and his livestock production officers (A. Nuriati and D. Sartika); and to Kusmono, Head of the Central Java Livestock Services, and his livestock production officers (Suharto and S. Kamarudin) for their excellent cooperation in the field trials conducted on farms.

Thanks are due to the Director of PAIR, BATAN, for giving stimulating support to the project. Appreciation is also extended to the first author’s colleagues in the Animal Nutrition Research Group of PAIR (Suharyono, T. Tjiptosumirat, B.H. Sasangka and R. Bahaudin) and to the technicians (T. Maryati, Nuniek and A. Syamsi) for their dedicated work in connection with the project.

The authors thank Mulyana, extension officer from the livestock services of Garut district, West Java, for his active participation in the introduction and transfer of the UMMB technology to small farmers in the district. Thanks also go to L.A. Sofian of the Institute of Agriculture, Bogor, for moral support.

REFERENCES


MODELLING AS AN AID TO NUTRITION RESEARCH

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United Kingdom

Abstract

MODELLING AS AN AID TO NUTRITION RESEARCH.

In many areas of research, including nutrition, the straightforward questions have now been answered and the speed of future progress will depend on successful collaboration between disciplines. Such collaboration is sometimes difficult, but can be helped by the adoption of a structured approach, such as that used in the development of mathematical models. Two examples of the use of mathematical modelling as a research tool in ruminant nutrition are considered. The first is the interface between the production of end products of digestion and their metabolism by the tissues. Absorbed nutrients undergo significant transformation by metabolism both during absorption across the gut wall and in the liver, and consideration of the published data suggests that metabolism by the visceral tissues needs to be considered separately from metabolism in the peripheral tissues. The lack of appropriate data to parameterize models stimulated a research programme on gut and liver metabolism. The second example relates to the control of intake and illustrates how the requirement for data to parameterize an intake model has identified the need to measure the daily patterns of voluntary intake, rumen volume and motility and concentrations of metabolites both in the rumen and entering the portal blood.

1. INTRODUCTION

The application of animal nutrition research in practice lies in the prediction of animal responses to changes in diet or feeding regime. This requires knowledge of the processes of ingestion, digestion and metabolism. These processes have been studied in detail over the years, but the tendency has been to concentrate on understanding the individual processes rather than on how they interrelate. Further progress requires study of the interactions between them.

As with many other areas of biological research, in which the major questions have been answered, but the ‘pieces of the jigsaw’ have not been put together, further progress will best be achieved by joining forces with scientists trained in other
disciplines. However, working in multidisciplinary teams is not always easy and there is a need for a structured approach to facilitate communication. Mathematical modelling, or quantitative representation of biological systems, provides such an approach which has been developed and tested through its application in other fields (e.g. in the plant sciences [1]). It is a multistage process which starts with a schematic representation (referred to as a flow diagram; see Fig. 1) of the components of the systems (boxes in Fig. 1) and the interactions between them (arrows in Fig. 1). Mathematical equations are then derived to describe the transfer of matter from one component to another, and the equations can be solved for different starting values using a computer. However, even if mathematical skills to write the equations are not readily available, the discipline of constructing a flow diagram helps to clarify hypotheses and to identify areas where information is lacking. The ideas generated by this exercise can then be fed back into an experimental programme, the results of which, in turn, can be used to update the flow diagram.

This process is described in the next section, followed by two sections which use examples of research topics in ruminant nutrition to illustrate how the development of mathematical models can enhance the value of experimental results, through helping to develop hypotheses from existing knowledge and in the design of further experiments to put these hypotheses to the test.
2. THE MODELLING PROCESS

2.1. General

Only a very brief description of the modelling process and its application to nutrition can be given here. For more details on the application of the modelling process to problems in agriculture, the book by France and Thornley [2] is recommended and for specific application to nutritional problems, the reader is referred to Refs [3, 4].

2.2. The flow diagram

The first step in model development is to define clearly the objective of the proposed model, particularly in relation to the types of outputs required. For example, for animal models, are the desired outputs at the whole animal, tissue or cellular level? For the diagram in Fig. 1, the objective is to predict carcass composition from absorbed nutrients. From this objective it is relatively straightforward to define the outputs as carcass fat and protein and then to work backwards to select the absorbed nutrient inputs which are required to synthesize these outputs. The major precursors of triglyceride fat are long chain and volatile fatty acids (VFAs), with glucose required for the glycerol component of the triglycerides and the supply of reducing factor (NADPH) for fatty acid synthesis, and energy required for both synthetic processes. Amino acids are the precursors of protein, with energy required for the formation of the peptide bonds. This representation is obviously simplistic in that other metabolites, together with genetic and endocrinological factors, will also affect the rates of fat and protein synthesis. However, one of the principles of modelling is to start with the simplest representation possible, and hence these components should be used for a first attempt. For simulations where this model results in poor predictions, other components may need to be added.

2.3. The equations

Taking the example of the fatty acid pool in Fig. 1, there is one arrow entering the box and one leaving it, i.e. one input from VFAs and one output to fat. The rate of change in the amount of fatty acids can therefore be calculated by subtracting the output from the input. The rates of synthesis of fatty acids from VFAs and of triglyceride from fatty acids are calculated on the basis of biochemical principles — Michaelis–Menten equations:

\[
V_{\text{max}} = \frac{1}{1 + K_m/[S]}
\]
where
\[ V_{\text{max}} \] is the maximum rate of the reaction;
\[ K_m \] is the affinity constant (the concentration of substrate at which the rate of the reaction is half maximum);
\[ [S] \] is the substrate concentration.

The effects of the secondary precursors (e.g. glucose and energy) are accounted for by adding additional \( K_m/[S] \) terms to the denominator, one for each secondary substrate. These flows of matter from one pool to another are generally calculated in molar units and standard stoichiometric relationships convert the output from the substrate pool into the input to the product pool. Further details on the types of equations used in metabolism models can be found in the mathematical statements of published models [5, 6].

2.4. Solution of the equations

The example illustrated in Fig. 1 is a relatively simple representation, but even that would have 20 equations which require solution. This is achieved most efficiently by converting the equations into a computer program, thus facilitating rapid estimation of the outputs to be generated for a whole range of inputs. Many whole animal models are programmed in the Advanced Continuous Simulation Language (ACSL), but where this software is not available other languages such as Fortran may be used.

3. INTERFACING DIGESTION AND METABOLISM MODELS

3.1. Theoretical considerations

The choice of inputs to represent in the example in Fig. 1 was based on the precursors required for the synthesis of the desired outputs. Similar objectives of wanting to explain differences in carcass composition from differences in end products of digestion have formed the basis for experimental programmes, and thus data on the flow of protein, carbohydrate and lipid into the duodenum and the production of VFAs within the rumen have been published. However, while trying to use these data as inputs for models, it became apparent that in many instances the experimental objectives were specific to protein or carbohydrate. Hence there are very few data sets where VFA production and the flow of protein, carbohydrates and lipid have been measured in animals on the same diets. Further, where these nutrients have been measured, summation of the energy they contribute rarely equals the estimates of digestible energy consumed.
TABLE I. SIMULATED PERCENTAGE CONTRIBUTIONS OF INDIVIDUAL TISSUES TO TOTAL ENERGY COSTS OF PROTEIN TURNOVER AND Na⁺,K⁺-TRANSPORT (ION TRANSPORT) IN GROWING LAMBS [10]

<table>
<thead>
<tr>
<th></th>
<th>Protein turnover</th>
<th>Ion transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal tract</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Muscle</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Liver</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Skin</td>
<td>24</td>
<td>3</td>
</tr>
</tbody>
</table>

Another deficiency in the use of digestion data to provide inputs to metabolism models is due to the fact that there is considerable metabolism of some of these nutrients during absorption. This has both qualitative effects in changing the profile of nutrients absorbed and quantitative effects in that 20% of whole body heat production in sheep can be accounted for as metabolism by the gut tissues [7]. A slightly lower proportion of heat production (16–18% in sheep [8]) is accounted for by the liver, further reducing the availability of nutrients to muscle, adipose tissue or mammary gland — the productive tissues. The representation in Fig. 1 appears to be over-simplistic, therefore, in relation to metabolism at the animal level, since it does not differentiate between tissues in terms of rates of metabolism. This raises the question of how many tissues need to be represented independently.

To answer this question, published information on the relative contributions of ten different organs and tissues to the two major energy utilizing processes (protein turnover and ion transport) in growing lambs was integrated [9] and a number of simulations were conducted [10]. The results (Table I) demonstrated that only the gut, liver and muscle contributed more than 10% each to Na⁺,K⁺-transport costs, but in addition to these tissues the skin made a significant (24%) contribution to protein turnover costs.

3.2. Experimental results

The theoretical study confirmed the gut and liver as major tissues for which data on the metabolism of specific nutrients are required, with the skin as a third tissue meriting further study, at least in sheep. At the AFRC Institute of Grassland and Environmental Research in Hurley, priority was given to the liver, although the diets fed were also used in experiments to measure gut metabolism at the University of Newcastle-upon-Tyne.
TABLE II. NET ABSORPTION OF METABOLITES FROM THE GASTRO-INTESTINAL TRACT (PORTAL PRODUCTION) AND NET PRODUCTION ACROSS THE LIVER (HEPATIC PRODUCTION) IN CATTLE EATING SILAGE [11]

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Portal production (mmol/min)</th>
<th>Hepatic production (mmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>12.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Propionate</td>
<td>4.81</td>
<td>-4.47</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.348</td>
<td>-0.209</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.60</td>
<td>-1.17</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.47</td>
<td>1.55</td>
</tr>
<tr>
<td>Amino acids</td>
<td>0.803</td>
<td>-0.592</td>
</tr>
<tr>
<td>Urea</td>
<td>-1.18</td>
<td>2.65</td>
</tr>
<tr>
<td>Ammonia</td>
<td>2.25</td>
<td>-2.20</td>
</tr>
</tbody>
</table>

The work on the liver aimed to measure the net appearance of major nutrients in the portal vein and their subsequent metabolism within the liver, in response to a variety of diets. The results in Table II for the net portal and hepatic production of metabolites in cattle offered silage diets published by Wilton [11] indicate the degree of variation in the metabolism of different nutrients within the liver, ranging from synthesis of glucose and urea to almost complete removal of the lactate, propionate and ammonia absorbed from the gut.

Wilton [11] also conducted an experiment in which ammonia was infused into the rumen of cattle offered a dried grass diet. These results may have particular relevance to the selection of components to represent in a metabolic model, since there was a pronounced trend for increasing levels of absorption of ammonia from the gut to increase the metabolism of amino acids by the liver (Table III). This suggests that for diets which lead to high rates of ammonia absorption, the efficiency with which amino acids are converted to body protein may be decreased owing to enhanced liver metabolism, and hence when simulating such diets with a model there may be a need to include ammonia as an independent component. This concept is currently being tested theoretically in a growth model [12].
TABLE III. NET ABSORPTION OF AMINO ACIDS FROM THE GASTRO-INTESTINAL TRACT (PORTAL PRODUCTION) AND NET PRODUCTION ACROSS THE LIVER (HEPATIC PRODUCTION) OF CATTLE DURING A 3 h INFUSION OF DIFFERENT LEVELS OF NH₄Cl [11]

<table>
<thead>
<tr>
<th>NH₄Cl infusion (mmol/min)</th>
<th>0</th>
<th>1.07</th>
<th>2.32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal production (mmol/min)</td>
<td>1.312</td>
<td>2.290</td>
<td>2.597</td>
</tr>
<tr>
<td>Hepatic production (mmol/min)</td>
<td>-0.204</td>
<td>-1.274</td>
<td>-1.986</td>
</tr>
</tbody>
</table>

4. DEVELOPMENT OF INTAKE MODELS

4.1. Theory of intake control

The previous section considered the prediction of production responses, starting either from absorbed nutrients or from dietary inputs. However, the problems associated with the prediction of the amount of feed which a given animal will consume have so far been ignored. Attempts have been made to predict intake from regression equations [13] and from complex representations of the minute by minute changes within the animal [14], and it is gradually becoming accepted that further improvements in the accuracy of prediction over a wide range of feeds will require continued development of the latter approach. A prerequisite to adopting this approach is the development of whole animal models such as that described above, but in addition to the prediction of metabolites arising from rumen digestion, such models will also need to incorporate an estimate of rumen volume.

Rumen volume is required for intake prediction since it has long been assumed that the intake of fibrous feeds by ruminants is limited solely by the maximum capacity of the rumen to contain a large volume of digesta [15]. Although more recently it is becoming accepted that other, metabolic, factors are also involved, the contribution of rumen ‘fill’ to intake control cannot be ignored. However, even the data to permit the representation of a so-called ‘physical’ limitation in a model are lacking. For example, how should the degree of ‘fill’ generated by a specific feed be estimated and what value should be used for maximum rumen capacity? Trying to answer these questions and assign appropriate parameter values led to the development of an experimental programme to measure the amount of digesta in the rumen of cattle offered forage diets.
FIG. 2. Rumen contents at various times of the day of steers eating silage (M. Gill, D.P. Poppi and A. Sargeant, unpublished observations).

FIG. 3. Rumen contents at various times of the day of dairy cows eating silage (J.D. Sutton and M. Gill, unpublished observations).
4.2. Experimental programme

The first experimental objective was to identify the factors governing the weight of digesta in the rumen in cattle offered different forages. Since the data are required for a model of voluntary intake, the measurements had to be made under experimental conditions where feed was freely available. Under this feeding regime, it could not be assumed that the weight of digesta in the rumen would be relatively stable over the 24 h, and thus the weight of digesta in the rumen was measured by complete manual emptying at a number of times relative to the offering of fresh feed.

The results for fresh and dry weights of digesta in steers (Fig. 2) and lactating cows (Fig. 3) offered silage diets demonstrate considerable variation over a 24 h period. This indicates that rumen capacity was not the major factor signalling the end of all individual meals. Indeed these data, in agreement with data from other countries (D.P. Poppi, unpublished observations), suggest that maximum rumen volumes tend to be achieved during the late afternoon or early evening. These results suggest a hypothesis based on different mechanisms predominating in the control of meal size at different times of the day. Given the lower rumen digesta weights immediately after the first meal, together with the usual pattern of a rapid increase in concentration of VFAs soon after the meal, it is suggested that the size of meals early in the feeding period may be controlled primarily by metabolic factors, e.g. concentrations of metabolites monitored by receptors in the rumen wall or in the liver [16, 17]. The consequences of this finding are the need for data on the daily pattern of variation

![Graph](image)

FIG. 4. *Cumulative dry matter intake of silage over a 24 h period.*
in the concentration of metabolites both within the rumen and entering the portal bloodstream. While there are many reports on the former in the literature, data on the pattern of metabolites reaching the liver are more limited, and are only now appearing in the literature (e.g. from Hurley [11] and the group at the United States Department of Agriculture, Beltsville, Maryland [18]).

The results also raise questions as to the factors limiting intake in the final part of the feeding period (i.e. just before fresh feed is offered), when rumen volume is also not at a maximum (Figs 2 and 3) but cattle appear reluctant to eat (Fig. 4). Both rumen and blood metabolite concentrations are low during this phase and therefore cannot be the cause of the inhibition, but work performed by the authors in collaboration with a physiologist (J.W. Sissons et al., Animal and Grassland Institute, United Kingdom, unpublished observations) demonstrated that the muscular activity of the rumen (estimated from myoelectric recordings) tended to be maximal during the early hours of the morning. Thus another mechanism, related to the physical removal of digesta from the rumen, may operate at this point in the feeding cycle.

Before further progress can be made in developing a model to predict intake, based on the individual control mechanisms, more information on the daily patterns of intake, rumen volume and motility and concentrations of VFAs in the rumen and of metabolites in the portal blood are required.

5. SUMMARY

Increasingly there is a need for research to concentrate less on single discipline approaches and to investigate problems using different combinations of disciplines. When integrating knowledge on processes occurring within the animal, nutritionists can benefit through collaboration with physiologists, biochemists and microbiologists. However, research involving multidisciplinary teams must be structured and there is a need for appropriate methodology to aid the process. The application of mathematical modelling techniques to the study of biological problems provides a structured framework which helps to clarify the objectives and the inputs required from each discipline, thus facilitating communication.

Such techniques have been applied at Hurley, leading to collaboration with biochemists on gut and liver metabolism and with physiologists on rumen function. While this work was stimulated by the development of models through to the simulation stage, benefits to the experimental programmes were obtained even at the stage of formulating flow diagrams. This discipline of describing on paper, in a structured manner, the interactions between components of a system can be applied to a range of nutritional problems and should aid both in the integration of existing knowledge and in the prioritization of research objectives.
REFERENCES


PREDICTING NUTRITIONAL STATUS OF RUMINANTS AND RESPONSES TO INPUTS AIMED AT OPTIMIZING FEED RESOURCE EFFICIENCY

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Abstract

PREDICTING NUTRITIONAL STATUS OF RUMINANTS AND RESPONSES TO INPUTS AIMED AT OPTIMIZING FEED RESOURCE EFFICIENCY.

Nutritional status is some measure of the extent to which current feeding meets the animal’s needs. Those needs depend in the long term on the animal’s genetically programmed potential path of growth and reproductive performance and in the short term on the extent to which its current condition diverges from its inherent target. To make responses of animals to inputs of feed more predictable, quantitative assessment of genotype and current condition is required, which calls for a more precise definition of genotype than has been conventional in most current nutritional systems. If properly calibrated, simple methods such as condition scoring of animals can be invaluable in practice for assessing an animal’s current state and, if combined with a quantitative description of potential growth, would be likely to enhance greatly the accuracy of predictions of food intake, diet selection and nutrient partition. Within this framework, the influence of the balance of glucogenic, lipogenic and aminogenic nutrients to sustain growth and lactation is considerable. The efficiency with which feed resources are used to sustain production depends both on supplying an appropriate balance of different major nutrient classes and on the animal’s inherent potential to perform. Evidence is presented that animals selected for high genetic merit for milk production in a temperate environment are both more biologically and economically efficient in terms of feed resource use in systems of production relevant to that environment. Future improvements in matching available feed resources to the needs of indigenous livestock will depend on greater attention to the assessment and improvement of the genotype and condition of animals as well as to better understanding of the qualities of feed.

1. INTRODUCTION

Nutritional status is a term which requires definition. It is some measure of the extent to which current feeding meets the animal’s needs. Those needs are determined principally by genotype but are modified by the animal’s current state and its physical environment. Thus, distortions to body composition alter needs (which are then geared to a return to a preferred composition) and the imposition of pregnancy,
and subsequently lactation, will further modify nutrient demand. These are long term or strategic aspects of nutritional status.

There is also the short term issue as to whether current nutrition is adequate. In this sense, nutritional status is a measure of the extent to which an animal is either under- or malnourished and is therefore capable of responding either to an increase in nutrient supply or to a redress of nutrient balance or of coping with a difficult environment. Alteration in the thermal environment in particular can place demands or constraints on thermogenesis. In such a definition there is no place for the concept of a surfeit of balanced nutrients. That is, if the animal in question is willing to eat a large amount of a perfectly balanced diet, then it both has need for and has capability to dispose of (through metabolism) those nutrients which have been absorbed from its gut.

Nutritional status is therefore inevitably bound up with the animal's inherent demand for nutrients, the nature of nutrients which are available from accessible food and the environment in which the animal is placed. In practice, the value of understanding nutritional status is to improve the management of animal performance through feeding. Many of the possible strategies for manipulating diet composition in order to improve animal performance are dealt with in other papers at this symposium. The purpose here is to present some thoughts on characteristics of animals themselves which may be important in determining the manner in which animals respond to available food and to consider some implications for improving the effectiveness of resource use. Gill and Beever [1] have outlined the values of a modelling approach in nutrition research. While research models may not be suited to field use, the logic of using a modelling approach to build robust frameworks to encapsulate current knowledge cannot be denied, and underlies this paper. Understanding of nutritional status should assist in improving our prediction of responses to feed. It is a matter of either biological or economic judgement as to whether changes in feeding practice and associated animal responses satisfy our criteria for optimal feed resource efficiency.

2. ANIMAL CHARACTERISTICS AND RESPONSE PREDICTION

The manner in which an animal responds to food which is available to it can be expected to depend on characteristics of the animal, of the available food and of the environment in which the food is presented. The characteristics of the animal which are important in determining response include some measures which appear to be easy to obtain (e.g. weight, milk yield and fibre growth) as well as those which are much less easy (degree of maturity, mammary gland size and genetic potential). There are two purposes in describing animal characteristics — to help in the prediction of the amount of food consumed and to help in predicting the partition of nutrients which are produced from food which has been consumed.
2.1. Animal size and food intake

The amount of food which growing ruminants will eat has, conventionally, been scaled to some function of weight. For a relatively digestible food, offered to 25 breeds of British cattle, age related patterns of intake in relation to scaled live weight have been found [2]. Between breeds, food intake scaled to weight$^{0.76-0.87}$ from age 4 to 10 months, but beyond 13 months, scaling was consistent with weight$^{0.73}$. Breeds of large mature size had a larger than average appetite at early ages and smaller breeds a lower than expected appetite at early ages.

For feeds (forages) of low digestibility, the rate of comminution of large fibre particles and passage of smaller particles will influence intake. Illius and Gordon [3] have used a modelling approach based on estimates of digestion kinetics to test the hypothesis that body weight influences the ability of an animal to obtain its energy requirements from forages of low digestibility. They concluded that large animals can obtain a greater proportion of their energy requirements from abundant poor quality food than can small animals and that maximum intake of metabolizable energy scales with weight$^{0.77}$ (i.e. slightly greater than the scaling of maintenance to weight$^{0.73}$). Thus animal weight figures largely in determining food intake with both relatively high and relatively low digestibility feedstuffs.

There may be genetic variation in the rate of passage of food particles through the gut [4]. Whether this correlates with size as Illius and Gordon might predict has yet to be established, but if independent of size it would point to an animal characteristic which has relevance to the individual's capability to survive and perform on a particular diet, especially when it is of lower nutrient density.

In milking animals the rate of milk secretion can also be expected to have a substantial effect on food consumption — although animal weight continues to have a substantial influence in most intake prediction equations which have been described [5, 6].

2.2. Body composition

Scaling to animal weight can be misleading as the composition of the animal's body can vary substantially. Maintenance requirements may be more closely related to body protein mass [7, 8] than weight because of the relatively low maintenance requirements of fat tissue. We [9, 10] have previously given descriptions of rates of maturing and fat:protein ratios (LPR) at a given stage of maturity ($u$) as:

$$\frac{du}{dt} = \text{rate of maturing} = Bu \ln u$$

$$\text{LPR}_u = \text{LPR}_w u^b$$

where $B$ is a genetically determined growth parameter and $b$ a genetically determined
fattening parameter, \( m \) denotes the mature body and \( t \) a time (which is before maturity has been reached); \( u \) is defined as a proportion of mature protein mass, i.e.

\[
u = \frac{P_t}{P_m}
\]

These descriptions apply to growth measured under conditions in which nutrition is not limiting. Quantification of \( B, b, P_m \) and \( \text{LPR}_m \) identifies a genotype.

Although not presented exactly in these terms some descriptions of growth for different sheep [11] and cattle [12] breeds in the United Kingdom have been given, and other general models of growth are available [13]. Such descriptions in quantitative terms will apply only to existing genotypes. Where selection programmes are under way to improve growth and carcass characteristics (e.g. Ref. [14]) reappraisal of growth characteristics would be needed after some generations. For poultry, at least, the Parkes model appears to cope reasonably with growth and food intake of genotypes differing substantially in rate of fattening [15]. But the important point is that without a description of ‘potential growth’ we lack a benchmark against which to judge current status and the potential to respond.

If, as seems reasonable, food intake is substantially determined by the animal’s drive to achieve a certain (genetically determined) rate of performance and body composition it would also be expected that prediction of food consumption would be improved if body composition, rather than weight only, were taken into account.

Food intake (of a highly digestible feed) could be expected to be the amount which the animal needs to eat in order to achieve its target rates of ash, protein and fat deposition at a given stage of maturity. Emmans [16] used data from a genetic selection experiment with Suffolk sheep [14] to compare actual food intakes with those predicted from (a) a sheep intake prediction equation [17], (b) an estimate [17] of metabolizable energy (ME) requirements to meet measured growth rates, and (c) estimated rates of protein and lipid accretion together with estimated parameter values for \( B, b, \text{LPR}_m \) and \( P_m \). Whilst the predictions from either of approaches (a) and (b) [17] were very poor, those based on approach (c) [16] were very good indeed. Knowledge of genotype, in quantitative terms, is therefore of great value in improving the predictability of food intake.

Weston [18] has used the concept of ‘relative energy deficit’ to discuss the extent to which rumen capacity can vary in animals offered poor quality feeds. Relative energy deficit is a conceptual measure of the extent to which (metabolizable) energy yield from food eaten falls short of the energy intake the animal is striving to achieve to follow its target performance. Rumen fill appears to be positively correlated with relative energy deficit. Post-ruminal supplementation with highly digestible nutrients has been found (for a given intake of poor quality forage) to decrease rumen fill and to increase the rate of rumen digesta passage in lambs [19]. This adds credence to the concept that knowledge of an animal’s ‘target’ performance is a necessary prerequisite of a full understanding of nutritional status.
For purposes of assessing nutritional status as much effort should be given to the assessment of animal characteristics as to the assessment of food characteristics. Such characteristics also have great value in defining selection objectives in breed improvement programmes.

2.3. Measurement of body composition in vivo

While it may be desirable to know current body composition in order to get a better understanding of current nutritional status the in vivo measurement of body composition is difficult. The two approaches which are most widely used are forms of ultrasonic scanning [20] or condition scoring [21, 22]. Both require calibration against direct measurements of composition after slaughter. The precision of the calibration and that of the values which are determined by scanning (usually the depth of fat and muscle at a defined lumbar position) determine the precision of the estimated body composition using scanning. Subject to these sources of error the techniques are quantitative but require relatively sophisticated equipment. Correlations between ultrasonically measured back fat depth and eye muscle area and actual body composition in sheep have been of the order of 0.6 [20] — use of computerized tomography can increase this precision to a correlation of approximately 0.9. Body condition scoring is a more subjective measurement and so is not strictly quantitative — although if carefully done by experienced operators, calibrations against direct measurements can be quite precise [23]. The big advantage of condition scoring is that no equipment is needed, only trained personnel.

Relationships between body fat and condition score (5 point scale) are not, however, constant across breeds. For example, relationships between condition score (CS) and body fat for Friesian and Hereford × Friesian cattle [23] were:

\[
\text{Friesian} \quad \text{fat (kg)} = 84.1 \text{ CS} - 71.1 \quad R^2 = 0.869
\]

\[
\text{Hereford} \times \text{Friesian} \quad \text{fat (kg)} = 52.6 \text{ CS} - 22.1 \quad R^2 = 0.906
\]

Practical application of this approach requires calibration against direct measurement of fatness for particular animal populations.

2.4. Nutritional status and diet selection

The relationship between rumen fill and ‘relative energy deficit’ [18], though difficult to quantify, shows that the ruminant animal will strive to achieve its target for performance when the feed resources available to it are limited in quality. If a variety of feeds are on offer, as is often the case under grazing or rangeland conditions, diet selection might be expected to be such as to minimize constraints to the achievement of the animal’s target performance.
That non-ruminants can display nutritional wisdom is now well established — although some care is needed in the design of experiments to test this issue [24]. When young pigs were made relatively fat or relatively lean by feeding single feeds of high or low protein:energy ratio [25] and were then offered a choice of the high and low nutrient density feeds the fat and thin animals differed markedly in their diet selection. In the course of the experiment the differences in body composition between the two groups of pigs were substantially reduced and, as this happened, their selected diets came to be more similar. Through some mechanism it therefore

<table>
<thead>
<tr>
<th>Species</th>
<th>CP concentration in feed</th>
<th>CP concentration in diet selected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feed 1</td>
<td>Feed 2</td>
</tr>
<tr>
<td>Sheep [27]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>234</td>
<td>180</td>
<td>199</td>
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<tr>
<td>234</td>
<td>123</td>
<td>177</td>
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<tr>
<td>234</td>
<td>66</td>
<td>166</td>
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<tr>
<td>180</td>
<td>123</td>
<td>150</td>
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<tr>
<td>180</td>
<td>66</td>
<td>141</td>
</tr>
<tr>
<td>123</td>
<td>66</td>
<td>111</td>
</tr>
<tr>
<td>Pigs [30]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>267</td>
<td>213</td>
<td>218</td>
</tr>
<tr>
<td>267</td>
<td>174</td>
<td>205</td>
</tr>
<tr>
<td>267</td>
<td>125</td>
<td>204</td>
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<tr>
<td>213</td>
<td>174</td>
<td>202</td>
</tr>
<tr>
<td>213</td>
<td>125</td>
<td>208</td>
</tr>
<tr>
<td>174</td>
<td>125</td>
<td>160</td>
</tr>
<tr>
<td>Poultry [31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>300</td>
<td>304</td>
</tr>
<tr>
<td>300</td>
<td>180</td>
<td>191</td>
</tr>
<tr>
<td>180</td>
<td>125</td>
<td>166</td>
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<tr>
<td>180</td>
<td>70</td>
<td>177</td>
</tr>
<tr>
<td>125</td>
<td>70</td>
<td>122</td>
</tr>
</tbody>
</table>
seems that the animal can recognize its fatness in relation to a preferred ‘target’ fatness and it has a nutritional drive to reinstate its target fatness if such is possible from available resources. Even when offered single feeds, cattle which are fat or thin will eat different amounts, which would be expected to adjust their fatness [26].

We are currently exploring further the relationships between body composition, genotype and food selection in both pigs and sheep. We have some indications that sheep are capable of making structured selections amongst foods of differing nutrient (protein) density [27] and that they are willing, at least to some extent, to work to defend choices which they have made freely [28]. However, it is not clear what drives the ruminant animal to make particular diet selections. In our experience (Table I) the protein density of a diet which sheep will select freely is high in comparison with conventional estimates of requirements of growing ruminants for protein [29]. In comparison with similar data from pigs [30] and poultry [31], though, the pattern of selection does strongly suggest that there is an animal rationale for the choices being made, although for ruminants the sensitivity of the animals in making structured selections seems to be less precise than in the case with pigs or poultry.

Under grazing or rangeland conditions it is well established that ruminants will select amongst various plant species — and different ruminant species will make different selections [32, 33]. It might be expected that some element of the differences in selection is geared to animal size [3] and to anatomical differences in the grazing machinery of different species (which itself may be allometrically related to size) [34].

The extent to which different proportions of plant species eaten confer a nutritional advantage is likely to be important in the animal’s overall strategy. Camels and goats display greater facility for selecting diets of high nutrient density than do cattle and donkeys [32]. The extent to which these differences are consistent with the size scaling approaches of Refs [2] and [3] presents an interesting issue for investigation.

Adoption of a choice-feeding approach to enable animals to select a diet which most closely meets their needs for achievement of ‘target performance’ may be a simple and effective way to allow sensible estimates of nutritionally unconstrained growth to be achieved. The diets on offer do, however, need to be of high digestibility.

2.5. Lactation and nutritional status

Functional lactation adds nutritional demands which are primarily driven by inherent capabilities to secrete milk. Differences in mammary cell number and secretory activity both feature in phenotypic variation in mammary secretion, the balance between these two elements differing somewhat amongst species [35].

The characteristics of the lactation curve which allow description of ‘potential’ might be given in a number of different ways [10, 36–38]. Genetic parameters which
describe the shape of the lactation curve are not yet available — but perhaps deserve
attention. Measures of genetic merit for performance (yield of major milk constitu­
tuents) are available for sires and for females in a number of countries. These criteria
for the assessment of progeny performance are reliable [39]. It would be an attractive
proposition to use such genetic descriptions to find ways to facilitate an understand­
ing of potential nutrient demands — and hence nutritional status in a given feeding
environment.

2.6. Body fatness and performance responses

Fatness of an animal at a weight, or at an age, has implications for the animal’s
needs. As already said, in pigs, body fatness affects dietary choice, when there is
freedom for such to be exercised [25].

Body fat can be used to advantage in production systems where feed resources
(and/or their associated costs) fluctuate substantially. Judicious use of low degrada­
bility (‘bypass’) protein with low energy density feeds can be used to manipulate car­
cass composition in animals previously maintained in fat condition [40].

In lactating animals (cows) body fatness, measured as condition score at calv­
ing, certainly influences the nature of the animal’s response to food in the early
stages of lactation [41-43]. Thin cows eat more food, although do not necessarily
yield more milk, than fat cows in early lactation.

<table>
<thead>
<tr>
<th>Back fat group: F1</th>
<th>F2</th>
<th>F3</th>
<th>Gross efficiency (MJ milk/MJ ME intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MY1</td>
<td>107</td>
<td>104</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.45</td>
<td>0.44</td>
<td>0.41</td>
</tr>
<tr>
<td>MY2</td>
<td>88</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>MY3</td>
<td>67</td>
<td>73</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>0.36</td>
<td>0.34</td>
</tr>
</tbody>
</table>
The manner in which body fat is used in support of lactation does, however, depend also on the propensity of animals to yield milk. In studies of lactational performance of cows of different genetic merit (for fat + protein yield) both milk production and gross energetic efficiency have been found to be greatest amongst high yielding cows which are relatively fatter at calving; but, conversely, and importantly, for cows of lower yield, the poorest milk yields and lowest efficiencies are found with cows which are fatter at calving (Table II) [44].

High genetic merit cows which are fat at calving use their reserves to sustain milk production; low merit cows which are fat at parturition tend to remain so, and their fatness may even compromise milk output.

2.7. Nutrient partition

Genotype, current body composition and nutrient balance all can influence nutrient partition. The exploitation of variance in production traits for purposes of genetic improvement recognizes the extent to which animals differ, both in the amount of food which they eat and in the manner in which nutrients from that food are used. So, for example, selection in sheep to increase size and leanness [14] chooses, for breeding, those animals which grow protein and deposit fat in different proportions, at a given age, from their contemporaries. Selection for yield of major milk solids (see later) does likewise. In these instances both intake and nutrient partition may vary. Broster [45] has nicely illustrated that there are big differences between individuals offered a fixed amount of food, in the manner in which nutrients are used; the main partition here is between use of nutrients for milk and body tissue deposition. But there are also differences between animals in the partition of energy and nutrients between different milk constituents (fat, protein and lactose) — hence variation in milk composition.

Apart from conventional methods for describing genetic merit, and the possible description of lactation curve parameters, there are no readily available measures of animal characteristics which will facilitate assessment of lactational potential (i.e. the relative partition of nutrients between milk and body). Juvenile predictors of merit such as the relative responsiveness of growth hormone release to standardized challenge using growth hormone releasing factor or thyroid stimulating hormone [46] show some promise, but are complex and are designed for sire selection, not current stock evaluation.

Simple ‘metabolic profiles’ using spot assessment of blood metabolic hormone and/or mineral concentrations have little value for assessing genetic merit [47–49] — but perhaps a little value for assessing nutritional status. However, this is mainly limited to identifying lack (or excess) of particular minerals, or a very general estimate of the adequacy of energy provision.

For a given genotype, current body composition will influence the partition of the next intake of food. If an animal is currently of a composition which fits its
growth path (including the lactating animal), partition of nutrients should be expected to allow further progression of that path. If the balance of nutrients provided is not ideally suited to that purpose, divergence from the preferred path can be expected. Conceptually, this helps identify the extent to which nutrient provision is 'well balanced' or not — but can only be interpreted as such if the animal's growth path has been identified. Alternatively, for an animal whose current body composition is different from the 'target' then partition of the next intake of nutrients might be expected to be used to return to the preferred growth path. It becomes possible for a food which is not well balanced for maintaining 'normal' growth and fattening to be apparently well balanced for redressing a previous distortion of body composition.

These are concepts, not mechanisms. The factors which are involved in the metabolic and physiological control of growth and of lactation have been copiously described [50-52].

3. OPTIMIZING FEED RESOURCE EFFICIENCY

3.1. Nutrient balance and efficiency

In growth it has long been argued [53] that in ruminants the balance of glucogenic and lipogenic nutrients made available through absorption determines the overall efficiency with which ME is used for growth. We [10, 54] have seen that feeds of equal metabolizability \( q \) can be used for growth of lambs with different energetic efficiencies, depending on the form of the major carbohydrate source in the diet, efficiency being lower with a feed (sugar beet feed) of low starch content in comparison with barley. Under these circumstances it appeared that the activity of the acetate-acetyl-CoA substrate cycle was enhanced when energetic efficiency for growth was reduced [55] although, by regression analysis, it appeared that the enhanced heat production with the higher fibre food was more related to energy costs of protein deposition than to fat deposition [54]. Subsequent studies have shown that the enhanced activity of the acetate-acetyl-CoA cycle is also sensitive to the rate of protein supply in the diet [56] and this is consistent with theories previously put forward [53].

In growing cattle, it has been shown that the inclusion of fish meal in diets based on straw enhances growth rate at constant digestible energy intake [57], which must imply an influence of additional protein supply on the energetic efficiency with which absorbed nutrients are being used. Further studies [58, 59] have shown that enhanced efficiencies of use of straw based diets by growing cattle consequent on the addition of either cereal and/or fish meal are closely associated with alterations in the supply of protein to the small intestine. The balance of nutrient provision
between glucogenic, lipogenic and aminogenic nutrients therefore has clear implications for the efficiency with which ME is used in growth.

As the energy cost of synthesizing protein is much higher than that of synthesizing fat [60], enhancement of amino acid supply to the growing animal holds possibilities either for increasing or for decreasing metabolic heat production. If the consequence of enhanced amino acid provision is to increase the rate of protein accretion, possibly with a reduction in fat deposition, heat production will be expected to increase with amino acid supply. Alternatively, if supply of amino acid results in more efficient use of fat precursors for fattening (the MacRae and Lobley [53] proposition) heat production would be expected to decrease.

The issue of nutrient balance is not restricted to growing animals. A general proposition on the influence of nutrient balance on lactational performance was given some years ago [61]. From studies with lactating rats we have been able to show that certain mixtures of fat:carbohydrate and protein appear to pose particular problems in allowing mothers to support adequate lactation [62, 63]. An initial description of a thermodynamic model to represent these events [64] suggests that the animal’s capacity to dispose of metabolic heat may be an important determinant in the associations between food consumption, secretion of milk and the use of body reserves. Such observations are, to some extent, consistent with those reported by Leng [65], who has suggested that, in dairy cattle in India, regulation of nutrient balance to the tissues may be a key factor in regulating metabolic heat and thereby allowing animals to consume diets at high rates and maintain high rates of production of milk, even in warm conditions.

4. GENETIC SELECTION AND FEED RESOURCE EFFICIENCY

By making genetic selections for improved rates of animal performance (growth rate, milk yield, etc.) it should be expected that nutritional efficiency would increase with the rate of performance on the basis that maintenance costs would be likely to be diluted. In the choice of appropriate selection objectives to meet the local market conditions for particular production systems, genetic correlations between a selected trait and an associated trait and the particular animal population from which selections are most suitably made are factors which make this broad generalization rather more complex in particular detail.

There are relatively few studies to show the long term consequences for efficiency of feed resource use which follow from genetic selection. Those best known to this author come from dairy cows [66, 67].

We are currently part-way through a major long term evaluation of the consequences of selection for high rates of milk solids output in dairy cows on the efficiency of production in two feeding systems and over the major part of the animal’s
TABLE III. LACTATIONAL PERFORMANCE, FOOD INTAKE, BIOLOGICAL EFFICIENCY AND A MEASURE OF PROFITABILITY IN DAIRY COWS OF AVERAGE (500) OR HIGH (700) COW GENETIC INDEX (CGI) AND MANAGED IN SYSTEMS OF FEEDING CHARACTERIZED BY A FORAGE:CONCENTRATE RATIO (DM BASIS) OF 45:55 (HIGH CONCENTRATE) OR 20:80 (HIGH FORAGE) (see text and Refs [68, 69] for details)

<table>
<thead>
<tr>
<th>Predicted CGI:</th>
<th>High forage</th>
<th>High concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>6030</td>
<td>7032</td>
</tr>
<tr>
<td>700</td>
<td>6164</td>
<td>700</td>
</tr>
</tbody>
</table>

| Milk yield (kg) | 5309 | 6030 | 6164 | 7032 |
| Fat + protein (kg) | 406 | 463 | 466 | 512 |
| Dry matter intake (kg) | 4010 | 4065 | 4408 | 4645 |
| Efficiency (MJ milk/MJ ME intake) | 0.367 | 0.414 | 0.366 | 0.388 |
| Margin over all food costs (£) | 599 | 732 | 643 | 759 |

productive life (lactations 1–3). Initial results from the first two years of this study are summarized in Table III [68, 69].

The table shows data generated from regressions of input and output traits on predicted cow genetic index (CGI; a measure of the genetic merit for fat + protein yield of the female). The particular values of 500 and 700 for CGI are chosen to represent cows of current average merit and those of high merit, respectively, in the United Kingdom. The two systems of feeding are each based on complete mixed diets containing grass silage:brewer’s grains:concentrate in the proportions (DM basis) 50:5:45 (high concentrate) or 75:5:20 (high forage) respectively. At the average rates of food intake achieved these systems approximate to the consumption of 2.5 or 1 t of concentrate per cow per lactation respectively. The results therefore apply to systems of production which are relevant to highly developed agriculture in temperate circumstances.

Clearly, however, in either system of production, animals of higher genetic merit yield more milk and milk solids (which is to be expected as yield of milk solids is the selection objective in this study), eat a little more food (more so in the higher concentrate feeding system) and are more biologically efficient. For milk values and feed costs which currently pertain in the United Kingdom, there is also a major advantage in economic efficiency with animals of higher merit.
A major challenge to the nutritionist is to ask what needs to be known about both food and the cow to make these results predictable. A particularly interesting outcome is the comparison between high merit cows on high forage and average merit cows on high concentrate. Secretion of milk fat plus protein (kg) is essentially equivalent, yet nutrient intake is clearly substantially greater with the high concentrate (lower merit) animals. Such an outcome could not be predicted from systems of response prediction which are solely substrate driven. The inherent characteristics of the cows are clearly of major importance in determining these outcomes.

5. SUMMARY/CONCLUSION

Both for a better understanding of nutritional status and for the prediction of responses to nutrients, nutritionists need to develop better quantitative descriptions of characteristics of animals which are important in determining the amount of food which can be eaten and the manner in which nutrients from that food will be used. Descriptions of animal growth and lactation which recognize genetic characteristics are needed and should be quantitative.

The measurement of the current body composition of animals is also important. Simple techniques such as condition scoring can be very effective in the field to address this issue but need careful calibration for each particular species and breed.

With sufficient descriptions of animal needs, progress towards effective response prediction will be possible. It will be most useful if such approaches recognize at an early stage their linkage to advances which can be made through genetic selection programmes. In this way, developments in nutritional science, which are substantially geared to improvements in response prediction, and in genetics can be brought together in ways which will benefit producers and which will lead to improvements in feed resource use.

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IMPROVING THE NUTRITIONAL STATUS OF SMALLHOLDER LIVESTOCK IN AGROSILVICULTURAL SYSTEMS IN SEMIARID SOUTHERN AFRICA

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Abstract

IMPROVING THE NUTRITIONAL STATUS OF SMALLHOLDER LIVESTOCK IN AGROSILVICULTURAL SYSTEMS IN SEMIARID SOUTHERN AFRICA.

Livestock are an important source of household food security in semiarid areas, as well as a source of cash income (through sales) and, in some instances, make significant inputs to food crop production through provision of draught power and manure. The productivity of the livestock is greatly constrained by the lack of good quality feed in the long dry season, particularly between the months of August and October. Planted forage legumes (e.g. groundnut hay, lablab, cowpea and pigeon pea) have been used as supplements to cereal crop stover with resultant increases in total dry matter intake. Often substitution, instead of supplementation, occurs. On-farm trials have shown an increase in milk yield due to addition of the legume stovers and/or sorghum bran. Supplementation with legume forage did not reduce post-partum anoestrus in smallholder flocks. Indigenous browsable tree species represent a potentially beneficial feed resource that is poorly utilized. Cattle and goats have been found to rely on these species during the critical dry season. However, very little research has been done on their quality and utilization by livestock and on their agronomic requirements with a view to improving their propagation.

1. INTRODUCTION

Livestock are an important component of household food security in semiarid southern Africa. Low (600 mm or less) and erratic rainfall coupled with fragile sandy soils often results in poor crop harvests. Livestock have several functions in these systems, including provision of draught power and manure for food crop production, supply of meat, milk and fibres, and raising of cash income through sales.

However, productivity of livestock in these areas is often constrained by the quantity and quality of feed available. The availability of green forage is seasonal and since most of it is unimproved native pasture, quality quickly declines at the end of the rainy season. Crude protein levels have been found to decrease from 10% in January to 3% or less in August–October [1]. The concomitant neutral detergent
insoluble fibre (NDF) increase results in low digestibility and intake of these pastures. Mineral and vitamin deficiencies also exacerbate the nutritional inadequacy of feed resources in these areas [2].

Smallholder livestock production systems in southern Africa are mainly extensive and are largely dependent on non-cultivated, non-harvested forages during the rainy season (November–March). In the dry season crop by-products augment the feed resource base available to livestock. Indigenous tree species play a prominent role as the only source of green forage during the dry season. Both crop by-products and browsable tree species are underutilized and unexplored in most of semiarid southern Africa.

Strategies for improving the nutritional status of smallholder livestock should aim at devising simple technological packages that increase the appropriate amount and balance of nutrients absorbed by the animal with minimum increase in cash outlay by the farmers. To achieve this an appreciation of the ruminant ecosystem is essential.

2. BASIC CONCEPTS OF THE RUMINANT ECOSYSTEM

Highly fibrous feeds, such as those available to the smallholder livestock, are mostly degraded in the rumen. The priority for improving the nutritional status of such livestock should therefore be the optimization of the fermentative processes in the rumen. This can be achieved by supplying sufficient microbial nutrients to ensure that the microbes grow efficiently and thus ferment the forages to the maximum possible. The microbial cells so produced are the main source of protein to the animal while the volatile fatty acids produced are the major source of energy. Microbes in the rumen require fermentable substrates such as cellulose, hemicellulose, starch and other sugars plus a source of nitrogen, minerals and some vitamins. Native mature pasture will provide fermentable substrates (often in sufficient quantities) but its use is limited by a low content of nitrogen, minerals and vitamins.

Additionally, cellulolytic microbes require pHs ranging from 6.2 to 6.8 and thus buffering the rumen to maintain these pH conditions is important. Tropical grasses have a low buffering capacity and supplementation with fast fermenting carbohydrates will result in reduction of pH to levels below the optimum for fibre digestion [3].

Furthermore, feeds that supply critical nutrients (such as protein, lipids and other glucogenic nutrients) which escape rumen fermentation to augment and balance the nutrients absorbed by the animals will result in improved productivity [4].

3. SUPPLEMENTARY FEEDING TO IMPROVE PRODUCTION

Use of drought tolerant legume forages as supplementary feed for livestock has been the main mode of improving the nutritional status of livestock in these areas.
Groundnut hay offered at graded levels to goats feeding on a basal diet of veld hay has been found to increase total dry matter intake, increase rate and extent of digestion and increase rate of passage [5]. The increase in total dry matter intake was, however, accompanied by a decrease in intake of the basal diet (Table I). This substitution effect is unfortunate since the groundnut hay is available in limited amounts compared with the veld hay and therefore should only be used as a supplement. A supplement in this context is taken as a feed that is added in small quantities to a basal diet in order to augment or balance deficient nutrients in the basal diet without reducing intake of the basal diet.

* Dolichos lablab has recently been introduced in the region and is being tested in on-farm experiments. Smallholder dairy farmers in Botswana have been encouraged to grow lablab on 1 ha of their land [6]. This is harvested and fed to lactating crossbred and indigenous cows at rates of 85:15 sorghum stover:lablab (by weight as fed). A trial to compare this feeding system with two others — one using un-supplemented sorghum stover and the other using moroko (sorghum bran), a by-product of a traditional processing technique for sorghum grain — showed higher intake and milk yield for the moroko system (Table II) [6]. Presumably moroko also supplied rumen undegraded glucogenic nutrients or lipids to the animals, as has been discussed for rice bran [4]. The lack of improvement in the lablab system was due to the low level of inclusion of lablab which was insufficient to increase rumen degradation of the stover. The level was based on realistic estimates of the lablab produced by the farmers.

<table>
<thead>
<tr>
<th>Groundnut hay on offer</th>
<th>Groundnut hay</th>
<th>Veld hay</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>135</td>
<td>130</td>
<td>300</td>
<td>430</td>
</tr>
<tr>
<td>270</td>
<td>230</td>
<td>300</td>
<td>530</td>
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<tr>
<td>405</td>
<td>330</td>
<td>230</td>
<td>560</td>
</tr>
<tr>
<td>900</td>
<td>670</td>
<td>130</td>
<td>800</td>
</tr>
</tbody>
</table>

TABLE I. DRY MATTER INTAKE OF GROUNDNUT HAY AND VELD HAY IN GOATS AS AFFECTED BY LEVEL OF GROUNDNUT HAY ON OFFER (g/d) [5]
TABLE II. INTAKE AND MILK YIELD OF SMALLHOLDER SIMMENTAL CROSS (SX) AND TSWANA (TS) COWS FED ON SORGHUM STOVER UNSUPPLEMENTED OR SUPPLEMENTED WITH *Dolichos lablab* OR SORGHUM BRAN [6]

<table>
<thead>
<tr>
<th></th>
<th>Sorghum stover</th>
<th>Sorghum stover (85%) + lablab (15%)</th>
<th>Sorghum stover (75%) + bran (25%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kg/d)</td>
<td>9.93</td>
<td>8.27</td>
<td>8.27</td>
</tr>
<tr>
<td>Milk yield</td>
<td></td>
<td>1.5</td>
<td>2.70</td>
</tr>
<tr>
<td>(kg/d)</td>
<td></td>
<td>8.27</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.21</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.21</td>
<td>2.1</td>
</tr>
</tbody>
</table>

TABLE III. INTAKE AND MILK YIELD OF SIMMENTAL CROSS (SX) AND TSWANA (TS) COWS FED SORGHUM STOVER SUPPLEMENTED WITH LABLAB HAY AND SORGHUM BRAN IN VARYING RATIOS [7]

<table>
<thead>
<tr>
<th>Diet</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>SX</td>
<td>2.46</td>
<td>2.46</td>
<td>2.50</td>
<td>2.24</td>
</tr>
<tr>
<td>TS</td>
<td>2.46</td>
<td>2.43</td>
<td>2.50</td>
<td>2.24</td>
</tr>
<tr>
<td>SX</td>
<td>2.43</td>
<td>2.43</td>
<td>2.50</td>
<td>2.24</td>
</tr>
<tr>
<td>TS</td>
<td>2.43</td>
<td>2.43</td>
<td>2.50</td>
<td>2.24</td>
</tr>
<tr>
<td>SX</td>
<td>2.50</td>
<td>2.50</td>
<td>2.24</td>
<td>2.24</td>
</tr>
<tr>
<td>TS</td>
<td>2.50</td>
<td>2.50</td>
<td>2.24</td>
<td>2.24</td>
</tr>
<tr>
<td>SX</td>
<td>2.24</td>
<td>2.24</td>
<td>2.24</td>
<td>2.24</td>
</tr>
<tr>
<td>TS</td>
<td>2.24</td>
<td>2.24</td>
<td>2.24</td>
<td>2.24</td>
</tr>
</tbody>
</table>

Dry matter intake (kg/100 kg live weight/d)
Milk yield (kg/d)

A subsequent trial [7] used a higher level of inclusion (Table III) and attempted to integrate both lablab and moroko as supplements. Intake was not significantly affected by treatment. However, milk yield in Simmental crossbred animals increased with the proportion of moroko, although the indigenous Tswana did not show such a response (Table III).
Goats are abundant in semiarid southern Africa and are mostly owned by smallholder farmers. Despite the high reproductive potential of the indigenous small stock, smallholder flocks have low reproductive performance [8, 9] with kidding intervals of up to 382 d. Nutrition has been cited as a possible cause, particularly in the dry season. In an attempt to improve the nutrition of does kidding in the dry season, farmers planted lablab intercropped with their maize/sorghum crop. The lablab was harvested and stored for supplementing does kidding in the dry season at 300 g/d per doe. Some of the does kidding at this time did not receive any supplement. Blood samples were collected weekly up to 20 weeks post-partum from all does kidding in this period and assayed for progesterone levels using the radioimmunoassay technique. Preliminary results indicated that none of the does were cycling 140 d post-partum, irrespective of provision of supplement [10]. It would appear that other environmental parameters could be involved in the resumption of cyclicity in does in addition to feed supply.

Other legume forages (e.g. pigeon pea and cowpea) have limited dry matter yield and their use as livestock feed has not been extensively researched in southern Africa.

<table>
<thead>
<tr>
<th>TABLE IV. BROWSE SELECTION BY GOATS IN DIFFERENT MONTHS OF THE YEAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree species</td>
</tr>
<tr>
<td>January</td>
</tr>
<tr>
<td>February</td>
</tr>
<tr>
<td>March</td>
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<tr>
<td>April</td>
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<td>May</td>
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<tr>
<td>June</td>
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<tr>
<td>July</td>
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<tr>
<td>August</td>
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<tr>
<td>September</td>
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<tr>
<td>October</td>
</tr>
<tr>
<td>November</td>
</tr>
<tr>
<td>December</td>
</tr>
</tbody>
</table>
TABLE V. BROWSE SELECTION BY CATTLE IN DIFFERENT MONTHS OF THE YEAR

<table>
<thead>
<tr>
<th>Tree species</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica</td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td>Leaves</td>
<td>Pods</td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acacia tortillis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dry leaves and pods</td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grewia bicolor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combretum apiculatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colophospermum mopane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Dichrostachys cinerea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pods</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

4. POTENTIAL OF BROWSE AS SUPPLEMENT FOR LIVESTOCK

Most research in southern Africa has used planted legume forages as supplementary feed in semiarid areas. Relatively less has been done with browse/tree species, and even less research has been done on the indigenous browsable tree species. A recent survey [11] indicated that both cattle and goats depend on browse for over six months of the year in semiarid areas of Zimbabwe (Tables IV and V). Preliminary laboratory analysis has indicated that the browsed tree species are high in crude protein and low in NDF content. An on-station trial to incorporate *Colophospermum mopane* leaves in the diets of goats did not result in intakes sufficient for maintenance of body weights of the animals [12]. However, goats and cattle on the veld utilize significant amounts of mopane [11]. This emphasizes the need for...
TABLE VI. EFFECT OF SEASON ON INTAKE AND QUALITY OF DIET SELECTED BY GOATS FORAGING ON A Colophospermum mopane WOODLAND IN A SEMIARID AREA OF ZIMBABWE [13]

<table>
<thead>
<tr>
<th></th>
<th>December to January</th>
<th>February to March</th>
<th>April to May</th>
<th>June to August</th>
<th>September to November</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake</td>
<td>45</td>
<td>59</td>
<td>35</td>
<td>55</td>
<td>51</td>
</tr>
<tr>
<td>(g/kg BW$^{0.75}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic matter</td>
<td>83</td>
<td>87</td>
<td>88</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td>(% DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>(% organic matter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>80</td>
<td>72</td>
<td>75</td>
<td>59</td>
<td>67</td>
</tr>
<tr>
<td>(% organic matter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>13</td>
<td>10</td>
<td>10</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>(% organic matter)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Further research on these species in order to understand the physicochemical principles underlying their utilization in the range. Nuclear techniques would be of great value in establishing nutrient flow in such studies.

There is a need to measure intakes of smallholder animals in the veld to determine feed quality and quantity. Using oesophageally fistulated goats, it has been shown that the animals select a high quality diet throughout the year (Table VI) but the dry matter intake is low [13]. Consequently, use of expensive protein supplements is not the solution. Improved intake of the available low quality roughages is required. Judicious use of the browsable tree species through proper management promises to be a better method. For instance, appropriate levels of supplementation have not been established. Some work has shown that feeding Acacia tortillis pods at 400 g/d improved animal productivity.

Research is still required on the processing of different tree parts as feed for livestock. Pods are available throughout the dry season but the animals feed on them unprocessed. It is likely that, despite their high nutritive value, their utilization is limited if fed unprocessed. Grinding A. tortillis pods resulted in increases in dry matter loss of the feed from nylon bags incubated in the rumen of goats grazing an area in which Acacia species were predominant (Table VII) (L.M. Sibanda, unpublished data, 1990).
TABLE VII. LOSS OF DRY MATTER (%) OF *A. tortillis* PODS FROM NYLON BAGS INCUBATED IN GOATS BROWSING *Acacia* AS AFFECTED BY PROCESSING

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground seeds</td>
<td>21</td>
<td>49</td>
<td>62</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>Unground seeds</td>
<td>12</td>
<td>25</td>
<td>32</td>
<td>35</td>
<td>36</td>
</tr>
</tbody>
</table>

Note: For ground seeds, \( p = 21.11 + 47.87(1 - e^{-0.073t}) \); for unground seeds, \( p = 12.42 + 23.22(1 - e^{-0.072t}) \), where \( p \) is the amount degraded at time \( t \).

It is known that tannins affect feed digestibility and the high crude protein levels of browsable indigenous tree species may not be available to the animal, depending on the types and quantities of tannins present. No detailed analysis on the tannin content of the indigenous browsable species has been found. Such knowledge would assist in selecting nutritive species which could be multiplied. It is noteworthy that in most countries in this region, exotic tree species like *Leucaena leucocephela* are being introduced before indigenous species have been evaluated.

5. CONCLUSION

Planted forage legumes that are drought tolerant have been used to improve productivity of livestock in semiarid areas. Very little research has been done on the use of indigenous browsable tree species, which are an important feed resource base.

REFERENCES


TECHNIQUES FOR MEASURING WHOLE BODY ENERGY EXPENDITURE OF WORKING ANIMALS: A CRITICAL REVIEW

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Abstract

TECHNIQUES FOR MEASURING WHOLE BODY ENERGY EXPENDITURE OF WORKING ANIMALS: A CRITICAL REVIEW.

All feasible methods for determining the whole body metabolism of draught animals are indirect and most involve measuring gaseous exchange. The relationship between gaseous exchange and energy metabolism is discussed and the open circuit system described. However, in its classic form it can be applied to draught animals only when they are resting in a respiration chamber or at work on a treadmill or circular race. Three portable devices for measuring the oxygen consumption of animals working in fields are described. All involve the use of an airtight face mask so that total respiratory volume can be measured and samples of inspired and expired air taken for analysis. Although all three devices work well in a technical sense, users often experience difficulty in getting experimental animals to behave normally when wearing the face masks and the measuring systems can become inaccurate if the animals start to pant. The theory and applicability of two tracer methods are discussed. Labelled carbon methods appear not to be very accurate and involve continuous infusion of label. They may be useful for determinations lasting a few hours. The double and triple labelled water methods may find application for measurements over one or two weeks now that several of the objections to the use of these methods on large ruminants have been met. However, both types of tracer method measure only CO₂ output, from which energy consumption has to be inferred, and the latter method is very expensive. Two other methods involve counting the number of heart beats and measuring the type and amount of physical activity of the animal. Both methods rely heavily on data from laboratory studies to link these parameters to energy expenditure. The validity of these methods and the techniques for collecting the relevant data from the animals are briefly discussed.
1. INTRODUCTION

Measurement of the heat production and/or energy consumption of animals, including human beings, has been a preoccupation of physiologists for over two hundred years [1]. Most of the early attempts were made simply to determine the source of animal heat [2, 3], but once it became firmly established that all heat produced by animals comes ultimately from the oxidation of food then such measurements were directed increasingly towards finding the energy costs to the animal of performing various functions. These functions fall into two broad categories, those associated with various aspects of digestion, metabolism and efficiency of utilization of food [4] and those associated with movement and the performance of work. The former group is generally easier to study because the experimental subject usually stays in one place. The latter by definition involves taking complex measurements while the animal is in motion.

In recent years there has been a resurgence of interest in the energy metabolism of working animals as it has become apparent that they will continue to provide much of the power used on farms in developing countries for the foreseeable future. However, the food energy requirements of such animals are large and land for grazing becomes scarcer as the human population grows [5]. A knowledge of the energy expenditure of such animals under as wide a range of conditions as possible is of great use in devising more efficient ways of employing them and of making the best uses of the food resources available.

2. DIRECT MEASUREMENT OF HEAT OUTPUT

Many methods have been devised to measure directly the heat produced by animals [6]. However, it seems unlikely that any of them would ever prove feasible or useful as a means of measuring the whole body energy metabolism of working animals.

3. INDIRECT MEASUREMENT OF WHOLE BODY ENERGY METABOLISM

All methods which have been applied to draught animals fall into the category of indirect measurement. As the term 'indirect' implies, the energy metabolism is calculated from other quantities which can be more easily measured rather than being measured directly. Chief amongst these is the gaseous exchange of the animal, that is its oxygen consumption and its production of carbon dioxide and, in the case of ruminants, methane. The relative proportions of the components of the gaseous
exchange can be used to infer the proportions of the major nutrients being oxidized by the animal and the actual amounts used to calculate the quantities of these nutrients.

3.1. The quantitative relationship between energy metabolism and gaseous exchange

The following formula for predicting energy metabolism from gaseous exchange may be derived using data drawn up by Brouwer [7], and it appears in theory to be applicable to adult draught animals [8]:

\[ H = 16.2C + 5.1P - 6.5U - 2.0M \]  

where

- \( H \) is the heat produced (kJ);
- \( C \) is the \( O_2 \) consumption (std L);
- \( P \) is the \( CO_2 \) production (std L);
- \( U \) is urinary nitrogen (g);
- \( M \) is the \( CH_4 \) production (std L).

Application of this formula to the 24 h metabolism of a 725 kg ox fed a low protein diet is shown in Table I, from which it can be seen that the last two factors, the methane and urinary nitrogen, have quantitatively little influence on the calculated energy consumption (0.7 and 0.5% respectively) and in most cases can be omitted.

Many of the methods used to measure energy metabolism of working animals in the field measure \( O_2 \) consumption only. In this case, energy consumption may still be calculated if assumptions are made regarding the ratio of \( CO_2 \) produced to \( O_2 \) consumed, also known as the respiratory quotient or RQ:

\[ RQ = \frac{CO_2 \text{ produced}}{O_2 \text{ consumed}} \]

Although the RQ can theoretically vary from 0.7 (oxidation of fat only, e.g. in starvation) to 1.3 (maximum production of fat from carbohydrate) the 24 h average RQ for adult ruminants fed around maintenance level is generally in the range of 0.8–1.0. In the case of draught animals, however, larger variations can occur during work, as shown in Fig. 1. In this case the RQ changed from about 1.0 after the animal’s morning meal to 0.7 after 6 h work. Since \( O_2 \) accounts for about 77% of the calculated energy expenditure, assuming an RQ of 1.0 would cause an error of 7.2% by the end of the day.
TABLE I. CALCULATION OF 24 h ENERGY CONSUMPTION OF A 725 kg OX FED AT MAINTENANCE ON A HIGH CARBOHYDRATE, LOW PROTEIN DIET

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>O₂ consumption</td>
<td>3053 L</td>
</tr>
<tr>
<td>CO₂ production</td>
<td>3002 L</td>
</tr>
<tr>
<td>CH₄ production</td>
<td>213 L</td>
</tr>
<tr>
<td>Urinary nitrogen (estimate)</td>
<td>50 g</td>
</tr>
</tbody>
</table>

Energy consumption

\[
E = (3053 \times 16.16) + (3002 \times 5.09) - (50 \times 6.5) - (213 \times 2.0) \text{ kJ}
\]

\[
E = 49336 + 15280 - 325 - 426 = 63865 \text{ kJ}
\]

Relative importance of the various factors

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>O₂</td>
<td>77.3%</td>
</tr>
<tr>
<td>CO₂</td>
<td>23.9%</td>
</tr>
<tr>
<td>CH₄</td>
<td>0.7%</td>
</tr>
<tr>
<td>Urinary N</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

FIG. 1. Oxygen consumption and CO₂ production (as a percentage of total airflow) and RQ of a 450 kg ox during a day's work.
Before using apparatus which measures $O_2$ only, therefore, it is advisable to take some short term measurements of $CO_2$ as well to find the range of RQ values likely to be encountered. This argument is even more important in techniques which measure $CO_2$ only. The $CO_2$ factor in Eq. (1) is only 24% of the total and changes of only 0.1 in RQ will produce an error of 10% in the calculated energy expenditure.

4. METHODS FOR MEASURING GASEOUS EXCHANGE IN DRAUGHT ANIMALS

4.1. The classic ‘open circuit’ system

The ‘open circuit’ method was one of the first to be devised [9] but could originally be applied only to animals in chambers as the equipment available for measuring gas concentrations required discrete samples of gas for analysis and each analysis took many minutes. It was therefore impossible to follow the rapid fluctuations in the rate of gaseous exchange characteristic of draught animals. The best that could be achieved was to take a continuous sample over an extended period to obtain an average value.

With the advent of modern methods of gas analysis with response times of fractions of a second it became possible to take measurements from working animals wearing ‘leaky’ face masks. The principle of the method, however, is the same as for animals in chambers. Air is passed over the animal at a constant rate and changes in gas concentrations between the ingoing and outgoing air are monitored. In any given period the gaseous exchange is therefore the product of the flow rate and the average concentration difference.

An example of this kind of apparatus which can be used for large draught animals is the one built by Lawrence at the Centre for Tropical Veterinary Medicine (CTVM) in Edinburgh [10] (Fig. 2). Here the constant airflow is provided by a multistage centrifugal pump driven by an induction motor. The air is drawn through a face mask worn by the animal when it is working, or through a respiration chamber when resting. The flow rate for a particular experiment is chosen so that the $CO_2$ concentration in the mixed expired air does not exceed 1% and therefore will not stimulate the animal’s respiration if rebreathed [11]. After drying, a sample of the mixed expired air is passed through one channel of a differential paramagnetic $O_2$ analyser while dried fresh air is passed through the other. Further samples are passed continuously through $CO_2$ and CH$_4$ infrared analysers. The amplified outputs from all these meters are sampled at 5 Hz by a modified personal computer. Values are averaged at suitable intervals and stored for subsequent calculations of gaseous exchange.

The advantages of such systems are that they are reliable and accurate and can be used for measurements over any length of time from a few minutes to days if a
FIG. 2. Classic 'open circuit' gas analysis system at the CTVM, Edinburgh, for use with large draught animals at rest or during work.
respiration chamber is also available. The main disadvantage is that the apparatus
is not portable. This means that when used for working animals, some arrangement
such as a treadmill or circular race must be made so that the animal can stay more
or less in one place. This also precludes the animal’s doing any normal agricultural
work such as ploughing, and although various aspects of the natural environment
such as soil condition and gradients may be simulated by using the circular race and
the treadmill respectively, the technique must remain essentially a ‘laboratory’ one.

4.2. Portable ‘breath by breath’ analysers

At present, ‘breath by breath’ apparatus appears to be the one most favoured
for use with draught animals in the field. No fewer than three systems have been
developed in recent years.

In contrast to the classic open circuit system, in which air is pumped through
a leaky face mask at a constant rate, in these devices the experimental animal wears
an airtight face mask fitted with inlet and outlet valves and a flow meter which mea-
sures the volume of each breath (Fig. 3). In some instruments a sample of expired
air is taken which is a constant proportion of each breath (Fig. 3(a)). The gas concen-
trations of this cumulative sample are thus the averages of the gas expired in a given
time. Total gaseous exchange is calculated from the change in gas concentrations
between the inspired air and the sample multiplied by the total flow. Energy expendi-
ture can be calculated after suitable corrections to the gas volumes have been made
for temperature, pressure and humidity. The principle of this kind of apparatus was first applied to humans [12] and has also been used for oxen by Clar at Hohenheim, Germany [13], as a preliminary to developing an apparatus of the type illustrated in Fig. 3(b).

In this type of apparatus each breath is analysed on the spot. This overcomes the major disadvantages of the proportional sampling devices, which are that (1) changes in metabolic rate during an experiment cannot be followed unless many samples are taken for analysis, and (2) the apparatus cannot be used very far away from a laboratory because it is difficult to preserve gas samples for more than a day or so without their composition changing.

However, the true breath by breath analysers also have intrinsic problems. For example, the response of the \( \text{O}_2 \) sensors and the flow meters must be very rapid even when a temporary reservoir of the sort shown in Fig. 3(b) is used. This usually limits the analysis to one component, with \( \text{O}_2 \) being the one of choice.

Homicke et al. [14] built an apparatus for use with horses. Airflow was detected using a strain gauge pneumotachograph and \( \text{O}_2 \) concentrations were monitored by a fast polarographic \( \text{O}_2 \) electrode. Signals from both sensors were transmitted by radio back to the laboratory for analysis by computer. Such a flow meter is good for this kind of application because it offers virtually no obstruction to the

FIG. 4. The Oxylog fitted to a cow: A, inlet valve and turbine flow meter; B, Oxylog signal processing unit; C, sample bypass.
passage of air. However, because there are no valves and the horse breathes both in and out through the flow meter there is no way to average the concentration of gases in the expired air even over a few breaths and thus the speed of response of the O\textsubscript{2} sensors becomes of paramount importance. Also, the flow meter tends to become contaminated by dust and moisture, which causes its response to become non-linear.

Because of the above problems this apparatus achieved only a moderate success. It remains, however, probably the only feasible approach for an animal whose metabolic rate can change as rapidly and to such an extent as that of a horse. Changes in the metabolic rate of working oxen are less dramatic (up to about 5 times maintenance level as opposed to 15 times for horses) and so the technical problems of breath by breath analysis for these animals are less acute.

The apparatus of Clar [13] mentioned earlier uses a mask made from transparent PVC on a model of a cow's head. It has one inlet valve (45 mm) on either side of the mask and a larger outlet valve (60 mm) in the middle. A PVC cap at the bottom of the mask can be opened to let out saliva and condensed water. The mask is strapped to the animal's head using a close fitting adapter. Between 2 and 3.5\% of the total expired air is drawn from a gas meter by a pump and stored in an aliquot collection bag. Subsequent CO\textsubscript{2} and O\textsubscript{2} concentrations in the samples are obtained by measuring their partial pressures with a blood gas analysis system.

Two other devices for measuring the O\textsubscript{2} consumption of working oxen have recently been developed.

Schroeter at the Imperial College of Science and Technology, London, working in conjunction with AFRC Engineering, Silsoe, Bedfordshire, United Kingdom, has developed a system in which flow rate is measured using a heated pneumotachograph [15]. This avoids problems caused by the condensation of water vapour from the animal's breath. Samples of expired air are passed over polarographic O\textsubscript{2} electrodes in a small reservoir which at any time contains samples from several previous breaths. The size of the reservoir was chosen so that the O\textsubscript{2} concentration changed sufficiently slowly for the electrodes (response: 90\% in 300 ms) to follow it accurately. Signal processing and collection of data are done by a microprocessor based data logger which forms part of the portable apparatus. Total airflow and O\textsubscript{2} consumption are calculated by computer after the data have been off-loaded. The apparatus is currently in regular use at the Holetta Research Station, Ethiopia, of the International Livestock Centre for Africa (ILCA), where it is being used to study the energy metabolism of working cows; it has also been used with camels in Morocco.

Lawrence and Dijkman, working at the CTVM in Edinburgh, approached the problem by adapting an apparatus, the Oxylog (P.K. Morgan Ltd, Kent, United Kingdom), which had originally been designed for use with human beings [16] and which has proved reliable in long term field trials and accurate compared with laboratory methods [17]. The Oxylog uses a turbine flow meter mounted on the inlet side of the face mask (Fig. 4).
There are two advantages to having the flow meter in this position. Firstly, it avoids the problem of condensation of water vapour from the animal's breath, and secondly, if the inlet volume is used to calculate O₂ consumption and hence energy production, this partially compensates for any inaccuracies caused by changes in RQ. The reason for this is that at low RQ values more O₂ is consumed than CO₂ is produced. This means that the volume of the exhaled air is less than the inhaled air. Using the volume of inhaled air in calculations will therefore overestimate O₂ consumption at low RQs. On the other hand the use of a constant factor to calculate energy consumption from O₂ consumption leads to an underestimation of energy at low RQs. Using values for the total inflow of air will therefore give more accurate values for energy consumption [18].

After each breath a small reciprocating pump takes samples of the air entering and leaving the mask. The samples are passed into separate reservoirs containing a solid desiccant which give 'running average' O₂ concentrations which are measured using two polarographic O₂ electrodes linked differentially. The electronic system calculates and displays total O₂ consumption and total volume of inspired air at STP after making corrections for atmospheric temperature, pressure and humidity. Other functions allow the display of O₂ partial pressure difference between the inlet and outlet, and minute volumes of O₂ consumption and airflow. All outputs can be linked to a data logger and recorded automatically (Fig. 5).

Several adaptations were necessary in order to use the Oxylog for oxen [19, 20]. Firstly, a mask was made to fit oxen which incorporated a saliva trap and allowed the ox to be guided either by a halter or by a nose ring. Initial attempts to seal the mask to the animal's face using foam rubber inside the mask proved unsatisfactory. The present seal consists of an annular cuff of 1 mm thick natural rubber which seals perfectly at a point just behind the animal's nose when the mask is pushed onto the face (Fig. 4). The basic frame of the mask is made from 10 mm plywood and is of a geometrically simple shape. This means that new masks to fit animals of different sizes can be made quickly, easily and cheaply.

Secondly, larger versions of the turbine flow meter were made. It was found possible to make scaled-up versions of this type of flow meter which gave good linear responses when calibrated using a reciprocating pump operated at different speeds to give a range of flow rates.

The capacity of the inlet and outlet valves was increased simply by increasing their number from one to three and nesting them in a larger tube. Finally, the tube connecting the mask to the Oxylog was fitted with a bypass so that only a fraction of the air passed the sampling point.

The ability of the modified system to measure O₂ consumption accurately was checked first of all by passing a known volume of air through the flow meter with the reciprocating pump whilst surrounding the O₂ electrodes with a standard gas of known composition. The results of one such test at different flow rates are shown in Fig. 6. Secondly, the whole system was checked against the standard open circuit
FIG. 5. Energy consumption calculated from $O_2$ consumption (EC), body temperature (BT) and mechanical power output (PO) over 3 h of an ox ploughing in Nepal. The $O_2$ consumption was continuously monitored using an Oxylog and all parameters were continuously recorded using a data logger.

Regression and 5% confidence limits of $O_2$ 'consumption' (L/min) calibration

$$Y = 0.008 + 0.234X, \quad R = 0.9996$$

FIG. 6. Results of a calibration test in which the $O_2$ electrodes of the Oxylog were surrounded by a standard gas and various ventilation rates were provided by a reciprocating pump.
TABLE II. COMPARISON OF MEASUREMENTS (LASTING AT LEAST 30 min) OF O$_2$ CONSUMPTION (L) ON THE OPEN CIRCUIT SYSTEM AND THE OXYLOG

<table>
<thead>
<tr>
<th>Open circuit</th>
<th>Oxylog</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>293.2</td>
<td>305.7</td>
<td>-4.3</td>
</tr>
<tr>
<td>285.5</td>
<td>276.3</td>
<td>3.2</td>
</tr>
<tr>
<td>263.3</td>
<td>250.1</td>
<td>5.0</td>
</tr>
<tr>
<td>244.9</td>
<td>231.2</td>
<td>5.6</td>
</tr>
<tr>
<td>223.5</td>
<td>217.9</td>
<td>2.5</td>
</tr>
<tr>
<td>198.0</td>
<td>192.0</td>
<td>3.0</td>
</tr>
<tr>
<td>197.3</td>
<td>189.1</td>
<td>4.1</td>
</tr>
<tr>
<td>194.8</td>
<td>203.7</td>
<td>-4.6</td>
</tr>
<tr>
<td>194.8</td>
<td>200.7</td>
<td>-3.0</td>
</tr>
<tr>
<td>177.3</td>
<td>179.7</td>
<td>-1.3</td>
</tr>
<tr>
<td>153.2</td>
<td>144.8</td>
<td>5.5</td>
</tr>
<tr>
<td>145.7</td>
<td>138.7</td>
<td>4.8</td>
</tr>
<tr>
<td>131.5</td>
<td>128.2</td>
<td>2.5</td>
</tr>
<tr>
<td>83.8</td>
<td>85.4</td>
<td>-1.9</td>
</tr>
</tbody>
</table>

Note: Average difference = 1.51%; SE = ±0.96.

TABLE III. EXAMPLES OF OXYGEN CONSUMPTION OF BUFFALOES PULLING CARTS IN COLOMBIA AS MEASURED BY THE OXYLOG

<table>
<thead>
<tr>
<th>Live weight (kg)</th>
<th>Standing O$_2$ consumption (L/min)</th>
<th>Distance walked (m)</th>
<th>Work done (MJ)</th>
<th>Total O$_2$ consumption (L)</th>
<th>Total measurement time (min)</th>
<th>Elapsed working time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>625</td>
<td>1.4</td>
<td>1127</td>
<td>0.5</td>
<td>349</td>
<td>118</td>
<td>19</td>
</tr>
<tr>
<td>805</td>
<td>1.8</td>
<td>1293</td>
<td>0.4</td>
<td>467</td>
<td>122</td>
<td>25</td>
</tr>
<tr>
<td>750</td>
<td>1.7</td>
<td>913</td>
<td>0.15</td>
<td>390</td>
<td>144</td>
<td>18</td>
</tr>
<tr>
<td>650</td>
<td>1.8</td>
<td>2005</td>
<td>1.3</td>
<td>618</td>
<td>157</td>
<td>44</td>
</tr>
<tr>
<td>760</td>
<td>2.1</td>
<td>815</td>
<td>0.2</td>
<td>468</td>
<td>162</td>
<td>26</td>
</tr>
<tr>
<td>740</td>
<td>1.8</td>
<td>785</td>
<td>0.6</td>
<td>347</td>
<td>90</td>
<td>20</td>
</tr>
</tbody>
</table>
system. To do this a cow was fitted with the Oxylog and all the exhaust gases from the mask and the Oxylog were passed into the open circuit system. Since the response of the latter to changes in \( \text{O}_2 \) concentration is relatively slow, each determination was made for at least 30 min. Under these conditions the results from the two methods agreed quite well (Table II).

Since these adaptations were made the Oxylog has been used successfully to measure \( \text{O}_2 \) consumption of working oxen in Nepal (Fig. 5) and of buffalo pulling carts in Colombia (Table III).

However well breath by breath analysers work in a technical sense, they present at least two intrinsic problems. The first is simply that many animals do not like wearing masks, however well designed and however little they impede the animal's breathing. In Nepal, readings were obtainable only from three out of six animals. Similar acceptance rates applied to the cows in the ILCA project in Ethiopia and the buffaloes in Colombia. The 'failures' either refuse to work steadily when wearing the mask even after repeated, patient attempts over many days or simply refuse to move altogether. Another problem is presented by animals that pant to keep cool. This means that although the ventilation rate through the mask goes up, the \( \text{O}_2 \) consumption remains fairly constant. The result is that the \( \text{O}_2 \) decrement in the airstream goes down, sometimes by as much as a factor of 2 (Fig. 7), and can reach values that are too low to measure accurately.

4.3. The metabolic rate monitor

The metabolic rate monitor (MRM), a portable flow-through meter designed by Webb and Troutman [21], has been shown to be accurate in the continuous measurement of \( \text{O}_2 \) consumption in humans, and might prove useful if it could be adapted for measurement of \( \text{O}_2 \) consumption of draught animals in the field.

The MRM consists of a mask through which air is drawn by a pump. The speed of the pump is controlled by a feedback loop activated by two polarographic \( \text{O}_2 \) sensors, one of which is in the airstream entering the mask and the other in the airstream leaving it. The loop adjusts the speed of the pump so that the difference in \( \text{O}_2 \) concentration is maintained at a fixed value (usually 1%). The method depends on having a pump which provides an airflow rate directly proportional to the power supply voltage. Total airflow and hence total \( \text{O}_2 \) consumption can then be found by integrating the applied voltage with respect to time and making suitable corrections for temperature and humidity of the airflow.

There is a slightly negative pressure in the mask which eliminates the need for it to be airtight. The absence of valves also means that there is virtually no obstruction to the animal's breathing. The accuracy of the instrument in the field, however, might be impaired by wind blowing into the mask or if the animal's breathing interferes with the smooth flow of air through the mask.
FIG. 7. The effect of panting on the partial pressure of oxygen ($pO_2$) in the expired air of a hot ox working on a day when the ambient temperature was 10–20°C and the weather cloudy at first and sunny later. (1 mmHg = 133.3 Pa.)

The MRM does not measure ventilation volume, nor does it give the $O_2$ consumption on a breath by breath basis, but the fact that total flow rate is adjusted automatically to give a constant $O_2$ decrement means that the instrument is equally accurate at all ventilation rates. The high ventilation rates seen in panting animals could thus be accommodated, making the MRM potentially applicable for use with working animals.

4.4. Tracer methods

4.4.1. Carbon dioxide entry rate

Because CO$_2$ is continuously produced and excreted, it forms a metabolic pool in the body. If labelled CO$_2$ is infused into this pool at a constant rate it will eventually reach an equilibrium concentration in the excreted CO$_2$ which depends on the rate of infusion of the label and the rate of excretion of endogenous CO$_2$.

If $x$ units of label are infused in one hour and the concentration of label in the excreted CO$_2$ is $y$ units/L, then the volume of CO$_2$ excreted in one hour is $x/y$ L or, expressed in terms of rates and concentrations,

$$a = \frac{b}{c}$$
where

\( a \) is the rate of CO\(_2\) excretion;

\( b \) is the rate of infusion of label;

\( c \) is the concentration of label in the excreted CO\(_2\).

This approach has been applied to cattle [22] and sheep [23]. Labelled CO\(_2\) was infused as NaH\(^{14}\)CO\(_3\) and the concentration of label determined in expired CO\(_2\) or in CO\(_2\) extracted from blood, urine or saliva. In general, comparisons of energy expenditure determined by CO\(_2\) entry rate and by direct measurement of gaseous exchange showed agreement to within 15–20%.

The main intrinsic source of error in the method is that the CO\(_2\) pool of the body is not homogeneous and physiologically consists of several interlinked pools in which the CO\(_2\) turnover rates are quite different. Also, there exist several CO\(_2\) 'fixing' reactions which can remove CO\(_2\) from the pool altogether. The errors caused by the inhomogeneity of the CO\(_2\) pool can be minimized by maximizing the length of time during which CO\(_2\) label is infused before sampling starts (Whitelaw [24] recommends 12 h), and the length of time during which samples are taken (24 h). It is important that the metabolic rate of the subject is relatively constant during the sampling period because the turnover rate of the CO\(_2\) pool (once every 1–2 h) is slow compared with the rate at which metabolic rate can change.

This last factor is one of the major objections to the application of this method to working animals. Bakrie [25] found that the method did not compare well with gaseous exchange measurements in working buffaloes. However, his method of measuring gaseous exchange was subject to some problems. The method applied to resting animals has been refined to the extent that the CO\(_2\) output of sheep could be measured to within 2–4% of the values obtained from gaseous exchange measurements [26]. The other major inconvenience of the method is the necessity of continuous and precise infusion of the labelled bicarbonate solution. White and Leng [27] devised a method which involved administering a single dose of labelled bicarbonate but it suffered from the disadvantages that body fluid had to be sampled much more frequently than in the continuous infusion method and mathematical analysis of the results was complex because of the different rates of turnover of the components of the CO\(_2\) pool.

4.4.2. Double and triple labelled water methods

The double and triple labelled water methods have not been applied to draught animals but they could be potentially useful.

Hydrogen is lost from the body mainly as water whereas oxygen is lost both in water and as part of the CO\(_2\) molecule. The oxygen atoms in body water and CO\(_2\) are kept in equilibrium mainly owing to the action of the enzyme carbonic anhydrase. If an animal is given a dose of water in which both the hydrogen and oxygen atoms
are labelled, the specific activity of the oxygen in the body will decrease faster than that of hydrogen. The difference in the two rates of decrease multiplied by the volume of the total body water (which may be estimated from the initial equilibrium specific activity) will give the rate of loss of CO₂.

The theoretical basis of this double labelled water method was originally worked out by Lifson et al. [28] and has been applied to a variety of animals from mice [29] to men [30].

In practice the method involves giving a dose of water enriched with the two non-radioactive isotopes deuterium and ¹⁸O and determining the concentrations of both isotopes at intervals in any body fluid such as saliva or urine. Measurements are taken first of all between 0 and 6 h to determine both the initial concentration of isotope and the total body water and then at intervals until the concentration of isotope has fallen to 25–12.5% of its initial value. In humans this usually means 10–14 d, by which time the ratio of the initial to final concentrations of the two isotopes is large enough to be accurately measured. The rate of loss of CO₂ from the body can then be determined and multiplied by the elapsed time to give the total amount of CO₂ produced.

The main disadvantages of the method are the costs of the isotope enriched water and the highly sophisticated analytical techniques required to determine the isotope enrichment. Also, the method measures CO₂ production only and assumptions have to be made as to the average RQ of the animal if the results are to be used to compute energy expenditure.

The main advantages are that the experimental animal does not have to be restrained in any way and that energy expenditure may be determined over a longer period than is normally possible by respiration calorimetry. Although not suitable for following hourly or daily changes in energy expenditure of draught animals, the method does appear to have a potential use for studying energy expenditure during the bouts of hard work that such animals often have to perform during the cultivation and harvest seasons.

There are, however, several theoretical and practical problems which have to be addressed before attempting to apply the double labelled water method to draught animals. Some of these problems arise because most draught animals are ruminants and others because of the generally large size and high levels of activity of these animals. Fortunately most of these problems have already been solved in other contexts.

The double labelled water method relies on the postulate that hydrogen is lost from the body only as water. In ruminants this is not true since substantial amounts of hydrogen are also lost in methane. Midwood et al. [31] found that the methane produced by sheep given water enriched with deuterium contained only 0.6536 as much deuterium per hydrogen atom as the urine over a wide range of methane production levels. They were thus able to formulate equations which permitted the calculation of CO₂ production provided that reasonable estimates of methane
production could also be made. Omission of the methane correction factors would have led to underestimations of CO₂ production of from 3.3 to 6.5% depending on the methane production level.

Another source of error is caused by differential fractionation of isotopes during any physical or chemical equilibrium process involving water. Chief among these processes are the evaporation of water during insensible perspiration, the equilibration of oxygen between water and CO₂ and the evaporation of water from the respiratory tract. Although most of these can be linked to CO₂ production and appropriate corrections made [32], the factor having the largest quantitative effect, the respiratory evaporation of water, can pose problems. This quantity correlates fairly well with CO₂ production in non-panting animals in temperate climates [32] but the same is unlikely to be true of hard working draught oxen in the tropics.

One solution is actually to measure evaporative water loss (insensible perspiration as well as respiratory evaporation) by introducing a third isotope into the water. Haggarty et al. [33] proposed the use of water labelled not only with ²H and ¹⁸O but also with either ¹⁷O or ³H. Tritium is much the cheaper option but has the disadvantage of being slightly radioactive. In the body the different isotopes fractionate to different (known) extents between the liquid and vapour phases during the evaporation of water. In the case of tritium, therefore, the change in the ratio of ²H to ³H in the body water can be used to assess the rate of loss of water by evaporation, which in turn can be used to correct for the differential fractionation of the ²H and ¹H isotopes during the same process. Similar reasoning can be used vis-à-vis the three oxygen isotopes if ¹⁷O is used.

Finally, the double labelled water method can be adversely affected by anything which effectively alters the amount of water hydrogen or water oxygen in the body after the start of the experiment. Sequestration of water hydrogen can occur through chemical incorporation into molecules such as proteins and, more especially, fats [34] although this is unlikely to be of importance in adult draught animals which are not growing. Of more likely relevance to studies with draught animals is the possibility of the amount of total body water at the beginning of an experiment being different from that at the end owing to factors such as dehydration.

None of the above problems appear insuperable, in which case the double/triple labelled water method would be potentially useful for the medium term measurement of the energy expenditure of draught animals. However, the method would first have to be proved against more conventional techniques, probably a combination of breath by breath analysis and open circuit chamber calorimetry.

Even with the technical problems removed, the main practical one remains the cost of the labelled water. Although for humans this dropped from an estimated US $3000 per dose in 1955 [29] to US $225 in 1982 [30] the cost of dosing draught animals at around US $1000 a time in sufficient numbers to obtain statistically reliable data remains prohibitive.
4.5. Correlation of energy consumption with heart rate

Good correlations between heart rate and energy expenditure have been achieved in humans within specific limits or for individual subjects [35]. The method has been found to be less reliable in draught animals.

Richards and Lawrence [36] produced a formula for the prediction of energy metabolism from heart rate in regularly trained draught cattle and buffaloes, when heart rate and energy expenditure were expressed relative to their respective resting values:

\[ EE = 24.94R - 16.25 \]  

where

- \( EE \) is the energy expenditure (W/kg\(^{0.75}\));
- \( R \) is the heart rate of the working animal/heart rate at rest.

This equation can be modified to measure energy expenditure over an extended period [8]. If \( n \) heart beats are recorded from a working animal in \( t \) minutes, the total extra energy used to do the work is:

\[ 24.94 \times 60 \times M^{0.75} \left( \frac{n - bt}{b} \right) (J) \]

where

- \( b \) is the heart rate when the animal is standing;
- \( M \) is the live weight of the animal (kg).

There are several problems in applying this method to animals in the field. Difficulties occur in assessing a basal heart rate, because of changes during the working period due to changes in fitness, recovery from previous work and anticipation of work to come. ‘Calibration’ of individual animals could allow more precise estimates of relative heart rate and hence energy expenditure to be obtained provided that facilities are available to ‘calibrate’ the animals concerned. Even so, animals may not show the same relationship in the field as in the laboratory [37]. Precise measurements of all heart beats over an extended period are technically difficult. Accumulation of sweat dislodges the electrodes and muscle action potentials interfere with the electrical recording apparatus. Even if these problems could be overcome, the confidence limits of Eq. (2) are such that energy consumption can never be estimated very accurately from relative heart rate [8].
4.6. A factorial method

A factorial method based on the extra energy used by draught animals to perform the basic types of movements involved in their work has been developed [38]. This has the following factors:

extra energy used for work =
energy for walking + energy for carrying loads + energy for pulling loads + energy for walking uphill

This formula may be expressed quantitatively as:

\[ E = AFM + BFL + \frac{W}{C} + \frac{9.81HM}{D} \]

where

- \( E \) is the extra energy used for work (kJ);
- \( F \) is the distance travelled (km);
- \( M \) is the live weight (kg);
- \( L \) is the load carried (kg);
- \( W \) is the work done whilst pulling loads (kJ);
- \( H \) is the distance moved vertically upwards (km);
- \( A \) is the energy used to move 1 kg of body weight 1 m horizontally (J);
- \( B \) is the energy used to move 1 kg of applied load 1 m horizontally (J);
- \( C \) is the efficiency of doing mechanical work (ratio of work done to energy used);
- \( D \) is the efficiency of raising body weight (ratio of work done raising body weight to energy used).

Applications of this formula to the energy expenditure of working oxen have been described [39, 40].

The objection to the use of this formula is that the factors \( A-D \) are derived from ‘laboratory’ experiments in which the animals work under conditions which are often very different from those found in the field. For example, the energy cost of walking is higher when animals walk on muddy surfaces than on hard surfaces [41]. However, the method has the advantage of being easily applicable to fairly large numbers of animals, and the quantities which have to be measured, such as distance travelled and work output, are often of interest for their own sake.

5. CONCLUSIONS

Although there are many methods available for measuring the whole body metabolism of large animals, relatively few have been applied to draught animals.
All the methods mentioned in this paper have inherent problems. Of the gaseous exchange methods, the classic open circuit devices are expensive and complicated to set up and the animals are restricted to a laboratory environment. Currently most effort is being put into portable breath by breath analysers, with three groups of workers having achieved a good level of success. However, such devices are complex and expensive and often difficult to calibrate and repair. Animals often refuse to tolerate wearing a face mask and the devices become inaccurate if the animal pants.

Of the two tracer methods discussed, only that for determining the CO₂ entry rate has been used with draught animals and then with indifferent results. Also, the requirement for the continuous infusion of label renders the method inconvenient for field studies. The double or triple labelled water method would appear to have potential usefulness for measurements lasting two to three weeks and would cause minimal disturbance to the experimental animal. However, there are several theoretical and practical problems which still have to be overcome, not least of which is the cost of the labelled water. Both tracer methods are inherently inaccurate as methods of measuring energy expenditure because they measure CO₂ production and not O₂ consumption.

Measurement of heart rate over any length of time is technically difficult and the results correlate poorly with energy expenditure even under laboratory conditions.

The factorial method also relies exclusively on factors derived from laboratory studies which may not be applicable in the field.

At the time of writing, the use of breath by breath analysers seems the most promising and versatile method for field studies on working draught animals but the choice of methods is wide and unforeseen theoretical and technical improvements may make other methods more attractive in the future.

REFERENCES


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ENVIRONMENTAL IMPLICATIONS OF ANIMAL AGRICULTURE: THE NEED FOR INTEGRATED SYSTEMS AND THE MODELLING OF ENERGY FLOW*

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Abstract

ENVIRONMENTAL IMPLICATIONS OF ANIMAL AGRICULTURE: THE NEED FOR INTEGRATED SYSTEMS AND THE MODELLING OF ENERGY FLOW.

The design of sustainable livestock strategies must be judged according to the likely impact on economic, ecological, ethological and sociological issues. Economic issues to be satisfied include international competitiveness in the price of the finished product, which requires maximizing comparative advantages of available natural resources. Ecological sustainability requires that the production system will result in (1) reduced emissions of the principal greenhouse gases carbon dioxide and methane, (2) reduced contamination of soil and water resources, (3) an effective control of soil erosion, and (4) integrated on-farm production of energy from renewable resources. Ethological concerns relate to potential effects of production systems on animal welfare and the safety and consumer acceptability (wholesomeness) of foods produced in such systems. Sociological issues require that employment opportunities be increased, especially for women, and that the production system encourages self-reliance with minimum dependence on outside inputs. Technologies for tropical animal agriculture which satisfy the above criteria should incorporate the following elements: (a) crops that have the maximum capacity to (i) incorporate atmospheric carbon dioxide into biomass, which in turn can be fractionated into components suitable for food, feed and fuel needs, (ii) fix atmospheric nitrogen, and (iii) maintain soil fertility; (b) selection of animal species (priority to be given to monogastric animals) and feeding and management systems (strategic supplementation of ruminants; multi- rather than special-purpose animals) which maximize product:methane ratios; (c) integration of crop, livestock and fuel production from biomass in systems which permit recycling of wastes and residues and cogeneration of fuel, food and feed. Integrated production of sugar cane, multipurpose trees and water plants, and their use for production of feed for pigs and sheep (or dual purpose cattle) and fuel satisfy the constraints outlined above. They are applicable in models suitable for family farms of 1–2 ha, for entrepreneurial farms of 20–25 ha and for an emerging agroindustry (the biomass

* This is an updated and expanded version of a paper entitled "Future strategies for livestock production in tropical Third World countries", first published in Ambio 19 (1990) 380–393.
refinery) offering renewable resources as alternatives to present fossil fuel based technologies for chemical and energy needs. Elements of the proposed systems are having increasing impact in many tropical countries.

1. THE CHANGING LIVESTOCK SCENE

The last five years have witnessed a marked change in the economic and political pressures to which livestock production is subjected in both industrialized and Third World countries. In most industrialized countries political support for farming has been weakened by food surpluses, the high degree of government subsidization in the agricultural sector and tariff barriers to food imports from developing countries. The resultant high prices have reduced demand for most agricultural products.

Farming systems throughout the world are threatened by:

— The increasing prices of fossil fuels (and the agrochemicals derived from them) due to decreasing reserves and political instability in the principal supplying countries;
— The concern over the increasing atmospheric and terrestrial contamination, much of which can be ascribed to agricultural activities;
— The increasing advocacy for free markets in agricultural products, greater discrimination by consumers and mounting concern for animal welfare.

The conduct of livestock production and the role of food products of animal origin have been influenced profoundly by these changes. As a consequence livestock production strategies are changing and will continue to change in response to these pressures.

In order to establish guidelines for a more sustainable development, steps must be taken:

— To identify the economic and political changes which have a bearing on livestock production systems,
— To design appropriate strategies to ensure that future contributions in animal production research will lead to the creation and adoption of livestock systems which are truly sustainable.

2. THE CONSTRAINTS TO LIVESTOCK PRODUCTION IN THE TROPICAL THIRD WORLD

The pressures facing livestock producers are of a diverse nature, encompassing political, socioeconomic, ethological and ecological issues. All these factors must be
considered in order to develop a strategy which will lead to the establishment of sustainable systems of production.

2.1. Economic pressures

All Third World countries require foreign exchange to finance their development. This must be earned by export of goods which usually will have their origin in the agricultural or industrial sectors. The first constraint is the need to allocate a high proportion of existing earnings to the servicing of foreign debt. The second pressure comes from international institutions such as the World Bank and the International Monetary Fund, which, by stipulating an ‘open, market economy’ as a prerequisite for financial assistance, effectively encourage imports, often of goods which can be produced by the target country. For industrial products, this policy is reasonably defensible as State intervention (subsidies) by the industrialized countries in this area is minimal, and prices mainly reflect true costs of production and are thus competitive. But this is not so in agriculture, especially for livestock products, since almost all industrialized countries provide some form of State support for their farmers. As a result export world market prices have little relation to true costs of production and ‘dumping’ of surpluses is commonplace, invariably to the disadvantage of Third World farmers. A strategy for livestock production in Third World countries must therefore have as its basis those features which provide true competitive advantage in the long rather than the short term. The classic example of short term folly is the experience in pig and poultry production in the Third World. Invariably this production has been based on imported cereal grains and protein meals. In the short term, production increased through the improvement in technological efficiency and consumers benefited. In the long term, financial pressures to limit imports, in order to conserve foreign exchange, either by direct taxes or simply the elimination of subsidies, have forced up production costs to the point where demand for the product has fallen relative to the increase in population. The dependency created by the imports also acted as a severe disincentive to the search for local alternatives.

In terms of natural resources — solar energy, water, soil, biological diversity and people — tropical countries have the basis for achieving an immense competitive advantage over the industrialized countries, which are almost exclusively located in temperate climatic zones. It is the use of these natural resources which must form the basis of their livestock strategy and government policy should be directed to this long term goal. An ‘open’ market in this case is not defensible. Protection is needed (a) because the competing imports are usually ‘subsidized’ and (b) to give time to develop the new systems which are needed in order to utilize the different, but potentially much more competitive, feed resources which can be produced in a tropical environment.
2.2. Environmental pressures

The overwhelming environmental issues are:

— The greenhouse effect, or global warming;
— Deforestation and grazing induced erosion;
— Contamination of soil and water resources because of industrial, and intensive farming, activities (e.g. mining and factory effluent entering waterways, and excessive use of chemical fertilizers and pesticides in agriculture).

2.2.1. Global warming

Global warming is considered to be caused mainly by increasing emissions of carbon dioxide and methane. The predictions of its effects include:

— Melting of the ice caps with an increase in sea levels and release of the trapped gases (there is considerably more methane in the ice caps than in the atmosphere), which will enhance the greenhouse effect;
— Chaotic weather changes, resulting in droughts or floods and eventually serious erosion;
— Decreased crop yields and decreased arable land availability (the coastal plains and river deltas are often the most fertile soils).

While there is debate about the degree and timing of climatic change likely to be induced by the accumulation of greenhouse gases, there is no doubt about the exponential rise in carbon dioxide and methane emissions. It is equally true that there are solutions to the problem, and that these are politically unwelcome especially to the industrialized countries, both old and new (it is their economic development which is responsible for most of the problems), because of the economic cost of the remedial measures that are needed.

A balanced view must be that every effort must be made to slow down the accumulation of the greenhouse gases. Policies that aim to do this must be supported, especially if it can be shown that these are not conflictive with development. What must be changed is the interpretation of development. The recent proposal of the United Nations Development Programme [1] to use a ‘human development index’ instead of GNP as a measure of human well-being is indicative of the kind of approach that is needed; one that will give positive credit, or the contrary, to presently unaccounted effects (in economic terms) of development on the Earth’s natural ecosystems.

From the point of view of livestock production, the issues are:

— How to reduce methane emissions per unit of animal product,
FIG. 1. The opportunity: photosynthesis and fossil fuel consumption [2].

— How to contribute to a situation in which atmospheric carbon dioxide concentration once again becomes stable, with production being balanced by utilization.

Inevitably this will mean a scenario in which fossil fuel is eventually replaced with energy derived from renewable resources. Direct fixation of solar energy in earthbound or spatial power stations may make sense in the industrialized world but not for financially poor Third World countries. Their participation in such endeavours will be more rewarding if they use to greater advantage their existing natural resources, which already give them an enormous advantage over temperate areas as they are the places where renewable biomass can be produced most rapidly and efficiently (Fig. 1).

2.2.2. Deforestation and grazing induced erosion

This issue has been discussed critically by Murgueitio [3]. The solution he proposes is eminently feasible — establish cropping systems which simulate the effect of the natural forest cover, and manage livestock in confinement. Because of their intensive use of land and labour, and low capital investment, such systems are to the advantage of the resource-poor farmer. They are in conflict with the interests of traditional graziers, but as these are in the minority and rarely live in the areas where the ecological damage is occurring, their undoubted short term political influence is likely to be overshadowed by the long term advantages to the majority, as has proved to be the case in the HADO project in Tanzania [4, 5].
2.2.3. Soil and water contamination

Many of the problems in this area are caused by modern agronomic practices which seek to maximize yield per unit area or per worker. Thus use of organic manures and biological pest control has given way to the short term advantages in convenience and efficiency associated with chemical fertilizers and pesticides. The ecological cost of such practices was not measurable nor visible in the short term. But it is now a cause of concern, especially in the industrialized countries, where the abuse of natural resources has reached levels at which the effects can be plainly seen.

For future researchers and decision makers in agriculture, the challenges are clear. An organically based agriculture, with crop rotations and mixed cropping systems, requires the involvement of livestock as recycling agents and restorers of soil fertility. Confinement systems permit maximum recovery of animal excreta, to be fermented in biodigesters prior to use of the effluent as a source of plant nutrients. Cropping systems which lead to fixation of atmospheric nitrogen will also be an integral part of organic agriculture. Predominant in such systems are likely to be legume trees, which can be used as forage; water plants with symbiotic N fixing algae such as *Azolla filiculoides*; and soil management practices such as mulching, which create favourable ecosystems for free-living organisms that fix atmospheric nitrogen [6].

2.3. Ethological issues

There is increasing concern that animal production be conducted in a way compatible with the well-being of the animals and the people who care for them and the wholesomeness of the final food product [7]. This concern is having its impact on the practice of animal production through three main avenues: government legislation, the market place and popular opinion. The positive side of this particular debate is that it has drawn attention to the idea that a ‘contented’ animal may well be more productive and efficient than one suffering stress.

2.3.1. Animal welfare

The major issue is the manipulation of animals to increase productivity but at a cost of increased stress to both the animals and their attendants. Increasingly affluent consumers in the industrialized countries are beginning to demonstrate their aversion to such practices and these trends are likely to develop to the point where food products that arise from animals subjected to stress will be discriminated against.

There are many examples of production induced stress, arising from the pressure to produce more at less cost:
— Much reproductive research in developed countries is directed to the implantation of additional embryos to increase prolificacy in animal species (e.g. cattle) biologically not adapted to such functions. The result is a dramatic increase in the incidence of retained placentas [8], causing stress to both the host animals and the persons responsible for managing them.

— Modern methods of managing pregnant sows dictate that they be housed and fed individually, since the feed allowance is so restricted as to cause psychological stress (the hunger syndrome), which is manifested in antisocial behaviour that makes group housing impossible.

— Cage housing of egg-laying birds is obviously stressful and is being legislated against in an increasing number of countries.

2.3.2. Natural food products

Parallel with the move to view animal welfare in the same light as human welfare, there is an increasing body of consumers concerned about the 'wholesomeness' and natural integrity of food. As purchasing power increases so does selectivity. Cattle treated with steroid hormones produce lean meat faster and more efficiently and therefore at less financial cost. But this argument is of little consequence to the affluent consumer unwilling to be exposed to unnecessary health hazards, and unimpressed by scientific analysis concerning permissible and minimum safety levels of the compound in question.

Somatropin, a hormone that can be produced industrially as a result of genetic engineering research, increases milk yields in cattle and leaness in pigs. But again the sceptical consumer may not be impressed and this concern, likely to be reflected in reduced demand for the product, is already causing many milk and meat marketing institutions to call for legislation prohibiting such practices.

The issue is not simply: is such food safe and/or of good nutritive value? When the consumer has adequate purchasing power, other issues come to the forefront and animal welfare and natural foods are proving to be popular rallying points.

Some of the practices mentioned here can already be observed in several Third World countries although consumer discrimination will take longer to develop since purchasing power is low. But future trends should not be ignored even in relatively protected home markets. In the export markets, discrimination is already a fact as witnessed by the refusal of the European Economic Community to import beef from North American cattle treated with anabolic hormones.

It is hoped that animal scientists in developing countries will study such trends and will decide that excessive interference in animal function, whatever its merit as a research topic, is not likely to be a profitable long term investment in a world where sustainability will count more than short term financial gain.
2.4. Sociological pressures

To be sociologically sustainable, livestock systems must aim to improve the quality of life of rural families, which mostly means increasing incomes. To this end the creation of sources of productive employment, especially for women and children, should have high priority. Integration of crop and animal agriculture, with diverse activities suitable for all members of the family, will facilitate the attainment of this goal. Livestock production, especially of milk and eggs and meat from small livestock species — sheep, goats, pigs and poultry — creates opportunities to add value to the products to be marketed and is an attractive activity for women and children.

3. ECOSYSTEMS THAT ARE PRODUCTIVE AND ENVIRONMENTALLY FRIENDLY

3.1. Crisis and opportunity

Attempting to emulate the classic modes of development established by the industrialized world has led many countries — developing and developed — to a crisis situation. However, an old Chinese saying insists that crisis is composed of two elements: the ‘danger’ — of destroying everything — and the ‘opportunity’ to make changes. It is the last element that should guide future development strategies.

*FIG. 2. Biomass productivity in different ecosystems [9].*
The great opportunity, and also the major challenge, is to learn how to exploit
the enormous potential of the Earth to produce renewable biomass, derived by
photosynthesis, and to manage it in a way that will meet future needs for food, feed
and fuel. Even with the existing vegetative cover, this capacity exceeds by a wide
margin the actual rate of consumption of fossil fuel (Fig. 2).

3.2. Productive ecosystems

The first step, in a strategy to capture solar energy in the form of biomass, is
to identify and exploit those ecosystems which are highly productive and environ­
mentally friendly and give rise to resources easily fractionated into components with
physiologically different characteristics (e.g. of high and low fibre content). The
cereal plant species which divide into grain and straw, and sugar cane, which can
be separated into sugar rich juice and bagasse, are examples of crops which are well
suited to this approach.

The most productive ecosystems are the tropical perennial crops, followed by
rainforests (Fig. 2). Annual crops in the tropics use solar energy less efficiently since
they present their maximum surfaces for photosynthesis during only limited periods
of the year. In regions with temperate climates, there is less difference between
perennial and annual crops since photosynthesis is at a low level, or even zero,
during the winter months.

3.2.1. Sugar cane

Sugar cane is the logical first choice among perennial crops [10] because:
— It is one of the most efficient plants in capturing carbon dioxide and trans­
forming it into biomass.
— It is easily fractionated into feed (the sugar rich juice) and fuel (the fibre).
— It tolerates extended dry seasons.
— It is highly resistant to pests and diseases.
— The technologies for cultivating and processing sugar cane are known in almost
all tropical countries.
— It has its maximum feed and fuel value in the dry season, when feed and fuel
shortages are most acute.
— It provides feed for monogastric animals (the sugar rich juice) as well as
herbivores (the residual fibre).

3.2.2. Trees and water plants

The complementary sources of tropical biomass, providing protein to balance
the carbohydrate in sugar cane, are trees and water plants. Trees should be a
component of all farming systems because:

— They are perennial.
— There are many species to choose from.
— The foliage is rich in protein, minerals and vitamins.
— Many species fix atmospheric N.
— They are deep-rooted and resistant to drought.
— They have multiple uses (fences, feed, wind breaks, etc.).

The advantages of trees and water plants are that they can provide a complementary source of protein with minimum inputs and at the same time help safeguard the environment. Trees are important reservoirs of carbon dioxide and are a renewable source of fuel and construction material as well as fulfilling other useful roles such as wind breaks and live fences. Incorporating them into grazing systems protects the soil against erosion and stimulates nutrient recycling.

*Leucaena leucocephala* has been the most publicized — receiving the accolade ‘wonder tree of the tropics’ [11]. But there are many other candidates in the existing tropical flora and with an even wider range of attributes than leucaena. For example, the ‘Nacedero’ tree (*Trichantera gigantea*), well known by farmers in coffee growing regions of Colombia, appears to be particularly appropriate for inclusion in integrated systems since the leaves are readily consumed by pigs and poultry as well as by herbivores [9]. It is also highly productive and, although not a legume, appears to have a symbiotic association with N fixing soil microbes, since it does not require N fertilizer.

Where rainfall and/or irrigation are adequate, water plants are also important sources of biomass and play complementary roles: a high rate of converting atmospheric N into protein (up to 9 t protein·ha⁻¹·a⁻¹), and protection of the environment by decontaminating (extracting nutrients) livestock and human wastes. The water fern *Azolla filiculoides* is the species which has had most impact with farmers [12] because of its high growth rate, relative ease of management and easy incorporation in feeding systems for pigs and ducks.

Another water plant with apparent potential in integrated production systems is the blue-green alga spirulina. Although most interest in this plant has been shown so far by human nutritionists, it can be adapted to grow well under farm conditions with the aid of biogas effluent as supplementary substrate, using the dissolved as well as atmospheric carbon dioxide [13].

3.3. Cultivate according to agroecological principles

Once the selection has been made of what appear to be the most appropriate ecosystems for biomass production, the next step should be to minimize inputs from inorganic sources, prevent the loss of plant nutrients from the soil and use biological methods to control weeds, pests and diseases [14].
3.4. Fractionate and recycle

3.4.1. Fractionation

The third step in the process of optimizing production and use of natural resources is to ensure that the biomass is processed in a way that will satisfy the contrasting needs of monogastric and ruminant animals for feed, and of energy.
The growth habit and composition of sugar cane facilitate the process of fractionation. The tops provide feed for ruminants, the juice is especially suitable for feeding to monogastric animals and the bagasse can be used as fuel (Fig. 3). Trees also lend themselves readily to fractionation — the foliage and/or fruits for feed and the branches for fuel.

3.4.2. Recycling

All production systems should incorporate recycling so that residues are managed as substrates for more production, rather than being treated as wastes. Integrated production systems are based on the premise that there are complementary activities to be performed by the different components within the system. The residues from one activity become the inputs for the next (Fig. 4).

3.5. Animal species for sustainable agriculture

Priorities must also be established for selecting animal as well as crop species. In this respect the pressures are:

— To reduce methane emissions (monogastric animals should have priority over the ruminant species),
— To encourage use of small rather than large herbivores (they are more efficient mainly because of greater selection capacity),
— To reduce fossil fuel use by promoting animal traction with multipurpose animals, such as cows and buffaloes.

4. PRODUCTION MODELS

Three models of production systems can be identified, corresponding to the needs of the family farm, the entrepreneur and industrial scale agroindustry (the biomass refinery).

4.1. The family scale model

The family scale model uses the products from sugar cane, trees and water plants to feed pigs, ducks and sheep; the unit (1–2 ha) is self-sufficient in energy, employing biodigesters and direct combustion of fibrous residues from the cane and the trees (Fig. 5).
FIG. 5. The family scale model.

FIG. 6. Production at the entrepreneurial level.
TABLE I. CROPS AND LIVESTOCK ON THE MEDIUM SCALE FARM

Crops
Sugar cane 15 ha
Agroforestry 5 ha
Ponds 2.25 ha

Livestock
75 dual purpose cows and followers
36 sows and progeny
4 buffaloes

Annual outputs

<table>
<thead>
<tr>
<th></th>
<th>(kg)</th>
<th>(US $)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig live weight</td>
<td>51 840</td>
<td>72 760</td>
</tr>
<tr>
<td>Milk</td>
<td>120 000</td>
<td>24 000</td>
</tr>
<tr>
<td>Cattle live weight</td>
<td>24 300</td>
<td>24 300</td>
</tr>
<tr>
<td>Electricity (kW·h)</td>
<td>100 000</td>
<td>5 000</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>126 060</td>
</tr>
</tbody>
</table>

Per hectare:
Live weight 3 310
Milk 5 200
Gross income 5 480

4.2. The entrepreneur

At the entrepreneurial level, the model can have many variations. Between 20 and 25 ha is a convenient size for the incorporation of a cattle unit dedicated to dual purpose production of milk and beef. Grazing is practised but always in association with tree crops. The principles of fractionation and recycling remain the same (Fig. 6). Stocking density and productivity per unit area are high (Table I).

4.3. The biomass refinery (cogeneration of fuel, food and feed from biomass)

The continuing, and deepening, fuel crisis will, it is hoped, be the stimulus for a new rural development model, based not on oil but on renewable biomass; and with an integrated approach aimed at satisfying the needs for fuel and food production and, at the same time, increasing job opportunities in rural areas.
FIG. 7. Sustainable agriculture: the biomass refinery (cogeneration of fuel, feed and food).

The concept is that of the 'biomass refinery', where the raw material is vegetative mass instead of oil, and where the transformations are into a range of products, including those presently derived from oil but with the added advantage of including food and feed as well as fuel.

New technologies are available for producing electricity from sugar cane fibre (bagasse and/or trash) using an integrated biomass gasifier and gas turbine generator to generate steam and electrical energy (Fig. 7). The advantages of the gas turbine (based on standard jet aeroplane engines) for thermal power generation are its low capital cost (compared with oil, coal, hydroelectric or nuclear configurations) and high thermodynamic efficiency [15].

The technology is particularly attractive when operated in a cogeneration mode with sugar and/or its derivatives as secondary products to the primary activity of electrical energy generation. The system permits the export of substantial amounts
TABLE II. INPUTS AND OUTPUTS AND ESTIMATED COSTS AND RETURNS FOR A COGENERATION UTILITY PRODUCING HIGH TEST MOLASSES AND ELECTRICAL ENERGY FROM SUGAR CANE
(harvest area would be about 5000 ha assuming annual stalk yields of 120 t/ha; adapted from Ref. [15])

<table>
<thead>
<tr>
<th></th>
<th>Cane stalks only (preburning of trash)</th>
<th>Stalks plus trash (no burning)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cane stalks (t/d)</td>
<td>1700</td>
<td>1700</td>
</tr>
<tr>
<td>Briquetted bagasse (t/d)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>289</td>
<td>289</td>
</tr>
<tr>
<td>Briquetted trash (t/d)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>371</td>
</tr>
<tr>
<td>High test molasses (t/d)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>340</td>
<td>340</td>
</tr>
<tr>
<td>(US $/a)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13 464 000</td>
<td>13 464 000</td>
</tr>
<tr>
<td>Electrical energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kW · h/t cane stalk)</td>
<td>258</td>
<td>700</td>
</tr>
<tr>
<td>(US $/a)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7 237 000</td>
<td>19 635 000</td>
</tr>
<tr>
<td>Total income (10&lt;sup&gt;6&lt;/sup&gt; US $/a)</td>
<td>20.701</td>
<td>33.099</td>
</tr>
<tr>
<td>Investment (10&lt;sup&gt;6&lt;/sup&gt; US $)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar factory</td>
<td>7.65</td>
<td>7.45</td>
</tr>
<tr>
<td>Gasifier/turbine&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18.45</td>
<td>49.5</td>
</tr>
<tr>
<td>Operating costs (10&lt;sup&gt;6&lt;/sup&gt; US $/a)&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed</td>
<td>0.492</td>
<td>1.3</td>
</tr>
<tr>
<td>Variable</td>
<td>0.15</td>
<td>0.5</td>
</tr>
<tr>
<td>Labour</td>
<td>0.108</td>
<td>0.3</td>
</tr>
<tr>
<td>Cane stalk&lt;sup&gt;g&lt;/sup&gt;</td>
<td>8.976</td>
<td>11.2</td>
</tr>
<tr>
<td>Briquetting (plus collection and transport for trash)&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.969</td>
<td>4.626</td>
</tr>
<tr>
<td>Total</td>
<td>11.695</td>
<td>17.926</td>
</tr>
<tr>
<td>Margin (income — costs) (10&lt;sup&gt;6&lt;/sup&gt; US $)</td>
<td>9.006</td>
<td>15.173</td>
</tr>
<tr>
<td>Margin as percentage of investment</td>
<td>34.5</td>
<td>26.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Assumes that amount of briquetted bagasse (15% moisture) is 17% weight of cane stalk.
<sup>b</sup> Assumes that amount of tops and trash (no burning) after field drying, collection and briquetting (15% moisture) is 22% weight of cane stalk.
<sup>c</sup> Price of high test molasses is set at US $120/t to be competitive with cereal grain.
<sup>d</sup> Sale price of electricity is assumed to be US $0.05/kW·h ex-factory.
<sup>e</sup> Generating capacity of gas turbine is 15 and 50 MW, respectively, for bagasse only and bagasse plus trash options.
<sup>f</sup> It is assumed that the factory works 330 days per year.
<sup>g</sup> Purchase price of cane stalk is US $16.00/t when trash is burned and US $20.00/t harvested green.
<sup>h</sup> Drying and briquetting of bagasse are estimated to cost US $1.25/GJ; collecting, transporting and briquetting of field dried trash are estimated to be US $1.35/GJ. The calorific value of dried (15% moisture) briquetted bagasse and trash is estimated to be 16.1 GJ/t.
of electrical energy (up to 700 kW·h/t cane stalk milled, when the trash as well as the bagasse is used as fuel for the gasifier) after satisfying the power and heat requirements of the factory for the production of sugar, alcohol or animal feed (high test) molasses.

The technology offers considerable environmental benefits with the potential for zero net carbon dioxide emissions, since the biomass feedstock (sugar cane) is a renewable resource. By producing high test molasses, there are further environmental and also economic benefits. High test molasses can be fed to pigs as a cheaper alternative to cereal grain (often imported); and by promoting pigs in place of cattle, emissions of methane per unit of meat produced are vastly reduced.

By harvesting the cane green, instead of after preburning, there are yet additional environmental benefits. Employment opportunities are also increased substantially since more cane cutters are needed to harvest green cane, while the collection, transporting and briquetting of the cane trash create more jobs.

Estimates of inputs and outputs, costs and returns for a cogeneration utility processing the cane from an area of 5000 ha are set out in Table II. The coefficients for processing of the cane bagasse and trash, gasification and power generation are taken from the paper by Ogden et al. [15]. The following two options are considered: (a) the standing cane is burned to facilitate harvesting (but at the cost of serious environmental contamination), and only the bagasse is available for gasification; and (b) the cane is harvested green and all the biomass (including tops and dry trash) is delivered to the factory.

Assuming that the investment in the factory, gasifier, turbine and generator is incurred in year 1, that operation begins in year 3 and that the project life is 20 years, then the internal rates of return are 26.8% and 21.3% for options with and without burning respectively.

The viability of biomass refineries will be determined by the principles of comparative advantage. Countries which presently have economically viable cane sugar industries are obvious candidates for the new technologies based on cogeneration.

5. POLICIES FOR SUSTAINABLE ANIMAL AGRICULTURE

5.1. The role of government

It can well be argued that governments in industrialized countries should play the lead role in promoting sustainable production systems, since it is the activities in these countries that are responsible for the major part of global greenhouse gas emissions and water and soil pollution. If support to agriculture in industrialized countries (subsidies) were directed to production of energy crops in ecologically benign systems, instead of food (that can be produced more economically in tropical
developing countries), there would be a reduced dependence on fossil fuel, stimulation of world trade and a general improvement in global well-being.

5.2. Research priorities

Agricultural research priorities in developing countries should emphasize areas such as biological control of weeds, diseases and pests, organic farming and more natural (less stressful) systems of feeding and management of farm livestock. The results of this interpretation of 'biotechnology' are likely to lead to more sustainable systems than the 'modern' biotechnology aimed primarily at gene modification with as yet quite unpredictable outcomes.

5.3. New approaches to training

Systems of technical training must also be changed when the goals are sustainability and not just productivity. The required approach will stress biology rather than animal or crop sciences, so that a holistic view is developed concerning the available resources and how these can best be used by present, and sustained for future, generations.

5.4. Communication

Inadequate communication — between people, institutions and countries — is one of the most serious constraints to sustainable development. North → North and North → South have been the dominant flow paths for information. But the needs, conditions and aspirations of the South are quite different from those of the North. Aid — financial and technical — is a poor substitute for knowledge about a country's indigenous resources and how to exploit them in the most rational way. The concepts of ecodvelopment and self-reliance will be strengthened by strengthening South ← South information flows [16].

6. CONCLUSIONS

There are convincing arguments why international funding agencies should now be directing most of their support to projects which are responsive to the issues discussed in this paper. In the present context, sustainable means that:

— Natural ecosystems are enhanced rather than threatened.
— Rural based social structures are strengthened rather than fragmented.
— Local resources are preferred and there is minimal dependence on inputs not produced directly on the farm.
— Livestock production technologies are increasingly directed to the reduction of stress at both animal and human level.

Research against this background may appear far removed from what is currently being published in the scientific journals in the industrialized countries, although already there are signs of impending change. What should be recognized is that research into sustainable systems is a unique opportunity for scientists in developing countries to establish their own priorities, to study new and different resources, and in so doing to set the groundwork for a future competitive advantage rather than the present dependency.

REFERENCES


SHORT CHAIN FATTY ACIDS IN THE FORESTOMACH OF CAMELS AND INDIGENOUS CATTLE, SHEEP AND GOATS AND IN THE CAECUM OF DONKEYS GRAZING A THORNBUSH SAVANNAH PASTURE

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Abstract

Concentrations of short chain fatty acids (SCFAs) were measured in the forestomach of camels and indigenous cattle, sheep and goats and in the caecum of donkeys grazing a thornbush savannah pasture. Total SCFA concentrations in camels were 40-90% higher than in ruminants. SCFA concentrations in the caecum of donkeys were about 30-40% lower. Seasonal variations in the quality of feed and differences in the feeding behaviour of the five animal species indicate considerable differences of microbial activity in the fermentation chambers. Concentrations of SCFAs in the fermentation chambers, on the other hand, showed only small seasonal variations; no differences between sheep and goats were found, although goats selected a diet of significantly higher digestibility in the dry season than sheep. The absorption of SCFAs through the epithelium of the reticulorumen of sheep and goats measured in the temporarily isolated forestomach was similar in the two species. The rate constants of SCFA absorption were 60-90% higher for propionate and butyrate than for acetate. Parallel measurements of forestomach fluid volumes and fluid turnover showed larger SCFA pools and higher flow rates of SCFAs (relative to body weight) in camels than in the ruminants. It is concluded that concentrations of SCFAs do not reflect actual SCFA production rates. Dilution rate, rumen fluid volume and absorption rate influence SCFA concentrations far more than production rate.

1. INTRODUCTION

In the forestomach and in the hindgut of herbivores carbohydrates are digested by microbial fermentation, and short chain fatty acids (SCFAs) are the major end products. The SCFAs contribute about 60-80% of the energy requirements in cattle and sheep fed conventionally [1-4]. Microbial fermentation of cell wall constituents in the hindgut of equines like donkeys contributes as well to a considerable extent to the energy supply, especially when the animals are fed a roughage diet [5].
Availability and digestibility of the different fodder plants vary considerably in many tropical and subtropical pastures. In addition, seasonal changes influence the digestibility of many plant species. During the dry periods feed quality of most of the tropical grasses is low and fibre content is high [6, 7]. These plants can be digested in the forestomach and in the hindgut only slowly, and thus production rates of SCFAs may be low.

The objectives of the present study were (1) to investigate concentration profiles of the SCFAs with respect to their diurnal and seasonal variations in the forestomach and in the caecum, respectively, of indigenous African livestock under traditional management on a thornbush savannah of northern Kenya, and (2) to estimate whether SCFA concentrations could be an indicator for differences in SCFA production. It will be concluded that differences in SCFA concentrations do not give useful hints about differences in microbial fermentation.

2. MATERIALS AND METHODS

2.1. Study area

Studies were carried out at a small research station situated on a holding ground near Isiolo, about 70 km north of the Equator. The station provided fenced paddocks as night enclosures for the animals and a deep borehole for water supply. The altitude is 1100 m a.s.l.; the mean annual rainfall at Isiolo is 510 mm, distributed in a bimodal pattern. Soils in the study area are predominantly volcanic in origin. The vegetation is that of a semiarid thornbush savannah dominated by various Acacia species with a sparse ground cover of annual grasses, herbs and dwarf shrubs. Along the seasonal watercourses there is Acacia woodland and bushes, dominated by Grewia species. Perennial grassland is found on about 15% of the study area [8, 9].

2.2. Characterization of pasture conditions

The bimodal distribution of the annual rainfall in the study area usually causes two growth periods of the vegetation each year, from March to June and November–December, followed by dry periods. However, rainfall events were irregular and unequally distributed over the study area. Therefore, the presence of certain indicator plants in the animals' diet was used to describe the actual pasture situation as dry, intermediate or green season [7, 9].

These indicator plants were annual grasses, Grewia spp. (G. tenax, G. villosa, G. bicolor), Tribulus terrestris and Vernonia cinerascens, which all grow after sufficient rainfall and gradually disappear from the animals' diet in the dry periods. Their presence in the diets was determined by feeding behaviour studies [7, 10] that were made at two week intervals throughout the study period from September 1985 to
December 1987. According to the frequency of observations of the indicator plants three pasture situations were distinguished: green season: more than 20 observations of indicator plants per hour of feeding time; intermediate season: 10–20 observations per hour; dry season: less than 10 observations per hour.

2.3. Animals and management

The studies were carried out with indigenous sheep (Somali Blackhead and Red Maasai, body weight (BW): 19–55 kg), goats (Small East African and Galla, BW: 23–47 kg), cattle (Small East African Zebu, BW: 185–375 kg), camels (Camelus dromedarius, BW: 350–620 kg) and donkeys (BW: 120–180 kg). Four male castrates of each breed or species were used to estimate SCFA concentrations in the forestomach or in the caecal fluid. Sheep and goats were fitted with rumen cannulae of 2 cm i.d. [11]. Cattle and camels were fitted with forestomach fistulae of approximately 8 cm i.d. [12]. The donkeys were fitted with caecum cannulae of 2 cm i.d. For estimation of SCFA absorption from the reticulorumens four sheep and four goats fitted with rumen fistulae (≈ 8 cm i.d.) were used. Surgery was performed under general anaesthesia using xylazine (Rompun, Bayer) or a combination of xylazine and ketamin (Ketavet, WDT) in donkeys with full aseptic precautions and local anaesthesia.

The experimental animals were herded in the study area together with others in a traditional manner throughout the year. A normal grazing day started at 08:00, was interrupted for sheep and goats for watering with a midday rest between 13:00 and 15:00, and ended at 18:00 when the animals returned to the night enclosures. Cattle, camels and donkeys were herded from 08:00 to 18:00 and watered at the end of the day.

2.4. Experimental procedure

Experiments were carried out repeatedly under different pasture conditions between September 1984 and December 1987 covering seven rainy seasons and respective dry seasons.

Forestomach and caecal fluid samples were taken to measure concentrations of SCFAs. Forestomach fluid volumes and flow rates of fluid from the forestomach were estimated by means of chromium-EDTA or cobalt-EDTA as non-absorbable fluid markers [9]. Forestomach fluid volumes and flow rates were estimated in camels, cattle, sheep and goats. Absorption of SCFAs from the reticulorumens of sheep and goats was measured using the technique of the washed and temporarily isolated reticulorumen [13].
2.4.1. SCFA concentrations in forestomach and caecal fluid

Fluid samples (10–20 mL) were taken at 2–4 h intervals for 16 or 24 h to estimate diurnal variations of SCFA concentrations. The samples were filtered through a tea strainer, and 5 mL aliquots were mixed with 0.5 mL of concentrated formic acid for acidification and transient storage. After transport to the home laboratory samples were centrifuged at 40 000g for 20 min. Measurements of SCFA concentrations were done in the supernatant by gas chromatography [11]. Mean daily concentrations of SCFAs were calculated after integration of the 24 h concentration profiles using a digitizing tablet (Jandel Scientific 2210).

2.4.2. Fluid volume and retention time of fluid in the forestomach

Preparation of Cr- and Co-EDTA was done according to Refs [14] and [15] respectively. Stock solutions were prepared to give a Cr or Co concentration of approximately 9 g·L\(^{-1}\). In sheep and goats 30 mL of the stock solution, and in camels and cattle 150 mL, were given as a single injection into the forestomach at 06:00. Subsequently, 20 mL samples of forestomach fluid were taken at 2–4 h intervals for 24 h for analysis of the marker. A tube inserted in the forestomach contents was used to withdraw fluid samples. After transport to the home laboratory the samples were centrifuged at 40 000g for 20 min. In the supernatant Cr and Co concentrations were determined by atomic absorption spectroscopy (Perkin-Elmer AAS 400).

2.4.3. Acetate production rates in the forestomach of camels

On two occasions during the green season and during the extremely dry season acetate production rates were measured in the four camels using a stable isotope technique [16]. A single injection of Cr-EDTA was used to estimate fluid volume and turnover of forestomach fluid. From the exponential decline of the concentration of \(^{13}\)C labelled acetate after single injection of the stable isotope and the total acetate concentration, acetate pool, acetate outflow rates and acetate production rates were calculated [17].

2.4.4. Absorption of SCFAs from the reticulorumen of sheep and goats

The technique of the washed and temporarily isolated forestomach [13] was used in sheep and goats to estimate the absorption of SCFAs through the reticulorumen wall. These experiments were carried out in the dry season only. In brief, the reticulorumen was emptied and washed free of contents using a buffer solution (Table I). The forestomach was isolated from saliva inflow by means of a saliva collector in the caudal oesophagus. Outflow was prevented using an inflatable balloon.
TABLE I. COMPOSITION (meq·L⁻¹) OF ARTIFICIAL FORESTOMACH FLUID USED IN ABSORPTION STUDIES

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (meq·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>60</td>
</tr>
<tr>
<td>Propionate</td>
<td>15</td>
</tr>
<tr>
<td>Butyrate</td>
<td>15</td>
</tr>
<tr>
<td>Na⁺</td>
<td>58</td>
</tr>
<tr>
<td>K⁺</td>
<td>80</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>1.5</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.5</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>19</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>25</td>
</tr>
<tr>
<td>H₂PO₄⁻</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

inserted in the omasal canal. Four litres of the buffer solution containing 4 mg of chromium as Cr-EDTA were prewarmed to 39°C and filled in the empty and isolated reticulorumen. Thereafter 5 mL samples were taken in 10 min intervals for 2 h and mixed with 0.5 mL of concentrated formic acid. Samples were analysed for SCFA and chromium concentrations. The actual volume \( V(t) \) of the buffer solution in litres at time \( t \) was calculated as

\[
V(t) = 4 \text{ L} \times [\text{Cr}]_0[\text{Cr}]_t t^{-1},
\]

where \([\text{Cr}]_0\) is the original chromium concentration in the buffer solution and \([\text{Cr}]_t\) the concentration at time \( t \). The pool size of SCFAs in the forestomach \( (A_i) \) was estimated as

\[
A_i = [\text{SCFA}]_i V_i,
\]

where \([\text{SCFA}]_i\) is the concentration of SCFAs at time \( t \).

2.5. Calculations

Mean retention time (MRT) of fluid in the forestomach: The equations

\[
\text{MRT} = \frac{1}{k} \quad \text{and} \quad k = (\ln C_0 - \ln C_t)t^{-1}
\]

were used, where \( k \) is the rate constant of marker elimination obtained by regression analysis, \( C_0 \) is the marker concentration at the time of injection, \( t \) is the time elapsed since marker injection, and \( C_t \) is the marker concentration at time \( t \).

Forestomach fluid volume: The amount of marker injected was divided by the marker concentration \((C_0)\) extrapolated to the time of injection by regression analysis.
Flow rate of fluid from the forestomach: Forestomach fluid volume was divided by the MRT of fluid in the forestomach.

SCFA pool size: Forestomach fluid volumes were multiplied by the mean SCFA concentrations.

Flow rate of SCFAs from the forestomach: The flow rates of fluid were multiplied by the mean SCFA concentrations of forestomach fluid.

Absorption rate of SCFAs from the temporarily isolated reticulorumen: The equation \( k_a = \frac{(\ln A_0 - \ln A_t)}{t} \) was applied, where \( k_a \) is the rate constant of SCFA absorption obtained by regression analysis, \( A_0 \) is the amount of SCFAs in the reticulorumen at the beginning of the experiment, \( t \) is the time elapsed since the beginning of the experiment, and \( A_t \) is the amount of SCFAs in the reticulorumen at time \( t \). The absorption rate was estimated as the mean SCFA concentration in the rumen fluid multiplied by \( k_a \).

Results are expressed as arithmetic means with standard deviations. Differences were tested for significance using a one way analysis of variance.

3. RESULTS

Mean total SCFA concentrations in the forestomach fluid of camels, cattle, sheep and goats and in the caecal fluid of donkeys in the three types of pasture situation are given in Fig. 1.

![Graph showing mean SCFA concentrations](image)

**FIG. 1.** Mean concentrations of short chain fatty acids (sum of acetate, propionate and butyrate) in the forestomach fluid of camels, cattle, sheep and goats, and in the caecal fluid of donkeys during dry, intermediate and green seasons. Significant differences between adjacent seasons are indicated by asterisks; level of significance: *** P < 0.001.
Camels showed higher SCFA concentrations than cattle, sheep and goats and donkeys \((P < 0.001)\) irrespective of the season. SCFA concentrations in camels were lowest in dry seasons \((140 \pm 22 \text{ mmol} \cdot \text{L}^{-1})\), significantly higher in intermediate seasons \((157 \pm 23 \text{ mmol} \cdot \text{L}^{-1})\) and highest in green seasons \((180 \pm 47 \text{ mmol} \cdot \text{L}^{-1})\). In cattle, a significantly higher SCFA concentration was measured during green seasons \((124 \pm 44 \text{ mmol} \cdot \text{L}^{-1})\) than for intermediate and dry seasons \((82 \pm 20 \text{ and } 77 \pm 10 \text{ mmol} \cdot \text{L}^{-1})\). In dry and intermediate seasons, cattle had the lowest SCFA concentrations amongst the forestomach fermenters, while in green seasons, higher concentrations were observed in cattle than in sheep and goats \((\text{average for sheep and goats: } 103 \pm 29 \text{ mmol} \cdot \text{L}^{-1}; P < 0.05)\). The mean total SCFA concentrations were generally slightly lower in goats than in sheep \((P < 0.05)\). In both animal species there were only slightly higher SCFA concentrations in the green than in the dry and intermediate seasons.

The mean SCFA concentrations in caecal fluid of donkeys were generally lower than SCFA concentrations in the forestomach fluid of camels, cattle, sheep and goats \((P < 0.001)\). As in cattle, significantly higher SCFA concentrations could be seen in the green season \((73 \pm 11 \text{ mmol} \cdot \text{L}^{-1})\) as compared with the intermediate and dry seasons \((\text{average: } 58 \pm 13 \text{ mmol} \cdot \text{L}^{-1}; P < 0.001)\).

The molar proportions of SCFAs in the forestomach fluid are shown in Table II. The molar proportion of acetate was higher in cattle during the dry \((77.6\%\) and intermediate seasons \((74.4\%)\) than in the other forestomach fermenters \((P < 0.001)\). In cattle, as in camels, the proportion of acetate was highest in the dry seasons and decreased significantly in the intermediate seasons \((P < 0.001)\). The lowest acetate proportions were observed in green seasons. Propionate and butyrate proportions, on the other hand, were highest in the green and lowest in the dry seasons.

In sheep, the proportion of acetate was only slightly lower in intermediate and green seasons than in dry seasons \((P < 0.01)\). The proportion of propionate increased at the same time. In goats, the proportions of the different SCFAs were almost independent of the season.

Donkeys showed higher acetate and lower butyrate proportions in the caecal fluid than was seen in forestomach fluid \((P < 0.001)\). As in the forestomach fluid of donkeys the proportion of acetate was highest in the dry seasons and declined in the intermediate and green seasons \((P < 0.001)\). This decline was compensated by an increase of the propionate proportion. The butyrate proportion, on the other hand, did not change markedly between the seasons, in contrast to the findings for cattle and camels.

Morning and evening concentrations of acetate, propionate and butyrate in the forestomach fluid are given in Fig. 2 to illustrate the diurnal variations of SCFA concentrations.

In the morning before animals went out for grazing no significant seasonal influence on acetate and propionate concentrations could be seen in any of the animal species. Morning butyrate concentrations were slightly higher in green than in inter-
TABLE II. MEAN RELATIVE MOLAR PROPORTIONS OF SCFAs IN THE FORESTOMACH FLUID OF CAMELS, CATTLE, SHEEP AND GOATS AND IN THE CAECAL FLUID OF DONKEYS (% OF TOTAL SCFA) DURING DRY, INTERMEDIATE AND GREEN SEASONS  
(n is the number of samples)

<table>
<thead>
<tr>
<th></th>
<th>Season</th>
<th>Acetate</th>
<th>Propionate</th>
<th>n-butyrate</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camels</td>
<td>Dry</td>
<td>72.0 ± 2.1</td>
<td>15.6 ± 2.0</td>
<td>9.4 ± 1.6</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>70.5 ± 2.5</td>
<td>15.9 ± 1.2</td>
<td>10.7 ± 1.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>66.9 ± 8.1</td>
<td>17.4 ± 4.5</td>
<td>11.8 ± 3.2</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>77.6 ± 1.8</td>
<td>13.8 ± 1.4</td>
<td>7.1 ± 1.7</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>74.4 ± 5.3</td>
<td>14.3 ± 2.5</td>
<td>8.4 ± 2.6</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>69.0 ± 3.8</td>
<td>16.8 ± 2.7</td>
<td>10.6 ± 1.4</td>
<td>27</td>
</tr>
<tr>
<td>Sheep</td>
<td>Dry</td>
<td>72.8 ± 2.9</td>
<td>15.0 ± 1.8</td>
<td>9.4 ± 1.6</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>71.7 ± 3.4</td>
<td>16.4 ± 2.4</td>
<td>8.6 ± 2.6</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>71.2 ± 3.5</td>
<td>16.1 ± 2.7</td>
<td>9.4 ± 1.7</td>
<td>112</td>
</tr>
<tr>
<td>Goats</td>
<td>Dry</td>
<td>71.8 ± 4.2</td>
<td>15.5 ± 2.8</td>
<td>9.5 ± 1.7</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>71.7 ± 3.1</td>
<td>15.9 ± 2.4</td>
<td>8.7 ± 2.5</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>71.9 ± 3.2</td>
<td>15.6 ± 2.7</td>
<td>8.7 ± 1.7</td>
<td>117</td>
</tr>
<tr>
<td>Donkeys</td>
<td>Dry</td>
<td>81.7 ± 4.4</td>
<td>13.6 ± 2.8</td>
<td>4.1 ± 1.6</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>78.9 ± 2.2</td>
<td>16.8 ± 1.8</td>
<td>4.3 ± 0.8</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>76.3 ± 2.4</td>
<td>18.7 ± 2.1</td>
<td>4.7 ± 0.7</td>
<td>30</td>
</tr>
</tbody>
</table>

mediate seasons in camels, cattle and sheep. In the evening, concentrations of SCFAs were generally higher than in the morning. However, in camels and in cattle this difference was significant in the green season only, while no increase of SCFA concentrations in the evening compared with the morning was seen in cattle during intermediate and dry seasons or in camels in the dry season.

In camels and cattle obviously only minor diurnal fluctuations of SCFA concentrations occurred in dry (camels) or dry and intermediate (cattle) seasons. In the green seasons (cattle) and in the green and intermediate seasons (camels) evening values were significantly higher than morning values, as was always the case in sheep and goats.
FIG. 2. Mean concentrations of short chain fatty acids in the forestomach fluid of camels, cattle, sheep and goats measured in the morning (05:00–08:00), before the animals went out for grazing, and in the evening (17:00–20:00) at the end of the grazing day in the dry, intermediate and green seasons.

Forestomach fluid volumes and SCFA forestomach pool sizes for camels, cattle, sheep and goats are given in Table III. The forestomach fluid volumes were larger in dry seasons than in intermediate and green seasons in all four animal species ($P < 0.001$). No significant differences between the fluid volumes in green and intermediate seasons were seen, except in camels where the volume in the green season ($40.0 \pm 4.3 \text{ L}$) was lower ($P < 0.01$) than in the intermediate season ($47.1 \pm 6.7 \text{ L}$). Owing to the larger forestomach fluid volumes, pool sizes of SCFAs
TABLE III. FORESTOMACH FLUID VOLUMES AND SCFA POOL SIZES OF CAMELS, CATTLE, SHEEP AND GOATS

<table>
<thead>
<tr>
<th>Season</th>
<th>Camels</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forestomach fluid volume (L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>58.0 ± 9.2</td>
<td>45.5 ± 11.1</td>
<td>5.9 ± 1.2</td>
<td>5.1 ± 1.5</td>
</tr>
<tr>
<td>Int.</td>
<td>47.1 ± 6.7</td>
<td>32.4 ± 10.9</td>
<td>4.4 ± 1.7</td>
<td>3.5 ± 1.1</td>
</tr>
<tr>
<td>Green</td>
<td>40.0 ± 4.3</td>
<td>34.4 ± 8.0</td>
<td>4.5 ± 1.0</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td>Forestomach SCFA pool size (mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>8.1 ± 1.3</td>
<td>3.5 ± 0.9</td>
<td>0.57 ± 0.12</td>
<td>0.45 ± 0.13</td>
</tr>
<tr>
<td>Int.</td>
<td>7.4 ± 1.1</td>
<td>2.7 ± 0.9</td>
<td>0.48 ± 0.19</td>
<td>0.34 ± 0.11</td>
</tr>
<tr>
<td>Green</td>
<td>7.2 ± 0.8</td>
<td>4.3 ± 1.0</td>
<td>0.44 ± 0.10</td>
<td>0.37 ± 0.09</td>
</tr>
<tr>
<td>Mean SCFA pool size relative to BW (mol·L⁻¹·kg⁻¹)</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

were larger in the dry than in the intermediate seasons. No significant differences between pool sizes in intermediate and green seasons occurred in camels, sheep and goats. In cattle, on the other hand, the SCFA pool size was highest in the green season (4.3 ± 1.0 mol) owing to the considerable increase of SCFA concentrations (Fig. 1) in cattle.

Flow rates of forestomach fluid and SCFAs from the forestomach into the lower gastrointestinal tract are shown in Table IV. The mean flow rates of fluid were generally higher in green seasons than in intermediate and dry seasons. This and the higher SCFA concentrations in the forestomach fluid resulted in greater flow rates of SCFAs from the forestomach SCFA pool in green seasons than in intermediate and dry seasons. The highest SCFA pool sizes and flow rates were observed in camels, followed by cattle, sheep and goats.

Acetate production rates in camels were measured during a green season and an extremely dry season. The fluid volumes and flow rates and the acetate concentrations, pool sizes, outflow rates and production rates in forestomach compartments 1 and 2 (C₁/₂) are given in Table V. When these measurements were done, feed was abundant in the green season and very reduced in the dry season.

Fluid volume, flow rates of fluid and of acetate, acetate concentration and acetate pool size correspond well with the average values shown for camels in the green season, when about 70% of the total SCFA is considered to be acetate (Tables II–IV). However, in the very reduced feeding situation forestomach fluid volume and
TABLE IV. MEAN FLOW RATES OF FORESTOMACH FLUID AND SCFAs FROM THE RETICULORUMEN INTO THE OMASUM OF CATTLE, SHEEP AND GOATS AND FROM FORESTOMACH $C_{1/2}$ INTO $C_3$ OF CAMELS

<table>
<thead>
<tr>
<th>Season</th>
<th>Camels</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate of fluid (mL·h⁻¹)</td>
<td>Dry</td>
<td>5015 ± 756</td>
<td>2949 ± 572</td>
<td>384 ± 90</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>5059 ± 750</td>
<td>3053 ± 856</td>
<td>402 ± 210</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>6542 ± 1528</td>
<td>3831 ± 547</td>
<td>507 ± 120</td>
</tr>
<tr>
<td>Flow rate of SCFAs (mmol·h⁻¹)</td>
<td>Dry</td>
<td>702 ± 106</td>
<td>227 ± 44</td>
<td>38 ± 9</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>795 ± 118</td>
<td>251 ± 71</td>
<td>46 ± 24</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>1176 ± 275</td>
<td>476 ± 106</td>
<td>56 ± 13</td>
</tr>
<tr>
<td>Mean flow rate of SCFAs relative to BW (mmol·h⁻¹·kg⁻¹)</td>
<td>1.67 ± 0.45</td>
<td>0.95 ± 0.37</td>
<td>1.27 ± 0.44</td>
<td>0.99 ± 0.29</td>
</tr>
</tbody>
</table>

TABLE V. FLUID VOLUMES AND FLOW RATES AND ACETATE CONCENTRATIONS, POOL SIZES, OUTFLOW RATES AND PRODUCTION RATES IN $C_{1/2}$ IN CAMELS DURING A GREEN SEASON ($n = 4$) AND A DRY SEASON ($n = 3$) [17]
(the camels weighed 420-515 kg in the green season and 450-560 kg in the dry season about one year later)

<table>
<thead>
<tr>
<th></th>
<th>Green season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid volume (L)</td>
<td>38.9 ± 2.1</td>
<td>39.8 ± 2.0</td>
</tr>
<tr>
<td>Flow rate of fluid (L·h⁻¹)</td>
<td>6.1 ± 0.7</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Acetate concentration (mmol·L⁻¹)</td>
<td>122.0 ± 6.2</td>
<td>111.4 ± 1.0</td>
</tr>
<tr>
<td>Acetate pool size (mol)</td>
<td>4.74 ± 0.37</td>
<td>4.48 ± 0.37</td>
</tr>
<tr>
<td>Acetate outflow rate (mmol·h⁻¹)</td>
<td>743 ± 90</td>
<td>415 ± 82</td>
</tr>
<tr>
<td>Acetate production rate (mmol·h⁻¹)</td>
<td>2234 ± 722</td>
<td>816 ± 178</td>
</tr>
</tbody>
</table>
flow rate of fluid were lower than those measured for the average dry season (Tables III and IV). Consequently acetate pool size and outflow rate were lower in this experiment than the values measured in the average dry season (Tables III and IV). The remarkable difference in acetate production rate between the green and dry seasons was not reflected by corresponding differences in the SCFA concentrations. A reduction of the acetate production by about 70% was accompanied by only a 10% lower acetate concentration. Using the data of Höller et al. [17], the acetate absorption from compartment C₁/₂ in the camels can be calculated. This leads to an estimated absorption rate of 1490 ± 675 mmol·h⁻¹ in the green season and 401 ± 181 mmol·h⁻¹ in the dry season.

FIG. 3. Absorption rates and rate constants of acetate, propionate and butyrate from the temporarily isolated reticulorumen of sheep and goats; measurements were done in the dry season.
Absorption rates of SCFAs from the forestomach of sheep and goats and the respective rate constants are given in Fig. 3. The rate constants of acetate, propionate and butyrate absorption were not different between the two animal species. Rate constants for propionate and butyrate absorption were about 60–90% higher than those for acetate absorption ($P < 0.001$). The estimated absorption rates, on the other hand, were higher for acetate (average for sheep and goats: $14.9 \pm 5.4$ mmol·h$^{-1}$) than for propionate ($5.39 \pm 2.4$ mmol·h$^{-1}$) and for butyrate ($3.45 \pm 1.20$ mmol·h$^{-1}$); this is certainly due to the higher concentration of acetate. No significant difference in the rate of SCFA absorption measured in the dry season could be seen when sheep and goats were compared.

4. DISCUSSION

The SCFA concentrations measured in the present study are in agreement with those reported by Williams [18] and Farid et al. [19] for camels, and with those found in other ruminants on a roughage based diet (reviewed by Bergman [4]). It is notable that SCFA concentrations in camels were about 30–40% higher than in the true ruminants. These higher concentrations correspond with a larger SCFA pool (Table III) and a higher flow rate of SCFAs from the forestomach (Table IV).

It was assumed repeatedly that concentrations of SCFAs estimated in forestomach fluid are an indicator for differences in SCFA production rates. Our findings clearly signify that such expected differences in microbial metabolism are not necessarily reflected by differences in concentrations of SCFAs in the forestomach.

Concentrations of SCFAs in the fermentation chamber depend mainly on:

(a) Production rates of SCFAs,

(b) The outflow rate of fluid from the fermentation chamber and the entry rate of fluid into the chamber,

(c) The fluid volume of the fermentation chamber,

(d) The absorption rate of SCFAs across the epithelium of the fermentation chamber.

These parameters may change rather independently from each other owing to variations in feeding conditions, and significant species differences exist. This will be discussed briefly with respect to the SCFA concentrations.

The flow rates of fluid from the forestomach relative to body weight were not different between ruminants and camels, averaging $12.1 \pm 0.38$ mL·h$^{-1}$·kg$^{-1}$ in the green season and $10.8 \pm 0.26$ mL·h$^{-1}$·kg$^{-1}$ in the dry season [9]. The flow rates are similar to the entry rate of fluid into the chamber under steady state conditions when absorption of fluid is neglected. The ileal flow into the caecum of ponies and horses fed conventionally is somewhat lower, 7.1–8.8 mL·h$^{-1}$·kg$^{-1}$ [20]. However, owing to marked differences in the volume of the fermentation chamber, fluid turnover rates in the chamber can be rather different.
Turnover of fluid (as a percentage of fluid volume per hour) was faster in the green season than the dry season in all animal species, and was higher in camels than in cattle, sheep and goats (Table VI). Higher fluid turnover causes a more rapid dilution of SCFAs in the compartment, and thus it should lower SCFA concentrations. SCFA concentrations had been, however, mostly higher when fluid turnover was high (Fig. 1).

The faster turnover may contribute to a low difference in SCFA concentrations between seasons, and thus may mask the higher production of SCFAs expected during the green season. Different fluid turnover rates also make an interspecies comparison difficult. In donkeys, for example, owing to the much smaller volume of the caecum compared with the forestomach, fluid turnover is faster, which may explain the lower SCFA concentrations (Fig. 1); but in the donkey also the production of SCFAs in the caecum is expected to be lower than in the forestomach owing to substantial pre-caecal digestion and absorption of carbohydrates.

Seasonal differences in the available amount and particularly the quality of feed plants on a semiarid pasture necessarily influence microbial SCFA production in the forestomach of ruminants and of camels. Studies of feeding behaviour and nylon bag digestibility trials carried out in parallel with the present studies showed that camels and goats are the more selective feeders and are able to obtain a diet that is easier to degrade than is the case for sheep, donkeys and cattle [7, 10]. Differences between animal species in the ability to select plants of higher degradability are greater in dry seasons; camels and goats are by far superior to cattle and donkeys in this respect. From the observed feeding behaviour we have to assume higher fermentation rates and thus higher production of SCFAs in the forestomach of camels and goats. Differences between cattle, sheep and goats were, however, not in any way reflected by the SCFA concentrations measured in the reticulorumen. Factors other than dilution in the forestomach must be involved.
Originally we had assumed that differences in absorption of SCFAs may explain the lower SCFA concentrations in goats than in sheep. However, absorption measured from the temporarily isolated reticulorumen did not show significant differences between these two small ruminant species (Fig. 3). At least in these two species absorption does not explain differences in SCFA concentrations.

Production of SCFAs in the forestomach had not been estimated in our study. However, in parallel studies production rates of acetate had been measured in the forestomach of camels during the dry and green seasons [17]. An almost threefold higher acetate production rate was measured in the green season compared with the dry season. The absorption of acetate from the forestomach was increased correspondingly. The acetate concentration in the forestomach fluid, on the other hand, did not vary significantly (Table V). These findings clearly indicate that concentrations of acetate in no way reflect the considerably higher SCFA production in the green season.

Under most conditions SCFA concentrations were higher in the evening after the end of the grazing period compared with the morning samples (Fig. 2). These differences are, however, small compared with the differences in microbial fermentation that have to be expected between the early morning and the late afternoon. Diurnal variations in dilution rate and in absorption of SCFAs from the forestomach may be an explanation.

In conclusion, concentrations of SCFAs in the fermentation chambers do not reflect actual production rates of SCFAs in interspecies comparisons or when various feeding conditions within one species are compared. Species differences and variations in dilution rate, rumen fluid volume and absorption rate influence SCFA concentrations far more than production rate.

REFERENCES


RADIOIMMUNOASSAY OF AFLATOxin M1 IN MILK

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Aflatoxin M1 is a 4-hydroxy derivative of aflatoxin B1, produced in the liver by the action of monooxygenases of the microsomal fraction of hepatocytes. It is excreted either in conjugated form with sulphates or glucuronates in bile and urine, or free in milk and urine.

Radioimmunoassay (RIA) is a suitable method for direct screening of aflatoxin M1 in fat free milk owing to its high sensitivity and accuracy and good reproducibility of results, as well as to the feasibility of automation. The RIA kit supplied by the Institute for Development and Use of Nuclear Techniques, Košice, is designed primarily for checking the quality of raw milk. It can also be used for investigating the effects of aflatoxin in dairy cows, especially in herds affected with increased embryonic mortality, and the resulting service returns and abortions.

FIG. 1. Aflatoxin M1 in milk from 25 farms.
Of the total of 753 samples collected from 25 farms, 742 were from individual cows and 11 from milk tanks. One quarter of the samples were obtained from seven cows. The samples were analysed by the standard method as described in the kit instructions (Fig. 1).

In 162 (21.51%) of the 753 samples examined, the concentration of aflatoxin M1 exceeded the limit set for baby milk food (> 0.1 µg/L) and in 3 of them the limit set for normal market milk was exceeded (> 0.5 µg/L).

Screening of aflatoxin M1 in a herd can be performed by examination of 30 to 50 milk samples collected and processed for the milk progesterone test. Our results have shown that in individual cows aflatoxin M1 was excreted in milk in concentrations easily measurable by the RIA.

Screening of aflatoxin M1 in skim milk is suitable for:

(a) Quality checks of raw milk,
(b) Investigations of the effects of aflatoxin on health and reproduction in animals,
(c) Detection of contamination of feedstuffs with mycotoxins.

IAEA-SM-318/14P

THYROID HORMONES INFLUENCE MANY BODY FUNCTIONS, INCLUDING MATURATION, GROWTH, METABOLISM AND LACTATION. THE OBJECTIVE OF THE STUDY DESCRIBED HERE WAS TO MEASURE PLASMA THYROXINE (T₄) AND TRIIODOTHYRONINE (T₃) AND RELATE THEM TO MILK T₄ AND T₃ AT DIFFERENT STAGES OF LACTATION IN DAIRY COWS AND BUFFALOES, AS WELL AS TO DETERMINE WHETHER T₄ AND T₃ IN MILK REPRESENT SIGNIFICANT AMOUNTS FOR TRANSFER TO THE OFFSPRING. T₄ AND T₃ WERE EXTRACTED FROM MILK BY THE METHOD OF AKASHA AND ANDERSON [1]. THEIR CONCENTRATIONS IN PLASMA AND MILK WERE ESTIMATED BY RADIOIMMUNOASSAY [2, 3] USING RIA KITS PROCURED FROM THE BHABHA ATOMIC RESEARCH
Centre, Bombay. The investigation was conducted on 10 crossbred cows and 12 Murrah buffaloes which were in their third to fourth lactation. Blood samples were collected at weekly intervals during the last month of pregnancy, at parturition, in the first month of lactation and subsequently at monthly intervals till the end of lactation. Milk samples were collected at weekly intervals during the first month of lactation and subsequently at monthly intervals till the end of lactation.

The plasma T4 and T3 levels in cows and buffaloes during pre-partum, parturition, and early, mid- and late lactation stages and changes in the concentrations of milk T4 and T3 during lactation are given in Tables I and II respectively. In both species the plasma T4 concentrations revealed a declining trend during the last month of pregnancy and approaching parturition, followed by a significant rise

**TABLE I. CONCENTRATIONS (MEAN ± SD, ng/mL) OF T4 AND T3 IN PLASMA OF CATTLE AND BUFFALOES**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Plasma T4</th>
<th>Plasma T3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Buffaloes</td>
</tr>
<tr>
<td>Pre-partum</td>
<td>74.2 ± 2.8</td>
<td>73.0 ± 1.8</td>
</tr>
<tr>
<td>Parturition</td>
<td>69.5 ± 6.2</td>
<td>75.0 ± 4.1</td>
</tr>
<tr>
<td>Early lactation</td>
<td>59.6 ± 2.6</td>
<td>59.4 ± 1.7</td>
</tr>
<tr>
<td>Mid-lactation</td>
<td>66.1 ± 3.6</td>
<td>51.2 ± 2.3</td>
</tr>
<tr>
<td>Late lactation</td>
<td>63.6 ± 4.4</td>
<td>60.2 ± 2.3</td>
</tr>
</tbody>
</table>

**TABLE II. CONCENTRATIONS (MEAN ± SD) OF T4 AND T3 IN MILK OF CATTLE AND BUFFALOES**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Milk T4 (ng/mL)</th>
<th>Milk T3 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Buffaloes</td>
</tr>
<tr>
<td>Early lactation</td>
<td>0.37 ± 0.03</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>Mid-lactation</td>
<td>0.26 ± 0.02</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>Late lactation</td>
<td>0.30 ± 0.02</td>
<td>0.29 ± 0.02</td>
</tr>
</tbody>
</table>


on the day of parturition. The plasma T₃ levels recorded during the pre-partum phase were lower than those recorded throughout the lactation period in both species. The decrease in plasma T₄ and T₃ during the pre-partum phase might be due to dilution by an increased blood volume during late pregnancy. Negative feedback control caused by the active foetal thyroid could be another factor [4]. After expulsion of the foetus, the negative feedback ceases to function and may cause the significant rise (P < 0.05) in plasma T₄ observed on the day of parturition.

The low plasma T₄ levels during early lactation were found to have a significant negative correlation with milk yield in both cows and buffaloes, which might be due to high demands from peak milk yield. Subsequently the plasma T₄ concentration rose to a peak during mid-lactation and thereafter dropped slightly during the late lactation period in cows. In buffaloes, however, plasma T₄ decreased during the mid-lactation phase, with the mean T₄ concentration being significantly lower (P < 0.05) than during early lactation. This could be attributed to the difference in the thermoregulatory mechanism between the two species. The winter season coincided with the mid-lactation phase of the buffaloes and the low T₄ levels were probably due to an increased demand by body tissues for thermogenesis. The secretion rate may be higher in winter [5] but increases in tissue uptake during winter probably overwhelm such an increase [6]. The significant rise (P < 0.05) in plasma T₄ during late lactation in buffaloes could be due to the onset of spring, during which the secretion rate probably does not fall very much and there is decreased demand for T₄ owing to milder ambient temperatures [6]. The plasma T₃ concentrations reflected a non-specific pattern during the course of lactation in both species, which is in agreement with the findings of Akasha et al. [7].

In cows the milk T₄ levels revealed a significant decline (P < 0.05) from early to mid-lactation (0.37 vs. 0.26 ng/mL), whereas the T₃ levels in milk increased during the corresponding period. The reverse pattern was observed in buffaloes.

The difference in the circulating levels of T₄ during lactation could be explained by the difference in the responsiveness to the environment between the two species. The results also support the contention of Akasha and Anderson [1] that though milk is not a significant source of thyroid hormone for the newborn, these levels may provide sufficient metabolic stimuli to ensure survival for newborn experiencing minimal thyroid function.

REFERENCES


IAEA-SM-318/28P

RELACION DEL COMPORTAMIENTO PRODUCTIVO Y REPRODUCTIVO CON LOS NIVELES DE TIOXINA, TRIIODOTIRONINA Y GLUCOCORTICOIDES DE VACAS HOLSTEIN EN EL TROPICO

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Se estudió el comportamiento reproductivo (intervalo parto-1er servicio (IP1S), parto-gestación (IPG) y servicios por gestación (SG)) de 68 vacas Holstein lactando (34 en cada estación del año) y el productivo de 180 vacas de la misma raza (90 en cada estación del año, correspondiendo 30 a cada trimestre de lactancia), en relación con las concentraciones séricas de tiroxina (T4) y triiodotironina (T3) medidas por RIA y glucocorticoides (F) determinados por CPB. Paralelamente se determinó la concentración de yodo en leche (I).

Las concentraciones de todos los indicadores fueron menores en la estación de verano (Fig. 1). Existió menor producción diaria de leche (p<0,001) en el verano (15,5 kg) en relación con el invierno (20,5 kg). Los niveles de I estuvieron por debajo de 25 μg/L. Se observó correlación (r = 0,361, p<0,001) entre los niveles de F y la producción lactea que aumenta en el segundo trimestre de producción (r = 0,619, p<0,001). No se encontró relación entre la producción y los niveles de hormonas tiroideas, aunque sí existió efecto de la etapa de la lactancia sobre estas hormonas (Fig. 2).
FIG. 1. Concentraciones de tiroxina (T4), triiodotironina (T3), yodo y glucocorticoides (F) en cada estación del año.

FIG. 2. Concentraciones de tiroxina (T4) y triiodotironina por trimestre de lactancia.

Existió asociación inversa entre T3-IPG \( r = -0.239, p<0.1 \) F-IPG \( r = 0.4587, p<0.001 \) y F-SG \( r = 0.465, p<0.001 \). En el verano resultaron de gran interés las asociaciones inversas T3-IPG \( r = -0.381, p<0.05 \) y T3-SG \( r = 0.363, p<0.05 \), siendo estas relaciones aún más fuertes al final de esta estación. Estas asociaciones no se encuentran en el invierno, por lo que se
concluye que las condiciones climáticas del verano afectan a los niveles de T3, T4 y F en la raza Holstein, lo que repercute de forma negativa tanto en su comportamiento productivo como reproductivo. Por lo tanto, es posible considerar el empleo de los niveles de estas hormonas en la selección de animales de esta raza más adaptados a las condiciones del trópico.

IAEA-SM-318/31P

RUMINAL AND INTESTINAL NYLON BAG DEGRADATION OF CELL WALL CONSTITUENTS OF GRASS SILAGES PRESERVED WITH DIFFERENT ADDITIVES

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The effect of ensiling additives on ruminal and intestinal nylon bag degradation of grass silage cell wall constituents was studied. Herbage, consisting of *Dactylis glomerata* and *Phleum pratense*, was ensiled with the following commercial additives in small scale silos of 3 m³:

A: AIV-2 containing 80% formic acid and 2% orthophosphoric acid (5.5 L/t);
B: Ensimax containing 50% Finfermex solution, 25% formic acid, 15.5% acetic acid and 9.5% hydrochloric acid (5.6 L/t);
C: Clampzyme containing cellulase and glucose oxidase (200 mL/t);
D: Without additive.

Silages supplemented with a 1:1 mixture of barley and oats were given (70:30 on dry matter basis) to provide maintenance, in a 4 × 4 Latin square, to four non-lactating cows, each fitted with a cannula in the rumen and duodenum.

Fresh, chopped grass silages were incubated in the rumen for 2, 4, 8, 16, 24, 48, 72 and 96 h. Intestinal degradation of the feeds was measured after 30 h of rumen incubation by the mobile bag method. The rumen disappearance values of organic matter (OM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were fitted to the equation $p = a + b(1 - e^{-ct})$ [1].

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FIG. 1. Degradability and digestibility in vivo of (a) organic matter and (b) neutral detergent fibre of grass silages preserved with different additives, A–D (described in text).

All silages were of good quality, containing no propionic or butyric acid. The lactic acid and water soluble carbohydrates contents (g/kg DM) of silages ensiled with A, B, C and D were 14, 74, 105, 106 and 64, 16, 23, 15 respectively. The NDF and ADF contents (g/kg DM) of the silages were 524, 509, 471, 501 and 310, 303, 275, 302 respectively, being clearly reduced in silage preserved with the cellulase-containing additive C.

There were no marked differences between silages in the maximum potential degradability in the rumen \((a + b)\) (mg/g) for OM (850, 851, 853, 862 respectively), but for NDF (792, 791, 777, 807) and for ADF (798, 801, 784, 817) the values for C were reduced compared with the other additives, probably owing to cellulase activity. The difference was significant \((P < 0.05)\) between silages C and D.
The subsequent degradation of feed residues (mg/g) in the intestine after 30 h of rumen incubation gave 112, 102, 112, 102 for OM; 64, 63, 63, 61 for NDF; and 64, 64, 59, 60 for ADF. The calculated values for total tract degradation (TTD) (ruminal disappearance + intestinal disappearance)/rumen-undegraded) were 720, 727, 737, 749 for OM; 584, 590, 574, 614 for NDF; and 598, 604, 578, 629 for ADF.

The intestinal OM, NDF and ADF degradations as proportions of TTD (mg/g) were 49, 45, 45, 38 for OM; 49, 48, 51, 41 for NDF; and 46, 46, 46, 37 for ADF. However, the differences between silages for the degradation parameters measured, except maximum potential degradability for NDF and ADF, were not statistically significant ($P > 0.05$).

When the TTD values of OM, NDF and ADF were compared with the digestibility coefficients of the same feeds measured by the conventional method [2], TTD values were on average about 2.3, 24.1 and 24.8% lower for OM, NDF and ADF respectively. The rather large difference for cell wall constituents was most probably due to too short an incubation time in the rumen (30 h) used with the nylon bag technique compared with the actual retention time of the cell wall fractions of 50–60 h, obtained on the respective grass silage diets [3]. However, the methods used gave a similar order of digestibility of feeds, so that the cell wall digestibility of C was lowest with both methods (Fig. 1).

**REFERENCES**


NUTRITIONAL STRATEGIES FOR THE BETTER UTILIZATION OF CROP RESIDUES IN MAURITIUS

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In Mauritius, a tropical sugar cane growing island, two distinct systems of cattle production at the national level have been identified:

(a) The village smallholder system, characterized by low inputs and ownership of a cow and a calf; and
(b) Large farms with more than 50 head.

A pilot project was executed in two groups of villages during which two types of concentrates (a locally compounded dairy concentrate of 17% crude protein with 30% sugar cane molasses, and imported cottonseed cake with 41% crude protein) were provided free to 88 lactating cows for a whole lactation of 301 d. The small farmers maintained their traditional management practices and fed their cows forages (sugar cane tops during the crop season, June–November, and a variety of grasses, shrubs, tree leaves, vegetable crop residues, etc., during the remaining months) according to their usual practice. The only new input in their daily routine was the addition of concentrates according to the desired production.

The results in terms of (1) milk production in general and by breed and (2) quantity of forage consumed support the idea that the small farmers have learned through generations of experience how to use crop residues very efficiently. The additional input of concentrate improved the whole ration and the cows performed very well, as shown in Tables I–III.

TABLE I. MEAN MILK PRODUCTION OF VILLAGE COWS

<table>
<thead>
<tr>
<th>Type of Concentrate</th>
<th>Mean Milk Production (kg/lactation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With zero or very little concentrate</td>
<td>1000–1200</td>
</tr>
<tr>
<td>With concentrates according to desired production (0.5 kg dairy concentrate or 0.25 kg cottonseed cake per litre)</td>
<td>2770 ± 65 (SE)</td>
</tr>
</tbody>
</table>
TABLE II. MEAN MILK PRODUCTION BY BREED\textsuperscript{a} WITH FEEDING OF CONCENTRATES

\begin{tabular}{lll}
\hline
 & \textit{n} & kg/lactation (± SE) \\
\hline
Creole\textsuperscript{b} & 23 & 2838 ± 224 \\
Friesian & 25 & 2679 ± 166 \\
Creole–Friesian cross & 40 & 2747 ± 119 \\
\hline
\end{tabular}

\textsuperscript{a} There was no significant difference in milk production between the breeds.
\textsuperscript{b} The local breed, a dual purpose type.

TABLE III. QUANTITY OF FORAGE CONSUMED, LIVE WEIGHT OF COWS AND DRY MATTER INTAKE WITH FEEDING OF CONCENTRATES

\begin{tabular}{lll}
\hline
 & Sugar cane tops & Mixed grasses \\
\hline
Fodder eaten (kg fresh weight/d) & 48.9 & 55.7 \\
Live weight (kg) & 353 & 332 \\
Total intake\textsuperscript{a} (kg DM/d) & 13.4 & 14.4 \\
Total intake\textsuperscript{a} (kg DM/100 kg LW) & 3.8 & 4.3 \\
\hline
\end{tabular}

\textsuperscript{a} Includes concentrate.

\textit{Strategy for the large farms}

Because of the large daily requirements of forage on the large farms and the inherent difficulties associated with selection of good quality fodder an alternative strategy is adopted to make maximum use of the ration. A daily supplement of molasses, which is a source of readily available energy, makes up for the deficiency in the forage.

Data from two Government farms (about 275 lactating cows) illustrate this point clearly. When the cows were given cottonseed cake at 3 kg/d and sugar cane tops or mixed grasses, milk production dropped from 7.5 to 4.0 kg/d; the cows were losing condition and had calving difficulties. When the dairy concentrate (at 6 kg/d) having 30\% molasses was given instead, milk production rose to 7.5 kg/d again and the cows recovered condition.
Conclusion

The above data point to the following facts:

(a) The village smallholder is right in the way he selects a variety of crop residues and forages for his cows.
(b) The input of a proper protein concentrate makes possible a more efficient use of the crop residues irrespective of breed.
(c) The correct strategy for the large farms is to use molasses to make more efficient use of the crop residues for milk production.

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RELATIONSHIP BETWEEN FOOD INTAKE, GROWTH, BLOOD HORMONES AND BODY COMPOSITION IN SARDI AND D'MAN LAMBS

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The D'Man sheep, a Moroccan breed known for its high prolificacy, possesses also a high relative growth rate which can be efficiently used in crossbreeding. Thus the absolute and relative growth rates between three and six months of age are respectively $144 \pm 7 \text{ g} \cdot \text{d}^{-1}$ and $15.8 \pm 0.6 \text{ g} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ in D'Man, 154 and 13.0 in Sardi, and 157 and 14.5 in Sardi × D'Man.
A study was undertaken to elucidate the physiological mechanisms behind the difference in relative growth rates between D’Man and Sardi sheep. The results showed that the higher relative growth rate in D’Man as compared with Sardi lambs was associated with higher voluntary food intake (191 vs. 174 ± 4 kcal·kg\(^{-0.75}\)·d\(^{-1}\))\(^1\) and higher plasma levels of triiodothyronine (1.35 vs. 1.12 ± 0.10 ng·mL\(^{-1}\)) and plasma insulin levels (23 vs. 17 ± 3 µU·mL\(^{-1}\)). Examination of body composition showed that D’Man lambs have much more internal fat than Sardi.

When offered food was adjusted to 1.5 times the maintenance requirements in both breeds (120 kcal·kg\(^{-0.75}\)·d\(^{-1}\)), plasma triiodothyronine and insulin decreased slightly, but the difference in hormone levels between D’Man and Sardi remained the same as when the animals were offered food ad libitum.

The results suggest that the hormonal capacity to metabolize energy intake is higher in D’Man lambs and this allows a relatively greater food intake and growth in comparison with Sardi lambs.

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**EFFECT OF POULTRY MANURE ON RUMEN FERMENTATION IN THE ANGORA GOAT**

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Experiments were conducted to study the effect of feeding poultry manure on rumen net volatile fatty acid (VFA) production rate, microbial protein synthesis, ruminal NH\(_3\)-N concentration and pH level. The main purpose of the study was to evaluate poultry manure as an alternative nitrogen source for Angora goats.

Three rumen cannulated Angora bucks of 40 kg average live weight and five years of age were used in a 3 × 3 Latin square design and each was fed daily: (I) no supplement; (II) 0.219 kg of poultry manure which is sun dried at about 30–35°C

\(^1\) 1 cal = 4.186 J.
for two days and ground; or (III) 0.146 kg of soybean meal. Each animal received a daily basal diet of 1 kg of chopped grass hay, 0.5 kg of barley and 0.02 kg of mineral mixture (no mineral mixture was added to the poultry manure supplemented diet). The goats were held in metabolism cages and fed by automatic feeding devices. The period of adaptation to the diet for each animal was 15 d. The net VFA production rate in the rumen was measured by single injection of $^{14}$C-acetate (50 µCi (1.85 MBq) in 50 mL) through the rumen cannula [1]. Microbial output from the rumen was measured by in vitro incubation of U-$^{(14)}$C-glucose with the rumen contents [2, 3]. The efficiency of microbial protein synthesis in the rumen was estimated as the amount of microbial nitrogen (MN) per kilogram of fermented carbohydrate in the rumen.

The mean estimated liquid volume of the rumen, net VFA production rate, microbial output estimated from incubation of $^{14}$C-glucose with rumen contents and from VFA production, estimated efficiency of microbial protein synthesis in the rumen, ruminal NH$_3$-N concentration and ruminal pH measurements for diets I, II and III are presented in Table I.

### Table I. Estimated Mean Rumen Liquid Volume, Fractional Outflow Rate, Net VFA Production Rate, Microbial Output, Efficiency of Microbial Protein Synthesis, NH$_3$-N Concentration and pH Level in the Rumen of Angora Goats (Average of 3 Goats) for Diets I, II and III

<table>
<thead>
<tr>
<th>Diet</th>
<th>I Basal</th>
<th>II Poultry manure supplement</th>
<th>III Soybean supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen liquid volume (L)</td>
<td>3.86</td>
<td>3.20</td>
<td>3.93</td>
</tr>
<tr>
<td>Fractional outflow rate (L/s)</td>
<td>0.33</td>
<td>0.53</td>
<td>0.57</td>
</tr>
<tr>
<td>Net VFA production rate (mol/d)</td>
<td>2.59</td>
<td>3.33</td>
<td>3.37</td>
</tr>
<tr>
<td>Estimated microbial output (g/d)</td>
<td>48.65</td>
<td>113.72</td>
<td>91.65</td>
</tr>
<tr>
<td>Estimated efficiency of microbial protein synthesis (g MN/kg fermented carbohydrate)</td>
<td>16.84</td>
<td>30.64</td>
<td>24.35</td>
</tr>
<tr>
<td>NH$_3$-N (mg/L)</td>
<td>177</td>
<td>271</td>
<td>436</td>
</tr>
<tr>
<td>pH</td>
<td>6.20</td>
<td>6.80</td>
<td>6.20</td>
</tr>
</tbody>
</table>
The differences between rumen liquid volumes for diets I, II and III were not significant ($P > 0.1$). The fractional outflow rates for diets II and III were similar but both were significantly different from the value obtained for diet I ($P < 0.01$). The same was true for net VFA production rates for diets I, II and III. Estimated mean values for microbial output and the efficiency of microbial protein synthesis in the rumen for the poultry manure and soybean supplemented diets (II and III) were significantly different from the estimated values for diet I. The highest NH$_3$-N concentration was observed for the soybean added diet (III). Also, the NH$_3$-N concentration for diet II was higher ($P < 0.01$) than that for diet I. The mean pH measurements on days 13, 14 and 15 for the poultry manure added diet (II) were higher ($P < 0.01$) than for diets I and III.

In conclusion, there was no significant effect of protein supplementation on net VFA production rate but the efficiency of microbial protein synthesis in the rumen of Angora goats was significantly ($P < 0.01$) increased. As a result poultry manure appears to be a promising alternative nitrogen source for the feeding of Angora goats.

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METABOLIC EFFECTS OF PROTOZOA IN THE RUMEN OF SHEEP

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Recent findings show that ruminal protozoa decrease the bioavailability of copper in sheep receiving soybean meal (SBM) but not in sheep receiving casein (CA) as a protein supplement [1]. Differences in metabolism of the two supplements by the protozoa were suspected as being a major factor in the interaction between ruminal protozoa, dietary protein and dietary Cu metabolism. This hypothesis was tested with two groups of four fauna-free wethers, each containing rumen and duodenal cannulae. One group received a CA supplemented corn silage diet and the other an SBM supplemented corn silage diet. Daily rations were fed in 12 equal portions at 2 h intervals. After the animals had received experimental diets for 14 d, sampling of ruminal fluid and duodenal digesta was carried out for 17 d. Each wether was inoculated with a mixed population of ruminal protozoa after the first 4 d of sampling.

Daily examinations of the ruminal fluid from each wether confirmed the protozoa-free status before the ruminal inoculation with protozoa. Thereafter, the protozoal population on both diets rapidly increased each day and reached normal levels of around $10^6$/mL after day 9. This was associated with an increase ($P < 0.01$) in ruminal pH and a decrease ($P < 0.01$) in total N concentration in ruminal fluid of wethers on both diets. There was, however, very little effect of protozoal population on the concentrations of ammonia N, α-amino N, sulphide and soluble Cu for wethers fed the CA diet, while there was a substantial increase in these N compounds, and a decrease in soluble Cu, due to faunation of wethers fed the SBM diet (Table I). It appears that the limited ability of protozoa to utilize ruminally soluble CA [2] was responsible for the lack of major changes for wethers fed the CA diet. However, the ability of ruminal protozoa to utilize ruminally insoluble SBM [2] resulted in the observed effects in wethers fed the SBM diet.

It is evident that protozoa ingest SBM and release amino acids and inorganic sulphur. The amino acids are metabolized by ruminal bacteria and additional S is released from the S-containing amino acids. Both S forms are rapidly reduced by the bacteria to sulphide, which forms insoluble complexes with Cu in the rumen and has a negative effect on Cu bioavailability [3]. However, CA is poorly metabolized by
TABLE I. MEAN CONCENTRATIONS OF NITROGEN, SULPHIDE AND SOLUBLE COPPER IN RUMINAL FLUID, AND DUODENAL FLOW OF AMINO ACIDS AND AMMONIA NITROGEN DURING 4 d BEFORE (FF) AND 9-13 d AFTER (FT) INOCULATION WITH RUMINAL PROTOZOA (PA) OF FAUNA-FREE SHEEP FED CASEIN OR SOYBEAN MEAL AS A PROTEIN (PN) SUPPLEMENT

<table>
<thead>
<tr>
<th></th>
<th>Casein</th>
<th>Soybean meal</th>
<th>SE</th>
<th>PN</th>
<th>PA</th>
<th>PN × PA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rumen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N (mg/100 mL)</td>
<td>183</td>
<td>151</td>
<td>189</td>
<td>168</td>
<td>11.4</td>
<td>NS</td>
</tr>
<tr>
<td>Ammonia N (mg/100 mL)</td>
<td>22.2</td>
<td>22.6</td>
<td>12.9</td>
<td>19.2</td>
<td>1.69</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Free α-amino N (mg/100 mL)</td>
<td>2.7</td>
<td>3.6</td>
<td>2.7</td>
<td>7.9</td>
<td>1.01</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Sulphide (μg/mL)</td>
<td>4.1</td>
<td>3.8</td>
<td>3.9</td>
<td>4.8</td>
<td>0.35</td>
<td>NS</td>
</tr>
<tr>
<td>Soluble Cu (μg/100 mL)</td>
<td>15.3</td>
<td>16.2</td>
<td>14.6</td>
<td>11.0</td>
<td>1.60</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Duodenal flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/kg organic matter intake)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total amino acids</td>
<td>114</td>
<td>82</td>
<td>137</td>
<td>109</td>
<td>7.5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Ammonia N</td>
<td>1.44</td>
<td>1.40</td>
<td>1.29</td>
<td>1.50</td>
<td>0.148</td>
<td>NS</td>
</tr>
</tbody>
</table>

protozoa; therefore, the protozoal requirement for N is to a large extent derived from an engulfment of ruminal bacteria. This results in a larger decrease due to faunation in the amino acid flow into the intestinal tract (Table I) of sheep fed the CA diet (28%) than of those fed the SBM diet (20%). However, bacterial protein when degraded by protozoa is mostly excreted as peptides, which are utilized by the remaining bacteria [4]. Therefore, the microbial S is not degraded to inorganic form, and cannot be reduced by bacteria to sulphide. This difference in sulphide production explains why protozoa decrease both the solubility of dietary Cu in the rumen and the concentration of Cu in the liver of sheep fed SBM supplements [1], and why protozoa do not have an effect on the dietary Cu metabolism when sheep are fed CA [1] or urea [5] supplements.
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ANIMAL REPRODUCTION

(Session 2)

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OPTIMIZING REPRODUCTIVE RATES IN CATTLE ON LOW QUALITY FORAGE BASED DIETS

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Abstract

OPTIMIZING REPRODUCTIVE RATES IN CATTLE ON LOW QUALITY FORAGE BASED DIETS.

In many of the world's tropical regions, cattle on low quality forage based diets are exposed to varying periods of undernutrition which reduce reproductive efficiency. In such groups of animals, temperate zone concepts of high annual calving rates are often incompatible with high levels of survival of pregnant and lactating cows. Hence, optimal biological and economic levels of reproductive performance can be lower than those accepted as satisfactory elsewhere. Herd modelling techniques offer a useful approach to determining optimal levels of reproductive performance when other production traits such as growth and survival are incorporated into models. Net reproductive rate (NRR) calculations provide a compact picture of the balance between fertility and survival in a population. NRR is a useful statistic for defining stages of reproductive wastage in cattle in developing countries, in order to best develop appropriate strategies for improvements in fertility. Nutritional effects on reproduction are frequently expressed in live weight and body condition changes, which, though imprecise measures, are useful practical indicators of nutrient status. Manipulation of pre-pubertal nutritional levels reduces target weights at conception and improves fertility in maiden heifers at mating at two years of age. For heifers and lactating cows, relationships of mating weight to fertility are linear and curvilinear respectively. Lactating cows are especially sensitive to both pre- and post-partum nutritional stresses. Techniques to minimize such stresses either through provision of extra nutrients or by reducing nutrient drain through restricting milk production, e.g. through early weaning or creep feeding, enhance subsequent fertility. Climatic variables influencing reproduction in cattle on low quality roughages include pasture growth days and timing and extent of dry season rainfall, all of which are useful predictors of re-conception rates, and which are useful aids in making nutritional management decisions. Finally, regardless of the production systems involved, evaluation of nutritional and management technologies needs to be considered in both biological and economic terms.

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1. INTRODUCTION

Reproduction, milk production, growth and survival are the most important production traits influencing the profitability and success of beef and multipurpose cattle units in the tropics. Where income is from sale of animals, high reproductive performance is directly related to high productivity. Where material is harvested from animals e.g. milk, high reproductive rates are important for optimal levels of output, while for draught animals optimal reproductive performance ensures a readily available cash reserve.

Cattle in almost all tropical regions have fibrous roughages as their major food source. Whether these roughages come from 'cut and carry' systems, from straw gathered post-harvest or from grazing, their diets are frequently subjected to seasonal fluctuations which influence reproductive rates. In many situations it is often difficult to distinguish between 'seasonal effects' and nutritional effects on reproduction. However, the two are interrelated, since nutritional variation is almost always a consequence of seasonal climatic variation. In higher latitudes, seasonal photo-periodic effects may be of consequence, but are of lesser importance in more equatorial environments.

In this paper, optimal levels of fertility for cattle on poor quality diets are briefly considered, and some recent developments in nutritional and management techniques which offer scope for optimizing reproduction are outlined. While major emphasis is placed on techniques applicable to grazing animals in the tropics, many of the technologies, with suitable modifications, have application in smallholder systems of production.

2. OPTIMAL FERTILITY LEVELS IN CATTLE

Most definitions of beef cattle fertility are given in terms of biological levels of reproductive performance considered to be optimal, e.g. pregnancy rates of 90% plus in beef cattle and inter-calving intervals of 380 d for dairy cattle. These definitions of optimal performance are largely influenced by our perceptions of reproductive management strategies based on concepts derived from herds in temperate areas of the world.

As an example, temperate zone concepts of an optimal level of fertility are directed to the production by a high proportion of cows of a viable calf every 12 months. For many tropical and subtropical regions, these levels of performance are little more than a fond hope. For many tropical environments, high levels of reproductive performance and high survival rates are not always compatible, and there is a need for a balance between fertility levels and survival rates.

Fertility levels need to be considered in economic as well as biological terms. For example, in many areas of northern Australia where environmental constraints
TABLE I. TARGETS FOR OPTIMAL REPRODUCTIVE PERFORMANCE OF BEEF CATTLE IN SOUTHERN (TEMPERATE) AND NORTHERN (TROPICAL) AUSTRALIA [1]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Temperate</th>
<th>Tropical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rate (%)</td>
<td>90-92</td>
<td>80-85</td>
</tr>
<tr>
<td>Loss from pregnancy to calving (%)</td>
<td>2-5</td>
<td>4-6</td>
</tr>
<tr>
<td>Calving rate (%)</td>
<td>85-90</td>
<td>—a</td>
</tr>
<tr>
<td>Loss from calving to weaning (%)</td>
<td>2</td>
<td>3-6</td>
</tr>
<tr>
<td>Weaning rate (%)</td>
<td>83-88</td>
<td>70-78</td>
</tr>
</tbody>
</table>

a Information on calving rates is rarely available for extensive herds in the tropics.

are severe, computer herd modelling indicates that provided calving rates are in excess of about 75%, other factors such as growth and survival are more important than fertility in influencing farm income [1]. One of the explanations for this is that in harsh environments, high levels of reproductive efficiency also increase the proportion of the herd highly susceptible to drought, i.e. pregnant and lactating breeders and weaners. Hence in adverse years, high breeder and weaner mortality rates can influence income over subsequent years.

Table I summarizes information on optimal reproductive performance targets for beef cattle in southern and northern Australia, i.e. environments classified as either temperate or tropical. It should be possible to estimate optimal targets for reproductive performance for dairy cattle in contrasting environments but I have been unable to locate appropriate data.

Reference was made earlier to the importance of considering survival rates as a critical production trait in harsh environments, and the basic incompatibility between high reproduction and high survival in such environments. One approach in taking both these traits into account is compilation of net reproductive rate (NRR) data for determining optimal fertility levels. NRR may be defined as the number of female offspring reaching mating age produced by each dam in her lifetime. This index, which gives a compact picture of the balance between births and deaths in a population, is a most useful tool for measurement of population changes, and allows some identification of the reasons for these changes. The technique can be applied to populations of animals maintained under a number of different management systems. Specified information on some parameters is required for computation, but other factors may be included to identify potential sources of wastage.
TABLE II. AN EXAMPLE OF COMPILATION OF NET REPRODUCTIVE RATES IN INDONESIAN CATTLE [2]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Factor influencing parameter</th>
<th>Proportion</th>
<th>Cumulative proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves born per breeding cow per year</td>
<td><em>Cows mated per year</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cows in oestrus</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Cows mated/cows in oestrus</td>
<td>0.85</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td><em>Calves born per cow mated</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cows pregnant/cows in oestrus</td>
<td>0.75</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Cows calved/cows pregnant</td>
<td>0.96</td>
<td>0.52</td>
</tr>
<tr>
<td>Survival, birth to first breeding</td>
<td>Live female calves born/calves born</td>
<td>0.48</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Female calves weaned/live female</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>calves born</td>
<td>0.96</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Live female calves at first</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>breeding/weaned</td>
<td>0.95</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td><em>Survival rate/year</em></td>
<td>0.95</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>(1 — slaughter rate)/year</td>
<td>0.86</td>
<td>0.18</td>
</tr>
<tr>
<td>Breeding life</td>
<td><em>Difference between average slaughter age</em></td>
<td>4.00</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>and age of first breeding (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>NRR</strong> =</td>
<td></td>
<td>0.75</td>
</tr>
</tbody>
</table>

The data presented in Table II are based on cattle fertility information collected in Indonesia in 1976 [2]. If NRR is less than 1.0 then reproductive rate is low to the extent that a dam does not produce a replacement female offspring during her lifetime. For the Indonesian data this appears to be true since the cattle population in that country is declining. In contrast, using beef cattle data derived from a number of sources in northern Australia, NRR approximates to 1.3.
Obviously compilation of such a reproductive performance table requires a knowledge of certain basic production indices. However, the table can be entered at a number of points, e.g. cows pregnant/cows mated or cows calved/cows pregnant, and NRR can still be calculated. The technique allows identification of potential sources of wastage. For example, the Indonesian data suggest two factors of importance, namely high annual slaughtering rates in breeding cows (14%) and a relatively short breeding life (4 years).

Another useful approach using NRR data is to measure population dynamics in large ruminants using the formula:

\[ r = \frac{\ln R}{LF} \]

where

- \( r \) is the rate of change in population;
- \( R \) is the NRR (0.75 in Indonesia example of Table II);
- \( LF \) is the generation length for females (5 years approximately for Indonesian data).

For the Indonesian data this indicates a decline in cattle numbers of approximately 5.5% per year (W.A. Pattie, University of Queensland, personal communication), a figure approximating calculated estimates. Similarly, estimates of future population size and time trends for certain changes in population size can be calculated from estimates of NRR [3].

In summary, optimal reproductive rates will vary between different types of cattle enterprises (dairy, beef) and between geographic zones. However, for most cattle on poor quality, seasonally variable forage based diets, high fertility and high survival rates are not always compatible, particularly during periods of nutritional stress. Hence levels of fertility regarded as biologically suboptimal by temperate zone standards can be economically and biologically acceptable in more severe environments.

3. NUTRITIONAL COMPONENTS AND NUTRITIONAL STATUS

A multiplicity of factors involved in feed intake regulation have been reviewed recently [4]. Basically, the voluntary intake of ruminants on low quality roughages is severely limited by a slow rate of fermentation in, and passage of digesta through the reticulorumen, being affected by forage factors such as fibre content and rate and extent of digestion. Animal factors determining forage intake include physiological state, degree of fatness and rumen metabolite levels.
The physiological effects of restrictions on intake are that in many situations reproducing animals suffer energy, nitrogen and sometimes mineral deficits. In the field, the effects of specific nutrient deficits on reproduction are only poorly defined, and hence it is probably more correct to think of animals as suffering from undernutrition rather than from specific nutrient deficiencies.

A characteristic of animals on forage based diets is the seasonal variations in live weight and live weight gain which frequently occur. For example, the typical sawtooth growth curve for cattle in tropical northern Australia is characterized by weight gains during the wet season, weight maintenance in the wet–dry transitional period and weight loss in the dry season. Because of these cyclical growth phases, annual live weight gains on native pastures in these regions may only be 100–130 kg. Since critical phases of the reproductive cycle, e.g. mating and calving, coincide with periods of either weight gain or weight loss, these effects can be profound in influencing reproductive performance, growth and, as a consequence, survival.

Easily measurable and practically relevant measures of the nutritional status of grazing animals have not been easy to define, nor have specific metabolic hormones or metabolites reflecting nutrient status been clearly established. Inadequate energy and/or protein intakes are reflected in changes in an animal’s live weight and condition. While both these parameters have been criticized as imprecise indices of nutrient status, they are, however, easy to measure, have practical relevance, and allow useful generalizations and recommendations to be made on live weight or condition scores needed to ensure optimum fertility, provided modifying factors such as age, lactation status and genotype are kept in mind.

TABLE III. EFFECTS OF BODY CONDITION AND LACTATION STATUS ON PREGNANCY RATE (%) IN MATURE COWS IN THREE NORTHERN AUSTRALIAN BEEF HERDS [6]

<table>
<thead>
<tr>
<th>Body condition</th>
<th>Score</th>
<th>Lactation status</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>3</td>
<td>Wet</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry</td>
<td>-</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>Backward store</td>
<td>4</td>
<td>Wet</td>
<td>68</td>
<td>61</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry</td>
<td>90</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>Forward store</td>
<td>6</td>
<td>Wet</td>
<td>66</td>
<td>77</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry</td>
<td>83</td>
<td>77</td>
<td>93</td>
</tr>
<tr>
<td>Fat</td>
<td>7+</td>
<td>Wet</td>
<td>90</td>
<td>87</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry</td>
<td>93</td>
<td>89</td>
<td>92</td>
</tr>
</tbody>
</table>
There is a need to recognize also that nutrition of breeding animals involves two aspects: long term nutrient status reflected in live weight and condition (static live weight), and short term nutrient status or live weight or condition change around mating (dynamic live weight). Both these factors and their interactions are important in considering correlations of nutrition with reproductive performance. It is also pertinent to remember that, depending on the stage within a particular physiological state, animals can tolerate periods of undernutrition without ill effects on subsequent reproductive performance, e.g. during early to mid-pregnancy [5].

Relationships of pre-mating live weight and body condition to fertility have now been well established for different breeds of cattle in different environments. Typical fertility/condition score relationships for Bos indicus cattle are shown in Table III. Most analyses of live weight/condition relationships to fertility [1, 5, 6, 7] indicate that these are linear for heifers and curvilinear for lactating cows, i.e. each increment of mating live weight increases probability of re-conception. Hence management systems need to be devised which ensure that both live weight and body condition at mating are sufficiently high to allow expression of optimal fertility levels. These aspects are considered in more detail below.

4. OPTIMIZING FEMALE REPRODUCTIVE RATES THROUGH NUTRITIONAL AND MANAGEMENT STRATEGIES

The deleterious effects of undernutrition at various physiological stages are represented by a range of syndromes, including: delayed puberty, resulting in delayed age at first calving; low calving rates resulting from low conception rates and/or high embryonic/foetal loss; and delayed re-conception patterns, resulting in extended inter-calving intervals and, in extreme cases, high cow/calf mortality rates. Many of these stages of loss can occur concurrently in groups of breeding females in both grazing and village situations.

The physiological mechanisms involved in many of these expressions of reproductive wastage are still poorly understood. For example, underfed lactating cows are frequently anoestrous but because of the complex hormonal control mechanisms we have yet to fully clarify whether nutritional effects are acting on the hypothalamus, the pituitary, the ovary or all three. This is a major area where endocrine studies involving isotopic techniques for determination of ovarian and pituitary hormones will play an increasingly important role.

In the following sections, nutritional effects on specific phases of the reproductive cycle in cows are discussed and some procedures for improvements are considered. While many of the examples quoted are derived from cattle studies under grazing situations, many of the principles involved are applicable to other environments and other management systems.
4.1. Pre-pubertal period

In *B. indicus* cattle there are marked year effects on weight-fertility relationships in heifers mated at two years of age [8, 9]. An explanation comes from recent studies [10] suggesting that pre-pubertal heifers which grow in their first dry season (from 6 to 12 months of age) have lower target weights at conception (~280 kg) than heifers which fail to grow (~320 kg). Additionally for animals of the same weight, those which grow as weaners are more fertile than those which lose weight, though these fertility differences decrease with increasing weight at two year old mating (Table IV).

The basic physiological mechanisms involved in these relationships have yet to be clearly defined. However, there is a transient phase of progesterone secretion in early life (4–7 months) which is likely to be an important cue for subsequent pubertal reproductive activity [11]. Nutritional signals at about this time may modulate these effects.

The practical applications of these findings are that dry season supplementation of weaner heifers to ensure continued growth between about 6 and 12 months of age will improve subsequent two year old fertility and improve survival in the current year [12].

<table>
<thead>
<tr>
<th>Weight at mating (kg)</th>
<th>Post-weaning growth rate (kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−0.1</td>
</tr>
<tr>
<td>200</td>
<td>21</td>
</tr>
<tr>
<td>250</td>
<td>52</td>
</tr>
<tr>
<td>300</td>
<td>82</td>
</tr>
</tbody>
</table>

4.2. Puberty

There is a vast amount of literature relating environmental effects to puberty in cattle [13]. Sexual maturation involves a complex interaction of endocrine factors which initiate development of the reproductive tract. The rate at which this occurs depends on both genetic and environmental factors, of which nutrition is an important component of the latter.
Both age and weight are important factors determining the timing of puberty and are closely related in that plane of nutrition determines growth rate and hence age at which pubertal weight is reached. In addition, other factors, including nutrition per se, influence puberty, it having been observed that increased nutritional intake [14] or severe nutritional restrictions [15] either induce or inhibit ovulation and oestrus even though heifer live weights are adequate.

Weight relationships to onset of puberty have led to the development of the target weight concept [5, 13] where, to ensure expression of puberty, heifers have to attain a genetically determined minimum weight. In general, this weight tends to be in the range of 250–280 kg for B. taurus breeds [13] and 260–300 kg for B. indicus breeds [8].

In cattle under extensive grazing conditions, as in tropical Australia and Latin America, joining times of heifers are largely dictated by seasonal and/or market considerations, rather than by an expected time (age) at which puberty will be attained. Hence in practical terms it is often more appropriate to consider weight at first mating and its relationship to fertility.

4.3. First mating weight relationships

Many studies in cattle have shown that heifer pregnancy rates increase with increasing weight at mating. As for puberty–weight relationships just discussed, these findings have led to the concept of target mating weights for heifers necessary to achieve optimal levels of fertility [5]. Most studies indicate general linear relationships between mating weight and fertility and also between body condition score and fertility [8, 16]. There is also evidence (see Table IV) that target mating weights tend to be lower in well grown than in poorly grown heifers.

In addition to weight effects, most studies of relationships of first mating weight to fertility indicate significant year effects on these correlations [9], implying that factors other than weight may be important. The significance of these year effects is discussed in subsequent sections. An additional implication is that there are carry-over effects of fertility at first mating on subsequent mating success. Lifetime reproductive levels are positively correlated with performance at first mating [17] and hence nutritional strategies which improve fertility at first mating will have long term reproductive and productive advantages.

4.4. Pregnancy

Two broad concepts need to be considered in discussing nutritional requirements during pregnancy. The first relates to the repartitioning of nutrients necessary to ensure placental and foetal growth, and to initiate mammary growth and preparation for subsequent lactation; the second relates to the effects of pre-partum
Table V. Birth weights of calves born to Brahman cross cows on poor quality forages either unsupplemented or supplemented during the last two months of gestation [19]

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Calf birth weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>22.0</td>
</tr>
<tr>
<td>Urea-sulphur</td>
<td>30.9</td>
</tr>
<tr>
<td>Urea-sulphur plus bypass protein (1 kg/d)</td>
<td>32.1</td>
</tr>
</tbody>
</table>

Nutritional plane on subsequent re-conception rates, an aspect considered in the following section.

In the cow during the first two trimesters of pregnancy, placental growth rate exceeds foetal growth rate; during the last trimester the situation is reversed and this is when nutritional stresses can affect foetal growth, birth weight and, in turn, calf survival [18]. While it is sometimes considered that nutritional stresses have to be very severe to induce foetal growth restrictions, this is not always the case, and an example of such effects is shown in Table V [19].

In B. indicus cows, placentome numbers are considerably less than in B. taurus cows [20], which could reflect either genotype differences or the often poor nutritional status of such genotypes. Whether the origin is genetic or non-genetic, recent studies indicate that foetal growth curves for B. indicus are lower than for B. taurus during the latter stages of gestation [21]. Such data also have added value in that correction factors for cow live weight can be derived to overcome complications in field experimentation caused by pregnancy and reproductive asynchrony when comparing cow live weights in nutrition-fertility experiments.

Quantitative information on relationships of calf birth weight to neonatal survival in tropical cattle is not readily available. However, as general relationships of birth weight to neonatal survival are common across all species, nutritional limitations during late pregnancy likely to reduce foetal growth and hence birth weight would reduce neonatal survival rates. An added consideration for many tropical cattle is that they are used for draught throughout gestation. However, as substantial repartitioning of nutrients to foetal growth does not occur until the last one to two months of gestation, work should be easily accommodated [22] but supplementation could be appropriate. As an example, data shown in Table VI indicate nutritional supplements that may be useful for pregnant animals on poor quality tropical forages to ensure appropriate live weight changes.
TABLE VI. THE EFFECT OF FEEDING UREA–SULPHUR AND BYPASS PROTEIN ON POOR QUALITY HAY INTAKE AND ON LIVE WEIGHT CHANGE OF PREGNANT BRAHMAN CROSS COWS DURING THE LAST TWO MONTHS OF GESTATION [19]

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Intake (kg/d)</th>
<th>Live weight change (kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>4.2</td>
<td>-0.81</td>
</tr>
<tr>
<td>Urea–sulphur</td>
<td>6.2</td>
<td>-0.31</td>
</tr>
<tr>
<td>Urea–sulphur plus bypass protein (1 kg/d)</td>
<td>8.1</td>
<td>+0.75</td>
</tr>
</tbody>
</table>

Since glucose provides the major energy nutrient for the growing foetus, a source of glucogenic energy may be highly beneficial to working pregnant animals. This could be achieved through increasing propionate production and/or supplementary feeding with bypass proteins [22].

4.5. Post-partum reproductive performance

Post-partum anoestrus is a normal physiological event but in many cattle on low quality forage based diets, a prolongation of this post-partum anoestrus interval is common, leading to long inter-calving intervals and frequently to calving only in alternate years.

The deleterious effects of undernutrition on post-partum reproductive performance have been well documented in cattle [5, 7, 8] and both pre- and post-partum nutrition are important. As with heifers, there are positive correlations between live weight, condition and fertility. These relationships tend, however, to be curvilinear, the curve inflection point being, for example, about 450 kg for Brahman cross cows [12, 23]. In addition to these static live weight effects on fertility, there is evidence from some but not other studies of positive effects of live weight change around mating on fertility. It is important to recognize, however, that such relationships are correlative rather than causal since underlying mechanisms of weight–fertility relationships have not been determined. Although live weight and condition are subjective measures of nutrient status, they do provide a useful predictive guide to the outcome of mating and are also useful for retrospective analysis of fertility data. Hence they have practical relevance in many field studies.

Randel [7] recently reviewed a series of experiments which indicated that pregnancy rates tended to be consistently lower in animals receiving either lower
protein or lower energy rations in the post-partum period. Similarly pre-calving levels of these nutrients also influenced subsequent conception rates. The field situation, however, is frequently a combined deficiency syndrome.

Reference was made earlier to the importance of between-year variations in reproductive performance. In a recent study of factors influencing pregnancy rates in Brahman herds in north Queensland, year effects were more important than weight effects in influencing pregnancy rates in lactating cows [24]. Detailed statistical modelling of these data [9] indicated that nutritional status in the dry season prior to calving and length of the dry season nutritional stress had major effects on subsequent reproductive performance of lactating cows. While such relationships are linear for younger animals, they are curvilinear for older cows, confirming previous information [7, 8] that younger lactating first-calf cows are more sensitive to nutritional stresses and hence have lower post-partum fertility than mature lactating animals.

Relationships of climatic variables to cow reproductive performance can also be of value to calculate minimum levels of such variables required to achieve various re-conception rates at the following mating. Using regression equations from the statistical models described [9], it was shown that pasture growth days and dry season rainfall need to be considerably higher, and timing of the seasonal break considerably earlier, for three year old cows to achieve similar re-conception rates to older cows (Table VII). The practical consequences are that nutritional supplementation is necessary in almost all years in north Queensland to ensure re-conception rates of at least 70% in three year old lactating cows. Supplementation would only be needed in about one third of years to achieve such levels in mature lactating cows. Similar modelling approaches could be used in a range of other environments to aid decision making on nutritional supplementation regimens.

While the physiological basis for the relationships of pre- and post-partum nutrition to fertility have yet to be fully clarified, there are now some leads. We know, for example [8, 11], that pre-partum energy supplementation with urea-molasses for as short a period as six weeks ('spike feeding') has significant effects on ovarian follicle populations up to 80 days post-partum, and that in first-calf lactating cows, consistent improvements in pregnancy rates of from 15 to 20% can be achieved by using this management strategy [12]. That such a response occurs in first-calf but not in mature cows is not surprising given the relationships indicated earlier. There are also indications from Indonesia (C. Hendratno, Centre for the Application of Isotopes and Radiation, Jakarta, personal communication) that urea-molasses block technology is being widely adopted to improve cow fertility in small village holder units. This example of technology transfer indicates the scope for application of basic principles with appropriate modifications to other management systems to optimize cattle fertility.

Of equal importance in influencing post-partum fertility are the combined effects of suckling and lactation. Williams [25] has recently reviewed the physio-
TABLE VII. MINIMUM LEVELS OF DRY SEASON CLIMATIC VARIABLES NEEDED TO ACHIEVE 50, 60, 70 AND 80% RE-CONCEPTION RATES FOR THREE YEAR OLD AND MATURE COWS IN THE SPEAR GRASS REGION OF NORTH QUEENSLAND [9]

<table>
<thead>
<tr>
<th>Re-conception rate (%)</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Three year old cows</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of seasonal break</td>
<td>11 Nov.</td>
<td>20 Oct.</td>
<td>28 Sep.</td>
<td>7 Sep.</td>
</tr>
<tr>
<td>Pasture growth days (Jul.–Nov.)</td>
<td>18</td>
<td>26</td>
<td>34</td>
<td>43</td>
</tr>
<tr>
<td>Rainfall (Jul.–Nov.) (mm)</td>
<td>148</td>
<td>207</td>
<td>265</td>
<td>324</td>
</tr>
<tr>
<td><strong>Mature cows</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of seasonal break</td>
<td>8 Jan.</td>
<td>21 Dec.</td>
<td>30 Nov.</td>
<td>3 Nov.</td>
</tr>
<tr>
<td>Pasture growth days (Jul.–Nov.)</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Rainfall (Jul.–Nov.) (mm)</td>
<td>5</td>
<td>38</td>
<td>79</td>
<td>135</td>
</tr>
</tbody>
</table>

logical effects of suckling as a regulator of post-partum reproductive function. In addition to exteroceptive stimuli from suckling influencing the post-partum reproductive process, nutrient partitioning into lactation also imposes an additional nutritional drain. Management techniques such as early weaning and creep feeding represent other approaches to minimizing tissue nutrient drain during lactation with the potential to improve fertility. Early weaning of calves from *B. indicus* cows [26] increased conception rates to 90% compared to a conventionally weaned group (60%) and also concentrated time of conception into the early wet season. Calf creep feeding strategies improved conception rates from 53 to 60%, presumably through reduced suckling activity and/or milk production enhancing ovarian function [26].

In summary, lowered fertility in lactating cows due to prolonged post-partum anoestrus interval mainly of nutritional origin is a widespread syndrome. Both direct (supplementation) and indirect (weaning, creep feeding) nutritional management techniques can minimize the problem. Their application will depend on the biological responses obtained and the net economic returns achieved.
4.6. Oestrous activity and oestrus synchronization

Nutritional influences on ovarian and oestrous activity are well documented in \textit{B. taurus} and \textit{B. indicus} cattle [27] and while such effects are often said to be seasonal, in reality these are mostly nutritional effects [5]. They are mediated through endocrine changes involving the uterus, ovary, anterior pituitary and hypothalamus. Hypothalamic dysfunction, through reduced gonadotrophin releasing hormone (GnRH) release, is the most likely primary mechanism [28]. Release of GnRH may also be influenced by oestrogen positive feedback mechanisms which themselves may be nutrition mediated. The nature of the nutritional signals modulating endocrine function is uncertain, but the somatotrophic axis may also be involved via insulin and insulin-like growth factors which alter ovarian response to gonadotrophins and which themselves may be mediated by growth hormone, which in turn is influenced by nutrition [29].

Responses to oestrus synchronization agents are also influenced by nutritional status [30], while conception rates to artificial insemination also tend to be less in lower live weight or lower conditioned cows. Hence in artificial breeding programmes nutritional planning and programming are often the key to success.

Finally, we need to recognize that nutritional influences on male reproductive function cannot be ignored, though space precludes any detailed discussion here. There is field evidence of seasonality of reproduction in bulls [8, 27] largely of nutritional origin. Pre-mating nutritional supplementation may improve bull fertility and thus improve overall herd fertility levels [8].

5. CONCLUSIONS

Each of the reproductive cycle components discussed contributes to the final measure of fertility, which is the production and rearing of a live calf to weaning by the majority of cows in a group or herd. Net reproductive rate is an important indicator of reproduction, since this gives an indication of the balance between reproduction and survival. Survival is an important production trait in the tropics and probably has a greater effect than realized on levels of herd fertility.

Difficulties of quantifying nutritional effects per se on reproduction have been highlighted. Information from climatic modelling exercises presented may point the way towards new nutritional management techniques and may also help provide an explanation for some of the physiological effects observed. Existing and new nutritional technologies have been indicated which will make possible improvements in fertility at first mating and in post-partum fertility.

Finally, we need to recognize that unless biological responses from nutritional interventions are economically acceptable, such practices will not be adopted. Hence
extensive field testing and economic analysis should form an integral part of nutritional technology evaluations, whether in extensive systems or in village smallholder situations.

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GENOTYPE AND ENVIRONMENT INTERACTIONS IN CATTLE IN THE TROPICS

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Abstract

GENOTYPE AND ENVIRONMENT INTERACTIONS IN CATTLE IN THE TROPICS.

Various factors affect reproductive performance in tropical cattle. The time of year when the animal is born, the season when breeding programmes are established, the breed and parity of the animals selected, management decisions and the body condition of the females prior to breeding are the most common factors that determine the success of farmers in the tropical areas of the world. Examples of these components are reviewed and areas for future research suggested.

1. INTRODUCTION

Lowland humid tropical regions of the world provide unique ecosystems much suited to breeds of cattle that are able to withstand inadequate levels of nutrition, extreme environmental conditions and exposure to numerous health hazards. For this reason, *Bos indicus* breeds are the most commonly found. However, in spite of a high level of adaptation, this type of cattle is generally considered to have poor reproductive performance [1]. Opinions differ as to the usefulness of introducing *B. taurus* breeds to the tropics as a way of improving production in this region. Unfortunately, few controlled studies are available which compare the performance of *B. taurus* breeds with that of *B. indicus* under similar tropical conditions. Many comparative studies, for instance, have the European cattle kept in the best part of the farm where they have the advantage over the Indian breeds from the nutritional, health and comfort points of view. Also, care should be taken when making these types of comparisons, so as not to compare a select population of the taurine breeds (i.e. only the few reaching calving) with a large population of Indian breeds that are regularly calving when raised under tropical conditions.
Examples of such care were found by Galina and Arthur [2] and Anta et al. [3] in the extensive recompilation and analysis of data available in the literature. They failed to observe important differences in reproductive performance of the various breeds that populate the tropics. Galina and Arthur [2] reviewed 162 studies where age at first calving and calving interval were calculated in various breeds of both cattle types. Average age at first calving for \emph{B. indicus} was 44.8 months and calving interval 15.2 months. Corresponding figures for the \emph{B. taurus} breeds were 32.3 months and 15.4 months respectively. Analysis of these data suggests that the reproductive performance of the \emph{B. taurus} breeds is similar to or perhaps slightly better than the \emph{B. indicus}, but as mentioned earlier, this could be the result of a biased comparison.

The above examples illustrate how difficult it is to make valid comparisons between breeds farmed in the same region. Taking management decisions then becomes a rather imprecise exercise which in turn affects the economic growth of farmers in the area.

The objectives of the present paper are twofold: (a) to illustrate how the environment regulates reproductive performance, and (b) to demonstrate the possible effects of nutrition, extreme ambient temperatures and management on the various breeds that populate the tropics.

2. EFFECTS OF TIME OF YEAR, AGE AND PARITY

There is ample evidence that the time of year when the replacement heifer is born and the season when breeding occurs in the mature herd exert an important influence on reproductive performance in the female. Many studies have shown that under range conditions, approximately 40% of the births will occur in the spring [4-7]. However, there are important considerations with respect to the type of breed chosen in which to measure this parameter. Figure 1, adapted from the work of Telleache [8], involving over one thousand calvings, illustrates how the environment interacts with the genotype. Whereas \emph{B. indicus} cattle tend to concentrate calving in the spring and summer, European cattle have a more widespread calving pattern, having their young in the autumn and winter. Cattle of European origin are found to be more sensitive to the yearly variations in environmental conditions than the corresponding Indian breeds. Interestingly, another two large studies carried out in Mexico [9, 10] pointed to the fact that the crossbreed between Charolais and Indobrazil gained from the resultant heterosis and performed similarly to the Indian breeds.

Figure 2, adapted from the work of Plasse et al. [11], projects the expected calving interval as affected by age and parity. In general, it can be said that the younger the female at first calving the poorer the performance. Dams performed best during their third, fourth and fifth calvings and later showed longer calving intervals.
FIG. 1. Distribution of calvings in Charolais, Indobrazil and the resultant crossbreed farmed under the same conditions (adapted from Ref. [8]).

FIG. 2. Calving interval in relation to age in Bos indicus type cattle (adapted from Ref. [11]).
Buck et al. [12], working in Botswana, confirmed that calving percentage in the herd rose from 69% at 2.5 years of age to a maximum of 82% at 6 to 7 years and then declined in cows over 8 years of age. This indicates that management decisions should take into consideration the average age of the herd to achieve maximum success in a breeding programme.

The application of nuclear and related techniques has allowed researchers in the field of animal reproduction to monitor endocrine patterns during the oestrous cycle in tropical cattle and to measure effects of environment on hormonal levels at different times of year. Galina and Arthur [13] reviewed this field of research and concluded that there are indications in the literature of a seasonal influence on hormonal production in tropical cattle, but more detailed studies are needed to clarify this important aspect of animal reproduction.

3. EFFECTS OF NUTRITION AND BODY CONDITION

There is considerable evidence that adequate feeding prevents large losses in body weight at the time of calving, thus reducing the time required to reinitiate ovarian activity. Studies measuring progesterone in either blood or milk have shown that the interval from calving to first ovulation can be drastically affected by poor nutrition [14]. Although this is well understood by producers in the tropical regions of the world, it is difficult for them to provide their animals with optimum nutritional levels owing to the variable conditions prevailing in these areas. Variability in the yearly onset of the rainy season, coupled with the overstocking of cattle in the dry season, affects the general body condition of the dam at the time of calving. This fact also influences performance in replacement heifers. In a recent review, Galina and Arthur [15] indicated that a common denominator with respect to the factors that advance or delay puberty was the nutrition plane of the animal during the growing period. Moreover, adequate nutrition can improve calving rate. Oliver and Richardson [16] found that only 67% of the herd will calve if the animals have a poor energy balance, whereas well fed cattle could have calving rates as high as 97%. Anta et al. [3] carried out an analysis of the results of different studies in Mexico, where animals were raised in confinement or under pasture conditions with or without supplementation. As can be observed in Table I, the worst performance was shown by animals farmed under range conditions.

Performance of cattle can be improved when adequate feeding is given. The improvement is more marked when the procedure is carried out for a suitable genotype. Figure 3, adapted from the work of Branton et al. [17], illustrates that supplementation can increase milk production almost twofold when the genotype chosen has more than 60% of a specialized dairy breed.
TABLE I. AVERAGES OF REPRODUCTIVE PARAMETERS IN CATTLE RAISED UNDER CONFINEMENT, AT GRAZING AND WITH SUPPLEMENTATION UNDER MEXICAN CONDITIONS.
(Values within parentheses indicate the numbers of studies.)

<table>
<thead>
<tr>
<th>Reproductive parameter (d)</th>
<th>Confinement</th>
<th>Grazing and supplementation</th>
<th>Grazing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at puberty</td>
<td>721&lt;sup&gt;ab&lt;/sup&gt; (1)</td>
<td>445&lt;sup&gt;a&lt;/sup&gt; (22)</td>
<td>661&lt;sup&gt;b&lt;/sup&gt; (9)</td>
</tr>
<tr>
<td>Age at first service</td>
<td>620&lt;sup&gt;a&lt;/sup&gt; (4)</td>
<td>741&lt;sup&gt;a&lt;/sup&gt; (15)</td>
<td>755&lt;sup&gt;a&lt;/sup&gt; (14)</td>
</tr>
<tr>
<td>Age at first conception</td>
<td>613&lt;sup&gt;a&lt;/sup&gt; (8)</td>
<td>867&lt;sup&gt;b&lt;/sup&gt; (12)</td>
<td>822&lt;sup&gt;b&lt;/sup&gt; (32)</td>
</tr>
<tr>
<td>Age at first calving</td>
<td>688&lt;sup&gt;a&lt;/sup&gt; (17)</td>
<td>1080&lt;sup&gt;b&lt;/sup&gt; (29)</td>
<td>1080&lt;sup&gt;b&lt;/sup&gt; (65)</td>
</tr>
<tr>
<td>Interval from calving</td>
<td>59&lt;sup&gt;a&lt;/sup&gt; (12)</td>
<td>63&lt;sup&gt;ab&lt;/sup&gt; (29)</td>
<td>94&lt;sup&gt;b&lt;/sup&gt; (43)</td>
</tr>
<tr>
<td>to first oestrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval from calving</td>
<td>84&lt;sup&gt;a&lt;/sup&gt; (29)</td>
<td>119&lt;sup&gt;b&lt;/sup&gt; (29)</td>
<td></td>
</tr>
<tr>
<td>to first service</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval from calving</td>
<td>140&lt;sup&gt;a&lt;/sup&gt; (11)</td>
<td>160&lt;sup&gt;a&lt;/sup&gt; (61)</td>
<td>150&lt;sup&gt;a&lt;/sup&gt; (86)</td>
</tr>
<tr>
<td>to conception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calving interval</td>
<td>437&lt;sup&gt;a&lt;/sup&gt; (19)</td>
<td>459&lt;sup&gt;a&lt;/sup&gt; (87)</td>
<td>438&lt;sup&gt;a&lt;/sup&gt; (113)</td>
</tr>
</tbody>
</table>

Note: Uncommon letters within rows indicate a significant difference ($P < 0.05$).

Some studies have shown that additional forage does not always improve conception rate in heifers, except when the food available is inadequate during their growing period, usually owing to poor rainfall. When this is the case conception rates can increase to 50% in the supplemented group [18]. Similarly, in a recent study [19], for Guzerat cows fed with the same balanced diet during the whole year no significant differences were found in the performance of the herd, yet a significantly greater proportion of cows exhibited oestrus in the spring, thus illustrating that even when adequate diet is provided environmental factors tend to predominate.
These results strongly suggest that supplementation is not an adequate procedure when carried out indiscriminately. Unfortunately, few studies contain adequate information to judge the body condition of the animal at the time of supplementation. As indicated by Moore and Roche [20], weight losses should be expressed as a percentage of mature weight and not as the number of kilograms that the animals have lost.

Nevertheless, from most of the information published and particularly in the case of studies carried out in developing countries, it is fairly obvious that supplementation of females during their growth and prior to or after calving will improve reproductive performance in the herd. However, researchers and producers alike have been faced with the challenge of making this management decision an attractive proposition for investors. The recent economic crisis felt by most of the countries in the tropical regions of the world has tended to orientate research towards minimum cost tactics for supplementation. Thus, investigations into the strategic addition of feedstuffs are needed in order to use the available resources more efficiently.
TABLE II. AVERAGES OF REPRODUCTIVE PARAMETERS IN CATTLE RAISED FOR BEEF, DAIRY AND DUAL PURPOSE UNDER MEXICAN CONDITIONS
(Values within parentheses indicate the numbers of studies.)

<table>
<thead>
<tr>
<th>Reproductive parameter (d)</th>
<th>Dairy</th>
<th>Beef</th>
<th>Dual purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at puberty</td>
<td>457&lt;sup&gt;a&lt;/sup&gt;</td>
<td>529&lt;sup&gt;a&lt;/sup&gt;</td>
<td>547&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(13)</td>
<td>(15)</td>
</tr>
<tr>
<td>Age at first service</td>
<td>586&lt;sup&gt;a&lt;/sup&gt;</td>
<td>691&lt;sup&gt;a&lt;/sup&gt;</td>
<td>729&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(20)</td>
<td>(12)</td>
</tr>
<tr>
<td>Age at first conception</td>
<td>602&lt;sup&gt;a&lt;/sup&gt;</td>
<td>779&lt;sup&gt;b&lt;/sup&gt;</td>
<td>885&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td>(26)</td>
<td>(23)</td>
</tr>
<tr>
<td>Age at first calving had</td>
<td>925&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1049&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1133&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(35)</td>
<td>(29)</td>
<td>(68)</td>
</tr>
<tr>
<td>Interval from calving to first oestrus</td>
<td>65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(35)</td>
<td>(35)</td>
<td>(50)</td>
</tr>
<tr>
<td>Interval from calving to first service</td>
<td>71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(16)</td>
<td>(25)</td>
</tr>
<tr>
<td>Interval from calving to conception</td>
<td>139&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(56)</td>
<td>(64)</td>
<td>(76)</td>
</tr>
<tr>
<td>Calving interval</td>
<td>435&lt;sup&gt;a&lt;/sup&gt;</td>
<td>437&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.462&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(73)</td>
<td>(78)</td>
<td>(112)</td>
</tr>
</tbody>
</table>

Note: Uncommon letters within rows indicate a significant difference ($P < 0.05$).

4. EFFECT OF CLIMATE

Productivity of cattle in the tropics is poor. Several papers confirm that, under the present conditions, about 30% of the replacement heifers will not reach weaning or branding [21, 22]. Furthermore, another 20% will not even calve for the first time prior to their release from the herd [23]. This situation gets worse when cattle of European origin are introduced into the tropics. Vaccaro et al. [24, 25] monitored
the production and life expectancy of Holsteins imported to Venezuela; 81.3% of the calves born from these animals died before one year of age, and the average number of calvings recorded for these animals during their lifetime in the herd was only 1.2.

As a consequence, crossbreeding of *B. taurus* and *B. indicus* breeds has emerged as a useful alternative for cattle production in the tropics. Nevertheless, a definite methodology has yet to be established. For example, Plasse [1] demonstrated that upgrading Criollo cattle to Zebus has been successful only up to the second generation. Cunningham and Syrstad [26], in an excellent review on crossbreeding techniques in *B. taurus* and *B. indicus* cattle raised for milk production, concluded that in the crossbreeding systems predictions depend on the estimated level of additive and heterosis effects. The accuracy of the prediction will depend on the validity of these estimates in the environment in which the system was implemented.

All this means that crossbreeding with a particular combination of breeds in one location may not be appropriate to another environmental situation. Similarly, Galina and Arthur [15] indicated that when crossbreeding *B. taurus* and *B. indicus*, the performance of the resulting crosses was in accordance with the amount of taurine genotype present in the animals. However, further crossbreeding of the resultant F1 gives unpredictable results. In this same study, it was found that interspecies crossbreeding did not favour reproductive performance and unspecified crossbred cattle showed the worst performance. Similar findings were reported by Anta et al. [3] in a large comparative study of dairy, beef and dual purpose cattle. The worst performance was obtained in the last type (Table II). In general, it is agreed that for adequate performance and endurance, it is desirable to have at least 25% of *B. indicus* blood in the crossbred animal. Nevertheless, reproductive efficiency is quite variable. This disparity in information could be due, among other things, to the effect of heat stress on cattle, this in turn affecting the cascade of reproductive hormones. However, this important concept requires investigation as some breeds are better adapted than others to tropical conditions (for review, see Ref. [13]). More information is needed on the environmental causes of poor reproductive performance of the various genotypes raised under tropical conditions, to be able to gain an insight into the physiological mechanisms responsible for these effects.

5. EFFECT OF MANAGEMENT

Martínez et al. [27], working in Mexico, observed that when European crossbred cattle were raised under two different management systems, cows with poor nutrition tended to calve in the spring (usually when fodder quality begins to improve) whereas those with adequate nutrition calved preferentially in the winter (when environmental conditions are more favourable for this type of cattle). This example shows how management policies are instrumental in improving reproductive performance in cattle. Another interesting example of how management deci-
sions interact with environmental conditions is the work reported by Fallas [28], who demonstrated that under the same feeding regime the highest milk producers in a European crossbred herd were also the cows with the best reproductive performance. This indicates that adaptability of cattle to the environment manifests itself in optimal productivity. Moreover, it illustrates the fact that adequate performance is closely related to the ability of the animal to adapt to the local environment, especially with respect to utilization of available food sources. One last example of management decisions affecting performance is described by Holness et al. [29], who found that temporary weaning significantly reduced the time from calving to conception. However, this effect was significantly more marked in the Mashona than the Afrikander cow. These data illustrate the fact that some breeds will benefit more than others from the application of a procedure to manipulate the reproductive performance of a herd.

6. CONCLUSIONS

The possible components of the distinct factors that influence the performance of cattle raised under tropical conditions are far from understood. More research is needed to determine the distinct roles of nutrition, breed, time of year, and age and body condition of the animal with respect to the heterogeneous farming conditions present in the area. As more research results become available, producers should be able to apply the findings to their own particular situation with the objective of increasing animal production. Furthermore, whatever new technique or procedure becomes available, a critical economic assessment should be performed prior to its implementation. Finally, researchers in developing countries should make a greater effort to develop viable procedures suitable for their own local environments instead of applying expensive imported techniques in places where the infrastructure and husbandry knowledge are poor. There is little doubt that such ill founded enterprises are unlikely to be successful.

REFERENCES


IMPROVING THE MANAGEMENT OF REPRODUCTION OF INDIGENOUS CATTLE IN THE SEMIARID AND SUBHUMID ZONES OF WEST AFRICA

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Zaria, Nigeria

Abstract

IMPROVING THE MANAGEMENT OF REPRODUCTION OF INDIGENOUS CATTLE IN THE SEMIARID AND SUBHUMID ZONES OF WEST AFRICA.

The traditional systems of management of reproduction of cattle indigenous to West Africa and various improvement strategies employed are reviewed. Strategies have been evolved to facilitate early attainment of puberty and first conception, reduce inter-calving intervals and control oestrus and the oestrous cycle. Among the strategies and techniques used to achieve various degrees of improvement in these reproductive parameters are improved nutrition, diagnosis and control of diseases, determination of hormonal profiles and semen characteristics, and administration of drugs as well as strategic management practices. More investigations are required for a full understanding of the factors influencing reproductive performance and identification of the most appropriate management practices necessary to improve the reproductive efficiency of cattle indigenous to West Africa.

1. INTRODUCTION

About 12 breeds of Zebu cattle classified as short- and longhorn types [1], 11 of the trypanotolerant breeds and 5 breeds representing crosses between Zebu and N'Dama or shorthorn types [2] have been identified in West Africa.

A major component of the productivity of livestock is their reproductive performance. Yet, there is no clear-cut management practice directed specifically towards improving the reproductive efficiency of the over 70% of cattle belonging to the small scale agropastoralists or pastoralists in West Africa, who adopt various production management systems with varying degrees of success. Many breeds of cattle indigenous to tropical Africa have lower reproductive rates than their temperate counterparts [3, 4]. These differences may be attributed to genetic or environmental and management influences among others. Attempts are being made to identify means of improving the productivity of these animals through diagnostic and interventionist measures. The diagnostic phase involves determination of basic routine management practices and monitoring of reproductive traits for the various breeds while the interventionist phase involves institution of improvement strategies.
<table>
<thead>
<tr>
<th>Breed</th>
<th>Age at puberty (months)</th>
<th>Age at first calving (months)</th>
<th>Duration of oestrus (h)</th>
<th>Length of oestrous cycle (d)</th>
<th>Length of gestation (d)</th>
<th>Post-partum interval (d)</th>
<th>Duration of uterine involution (d)</th>
<th>Calving interval (months)</th>
<th>Calving rate (%)</th>
</tr>
</thead>
</table>

TABLE I. REPRODUCTIVE PARAMETERS FOR SOME INDIGENOUS WEST AFRICAN CATTLE
This paper reviews the available information on the reproduction and management strategies adopted for the improvement of reproductive efficiency in cattle indigenous to the semiarid and subhumid zones of West Africa.

2. TRADITIONAL METHODS OF MANAGEMENT OF REPRODUCTION

2.1. Herd management

The transhumance practice, being a common feature of cattle management in West Africa, makes certain reproductive management practices almost non-existent. Animals of all ages and sexes are usually herded together, with bulls running with the females all year round [5, 6], thus precluding planned mating.

2.2. Calf management

Some degree of calf management to enhance milk production by the dam is practised in slightly different ways. While the Fulanis of Nigeria merely allow calves to suckle to enhance milk let-down [5], the Jayaraji of Mali have a distinct management policy for calves [6]. Calves are separated and grazed separately from their dams in the morning and allowed to suckle in the evening before milking of the dams and remain with their dams until the following morning. Weaning of the calves is achieved either by transfer to another herd or by muzzling with a band of thorns [7]. In contrast, 'self-weaning' by the calves is practised by the Fulanis of Nigeria.

3. FEMALE REPRODUCTION

A summary of available information on reproductive performance of some indigenous breeds of cattle in West Africa is presented in Table I. While some of these data were obtained from surveys and records, a few others were based on studies involving measurement of reproductive hormones. In this regard, the radioimmunoassay (RIA) technique has been fairly widely used to determine progesterone (P4) levels for the different phases of the reproductive cycle of Zebu cattle in West Africa.

3.1. Age at puberty and first calving

Although reports on age at puberty are scanty [8-11] the few available and those on age at first calving [4, 6, 7, 11-30] indicate that these animals do not reach puberty early. Nutritional and genetic factors have been advanced as possible causes of this.
FIG. 1. Serum progesterone profile in Bunaji cows during the oestrous cycle [35].

FIG. 2. Variations in plasma progesterone concentration during the oestrous cycle (a, b) and during two successive short oestrous cycles (c) [62].
3.2. Oestrus and oestrous cycle

Reports on the duration of oestrus indicate a range of 3.1–12.6 h [31–34]. At the lower limit these observations are different from values reported for temperate breeds of cattle but are similar at the upper limit [58, 59].

Measurement of blood P₄ has been used to determine oestrous cycle length and characterize P₄ profiles during the oestrous cycle [35, 60, 61] (Fig. 1). In a study of the oestrous cycle of Bunaji cows, the duration of the cycle was found to be within the normal range of 18–22 d while the range of P₄ levels was from 0.2 ± 0.4 ng/mL at oestrus to 5.3 ± 2.3 ng/mL at mid-cycle [35]. Variations in P₄ concentrations were found among Zebu cows [62]. For example, while peak concentration in some cows did not exceed 1 ng/mL, in others it was as high as 7–9 ng/mL. Some of these animals also experienced short oestrous cycles with low P₄ levels (Fig. 2(c)). This phenomenon may be an important factor influencing the endocrine events controlling ovulation and intensity of oestrus in these animals.

3.3. Gestation

The gestation lengths both for the Sudanese (Macinanke) cattle of Mali [6] and for the Bunaji and Sokoto Gudali of Nigeria [35, 36] are within the normal values for cattle. Progesterone concentrations in Bunaji cows fluctuated between mean values of 6.6 ± 2.1 and 10.7 ± 3.3 ng/mL from 30 d of gestation to about 3 d pre-partum, when a sharp decrease to 1.2 ng/mL occurred, reaching 0.5 ng/mL at parturition (Fig. 3) [35]. Again, variations in P₄ levels among cows were reported. Higher levels of P₄ were measured during gestation than during the oestrous cycle owing to increased activity of the corpus luteum in addition to placental secretion of P₄.

3.4. Post-partum period

Rectal palpation [43] and P₄ levels measured during the post-partum period [39–41] have been used to determine intervals from calving to ovulation, anoestrus and silent oestrus [42] and diagnose early pregnancy [63, 64] in Zebu cattle. Intervals from calving to ovulation varied from 15 to 210 d, depending on breed or individual animal variation, animal management and investigator [10, 37–44]. About 29.4% and 44.1% of post-partum Bunaji cows in a herd exhibited silent oestrus and anoestrus, respectively, during the first 90 d following parturition (Fig. 4) [42]. The average durations of the first, second and third oestrous cycles were 18.7 ± 4.3, 23.9 ± 11.1 and 20.4 ± 8.6 d, respectively, for silent oestrus cows. These were, however, comparable with the corresponding durations of 19.1 ± 6.9, 21.1 ± 10.9 and 20.6 ± 7.4 d for normal oestrous cows. The implication of this finding is the
FIG. 3. Serum progesterone profile in Bunaji cows during gestation [39].

FIG. 4. Serum progesterone concentrations in Bunaji cows indicating (a) silent oestrus and (b) anoestrus during the post-partum period [42].
apparent delay caused in rebreeding such animals under an artificial insemination (AI) programme and its subsequent effect on the calving interval.

Reports on uterine involution indicate that the duration ranges from 16 to 35 d for indigenous West African cattle [37, 38, 40, 43-46], depending on the method of determination. Determinations based on clinical involution as monitored by rectal palpation usually indicated longer durations than those based on gross measurements and histological studies [45]. Generally, uterine involution does not appear to constitute a problem in the productivity of these animals.

3.5. Calving intervals and rates

The reported inter-calving intervals for cattle in the region ranged from 11 to 29 months, as indicated in Table I [12, 13, 15, 18-24, 28-30, 48, 49]. Most of these intervals are obviously longer than the desired interval of 12-13 months required for good productivity.

The calving rates [5, 12, 13, 23, 26, 50-57] also vary with breed, location of study and investigator. The reported values ranged from 35% for the N'Dama and Lagune to 85% for the Baoule. The wide range seems to indicate that improved management could increase these calving rates.

4. MALE REPRODUCTION

Despite the importance of the male in animal productivity very limited attention has been given to the study of bulls in West Africa. A few reports on histological studies of the genitalia to determine puberty are available, however [65, 66]. Spermatozoa were observed in large numbers in both the seminiferous tubules and portions of the epididymis from 60 weeks of age in Bunaji bulls [67]. The work reported in Ref. [66] indicated that spermatogenesis commenced in Bunaji bulls at 13 months but consistently large gonadal and epididymal sperm reserves first became apparent at about 17 months, when sexual maturity could be said to have been attained. Bunaji bulls reached puberty 7 weeks earlier than Sokoto Gudali bulls but there was no difference between the two breeds in their scrotal circumference (Table II) [67].

Seasonal influence on the ejaculate characteristics of bulls has also been observed [68, 69]. Ejaculate volume, sperm concentration, and the percentages of morphologically normal and of live spermatozoa were significantly higher in the rainy than in the dry season (Table III). Seasonal effects attributed to changes in humidity were also reported for Baoule [70] and N'Dama [71] bulls. It was indicated in Ref. [71] that sperm motility, concentration and abnormalities increased with humidity, with more than 50% of the abnormalities being proximal and terminal cytoplasmic droplets. This indicates the adverse effects of humidity on sperm maturation.
TABLE II. RELATIONSHIP BETWEEN PUBERTAL CHARACTERISTICS OF VARIOUS BREEDS OF BULLS [67]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Breed mean ± SD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bunaji (White Fulani)</td>
<td>Sokoto Gudali</td>
<td>Friesian x Bunaji</td>
</tr>
<tr>
<td>Age (weeks) at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First motile sperm</td>
<td>62.14 ± 4.09^a</td>
<td>70.6 ± 4.59^b</td>
<td>61.29 ± 10.63</td>
</tr>
<tr>
<td>50 x 10^6 sperm</td>
<td>66.79 ± 1.01^a</td>
<td>73.4 ± 2.16^b</td>
<td>62.43 ± 10.70</td>
</tr>
<tr>
<td>Weight (kg) at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First sperm</td>
<td>147.0 ± 13.9</td>
<td>156.6 ± 7.2^c</td>
<td>134.0 ± 13.9^d</td>
</tr>
<tr>
<td>50 x 10^6 sperm</td>
<td>154.3 ± 8.7</td>
<td>157.0 ± 11.0</td>
<td>155.0 ± 31.1</td>
</tr>
<tr>
<td>Scrotal circumference (cm) at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First motile sperm</td>
<td>21.3 ± 2.4</td>
<td>21.0 ± 1.8</td>
<td>19.1 ± 3.2</td>
</tr>
<tr>
<td>50 x 10^6 sperm</td>
<td>21.8 ± 1.7</td>
<td>22.5 ± 1.8</td>
<td>19.1 ± 3.2</td>
</tr>
<tr>
<td>Heart girth (cm) at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First motile sperm</td>
<td>129.3 ± 8.4</td>
<td>132.6 ± 4.6^e</td>
<td>122.5 ± 6.8^f</td>
</tr>
<tr>
<td>50 x 10^6 sperm</td>
<td>133.0 ± 2.2</td>
<td>134.0 ± 3.6</td>
<td>125.0 ± 12.7</td>
</tr>
</tbody>
</table>

Note: Data with different superscripts within each row are significantly different: ab, cd, gh: \( P < 0.01; \) ef: \( P < 0.05.\)

5. STRATEGIES FOR IMPROVING REPRODUCTION

A number of strategies have been adopted to improve the reproductive performance of the indigenous cattle of West Africa, with varying degrees of success.

5.1. Diagnosis and control of reproductive diseases

The major reproductive diseases that have been identified as contributors to the low productivity of the indigenous West African livestock are brucellosis, campylo-
TABLE III. SEASONAL EFFECTS ON EJACULATE CHARACTERISTICS OF AI BULLS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Season</th>
<th>LSD(0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Rainy</td>
</tr>
<tr>
<td>Number of ejaculates</td>
<td>254</td>
<td>303</td>
</tr>
<tr>
<td>Semen volume (mL)</td>
<td>3.42 ± 0.68</td>
<td>5.80 ± 0.41*</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>78.65 ± 2.28</td>
<td>80.57 ± 2.40NS</td>
</tr>
<tr>
<td>Sperm concentration (10^9/mL)</td>
<td>0.970 ± 0.11</td>
<td>1.742 ± 0.11*</td>
</tr>
<tr>
<td>Total spermatozoa (10^9/ejaculate)</td>
<td>3.317 ± 0.21</td>
<td>10.104 ± 0.51*</td>
</tr>
<tr>
<td>Sperm defects (%)</td>
<td>14.045 ± 1.79</td>
<td>6.456 ± 0.66*</td>
</tr>
<tr>
<td>Dead sperm (%)</td>
<td>17.66 ± 0.90</td>
<td>15.391 ± 1.63*</td>
</tr>
</tbody>
</table>

* Significant \( (P < 0.05) \).
NS Not significant.

bacteriosis and trypanosomiasis. These organisms have been implicated in infertility and abortion problems in livestock.

In an attempt to determine the prevalence and incidence of bovine brucellosis in the region, several serological surveys of various categories of cattle have been undertaken [72-77]. The results of these surveys indicate that brucellosis is a significant problem in livestock production in West Africa. The control of this disease has been by vaccination, culling of infected animals and isolation of exposed herds. These measures have yielded some degree of success.

The diagnosis of campylobacteriosis [78] in institutional farms, where management is better than in traditionally managed farms, indicates that this problem may be serious in West Africa. More surveys need to be undertaken to ascertain the level of infertility caused by this disease in the region.

Apart from diagnosis, the effects of trypanosomiasis on both female and male reproduction have been investigated [79-86]. *Trypanosoma congolense* caused abortions in Baoule and N'Dama cows at 3-4 and 5-6 months of gestation respectively [81], while *T. vivax* caused abortion at 7 months in Bunaji heifers [80]. Infection of bulls with *T. vivax* and *T. congolense* for 12 weeks increased the mean reaction time of semen collection from 15 to 75 and 64 s respectively [84]. Infections also decreased semen volume and sperm concentration while increasing sperm abnormalities [82, 84, 86] and causing various grades of lesions in the reproductive
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organs [85]. This disease is controlled through eradication of the vector, the tsetse fly. Prophylactic treatment of animals has, however, been used as a control measure by some farmers.

Other diseases that have been associated with detrimental effects on male reproduction are besnoitiosis [87, 88] and dermatophilosis or cutaneous streptothricosis (Kirchi) [89]. The cysts of *Besnoitia besnoiti* caused mild testicular degeneration [88] while dermatophilosis caused scrotal insulation, resulting in cessation of spermatogenesis and thus sterility [89]. Treatment of dermatophilosis with long-acting Terramycin [90] and control by dipping in acaricides have not yielded any appreciable success. Culling of infected animals appears to be the best control measure for now.

5.2. Improved nutrition

Reports on the effect of level of nutrition on the onset of puberty indicate that increased protein in the diet accelerated age and weight at puberty of Zebu heifers (Table IV) [11]. Also, young Bunaji bulls and Friesian–Bunaji crossbred bulls on

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Pubertal trait</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (d)</td>
<td>Body weight (kg)</td>
<td>Pelvic area (cm²)</td>
<td>Growth rate (kg/d)</td>
</tr>
<tr>
<td><strong>Ration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High protein</td>
<td>570.4ᵃ</td>
<td>207.1ᵃ</td>
<td>128.5</td>
<td>0.58 ± 0.06ᵃ</td>
</tr>
<tr>
<td>Medium protein</td>
<td>640.8ᵇ</td>
<td>187.0ᵇ</td>
<td>124.3</td>
<td>0.41 ± 0.04ᵇ</td>
</tr>
<tr>
<td>Low protein</td>
<td>704.2ᶜ</td>
<td>161.7ᶜ</td>
<td>124.7</td>
<td>0.12 ± 0.07ᶜ</td>
</tr>
<tr>
<td><strong>Breed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Fulani</td>
<td>634.9</td>
<td>187.1</td>
<td>127.9</td>
<td></td>
</tr>
<tr>
<td>Sokoto Gudali</td>
<td>641.9</td>
<td>183.4</td>
<td>123.8</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>65.7</td>
<td>17.2</td>
<td>13.9</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data with different superscripts within columns in each subclass differ significantly (P < 0.05).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bunaji</th>
<th>Friesian × Bunaji</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low protein (10 bulls)</td>
<td>High protein (11 bulls)</td>
</tr>
<tr>
<td></td>
<td>High protein (11 bulls)</td>
<td>Low protein (10 bulls)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>208.67 ± 14.47^a</td>
<td>239.64 ± 12.54^b</td>
</tr>
<tr>
<td></td>
<td>265.46 ± 18.10^c</td>
<td>316.13 ± 14.05^d</td>
</tr>
<tr>
<td>Body condition score</td>
<td>3.65 ± 0.37^a</td>
<td>5.12 ± 0.20^b</td>
</tr>
<tr>
<td></td>
<td>3.57 ± 0.45^a</td>
<td>5.00 ± 0.0^b</td>
</tr>
<tr>
<td>Scrotal circumference (cm)</td>
<td>21.47 ± 0.95^a</td>
<td>24.17 ± 1.01^b</td>
</tr>
<tr>
<td></td>
<td>28.25 ± 1.64^c</td>
<td>30.25 ± 1.18^d</td>
</tr>
<tr>
<td>Average daily gain (kg)</td>
<td>0.24 ± 0.01^a</td>
<td>0.31 ± 0.02^b</td>
</tr>
<tr>
<td></td>
<td>0.32 ± 0.02^b</td>
<td>0.45 ± 0.02^c</td>
</tr>
<tr>
<td>Semen volume (mL)</td>
<td>3.90 ± 0.54^a</td>
<td>5.10 ± 0.86^b</td>
</tr>
<tr>
<td></td>
<td>3.60 ± 1.01^a</td>
<td>5.25 ± 1.12^b</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>70.10 ± 2.14^a</td>
<td>74.04 ± 5.13^b</td>
</tr>
<tr>
<td></td>
<td>70.08 ± 1.69^a</td>
<td>77.50 ± 4.01^b</td>
</tr>
<tr>
<td>Sperm concentration (10^6/mL)</td>
<td>70.41 ± 10.15^a</td>
<td>160.50 ± 9.18^c</td>
</tr>
<tr>
<td></td>
<td>110.18 ± 6.14^c</td>
<td>190.73 ± 11.83^d</td>
</tr>
<tr>
<td>Total spermatozoa (10^9/ejaculate)</td>
<td>270.65 ± 4.11^a</td>
<td>820.38 ± 7.62^c</td>
</tr>
<tr>
<td></td>
<td>510.08 ± 7.37^b</td>
<td>1000.18 ± 10.03^d</td>
</tr>
</tbody>
</table>

Note: Data in rows with different superscripts are significantly different (P < 0.05).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of heifers</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Number pregnant:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 60 d</td>
<td>2 (11.1)</td>
<td>2 (11.1)</td>
<td>0 (0)</td>
<td>3.5</td>
</tr>
<tr>
<td>at 90 d</td>
<td>10 (58.8)</td>
<td>5 (27.8)</td>
<td>3 (16.7)</td>
<td>7.4 ($P &lt; 0.05$)</td>
</tr>
<tr>
<td>at 120 d</td>
<td>10 (58.8)</td>
<td>6 (33.3)</td>
<td>3 (16.7)</td>
<td>6.8 ($P &lt; 0.05$)</td>
</tr>
<tr>
<td>Mean body weight ± SD at conception (kg)</td>
<td>240.0 ± 22.7</td>
<td>240.0 ± 16.4</td>
<td>248.0 ± 29.1</td>
<td></td>
</tr>
<tr>
<td>Mean age ± SD at conception (d)</td>
<td>624.3 ± 94.3$^a$</td>
<td>759.7 ± 103.3$^b$</td>
<td>930.8 ± 78.7$^c$</td>
<td></td>
</tr>
</tbody>
</table>

Note: Means with different superscripts within each row are significantly different ($P < 0.05$).
high protein diets had larger scrotal circumference than those on low protein diets [91]. Semen volume, percentage sperm motility, sperm concentration and total spermatozoa were significantly higher for bulls on high protein than those on low protein diets (Table V). Similar results were obtained when N'Dama bulls were supplemented with 4 kg/d of a mixture of milled andropogon, rice bran and groundnut cake [71]. These effects could be attributed to the effect of nutrition on production of gonadotrophin by the anterior pituitary to stimulate the production of testosterone and semen.

Pregnancy rates of Zebu heifers on different levels of protein indicated a significant advantage in favour of high protein (Table VI) [11]. Although these heifers conceived at different ages their weights were similar. This implies that nutrition can accelerate the age at first conception by accelerating attainment of the target weight for conception.

Zebu cows that received supplementary concentrate post-partum had their first follicle 2 days before and also ovulated 18 days earlier than those that only grazed [43].

5.3. Strategic management practices

5.3.1. Control of oestrus and oestrous cycle

The signs of oestrus are less overt in tropical breeds of cattle than in temperate breeds. Therefore the efficiency of heat detection in these animals is poor and thus there is a need for artificial control of oestrus, especially in an AI programme.

Oestrus synchronization has been achieved with progestagens in the form of the progesterone releasing intravaginal device (PRID) [92] and prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) or its analogue [93–95] in Zebu cattle. Single or double injections of PGF$_{2\alpha}$ have been used with varying results. Adeyemo et al. [93] reported 100% synchronization with a single injection of PGF$_{2\alpha}$ and 61% conception to a single insemination 80 h after synchronization in Bunaji heifers. When two injections of 500 µg of cloprostenol (Estrumate, ICI) were administered 11 d apart, 8 and 13 out of 17 heifers were synchronized in oestrus after the first and second injections respectively (Table VII) [94]. Nine of the 13 heifers confirmed to be in oestrus ovulated between 72 and 96 h after the second injection. When 25 mg quantities of Dinoprost tromethamine (Lutalyse, Upjohn Co., Kalamazoo, Michigan, United States of America) were used also as double injections 11 d apart, responses of 30 and 68% to the first and second injections were reported [95]. The results of the two studies indicate a better response to cloprostenol than Dinoprost tromethamine by Bunaji heifers.
<table>
<thead>
<tr>
<th>Breed</th>
<th>Number of heifers</th>
<th>Ovarian status</th>
<th>Uterine tone</th>
<th>Crystallization patterns in mucus</th>
<th>Mucus discharge</th>
<th>Confirmed in oestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Palpable structures</td>
<td>Mature follicles</td>
<td>Corpora lutea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After first injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Fulani</td>
<td>12</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Sokoto Gudali</td>
<td>2</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Friesian × Zebu</td>
<td>3</td>
<td>—</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>After second injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Fulani</td>
<td>12</td>
<td>—</td>
<td>4</td>
<td>8</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Sokoto Gudali</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Friesian × Zebu</td>
<td>3</td>
<td>—</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>1</td>
<td>7</td>
<td>9</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>
TABLE VIII. PREGNANCY RATES OF BUNAJI COWS TO ARTIFICIAL INSEMINATION AT DIFFERENT TIMES FOLLOWING STANDING OESTRUS [96]

<table>
<thead>
<tr>
<th>Interval from standing oestrus to AI (h)</th>
<th>Number of cows</th>
<th>Cows pregnant</th>
<th>Services per conception</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20 11 56</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20 13 65</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>20 14 70</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>20 12 60</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>20 12 60</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100 62 63</td>
<td>1.56</td>
<td></td>
</tr>
</tbody>
</table>

Note: For all data $P > 0.05$.

5.3.2. Artificial insemination

Artificial insemination as a means of genetic upgrading of the indigenous cattle has also been adopted with the intention of improving their productivity. Although this technique is now routine in Europe and the USA, its use in West Africa is still very limited. Studies have been carried out to determine the optimum time for AI following natural [96] and synchronized [95] oestrus. Pregnancy rates of Bunaji cows to AI at different times following natural oestrus ranged from 56% to 0 h to 70% at 12 h (Table VIII) [96] while the conception rates to AI at fixed times following synchronization with Dinoprost tromethamine ranged from 20 to 54% [95]. These results call for further investigations and trials to achieve a higher fertility rate to AI following synchronization of the indigenous cattle.

5.3.3. Other strategic management practices

The effect of season on pregnancy rates to AI has been investigated [97], as has the effect of suckling [39]. Supplementation with concentrate rations during the dry and rainy seasons improved the pregnancy rate of Zebu cattle by 26.5% while insemination during the rainy season resulted in higher pregnancy rates than during the dry season for both supplemented and unsupplemented animals (Table IX).
TABLE IX. PREGNANCY RATES OF GROUPS OF ANIMALS CLASSIFIED BY MANAGEMENT AND SEASON OF BREEDING

<table>
<thead>
<tr>
<th>Season</th>
<th>Supplemented</th>
<th></th>
<th>Unsupplemented</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inseminations</td>
<td>PR (%)</td>
<td>Inseminations</td>
<td>PR (%)</td>
<td>Inseminations</td>
</tr>
<tr>
<td>Wet</td>
<td>415</td>
<td>67.5</td>
<td>228</td>
<td>35.1</td>
<td>643</td>
</tr>
<tr>
<td>Dry</td>
<td>145</td>
<td>35.9</td>
<td>59</td>
<td>23.7</td>
<td>204</td>
</tr>
<tr>
<td>Total</td>
<td>560</td>
<td>59.3</td>
<td>287</td>
<td>32.8</td>
<td>847</td>
</tr>
</tbody>
</table>

Note: $\chi^2$ for season: 33.4 ($P < 0.001$); $\chi^2$ for management: 34.1 ($P < 0.001$).

* Wet and dry seasons refer to the months of May–October and November–April respectively.
TABLE X. MEAN CALVING INTERVALS, POST-PARTUM INTERVALS TO CONCEPTION AND PREGNANCY RATES AT 60-90 d POST-PARTUM FOR SUCKLED AND NON-SUCKLED BUNAJI COWS [39]

<table>
<thead>
<tr>
<th></th>
<th>Suckled</th>
<th>Non-suckled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Calving interval (d)</td>
<td>$512.5 \pm 22.6^a$</td>
<td>$352.5 \pm 18.8$</td>
</tr>
<tr>
<td></td>
<td>(327-977)</td>
<td>(308-491)</td>
</tr>
<tr>
<td>Post-partum interval to conception (d)</td>
<td>$232.5 \pm 15.2$</td>
<td>$72.6 \pm 8.5$</td>
</tr>
<tr>
<td></td>
<td>(47-697)</td>
<td>(28-138)</td>
</tr>
<tr>
<td>Number of cows that conceived between 60 and 90 d</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

$^a$ Standard deviation.

Suckled Bunaji cows conceived later and had longer calving intervals than non-suckled cows, while more non-suckled cows conceived between 60 and 90 d post-partum than suckled cows (Table X). As encouraging as this result may appear, it should be noted that the advantage of early weaning could easily be marred by the problem of calf survival under the present level of management of these cattle.

6. CONCLUSION

The results discussed in this review show that even though a relatively high number of investigations have been carried out on the reproductive capabilities and strategies for improvement of cattle indigenous to West Africa, much more research is required to improve the reproduction of these animals.

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TECHNIQUES FOR IMPROVING LIVESTOCK PRODUCTION IN DEVELOPING COUNTRIES

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Abstract

TECHNIQUES FOR IMPROVING LIVESTOCK PRODUCTION IN DEVELOPING COUNTRIES.

The reproductive potential of domestic animals is rarely fully realized, even under the best management conditions available in developed countries. More efficient reproduction would ensue if puberty occurred at the earliest possible age, if mating always resulted in pregnancy, if embryonic and neonatal mortality were minimized, if the maximum number of offspring resulted from each pregnancy, and if the interval between parturitions were as brief as physiologically possible. If the sex of offspring could be preselected, herd composition and reproductive performance could be influenced. Reproductive technology can be employed to address all of these issues. Traditional and modern methods have been used to improve livestock reproduction in the developing world. Observation of the frequency of parturition and the occurrence of oestrus, measurement of scrotal circumference and palpation of ovarian structures are traditional means of determining reproductive status. If coupled with rigorous record keeping, these methods can increase reproductive efficiency. Of the modern methods, ultrasonographic observation of reproductive organs can provide valuable information about ovarian function and embryo mortality. Hormonal measurements, particularly progesterone radioimmunoassay (RIA), have been useful for the determination of reproductive status. The gonadotrophic hormones, luteinizing hormone and follicle stimulating hormone, can be determined in animals by double antibody RIA. These measurements are valuable for studies of puberty and post-partum anoestrus. The ovulation rate is the principal limitation on the number of offspring produced by a species. Immunization of domestic animals against ovarian inhibitory substances may serve to increase this rate. The selection of female offspring can be achieved by molecular biological methods. The most promising method is determination of male embryos by amplification of a DNA sequence of the Y chromosome.

1. INTRODUCTION

Reproduction of a species is central to its evolutionary survival. It can be argued that successful and efficient reproduction is the most important aspect of the production of domestic animals. In spite of its importance, maximal reproductive
performance is rarely achieved for any domestic species under any management conditions. In the developing world, the lack of technology, training and appropriate animal nutrition as well as numerous other factors limit the reproductive rates of domestic animals. This restriction becomes more pronounced when combined with the annual limitations in rainfall and consequent forage unavailability.

A number of factors can be associated with the failure to achieve full reproductive potential by domestic mammals. These include: late attainment of puberty in both males and females, inadequate production of gametes by both sexes, unsuccessful fertilization, low rate of embryo and foetal survival, a long interval between parturition and return to gestational capability, and some seasonal characteristics of reproduction. A principal constraint is lack of information about these processes in a particular herd or species. In most species, the limiting factor is the number of young carried by the female. In some species, particularly dairy cattle, there is a compelling rationale for the selection during the pre-natal period for female animals as a strategy to improve the efficiency of production.

Husbandry practices and technology of various sorts have been employed to determine reproductive status and to increase reproductive success in domestic animals. The purpose of this paper is to consider the benefits, drawbacks and potential of some of these practices.

2. TRADITIONAL METHODS FOR EVALUATION OF REPRODUCTIVE PARAMETERS

The determination of reproductive parameters of domestic animals in developing countries is commonly restricted to observation by a herdsman or owner. Mating, the occurrence of oestrus and the attainment of puberty are evaluated qualitatively, or not at all, and accurate records are infrequently kept.

In some species, male reproductive status can be evaluated by measurement of scrotal circumference [1]. If a sufficient base of data for a breed or species exists to relate the observed values to breeding capability, valid inferences can be made about reproductive fitness, reproductive seasonality and age of attainment of puberty.

Palpation of ovaries per rectum is the usual method of determination of reproductive status of cattle, and has also been used in water buffalo [2]. Where detectable, the presence of a corpus luteum (CL) on the ovary is usually accepted as an unequivocal indication of ovulatory capability. The skill of the operator is the major limitation in the assessment, and populations of ovarian follicles and small CL are usually not reliably detected.
3. ECHOGRAPHY

In cows and horses, ultrasonography of the reproductive tract has developed as a research and diagnostic tool. It confers the advantage of allowing for the instant and non-invasive determination of ovarian status and embryonic vitality. Serial ultrasonographic determination resolved a number of questions about the nature of follicular events in cattle, particularly whether follicles developed at random or in waves [3, 4]. Bovine embryos can be detected as early as day 10 of gestation, and serial ultrasonography is valuable to studies of embryonic mortality in this species.

Ultrasonography is not widely used in developing countries for the assessment of reproductive status, because of the capital cost of the ultrasound apparatus and the level of skill required for accurate diagnosis. Anatomical constraints render rectal echography impossible in smaller species. Transabdominal ultrasound examination is much less reliable, and difficult to accomplish in species such as the pig in which the abdominal fat is thick.

4. HORMONE MEASUREMENT

The capability to determine the levels of reproductive hormones in biological fluids has revolutionized reproductive biology over the last twenty years. In many domestic species, in-depth understanding of most aspects of reproduction, including puberty, ovulation, pregnancy and anoestrus, has emerged from thousands of studies employing radioimmunoassay (RIA) to determine circulating hormone levels. More recently, enzyme linked immunosorbent assay (ELISA) has proven useful, particularly for rapid, semiquantitative determination of steroid hormone levels. Most of the physiological parameters pertinent to successful reproduction can be evaluated by RIA or ELISA of gonadal or pituitary hormones. The hormones which will provide the most useful information are luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, oestrogens and testosterone.

Puberty in bull calves and ram lambs is characterized by gradual changes in the secretion of LH and testosterone. As both of these hormones are secreted in an episodic mode, both the frequency and amplitude of secretory pulses are important to determine the reproductive state of males. Nevertheless, it is possible to establish minimum values of testosterone that must be attained for a particular species for puberty to have been reached by males. Puberty in the females of species which ovulate spontaneously is marked by the presence of a CL, which can be readily detected by RIA or ELISA for progesterone. As in the male, LH is secreted episodically, and LH pulse frequency increases as puberty approaches in the heifer [5]. Multiple samples are thus required to provide any useful information from LH levels.
Sheep, goats, camelids and other large domestic species exhibit seasonality in their patterns of breeding. Hormone analysis is a useful means of studying this phenomenon [6] as well as the interactions between seasonal breeding and puberty [7], and is helpful in selection of individual animals for longer breeding seasons. Progesterone, testosterone and LH are the best candidates for these evaluations.

It is estimated that 90% of bovine ova are fertilized following natural mating or artificial insemination, and that 55% or fewer of the cows calve as a result of this fertilization [8]. The embryonic mortality has been attributed to factors intrinsic to the embryo and to extrinsic factors associated with the uterine environment or due to xenobiotics or malnutrition [9]. The rate of embryonic mortality is also high in other species, including the alpaca, in which there is a loss of approximately 65% of fertilized embryos [10]. Much of the embryonic loss occurs within the first weeks following fertilization, and, as the embryo is not present to interfere with subsequent follicular growth and ovulation, normal cycles ensue. Early embryonic mortality is best diagnosed by ultrasonography. Embryonic loss which occurs three or more weeks after breeding can be identified by examination of the inter-oestrus interval; i.e., intervals in excess of the expected oestrous cycle are indicative of a pregnancy which failed. Because the persistence of the CL depends on the presence of a viable embryo in most artiodactyls, assay of progesterone is a good means of evaluating embryo mortality.

Evaluation of milk progesterone profiles from a large number of dairy cows suggests that the levels of this hormone diverge in pregnant and non-pregnant animals as early as day 10 after oestrus [11]. Samples from pregnant cows are higher than those in non-pregnant animals between days 18 and 21 after service, because the latter group will have undergone luteolysis and should be in oestrus during this interval [12]. Milk progesterone assay at the time of next expected oestrus has been shown to be accurate in detection of pregnancy in 85% or more of animals studied [12, 13]. Oestrone sulphate, produced by the placenta, is another reproductive hormone which may have utility in detection of pregnancy. Its concentrations are elevated in pigs after day 20 of pregnancy [14], and between days 60 and 90 in sheep and goats [13].

The frequency of parturition in the Bovidae is a major constraint to their production under most conditions of management. The loss of potential production is exacerbated by harsh climate, forage unavailability or other nutritional stress. Post-partum periods in domestic animals can include periods of anoestrus as well as periods in which expression of behavioural oestrus does not occur in animals undergoing ovarian cycles [15]. Analysis of the reproductive events that occur (or do not occur) can be achieved by determination of reproductive hormones, especially LH and progesterone.

RIA technology became widely used in the 1970s in developed countries, and assays were developed for virtually every hormone which was available in sufficient quantity and of sufficient purity to allow for antibody production [16]. All were
based on competition for binding to the antibody of native hormone and radioactively labelled hormone, with the latter present in excess. A number of strategies have been employed to separate antibody bound from unbound labelled hormone. These include precipitation of the first antibody with a second antibody, which is the most common method for protein hormones, and separation of unbound small molecules by charcoal extraction, which is commonly used for steroids and prostaglandins. Both of these methods require centrifugation at lower than ambient temperature. Solid phase assay systems, where the antibody is bound to the tube, can be employed at ambient temperature and require no centrifugation, and have proven to be adaptable to determination of reproductive hormone concentrations in developing countries.

4.1. Measurement of progesterone in developing countries

Through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, assay systems have been established for reproductive hormones in developing countries. The most successful has been a solid phase progesterone assay in which a commercial antibody coated tube (Diagnostic Products Corporation) and $^{125}$I-progesterone are employed. The system is repeatable, generally robust and has been used successfully to determine progesterone levels in serum, plasma and milk in a wide variety of animals and conditions. It is functional through a range of temperatures and tolerant of shipping conditions. It has been seen to fail under two conditions: firstly, when the pH of the buffer added to the standards is low (<5.0) no standard curve ensues. The second problem, which is more frequently encountered, is due to the short half-life of $^{125}$I, which can render the assay system ineffective if it is held up for weeks in customs or during shipment. Other failures can be related to sample handling. Progesterone in milk is unevenly distributed between the aqueous and organic phases, and samples from whole milk can provide spurious results. Progesterone is rapidly degraded in whole blood samples at ambient temperatures. To preserve progesterone integrity in serum and plasma, the samples must be quickly cooled and cellular elements removed, an operation which can be logistically difficult under field conditions.

ELISA is based on the same principle of antigen–antibody interaction and competition as RIA, with the simple difference that the marker antigen is labelled with an enzyme instead of a radiolabel. The end point is the spectrophotometric measurement of a colour reaction which is indicative of the quantity of enzyme present. ELISA is usually performed in solid phase with the antibodies plated to the wells of microtitre plates. As with RIA, ELISA has been developed for a wide range of hormones. Progesterone ELISA has been easily adapted to use in developed countries. Preliminary studies demonstrate that differences associated with pipetting of reagents for the enzymatic and colorimetric reactions resulted in highly significant differences in the final results (J.C.B. Plaizier, B. Rogovic and B.D. Murphy, unpublished data). A comparison was made of ELISA with RIA for milk progesterone in Euro-
pean and African laboratories (J.C.B. Plaizier, unpublished data). Intra-assay variation, which is characteristically 1–9% in RIA, ranged from 10 to 54% in ELISA in African laboratories. This study further indicated that the ELISA kits were unstable and became less reliable when the intervals between shipping and receipt were more than one week. Thus, current technology is not sufficiently developed for the measurement of progesterone by ELISA under the less than optimal conditions present in developing countries.

4.2. Gonadotrophic hormones

As noted above, the measurement of LH and FSH in samples taken at frequent intervals has the potential to provide valuable information about the reproductive status of domestic animals. Gonadotrophic hormones are proteins which vary in primary structure among species [17]. Surface epitopes of these hormones appear to be highly conserved; thus, species specificity of the first antibody is usually not important. Double antibody RIA is the most common procedure by which these hormones have been measured [16]. First antibodies and hormones for iodination and standards are supplied by the agencies in developed countries, usually the National Institutes of Health in the United States of America. Purified hormones are labelled with $^{125}$I, usually on the tyrosine residues, by an oxidation reaction [18]. Labelled hormone and free iodide are separated by gel exclusion or ion exchange chromatography. Second antibodies against the $\gamma$-globulin of the species in which the first antibody was raised are used for precipitation of the first antibody–antigen complex.

Double antibody RIA for both LH and FSH has been successfully established in laboratories in developing countries. These methods require higher levels of training and technology relative to those required for solid phase progesterone assay. Amounts of radioactivity are two or more orders of magnitude higher than in the progesterone RIA because protein hormones are usually labelled on site. Problems such as incorrect pH and inappropriate molarity of buffers, which interfere with progesterone assay, can also disrupt double antibody RIA. The most common failures occur in either the iodination or the second antibody precipitation steps. The labelled hormone functions best at optimal specific activity, usually 20–60 $\mu$Ci/$\mu$g (0.74–2.22 MBq/$\mu$g), or approximately 0.3–1.0 atom of $^{125}$I per molecule of hormone. Over-iodination can result in radiolysis of the hormone, while underiodination reduces precision [19]. Precipitation of the antigen–antibody complex by second antibody may fail owing to a variety of complications. These include inadequate centrifugation equipment, inappropriate titre or loss of activity of second antibodies, loss of the pellet when the supernatant is aspirated, and high non-specific binding.
5. NEW TECHNOLOGY

In ruminant species, the reproductive potential of a herd is limited by the number of females, as the reproductive capacity of the female is much lower than that of the male. Increasing the reproductive capacity of females and preselection for female progeny are means by which livestock production can be rendered more efficient. Technology is currently evolving to achieve both of these objectives.

5.1. Increasing reproductive capacity of females

The factor limiting the number of offspring in ruminants is the number of ovulations which occur at each oestrous cycle. Studies of superovulation have demonstrated that the administration of FSH will increase the population of ovarian follicles that attain pre-ovulatory status [20]. Endogenous levels of FSH are limited by feedback control by elements from the ovary, including steroids and proteins. Immunization of female animals against these elements may be a viable strategy to elevate endogenous FSH, thereby increasing ovulation rate.

Inhibin is a heterodimeric gonadal glycoprotein first purified from porcine follicular fluid [21]. Since its isolation, the complementary DNA has been cloned and the primary structure deduced. The $\alpha$ subunit has been produced by recombinant means [22, 23]. Immunization of ewes with recombinant $\alpha$-inhibin resulted in increased circulating levels of FSH, increased FSH responses to GnRH and increased ovulation rates [22, 24, 25].

Follistatin is a monomeric glycoprotein from the ovary which has no structural similarity to inhibin, but is a potent and specific inhibitor of FSH secretion by the pituitary. The cDNA for follistatin has been cloned and the nucleotide and amino acid sequences are known [26]. In our laboratories, the coding region for the follistatin gene (1005 bp) has been amplified using the polymerase chain reaction (C.E. Lindsell et al., unpublished data). Expression of this protein in vitro is under development in aid of producing an antigen for immunization of domestic animals.

5.2. Preselection for female offspring

Selection for offspring of one sex or the other has economic value. The separation of sperm bearing $X$ and $Y$ chromosomes is expected to be the best method for preselecting sex as it can be performed on ejaculated sperm prior to artificial insemination. Numerous methods have been promoted for separation of $X$ and $Y$ sperm on the basis of density, electric charge, motility and cell surface antigens [27, 28]. No scientific validation currently exists for any of these schemes. Some promise may be present in methods by which separation by immunoaffinity chromatography is based on the presence of the putatively male specific H-Y antigen on the sperm surface [29]. There is some question about whether the H-Y antigen
is restricted to Y bearing sperm [28]. There are two obstacles at this time to development of sperm separation procedures. Total separation may not be possible as X and Y bearing spermatozoa may share phenotypic characteristics owing to the cytoplasmic bridges which are present during spermatogenesis [30]. Secondly, no method has yet been developed which does not reduce the viability of the sperm population to be inseminated [28].

Determination of the sex of embryos and the selective transplantation of one sex or another is a means by which the sex ratio of a herd can be altered. Early successes were achieved by removal of cells from bovine embryos after hatching from the zona pellucida. These cells were cultured and karyotypes analysed for the presence of the Y chromosome. This method, although successful, is cumbersome [31]. A second approach under development is the detection of H-Y antigen expressed on the surface of embryonic cells [32].

The most recent scheme for discovery of the sex of embryos consists of determination of sequences of DNA peculiar to the Y chromosome. The sex of human embryos from parents with genetic proclivity for conception of children with specific X linked disorders has been determined [33]. The method consists of in vitro fertilization and embryo maturation, and removal of one cell by biopsy at the 6-8 cell stage of the embryo. The polymerase chain reaction (PCR) is then employed to amplify a 149 bp region of the DNA specific to the Y chromosome, and the consequent nucleic acids are separated by electrophoresis. This procedure has been applied to the determination of the sex of domestic animals. The successful sexing of sheep embryos by means of PCR of a 124 bp segment of the ovine Y chromosome has been reported, a procedure that was completed within 3 h [34]. Herr and Reed [35] have recounted the feasibility of similar methods for sexing of bovine embryos.

The embryo sexing technology of the 1990s has the potential to increase production of domestic livestock. Successful employment of the most promising method, PCR amplification of Y specific DNA sequences, requires a contamination free laboratory environment and careful exclusion of all sources of DNA extraneous to the embryonic cells [35]. Given the problems that beset developing countries in the establishment and maintenance of laboratory facilities, it is clear that embryo sexing procedures are beyond the technical scope of most laboratories at this time. With the development of DNA laboratories for detection of diseases in developing countries may come the possibility for sex selection of domestic animals.

6. SUMMARY AND CONCLUSIONS

Livestock production in developing countries is constrained by a variety of factors, including forage unavailability, poor breeding stock and inefficient reproduction. Current technology has the potential to reduce the inefficiency of reproduction, principally by providing reproductive information. Age of puberty,
rates of embryonic mortality, characteristics of post-partum anoestrus and the sex of embryos can all be determined by existing analytical methods. Some technology, particularly ultrasonography and RIA, has been successfully established in developing countries and has improved reproductive success. Other methods, including ELISA for hormones and PCR for Y specific DNA segments, are beyond the general capability of laboratories outside developed countries. The challenge we face is to optimize the methods which are currently established, and to work towards development and simplification of new technology.

REFERENCES


CONTRIBUTION OF THE RADIOIMMUNOASSAY TECHNIQUE TO KNOWLEDGE OF THE REPRODUCTIVE PHYSIOLOGY OF SOUTH AMERICAN CAMELIDS

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Abstract

CONTRIBUTION OF THE RADIOIMMUNOASSAY TECHNIQUE TO KNOWLEDGE OF THE REPRODUCTIVE PHYSIOLOGY OF SOUTH AMERICAN CAMELIDS.

The review describes several experiments conducted in order to characterize important reproductive parameters in alpacas and llamas. Nuclear techniques such as radioimmunoassay for measuring reproductive hormones have provided comprehensive knowledge of various reproductive functions and aided the design of rational breeding systems. Profiles of the major steroid hormones and prostaglandins as well as the gonadotrophic hormones in milk, blood plasma or urine have been used to monitor normal ovarian activity and heat symptoms, ovulation, luteal activity in non-pregnant females and following fertile mating, maternal recognition of pregnancy, early embryo death, pregnancy, parturition, post-partum and pregnancy diagnosis in the female. In the male, plasma testosterone levels were determined in an attempt to define the seasonality of reproduction of these domestic animals in the Southern Hemisphere.

1. INTRODUCTION

In South America there are two domesticated species of camelids, the alpaca and llama. These animals are native to a fragile, high altitude ecosystem and are perhaps the most environmentally gentle ruminant livestock in the world. In Peru, peasant communities own 80% of the domestic camels (total population 4.5 million), which serve as mobile rural banks. The fibre accumulating on their backs is often safer than capital in the bank. When the need arises, they can be sheared and the fibre sold. Surplus males and old females are the only source of meat available to the inhabitants of the High Andes and the skin is used for handicraft work and clothing. Increasing camelid productivity will directly benefit South America’s most destitute. A major constraint on camelid production is lack of reliable data on reproduction. The existing management systems utilized with alpacas and llamas are extrapolations of those techniques developed for other species, particularly sheep,
even though the reproductive physiology of camelids differs greatly from that of sheep. In fact, the current birth rate of alpacas is only around 50%. This situation is due in part to the lack of knowledge in camelid reproductive physiology; the need for developing knowledge on camelid reproduction and management is thus quite apparent.

The introduction of radioisotopic techniques for studying endocrinological reproductive functions in South American camelids has made it possible to carry out measurements, in small amounts of blood, of hormone levels which control normal physiological events. This paper presents the results of many studies carried out on domestic camelids (alpaca and llama) by Peruvian and foreign researchers using radioimmunoassay (RIA) techniques in most cases, as well as related techniques.

2. REPRODUCTIVE HORMONES IN FEMALES

2.1. Normal ovarian activity and heat symptoms

Female alpacas and llamas do not have oestrous cycles like other domestic species. In these animals oestrus and ovulation are not manifested in a repetitive, cyclic and predictable fashion as is known to occur in some other species (spontaneous ovulators). Since copulation is ordinarily a necessary prelude to ovulation, the South American camelids (domestic and wild) have been classified as reflex or induced ovulators.

The release of anterior hypophyseal ovulating hormone into the bloodstream is brought about as a result of the copulating act, probably via one or more neural pathways. Previous studies of the duration and periodicity of oestrus in alpacas [1] suggest that there is considerable variability between individuals; these studies were done using sexual behaviour as a main criterion for delineating the relatively long periods of sexual receptivity (oestrus) and short periods of non-receptivity (anoestrus). On the basis of laparoscopic examination, the average time required for follicle development (3–5 d), maintenance (2–8 d) and regression (3–5 d) was 12 d [2] (Fig. 1). Later, Bravo et al. [3] determined ovarian follicular dynamics in adult llamas by ultrasonography, by rectal palpation and by analysis of oestradiol-17β (E$_2$) and oestrogen conjugates of plasma and urine, as well as analysis of plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH). E$_2$ was determined by RIA following the protocol of Shille et al. [4] and the sensitivity of the assay was 6 pg/mL. Oestrogen conjugates were determined in plasma and urine samples by enzyme immunoassay (EIA) following the technique described by Czekala et al. [5], and the sensitivity of the assay was 1.6 pg/well. FSH was measured in plasma by use of a heterologous RIA [6] and LH concentrations were determined in plasma by use of a heterologous RIA validated for the llama [7].
FIG. 1. Alternate follicular growth in left (□) and right (■) ovaries from adult alpacas at different time intervals and seasons: (a) 3 d intervals during the breeding season; (b) 5 d intervals during the non-breeding season. Arrows indicate sexual receptivity (+) by the female.
Progesterone (P₄) analysis of plasma was used to verify or exclude the presence of a corpus luteum (CL).

From analysis of all the information obtained (hormones, ultrasonography and behaviour), the interval between ovarian follicle waves was found to average 11.1 d (Fig. 2). Plasma oestradiol and oestrogen conjugate concentrations were positively associated (P < 0.05) with follicular activity; urinary oestrogen conjugate concentrations best reflected ovarian follicular dynamics (P < 0.001). Daily FSH concentrations in plasma were not correlated with follicular activity and LH concentrations in plasma were low in all animals throughout the study, indicating that oestrogen from developing ovarian follicles does not induce the release of LH. Recently, Adams et al. [8] studied the effect of lactational and reproductive status on interwave interval; successive dominant follicles emerged at intervals of 19.8 ± 0.7 d in unmated and vasectomy-mated llamas and 14.8 ± 0.6 d in pregnant llamas. Lactation was associated with an interwave interval that was shortened by 2.5 ± 0.05 d compared with a non-lactating group.

2.2. Ovulation

The alpaca, llama and vicuña show induced or reflex ovulation [1, 9–11]. The same mechanism has been found in the Old World camels [12, 13]. The minimum time from copulation to ovulation has been estimated to be 26 h following natural mating and 24 h after injection of human chorionic gonadotrophin (HCG) (500–700 IU, intramuscular) [1]. Sumar et al. [14], using a laparoscopic technique,
found that 48.8% ovulated between 26 and 30 h, 28.8% ovulated between 30 and 72 h and 22.5% failed to ovulate; yearling alpacas accounted for much of the lack of ovulation.

Investigations on the llama using ultrasonography revealed that ovulation occurred on average 1.8 d after a single mating (3 of 9 llamas ovulated within one day after breeding [8]). Using the same technique, Sumar [15] reported that ovulation in alpacas occurred on average 36 h after a single mating (range: 26–50 h). Ovulation was also induced using ovine LH or gonadotrophin releasing hormone (GnRH) [9, 16].

There are some indications that females ovulate without coital or exogenous hormonal stimulus, especially after being kept isolated from males and then being exposed to them, but without penis intromission. This is probably caused by the presence of the male on isolated females and the fact of some type of physical contact between male and female during the courtship [8, 17, 18]. It has also been shown that both alpaca and bull semen can induce ovulation in alpacas and llamas by deep introduction of the semen into the vagina of an oestrous animal [19], suggesting the presence in alpaca and bull semen of an 'ovulation induction factor' (OIF) of unknown biochemical composition. The same mechanism has been found in the Chinese camel [20].

| TABLE I. EFFECT OF DIFFERENT OVULATION INDUCTION STIMULI IN ALPACAS AND LLAMAS (J. Sumar et al., unpublished data) |
|---------------------------------|-----------------|-----------------|-----------------|
| Stimulus                        | Alpacas         | Llamas          |
|                                 | n | Observations | % | n | Observations | % |
| A. Vasectomized mating          | 5 | 4            | 80 | 3 | 3            | 100 |
| B. Fertile mating on ligated oviduct of female | 5 | 5 | 100 | 5 | 5 | 100 |
| C. Intravaginal bull semen      | 11 | 4           | 36.4 | 5 | 0 | 0 |
| D. Intravaginal alpaca semen    | 10 | 6           | 60 | 8 | 5 | 67.5 |
| E. Exposure to a male: no coitus| 7 | 0            | 0 | 5 | 3 | 60 |
| F. Control: isolated from males | 10 | 1           | 10 | 10 | 1 | 10 |
| Total:                         | 48 |             |   | 36 |   |   |
TABLE II. MEAN PROGESTERONE LEVELS (nmol/L) IN PERIPHERAL BLOOD IN ALPACAS AFTER DIFFERENT STIMULI FOR INDUCTION OF OVULATION
(J. Sumar et al., unpublished data)

<table>
<thead>
<tr>
<th>Days post-stimulus</th>
<th>Stimulus</th>
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<td>14.0</td>
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<tr>
<td>9</td>
<td>16.8</td>
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<td>10</td>
<td>6.4</td>
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<tr>
<td>12</td>
<td>0.1</td>
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<td>14</td>
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<tr>
<td>16</td>
<td>0.2</td>
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<tr>
<td>18</td>
<td>0.2</td>
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</tbody>
</table>

* The first number in brackets is the number of females treated and the second number is the number of females ovulating.

In another study, P₄ concentrations were determined after different ovulation induction stimuli to obtain evidence that ovulation had occurred and, subsequently, to elucidate CL development and regression (J. Sumar et al., unpublished data). For this work, 48 adult female alpacas and 36 adult female llamas in heat were distributed in the following groups: A, mated with vasectomized males; B, females with a ligation on the oviducts (sterile) mated to fertile males; C, intravaginal deposition of bull semen; D, intravaginal deposition of alpaca semen; E, exposure to fertile males without penis intromission; and F, control females, isolated from males. The results are shown in Tables I-III. In general the results show different mechanisms of ovulation in both camelid species, with the formation of a CL and the typical sterile-mated
### TABLE III. MEAN PROGESTERONE LEVELS (nmol/L) IN PERIPHERAL BLOOD OF LLAMAS AFTER DIFFERENT STIMULI FOR INDUCTION OF OVULATION

*(J. Sumar et al., unpublished data)*

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<td>1.6</td>
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<td>0.6</td>
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<td>18</td>
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<td>0.4</td>
<td>0.6</td>
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<td>5.6</td>
</tr>
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</table>

*a The first number in brackets is the number of females treated and the second number is the number of females ovulating.

Profile of P₄ secretion; however, in mating with a fertile male mechanical stimulation of the genital tract by the penis together with the OIF of the semen interact to maintain the secretion of P₄ from the CL 1–2 d longer than either stimulus alone. In this experiment, as well as in other studies [21, 22] in alpacas, those females that failed to ovulate had no CL and consequently had basal P₄ levels.

The time course for secretion of LH and E₂ following a single copulation was studied in the llama [7]. LH was assayed by RIA using a monoclonal antibody against the β subunit of bovine LH (heterologous assay). A significant increase in LH concentration (1.1 ng/mL) was observed by 15 min after the onset of copulation, with the peak of the pre-ovulatory surge of LH occurring at 2 h (4.37 ± 1.54 ng/mL);
values were basal by 7 h after copulation (0.96 ng/mL). The $E_2$ was determined by RIA, following copulation, at 4 h intervals through 24 h and then daily for up to 10 d; the values were unchanged through 18 h after copulation (12.0 pg/mL), tended to decline at 22 h (8.9 pg/mL) and were significantly lower than the 18 h values by 48 h (5.5 pg/mL) after copulation. $E_2$ values remained unchanged from 2 d through to 10 d after copulation.

In alpacas, multiple ovulation occurred in 3–10% of the cases after natural mating and in 9–20% of the cases with the application of gonadotrophins, but twins born alive are extremely rare [21]. The ovulatory activity indicated by the presence of a CL of pregnancy was always in favour of the right ovary. However, the magnitude of the difference in frequency between left and right ovary varied in the several studies [21].

2.3. Luteal activity in non-pregnant females

CL function was studied in non-pregnant and pregnant alpacas [23, 24]. In one experiment, ovulation was induced by sterile mating or single intramuscular injection of 750 IU of HCG. The results showed that the CL of the alpaca undergoes rapid development after ovulation, reaches its maximum size and secretory activity at Days 8–9, and in the absence of pregnancy starts to decline sharply in both size and secretory activity on Day 12. Regression of the CL was complete in most cases by Day 18. $P_4$ levels after sterile service have been studied in the milk of alpacas and llamas [21]; the luteal phase was characterized by an increase in milk $P_4$ around Day 6, reaching a maximum concentration of 14 nmol/L by Day 9, with an abrupt decrease between Days 10 and 11 post-mating (Table IV). The levels of $P_4$ in the milk of alpacas and llamas after sterile mating parallel the concentration of this hormone in the blood.

Studies [25] were recently conducted in order to document the secretory profiles of PGF$_{2\alpha}$, $P_4$ and $E_2$ in the llama and alpaca following sterile mating. The results show that vasectomized mating will induce ovulation and a short luteal phase with $P_4$ secretion yielding a maximum blood concentration of 10–20 nmol/L at 7–8 d after copulation. A rapid decline in $P_4$ occurs on Days 9–10 in connection with repeated surge releases of PGF$_{2\alpha}$. Oestradiol concentrations were 100–200 pmol/L shortly after mating. During the luteal phase they were around 20–40 pmol/L but rose to 40–60 pmol/L following luteolysis, at which time the animal resumed sexual receptivity (Fig. 3).

Thus, the alpaca and llama appear to have a reproductive advantage compared with other induced ovulators, in that the short luteal phase after sterile mating allows for a more rapid return to a potentially fertile state.

One important physiological effect of $P_4$ in some species is the suppression of oestrus. In some domestic animals, such as the sow, ewe and mare, sexual receptivity usually wanes within a few hours after ovulation, presumably owing to $P_4$
TABLE IV. MILK PROGESTERONE LEVELS (nmol/L, MEAN ± SD) AFTER STERILE-MATING OVULATIONS IN ALPACAS AND LLAMAS [21]

<table>
<thead>
<tr>
<th>Day</th>
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<th>Llamas (n = 11)</th>
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<tr>
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<tr>
<td>16</td>
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</table>

\(^a\) Day of mating.
\(^b\) ND: not detectable.

production by a newly formed CL. With the alpaca and llama, in contrast, on the average, sexual receptivity continued to be manifested (2-3 d after ovulation); this is because the formation of the CL lasts up to 5 d and no significant increases in circulating P\(_4\) are found (J. Sumar, unpublished data). Nevertheless, receptivity or heat in the female cannot be interpreted as being strictly associated with an absence of P\(_4\) secretion. This and other studies confirm the absence of true pseudopregnancy in alpacas and llamas.

### 2.4. Luteal activity following fertile mating

In most cases, a fertile mating results in the formation of a CL of pregnancy which secretes P\(_4\) throughout the gestation period. The mean P\(_4\) concentration in the
milk of pregnant alpacas was studied from the day of mating (non-detectable) to Day 14 (14.35 nmol/L) [26]. Compared with the pattern of P₄ concentration shown by females mated with a sterile male, in this case the P₄ concentration at Day 8 post-service showed a tendency to be higher in pregnant animals. By Day 10 the levels in pregnant alpacas were on average 12.5 nmol/L, showing a progressive increase up to Day 14. Similar results have been found for the blood plasma of alpacas and llamas after fertile mating [22]. In this later study P₄ levels of 16–17 nmol/L were found at 9 d after mating, with levels of 14–18 nmol/L at 16 d, remaining more or less constant until 30 d of pregnancy, the time course of this experiment (Table V). The P₄ concentrations showed a high variability between animals, especially in llamas.

Adam et al. [27], working in the United Kingdom, reported that the P₄ concentrations in the llama increased after mating and remained high if conception had occurred: 6–12 ng/mL in the first four months of pregnancy and 5–9 ng/mL in the fifth to ninth month of the 11 month gestation. In a recently published paper [28], the authors indicated that plasma P₄ concentrations had increased by Day 5 after mating and remained elevated (>2.0 ng/mL) throughout most of the pregnancy.

Several authors have suggested that not all the fertile matings ended in pregnancy; in many cases, failure of fertilization or very early embryo death is very common. The causes of this phenomenon are discussed in Section 2.6.
TABLE V. BLOOD PROGESTERONE LEVELS (nmol/L) IN PREGNANT ALPACAS AND LLAMAS DURING THE FIRST 30 d [22]

<table>
<thead>
<tr>
<th>Day</th>
<th>Alpacas (n = 5)</th>
<th>Llamas (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min.</td>
</tr>
<tr>
<td>1</td>
<td>0.32</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>2.46</td>
<td>1.2</td>
</tr>
<tr>
<td>8</td>
<td>18.50</td>
<td>9.3</td>
</tr>
<tr>
<td>9</td>
<td>16.34</td>
<td>10.4</td>
</tr>
<tr>
<td>10</td>
<td>13.70</td>
<td>9.6</td>
</tr>
<tr>
<td>11</td>
<td>12.84</td>
<td>6.8</td>
</tr>
<tr>
<td>12</td>
<td>16.00</td>
<td>11.9</td>
</tr>
<tr>
<td>13</td>
<td>17.36</td>
<td>12.4</td>
</tr>
<tr>
<td>14</td>
<td>16.94</td>
<td>11.0</td>
</tr>
<tr>
<td>15</td>
<td>12.32</td>
<td>8.9</td>
</tr>
<tr>
<td>16</td>
<td>14.46</td>
<td>9.1</td>
</tr>
<tr>
<td>17</td>
<td>15.38</td>
<td>9.7</td>
</tr>
<tr>
<td>18</td>
<td>12.74</td>
<td>8.1</td>
</tr>
<tr>
<td>19</td>
<td>16.60</td>
<td>8.5</td>
</tr>
<tr>
<td>20</td>
<td>16.70</td>
<td>8.0</td>
</tr>
<tr>
<td>25</td>
<td>13.00</td>
<td>10.6</td>
</tr>
<tr>
<td>30</td>
<td>14.06</td>
<td>11.0</td>
</tr>
</tbody>
</table>

2.5. Maternal recognition of pregnancy

After fertilization, the ova remain in the fallopian tubes for about 3–4 d and then enter the uterus at the 32 cell to morula stage (J. Sumar, unpublished observations). Long before becoming elongated and attached, the blastocyst is able to prevent the 'sudden' regression of the CL, possibly by Days 7–8 after mating. This may be effected by inhibition of the release of uterine luteolysin (PGF₂α), which occurs in sterile mating of females between Days 8 and 12 [25]. An embryo must be in the left uterine horn on Days 7–8 after mating in order for the CL to be maintained; the embryo is directly or indirectly luteotrophic. The critical period when the
conceptus must signal its presence to allow pregnancy to be established is called 'maternal recognition of pregnancy' [29]. If the conceptus fails to signal its presence at exactly the correct time, the function of the CL is terminated by the luteolytic action of PGF$_{2\alpha}$ from the uterus. The CL of pregnancy secretes P$_4$, assuming this function throughout the pregnancy period in alpacas and llamas [30].

The exact time of implantation of the embryo is not established in South American camelids. Fernández-Baca [31] argues that it is probably around Day 21 after mating, although there are no published histological studies of the attachment of the foetal membranes. However, Johnson [32] suggests that the time of implantation is probably similar to that of the horse, beginning at about Day 30 and complete by Day 90.

The plasma P$_4$ changes associated with the first 30 d of pregnancy in the alpaca and llama have been described [22]; in pregnant alpaca females the first significant increase occurred around Day 4 or 5 after mating, with peak levels on Day 8. However, a transient decrease in P$_4$ concentration occurred on Days 9–11, with a subsequent increase on Day 12 onwards. This period is coincident with the so-called 'rescue of the corpus luteum' in response to pregnancy, since in sterile mating females [25] P$_4$ levels began to drop on Day 9.

2.6. Early embryo death

During the first two months of gestation, embryo mortality is higher in alpacas than in other domestic species, and seems to be a serious reproductive problem of this domestic animal. One field trial designed explicitly to study embryonic mortality [23] revealed that more than 70% of the ova recovered 3 d after mating were in the process of dividing, but only 50% of the fertilized ova survived beyond 30 d of gestation. Furthermore, other researchers [33] studying reproductive wastage in single-mated female alpacas of different ages and reproductive status suggested that the overall reproductive wastage (ova, embryo and foetal loss) was 83.2%.

Table VI shows the mean levels of plasma P$_4$ in female alpacas and llamas that were mated with fertile males. It is interesting to note that the P$_4$ concentration on Day 8 post-service showed a tendency to be higher in pregnant than in non-pregnant animals ($P < 0.05$). Whether these levels are higher in pregnant animals owing to the presence of a live embryo or lower in non-pregnant animals because of the incapability of the CL to secrete P$_4$ is still unknown. Further studies are required on this matter.

The precise cause of early embryonic death is unknown, but nutritional constraints, hormonal imbalance and chromosomal aberrations may be the principal factors. Specific reproductive infections have not been identified as a cause of embryo mortality. Diverse studies have established that pre-natal loss attains important levels in all farm species and that the bulk (two thirds) of this considerable loss occurs during the early embryonic stages that precede implantation [34].
TABLE VI. MEAN PLASMA PROGESTERONE LEVELS (nmol/L) IN FEMALE ALPACAS AND LLAMAS THAT WERE MATED WITH FERTILE MALES [22]

<table>
<thead>
<tr>
<th>Day</th>
<th>Alpacas (n = 12)</th>
<th>Llamas (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant</td>
<td>Non-pregnant</td>
</tr>
<tr>
<td>1</td>
<td>0.32</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>2.46</td>
<td>1.46</td>
</tr>
<tr>
<td>8</td>
<td>18.50</td>
<td>12.03</td>
</tr>
<tr>
<td>9</td>
<td>16.34</td>
<td>3.20</td>
</tr>
<tr>
<td>10</td>
<td>13.70</td>
<td>0.76</td>
</tr>
<tr>
<td>11</td>
<td>12.84</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>16.00</td>
<td>—</td>
</tr>
</tbody>
</table>

2.7. Pregnancy, parturition and post-partum period

Progesterone profiles during all of gestation, parturition and the post-partum period in alpacas and llamas have been determined by RIA techniques [35]. The P₄ profile during early gestation (30 d) has been described already. From the second month of gestation to two weeks before parturition, the P₄ levels decreased slowly to 6.29 and 8.77 nmol/L in alpacas and llamas respectively. Between Day 16 and parturition, P₄ concentration declined significantly (P < 0.001) from a mean of 8.85 to 2.42 nmol/L in alpacas and from 6.38 to 2.90 nmol/L in llamas. Between Day 1 post-partum and the end of the first week, the P₄ concentrations declined from 1.26 ± 0.52 and 1.00 ± 0.38 nmol/L to 1.13 ± 0.24 and 0.97 ± 0.19 nmol/L in alpacas and llamas respectively. During the second week post-partum, P₄ concentrations remained at basal levels and did not change significantly (P > 0.05).

One study done in the United States of America on llamas [28] using RIA techniques described the changes in plasma P₄, triiodothyronine, thyroxine and cortisol, as well as serum E₂ and total oestrogen concentrations, during pregnancy, parturition and the early post-partum period. More recently, also using RIA techniques, changes in P₄, PGF₂α and oestrone sulphate during all of pregnancy, parturition and the post-partum period were studied in alpacas and llamas (J. Sumar et al., unpublished data).
The length of gestation in alpacas of the Huacaya and Suri breeds was 341 and 345 \(d\) respectively [1]. In llamas the recorded gestation length was 346 ± 8 \(d\) (327–357 \(d\)), with no significant differences due to reproductive status (uniparous vs. pluriparous) or to the sex of the 'cria' or newborn alpaca or llama [36].

2.8. Pregnancy diagnosis

Several methods of diagnosis to assess pregnancy in alpacas and llamas have been described. Some of them are based on oestrous behaviour, rectal palpation, \(P_4\) levels in milk and blood plasma, ultrasound (A-mode) and ultrasonographic techniques (B-mode) [26, 32, 37]. The hormonal method (\(P_4\)) will be discussed here.

A striking difference in milk \(P_4\) concentrations between non-pregnant and pregnant alpacas can be observed at 10–12 \(d\) after mating, and it was suggested that this difference might furnish the basis of an early pregnancy test [26]. Plasma \(P_4\) changes during the first 30 \(d\) of pregnancy have been described [22]. Plasma \(P_4\) concentrations in the alpaca increased on Day 5 after mating (2.3 nmol/L), reached a peak level by Day 9 (16.0 nmol/L) and remained elevated (> 12 nmol/L) during the first 30 \(d\) of pregnancy. The \(P_4\) pattern was similar in the llama, but levels were slightly higher. Llamas and alpacas that failed to ovulate, as well as those that failed to conceive, showed basal levels of \(P_4\) on Day 12 and later after mating (Table VI).

In this study, pregnancy diagnosis was based on levels of 6.0 nmol/L and greater as indicating pregnancy and less than 6.0 nmol/L as indicating non-pregnancy. Pregnancy diagnosis in domestic camelids could be done in females 12 \(d\) after mating, but because the South American camelids have a high embryonic death rate prior to 60 \(d\) of gestation, it was considered necessary to take an extra sample at Day 30 or 60 in order to confirm the gestation or detect the embryonic loss.

<table>
<thead>
<tr>
<th>Month</th>
<th>Alpacas</th>
<th>Llamas</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>1142.50 ± 108.27</td>
<td>208.00 ± 52.69</td>
<td>0.001</td>
</tr>
<tr>
<td>June</td>
<td>992.50 ± 388.00</td>
<td>37.75 ± 14.90a</td>
<td>0.05</td>
</tr>
<tr>
<td>September</td>
<td>877.50 ± 91.32</td>
<td>291.25 ± 74.84b</td>
<td>0.001</td>
</tr>
<tr>
<td>December</td>
<td>2445.00 ± 694.82</td>
<td>362.25 ± 73.73c</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Note: Mean values with different superscripts are significantly different. a, \(P < 0.05\) in relation to March; b, \(P < 0.02\) and c, \(P < 0.01\) in relation to June.
In a herd of 138 female llamas (71 yearlings and 67 parous), blood plasma was taken 15 d after an 'alternate' breeding period of 45 d (60 d after joining males and females); the results of P₄ analysis were compared with rectal palpation done two months later, and the total gestation rate was 92.7% [38]. Ten females had high levels of P₄, but later, at rectal palpation, they were found to be non-pregnant. The occurrence of false positive predictions related to high blood P₄ concentrations is probably due to early embryonic loss, according to several authors; no false negative cases were recorded [23, 33].

In the USA [32] most laboratories reported values of greater than 1 ng/mL of P₄ in serum taken 21 d after the last exposure to a male as being consistent with pregnancy in llamas.

3. REPRODUCTIVE HORMONES IN THE MALE

3.1. Seasonal pattern of testosterone secretion in the male

An attempt was made to define the seasonality of reproduction in male alpacas and llamas in the Southern Hemisphere (Peru) by measurement of testosterone concentrations in the blood [39]. Plasma testosterone content was determined by the RIA technique. Results showed that the two species exhibited a marked elevation of plasma testosterone concentrations during spring and summer months (breeding season), while lower levels occurred in autumn and winter months (Table VII). It is suggested that these seasonal variations resulted primarily from changes in environmental factors, especially food availability.

ACKNOWLEDGEMENTS

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PURIFICATION, CHARACTERIZATION AND SETTING UP OF IMMUNOASSAYS OF CAMEL PITUITARY HORMONES

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Abstract
PURIFICATION, CHARACTERIZATION AND SETTING UP OF IMMUNOASSAYS OF CAMEL PITUITARY HORMONES.

The authors have undertaken the purification and physicochemical, immunological and biological characterization of luteinizing hormone, follicle stimulating hormone, growth hormone and prolactin from the dromedary and have developed different assays suitable for their measurement in the circulation of this animal. It is particularly important to make camel hormone standard preparations available for the sake of interlaboratory comparison. The availability of these standards and of corresponding immunoassays that can be performed without expensive and/or uncommon equipment will permit substantial progress in the study and management of this species.

1. INTRODUCTION

The reproductive performance of the camel has to be improved to exploit fully its ability to survive in drought areas. Indeed, camel farming is currently precarious and uneconomical as females only calve for the first time at five years of age and males do not become sexually active before they are six years old. Moreover, female camels have a very low fertility compared with other domestic animals. These characteristics stress the importance of studying the reproductive physiology of this species in order to improve its efficient farming in drought stricken areas. Improvements require better knowledge of the endocrine controls of reproduction at the molecular and cellular levels in order to develop efficient new methods of fertility control.
In this regard, we have undertaken the purification and physicochemical, immunological and biological characterization of luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH) and prolactin (PRL) from the dromedary and have developed different assays suitable for their measurement in the circulation of this animal.

2. PURIFICATION OF PITUITARY GLYCOPROTEIN AND PROTEIN HORMONES

Owing to the relative scarcity of available dromedary pituitaries, we set up protocols of purification [1-3] permitting the simultaneous isolation of the four hormones of interest and, potentially, of other hormones and factors (thyroid stimulating hormone (TSH), follicle growth factor (FGF), etc.).

One hundred and fifty glands (165 g) were collected less than thirty minutes after death and immediately frozen on dry ice. They were ground in 500 mL of acetone at −15°C. The acetone dried residue (32.4 g) was then suspended and agitated for 1 h at 4°C in 400 mL of 0.15M ammonium acetate (pH4.7) in order to extract LH and FSH activities. After centrifugation the supernatant was recovered and the residue was re-extracted in 400 mL of the same buffer at pH5.8. The supernatant obtained after centrifugation was added to the first one and this solution (SI: 730 mL) was the starting material for LH and FSH purifications.

The residue was ground in water, frozen, lyophilized and weighed (17 g). It was then ground in 1.8 L of water and the pH of the suspension was brought to 10.0 with 1N NaOH. After 30 min at 4°C under agitation, the supernatant solution (S2: 1500 mL) was recovered by centrifugation and used for GH and PRL purifications.

The flow sheet of the purification of all four hormones has been previously reported [4].

3. CHARACTERIZATION OF CAMEL PITUITARY HORMONES

3.1. Luteinizing hormone

In contrast to LHs from all other species known so far, highly purified camLH was found to exhibit no charge polymorphism. Its pI was found to be 7.9 by chromatofocusing. The acid dissociation of camLH was studied at a hormone concentration of 25 μg/mL (8.9 × 10⁻⁷M). Its half-dissociation at equilibrium occurs at pH4.18 ± 0.02 at 37°C [1]. We determined the N-terminal sequences of its α and β subunits [5] up to the 53rd and 67th residue respectively and found that they are very close to the sequences of their porcine counterparts.
The biological activity of camLH was studied in homologous radioreceptor assays (RRAs) using porcine or camel testicular binding fractions and $^{125}\text{I}pLH$ or $^{125}\text{I}camLH$ respectively. In both cases, camLH was found to be 50-60% as active as pLH with a $K_a$ of $1.1 \times 10^{-10}\text{M}$. In the in vitro stimulation of testosterone production by rat Leydig cells, camLH was also found to be half as active as pLH, indicating that the two hormones have similar efficiencies in transmembrane signalling after their binding to the LH receptors.

### 3.2. Follicle stimulating hormone

Camel FSH was found to exhibit charge polymorphism like the FSHs from all the species tested so far. By chromatofocusing, at least nine isoforms of camFSH can be revealed with pIs ranging from 3.0 to 5.5. The acid dissociation of camFSH was studied at a hormone concentration of $25 \mu\text{g/mL}$ ($7.4 \times 10^{-7}\text{M}$). Half-dissociation at equilibrium at $37^\circ\text{C}$ occurred at pH 4.8. This value is 0.6 higher than that of camLH, indicating that the quaternary structure of camFSH is slightly less stable than that of camLH. The same observation was made for equine LH and FSH but the half-dissociation pH of eFSH was more than 2 units higher than that of eLH.

The activity of highly purified camFSH was measured in an RRA using camel testicular binding fraction and $^{125}\text{I}oFSH$. Its activity was $30.7 \times \text{NIH FSH SI}$ in this system.

### 3.3. Growth hormone

Camel GH exhibits no charge or size polymorphism. Its molecular weight determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis was found to be 22 300. Its isoelectric point in isoelectric focusing was found to be 6.7. Moreover, when camGH was submitted to affinity chromatography on ConA-sepharose, no material was retained, indicating that there is probably no glycosylated camGH in this preparation.

We determined the N-terminal amino acid sequence of camGH, which was found to be very similar, but not identical, to that of porcine GH [2].

The in vitro binding activity of camGH was estimated by RRA using late pregnant rabbit liver membrane preparations and $^{125}\text{I}oGH$. It was found to be 39% that of pure oGH.

### 3.4. Prolactin

Size polymorphism of camPRL was observed and appeared to be due to the presence or absence of an oligosaccharide moiety. The N-terminal amino acid sequences of these two isoforms were determined and a potential site of glycosylation was found in position 31 [3]. In the sequence of the non-glycosylated camPRL, an
Asn residue is present in position 31. No amino acid residue was identified in this position for the glycosylated form, indicating that it bears a carbohydrate moiety. Afterwards, we completed the amino acid sequence of camPRL [6] and found that this Asn 31 residue is the sole site of glycosylation.

The binding activities of camPRL-A (non-glycosylated) and camPRL-CII (glycosylated) were measured on a membrane fraction from rabbit mammary gland with $^{125}$IoPRL S13 (NIADDK, National Institutes of Health, United States of America). The latter form exhibited approximately 20% the activity of the non-glycosylated camPRL-A. In the in vitro bioassay specific for PRL and using Nb2 cell proliferation assay, glycosylated camPRL was also largely less active than the non-glycosylated form.

4. SETTING UP OF IMMUNOASSAYS OF CAMEL PITUITARY HORMONES

4.1. Luteinizing hormone

Three different immunoassays of camLH were developed in our laboratory: a radioimmunoassay (RIA), a competitive enzyme linked immunosorbent assay (ELISA) and a sandwich ELISA [7].

An anti-camLH serum was raised by immunizing a rabbit first with ovine LH and subsequently with highly purified camLH. IgGs from this antiserum were purified by ion exchange chromatography on DEAE-Trisacryl (IBF, France) and an aliquot of these anti-camLH IgGs was conjugated to horseradish peroxidase (HRP) using glutaraldehyde.

For the RIA, camLH was labelled with $^{125}$I using iodogen as the catalyst. Standards and unknowns (100 µL) were mixed in tubes with the antiserum (50 µL) at an initial dilution of 1/80 000 and incubated for 48 h at 4°C. Then the radioactive hormone (50 µL; 15-20 000 counts/min) was added to each tube and the incubation was continued for 16 h at 4°C. Finally, 50 µL of an ovine anti-rabbit IgGs serum were added for precipitation of the anti-LH IgGs. After 30 min at room temperature, the free and bound hormone molecules were separated by centrifugation and the radioactivity of pellets was measured in a multiwell gamma counter. The detection limit of this assay is less than 0.1 ng/tube (i.e. less than 1 ng/mL of sample). Because the use of RIAs is restricted to well equipped and authorized laboratories owing to the application of radioactive isotopes, this kind of assay is not suitable for field trials in remote areas. Therefore, we decided to develop an ELISA for LH as well as for the other pituitary hormones.

For the competition ELISA, standards and unknowns (200 µL) were mixed in tubes with the antiserum (200 µL) at a final dilution of 1/20 000. After 3 h at 37°C, 100 µL aliquots were transferred to wells (96 well microtitre plates) previously
coated with porcine LH and saturated with bovine serum albumin (BSA) just before use. After 1 h at 37°C, the wells were emptied and washed. Then, anti-camLH antibodies bound to coated pLH were detected with an anti-rabbit IgGs antibody conjugated with HRP. After three washings, HRP activity was measured using orthophenyldiamine (OPD) as the substrate and optical density measurement of the product at 492 nm with a plate reader. This assay is very specific but because its detection limit is only 8 ng/mL it is not suitable for the measurement of basal LH concentrations in the plasma of animals. However, it is a very convenient tool for the monitoring of LH activity during the purification of dromedary hypophyseal hormones.

For the sandwich ELISA, wells of 96 well microtitre plates were coated with 0.4 µg of anti-camLH IgGs in 100 µL of buffer at pH9.6 and saturated with BSA just before use. Standard or unknown samples (100 µL) were added to the wells and incubated for 16 h at 37°C. After three washings, anti-camLH conjugated with HRP was added and incubated for 3 h at 37°C. After three washings, bound HRP activity was measured as described above.

This sandwich ELISA was found to be highly specific for camLH since all other tested dromedary hormones (FSH, GH and PRL) showed less than 0.1% cross-reaction. The detection limit for camLH was less than 0.1 ng/mL and largely sufficient for the measurement of expected basal concentrations of circulating LH in the dromedary.

4.2. Follicle stimulating hormone

For the time being, only a heterologous RIA has been set up for camFSH using an anti-oFSH antiserum and ¹²⁵I-oFSH. The protocol is essentially the same as that described above for camLH. This RIA is extremely specific for FSH since camFSH and oFSH had similar activities while camLH, oLH and ratTSH only exhibited 0.1%, 0.2% and 0.4% cross-reaction, respectively, compared with oFSH; this may be due to low residual contamination by FSH.

As for the other hormones, it would be of interest to make a camFSH ELISA available whose use is not limited to well equipped laboratories. We are currently working to set up such an assay.

4.3. Growth hormone

CamGH can be measured by a RIA for porcine GH in which both the antigen (pGH i071; 1.0 × USDA B1) and the anti-pGH serum raised in the rabbit (i571) are of commercial origin (UCB Bioproducts, Brussels). Highly purified pGH was used for iodination. The antiserum was used at a final dilution of 1/8000 with 15 000 counts/min of radiiodinated pGH and 0.1–1000 ng of the different fractions in a total volume of 250 µL of phosphate buffer saline (PBS) containing 0.5% BSA.
these conditions, half-inhibition of $^{125}$IpGH binding to the antiserum (ED50) was obtained with 1.4 ng pGH/tube or only 0.82 ng camGH/tube. The activity of our camGH in this assay was then 1.7 times higher than that of highly purified pGH. This assay was highly specific for GH since camPRL only cross-reacted by 2% compared with camGH. The detection limit for camGH was as low as 0.2 ng/tube, much below the expected circulating concentrations of this hormone in the dromedary.

4.4. Prolactin

During its purification, camPRL was monitored by a heterologous RIA using highly purified pPRL and anti-ovine PRL antiserum (A28) raised in the guinea pig. After its isolation, three different homologous immunoassays (RIA, competition ELISA and sandwich ELISA) of camPRL were developed.

For the RIA, the antiserum raised in the rabbit was used at an initial dilution of 1/10 000 with $^{125}$IcamPRL as the tracer. The antiserum and samples were preincubated for 3 d at 4°C before the radioiodinated hormone (20 000 counts/min) was added. The incubation was then continued for 16 h at room temperature. After immunoprecipitation of bound hormone the radioactivity of the pellets was measured. The specificity of this assay is good since camGH exhibited only 1% cross-reaction and camLH and camFSH were undetectable even at the highest concentrations used. The detection limit for camPRL was found to be 0.8 ng/mL.

For the competition ELISA, 96 well microtitre plates were coated with camPRL (pH 9.6, 37°C, 1 h) and saturated with BSA. The samples and the antiserum at a final dilution of 1/6400 were incubated overnight at room temperature. Aliquots of this medium were transferred to the wells and incubated for 1 h at 37°C. After three washings, the bound anti-camPRL IgGs were reacted with sheep anti-rabbit IgG conjugated to HRP and detected at 492 nm after the reaction with OPD. This assay is very specific but its detection limit is much too high (40 ng/mL) for detection of the hormone in the plasma.

For this reason, we undertook the development of a sandwich ELISA. For this, anti-camPRL serum IgGs were purified on DEAE-Trisacryl and a quantity of them were conjugated to HRP with glutaraldehyde. The wells were coated with anti-camPRL IgGs and after saturation and washings the samples were incubated in the wells for 16 h at 4°C. After three washings, anti-camPRL IgGs conjugated to HRP were added and incubated for 1 h at 37°C. After three washings, the amount of bound HRP was determined using OPD as the substrate as described above. This sandwich ELISA of camPRL is highly specific and its detection limit (0.8 ng/mL) is much better than that of the competition ELISA and identical to that of the RIA. Therefore, it is convenient for the follow-up of this hormone in the circulation of animals.
5. CONCLUSION

Our studies have led to the purification and physicochemical characterization of LH, FSH, GH and PRL from the dromedary and we have set up several assays for each hormone (in vitro bioassays, RRA, RIA, ELISA) allowing the thorough study of their structure–function relationships as well as the measurement of their concentrations in the circulation. In particular, we have focused our efforts on the setting up of assays that can be performed using portable battery powered ELISA plate readers in remote areas where no suitable facilities exist. It would be very useful if the preparation and distribution of hormone standards for this species were organized in order to permit reliable measurements in different laboratories all over the world.

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INTERACTIONS NUTRITION–REPRODUCTION CHEZ LES PETITS RUMINANTS EN MILIEU MEDITERRANEEN

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Abstract–Résumé

NUTRITION–REPRODUCTION RELATIONSHIPS IN SMALL RUMINANTS IN MEDITERRANEAN CONDITIONS.

A series of experiments were carried out to study the influence of the nutritional level and physical condition of Barbary ewes on their sensitivity to the male effect, reproductive performance and the intensity of seasonal anoestrus. The results show that undernourishment of ewes during gestation and/or lactation leads to a deterioration of their physical condition, increases the intensity of seasonal anoestrus and alters the quality of their corpus luteum (corpora lutea) induced by the male effect. These effects persist even when these females are overfed between the dry period and the introduction of rams in spring. However, improvement in the nutritional level of such ewes before mating appreciably raises their ovulation, fertility and prolificacy rates.

1. INTRODUCTION

Chez les ovins, l’introduction des béliers dans un troupeau de femelles en anoestrus saisonnier et préalablement isolées de ces derniers provoque l’apparition de l’œstrus chez la plupart d’entre elles [1]. Ce phénomène de l’effet bélier, ou “effet mâle”, est décrit dès 1944 par Underwood et al. [2] sur des brebis de race...
Mérinos. Certains travaux ont permis d'élucider la nature des informations sensorielles impliquées dans ce phénomène et suggèrent que les stimulations olfactives sont suffisantes pour l'obtenir [3–6].

L'emploi de cette méthode peut être avantageuse dans le cas des systèmes extensifs de production pour obtenir les agnelages à des saisons où les disponibilités alimentaires sont importantes pour l'élevage des jeunes. L'alimentation lors de la lutte peut alors être un facteur limitant.

La réponse des brebis de race Barbarine à grosse queue à l'effet mâle a donc été étudiée au printemps, période habituellement souhaitée pour la lutte, chez des femelles recevant différents niveaux d'alimentation; les disponibilités alimentaires peuvent en effet varier fortement d'une année à l'autre ou d'une région à une autre de la Tunisie.

2. INFLUENCE DU POIDS VIF ET DE SON EVOLUTION SUR LA REPONSE A L'EFFET MALE

2.1. Matériel et méthodes

L'expérience est réalisée sur 122 brebis sèches, âgées de 3 à 6 ans (âge moyen: $4.5 \pm 1,1$ ans). Neuf semaines avant la date retenue pour l'introduction des mâles, elles sont réparties en 5 groupes en fonction de leur poids vif. Différents régimes alimentaires sont alors distribués de façon à maintenir, augmenter ou diminuer le poids vif des brebis avant l'introduction des béliers:

— Groupe I: 25 brebis légères (39 kg) gardant un poids vif constant
— Groupe IL: 24 brebis légères (39 kg) gagnant du poids (7 kg)
— Groupe LL: 25 brebis lourdes (52 kg) gardant un poids vif constant
— Groupe LI: 24 brebis lourdes (52 kg) perdant du poids (7 kg)
— Groupe MM: 24 brebis moyennes (46 kg) gardant un poids vif constant.

Les quantités d'aliments distribués et refusés sont contrôlées quotidiennement pour chaque groupe. Les quantités de fourrages grossiers sont ajustées toutes les semaines en fonction de l'évolution du poids vif moyen de chaque groupe.

L'activité ovarienne de toutes les brebis au cours des 3 semaines précédant l'introduction des mâles est contrôlée par l'estimation du niveau de progestérone plasmatique (3 fois/semaine). Après l'introduction des béliers ($J_0$), elle est contrôlée par cœlioscopie des ovaires à $J_4$, $J_9$ et 4 à 7 jours après l'apparition du premier oestrus.

L'introduction des mâles a lieu le 9 mai à raison de 4 béliers entiers dans chaque groupe. La détection des oestrus est effectuée deux fois par jour, matin et soir, et toute femelle en chaleur est saillie 12 et 24 heures après le début de l'oestrus.
2.2. Résultats

2.2.1. Consommations alimentaires et évolution du poids vif

Les animaux des groupes IL et LL consomment la totalité de l’aliment concentré distribué (400 et 200 g/tête/jour respectivement). Les quantités ingérées de foin sont de 1,2; 1,4; 1,0 et 1,1 kg/tête/jour pour les groupes II, IL, LL et MM respectivement. La consommation moyenne de paille par les femelles du groupe L1 est de 0,8 kg/tête/jour.

Les poids vifs des brebis appartenant aux groupes LL, IL et MM restent pratiquement constants jusqu’à la date d’introduction des béliers. Les femelles du groupe LL, soumises à une restriction alimentaire très sévère, perdent en moyenne 7,3 ± 2,0 kg pendant la même période (-116 g/jour). En revanche, celles du groupe IL gagnent du poids (+105 g/jour). Leur gain de poids moyen est de 6,6 ± 2,5 kg. En dépit de leurs états corporels très différents au début de l’expérience, les femelles des groupes MM, IL et LL ont sensiblement un même poids vif moyen voisin de 46 kg.

2.2.2. Activité ovarienne

Le poids vif au début de l’expérience affecte significativement \( P < 0,01 \) les pourcentages de femelles ayant une activité ovarienne spontanée avant l’introduction des mâles (II et IL: 6,1%; L1 et LL: 40,8%). La proportion de femelles cycliques dans le groupe MM est de 12,5%. Elle n’est pas significativement différente de celles des autres groupes.

Le poids vif des brebis à la date d’introduction des béliers et son évolution au cours des 9 semaines précédentes n’ont aucune influence significative sur leur activité ovarienne avant le début de la lutte.

Dans le cas des brebis non cycliques, l’ovulation est induite dans les 3 jours qui suivent l’introduction des mâles chez la plupart des femelles des groupes LL (87,5%), IL (91,3%) et MM (90,5%). La sous-alimentation sévère diminue la réponse à l’effet bélier (tableau I). C’est ainsi que l’ovulation n’est induite que chez 76,9% des brebis du groupe L1 et 65,2% de celles du groupe IL. Cependant, seule la dernière proportion est significativement \( P < 0,05 \) inférieure à celles des lots IL et MM.

La durée du premier cycle ovarien induit à la suite de la stimulation par les béliers peut être normale (16,5 ± 1,5 jours) ou courte (5,3 ± 0,7 jours). Le poids vif des brebis 9 semaines avant l’introduction des mâles a une influence considérable sur la qualité de leur ovulation induite. En effet, la fréquence des premiers cycles ovariens de courte durée est significativement \( P < 0,01 \) plus élevée dans les groupes II (53,3%) et IL (76,2%) que dans les groupes LL (21,4%) et L1 (20,0%). La proportion de femelles montrant une régression prématurée des corps jaunes...
TABLEAU I. CARACTERISTIQUES DE LA REPONSE DES BREBIS A L’EFFET MALE

<table>
<thead>
<tr>
<th>Groupe</th>
<th>Femelles non cycliques</th>
<th>Femelles ovulantes (%)</th>
<th>Cycles courts (%)</th>
<th>Taux ovulation (%)</th>
<th>Taux oestrus (%)</th>
<th>Fertilité (%)</th>
<th>Prolificité (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>l</td>
<td>23</td>
<td>65,2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53,3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60,9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26,1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100,0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>lL</td>
<td>23</td>
<td>91,3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76,2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91,3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78,3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116,7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>lL</td>
<td>13</td>
<td>76,9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>20,0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61,5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38,5&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>100,0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LL</td>
<td>16</td>
<td>87,5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21,4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75,0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>62,5&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>110,0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MM</td>
<td>21</td>
<td>90,5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31,6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90,5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66,7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>114,3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Les résultats présentant des lettres identiques ne sont pas significativement différents au seuil 5%.

Induits dans le groupe MM est de 31,6%. Elle n’est significativement différente ($P < 0,05$) que de celles des groupes lL et lL (tableau I).

L’évolution du poids vif des brebis n’a aucune influence significative sur la durée de leur premier cycle ovarien. D’ailleurs, quelle que soit l’évolution ultérieure du poids vif, il existe, 9 semaines avant l’introduction des mâles (tarissement), un poids seuil de 42,7 kg (obtenu par analyse discriminante) qui permet de prévoir le pourcentage de femelles dont le premier cycle ovarien induit est de courte durée. Au dessous de ce poids seuil, 67% des femelles ont un cycle court. Au dessus, le premier cycle ovarien induit est de durée normale dans 73% des cas.

2.2.3. Taux d’ovulation

Le poids vif des brebis ne semble pas avoir d’impact significatif sur les taux d’ovulations induites. En effet, ces derniers sont sensiblement identiques dans les groupes lL (1,27) et LL (1,29). Celui du groupe MM est légèrement plus élevé (1,42). En revanche, la variation de poids vif des femelles entre le tarissement et le début de la lutte a un effet marqué sur leur taux d’ovulations induites (tableau I). Cette influence apparaît à travers la différence observée entre les groupes lL et Ll. Le taux d’ovulation du premier est le plus élevé (1,43) et celui du second est le plus faible (1,10).

Pour l’ensemble des femelles, le taux d’ovulation induite est significativement ($P < 0,05$) plus élevé (1,33) que celui de l’ovulation suivante survenant à la suite d’un cycle court (1,15) ou normal (1,14).
2.2.4. Apparition de l'œstrus

L’ovulation induite est toujours silencieuse. De même, les ovulations qui apparaissent à la suite d’un cycle ovarien de courte durée ne sont accompagnées d’œstrus que dans 8,6% des cas. L’œstrus survient vers J\textsubscript{17} ou J\textsubscript{23} lorsque le premier cycle ovarien est normal ou court respectivement.

L’apparition de l’œstrus chez les femelles non cycliques avant l’introduction des béliers semble dépendre à la fois de leur poids vif au moment du tarissement et de son évolution jusqu’au début de la lutte. Les proportions de brebis manifestant un comportement d’œstrus au cours des 27 premiers jours suivant l’introduction des mâles varient suivant les groupes de 60,9 à 91,3% (tableau I).

2.2.5. Fertilité et prolificité au premier œstrus

Le poids vif des brebis a une influence très marquée sur leur fertilité apparaînente (nombre de mises bas pour 100 brebis mises à la lutte). Elle est significativement plus faible (\(P < 0,01\)) chez les brebis légères du groupe II (26,1%) que chez celles des groupes LL (62,5%) et MM (66,7%).

Elle est également affectée par la variation de poids. En effet, le gain de poids se traduit par une amélioration significative (\(P < 0,01\)) de leur fertilité apparaînente à l’œstrus induit (78,3%) par rapport à celle du groupe II (26,1%).

La sous-alimentation sévère prolongée (II) ou plus récente (I\textsubscript{I}) a un effet néfaste sur la prolificité des femelles au premier œstrus (nombre d’agneaux nés pour 100 brebis mettant bas).

Effectivement, toutes celles des groupes II et I\textsubscript{I} ont des portées simples (tableau I). Le taux de prolificité est de 110, 114 et 117% dans les lots LL, MM et I\textsubscript{I} respectivement.

3. INFLUENCE DU NIVEAU ALIMENTAIRE AVANT ET APRES LA MISE BAS SUR LA REPONSE A L’EFFET MALE

3.1. Matériel et méthodes

Cent quarante brebis adultes (3 à 6 ans) de race Barbarine du troupeau expérimental de la Station de Bou Rébiâa sont réparties en 4 lots selon leurs niveaux alimentaires pendant les 12 dernières semaines de gestation et les 18 premières semaines d’allaitement:

— lot HH: brebis recevant un haut niveau alimentaire avant et après la mise bas
— lot HB: brebis recevant un haut niveau alimentaire avant la parturition et bas pendant la lactation
— lot BH: brebis recevant un niveau alimentaire bas avant la parturition et haut pendant la lactation
— lot BB: brebis recevant un niveau alimentaire bas avant et après la parturition.

Le poids vif moyen des brebis au début de l’expérience est de 51 kg. Les animaux sont pesés une fois par semaine ainsi que 24 heures après la mise bas. Les agnelages ont lieu au cours de la première quinzaine du mois d’octobre. Les femelles vides, ayant donné naissance à des agneaux mort-nés ou ayant perdu leurs produits au cours des deux premiers mois d’allaitement sont éliminées de l’expérience.

Les brebis sont maintenues en bergerie et les quantités d’aliments offertes et refusées sont pesées quotidiennement. Les quantités d’aliments distribuées sont ajustées en fonction de l’évolution du poids vif moyen de chaque lot. Après le sevrage (28 février), le poids vif moyen des brebis des 4 lots est ramené à une valeur sensiblement identique en début de lutte (30 avril). Ce poids vif moyen est de l’ordre de 47 kg.

L’activité ovarienne des brebis est contrôlée par une série d’examens cœlioscopiques $J_{1}$, $J_{4}$, $J_{9}$ et 8 à 12 jours après l’apparition du premier cœstrus, $J_{0}$ étant le jour de l’introduction des béliers (30 avril). Ces derniers sont utilisés à raison de 1 pour 10 femelles.

La détection des chaleurs et la lutte sont réalisées selon la méthode décrite dans la première expérience.

3.2. Résultats

3.2.1. Evolution du poids vif

Le poids vif des femelles bien nourries avant la mise bas (HH et HB) augmente d’une façon très importante jusqu’à la parturition (6 kg), tandis que celui des femelles sous-alimentées (BB et BH) reste pratiquement constant. La variation de poids des femelles pendant la phase d’allaitement est significativement ($P < 0.01$) affectée par leur niveau alimentaire post-partum. Cette variation dépend également du niveau alimentaire pré-partum.

En effet, la perte de poids est plus faible chez les brebis soumises à une restriction alimentaire avant la mise bas puisqu’elle est de 3 kg dans le lot BB et que les brebis du lot BH gagnent en moyenne 3 kg. Les femelles bien alimentées pendant la gestation et sous-alimentées pendant la lactation accusent une chute très importante de leur poids vif (HB: 10 kg). Celles du lot HH gardent un poids vif constant.

3.2.2. Activité ovarienne

La sous-alimentation à long terme avant et/ou après la mise bas réduit de moitié le pourcentage de femelles cycliques avant l’introduction des béliers (tableau II).
TABLEAU II. CARACTERISTIQUES DE LA REPONSE DES BREBIS A L'EFFECT MALE

<table>
<thead>
<tr>
<th>Lot</th>
<th>Nombre de brebis</th>
<th>Brebis non cycliques</th>
<th>Cycles courts (%)</th>
<th>Taux ovulation</th>
<th>Fertilité (%)</th>
<th>Prolificité (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH</td>
<td>32</td>
<td>11</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>130&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HB</td>
<td>26</td>
<td>18</td>
<td>67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>118&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BH</td>
<td>31</td>
<td>20</td>
<td>75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BB</td>
<td>21</td>
<td>14</td>
<td>79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>130&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Les résultats présentant des lettres identiques ne sont pas significativement différents au seuil 5%.

En effet ce pourcentage est relativement faible chez les brebis ayant souffert de la sous-alimentation à un stade quelconque de leur cycle de reproduction (BB: 33%; HB: 31%; BH: 35%). Il est environ le double (66%) chez les femelles bien alimentées (HH).

L'ovulation est induite chez la totalité des femelles des 4 lots au cours des 3 jours qui suivent l'introduction des béliers. La sous-alimentation sévère des brebis pendant la gestation et/ou la lactation ne semble pas affecter leur réponse à l'effet mâle à condition de les bien nourrir entre le sevrage et la lutte.

L'ovulation induite est suivie chez la totalité des femelles d'une seconde ponte ovarienne. Cette deuxième ovulation se produit, chez un grand nombre d'entre elles, à la suite d'une régression prématurée du corps jaune induit, donnant naissance à des cycles ovariens de courte durée (tableau II). La fréquence de ces cycles courts est plus élevée dans les lots BH (75%), HB (67%) et BB (79%) que dans le lot HH (45%). Néanmoins, les différences entre les 4 lots ne sont pas significatives.

3.2.3. Taux d'ovulation

Les brebis des 4 lots, ayant été ramenées au même poids vif au moment de la lutte, présentent des taux d'ovulations induites différents. Cette différence apparaît nettement entre les lots BB (1,79) et HH (1,27). Les valeurs de ce taux sont intermédiaires dans les lots HB (1,35) et BH (1,50). A l'oestrus induit, le taux d'ovulation est de 1,31; 1,36; 1,12 et 1,30 dans les 4 lots respectivement.
3.2.4. **Fertilité et prolificité**

La fertilité apparente varie de 77 à 100% dans les 4 lots (tableau II). Le plus faible taux est observé chez les brebis du lot BB. Ces résultats montrent que la sous-alimentation prolongée de part et d'autre de la mise bas provoque une diminution importante de la fertilité même si les brebis sont suralimentées entre le sevrage et la lutte.

La prolificité des femelles des 4 groupes varie de 118 à 142%; la valeur la plus élevée est observée dans le lot BH et la plus faible dans le lot HB. Néanmoins, ces différences ne sont pas statistiquement significatives.

4. **INTENSITE DE L’ANOESTRUS SAISONNIER**

4.1. Matériel et méthodes

Pour caractériser l'intensité de l'anœstrus saisonnier chez les brebis de race Barbarine, nous avons été amenés à calculer les régressions entre les pourcentages de cycles ovariens de courte durée chez les femelles dont l'ovulation est induite par effet mâle au printemps et leur poids vif au moment du tarissement (environ 2 mois et demi avant la lutte) ainsi que les pourcentages de femelles non cycliques juste avant la stimulation. Dans ce but, les données relatives à 20 lots de brebis utilisés entre 1981 et 1989 totalisant un effectif de 315 individus sont analysées.

4.2. Résultats

La proportion de femelles anovulatoires au printemps est étroitement liée à leur état corporel au moment du tarissement au début du mois de mars. En effet, cette proportion est négativement corrélée au poids vif à ce stade physiologique \((R = -0,58; P < 0,05)\). La droite de régression liant ces deux variables est la suivante:

\[
Y = -2,57X + 178,46
\]

avec:

\[
Y = \text{proportion de brebis non cycliques}
\]

\[
X = \text{poids vif des brebis au moment du tarissement en kg}
\]

La qualité du corps jaune induit par effet mâle au printemps dépend également du poids vif des femelles stimulées. C'est ainsi que le coefficient de corrélation entre le pourcentage de cycles ovariens de courte durée (6 jours environ) et le poids vif
des femelles à la fin de la phase d'allaitement est de $-0.71 (P < 0.01)$. La droite de régression entre les deux critères est:

$$Y = -2.48X + 157.57$$

avec:

$Y = \text{pourcentage de cycles courts}$
$X = \text{poids vif des brebis au moment du tarissement en kg}$

Il existe également une liaison étroite entre le même pourcentage de cycles ovariens de courte durée et la proportion de femelles non cycliques au moment de l'introduction des mâles ($R = 0.49; P < 0.05$). L'équation de régression entre les deux critères en question est:

$$Y = 0.39X + 26.87$$

avec:

$Y = \text{pourcentage de cycles courts}$
$X = \text{pourcentage de brebis non cycliques}$

5. DISCUSSION

5.1. Induction de l'ovulation par effet mâle

La sous-alimentation sévère à long terme des brebis pendant la gestation et/ou pendant la lactation ne semble pas affecter leur réponse à l'effet mâle à condition de les suralimenter après le tarissement (expériences 1 et 2). En revanche, cette réponse, exprimée en pourcentages de brebis ovulant par effet mâle, diminue en cas de sous-alimentation entre le tarissement et l'introduction des béliers (groups II et LI de l'expérience 2).

Les faibles niveaux alimentaires seraient en mesure d'entraîner une déficience hypothalamique en GnRH chez les femelles [7, 8]. L'état réfractaire à l'effet mâle des brebis sous-alimentées pourrait également résulter d'une modification de leur sensibilité hypophysaire à l'action du GnRH [7].

5.2. Durée du premier cycle ovarien induit par effet mâle

Un faible poids vif au moment du tarissement (expérience 1) ou une sous-alimentation sévère avant et/ou après la mise bas entraîne une augmentation importante de la proportion de femelles dont le premier cycle ovarien induit par effet mâle

Nos résultats témoignent de l’existence d’une liaison étroite entre la proportion de cycles ovariens de courte durée et l’intensité de l’anœstrus saisonnier, ce dernier étant exprimé en termes de poids vifs ou de pourcentages de femelles anovulatoires avant la stimulation par les mâles. En effet, les coefficients de corrélation entre le premier critère et les deux seconds sont significatifs (−0,71 et 0,49 respectivement).

5.3. Intensité de l’anœstrus saisonnier

Chez les petits ruminants, la proportion de femelles spontanément cycliques au printemps peut traduire l’intensité de l’anœstrus saisonnier des brebis [11] et des chèvres [12]. La cyclicité ovarienne des brebis de race Barbarine à cette période de l’année dépend essentiellement de leur état corporel lors du tarissement au début du printemps. Cet état corporel traduit le poids vif des animaux (première expérience) qui résulte lui-même de leur niveau alimentaire pendant la gestation et/ou la période d’allaitement (deuxième expérience). C’est ainsi que les plus faibles pourcentages de brebis cycliques se rencontrent parmi les animaux les plus légers au moment du sevrage des agneaux et ceux qui ont souffert d’une sous-alimentation sévère avant et/ou après la parturition. Nos résultats montrent que la liaison entre l’intensité de l’anœstrus saisonnier, représentée par la proportion de femelles anovulatoires dans un groupe au moment de l’introduction des béliers, et le poids vif moyen du même groupe est étroite (\( R = -0.58; \ P < 0.05 \)).

Il est admis que les effets d’une sous-alimentation sévère et d’un amaigrissement excessif peuvent se traduire soit par un début tardif de la saison sexuelle [13], soit par une fin précoce de cette même saison [7, 14, 15].

5.4. Taux d’ovulation

Les résultats de l’expérience 1 montrent que le taux d’ovulation induite ne semble pas être affecté par le poids vif des brebis puisqu’il est strictement identique dans les groupes II et LL. De même, les groupes IL et MM ont des taux d’ovulation plus élevés que celui du groupe L1 alors que le poids vif moyen des trois groupes est identique au moment de l’introduction des mâles. Les résultats de l’expérience 2 suggèrent aussi que le taux d’ovulation est en relation avec l’évolution du poids vif avant le début de la lutte. La fréquence des ovulations multiples en race Barbarine dépend donc beaucoup plus de la variation du poids que du poids vif lui-même. Cette absence de l’effet dynamique du poids vif sur le taux d’ovulation des brebis est en contradiction avec les résultats de nombreux auteurs [16–20].
5.5. Fertilité et prolificité

Les effets statique et dynamique du poids vif des femelles de race Barbarine sur leur fertilité et leur prolificité sont identiques à ceux observés chez d'autres races ovines [21-25].

6. CONCLUSION

Les résultats de cette étude montrent que l'intensité de l'anœstrus saisonnier des femelles ovines est liée dans une large mesure à leur état corporel à la fin de la période d'allaitement au début du printemps. Cet état corporel dépend évidemment de leurs conditions alimentaires de part et d'autre de la parturition. Une sous-alimentation sévère à l'un de ces stades entraîne une détérioration des caractéristiques de reproduction à contre saison des brebis.

REFERENCES

DETERMINISME PHYSIOLOGIQUE DES VARIATIONS SAISONNIERES DE L'ACTIVITE OVARIENNE CHEZ LA BREBIS EN MILIEU SAHELIEN

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Abstract–Résumé

DETERMINISME PHYSIOLOGIQUE DES VARIATIONS SAISONNIERES DE L'ACTIVITE OVARIENNE CHEZ LA BREBIS EN MILIEU SAHELIEN.

Deux types d’anomalies de la fonction ovarienne limitent la fécondité de la brebis en milieu sahélien: (1) les corps jaunes persistants, (2) l’anœstrus saisonnier. Ces anomalies apparaissent chez 52% des animaux dans un troupeau; elles coïncident avec la période de l’année où l’amplitude thermique journalière est maximale (décembre à avril). Les animaux qui manifestent ces anomalies ont une plus grande variation de leur température rectale (respectivement 1,98°C ± 0,39 et 1,13°C ± 0,10), transpirent moins (2,4 mg·h⁻¹·cm⁻² et 3,7 mg·h⁻¹·cm⁻²), ont un faible rythme de sécrétion de l’hormone lutéinisante (LH) (1 pulse/4 h et 1 pulse/1,6 h), un niveau d’œstradiol 17β sécrété plus bas (3,6 ± 0,5 pg/mL et 11,5 ± 1,5 pg/mL) et un index mitotique de la granulosa plus faible (11,9 x 10⁻³ et 17,8 x 10⁻³) par rapport aux autres brebis qui sont cycliques. Ces résultats suggèrent qu’il y a une forte interaction entre les mécanismes qui contrôlent la fertilité et ceux qui assurent la régulation thermique chez la brebis au Niger et plus généralement chez les animaux soumis à un stress thermique important: le rythme endogène de sécrétion de LH qui gouverne l’ovulation est perturbé chez les animaux qui ont une mauvaise régulation thermique avec l’environnement pendant le cycle œstral.
1. INTRODUCTION

En milieu tropical sahélien, la réduction de la fécondité chez la brebis est due entre autres à l’apparition d’un anœstrus saisonnier et de corps jaunes persistants au moment de l’année où l’amplitude thermique journalière est la plus élevée (décembre à avril) [1, 2].

Cet anœstrus est caractérisé par des ovulation fréquemment silencieuses et irrégulières; le cycle lutéal devient court et la phase folliculaire longue. Les corps jaunes persistants sont associés à l’absence d’œstrus et d’ovulation à la présence de phase lutéales anormalement longues (figure 1). Ces deux types d’anomalies de l’activité ovarienne constituent probablement des formes d’adaptation de la brebis à son environnement climatique difficile. L’étude suivante a pour objectif de comprendre les mécanismes endocriniens mis en jeu dans le contrôle de la reproduction chez la brebis en milieu sahélien.

2. REGULATION INTERNE

2.1. Variations saisonnières des teneurs plasmaliques des hormones hypothalamiques et de leur rétrocontrôle

Les changements de la sécrétion tonique des gonadotropines déterminent les variations saisonnières de la fonction ovarienne chez la brebis. Les anomalies ovariennes qui apparaissent chez la brebis Peul sont-elles la conséquence d’une déficience de la sécrétion de prolactine, de l’hormone folliculo-stimulante (FSH), de l’hormone lutéinisante (LH)?

2.1.1. Variations circannuelles de la prolactine

La concentration moyenne mensuelle de la prolactine suit une variation saisonnière parallèle à la durée du jour et à la température [3]. Les concentrations maximales sont observées en avril/mai quand la durée du jour est la plus longue et la température moyenne est la plus élevée (figure 2). Les concentrations minimales coïncident avec les jours les plus courts et les températures les plus basses en déc./jan.

Ainsi donc, en période d’anœstrus et de corps jaunes persistants, les niveaux de prolactine sont faibles (déc./jan.) et élevés (février à avril); ceci montre que l’apparition des anomalies ovariennes est relativement indépendante du niveau de sécrétion de la prolactine.
FIG. 1. Evolution du niveau de progestérone: a) au cours de deux cycles œstraux normaux, b) au cours d'un cycle à phase lutéale prolongée, c) au cours de deux allongements de l'intervalle entre deux phases lutéales ($J_0 = \text{œstrus}$).
2.1.2. Variations circannuelles de LH

Contrairement à la prolactine, LH n’est pas sécrétée de façon continue mais sous forme pulsatile [4]. Chez la brebis Peul, l’amplitude de la durée des pulses de LH ne changent pas au cours de l’année: seule la fréquence varie (tableau I): elle est 2,5 fois supérieure en saison sexuelle qu’en période d’arrêt de l’œstrus et de l’ovulation. Mais le rythme de sécrétion de LH est dépendant de l’activité intrinsèque et de la sensibilité du complexe hypothalamo-hypophysaire aux stéroïdes ovariens [5]. Y a-t-il alors une variation saisonnière de ces paramètres hypothalamo-hypophysaires qui contrôlent la libération de LH?

Activité intrinsèque et sensibilité à l’œstradiol du complexe hypothalamo-hypophysaire

L’activité intrinsèque de complexes est mise en évidence chez l’animal ovariectomisé [6]. Il y a baisse de fréquence d’apparition des pulses et de LH sans
modification d'amplitude, ni de durée en période d'anomalies ovariennes par rapport à la saison sexuelle (tableau I).

La sensibilité du complexe hypothalamo-hypophysaire aux stéroïdes ovariens est appréciée à travers les effets de la castration et de l'injection d'œstradiol sur le niveau de LH. L'élévation relative du niveau de LH après ovariectomie, l'intervalle entre le moment de la castration et l'augmentation significative du niveau de LH suivant l'ovariectomie [5, 7], la diminution du niveau de LH après injection d'œstrogène à des animaux castrés et l'intervalle entre le moment de l'injection et le début d'une décroissance significative de LH [8] sont les critères d'évaluation de la sensibilité du complexe hypothalamo-hypophysaire aux stéroïdes ovariens.

Ces paramètres ne subissent pas de variation saisonnière significative chez la brebis Peul (tableau II). Ceci suggère que la sensibilité du complexe hypothalamo-hypophysaire aux stéroïdes ovariens ne change pas suivant les périodes favorables et défavorables de l'activité sexuelle. Ainsi donc, contrairement aux brebis de race européennes [7, 8], on n'observe pas d'augmentation de l'insensibilité du rétrocontrôle du système hypothalamo-hypophysaire aux œstrogènes chez la brebis Peul en période d'arrêt de l'œstrus et de l'ovulation.

TABLEAU I. VARIATIONS SAISONNIERES DE LA PULSATILITE DE LH CHEZ LES BREBIS NORMALES ET CASTREES DE RACE PEUL AU NIGER

<table>
<thead>
<tr>
<th>Traitement et mois</th>
<th>Pulse</th>
<th>Amplitude (ng/mL)</th>
<th>Durée (min)</th>
<th>Fréquence (pulse/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brebis normale</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septembre (saison sexuelle)</td>
<td></td>
<td>3,2 ± 0,1</td>
<td>64,0 ± 5,1</td>
<td>1/1,6</td>
</tr>
<tr>
<td>Mars (arrêt œstrus et ovulation)</td>
<td></td>
<td>3,2 ± 0,1</td>
<td>65,0 ± 15,0</td>
<td>1/4,0</td>
</tr>
<tr>
<td>Brebis castrée</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septembre (saison sexuelle)</td>
<td></td>
<td>5,5 ± 0,3</td>
<td>62,5 ± 4,8</td>
<td>1/1,3</td>
</tr>
<tr>
<td>Mars (arrêt œstrus et ovulation)</td>
<td></td>
<td>5,6 ± 0,2</td>
<td>57,2 ± 4,8</td>
<td>1/2,0</td>
</tr>
</tbody>
</table>
TABLEAU II. EFFETS DE LA CASTRATION ET DE L'INJECTION D'ŒSTRADIOL SUR LES PARAMETRES DE SECRETION DE LH ET DE FSH

<table>
<thead>
<tr>
<th>Paramètre</th>
<th>Novembre (saison sexuelle)</th>
<th>Février/mars (arrêt ovulation et œstrus)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH</td>
<td>FSH</td>
</tr>
<tr>
<td><strong>Castration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niveau avant castration (ng/mL)</td>
<td>0,91 ± 0,15</td>
<td>4,7 ± 0,7</td>
</tr>
<tr>
<td>Niveau après castration (ng/mL)</td>
<td>1,64 ± 0,4</td>
<td>8,2 ± 1,1</td>
</tr>
<tr>
<td>Taux accroissement (%)</td>
<td>80</td>
<td>74</td>
</tr>
<tr>
<td>Intervalle castration</td>
<td>12,85 ± 3,1</td>
<td>17,4 ± 7,4</td>
</tr>
<tr>
<td>augmentation hormone (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Injection œstrogène</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niveau avant injection (ng/mL)</td>
<td>4,1 ± 0,3</td>
<td>47,7 ± 10,9</td>
</tr>
<tr>
<td>Niveau après injection (ng/mL)</td>
<td>2,3 ± 0,2</td>
<td>20,9 ± 0,03</td>
</tr>
<tr>
<td>Taux de diminution (%)</td>
<td>-44</td>
<td>-56</td>
</tr>
<tr>
<td>Intervalle injection</td>
<td>3,9 ± 0,6</td>
<td>4,0 ± 0,2</td>
</tr>
<tr>
<td>chute hormone (h)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.1.3. Variations interannuelles de FSH

Les concentrations circadiennes de FSH sont les plus basses à 20 h et les plus élevées à 24 h (figure 3). La concentration de FSH est à tout moment supérieure en période d’anomalies ovariennes à ce qu’elle est en saison sexuelle. Cette élévation du niveau de FSH est due probablement à une modification de l’activité intrinsèque et/ou de la sensibilité du complexe hypothalamo-hypophysaire aux stéroïdes ovariens [9].
Activité intrinsèque et sensibilité à l’œstradiol du complexe hypothalamo-hypophysaire

Il n’y a pas de variation saisonnière significative ($P > 0,05$) du niveau de FSH au cours de l’année chez la brebis Peul castrée ($19,6 \pm 0,6$ ng/mL en saison sexuelle et $18,5 \pm 0,5$ ng/mL en période d’anomalies ovariennes). La libération de FSH est en partie sous le contrôle du GnRH hypothalamique mais certaines cellules hypophysaires sont capables de libérer FSH même après déconnexion de l’hypothalamus [9, 10]. On peut penser que les systèmes qui contrôlent la libération hypophysaire de FSH ne subissent pas de variation saisonnière dans leur fonctionnement chez la brebis Peul. En utilisant les mêmes critères d’évaluation de la sensibilité du complexe hypothalamo-hypophysaire aux stéroïdes que dans le cas de LH, on n’observe pas de variation saisonnière du rétrocontrôle des stéroïdes ovariens sur la libération de FSH (tableau II). La libération de FSH par l’hypophyse est également influencée par l’inhibine d’origine folliculaire [11]; or, il n’y a pas de variation saisonnière de la capacité de l’inhibine à réduire le niveau de FSH chez la brebis [12]. Il est probable que l’élévation du niveau de FSH en période d’anestrus chez la brebis Peul est due à une faible sécrétion d’œstrogène (et d’inhibine) d’origine folliculaire.

**FIG. 3.** Evolution circadienne du niveau de base moyen de FSH en saison sexuelle (juin) et en période d’arrêt de l’aestrus et de l’ovulation (février).
2.1.4. Conclusion

Il existe chez la brebis Peul en zone sahélienne un rythme saisonnier des sécrétions de prolactine, LH et FSH. L'évolution circannuelle de la prolactine suit les variations de la photopériode, et celle de LH est contemporaine de l'augmentation de l'amplitude thermique journalière. La libération de ces hormones hypophysaires intervenant dans la reproduction semble dépendre directement des facteurs climatiques. Le rythme saisonnier de libération de FSH est influencé par un rythme saisonnier de sécrétion d'œstrogène (et d'inhibine) d'origine folliculaire. La production d'œstrogène est elle-même soumis au contrôle de LH.

L'activité sexuelle chez la brebis Peul, vivant sous le climat sahélien, est donc contrôlée par le rythme endogène de sécrétion de LH.

2.2. Evolution des hormones ovariennes

2.2.1. Progestérone

Les corps jaunes provenant d'ovulations en période d'anœstrus secrètent peu de progestérone par rapport aux corps jaunes des cycles œstraux normaux (respectivement 1,7 ± 0,1 ng/mL et 2,4 ± 0,2 ng/mL). Cette faible sécrétion de progestérone est probablement associée à un soutien insuffisant du corps jaune par les gonadotropines [13, 14]; en effet, nous avons observé chez la brebis Peul une baisse du niveau et du rythme de sécrétion de LH en période où apparaissent ces anomalies ovariennes.

2.2.2. Oestradiol 17β

Le niveau moyen d'œstradiol 17β est plus faible pendant une phase lutéale prolongée que lors d'une phase lutéale normale ou d'une phase folliculaire longue (respectivement 2,4 ± 0,4 pg/mL; 3,8 ± 2,4 pg/mL et 4,9 ± 0,8 pg/mL). L'activité œstrogénique des follicules ovariens est donc la plus faible lors d'une phase lutéale prolongée; le niveau d'œstradiol 17β est alors comparable à celui d'une brebis dont l'ovaire a été irradié aux rayons X [15]. Ce faible niveau d'œstradiol ne permet pas aux prostaglandines F2α d'être lutéolytiques chez la brebis [15].

2.3. Croissance folliculaire

2.3.1. Croissance folliculaire au cours de l'anœstrus

La comparaison des effectifs folliculaires (normaux et atrétiques) montre deux types de réponse:
— l’anœstrus est caractérisé par des effectifs folliculaires comparables à ceux observés pendant la saison sexuelle, mais la taille moyenne des follicules est plus faible qu’en saison sexuelle (1,75 ± 0,11 mm contre 1,29 ± 0,09 mm);
— l’anœstrus est marqué par des follicules atrétiques plus nombreux qu’en saison sexuelle; la taille moyenne des petits follicules en croissance (diamètre < 0,82 mm) n’est pas différente de manière significative entre saison sexuelle et anœstrus; par contre, la taille moyenne des gros follicules (diamètre > 0,82 mm) est plus grande en saison sexuelle (1,7 ± 0,10 mm) qu’en anœstrus (1,54 ± 0,17 mm).

La vitesse de croissance des follicules est significativement plus grande en saison sexuelle qu’en période d’anœstrus (respectivement 17,8 x 10^{-3} et 11,9 x 10^{-3}). Par contre, la croissance de l’antrum n’est pas modifiée selon la saison (73 ± 2 et 71 ±4 respectivement).

2.3.2. Croissance folliculaire au cours d’un dioœstrus prolongé

La taille moyenne du plus gros follicule mesuré après un corps jaune persistant de 23 jours est de 1,30 ± 0,11 mm, alors que, pour un cycle à phase folliculaire prolongée, la taille est de 3,15 ± 0,48 mm et, pour un cycle normal, elle est égale à 3,97 ± 0,30 mm.

L’absence de follicules de grande taille est due probablement à un ralentissement de la vitesse de croissance puisque l’importance numérique de l’atrésie ne change pas de manière significative au cour d’un dioœstrus normal ou prolongé (respectivement 21% et 18%).

3. REGULATION EXTERNE PAR LA TEMPERATURE

3.1. Evolution circadienne de la température rectale

Un enregistrement toutes les heures pendant 24 h de la température rectale a été effectué sur des brebis cycliques et celles présentant des anomalies ovariennes, en décembre où l’amplitude thermique journalière est importante (15°C). Les animaux qui manifestent ces anomalies ont une plus grande variation circadienne de leur température rectale que les brebis cycliques (respectivement 1,98°C ± 0,39 et 1,13°C ± 0,10). La variation circadienne normale de la température rectale chez la brebis est de 0,8°C en moyenne [16].

3.2. Activité des glandes sudoripares

L’activité des glandes sudoripares des brebis cycliques et de celles présentant des arrêts de l’ovulation et de l’œstrus a été appréciée au moment le plus chaud de la journée, entre 13 et 19 h, à l’aide de capsules déshydratantes. A partir des résultats
obtenus, il apparaît que les animaux cycliques transpirent plus que ceux qui manifestent une anomalie ovarienne (respectivement 3,7 mg·h⁻¹·cm⁻² et 2,4 mg·h⁻¹·cm⁻²).

La transpiration est un des mécanismes essentiels de lutte contre la chaleur chez la brebis [17–19]. Tout se passe comme si les animaux cycliques ont besoin d'accroître leur transpiration pour maintenir la variation de leur température corporelle dans les limites normales.

4. MECANISME D'INDUCTION D'UN CORPS JAUNE PERSISTANT ET D'UNE PHASE FOLLICULAIRE PROLONGEE

La chute du niveau de LH est consécutive à une élévation anormale de la température rectale des brebis en réponse à une augmentation de l'amplitude thermique journalière. Ceci a déjà été montré par d'autres auteurs chez la brebis [16, 18, 20] et la vache [21]. Cette action de la température sur la libération de LH est assurée probablement par des neurotransmetteurs comme la noradrénaline ou l'acide gamma aminobutyrique [18, 22].

La diminution de la sécrétion pulsatile de LH provoque un ralentissement de la vitesse de croissance des follicules et/ou l'atresie des gros follicules en phase de croissance terminale; elle a également pour conséquence une baisse du niveau circulant de l'œstradiol d'origine folliculaire. Il a été montré que le niveau circulant de ce stéroïde influence l'action lutéolytique des prostaglandines sur le corps jaune [15, 23, 24] en modifiant le rapport PGF/PGE [14, 25, 26].

Le mécanisme endocrinien suivant peut alors être proposé (figure 4). L'apparition d'un corps jaune persistant ou d'une phase folliculaire prolongée dépend probablement du niveau d'œstradiol sécrété par l'ovaire. Si la baisse du niveau de l'œstradiol consécutive à la diminution de LH est:

— peu importante, mais insuffisante pour provoquer une décharge préovulatoire de LH, elle induira une phase folliculaire prolongée;
— plus importante en inhibant la sécrétion des prostaglandines F2, elle aboutit à la formation d'un corps jaune persistant.

![Figure 4](image_url)
5. CONCLUSION

Chez la brebis Peul, il existe des individus plus ou moins sensibles au climat sahélien. Les animaux les plus sensibles réagissent aux variations circadiennes de la température ambiante par un accroissement de leur température rectale et une transpiration cutanée modérée par rapport aux autres brebis. Ces deux types de réaction se traduisent normalement par une économie de la dépense d’énergie dans la lutte contre le chaud [18]. Ceci peut être considéré comme une forme d’adaptation de ces animaux à un environnement écologique difficile caractérisé par un manque chronique d’eau et de ressources alimentaires.

Dans le domaine de la reproduction, la réaction des animaux à ces variations de température se traduit par une baisse momentanée de la fécondité, donc des accouplements (décembre à avril) dès lors que ces accouplements auraient pour conséquence de faire naître les jeunes à un moment de l’année peu favorable à leur survie. Ceci est vérifié par des faits expérimentaux rapportés par Haumesser et Gerbaldi [27] qui montrent que la fréquence maximale des agnelages naturels chez la brebis Peul se situe en septembre/octobre, donc en période d’abondance des pâturages.

On peut donc suggérer que la sélection des animaux sensibles aux variations circadiennes de la température ambiante peut être une des solutions pratiques qui permettront l’adéquation entre la production d’agneaux et les disponibilités alimentaires au Sahel. Ceci constitue également une forme d’adaptation de la brebis Peul à son milieu.

REFERENCES


CONTROL AND MANIPULATION OF FACTORS AFFECTING SMALL RUMINANT REPRODUCTIVE PERFORMANCE IN TROPICAL CONDITIONS.

In tropical areas, reproductive efficiency in small ruminants is affected by various factors, including season, rainfall, male effect, nutritional state or corporal condition (CC) and physiological factors such as post-partum interval, milk production, lactational stage and suckling. Animals with good CC show early puberty, shorter anoestrous periods and better response to male effect and synchronized treatment than animals with low CC; reproductive efficiency is increased by 33% in sheep with CC over 2 (on a scale of 0 to 5) and lambing is 20% lower in the dry than in the rainy season. These data suggest that seasonal sexual inhibition is not too deep in a tropical environment. The response to male effect is good in the different seasons but in animals with low CC this effect is not enough to compensate for the negative influence of undernutrition. Hormonal treatments are effective if animals have adequate CC but the cost is too high. In animals with low CC these treatments must be combined with nutritional flushing. Male introduction at the beginning or 48 h before the end of treatment improves efficiency. Oestrus-ovulation dissociation is observed in tropical sheep after hormonal synchronization and male introduction (9.3%); this is higher in animals with low CC (11.9 vs. 6.8%). Embryo mortality in synchronized goats was affected by nutritional state, being twice as frequent in goats with low CC (26.3 vs. 11.5%). In tropical conditions, appropriate use of male effect and CC can improve reproductive performance by strategic programming of breeding and parturition during the season with better conditions and pasture availability.
anestro más cortos y una mejor respuesta al efecto macho y al tratamiento sincronizado que los animales con baja CC superior a 2 (en una escala de 0 a 5) y la parición es un 20% más baja en la temporada seca que en la de lluvia. Estos datos parecen indicar que la inhibición sexual estacional no es muy marcada en un medio tropical. La respuesta al efecto macho es buena en las diferentes temporadas pero en animales con baja CC este efecto no basta para compensar la influencia negativa de la desnutrición. Los tratamientos hormonales son eficaces si los animales tienen una CC adecuada pero el costo es demasiado alto. En animales con baja CC estos tratamientos deben combinarse con suplementación nutricional. La introducción del macho al inicio o 48 horas antes del final del tratamiento mejora la eficiencia. En ovejas tropicales se observa falta de ovulación en el celo después de la sincronización hormonal e introducción del macho (9,3%); este procentaje es más elevado en animales con baja CC (11,9% frente a 6,8%). La mortalidad embrional en cabras sincronizadas es afectada por la condición nutricional, siendo dos veces más frecuente en cabras con baja CC (26,3% frente a 11,5%). En condiciones tropicales, la utilización adecuada del efecto macho y la CC pueden mejorar el rendimiento reproductivo mediante la programación estratégica de las épocas de cubrición y parto en la estación que ofrezca mejores condiciones y disponibilidad de pastos.

1. INTRODUCCION

Las regiones tropicales caracterizan un complejo de situaciones ambientales, culturales y socio-económicas que influyen en el desarrollo de programas de control y mejora de los pequeños rumiantes locales. Los ovejas y cabras criollas no forman una raza definida, pero constituyen una inmensa reserva de recursos genéticos debido a su rusticidad y adaptación a las zonas áridas y semiáridas. Entre el 80 y el 95% de estos animales se encuentra en explotaciones extensivas de manejo tradicional bajo pastoreo, sin suplemento y con presencia permanente del macho. Poseen una baja, aunque singular capacidad productiva de leche y carne, cuyo potencial no ha sido explotado técnicamente; sólo se ordeña el 40% de las cabras en un 45% de las fincas, con una producción de 200 a 400 g/d de leche durante 120 a 150 d [1].

El manejo reproductivo busca el incremento de la productividad y la disminución de los períodos improductivos, al ajustar la estacionalidad sexual y el comportamiento reproductivo al control de los factores que los regulan. Algunos factores básicamente ambientales, como la duración del día, la temperatura y las precipitaciones, son difíciles de controlar en el medio tropical; otros, como el amamantamiento, la presencia del macho y la alimentación, son factibles de ser modificados aunque, al actuar muchas veces interrelacionados, complican su manejo. Estos factores afectan a estadios reproductivos tales como pubertad, estacionalidad y ciclicidad ovárica, gestación y parto, fertilidad y prolificidad y pérdida embrionaria y neonatal [1].
Las hembras tropicales tienen gran capacidad de respuesta al efecto macho en distintas épocas [2–4]. Su introducción en los rebaños permite una rápida reanudación de la actividad ovárica y sexual de casi la totalidad de animales, siempre que se encuentren en buen estado nutricional [5]. La alimentación constituye el principal factor modulador del comportamiento reproductivo de ovejas y cabras tropicales que poseen habitualmente una limitada, discontinua y desequilibrada disponibilidad de alimentos. La variación estacional de las lluvias y pastos es la que decidirá básicamente los cambios del estado nutricional y de las reservas grasas y energéticas [5], los cuales pueden ser evaluados por la condición corporal (CC). La CC se califica desde 0 (muy delgado) hasta excepcionalmente 5 (muy graso), por palpación a nivel lumbar y esternal [6] y en períodos estratégicos como pubertad, preparto, parto, servicio o al iniciar tratamientos de sincronización. Una disminución de las reservas y de la CC afectará a las funciones productivas y reproductivas; los animales con pobre CC aumentan sus reservas al igual que su actividad sexual al empezar las lluvias y crecer los pastos, o al ser sometidos a una suplementación alimenticia [1, 4].

Dado que todos los estadios fisiológicos reproductivos, al igual que las respuestas a las técnicas de control y manejo de la función reproductiva de los pequeños rumiantes, dependen principalmente de la alimentación, del estado corporal y de la presencia del macho, este trabajo destacará esa importante relación y su aplicación para la mejora del comportamiento y eficiencia reproductivos de las ovejas y cabras de los trópicos americanos.

2. SIGNIFICADO Y CONTROL DE LA CONDICION CORPORAL Y DEL “EFECTO MACHO”

2.1. Pubertad

Un crecimiento atrasado debido a condiciones de manejo deficientes y prolongadas, especialmente de tipo nutricional, ocasiona una baja CC y un retraso de la pubertad (Cuadro I). Según la época, la deficiencia será compensada por la presencia de pastos, y de ahí viene el importante papel de las lluvias en el inicio de la pubertad. La edad de pubertad de las ovejas varía entre 286 y 410 d, con un peso de 21 kg (40–60% del peso adulto) [7]; la de las borregas West African (WA) y Barbados Barriga Negra, entre 239 y 412 d, con pesos de 19,4 y 19,2 kg, cuya diferencia se debe más a efectos nutricionales que raciales. Para cabritonas criollas fue de 371 d y 26,8 kg [7]. Para los pequeños rumiantes tropicales se recomienda un peso al servicio de 24 kg (65–75% del peso adulto), el cual puede lograrse en 11 o 12 meses [1] o incluso desde 6 a 8 meses, para alcanzar un 93% de celos antes de los 11 meses [8].
CUADRO I. EFECTO DE LA SUPLEMENTACION A PARTIR DEL DESTETE O PUBERTAD Y DE LA CONDICION CORPORAL SOBRE LA PUBERTAD Y LA EFICIENCIA REPRODUCTIVA DE BORREGAS WEST AFRICAN EN UNA ZONA TROPICAL DE VENEZUELA [13]

<table>
<thead>
<tr>
<th>Momento del pastoreo + suplemento</th>
<th>Pubertad</th>
<th>Fertilidad</th>
<th>Prolificabilidad</th>
<th>1er parto</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N°.</td>
<td>Edad (días)</td>
<td>Peso (kg)</td>
<td>(%)</td>
</tr>
<tr>
<td>Destete</td>
<td>24</td>
<td>259&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24</td>
<td>87,5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pubertad</td>
<td>26</td>
<td>321&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23</td>
<td>80,7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Condición corporal**

<table>
<thead>
<tr>
<th></th>
<th>N°.</th>
<th>Edad (días)</th>
<th>Peso (kg)</th>
<th>(%)</th>
<th>(crías/parto)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>13</td>
<td>301&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21</td>
<td>76,9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-3</td>
<td>20</td>
<td>280&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24</td>
<td>85,0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>&gt;3</td>
<td>17</td>
<td>295&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24</td>
<td>88,2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Promedios                          | 50       | 291        | 23     | 84,1    | 1,19          | 42  | 460           |

<sup>a-b</sup> Cifras con literal o exponente diferente varían significativamente (P < 0,05).

Los intentos para adelantar la pubertad de las borregas tropicales pueden estar afectados por una baja tasa de crecimiento, la cual es posible superar con un suplemento nutricional que proporcione la energía necesaria para el desarrollo corporal y lograr el peso crítico de pubertad 60 a 120 d antes, o incluso reducir la edad en un 12%, o entre el 14 y el 27% en explotaciones extensivas, o con pastizales mejorados de acuerdo a la época [8-10]. Un programa de alimentación a partir de la pubertad puede compensar el efecto de una pubertad atrasada. Parecería que la subalimentación prepuberal temporal no tiene excesivas consecuencias sobre el posterior comportamiento reproductivo de las ovejas tropicales [11].

La presencia permanente del macho en rebaños caprinos ocasiona la aparición de partos tempranos (al año de edad, en lugar de la media de 18 meses), mientras que la introducción de machos en rebaños libres adelanta la primera ovulación de cabritonas de crecimiento normal o atrasado [1], como se ha señalado en corderas manchegas [12] o pelibuey, a las cuales el macho logra inducir celos fértiles en un 89,2% de los ciclos, 18 a 27 días después [4]. Se destaca el efecto beneficioso de la CC y del peso, al lograrse los mejores resultados con borregas puberales que pesan más de 24 kg [4]. Los tratamientos hormonales de progesterona o progestágenos adicionados o no de PMSG han permitido inducir y adelantar la reproducción de animales puberales que han alcanzado el peso y la CC adecuados [13, 14].
Es frecuente la disociación celo-ovulación de las cabritonas y borregas tropicales, registrándose elevada la tasa de ovulaciones silenciosas, celos anovulatorios y ciclos cortos al inicio de la pubertad [7], como se ha señalado para ovejas javanesas [15] y para cabras criollas en Guadalupe [16].

2.2. Estacionalidad y épocas de servicio

La programación estratégica de las mejores épocas para el nacimiento y supervivencia de las crías debe considerar las variaciones estacionales en la frecuencia y magnitud de los celos, al igual que sus efectos sobre la fertilidad y prolificidad. El concepto de estacionalidad es relativo, pues aun las razas más estacionales presentan alguna actividad fuera de las épocas sexuales [1, 17]. En explotaciones con habitual buen manejo y alimentación se observa actividad ovárica con ovulaciones y fecundaciones a través del año [16–19], detectada por los niveles de progesterona y observación ovárica. No obstante, siempre se señalan dos o tres épocas anuales de mayor actividad sexual y fertilidad que les diferencian de la clásica estacionalidad fotoperiódica de ovejas [11, 18] y cabras [17, 19, 20]. En ambientes tropicales, esa aparente desestacionalidad, variable en su inicio y duración entre rebaños próximos y aun dentro del mismo rebaño en distintos años, sería causal de las épocas de partos y de la amplia dispersión del intervalo entre partos, que fluctúa entre 6 y 12 meses [1].

CUADRO II. COMPORTAMIENTO Y EFICIENCIA REPRODUCTIVA SEGÚN LA CONDICION CORPORAL DE OVEJAS TROPICALES EN EL MOMENTO DE SU INCORPORACION AL SERVICIO NATURAL [5]

<table>
<thead>
<tr>
<th>Condición corporal (0/5)</th>
<th>Nº de ovejas</th>
<th>Exhibición del celo (%)</th>
<th>Fertilidad (F) (1° Serv.) (%)</th>
<th>Prolificidad (P) (1° Serv.) (crías/parto)</th>
<th>Fecundidad (F × P) (%)</th>
<th>Eficiencia condición corporal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>7</td>
<td>71,4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60,0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,33</td>
<td>79,8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57,0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1–2</td>
<td>14</td>
<td>92,9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61,5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,25</td>
<td>76,9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71,4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2–3</td>
<td>19</td>
<td>100,0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68,4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,23</td>
<td>84,1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>84,1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>&gt;3</td>
<td>11</td>
<td>90,9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70,0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,29</td>
<td>90,3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82,1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Promedios</td>
<td>51</td>
<td>92,2</td>
<td>66,0</td>
<td>1,26</td>
<td>83,2</td>
<td>76,7</td>
</tr>
</tbody>
</table>

<sup>a–b</sup> Cifras con literal diferente varían significamente (P < 0,05).
CUADRO III. EFECTO MACHO EN CABRAS CRIOLLAS: INFLUENCIA DE LA EPOCA DEL AÑO Y DE LA CONDICION CORPORAL SOBRE LA INDUCCION DEL CELO Y EFICIENCIA REPRODUCTIVA

(20 cabras por tratamiento en dos rebaños libres del macho durante 2 meses) [23]

<table>
<thead>
<tr>
<th>Epoca reproductiva</th>
<th>Condición corporal (Prom.)</th>
<th>Inducción del celo (%)</th>
<th>Fertilidad (%)</th>
<th>Prolificidad (crías/parto)</th>
<th>Eficiencia del efecto macho (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-8 d</td>
<td>1-30 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inicio época seca (enero-febrero)</td>
<td>1,7</td>
<td>10</td>
<td>29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>1,00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epoca seca (marzo-abril)</td>
<td>1,5</td>
<td>20</td>
<td>50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epoca sexual menor (mayo-junio)</td>
<td>2,5</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Testigos</td>
<td>2,6</td>
<td>15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epoca sexual mayor (septiembre-octubre)</td>
<td>3,1</td>
<td>75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Testigos</td>
<td>3,0</td>
<td>30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>-</sup><sup>c</sup> Cifras con estos literales varían significativamente (P < 0,05).
<sup>a</sup><sup>-</sup><sup>c</sup> Cifras con estos literales varían de forma altamente significativa (P < 0,01).

Luego de un período de escasez de pastos, que ocasiona lapsos de anestro de profundidad (% de hembras que ciclan espontáneamente) y duración variables, es la alta dependencia de la actividad sexual estacional de la aparición de las lluvias la que modulará el efecto de la mejora nutricional y del estado corporal de ovejas y cabras tropicales [5, 11, 17, 20], aunque debe reconocerse que la mayor parte de los trabajos que concluyen esa idea no vienen acompañados de estudios sobre disponibilidad, calidad y digestibilidad de los pastos. Se ha señalado una alta correlación entre los celos y las lluvias del mes que precede a la fecundación en cabras bien alimentadas [21] o más celos en ovejas durante las estaciones de lluvia y prelluvia [22].

La condición corporal en el momento de la incorporación al servicio de ovejas tropicales influencia el comportamiento y eficiencia reproductivos; se encuentran disminuídos en animales con CC ≤ 2, pero las respuestas son más elevadas en ovejas con una CC ≥ 2 ó 3, al lograr una fecundidad del 84 y 90% [5] (Cuadro II).
En rebaños tradicionales, al estar ambos sexos en contacto permanente, la percepción y el comportamiento sexual de las cabras resultan afectados. Aparentemente, se induce un estado refractario de la actividad sexual que favorece la acción de otros factores ambientales, como la alimentación sobre la agrupación estacional de los celos [1]. En rebaños con buena CC, la introducción del macho induce una rápida y sincrónica reanudación del celo y ovulación [5]. Es posible que la diferente CC promedio de dos rebaños con 2,4 y 3,3 influya en la respuesta al efecto macho, ya que se obtiene una diferente y significativa eficiencia del 45 y 85% [23]. El macho en rebaños con mala CC y escasa ciclicidad parece inducir una mayor frecuencia de primeras ovulaciones deficientes y cuerpos lúteos débiles de corta actividad, que parece ser poco importante con animales tropicales [7, 14, 21]; estos ciclos cortos son seguidos de un celo normal, incluso 25 a 26 días después de la introducción del macho en rebaños de cabras criollas durante la época seca [2].

CUADRO IV. EFECTO MACHO EN OVEJAS WEST AFRICAN EN LA ZONA TROPICAL: INFLUENCIA DE LA EPOCA Y DE LA CONDICION CORPORAL SOBRE LA INDUCCION DEL CELO Y LA EFICIENCIA REPRODUCTIVA (65-100 días posparto) [5]

<table>
<thead>
<tr>
<th>Epoca del año</th>
<th>Condición corporal</th>
<th>N° de observ.</th>
<th>Exhibición de celos (%)</th>
<th>Fertilidad (crías/parto)</th>
<th>Prolificidad (%)</th>
<th>fecundidad (%)</th>
<th>Eficiencia efecto macho (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seca</td>
<td>&lt;1</td>
<td>12</td>
<td>33,3a</td>
<td>50,0a</td>
<td>1,00</td>
<td>16,7a</td>
<td>16,7a</td>
</tr>
<tr>
<td>(dic.-feb.)</td>
<td>1-2</td>
<td>18</td>
<td>55,5a</td>
<td>70,0b</td>
<td>1,29</td>
<td>38,9a</td>
<td>50,1a</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>13</td>
<td>84,6b</td>
<td>72,7b</td>
<td>1,50</td>
<td>61,5b</td>
<td>99,3b</td>
</tr>
<tr>
<td></td>
<td>&gt;3</td>
<td>9</td>
<td>88,9b</td>
<td>75,0b</td>
<td>1,10</td>
<td>66,7b</td>
<td>100,0b</td>
</tr>
<tr>
<td>Promedios</td>
<td>52</td>
<td></td>
<td>63,5*</td>
<td>69,7*</td>
<td>1,39*</td>
<td>44,3*</td>
<td>61,5**</td>
</tr>
<tr>
<td>Prelluvia</td>
<td>&lt;1</td>
<td>7</td>
<td>42,9a</td>
<td>66,7a</td>
<td>1,00</td>
<td>28,6a</td>
<td>28,6a</td>
</tr>
<tr>
<td>(abr.-may.)</td>
<td>1-2</td>
<td>16</td>
<td>62,5ab</td>
<td>70,0a</td>
<td>1,43</td>
<td>43,8ab</td>
<td>62,6*</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>20</td>
<td>90,0b</td>
<td>83,3b</td>
<td>1,67</td>
<td>75,0b</td>
<td>125,2b</td>
</tr>
<tr>
<td></td>
<td>&gt;3</td>
<td>15</td>
<td>86,7b</td>
<td>84,6b</td>
<td>1,64</td>
<td>73,3b</td>
<td>120,3b</td>
</tr>
<tr>
<td>Promedios</td>
<td>58</td>
<td></td>
<td>75,9*</td>
<td>79,5*</td>
<td>1,57*</td>
<td>60,3*</td>
<td>94,7**</td>
</tr>
</tbody>
</table>

a-b. * Cifras con estos literales varían significativamente (P < 0,05).
** Cifras con estos literales varían de forma altamente significativa (P < 0,01).
Un pobre estado nutricional podría interferir la respuesta al efecto macho [24], el cual no sería estimulo suficiente para compensar una prolongada subalimentación, en especial durante la época seca o de anestro más profundo [23]. Rebaños con bajas CC (medias de 1,7 ó 1,5) sólo exhiben respuesta al macho en el 35 y 50% de los casos, mientras que a partir del inicio de las lluvias (con CC de 2,5 y 3) la respuesta resulta más rápida y eficiente (Cuadro III). En la estación principal, con óptima CC, la inducción del celo y la eficiencia reproductiva por el efecto macho son mayores que las observadas en los testigos sin introducción de machos (115% frente a 90%) [23].

En forma similar, ovejas WA con baja CC manifestaron una pobre respuesta de celo luego de 30 días de introducción de los machos [5]; la respuesta se eleva del 33% para CC de 1 hasta el 85 y 89% para CC superiores a 2 y 3, para obtener una eficiencia del efecto macho del 100% frente al 17% y del 120% frente al 19% en ovejas con pobre CC en época seca y de prelluvia, respectivamente (Cuadro IV). Estos datos parecen señalar una inhibición estacional de la actividad sexual, más o menos profunda [2, 3]; la suplementación previa a los servicios de ovejas pelibuey mejora la CC y la respuesta al efecto macho [4]. Es posible que las malas condiciones de alimentación bloqueen las descargas hipofisarias, ya que mantienen una mayor sensibilidad el efecto “feedback” de los esteroides.

2.3. Posparto

Después del parto, los pequeños rumiantes tropicales entran en anestro, y los perfiles de progesterona han permitido conocer los períodos de inactividad, transición y reinicio de la cíclicidad ovárica [7, 16, 18]. La duración del anestro posparto depende del manejo nutricional previo y de la CC al parto y está regulado tanto por la época, el estadio de lactación y las crías amamantando como por la presencia del macho [11, 25].

En ovejas como en cabras tropicales no suplementadas, se aprecia el efecto de una CC inadecuada al parto [5], al alargar significativamente el período de inactividad de 48 a 68 d en ovejas y de 56 a 92 d en cabras, cuando se comparan CC de 1 contra 2-3, a la vez que disminuye la fertilidad y prolificidad (Cuadro V). Mientras no se recupere el peso y la CC, se mantiene el anestro en la mayoría de las hembras, lo que demuestra el papel decisivo del estado nutricional en el reinicio de la cíclicidad posparto de las hembras tropicales.

Una consecuencia de la pobre CC al parto serían los problemas de supervivencia de las crías, debido a los bajos pesos al nacer y a una tasa menor de crecimiento; el desarrollo fetal prioritario se abastece a partir de las reservas corporales de la madre, lo que ocasiona un mayor deterioro del peso y de la CC, a la vez que deteriora el comportamiento maternal de las ovejas [26].
CUADRO V. INFLUENCIA DE LA CONDICIÓN CORPORAL EN EL MOMENTO DEL PARTO SOBRE EL COMPORTAMIENTO Y LA EFICIENCIA REPRODUCTIVA DE OVEJAS Y CABRAS TROPICALES NO SUPLEMENTADAS [5]

<table>
<thead>
<tr>
<th>Especie</th>
<th>Condición corporal al parto</th>
<th>Nº parto-servicio (%)</th>
<th>Intervalo</th>
<th>Fertilidad (%)</th>
<th>Prolific. (crías/parto)</th>
<th>Crecim. (g/d)</th>
<th>Mortalidad crías (0-30 d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovejas</td>
<td>&lt;1</td>
<td>16</td>
<td>68</td>
<td>56,3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2180&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20,0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>25</td>
<td>59</td>
<td>72,0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2345&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9,5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>33</td>
<td>48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72,7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,17&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>&gt; 3</td>
<td>48</td>
<td>56&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71,4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,20&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>66,7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,42&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>11,8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1,47&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>31</td>
<td>56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77,4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5,3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
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<td>58&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>101&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6,7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-b</sup> Cifras con literal diferente varían significativamente (P < 0,05).

3. CONTROL Y MANEJO HORMONAL DE LA REPRODUCCION DE OVEJAS Y CABRAS

La sincronización del celo y la ovulación de ovejas y cabras del medio tropical ha mostrado una respuesta efectiva, tanto con la aplicación de tratamientos hormonales [13, 27] como empleando el efecto macho [4, 21, 23]. Una aparente desestacionalidad y un período corto de anestro, de escasa profundidad, han favorecido su desarrollo, en especial el vinculado a la difusión de genotipos mejoradores a través de la inseminación artificial [14]. Antes de adoptar algún tratamiento es importante determinar el comportamiento de los rebaños y sus características dentro de cada sistema, el período parto-tratamiento y el estado nutricional, así como las implicaciones climáticas y forrajeras durante la época de tratamiento y parto.

La administración de hormonas asegura el momento de la sincronización del celo asociado con la ovulación, aunque su empleo en explotaciones tradicionales ocasiona inversiones que no siempre revierten económicamente [14]. La progesterona o, mejor aún, los progestágenos por vía vaginal o subcutánea han sido utilizados...
con éxito en hembras cíclicas o en anestro [13]; se consideran propulsores de la actividad estrual, ya que bloquean la ovulación por retroacción negativa [28]. Pueden adicionarse con gonadotropinas séricas (PMSG) especialmente para animales no cíclicos, para contrarrestar la baja actividad hipofisiaria o cuando se desea mejorar la prolificidad; la PMSG asegura una descarga más precisa de LH y asocia la ovulación con el comportamiento del celo [28]. En ocasiones no es recomendable el uso de PMSG, especialmente en dosis altas; una variable respuesta supervolutoria y de prolificidad parece ser contraproducente, ya que ocasiona un incremento de la mortalidad embrionaria y aun neonatal [13].

El tratamiento progestativo simula la duración de la fase luteal de 12-14 d en ovejas o de 19-21 d en cabras. Un tratamiento largo resulta algo perjudicial para el transporte espermático y la fertilidad en las cabras [28]; aunque aumenta la tasa de ovulación, disminuye su eficiencia debido a una menor fertilización o a una mayor pérdida de óvulos y embriones [14]. La reducción del tratamiento a un lapso menos crítico de 11-12 días, con la adición de prostaglandinas en el momento de la PMSG, ha favorecido una mejor sincronización en cabras; al asociar la descarga de LH y la ovulación con el celo y regular la tasa de ovulación y duración de la fase luteal, las prostaglandinas causan una menor alteración de los ciclos y pérdidas embrionarias [14]. La aplicación de implantes subcutáneos de 3 mg de Norgestomet asociado con PMSG a cabras tropicales permite alcanzar un buen control del ciclo y mejorar la fertilidad [13].

Las prostaglandinas sólo inducen celo ovulatorio a las hembras cíclicas cuando son utilizadas en la fase luteal. Para controlar el momento de ovulación se requieren dos inyecciones a intervalo de 8-11 d, mostrando dispersión de celos en 5 d y menor eficiencia en cabras tropicales [27]. La inyección de prostaglandinas por vía submucosa intravulvar a cabras criollas permite disminuir las dosis habituales de 125 a 40 µg de cloprostenol y de 25 mg a 6,25 mg de lutalyse, sin afectar a la respuesta de celos, fertilidad y prolificidad, si se realiza en épocas adecuadas y con animales en buenas CC [29].

El efecto macho se ha combinado con un tratamiento previo de 20 mg de progesterona para ovejas pelibuey [4], buscando corregir los bajos niveles de progesterona, cuerpos lúteos de mala calidad y los ciclos cortos poco fértiles inducidos inicialmente por el macho [24]; el celo así sincronizado es seguido por una función luteal normal asociada con buena fertilidad, incluso en ovejas en lactación [24] y de buena CC [30]. En cabras con buena CC tratadas con FGA se ha sustituido la PMSG por el efecto macho, sin diferir del efecto macho sólo [13, 23]; la fertilidad es más elevada en la primera ovulación como consecuencia de una mejor asociación celo-ovulación y la eliminación de ciclos cortos [16]. Igualmente, en cabras criollas se ha vinculado la introducción del macho 48 h antes de finalizar el tratamiento progestágeno con una óptima sincronización en 48-72 h y una eficiencia de 135% frente a 95% para animales tratados sin PMSG [23], ratificando reportes sobre ovejas lactantes [24].
4. CONTROL DE LOS PRINCIPALES FACTORES QUE ALTERAN LA RESPUESTA REPRODUCTIVA

4.1. Alimentación y condición corporal

El suplemento alimenticio mejora la CC y favorece una respuesta ovulatoria más eficiente de los animales tropicales sincronizados. Los tratamientos hormonales en épocas secas y hembras con baja CC producen habitualmente una pobre inducción de celos y fertilidad [13]. Cabras tratadas con FGA + PMSG exhiben un comportamiento deficiente en caso de CC menores de 2; al mejorar la CC (de 2,1 a 2,7) aumenta la eficiencia, que es máxima en caso de animales con mejores CC [31]. La suplementación con concentrado durante tres semanas al inicio del tratamiento de cabras criollas favorece la eficiencia de éste (135% contra 38% en testigos no suplementados), e incluso la frecuencia de cabras en celo (95% contra 53% resp.) [31]. Este efecto de la CC se confirma con cabras sincronizadas servidas bajo monta natural [5]: aquellas con CC inferior a 2 muestran menor eficiencia (109% frente a 65% con cabras en CC superior a 2), siendo muy similar la respuesta de ovejas WA, en las cuales una CC mayor de 3 al tratamiento mejora la eficiencia de la sincronización (Cuadro VI). Tanto para cabras como para ovejas, la influencia de la baja CC persiste habitualmente a lo largo de la gestación y se evidencia en una significativa mayor tasa de mortalidad neonatal [5].

4.2. Intervalo posparto y estadio de lactación

El lapso entre parto y servicio sincronizado influye en la respuesta del tratamiento. Un intervalo óptimo debe fluctuar entre 45 y 75 d para lograr un período entre partos de 7-8 meses en animales tropicales [13]. Lapsos menores disminuyen de forma significativa su eficiencia y fecundidad: el medio uterino parece ser menos favorable para mantener una gestación temprana [14]. Las cabras responden con buena eficiencia a partir de los 60 d posparto y, mejor aún, después de tres meses, cuando se consigue una eficiencia reproductiva del 135% e intervalos entre partos de 8-9 meses [31].

La influencia del intervalo posparto se confunde con la del estadio de lactación, el cual también influye la respuesta al tratamiento de ovejas WA lactantes [32]. Con un estadio de lactación menor de 45 d, la eficiencia es baja; la prolificidad mejorará directamente con el avance de la lactancia y disminución de la producción; con un estadio de entre 45 y 60 d de lactancia la fertilidad y prolificidad alcanzan 86% y 1,56 [11]. La menor respuesta de fertilidad en los tratamientos posparto tempranos ha sido atribuida a posibles alteraciones de la ovulación y del transporte espermático a nivel cervical [28].
CUADRO VI. INFLUENCIA DE LA CONDICIÓN CORPORAL SOBRE LA EFICIENCIA DEL TRATAMIENTO FGA + PMSG EN OVEJAS Y CABRAS CRIOLLAS Y MESTIZAS SERVIDAS POR MONTA NATURAL DURANTE LA ESTACIÓN SEXUAL MENOR EN EL MEDIO TROPICAL (MAYO-JUNIO) [5]

<table>
<thead>
<tr>
<th>Especie</th>
<th>Condición corporal al tratam.</th>
<th>N°</th>
<th>Exhibición de celos (%)</th>
<th>Fertilidad 1° serv. (%)</th>
<th>Prolífic. 1° serv. (crías/parto)</th>
<th>Eficien. cond. corp. (%)</th>
<th>Mortalidad crías (0-30 d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovejas</td>
<td>&lt;1</td>
<td>11</td>
<td>54,5 b</td>
<td>50,0 b</td>
<td>1,00 b</td>
<td>27,3 c</td>
<td>66,6 c</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>17</td>
<td>88,2 a</td>
<td>60,0 b</td>
<td>1,11 b</td>
<td>58,7 b</td>
<td>30,0 b</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>13</td>
<td>92,3 a</td>
<td>75,0 a</td>
<td>1,22 a</td>
<td>84,4 a</td>
<td>18,2 a</td>
</tr>
<tr>
<td></td>
<td>&gt;3</td>
<td>8</td>
<td>100,0 a</td>
<td>75,0 a</td>
<td>1,33 a</td>
<td>99,8 a</td>
<td>12,5 a</td>
</tr>
<tr>
<td>Cabras</td>
<td>&lt;1</td>
<td>14</td>
<td>71,4 b</td>
<td>70,0 b</td>
<td>1,20 a</td>
<td>64,5 a</td>
<td>22,2 a</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>21</td>
<td>90,5 a</td>
<td>84,2 a</td>
<td>1,44 b</td>
<td>109,7 b</td>
<td>8,7 b</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>11</td>
<td>100,0 a</td>
<td>72,7 b</td>
<td>1,50 b</td>
<td>109,1 b</td>
<td>8,3 b</td>
</tr>
<tr>
<td></td>
<td>&gt;3</td>
<td>8</td>
<td>75,0 b</td>
<td>83,3 a</td>
<td>1,60 b</td>
<td>100,0 b</td>
<td>12,5 b</td>
</tr>
</tbody>
</table>


a-b, b-c: Cifras con estos ligerares varían significativamente (P < 0,05).
a-c: Cifras con estos literalas varían de forma altamente significativa (P < 0,01).

4.3. Nivel de producción y amamantamiento

Son evidentes los efectos negativos de la lactación, intensidad del amamantamiento y presencia de la cría sobre la reanudación de la actividad posparto de los animales tropicales [1], los cuales afectan también a los resultados de los tratamientos de control del ciclo [32, 33]. Cabras tratadas con FGA + PMSG durante la estación sexual no ven afectada su eficiencia cuando la producción de leche en los 100 primeros días es inferior a 400 g/d; al superar esos límites hay una disminución de la fertilidad y aumento de los servicios. La eficiencia al tratamiento es del 64 y 70% para producciones superiores a 50 y 40 kg y aumenta significativamente al 94% para producciones menores [33]. Esa baja respuesta coincide con informes sobre ovejas Prealpes y Sardas, en las cuales una producción superior a 500 y 1100 g/d resp. disminuye la tasa de celos y fertilidad luego de la sincronización [34]; esa caída sería atribuible a una modificación de la secuencia endócrina, que atrasa la descarga preovulatoria de LH, lo que, a su vez, reduciría el transporte y la supervivencia espermática [34].

El amamantamiento continuo atrasa el reinicio de la ciclicidad posparto, a la vez que reduce en un 30% la respuesta de sincronización y eficiencia de ovejas
tropicales, al alcanzar un 138% y un 106% en ovejas sin y con amamantamiento de las crías [32]. Este efecto puede estar relacionado con un medio uterino menos favorable y fases luteales de corta duración en las ovejas que amamantan [32]; la deficiente función luteal estaría asociada con la ausencia de retroacción negativa ovárica. La inactividad ovárica se encuentra más relacionada con la presencia de la cría y con el frecuente amamantamiento que con el nivel de leche producido, ya que el reestablecimiento de la fertilidad será más tardío en las ovejas que amamantan más de una cría y más precoz en las ovejas secas [32]. Este efecto antagónico del amamantamiento sobre la reproducción puede ser atenuado en las ovejas separando temporalmente la cría 48 a 72 h antes de terminado el tratamiento hormonal; la eficiencia es entonces del 141% frente al 106% alcanzado con hembras que amamantan [32].

Luego de los tratamientos de control reproductivo se han detectado, a partir de los perfiles de progesterona, algunos problemas de disociación celo-ovulación del 9,3%, la cual es más elevada en hembras con pobre CC (11,9 contra 6,8%); los celos anovulatorios, ovulaciones silenciosas y ciclos cortos fueron del 5,8%, 3,5% y 15,1% respectivamente. Este efecto no se observó en cabras criollas de pobre CC, sincronizadas sin efecto macho (Cuadro VII).

CUADRO VII. EFECTO DE LA CONDICION CORPORAL SOBRE LA DISOCIACION CELO-OVULACION Y CICLOS CORTOS EN OVEJAS CRIOLLAS Y MESTIZAS SINCRONIZADAS CON FGA (14 DIAS) E INTRODUCCION DEL MACHO AL INICIO DE LA ESTACION SEXUAL [35]

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<td>(%)</td>
<td>(N°)</td>
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CUADRO VIII. ALTERACIONES DEL CICLO Y POSIBLES PERDIDAS EMBRIONARIAS EN CABRAS CRIOLLAS SINCRONIZADAS E INSEMINADAS: EFECTO DE LA CONDICION CORPORAL EN EL MOMENTO DEL SERVICIO [35]

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<td>Menor de 2,5 (%)</td>
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</tr>
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<td></td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
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Alteraciones del ciclo (63 observ.)

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<td>1,6</td>
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<td>3,2</td>
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<tr>
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<td>3,2</td>
<td>7,9</td>
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</tbody>
</table>

Muchos intentos para aumentar la tasa de ovulación y prolificidad a través de tratamientos hormonales tienen una respuesta limitada, debido a una temprana mortalidad embrionaria [35]. Se desconoce su frecuencia en los primeros días después del servicio de cabras criollas, pero entre los días 21-28 y 29-35 se observan pérdidas del 11,1 y 6,7% resp. (Cuadro VIII). Este factor, que limita el mantenimiento de la gestación inicial, puede deberse a una pobre calidad de la ovulación o a una función inadecuada del cuerpo lúteo, debida posiblemente a una supresión incompleta de la señal luteolítica del útero [35]. Con el tratamiento de FGA+PMSG aplicado a cabras se ha señalado una respuesta más errática que con el tratamiento corto adicionado de prostaglandinas [14]; se observa una descarga de LH antes del propio celo sincronizado y, a pesar de una mayor tasa de ovulación, la prolificidad será menor debido probablemente a fallas ovulatorias y de la fertilización, que pueden ocasionar una mortalidad embrionaria temprana. No debe descartarse un posible efecto de la deficiencia nutricional que pudiera afectar al equilibrio endocrino materno y a la calidad de los óvulos, ya que las pérdidas entre 21 y 35 d después del servicio sincronizado se duplican en las cabras con pobre CC (26,3% contra 11,5%) (Cuadro VIII).
En conclusión, el control de la reproducción de cabras y ovejas tropicales permite disminuir los períodos improductivos, adelantando la edad de pubertad y reini-ciando una cíclicidad temprana, con el consiguiente aumento de la fertilidad y la frecuencia de los partos. Es conveniente establecer una estrategia en el manejo del estado nutricional a través del control de la CC en períodos previos a la pubertad, servicio, parto y tratamientos hormonales, evaluando incluso los beneficios económicos y productivos de una alimentación suplementaria. Una buena CC es suficiente para aprovechar la acción estimulante del efecto macho y atenuar los factores desfavorables que afectan al comportamiento de los pequeños rumiantes de las regiones tropicales.

REFERENCIAS


Abstract

RECENT TECHNIQUES FOR IMPROVING REPRODUCTIVE EFFICIENCY OF LIVESTOCK.

Radioimmunoassay (RIA) techniques for the measurement of reproductive hormones have been developed over the past two decades. These have contributed enormously to the understanding of reproductive physiology and to the application of fertility programmes in the field. For example, the measurement of progesterone in milk or blood of cows is a widely used technique to monitor ovarian function and to determine fertility parameters, e.g. for pregnancy diagnosis. RIA techniques have also found major application in detailed study of biochemical and physiological mechanisms controlling reproductive processes. For example, the RIA of the gonadotrophin hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH), oestradiol-17\beta, progesterone, prostaglandins and more recently inhibin has been a basis for understanding the control of ovarian function. The major biological and management constraints to optimal livestock fertility are ovarian and behavioural anoestrus, failure of pregnancy and suboptimal litter size. The physiological background of these problems is briefly reviewed together with techniques being developed to alleviate their effects.

1. INTRODUCTION

Even in the most developed countries, reproductive performance of farm animals, particularly of ruminants, is poor. For example, in the United Kingdom the calving rate of dairy cows to first insemination is of the order of only 55–60%. Furthermore, few herds meet the target optimum calving frequency of one calf per cow per year. In the developing countries the problems are compounded by adverse climatic conditions, e.g. heat [1] and often poor nutrition. Similarly the use of animals for draught purposes may have adverse effects on their reproductive performance [2, 3]. Also, less may be known about the physiology of these species, e.g. Bos indicus and buffalo compared with B. taurus [4]. Such poor reproductive performance can have serious economic consequences. For example, in the United Kingdom the cost of extended calving intervals has been estimated to be up to £3 per cow per day.
Radioimmunoassay (RIA) techniques for the measurement of reproductive hormones have been developed over the past two decades. These have contributed enormously to the understanding of reproductive physiology and to the application of fertility programmes in the field. For example, the measurement of progesterone in milk or blood of cows is a widely used technique to monitor ovarian function and to determine fertility parameters, e.g. for pregnancy diagnosis. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has developed and validated a simple, robust RIA kit. About 3000 kits are distributed annually from the International Atomic Energy Agency's laboratory in Seibersdorf, Austria, to 400 scientists in developing countries [5]. Such programmes are greatly assisting the measurement of animal fertility in developing countries and are enabling the precise causes of infertility problems to be investigated in detail.

RIA techniques have also found major application in detailed study of biochemical and physiological mechanisms controlling reproductive processes. For example, the RIA of the gonadotrophin hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH), oestradiol-17β, progesterone, prostaglandins and more recently inhibin has been a basis for understanding the control of ovarian function.

The present paper discusses the application of immunoassay to hormone measurements in reproduction and recent developments in the manipulation and enhancement of animal fertility.

2. USE OF PROGESTERONE CONCENTRATIONS TO MONITOR REPRODUCTIVE STATUS

As plasma progesterone directly reflects the function of the corpus luteum it is a precise indicator of ovarian function and has been used to monitor pregnancy, oestrous cycles and anoestrus. Absorption of plasma progesterone by the mammary gland has been demonstrated in several species and concentrations in milk parallel those in plasma. Consequently, the assay of progesterone in milk has been particularly useful for monitoring reproductive activity, especially in cows (e.g. Ref. [6]).

Typical progesterone profiles for non-pregnant and pregnant cows are shown in Fig. 1. During pregnancy the corpus luteum is maintained beyond the normal 16–18 days and this fact is the basis of using progesterone as a means of early pregnancy diagnosis. Unfortunately, high progesterone concentrations between days 18 and 24 are not specific to pregnancy, leading to accurate diagnosis only in about 85% of cases. Conversely, the diagnosis of non-pregnancy from low progesterone levels at this time is almost 100% accurate. This allows the early identification of non-pregnant cows so that they can be treated accordingly. Pregnancy can be
confirmed in cattle at later stages using oestrone sulphate [7]. RIA of oestrone sulphate has also been successfully used to diagnose pregnancy in sows [8].

The fall in progesterone concentrations at the end of the luteal phase has been monitored by some workers to determine the day of insemination without observation for oestrus. Cows inseminated on this basis have shown normal fertility levels in several studies [9]. Progesterone profiles can be constructed for animals from repeated samples to monitor their ovarian activity over periods of time to determine normal and deviant patterns of activity [6]. Progesterone concentrations can also be used to differentiate certain kinds of ovarian cystic condition (e.g. Ref. [10]).

3. RADIOIMMUNOASSAY OF OTHER HORMONES

The principle of RIA has been applied to other reproductive hormones, allowing detailed study of physiological mechanisms and thus a better understanding of reproductive processes. The example of LH is discussed below. It has become apparent in recent years that hormone secretion patterns vary over the very short term as well as the long term and therefore the nature of the information required will determine the sampling frequency necessary. For example, seasonal trends in LH concentrations can be identified from thrice-weekly samples in sheep whilst samples taken at a minimum of every 2–3 h are necessary to identify the pre-ovulatory surge. If episodic changes in hormone patterns are required then samples are required every few minutes [11].

FIG. 1. Milk progesterone concentrations before and after insemination (day 0) in cows which did (●) or did not (○) conceive to that insemination. Each point is the mean ± SEM of 40 observations [6].
Analysis of detailed changes in endogenous LH release in sheep, cattle and pigs suggested that the development of a critical pattern of episodic LH secretion is associated with the stimulation of the final phases of ovarian follicle development [11]. Further evidence for such a functional relationship between these events has come from experiments in which episodic LH secretion induced by gonadotrophin releasing hormone (GnRH) was also effective in stimulating ovarian activity in sheep and cattle [12, 13].

4. CAUSES OF REPRODUCTIVE INEFFICIENCY

Functional reproductive problems, in the absence of infection, can be classified into four categories. They are:

1. Failure to undergo ovarian cycles, known as true anoestrus;
2. Failure to exhibit oestrus at the appropriate time (behavioural anoestrus);
3. Failure to conceive when mated or inseminated;
4. Small litter size in litter producing animals or low fecundity.

These four areas will be considered in turn with particular reference to recent research to alleviate their effects, if not entirely to overcome them.

4.1. Anoestrus

Lack of ovarian activity or true anoestrus occurs in three states: before puberty, post-partum and, in some species, on a seasonal basis. The final endocrine pathway, i.e. suppressed gonadotrophin secretion, particularly pulsatile LH release, appears to be common in these three conditions. However, their causes and primary mechanisms appear to be different. Seasonal anoestrus will not be considered here but has been extensively reviewed recently [14].

Considerable research effort has been expended in understanding the endocrine mechanisms involved in controlling the time of puberty [15, 16]. A failure of pulsatile LH secretion is involved but attempts to induce puberty in young heifers by restoring this using repeated injections of GnRH or prolonged infusions have met with only limited success [17, 18]. The attainment of puberty is known to be related closely to body weight [19], with typical European breeds of cattle reaching puberty at around 250–300 kg usually at 7–12 months. Puberty appears to occur later in Zebu cattle and delays are also well recognized in buffalo [4], all probably associated with poor weight gain during pre-pubertal life. There seems little prospect of immediate pharmacological answers to delayed puberty but resolution most likely lies with good management and improved nutrition.
4.1.1. Post-partum anoestrus

The post-partum period in farm animals is characterized by lactation and a lack of ovarian activity. In normal cattle, this acyclic period is generally shorter in dairy animals than in suckled beef cows. For example, 95% of dairy cows under farming conditions in the United Kingdom had resumed ovarian cycles by day 50 post-partum [6], whereas the equivalent figure for beef cows was 40% [20]. In the sow, lactation is associated with anoestrus for up to six weeks, with oestrus and ovulation occurring within three to ten days after weaning [21]. If ewes lamb in the breeding season the anoestrous period is likely to be three to six weeks [22].

A variety of genetic, environmental and husbandry factors may act on the pregnant and post-partum animal to influence ovarian activity post-partum. These include nutrition, suckling, season, climate and disease. One or a combination of these factors may result in extended periods of ovarian acyclicity post-partum possibly involving common final endocrine mechanisms. However, despite intensive study the exact interactions of these factors with the neuroendocrine system are poorly understood. We have recently reviewed the endocrine aspects of lactational anoestrus in farm animals [23].

Inhibitory environmental stimuli in some way result in reduced hypothalamic GnRH secretion, a lack of LH secretion and consequently lack of ovarian follicle development.

Over the years, many workers have examined methods of inducing ovulation in anoestrous animals but none has yet been totally effective in deeply anoestrous animals. Our work has concentrated on the possibility of mimicking hypothalamic GnRH secretion by giving repeated intravenous injections of the exogenous decapptide for up to 48 h in post-partum beef cows [13, 24]. This was moderately effective in that ovulation was induced in about two thirds of the animals.

In post-partum sheep, hourly injections of 100 ng GnRH for 48 h induced pre-ovulatory LH surges and ovulation, but luteal function was deficient [25]. Using a modified treatment regimen, Wright et al. [26] induced ovulation in 11/15 ewes by injecting GnRH three-hourly for 24 h, two-hourly for 24 h and hourly for 24 h. Luteal function was normal in most ewes and the authors concluded that the ovary required a period of stimulation by LH pulses before ovulation in order to ensure normal luteal function.

Oestrus and ovulation were induced in both lactating and weaned sows using hourly or two-hourly injections of GnRH [27, 28]. In the lactating sows, 3/6 receiving 2.5 μg GnRH every 2 h and 6/6 receiving 1.5 μg/h showed oestrus whereas none of the controls did so.

If such a technique were consistently successful it would clearly have considerable potential in several species; however, automatic pulsatile administration is currently technically impractical. Therefore later studies have investigated the possibility of using continuous administration of GnRH to achieve the same result. This
has also raised the question of the physiological necessity of a pulsatile pattern of endocrine signalling.

To our knowledge continuous infusion of GnRH has not been reported for the post-partum ewe but it has been for the seasonally anoestrous ewe. Constant infusion of 125 or 250 ng GnRH per hour for 48 h resulted in elevated plasma LH concentrations, eventual pre-ovulatory surges and ovulation [29]. However, a period of progesterone pretreatment was required to ensure that viable corpora lutea were formed. Studies with continuous infusion of GnRH in sows are limited but Britt et al. [21] reported that lactating sows infused with 2.5 µg/h for 9 d did not exhibit oestrus until after weaning.

In our most recent experiments in cattle [24] post-partum beef cows received 1.0, 2.5 or 5.0 µg GnRH every 2 h for 48 h either as 24 two-hourly repeated injections or by continuous infusion. Pre-ovulatory LH surges occurred in half the cows receiving repeated injections and 8/10 of the cows receiving the two highest doses by continuous infusion.

It appears to date that low dose GnRH treatments are more consistently successful in inducing ovulation in the sow [21] and ewe [26] than in the cow [24], although this might simply reflect differences in the treatment regimen. If low dose GnRH treatments are to be applied in practice then a subcutaneous delivery system would be necessary. Recent experiments in heifers [18] suggest that this is feasible.

It is considered unlikely that practical application of this type of work will progress unless a suitable subcutaneous delivery system becomes available. McLeod et al. [30] experimented with a number of controlled release vehicles for GnRH and found implant devices made from polyhydroxy-butyric acid to be most successful. On the other hand oil based depot formulations were found to give insufficient control over release for these purposes.

Some uncertainty has surrounded the subject of continuous infusion of GnRH compared with repeated injections. Continuous intravenous infusion of 2.5 µg GnRH per hour in sheep [31] resulted in a 50% decrease in pituitary GnRH receptor concentrations after 24 h, although these were restored within 6 h after the end of infusion. In cows, infusion of 20 µg GnRH per hour resulted in an initial increase in plasma LH concentrations followed by a return to pre-treatment levels after 48 h [32]. After 14 d of infusion the pituitary was still able to respond to a 10 µg GnRH challenge but at a reduced magnitude. This is also suggestive of a reduction of pituitary receptors or at least some form of desensitization. Nevertheless, as shown above, continuous infusion has been used to induce ovulation successfully in some circumstances and it may be that such desensitization is more an effect of dosage rather than of the mode of administration per se.

4.2. Oestrus and its detection

As indicated above, failure of oestrus is an important cause of poor fertility. However, perhaps a more common phenomenon is the failure to detect oestrus. Any
progress in this field in the short term is likely to come via further development of diagnostic kits. A number of kits for enzyme linked immunosorbent assay (ELISA) of progesterone are now available commercially and may be applied to this problem as discussed above. Future progress may be made in detecting subtle hormonal or other changes which are predictive of the timing of ovulation as opposed to oestrus. This would be more appropriate for the timing of inseminations. It is our view that little substantial improvement will be made to existing techniques in this area in the near future.

4.3. Establishment of pregnancy

The ability to conceive is clearly vital in determining reproductive performance. A low pregnancy rate to first service is a major cause of poor reproductive performance. In the absence of specific infectious disease it has been shown that the major problem here, at least in cattle, is early embryonic death [33], normally occurring before day 25 after service. The exact cause (or causes) of embryonic death is unknown but it is circumstantially related to premature regression of the corpus luteum. In other words the corpus luteum is normally maintained for the whole of gestation and early embryonic mortality is associated with its early loss. This results in a decrease in progesterone concentrations, allowing the animal to return to oestrus, probably at the normal time.

Before examining methods of reducing embryo mortality it would be useful to briefly review new findings in relation to the establishment of pregnancy. In the non-pregnant animal prostaglandin \( F_{2\alpha} \) secreted by the endometrium causes regression of the corpus luteum. This is illustrated in Fig. 2.

There is evidence that oestriadiol-17\( \beta \) from developing ovarian follicles stimulates the synthesis of receptors for oxytocin on endometrial cells. Oxytocin, now known to be of luteal origin [34], binds to these receptors, thereby stimulating the synthesis and secretion of PGF\( _{2\alpha} \). Early in the pregnancy of a normal ruminant, a protein of molecular weight of approximately 18 000 daltons is secreted by the embryo, which has an antiluteolytic effect. This has been termed ovine or bovine trophoblast protein 1 (e.g. oTP1). It has recently been shown [35] that the amino acid sequence of oTP1 is 70.3% similar to bovine \( \alpha_2 \)-interferon. Further studies have shown that oTP1 and bTP1 bind to receptors on the endometrium and intra-uterine infusion of trophoblast proteins or recombinant interferon can extend the length of the luteal phase in non-pregnant animals [35, 36]. This work has led to the tentative conclusion that some embryo mortality may be caused by a failure of some embryos to produce sufficient trophoblast protein. These early results clearly offer the exciting possibility of using recombinant trophoblast protein to prevent or reduce embryo mortality in domestic animals.
4.3.1. Improvement of pregnancy rate

A number of methods have been used in the field to improve pregnancy rates in cattle. A review of the literature on comparisons of progesterone concentrations in pregnant and non-conceiving cows before and after insemination [37] concluded that the data were conflicting and inconclusive in all respects. Similarly, progesterone supplementation during early pregnancy has given equivocal results but does seem to be effective where control pregnancy rates are particularly low, i.e. 40% or less.

Also, there have been numerous studies where either chorionic gonadotrophin (LH-like) or GnRH has been administered on the day of service but again the overall results are equivocal.

A somewhat different approach was used by MacMillan et al. [38] to attempt to support the corpus luteum when it becomes susceptible to the luteolytic mechanism, i.e. approaching day 16 after oestrus in the cow. Treated cows (approximately 225) received a single injection of 10 µg of buserelin (synthetic analogue of GnRH) on day 11, 12 or 13 after AI. Treated and control cows were palpated at 6 to 9 weeks to determine pregnancy status. Cows returning to service were reinseminated. Pregnancy rates at 6 to 9 weeks were 72.4% and 60.9% for the treated and control cows respectively. Of those cows returning to service the pregnancy rates to second service were 85.1% and 69.5% for the treated and control groups. The differences between groups were highly significant on both occasions. In further work [36]
TABLE I. EFFECT OF BUSERELIN TREATMENT ON DAY 12 AFTER INSEMINATION ON PREGNANCY RATE IN COWS [39]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treated cows</th>
<th>Control cows</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rate to first service</td>
<td>65.4%</td>
<td>53.4%</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Pregnancy rate to repeat services</td>
<td>59.4%</td>
<td>52.9%</td>
<td>NS</td>
</tr>
<tr>
<td>Days from calving to conception</td>
<td>85.3</td>
<td>91.4</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Percentage barren cows</td>
<td>5.3</td>
<td>10.2</td>
<td>NS</td>
</tr>
<tr>
<td>Number of services per conception</td>
<td>1.58</td>
<td>1.88</td>
<td>NS</td>
</tr>
</tbody>
</table>

non-pregnant cows were injected at 3 d intervals from day 12 of the cycle to day 48 with 10 \( \mu \text{g} \) of buserelin. The corpora lutea of these animals were maintained for the duration of treatment. The results of MacMillan et al. [38] have now been confirmed under farming conditions in the United Kingdom [39] (see Table I), with an increased pregnancy rate in treated cows of about 12%. Similar improvements have also been achieved by buserelin treatment of sheep during the luteal phase [40].

It has been suggested [36] that buserelin acts in these situations by disrupting normal waves of ovarian follicular growth and hence oestradiol secretion during this period, resulting in a failure of synthesis of the oxytocin receptor, a necessary step for the luteolytic mechanism (see Fig. 2). It is also likely that accessory corpora lutea are formed which may boost progesterone production.

4.4. Improving fecundity

The increase of ovulation rate, thereby resulting in increased litter size or fecundity, has long been a goal of reproductive scientists. A number of approaches have been used, including gonadotrophins, particularly those with FSH-like activity. The use of such gonadotrophins has been an essential part of embryo transfer programmes. Other methods which will be briefly discussed here include immunization against steroids and inhibin.

4.4.1. Immunization against steroids

Ovarian steroids exert a negative feedback or suppressive influence on gonadotrophin secretion (Fig. 3). It has been found that immunization of sheep against a variety of steroids will increase ovulation rate and subsequent lambing percentage [40]. Active immunization against androstenedione, an oestrogen precursor,
was one of the most effective methods and is the basis of the product Fecundin (Cooper-Pitman-Moore, Crewe, UK). Field trials on over 3000 treated ewes in the United Kingdom showed a mean increase in lambs reared of about 25% [41]. However, there was a considerable variation in response between farms from −2 to +49%, suggesting interactions with breed, nutrition and other factors. Consequently the product has not been a commercial success in the United Kingdom. Contributory factors may be the relative sophistication of such a technique and the very precise management required to obtain the maximum benefit. In our view such products will have to be extremely robust to perform consistently well in the field and this may present a major difficulty in utilizing such technology in many countries. The consistent production of twin ovulations in cattle using steroid immunization techniques has not yet proved possible.

4.4.2. Immunization against inhibin

Inhibin is a gonadal peptide produced in both the male and female which selectively suppresses FSH secretion (see Fig. 3). In the female it is secreted by the follicular granulosa cells [42] and accumulates in the follicular fluid [43].

Inhibin from sheep and cattle has a molecular weight of 32 000 daltons and consists of two subunits, α and β. It has been shown [44] that the larger follicles (>0.5 mm) are the main source of inhibin in sheep. Administration of inhibin,
usually in the form of charcoal treated follicular fluid (to remove steroids), from various species results in a suppression of FSH in plasma usually after a delay of 2–3 h [45]. In intact ewes the initial suppression of FSH secretion is followed by a rebound increase [46].

The effects of inhibin may be exploited by immunizing animals against inhibin and increasing FSH secretion. Immunization against fractions of follicular fluid containing inhibin activity has been carried out [47, 48]. This resulted in a transient increase in mean ovulation rate from 1.2 to 2.3 in a small number of ewes, which was restored in subsequent years following booster doses. Recent studies have also shown that a controlled increase in ovulation rate can be induced by immunization against inhibin in cows [49] and in pigs [50].

5. CONCLUSIONS

Selected areas of recent research into the physiology of reproduction of farm animals have been briefly reviewed. The application of biotechnology will bring exciting prospects for the manipulation and enhancement of animal fertility, particularly through the synthesis of peptides with hormonal activity and the development of novel immunogens. However, drug delivery and vaccine adjuvant systems should be developed in parallel as far as possible with physiological knowledge, so that the new findings can be applied at the earliest opportunity.

Finally, two notes of caution in view of past experience. The introduction of sophisticated biotechnological treatments to improve fertility may not be appropriate unless certain minimal management, disease control and nutritional conditions can be met. Notwithstanding, very reliable and robust products will be required to be successful in the hostile field environment.

REFERENCES


APPLICATIONS OF MOLECULAR BIOLOGY TO UNDERSTANDING GENOTYPE-ENVIRONMENT INTERACTIONS IN LIVESTOCK PRODUCTION

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Abstract

APPLICATIONS OF MOLECULAR BIOLOGY TO UNDERSTANDING GENOTYPE-ENVIRONMENT INTERACTIONS IN LIVESTOCK PRODUCTION.

There is a continual consumer demand to increase the quality and total yield from livestock production systems. One way this can be achieved is to exploit the natural variation that exists within and across different animal breeds by selecting the genotypes that produce offspring well suited to a particular environment. In this context the environment may be the physical climate, the management system, nutritional level or perhaps disease and parasite loads. The genetic differences between animals may be reflected in measurable differences in performance in a given environment or in changes in the relative performance of genotypes in different environments. The basis of these genotype-environment interactions is the underlying genotype or combination of genes. New techniques in molecular biology allow the direct assessment and manipulation of animal genotypes using methods such as DNA marker analysis, sequencing, gene mapping and transgenesis. These techniques allow the identification, localization, analysis of interaction and alteration of individual genes, and in this way will greatly expand the understanding of genotype-environment interaction and help identify ways of optimizing animal breeding programmes.

1. INTRODUCTION

The domestication of livestock was carried out by early man to decrease the effort required to harvest animal products. It soon became apparent that by selecting particular animal types and crossbreeding them with others, improvements could be made in virtually every aspect of animal production. This exploitation of different animal genotypes has been carried through to the present day where advanced breeding techniques still seek the optimal combinations of animals to suit each new environment. The selection of the 'correct' genotype for the desired breeding structures is still relatively uncontrolled as the animal phenotype is influenced by the environment and so masks its genetic make-up. The discovery in 1953 of the structure of DNA quickly led to the notion that a direct analysis of the DNA would
provide an uncompromised assessment of animal genotypes. The discipline of molecular biology has developed, and can reveal the basic nucleotide variability of genomic DNA. It is this variation that underpins the ability of different genotypes to respond to different environments in different ways. This paper examines the basis and implication of genotype–environment interactions and the DNA techniques which can be used to gain an understanding of genotype structure and function.

2. GENOTYPE-ENVIRONMENT INTERACTIONS

2.1. Definitions

The absolute performance (or phenotype) of an individual is determined by the genetic make-up (genotype), the level of the environment plus any interaction between the genotype and the environment. The term genotype may refer to an individual animal or the average genotype of a strain or breed. In this context, environment encompasses not only climatic and physical factors but also management, nutrition, health and husbandry factors. It therefore includes the entire production environment.

Two types of genotype–environment interactions can occur. Firstly, the differences between genotypes in either performance or estimated genetic merit can vary between environments even though the genotypes rank in the same order in all environments. Secondly, the ranking of genotypes can vary across environments. In practical terms, the second type of interaction is more important because the optimum choice of breed, strain or animal (e.g. bull) will depend on the nature of the production environment, where performance in more than one environment is a consideration. It is therefore important to know when genotype–environment interactions are likely to be biologically and economically significant. In particular, it is necessary to define which factors in the environment will result in interactions. Alternatively, the sensitive components of the underlying genotype of specific traits need to be determined. Ultimately this amounts to pinpointing individual genes which confer advantages/disadvantages in different production environments. Definition at the gene level is a substantial task but, as will be discussed, molecular biology techniques have developed to the point that studies at the gene level are now possible.

2.2. Implications

Where genotype–environment interactions (G × E) exist, different genotypes are required for optimum performance in different environments. Superior animals in one environment will therefore not be superior in other environments. Traditionally, it has been recommended that breeding programmes be carried out in the same
environment for which the superior animals are to be used [1]. However, in practice it is usually expensive and time consuming to duplicate genetic improvement programmes across a number of environments. In addition, there are substantial economies of scale (within reason) from larger breeding programmes. Therefore, once the genetic basis of G x E is understood it may be more attractive to restrict the number of environments in which breeding programmes are conducted and to select animals from within these populations for use in other environments. Such animals will be chosen on the basis of their assessed genetic make-up for traits which are important in particular environments. Clearly the desirable background genotype, e.g. breed/strain, will need to be common to all environments.

2.3. Evidence

G x E can be expected to be of most significance when the range of genotypes or environments is very large. With intensively housed animals such as pigs and chickens, feeding and disease control is largely standardized. Consequently most studies have concluded that G (breed/strain) x E are important (e.g. Refs [2, 3]) and there is little scope for variation from economic options. Even when progeny of 19 sires were compared in environments as extremely different as Berlin and Kuala Lumpur, the statistically significant G x E for a range of egg production traits were minor in magnitude [4].

In dairy cattle, where variability in production environments in developed countries is relatively small, perhaps with the exception of feeding systems, there has been a lack of significant G (sire) x E, e.g. within the United States of America [5] or within Europe [6]. On the other hand, G (sire) x E for Holstein–Friesian cows across temperate and tropical environments have been reported [7], with a significant negative correlation between estimated breeding values in India and the USA. Because of the confounding of environmental factors it was not possible in this study to say which factors, e.g. climate, feed quality or milking systems, were responsible for the G x E.

The most significant G x E will occur where animal management is minimal and their genetic make-up determines how animals seek out forage and combat disease and parasites. Under these conditions, genetic variability is broadened and the scope for interaction with environmental factors increased. Given that beef cattle are raised under a wide range of conditions and are represented by a wide range of genotypes from Bos taurus through Sanga types to B. indicus breeds, it is not surprising that G x E have been most studied in this group. In a series of studies [7, 8], the performance of B. indicus, B. taurus and crossbred cattle was observed in a range of environments. In the most benign environment, where most environmental stresses were controlled, B. taurus animals grew fastest. However, where animals grew in the presence of heat stress, internal parasites, external parasites (ticks, buffalo fly) and fluctuating nutritional levels, B. indicus cattle were superior in
growth. There were also changes in ranking of *B. indicus* and the crossbred groups across environments, so the G × E also occurred within *B. indicus* type cattle. It was suggested that the G × E were associated with differences in resistance to environmental stresses which cause a reduction in feed intake.

Although there have been relatively few studies of G (sire) × E in beef cattle, even in (sub)tropical environments, such interactions should be expected on the basis of the G (breed) × E results discussed above. This is especially likely since for most traits the variation within *B. indicus* or *B. indicus* crossbreeds is greater than the variation between breeds.

As alluded to earlier, many of the same traits which are responsible for G × E across environments will be responsible for differences in performance of different genotypes within an environment. This is reflected in positive within-genotype genetic correlations between production traits such as growth and measures of resistance to environmental factors such as heat, ticks and internal parasites [9]. In environments where the stresses are present and not artificially controlled, genetic resistance to the stresses will be associated with productivity. Therefore by studying the genetic basis of resistance traits in an environment where variation is exhibited, it will be possible to develop strategies to identify superior animals for other environments.

Recombinant DNA techniques permit the study of genetic variation at the gene level. With appropriate experimental approaches it is possible to identify individual genes which are responsible for differences in production both within and across environments. The molecular genetic approach will now be outlined.

3. ANALYSIS OF GENOME VARIATION

Early work on the detection of genome variation focused on the analysis of serum protein and blood type variation, but this was found to be impractical. Protein systems lacked adequate polymorphism, genome coverage was low and there was a requirement for the gene to be expressed to make detection possible. The development of methods to handle DNA directly led to the discovery of restriction fragment length polymorphisms (RFLPs) [10], which provided a direct measurement of genome variability in nucleotide structure. It quickly became apparent that these variable length DNA fragments could act as DNA markers for a section of the genome, and this made it possible to follow the segregation of each locus. Following the development of the RFLP, minisatellite and microsatellite marker systems have been developed, and together with sequence analysis and genomic mapping these have greatly boosted the power of genome analysis.
FIG. 1. RFLPs are generated by digesting genomic DNA with a restriction enzyme (E). In (a) the variable lengths of the fragments are produced by the formation of a new E enzyme site on chromosome 4A of the homologous pair, and in (b) by a deletion event on chromosome 6B. After restriction enzyme digestion, the DNA is electrophoresed on agarose gel, blotted onto a nylon membrane and then hybridized to a radioactive DNA probe. After excess probe has been washed off, the membrane is exposed to X ray film to produce the RFLP image shown in (c), in which each allele of the homologous chromosomes can be recognized.
3.1. Restriction fragment length polymorphisms

RFLPs are generated by allowing bacterial restriction enzymes to cut genomic DNA. Nucleotide variation at the enzyme target site or insertions or deletions of DNA sequences within two enzyme sites produce fragments of variable length at the locus (Fig. 1). The cut genomic DNA is size sorted by electrophoresis in agarose gels and then transferred to nylon support membranes. The specific DNA fragments of interest can be highlighted on the membranes by hybridization binding to radioactive DNA probes. RFLPs will separate the two alleles of any gene, which allows each allele to be followed within a pedigree if one animal is heterozygous at the locus. If both parents are homozygous or both parents and offspring are heterozygous the locus becomes uninformative. This limitation becomes more pronounced in domestic animals where inbreeding has produced a reduced genomic variability in comparison with humans [11].
RFLP systems can be made more informative by altering the analysis technique to produce multiple alleles. This can be achieved in two ways. (a) By using a long DNA probe (e.g. 50 kbp), a haplotype of many restriction fragments may be revealed with the same enzyme (Fig. 2) [12]. (b) The polymerase chain reaction (PCR) [13] is used to generate a fragment in the region of interest, which is then

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**FIG. 3.** (a) Variable length fragments at a specific locus can be produced by digesting a polymerase chain reaction (PCR) product with numerous restriction enzymes simultaneously (e.g. E, B, X and M). The size variability of the fragments is produced by the gain or loss of enzyme sites (such as the loss of enzyme site B on chromosome 11A), or by deletion/addition events. (b) The fragments (e.g. numbers 1 to 8) are radioactively labelled by $^{32}$P or $^{35}$S incorporation in the PCR and are visualized on high resolution acrylamide gel. Pr. A and Pr. B are PCR primers.
FIG. 4. (a) DNA variability at minisatellite loci is produced by the presence of different numbers of sequence blocks repeated in tandem in each allele. By digestion of the DNA at restriction enzyme sites (E) outside the full repeat unit, the fragments can be resolved on agarose gel (b). When high stringency hybridization and membrane washing conditions are used only two alleles at the locus specific to the DNA probe are seen (b1). Under low stringency conditions, alleles from additional, closely related loci are also seen, which results in a multibanded ‘fingerprint’ pattern (b2).

cut with numerous high cutting frequency restriction enzymes. The resulting multifragment pattern can be visualized on a high resolution acrylamide gel (Fig. 3). Both procedures do, however, have practical drawbacks. In haplotyping, the large DNA probe often contains highly repetitive DNA which masks the RFLP signal, and the multicut PCR method requires the nucleotide sequence of the marker region to be known. In many cases, the difficulties of RFLP systems can be overcome by simply using DNA markers that are more polymorphic (see below).

3.2. Minisatellite systems

Minisatellites are fragments of DNA characterized by the tandem repetition of a sequence usually around 25 bp in length [14]. Each repeat sequence has a core that
is well conserved, with more variable flanking sequences. At any locus different numbers of the repeat sequence may be present, which produces alleles of different sizes when a restriction enzyme cuts outside the entire group of repeats. Again the DNA is visualized by gel electrophoresis, blotting and DNA probing (Fig. 4). Variation in the number of repeats can be high, with some loci in humans being heterozygous in 99% of individuals [15]. In cattle the mean heterozygosity of 50 minisatellite loci was found to be 51% [12]. Minisatellite loci exist in high numbers in any genome, and there is a strong sequence similarity between many of these loci. Thus when the genomic DNA is visualized by a minisatellite probe at low DNA hybridization stringencies, many loci become visible simultaneously (Fig. 4(b2)). This results in a DNA fingerprint, which permits the absolute identification of animals, and a means of rapidly screening genomes to find DNA markers that are linked to productive genes. When used at high stringency, minisatellite probes reveal only two alleles, but with a high probability that the locus is heterozygous and with different length alleles in each parent in a pedigree. This greatly expands the amount of genotype information available for analysis of genetic linkage between a DNA marker and a production trait. One possible limitation to minisatellite systems is a non-random distribution of genomic loci, with a bias towards chromosome telomeres [16, 17]. There may be some regions of a genome which do not have minisatellite sequences.

3.3. Microsatellite systems

DNA microsatellites are highly variable polyallelic systems composed of DNA repeated in tandem at each locus [18, 19]. The tandem repeats in microsatellites are usually simple dinucleotides (such as (CA)n) with each dinucleotide repeated around ten times. The length of each allele is determined by PCR analysis using unique oligonucleotide primers flanking the repeat sequence. The DNA products are visualized on sequencing gels (Fig. 5). There are thought to be around 100 000 microsatellite loci in any animal genome [19], which means that any position on the genome lies within 25–50 kbp from a microsatellite. With such a widespread genome coverage and the ability to run up to five microsatellite loci in a single 'multiplexed' PCR, microsatellite systems have an analytical power approaching the level of minisatellite systems.

3.4. Sequence analysis

DNA sequencing provides the highest level of resolution that can be obtained in genome analysis [20]. Sequence analysis provides the fundamental structure of gene systems, and allows an understanding of how nucleotide variation can influence gene expression. It is not practical, however, to use DNA sequencing to analyse comparative nucleotide variation in large animal populations. The task would be
FIG. 5. (a) Microsatellite loci are characterized by the presence of simple dinucleotide tandem repeats (e.g., CACA...) which vary in number on each allele. PCR using locus specific oligonucleotide primer pairs (Pr. A and Pr. B) and incorporating a radioactive nucleotide in the reaction will show the DNA length variation when the PCR product is run out on acrylamide sequencing gel (b1). Multibanded patterns representing alleles from other loci can be produced by combining several primer sets in the one PCR (b2).

simply too large. The analysis of gene sequence, particularly in relation to gene expression, is, however, a vital tool in the development of novel transgenic animals, in which induced nucleotide changes can produce artificial animal variation.

3.5. Genomic mapping

Genomic mapping requires the assignment of DNA markers onto chromosomes. This can be done using a combination of physical mapping methods such as in situ hybridization [21], somatic cell hybrid panels [22], pulsed field gel electrophoresis (PFGE) [23], and genetic mapping methods which measure the rate of genetic recombination between DNA markers [24]. Genomic mapping has two
significant roles in the analysis of genome variation. Firstly, it provides the foundation for trait analysis by DNA marker linkage by allowing the selection of markers from the map to ensure that they are appropriately spaced and that the entire genome is covered satisfactorily. In this way, all the major loci that represent the trait can be resolved. Secondly, genome maps provide detail on gross chromosome rearrangements that may result in phenotypic change, and also on vertebrate chromosome conservation which can provide information on the localization of functionally important genes. Mapping information is gross on a molecular scale, but it does provide important detail on the relationship of animal genotype to animal function, and it does localize regions that can be further investigated using more practical methods such as DNA marker analysis.

4. FAMILIES AND LINKAGE

It has been shown above that it is possible to produce polymorphic DNA markers and, with genetic mapping programmes, to localize the relative positions of these DNA markers on animal chromosomes. A saturated genetic map will contain enough DNA markers to allow the detection of linkage to any specific gene of interest. What must now be produced are families in which the required gene system is effectively segregated, and then these must be screened with the DNA marker probes. The ability to associate a marker with a trait depends on the size of the effect of the trait, the proximity of markers to the major gene(s), allelic frequencies of the markers and gene(s), breeding structure and population size [25]. Where genes have a measurable phenotypic effect family material is simple to collect in livestock. Where individual gene effects are not discernible at the phenotypic level, it may be necessary to set up special matings. Crossing of breeds or strains which have evolved in different environments will provide populations (families) in which segregation of these genes can be identified with genetic markers. The objective is to analyse families in which one or both of the parents is heterozygous for the gene in question. Crosses of widely divergent breeds or strains is a possible approach. Another approach is to analyse crosses of lines selected for specific traits on the assumption that selection would have increased the frequency of major genes in some cases to fixation. There are also in existence some multibreed populations which are the result of mixing divergent breeds, and these should possess most of the variants of particular genes.

Single gene production traits present a relatively uncomplicated analysis, where the numbers of offspring to be screened are low (e.g. several hundred) and the genotype determination of the animals is usually unambiguous. Analyses of this type are currently under way for a number of livestock traits, for example Pompe’s disorder, the Poll and MH genes in cattle [26, 27], the high fecundity gene in Merino sheep [28] and the HAL gene in pigs [29]. The analysis of more complex, multigenic
traits such as parasite resistance, heat tolerance, meat quality and growth rate is understandably more difficult. Family sizes become much larger (e.g. thousands of animals) and the offspring are invariably half-sibs, phenotype data are less precise, and the statistical linkage analysis is less able to distinguish significant associations, and more likely to divulge time consuming false positive linkages. The biometric methods required for multigene linkage analysis are still being refined [30–32], with emphasis being directed towards maximizing the information content of DNA markers, reducing the number of offspring in a pedigree that need to be assessed, and ensuring that important linkages are not overlooked. Progress in this area has

**FIG. 6. Example of a DNA marker test. RFLPs allow the two gene alleles to be identified and followed through a pedigree. Carriers of a specific allele can be rapidly diagnosed.**
reached a point where it is technically and economically feasible to map traits in large livestock species. At present the main limitation is the sufficient development of genome maps for each species.

5. DIAGNOSTIC TESTING

The outcome of gene mapping, DNA marker development and gene linkage analysis is the production of diagnostic tests for important genes. Typically, a diallelic DNA marker will be able to establish an animal's genotype for a specific gene with up to 100% accuracy (Fig. 6). When appropriate, the application of PCR based technology will enable these tests to be carried out on very large populations rapidly and inexpensively using small amounts of DNA extracted from blood or tissue. Screening can even be carried out on a newly formed embryo, without affecting viability, by amplifying the target sequences using PCR [13]. Because the direct detection of genes using gene probes does not depend on gene expression, it will permit early selection of animals and will be especially useful for traits such as reproductive performance and parasite/disease resistance. Furthermore, selection for sex limited traits such as milk production could be carried out in males to reduce the number of sires evaluated by the expensive and time consuming progeny testing procedure. It is clear that one of the foremost advantages of industrial application of DNA marker technology in animals will be the ability to increase both the speed and accuracy of animal selection.

6. ARTIFICIAL PRODUCTION OF GENOME VARIATION

The generation of variation in animal populations is largely produced by natural mutations which are exploited by selective breeding. The development of artificial (transgenic) genomes by the microinjection of DNA into early embryos [33] and subsequent technical improvements to allow a more accurate access to DNA sequences in the genome [34] have created the opportunity to produce 'controlled' genome variation. The production potential of this technique is enormous but the power has yet to be harnessed effectively. The primary constraints in the development of productive transgenic domestic livestock have been in the accuracy and control of foreign DNA insertion, control of foreign gene expression, the effect of the genomic DNA surrounding the foreign gene, a lack of understanding of the basic physiology of gene systems, and even in the choice of suitable gene systems to manipulate [35]. Nevertheless, several studies have been produced outlining the production gains that can be made from the development and utilization of artificial variation in livestock [36, 37]. There is certainly a need to gain a much better assessment of how natural genotypes react with any given environment, before effective gains can be made from artificial genotype variation.
7. CONCLUSIONS

The livestock breeding industry will most certainly benefit from the development of new technologies that should allow greater utilization of the animal types and environments currently available. These technologies will provide a means of detecting desirable and undesirable genetic variation in natural populations and could eventually result in 'designer animals' to exploit unused land zones and new husbandry systems. On both fronts the technology is still in its infancy, with an expected interval of at least ten years before numerous major commercial applications become evident. This work will also produce a great deal of fundamental knowledge on the gene composition and control of complex production traits, and this information will be of immense benefit to our understanding of how different genes will react when combined in any environment.

REFERENCES


EFFECT OF GENOTYPE X MANAGEMENT INTERACTIONS ON REPRODUCTIVE AND PRODUCTION EFFICIENCY OF CHIOS EWES UNDER AN ACCELERATED BREEDING SYSTEM

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Efficiency of production from sheep could be increased effectively by increasing the rate of output. The use of prolific breeds in accelerated breeding systems has been the subject of a number of investigations [1, 2]. The present study involved the Chios breed, which combines some of the qualities required for such systems [3] with high milk production, an important trait in most Mediterranean countries.

A total of 145 Chios ewes were utilized in a five year accelerated breeding system, producing 464 lambings, averaging 2.67 lactations (range 1–7) per ewe. A group of 119 Chios ewes was maintained concurrently, and was managed to lamb once a year, as a control. The study was initiated in 1978 with the first mating season being concurrent in the two groups (April). Mating within the accelerated breeding system was practised throughout the year, with the exception of January and February. All ewes in this system were exposed to rams for rebreeding following weaning (42 ± 3 d post-partum), with the exception of those lambing in November and December, which had to await rebreeding until April. All ewes, regardless of the system, were hand mated and oestrus was detected twice-daily using vascctomized teaser rams. No exogenous hormones were used for oestrus synchronization or induction.

All ewes, regardless of breeding system, were kept indoors throughout the year and minimum grazing was practised. They were mainly fed on concentrates and hay according to requirements for the various phases of the production cycle. All records were kept on an individual basis except feed records (group data). Milk production was computed from monthly test day records following weaning and reflects commercial yield. Replacements were selected within the systems such that
TABLE I. PERFORMANCE OF EWES UNDER TWO SYSTEMS OF BREEDING

<table>
<thead>
<tr>
<th>Trait</th>
<th>System of breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Accelerated</td>
</tr>
<tr>
<td>Litters/ewe</td>
<td>3.00&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total abortions</td>
<td>28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lambs born/ewe</td>
<td>5.63&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lambs born live/ewe</td>
<td>5.00&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Litter weight at birth/ewe (kg)</td>
<td>19.5&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lambs weaned/ewe</td>
<td>4.54&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Litter weight at weaning/ewe (kg)</td>
<td>58.4&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total days in milk/ewe</td>
<td>347&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total milk yield/ewe (kg)</td>
<td>388.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Means with different letters in the same row are significantly different: lower case letters: $P < 0.05$; upper case letters: $P < 0.01$.

A replacement rate of 30% was maintained each year. All rams were replaced following one full calendar year of use.

Ewe fertility in the accelerated breeding system, although lower than that of the control group, could be considered satisfactory (Table I). Differences in conception rate between mating seasons reflect, probably, seasonal fertility [4, 5]. Moreover, the higher productivity of a highly productive dual purpose breed might have affected fertility by accentuating production pressure on the ewe.

The lambing interval realized in the present study (294 d), although higher than anticipated (8 months), appears to be a more realistic target for dual purpose breeds. Ewes in the accelerated lambing system produced more lambs with a higher total lamb weight per ewe at weaning than those in the control system, despite having twice the abortion rate. Total milk production per ewe, although somewhat higher in the accelerated breeding system (difference 54 kg), was not significantly different from that of ewes in the control system.

It was expected that ewe productivity would increase since the unproductive period in the ewe's annual production cycle was shortened. Better feeding and management are necessary prerequisites, since much higher pressure is exerted on the ewe. The use of prolific breeds seems to add some advantage when accelerated breeding systems are considered.
REFERENCES


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RIA MEASURED TESTOSTERONE PRODUCTION AS AN EVALUATIVE PARAMETER IN AN IN VITRO BIOASSAY FOR MALES

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This presentation deals with the possibilities of exploiting testosterone production by a suspension of testis cells stimulated by gonadotrophin.

The method used is as follows. Mouse testis was split by collagenasis and the released cells were stimulated by pregnant mare serum gonadotrophin (PMSG) (Bioveta, Czechoslovakia) at concentrations of 0, 7.8, 31.2, 62.5, 125 and 250 IU/L. Testosterone production was measured in the medium by radioimmunoassay (RIA) using rabbit antiserum (Veterinary Research Institute, Brno) and $^{125}$I-testosterone (Institute for Radioecology and Nuclear Technique Utilization, Košice).

There are several possibilities for using a suspension of mouse testis cells:

(a) Routine checks of luteinizing hormone (LH) activity of production batches of PMSG. The tested sample is compared with a declared standard and its relative activity (%) is calculated.
(b) Determination of bovine serum LH activity. Gonadotrophin is replaced by samples of bull serum for cell stimulation. Testosterone production in vitro shows the biological LH activity of the tested serum. A relative expression is used for comparison of testosterone levels from several experiments.

(c) Assessment of in vivo effects on gonadal functions. The influence of acute and chronic stress on testosterone production in mice was investigated in vitro (Fig. 1). The intensity of stress was assessed by determination of the concentration of 11-hydroxycortico-steroids in mouse serum. Testosterone concentrations were determined by RIA in serum and in the medium.

(d) Estimation of direct effects of endogenous and exogenous substances on cell suspensions.

(e) Post-mortem estimation of the function of bull and goat testicular tissue.

(f) Comparative testosterone production estimations among affected groups of bulls.

This method was also applied to bull testis tissue removed at slaughter. In one test, the ability of bull testis cells, obtained by this method, to respond to gonadotro-
phin stimulation was proved. A second test consisted of the comparison of testosterone production by a group of bulls with spermatological disorders, another group of bulls with an inflammation process in the testis and a control group.

In summary, in vitro bioassay using a suspension of testis cells followed by RIA determination of testosterone is a suitable experimental laboratory method with a relatively wide range of applications.

**BIBLIOGRAPHY**

(in Czech)


**IAEA-SM-318/11P**

**RELATIONSHIP BETWEEN LIVER FUNCTION AND PROGESTERONE CONCENTRATION IN MILK OF DAIRY CATTLE**

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Veterinary endocrinology in farm animals in Czechoslovakia deals mainly with determination of progesterone in milk and blood of cattle and to a lesser extent with determination of oestradiol, oestrone, testosterone, thyroxine and triiodothyronine. The evaluation of luteinizing hormone, follicle stimulating hormone, prolactin, insulin and cortisol is performed by several laboratories as part of their research work. However, in routine diagnostics only the radioimmunoassay (RIA) of progesterone, aflatoxin M1 and polychlorinated biphenyls (PCBs) in milk plays an important role.
The RIA of these substances is used in eight specialized laboratories following the recommendations of the Veterinary Research Institute concerning both methods and interpretation of results. The laboratories can offer complex and cheap services using sample preparation units, high throughput counters, computers for evaluation of the results and direct input of the results to a computerized system for management of reproduction and health care. This computerized system is used for more than 150,000 dairy cows, representing almost 10% of all dairy cows in Czechoslovakia. The system allows us to evaluate the results of progesterone determination in hundreds of herds, with more than 100,000 samples being analysed per year.

Milk progesterone determination helps to concentrate the attention of herds­men and veterinarians on cows for which there is a small probability of successful insemination owing to the high progesterone concentration on the day of insemination or to the low concentration three weeks after insemination. RIA of progesterone allows the monitoring of ovarian function during the post-partum period and during reproductive failures, and improvement of the diagnosis of ovarian disorders. The determination also facilitates the checking of therapy results. The relation between progesterone concentration and conception rate after first insemination has been proved. Our results have also indicated the relationship between the biological half-life of progesterone, the concentration of this hormone in milk and the pregnancy rate after the first insemination. On one farm the half-life of progesterone was 20 min, the pregnancy rate 33% and the progesterone concentration 1.33 ng/mL of fat free milk. On two other farms the physiological half-lives were 26 and nearly 28 min, the pregnancy rates 51 and 57% and progesterone concentrations 2.77 and 2.70 ng/mL. On the last two farms repeat breeders had a significantly shorter biological half-life than cows that conceived after one or two inseminations.

It is well known that the steroid metabolism in the liver is influenced by microsomal enzymes induced by some toxic substances, e.g. PCBs. Therefore, we examined the relationships between reproductive parameters, milk and blood progesterone concentrations, environmental pollution and contamination of the animals.

<p>| TABLE I. PROGESTERONE IN MILK AND BILE ACIDS IN SERUM OF PREGNANT COWS AFFECTED BY POLYCHLORINATED BIPHENYLS |
|---------------------------------------------------------------|-------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Number of cows</th>
<th>PCBs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCBs in milk (ng/kg of fat)</td>
<td>0.38</td>
<td>0.08</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>0.85</td>
<td>3.2</td>
</tr>
<tr>
<td>Bile acids (µmol/L)</td>
<td>35.6</td>
<td>14.2</td>
</tr>
</tbody>
</table>
with compounds which could impair steroid hormone metabolism. Our results indicated the progressive decrease of progesterone concentration in milk during three successive years. The reason for this decrease could be both failure of corpus luteum function due to the impaired conditions, e.g. insufficient \( \beta \)-carotene concentration in the ration or a deficiency in some precursors, and the acceleration of progesterone degradation due to liver injury. The causes of this phenomenon have to be carefully studied, because they are important not only for the correct interpretation of the results of progesterone determination but also for animal health and the quality of milk and meat.

The biological half-life or metabolic clearance of progesterone was determined by progesterone assays of five to six blood samples collected within 90 min after intravenous administration of 100 mg of progesterone (Agolutin, Spofa, Czechoslovakia). Preliminary results show extraordinarily short biological half-lives at some farms.

The low concentrations of progesterone and its shorter biological half-life have been found in cows affected by PCBs. Progesterone in blood and milk and PCBs in milk fat and body fat were determined by RIA. Liver function was followed by RIA of bile acids in blood serum. All the differences shown in Table I had high statistical significance. We have proved also the negative relationship between milk progesterone concentration and the concentration of PCBs in milk fat. According to some published results the decrease of immunocompetency of animals under the influence of PCBs could be expected. We supposed the same in our group of experimental cows. However, the increase of antibodies against ovalbumin after administration of this protein was higher in animals with PCBs than in the control group of healthy cows.

Nevertheless, the concentration of progesterone in cow milk can be used as an indirect indicator of metabolic disorders that can be caused by intoxication, e.g. with PCBs. The RIA of PCBs, developed in our institute, can be used for relatively simple screening of these toxic compounds not only in cow milk but in human milk as well. According to our results this analysis is indicated in all cases of unexplained low concentrations of progesterone in cows during dioestrus or pregnancy.
PROGESTERONE PROFILES AND SAMPLING FREQUENCY

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For the purpose of studying the sampling frequency necessary for accurate evaluation of progesterone profiles of post-partum dairy cows, daily milk samples were collected from 20 Icelandic dairy cows for 100 d starting on the day of calving.

The hormone was determined in the fat free part of the milk by a radioimmunoassay (RIA) technique. Sheep anti-progesterone antibody diluted to 1:20 000 was used as well as tritiated progesterone as tracer and dextran coated charcoal to separate free progesterone from bound antibody.

The ovarian cycles lasted from 8 to 26 d with the luteal phase lasting from 6 to 19 d and the inter-luteal phase from 2 to 8 d. Progesterone spikes lasting less than 48 h were encountered during both the luteal and inter-luteal phases. A drop in progesterone value from the luteal phase plateau lasting less than 48 h was also found. A cow with luteal cysts had progesterone values that fluctuated between 1.5 and 6 nmol/L over a period of 42 d. The progesterone level in a cow with pyometra fluctuated between 3 and 13 nmol/L over a period of 28 d. Another cow suffering from pyometra showed a slow rise (lasting 14 d) in progesterone concentration to a plateau that lasted 7 d, followed by luteolysis and ovulation.

A drop in progesterone lasting 24–96 h was also encountered 11–20 d postconception.

The results indicate that for evaluating the precise length of the ovarian cycle as well as the length of the luteal and inter-luteal phases, daily samples have to be collected. With less frequent sampling short term fluctuations in ovarian activity may be missed and the picture of long term activity may be distorted (Figs 1 and 2). Sampling every third or even every fifth day suffices for a rough estimate of the length of the ovarian cycle and its various phases in healthy cows with normal cycles. If the ovarian activity is abnormal owing to diseases or cysts (follicular or luteal), infrequent (e.g. every fourth or fifth day) sampling may give an incorrect picture of the ovarian activity.

Owing to the variations in progesterone values and in the lengths of the luteal and inter-luteal phases one sample is usually not enough for establishing the time of oestrus. Two to four daily samples are usually needed.
FIG. 1. Progesterone profile of dairy cow 853 as obtained by sampling every fifth day from calving until 100 d post-partum.

FIG. 2. Progesterone profile of dairy cow 853 as obtained by daily milk samples.
For the confirmation of pregnancy no less than three samples should be collected at 2–3 d intervals 18–24 d after service.

When evaluating the effects of diseases like ovarian cysts and metritis on ovarian activity, samples taken every third or fourth day should suffice but daily samples give a more precise picture. The same is true when evaluating the time of early embryonic death.

The drop in progesterone concentration soon after conception may be interpreted as a return to oestrus, which would be the case if the embryo could not interrupt the oestrous cycle at this point and hinder luteolysis. This drop in progesterone concentration has been reported by Claus et al. [1] and Schallenberger et al. [2].

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OESTROGENS IN RUMINANT ANIMAL FEEDS AND THEIR EFFECT ON FERTILITY IN CATTLE

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There are two principal sources of oestrogen in ruminant animal feeds which can adversely affect fertility: (1) non-steroidal oestrogenic compounds present in plants (phytoestrogens), and (2) steroidal oestrogens present in poultry manure. The principal phytoestrogens are coumestrol, formononentin, daidzein and genistein. These compounds are measured using a combination of radioreceptor assay and high pressure liquid chromatography. The principal sources of these compounds in animal feed are soya, alfalfa and clover. Feeding of these highly oestrogenic feeds can result in infertility due to cysts, nymphomania and cessation of cycling. Phytoestrogen content of plants is elevated by environmental factors such as exposure to fungi and
irrigation with treated sewage water. Alfalfa fields irrigated with treated sewage water had a coumestrol content of $44 \pm 16 \mu g/g$ as opposed to control alfalfa which contained $13 \pm 3.4 \mu g/g$. When alfalfa plants were grown with or without exogenous oestrogen under controlled conditions, the oestrogen treated plants had a fivefold elevation in coumestrol content compared with the controls. Therefore, the factor in treated sewage water which results in elevation of phytoestrogen content is steroidal oestrogen ($16.7 \pm 5.5 \text{ ng/100 mL;}$ $N = 5$; range 2.3–36.2 ng).

A survey of chicken manure prior to use from 28 farms in 1990 indicated that oestrogen was present in a concentration of $44.3 \pm 6.6 \text{ ng/g;}$ ($N = 28$; range 10–145 ng), as well as appreciable amounts of testosterone ($267 \pm 29 \text{ ng/g;}$ $N = 26$; range 10–591 ng/g), as determined by radioimmunoassay. We previously determined that chicken manure containing high amounts of oestrogen ($> 300 \text{ ng/g}$) can result in premature udder formation but this is not known to affect fertility. It was also found that chicken manure containing high amounts of testosterone ($> 400 \text{ ng/g}$) can cause delayed puberty in heifers characterized by small ovaries and enlarged clitori. In a field experiment in which 9 mature heifers were fed 2.9 kg/d of chicken manure containing over 200 ng/g of testosterone, 8 of the cows stopped cycling within one month of feeding.

In summary, our data demonstrate that steroidal oestrogens from human or animal sources can adversely affect fertility in cattle in two ways: (1) indirectly by increasing the phytoestrogen content of plants, and (2) directly by feeding of chicken manure. It is therefore recommended (a) that alfalfa grown with irrigated sewage water should be tested prior to use and that the coumestrol level should not exceed $20 \mu g/g$, and (b) that chicken manure should be evaluated prior to use and that the oestrogen and testosterone levels should not exceed 100 ng/g.

IAEA-SM-318/16P

EFFECT OF NUTRIENT AVAILABILITY ON LH SECRETION IN PRE-PUBERTAL GILTS AND LACTATING SOWS

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Università di Bologna,
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There is consistent evidence that nutrition can influence the reproductive efficiency of farm animals. The mechanism by which this influence is exerted is, however, largely unknown. The present research, consisting of two experiments,
was thus designed to describe the pulsatile secretion of luteinizing hormone (LH) in pre-pubertal gilts and lactating sows fed different diets.

In the first experiment four Large White gilts with an average weight of 74 kg housed in individual crates were used. After a 7 d period required by the gilts to become accustomed to the new environment the animals were anaesthetized and then fitted with Silastic chronic jugular cannulae. One week later the animals, fed ad libitum (about 7500 kcal ME/d), were bled at 15 min intervals for 24 h. The same sampling protocol was followed after 4 and 7 d. The animals were then fed a restricted diet meeting only the energy requirements for maintenance (about 2800 kcal ME/d). On the 4th, 7th and 20th days from the beginning of the restricted diet the animals were bled at 15 min intervals for 24 h. After 20 d of restricted diet the animals were again allowed to feed ad libitum and blood sampling was carried out on the first day of ad libitum feeding and 5 d later. Plasma levels of LH were measured by radioimmunoassay. Gilts fed ad libitum, either before or after the restricted diet period, showed an episodic secretion of LH with a pulse frequency of 2.8/12 h during the day (6 a.m. to 6 p.m.) and 3.5/12 h during the night (6 p.m. to 6 a.m.). The LH pulse amplitude ranged between 0.5 and 2 ng/mL. After 4 d of restricted diet the pulsatile secretion of LH during the day was inhibited in 2 gilts out of 4 (mean pulse frequency 0.7/12 h) and after 7 d it was totally eliminated in all the gilts. By contrast the pattern of LH secretion during the night was not influenced by the nutritional level and even after 20 d of restricted diet it was similar to that recorded during feeding ad libitum (3.2 pulses/12 h).

In the second experiment four lactating sows were used 14 d post-partum. The animals were fitted with chronic jugular cannulae and blood samples were collected at 15 min intervals for 12 h (6 a.m. to 6 p.m.) at 21, 23, 25, 28 and 29 d after parturition. Two sows were supplied with 9500 kcal ME/d throughout lactation whereas the other two animals, beginning from the 22nd day of lactation, were supplied with 17 100 kcal ME/d. Weaning was carried out 29 d post-partum. Lactating sows fed a low energy diet were characterized by a low frequency LH pulsatile secretion. The pulse frequency increased after feeding a high energy diet to an average of 1.25 pulses/12 h 25–28 d post-partum, compared with 0.25 pulse/12 h observed in the sows fed on the low energy diet. The influence of the level of energy intake was even more marked at weaning, when pulse frequency sharply increased (4 pulses/12 h) in sows fed on the high energy diet while in restricted animals no significant change in the LH episodic secretion was recorded.

In conclusion, these data demonstrate that the pulsatile secretion of LH is dependent on nutrition both in pre-pubertal gilts and in lactating sows. The influence of nutrition is limited to the daytime, whereas during the night the mechanism controlling LH secretion seems to escape the influence of nutrition.

1 cal = 4.186 J. ME: metabolizable energy.
It is noteworthy that a negative energy balance during lactation prevents the increase in LH secretion which normally occurs at weaning, which represents the major stimulus for the resumption of ovarian activity after weaning.

ACKNOWLEDGEMENT

This work was supported by MURST and Regione Emilia Romagna – CRPA.

IAEA-SM-318/17P

DIRECT RIA OF PROGESTERONE IN WHEY FOR FERTILITY CONTROL OF DAIRY COWS

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A simple, sensitive and direct radioimmunoassay (RIA) method for the determination of low concentrations of progesterone (P₄) in whey has been developed and employed to assist in the management of low fertility cows.

1. MILK SAMPLE COLLECTION AND PROCESSING

Milk samples were taken separately from milking time. It was found preferable to take samples late in the afternoon as this gave a better agreement with oestrus detection. Milk samples were taken every 3–4 d, starting 30–40 d after calving and continuing until the cow was clinically diagnosed as pregnant.

The milk, sampled directly into tubes containing rennet (3200 UR/mL of milk¹), was stored in a refrigerator at 4°C until it was sent to the laboratory. The tubes were incubated at 40°C for 30 min to complete the clotting process and then centrifuged at 1500g for 10 min. After aspiration of the fatty supernatant, 100 μL of whey were taken for radioimmunological analysis of P₄.

¹ UR: units of rennin activity.
2. RIA PROCEDURE

The assay was performed in 3 mL polystyrene tubes. To each tube were added 50 µL of 0.05M PBS-EDTA at pH7.5 (0.1% BSA), containing 30 pg of tritiated P₄ (Amersham), and 50 µL of PBS-EDTA diluted antiserum (anti-11-α-OHP₄-HS-BSA, 1:2500). The final incubation volume was made up to 300 µL with the same buffer. After mixing on a vortex for 1 min, the tubes were incubated overnight at 4°C. The standard curve was constructed with 10, 20, 40, 100, 200 and 400 pg of standard P₄ (SIGMA) added to the whey from cows in heat, stripped with 1% charcoal PBS-EDTA solution. After overnight incubation, 1 mL of a buffered suspension of 0.25% charcoal:0.02% dextrane was added to each tube. After further incubation for 15 min at 4°C, the tubes were centrifuged for 7 min at 3000g and the supernatant was decanted into vials containing 4 mL of scintillation fluid (Ultima Gold, Packard) and beta-counted.

3. ASSAY PERFORMANCE

3.1. Analytical reliability

Matrix effects. In order to evaluate the P₄ concentration without the influence of interfering compounds, some of the whey samples were also extracted with petroleum ether (200 µL whey + 8 mL petroleum ether). The results obtained showed significant differences in P₄ concentrations between extracted and unextracted samples (Table I).

Sensitivity. The sensitivity, defined as the quantity of P₄ which reduces by 10% the zero binding capacity (B/B₀), was 70.00 ± 0.63 pg/mL (X ± SEM) (n = 20).

Precision. The method’s precision, evaluated by repeated assay (n = 20) of whey samples taken from both oestrous and pregnant cows, was expressed as a coefficient of variation. The inter-assay CVs were 18.84 and 8.92%, and the intra-assay CVs were 16.28 and 7.62% respectively.

P₄ analysis was performed immediately after sampling and after the milk samples had been stored for 7 d at 4°C. No significant differences in P₄ concentrations were observed (Table II).

There was also no significant effect of keeping the milk samples at 40°C for 6 h (Table III).

Whey from a pregnant cow was progressively diluted with equal volumes from a cow in oestrus. The correlation between expected and observed concentrations was excellent (R = 0.9942). There was also a very good degree of parallelism between the standard and dilution curves.
POSTER PRESENTATIONS

TABLE I. P₄ CONCENTRATIONS (X ± SEM, pg/mL) IN WHEY EITHER EXTRACTED IN PETROLEUM ETHER OR ASSAYED DIRECTLY

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Extracted</th>
<th>Direct</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>High level</td>
<td>10</td>
<td>873.4 ± 23.2</td>
<td>746.2 ± 39.9</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Low level</td>
<td>10</td>
<td>155.9 ± 9.1</td>
<td>60.6 ± 2.7</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

TABLE II. P₄ CONCENTRATIONS (X ± SEM, pg/mL) ASSAYED EITHER IMMEDIATELY AFTER SAMPLING OR AFTER 7 d AT 4°C

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>High level</td>
<td>10</td>
<td>692.77 ± 24.1</td>
<td>784.12 ± 31.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

TABLE III. P₄ CONCENTRATIONS (X ± SEM, pg/mL) AFTER INCUBATING WHEY AT 40°C FOR 6 h

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>0 h</th>
<th>6 h</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>High level</td>
<td>10</td>
<td>525.06 ± 11.39</td>
<td>539.96 ± 48.07</td>
<td>NS</td>
</tr>
<tr>
<td>Low level</td>
<td>10</td>
<td>36.39 ± 2.98</td>
<td>34.97 ± 3.27</td>
<td>NS</td>
</tr>
</tbody>
</table>

4. FIELD APPLICATION

The method was employed for assisting reproductive management and to assess normal luteal function in dairy cows.

*Control of luteal function.* The P₄ values measured in cycling cows were 450 ± 151 pg/mL in dioestrus and 70 ± 60 pg/mL in oestrus (n = 10).

*Pregnancy test.* Whey samples taken 20–21 d after artificial insemination (AI) were used to discriminate between pregnant and non-pregnant animals. It was found that P₄ levels lower than 120 pg/mL at days 20–21 indicated a non-pregnant status.
The accuracy for the cows diagnosed as 'not pregnant' was 100%, compared with 82.8% for the 'pregnant' ones.

Control of AI time. A study of 500 AIs showed that 83.0% of inseminations took place with P₄ levels below 50 pg/mL, 9.0% between 51 and 100 pg/mL, 3.0% between 101 and 150 pg/mL and 5% above 150 pg/mL. AIs performed with P₄ levels lower than 100 pg/mL gave 43.04% clinically confirmed pregnancies; between 101 and 150 pg/mL this number was only 20%. No pregnancies were recorded with P₄ concentrations greater than 150 pg/mL.

It was found that about 10% of the AIs were performed on non-cycling animals, even though normal cyclical activity had been diagnosed by rectal palpation. Embryonic losses were assumed in 7% of correctly performed AIs.

ACKNOWLEDGEMENT

This work was supported by a MURST (40%) grant.

IAEA-SM-318/18P

INFLUENCE OF CLIMATIC HEAT ON OVARIAN CYCLICITY OF SWAMP BUFFALO

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Malaysian Agricultural Research and Development Institute,
Serdang, Malaysia

The influence of climatic heat on ovarian cyclicity and adaptive response of swamp buffalo was studied using 28 cycling buffaloes aged 3–4 years and weighing around 400 kg. Each animal was fed with 7 kg/d of concentrate, containing 50% palm oil mill effluent and 50% palm kernel cake, plus 4 kg/d of cut guinea grass. Water and salt lick were provided at all times. Ovarian cyclicity was determined by monitoring plasma progesterone level in samples taken every 5 d. The animals were divided randomly into four treatment groups: shade + wallow (S-W), no shade + wallow (NS-W), shade + no wallow (S-NW) and no shade + no wallow (NS-NW). The experiment was conducted for a period of three months each during the rainy and dry seasons. The climatic variables are shown in Table I.
TABLE I. MEANS (± SD) OF ENVIRONMENTAL PARAMETERS IN THE SHADE AND IN THE OPEN DURING THE RAINY AND DRY SEASONS

<table>
<thead>
<tr>
<th></th>
<th>Rainy season</th>
<th></th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open</td>
<td>Shade</td>
<td>Open</td>
</tr>
<tr>
<td>Max. black globe temp. (°C)</td>
<td>40.2 ± 3.0&lt;sup&gt;b&lt;/sup&gt; 35.1 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.9 ± 5.0&lt;sup&gt;c&lt;/sup&gt; 37.8 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Max. dry bulb temp. (°C)</td>
<td>31.4 ± 1.7&lt;sup&gt;a&lt;/sup&gt; 31.8 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.8 ± 1.7&lt;sup&gt;c&lt;/sup&gt; 32.4 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>79 ± 15</td>
<td>80 ± 10</td>
<td>73 ± 12</td>
</tr>
<tr>
<td>Sunshine (h)</td>
<td>5.5 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.3 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Rainfall (mm/d)</td>
<td>15 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Note: Means with different superscripts between columns are significantly different (P < 0.05).

During the rainy season, animals under all four treatments were able to maintain similar heat balances, as indicated by the similarity in their water turnover rates (Table II). These values were similar to the values reported in buffaloes reared under oil palm plantation conditions [1]. Owing to low heat stress, all the buffaloes were able to maintain ovarian cyclicity. This was shown by the percentage of animals which could maintain cyclic plasma progesterone levels (0.5–2.5 ng/mL) for a period of 20–28 d (Table II). The mechanism by which the animals maintained the heat balance, however, differed between the different groups. The NS-NW group maintained the heat balance by increasing the metabolic rate (higher level of thyroid hormones) and the S-W group by having the lowest metabolic rate, while the other groups had intermediate thyroid hormone levels.

Climatic heat stress was more acute during the dry season than during the rainy season (Table I) and was found to affect ovarian cyclicity. The percentage of animals which could maintain ovarian function under NS-NW was lower (Table II) than under S-W. Wallowing alone (NS-W group) was insufficient to maintain the ovarian activity of all buffaloes. During sunny days, the animals in the S-W group tended to avoid direct solar radiation by going into the shade. They also wallowed when the body heat reached the upper limit of comfort temperature. The animals in the S-NW treatment tended to put their feet in the water despite being protected from direct solar radiation. However, results on progesterone profiles indicated that the number
## TABLE II. OVARIAN CYCLICITY AND SOME PHYSIOLOGICAL RESPONSES (MEAN ± SD) OF BUFFALOES TO DIFFERENT LEVELS OF ENVIRONMENTAL STRESS DURING THE RAINY AND DRY SEASONS

<table>
<thead>
<tr>
<th></th>
<th>Shade + wallow</th>
<th>No shade + wallow</th>
<th>Shade + no wallow</th>
<th>No shade + no wallow</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rainy season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>466 ± 20</td>
<td>477 ± 15</td>
<td>479 ± 20</td>
<td>460 ± 10</td>
</tr>
<tr>
<td>Cycling (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Water balance:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turnover rate (mL·d⁻¹·kg⁻⁰.₈²)</td>
<td>280 ± 20</td>
<td>285 ± 35</td>
<td>290 ± 40</td>
<td>277 ± 25</td>
</tr>
<tr>
<td>Body water (% of body weight)</td>
<td>75 ± 5</td>
<td>78 ± 10</td>
<td>80 ± 7</td>
<td>72 ± 9</td>
</tr>
<tr>
<td>Half-life (d)</td>
<td>5.9 ± 1.0</td>
<td>5.1 ± 0.9</td>
<td>5.5 ± 1.1</td>
<td>6.2 ± 1.3</td>
</tr>
<tr>
<td>Thyroid hormones (ng/mL):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>0.63 ± 0.10ᵃ</td>
<td>0.77 ± 0.25ᵇаб</td>
<td>0.81 ± 0.17ᵇ</td>
<td>0.91 ± 0.12ᶜ</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>31.7 ± 1.2ᵃ</td>
<td>36.5 ± 2.1ᵇ</td>
<td>37.0 ± 2.2ᵇ</td>
<td>39.9 ± 1.7ᶜ</td>
</tr>
<tr>
<td><strong>Dry season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>475 ± 15</td>
<td>480 ± 25</td>
<td>460 ± 31</td>
<td>470 ± 18</td>
</tr>
<tr>
<td>Cycling (%)</td>
<td>85ᵈ</td>
<td>57ᶜ</td>
<td>42ᵇ</td>
<td>28ᵃ</td>
</tr>
<tr>
<td>Water balance:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turnover rate (mL·d⁻¹·kg⁻⁰.₈²)</td>
<td>355 ± 15ᵇ</td>
<td>300 ± 23ᵃ</td>
<td>337 ± 19ᵇab</td>
<td>450 ± 21ᶜ</td>
</tr>
<tr>
<td>Body water (% of body weight)</td>
<td>77 ± 7ᵇ</td>
<td>75 ± 9ᵇ</td>
<td>70 ± 5ᵇ</td>
<td>65 ± 4ᵃ</td>
</tr>
<tr>
<td>Half-life (d)</td>
<td>4.3 ± 0.7ᵇ</td>
<td>3.8 ± 0.9ᵇ</td>
<td>4.0 ± 0.4ᵇ</td>
<td>3.0 ± 0.7ᵃ</td>
</tr>
<tr>
<td>Thyroid hormones (ng/mL):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>0.75 ± 0.1ᵃ</td>
<td>0.83 ± 0.17ᵇ</td>
<td>0.90 ± 0.09ᵇᶜ</td>
<td>0.94 ± 0.09ᶜ</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>39.3 ± 4.1ᵇ</td>
<td>39.8 ± 2.1ᵇ</td>
<td>38.0 ± 1.7ᵃ</td>
<td>43.1 ± 2.2ᶜ</td>
</tr>
</tbody>
</table>

Note: Within a season means among treatments with different superscripts are significantly different (P < 0.05).
of cycling animals was lower than in the NS-W group. Protecting the animals from direct solar radiation was therefore not sufficient to reduce the heat load. The animals need to wallow to lower their body temperature when the environmental temperature is too high. The mechanism by which heat stress affects reproduction in buffaloes could be similar to the mechanism found in cattle [2].

The buffaloes responded to high heat load by increasing their metabolic rate through increased thyroid activity. Those receiving the highest heat stress (NS-NW) had the highest activity while those receiving the least heat stress (S-W) had the lowest activity [3]. Water turnover rates of the animals in all groups during the dry season were higher (Table II) than in the rainy season. The buffaloes which were denied wallowing mobilized their body water to meet the high water requirement for heat dissipation.

It is concluded that ovarian cyclicity of swamp buffaloes was depressed by climatic heat during the dry season. This effect can be reduced by providing both shade and wallow.

REFERENCES


1. INTRODUCTION

The length of the calving interval is economically important in dairy animals because it affects milk yield per cow per day of herd life and the number of replacements. The interval from calving to resumption of cyclic ovarian activity is one of the factors that determine the length of the calving interval. The enzyme immunoassay (EIA) technique has been used to investigate the influence of calving season and parity on the resumption of ovarian cyclicity in the post-partum period of Nili-Ravi buffaloes as assessed by milk progesterone profiles.

The aim of the present study was to describe the reproductive events during the post-partum period from the point of view of ovarian activity. The observations were based on functional activity (milk progesterone concentration) rather than on the morphological aspects (rectal palpation). The oestrus observation records of the farm, in Rawalpindi, Pakistan, were also used in order to correlate oestrous behaviour with the functional activity of the ovaries. Individual progesterone values were used to construct a profile which was considered to reflect ovarian functional activity.
TABLE I. NORMAL AND ABNORMAL ANIMALS AS JUDGED BY PROGESTERONE PROFILES, AND MEAN INTERVALS FROM PARTURITION TO FIRST OVULATION AND FROM PARTURITION TO FIRST OESTRUS IN THE NORMAL ANIMALS

<table>
<thead>
<tr>
<th></th>
<th>Number of animals</th>
<th>Normal</th>
<th>Parturition to first ovulation (d)</th>
<th>Parturition to first oestrus (d)</th>
<th>Abnormala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Summer</td>
<td>30</td>
<td>24</td>
<td>39.8 ± 20.5</td>
<td>70.5 ± 21.2</td>
<td>4</td>
</tr>
<tr>
<td>Autumn</td>
<td>30</td>
<td>26</td>
<td>29.1 ± 16.3</td>
<td>57.5 ± 18.2</td>
<td>1</td>
</tr>
<tr>
<td>Winter</td>
<td>30</td>
<td>29</td>
<td>36.3 ± 25.6</td>
<td>73.2 ± 17.8</td>
<td>1</td>
</tr>
<tr>
<td>Spring</td>
<td>30</td>
<td>22</td>
<td>43.6 ± 14.7</td>
<td>67.8 ± 18.1</td>
<td>7</td>
</tr>
<tr>
<td>Parity 1</td>
<td>60</td>
<td>47</td>
<td>44.5 ± 23.6</td>
<td>74.9 ± 24.4</td>
<td>10</td>
</tr>
<tr>
<td>Parity 2 and higher</td>
<td>60</td>
<td>54</td>
<td>30.6 ± 15.9</td>
<td>61.4 ± 19.4</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>101</td>
<td>36.9 ± 20.7</td>
<td>67.83 ± 21.0</td>
<td>13</td>
</tr>
</tbody>
</table>

a A, true anoestrus; B, persistent corpus luteum; C, cystic ovaries.

2. RESULTS AND DISCUSSION

In this study only 55.0% of the dairy buffaloes had resumed ovarian cyclic activity by 40 d post-partum. A post-partum profile in which ovarian cyclicity was resumed within 100 d post-partum was considered as normal. Results showed that 84.2% of the buffaloes were normal by this definition. In earlier studies, 62-78% of the buffaloes had resumed ovarian cyclicity within 90 d post-partum [1, 2]. It appeared that in comparison with dairy cows the onset of ovarian cyclicity is much longer in buffaloes. For example, nearly half of the dairy cows resume ovarian cyclic activity within 20 d of calving and this increases to more than 90% by 40 d [3].

The number of anoestrous buffaloes is abruptly reduced in the groups of autumn (August–October) and winter (November–January) calvers, and then it increases again in the group of spring (February–April) calvers. Statistical analysis of the normal progesterone profiles showed that autumn and winter were the preferred calving seasons for early resumption of ovarian cyclicity.

In spite of earlier resumption of ovarian activity in winter calvers the parturition to first oestrus interval was found to be the longest (Table I). This can
only be explained by a high incidence of silent heat or missed oestruses, which is supported by the observed maximal difference between parturition to first oestrus and parturition to first ovulation in winter calvers (Table I). This highlights the urgent need for accurate and practical methods for oestrus detection in buffaloes.

Suboestrous winter calvers have a high probability of becoming truly anoestrous in the following spring and summer. Therefore, fertility control during the winter season will certainly reduce calving intervals. Particularly in winter adequate oestrus detection is essential as the animals are kept in confinement.

El-Belly et al. [4] observed in dairy buffaloes that during the post-partum period plasma progesterone decreased gradually and reached baseline values after day 15 post-partum, when the residual corpus luteum of pregnancy had completely regressed in all the animals studied. We observed elevated levels of progesterone after calving in 27.3% of the animals and these levels reached the detection limit at 7.6 ± 2.9 d post-partum. In buffaloes, the corpus luteum of pregnancy takes 2-45 d to regress completely [5, 6]. However, in cattle the corpus luteum regresses more rapidly [7]. The longer time required for the corpus luteum to regress in buffaloes might be due to a decline in concentrations of prostaglandin in the blood one day after parturition [8], whereas in cows a level similar to that at parturition is maintained up to 4-5 d post-partum [9].

REFERENCES

INFLUENCE OF REPEATED INFUSIONS OF NOREPINEPHRINE ON THE SECRETION OF PROGESTERONE AND OVARIAN OXYTOCIN IN CATTLE

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Although the corpus luteum (CL) of many species is devoid of any innervation, a stimulatory effect of catecholamines on the secretion of progesterone from bovine luteal cells and both progesterone and oxytocin (OT) from bovine granulosa cells has been observed in in vitro studies. We have found recently [1] in in vivo experiments that norepinephrine (NE) releases ovarian OT in amounts comparable with that released by prostaglandin F2α [2]. Since OT and progesterone are released concomitantly in the mid-luteal phase, OT is suggested to be involved in ovarian steroidogenesis. Furthermore, ovarian OT is proposed to be a trigger for luteolysis at the end of the oestrous cycle of ruminants. Therefore, the objective of our studies was to investigate whether NE can deplete the bovine CL of OT and thus to determine its physiological importance.

Four mature heifers (380–450 kg body weight) each with a developed CL confirmed by rectal palpation were injected with the PGF2α analogue Oestrophan (Spofa) to facilitate oestrus synchronization. On day 10 of the oestrous cycle catheters were inserted into the aorta abdominalis through the coccygeal artery for infusion of either saline or NE (0.3 μg · kg⁻¹ · min⁻¹). The tip of each cannula was positioned cranially to the origin of the ovarian artery. Thus the infused drug could be transported in the bloodstream directly into the reproductive tract. Jugular veins were cannulated for blood sample collection.

On day 11, NE was infused three times. After each NE treatment saline was given for 1 h by the same means. On day 12, NE was infused in the same manner and 1 h after the last infusion the PGF2α analogue (500 μg) was injected (Fig. 1). Five minutes after the start of NE infusion a rise (P < 0.01) of OT was observed which usually preceded the elevation of progesterone. Significant correlation (P < 0.01) between the two hormones was also ascertained during NE infusion and after PGF2α injection. These observations may suggest some relationship between ovarian OT and progesterone release/synthesis. A decreasing time dependent effect of NE on the release of OT was found. Large amounts of OT released after each
FIG. 1. Concentrations of progesterone (●) and oxytocin (○) in individual heifers infused with norepinephrine (0.3 μg·kg⁻¹·min⁻¹) (horizontal lines) on (a) day 11 and (b) day 12 and injected with PGF₂α analogue on day 12 (arrow).

NE infusion suggest that catecholamines can influence the components of the post-translational processing pathway of OT synthesis in the bovine CL. Thus NE does not appear to be useful for depleting the CL of OT. Secretion of OT after PGF₂α injection indicates that NE and PGF₂α cause the release of this peptide independently of each other.

ACKNOWLEDGEMENT

This work was supported by the Polish Academy of Sciences (Project No. RPB 05.2).
REFERENCES


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STUDIES ON REPRODUCTION IN INDIGENOUS BUFFALOES AND CATTLE IN SRI LANKA

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Indigenous buffaloes and Zebu cattle are the predominant types of animal on rural farms in Sri Lanka. Reproductive patterns of buffaloes have been well characterized [1, 2] and a major cause of low reproductive efficiency was found to be prolonged post-partum anoestrus, which appears to be the result of poor nutrition and frequent suckling by calves.

Analysis of data on calvings and conceptions of Lankan buffaloes was done over a five year period. The average calving interval was 504.46 ± 117.21 d and the calving was seasonal with 81% of calvings occurring between October and February. Calving to conception interval was short in animals which calved early during the calving season, being only 127 ± 83.99 d compared with 258.38 ± 135.35 d for animals calving late in the season. The calvings coincided with the onset of northeast monsoon rains.

Manipulation of suckling management and hormonal treatment methods were used in attempts to improve post-partum fertility. First, 30 buffaloes were randomly assigned to the treatments shown in Table I. Both complete weaning and limited suckling resulted in early onset of ovarian activity compared with ad libitum suckling. However, the completely weaned group suffered heavy calf mortality (72%).
### TABLE I. EFFECT OF DIFFERENT SUCKLING MANAGEMENT PRACTICES ON PREGNANCY RATES

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Number pregnant at 90 d (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum suckling</td>
<td>9</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Limited suckling from 30 d post-partum</td>
<td>10</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Complete weaning at 45 d post-partum</td>
<td>11</td>
<td>8 (72)</td>
</tr>
</tbody>
</table>

### TABLE II. EFFECTIVENESS OF DIFFERENT HORMONAL TREATMENTS IN INDUCING OESTRUS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Number pregnant at 90 d (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH (2 × 250 µg, 24 h apart)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>FSH (2 × 4 mg, 12 h apart) + GnRH (2 × 250 µg, 24 h apart)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>PRID (for 11 d) + PMSG (700 IU at PRID removal)</td>
<td>14</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>1 (9)</td>
</tr>
</tbody>
</table>

In a second trial 52 buffaloes were assigned as follows: (1) ad libitum suckling from birth; (2) limited suckling from day 7 post-partum; (3) complete weaning on day 60 post-partum; and (4) complete weaning on day 75 post-partum. Preliminary results indicate that limited suckling from 7 d post-partum appeared to be as effective as complete weaning on day 60 or 70 post-partum. The limited suckling did not appear to have adverse effects on growth of the calves.

Several trials were conducted to assess the suitability of synthetic gonadotrophin releasing hormone (GnRH), GnRH with follicle stimulating hormone (FSH) priming and a combination of progesterone releasing intravaginal device (PRID) and
pregnant mare serum gonadotrophin (PMSG) to induce oestrus in buffaloes. Results are given in Table II. Treatment of post-partum anoestrous buffaloes with short term progestogen in combination with PMSG was effective in inducing oestrus.

Since information on reproductive patterns of Zebu cattle in Sri Lanka is scarce, an island-wide survey and several case studies were undertaken. Preliminary results indicate that Lankan Zebu animals mature slowly and are low producers of milk and meat, but can thrive under very harsh environmental conditions, particularly on low quality herbage. A case study done in the dry zone of Sri Lanka indicated that age at first calving is 4–5 years and calving interval ranges from 1 to 2 years with an annual calving rate of about 60–65%.

To determine the post-partum interval and gestation length ten pluriparous Lankan Zebu animals were studied by weekly blood sampling and rectal examination until pregnancy, with monthly blood sampling thereafter until eight months of pregnancy and weekly blood samples until parturition. The traditional calf rearing method was suckling once a day from 7 d post-partum. The mean (± SD) interval from calving to first observed oestrus was 53.8 ± 44.03 d, from calving to first progesterone peak was 40.00 ± 39.37 d and from calving to conception was 67.80 ± 56.09 d. These limited results indicate that Lankan Zebu cattle are very fertile though they are poor producers of milk.

REFERENCES

A study was carried out to examine the feasibility of inducing breeding activity in Angora goats at approximately 40 d post-partum. The treatment was imposed in May, a period of deep seasonal anoestrus for this breed.

Thirty pluriparous Angora does, aged three to four years, were selected from a flock of goats kept at Lalahan, Ankara (39°57' N, 32°53' E). They were treated with intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Veralix, Upjohn, United Kingdom) for 14 d. An intramuscular injection of 500 IU of pregnant mare serum gonadotrophin (PMSG) (Folligon, Intervet, Holland) was given at sponge removal.

Oestrus detection was performed twice-daily (at 09:00 and 18:00) using three bucks. Since Angora bucks lack libido outside the normal breeding season (November to December), each animal was given 25 mg of testosterone (Sustanon '100', Organon, Turkey) plus 1000 IU of PMSG every 3 d for 20 d. All the does came on heat and exhibited standing oestrus between 48 and 60 h after sponge removal. Twenty does were mated by the bucks at supervised services when oestrus was first detected and again 12 h later, as is conventional practice. Each of the other ten does was artificially inseminated twice using pre-deep-frozen Angora buck semen in skimmed milk diluent (300 × 10^6 spermatozoa/0.5 mL straw/dose) as deep as possible into the cervix [1].

For early pregnancy (or non-pregnancy) diagnosis progesterone levels in the peripheral serum samples were determined by direct radioimmunoassay on day 20 following breeding [1]. The sensitivity of the assay was 8 pg/mL. The inter-assay variation was 9.9, 5.1 and 4.1% for the low, medium and high levels of progesterone, respectively, and the intra-assay variation was 4.0%.
TABLE I. KIDDING PERFORMANCE OF ANGORA GOATS FOLLOWING INDUCED BREEDING ACTIVITY

<table>
<thead>
<tr>
<th>Type of insemination</th>
<th>Number of does</th>
<th>Pregnant does (with progesterone on day 20)</th>
<th>Does that aborted (2-3 months)</th>
<th>Does that kidded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial insemination</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Hand mating</td>
<td>20</td>
<td>9</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

While the pregnant goats revealed the expected sustained high progesterone concentrations, the non-pregnant animals had values of less than 1.0 ng/mL progesterone. The conception rates of the naturally mated and the artificially inseminated animals were 47 and 30% respectively. However, these figures were reduced to 35 and 10%, respectively, for the percentage of does that carried their pregnancy to term because two does from each treatment aborted at two to three months after conception.

The data obtained are presented in Table I.

REFERENCE

A radioimmunoassay (RIA) technique was developed for the determination of 13,14-dihydro-15-keto-PGF$_{2\alpha}$ (PGFM) in goat plasma. PGFM immunogen was synthesized by coupling PGFM to bovine serum albumin (BSA) for producing antiserum as described by Axen [1]. The ratio of binding was 28.9 mol PGFM/mol BSA. Six female rabbits, aged five months, were injected with the immunogen at three week intervals. The highest titre observed, which was obtained at the final bleeding, was 1:30 000. The association and dissociation constants of antiserum were determined as $K_a = 4.1 \times 10^{10}$ M$^{-1}$ and $K_d = 2.44 \times 10^{-11}$ M, respectively, from a Scatchard plot [2] (Fig. 1).

**FIG. 1. Scatchard plot of PGFM antiserum (dilution: 1:30 000 in 0.5 mL; a = 1.2, b = -0.041, $R^2 = 0.962$).**
Three angora goats, aged three years, were bled three times a day for the determination of PGFM during the oestrous cycle in the breeding season. In order to show cyclicity, progesterone concentrations were assessed by validated RIA [3]. PGFM was analysed by the direct RIA technique of Meyer et al. [4] using $^{3}$H-PGFM (157 Ci/mmol (5.8 TBq/mmol), Amersham, United Kingdom). Radioactivity was measured in Rialuma (J.T. Baker, Netherlands) with a Packard TRI-CARB 1550 LC analyser with an efficiency of 59.9%. The detection limit of the test was 62 fmol/mL. Inter- and intra-assay coefficients of variation were 16.4 and 11.7% respectively. The results of one representative goat are shown in Fig. 2.

In conclusion, the produced antibody can be effectively used in RIA for the quantitative determination of low levels of PGFM in goat plasma.

REFERENCES

Teniendo en cuenta la baja fertilidad de las vacas Holstein Friesian en los meses de verano en las regiones de clima tropical y subtropical [1], nos propusimos, en un rebaño de 504 vacas Holstein puras, evaluar las características productivas y reproductivas de los animales que se gestaban de forma repetida en los meses de mayo a septiembre y su relación con los niveles de cortisol (F), temperatura rectal (TR) y frecuencia respiratoria (FR) medidos entre los 7 y 9 meses de edad bajo condiciones de stress de calor.

Los animales se clasificaron a partir de los dos primeros partos en tres grupos: 0) ninguna gestación en verano, 1) una de las dos veces se gestaron en verano; y 2) las dos veces se gestaron en verano. Los niveles de producción de leche (PL), período de servicio (PS) e intervalo entre partos (IPP) se tomaron de los registros de producción individuales, el F se midió por RIA y el cálculo estadístico por análisis de varianza.

El 62,2% de las vacas se gestó por primera vez en verano y de éste el 51,4% volvió a gestarse en esta estación, de forma que el 34,9% se gestó las dos ocasiones en verano (grupo 2), el 25,8% las dos veces en invierno (grupo 0) y el 39,3% de forma alterna en ambas estaciones (grupo 1). Los animales de los grupos 0 y 2 tuvieron promedios menores de PS (p<0,01) que los del grupo 1. No hubo diferencias en la PL entre los tres grupos, sin embargo el grupo 2 tuvo mayor productividad (PL/IPP) que los grupos 1 y 0 (p<0,01) (Fig. 1). También, los animales del grupo 2 presentaron ante el stress de calor en edades tempranas los menores valores de F, TR y FR (p<0,01) (Fig. 2).

Estos resultados muestran un mejor comportamiento productivo de las vacas que se gestan de forma repetida en el verano, debido a una mayor disponibilidad de alimentos en esta época del año, lo que corrobora resultados previos [2] y demuestra una mejor capacidad adaptativa al clima de trópico cálido húmedo así como la posibilidad de su detección a edades tempranas a través de indicadores fisiológicos.
**FIG. 1.** Comportamiento productivo de vacas Holstein según la estación del año en que ocurre la gestación.

**FIG. 2.** Comportamiento adaptativo de hembras Holstein según la estación del año en que se gestan.

**REFERENCIAS**


EFFECTS OF MELATONIN IMPLANTS ON OVARIAN ACTIVITY AND FERTILITY OF ANOESTROUS EWES AND GOATS

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It is well known that melatonin administered chronically to anoestrous ewes is effective in bringing forward the breeding season if animals have experienced a period of long days prior to the beginning of the treatment [1–3]. Different results have been obtained with a similar treatment in goats [4], which therefore seem to be less sensitive to the pineal hormone. The aim of the experiment described here was to investigate the effectiveness of melatonin biodegradable pellets in stimulating ovarian activity and in improving reproductive efficiency in these species. On 20 June 1990, 40 pluriparous lactating Saanen goats and 40 Suffolk cross ewes were subcutaneously implanted with one melatonin pellet (18 mg; Regulin, Schering); an additional group of 40 animals (20 for each species) was used as control. On 30 July fertile males of both species were introduced into the two flocks. Fertility was recorded at parturition; the approximate date of fertile mating was established by assuming a gestation length of 150 d in both species. At the end of January, pregnancy diagnosis was performed by echography on all the ewes that had not yet delivered. Beginning on 5 June (two weeks before the treatment started), ten melatonin implanted and ten control animals for each species were bled weekly till 18 September; sample progesterone plasma levels were determined in each by enzyme immunoassay. Animals were considered to be cycling when progesterone plasma concentrations exceeded 1 ng/mL for at least two consecutive samples. Data related to the onset of ovarian activity in both species (treated and control) are presented in Table I. The reproductive parameters are shown in Tables II and III.

Melatonin implants do not seem to be effective in advancing the onset of ovarian activity (monitored by progesterone plasma determination) in either ewes or goats, even if the mean conception date is brought forward (by about 11 d), at least in goats. The main differences between treated and control animals are related to fertility, which was higher in melatonin implanted than in control females (85% vs. 80% and 100% vs. 75% in ewes and goats respectively).
TABLE I. DAYS BETWEEN BEGINNING OF MELATONIN TREATMENT AND ONSET OF OVARIAN ACTIVITY

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days ($\bar{X} \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated ewes ($n = 10$)</td>
<td>29.3 $\pm$ 16.7</td>
</tr>
<tr>
<td>Control ewes ($n = 9$)</td>
<td>34.7 $\pm$ 13.4</td>
</tr>
<tr>
<td>Treated goats ($n = 10$)</td>
<td>63.1 $\pm$ 10.4</td>
</tr>
<tr>
<td>Control goats ($n = 7$)</td>
<td>64.0 $\pm$ 5.7</td>
</tr>
</tbody>
</table>

* One ewe and three goats did not show any ovarian activity till 18 September.

TABLE II. REPRODUCTIVE PARAMETERS OBSERVED IN MELATONIN TREATED AND CONTROL EWES

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Lambing before 29 Jan. (%)</th>
<th>Mean date of fertile matings</th>
<th>Ewes diagnosed as pregnant on 29 Jan. (%)</th>
<th>Ewes that did not conceive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated ($n = 40$)</td>
<td>57.5</td>
<td>28 Aug.</td>
<td>27.5</td>
<td>15</td>
</tr>
<tr>
<td>Control ($n = 20$)</td>
<td>55.0</td>
<td>3 Sep.</td>
<td>25.0</td>
<td>20</td>
</tr>
</tbody>
</table>

TABLE III. REPRODUCTIVE PARAMETERS OBSERVED IN MELATONIN TREATED AND CONTROL GOATS

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Kidding before 29 Jan. (%)</th>
<th>Mean date of fertile matings</th>
<th>Goats diagnosed as pregnant on 29 Jan. (%)</th>
<th>Goats that did not conceive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated ($n = 40$)</td>
<td>90.0</td>
<td>15 Aug.</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Control ($n = 20$)</td>
<td>55.0</td>
<td>26 Aug.</td>
<td>20</td>
<td>25</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENT

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REFERENCES


ANIMAL HEALTH
(Session 3)

Chairmen

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Mali

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FAO/IAEA
Abstract

DIAGNOSIS OF ANIMAL DISEASES USING NUCLEAR AND RELATED TECHNIQUES: DEVELOPMENTS AND TRENDS.

Nuclear techniques such as radioimmune precipitation, radioimmunoassay, DNA cloning and amino acid sequencing have led to a greater understanding of protein structure and function, antigenic variation and the immune response to infection. Knowledge gained from the use of this technology has led to the development of improved diagnostic assays. Although radioimmunoassay has been used for animal disease diagnosis for many years, more recently it has been replaced by the enzyme linked immunosorbent assay (ELISA). The ELISA offers advantages in speed of reading and longer reagent shelf life and obviates the use of radiochemicals. This is particularly important in developing countries, which may have no facilities for storage, handling and disposal of radioactive materials. In the case of rinderpest diagnosis, taken as an example, the virus neutralization test was replaced by a simple indirect ELISA for seromonitoring throughout the Pan-African Rinderpest Campaign. In the near future, this will be replaced by a competitive ELISA using a rinderpest specific monoclonal antibody, which will offer significant advantages in sensitivity and specificity. In the future it may be possible to replace the rinderpest antigen with vector expressed proteins or synthetic polypeptides. More recent developments such as the ‘amplified’ ELISA and the use of fluorogenic and bioluminescent substrates may further improve disease diagnosis. The knowledge gained from the use of modern technology is essential to the development of improved diagnostic assays, which in turn will lead to improved disease diagnosis and control.

1. INTRODUCTION

Following the development of radioimmunoassay (RIA) and the enzyme linked immunosorbent assay (ELISA), disease diagnosticians were divided into three camps: those who still only had faith in ‘traditional’ assays such as virus neutralization, complement fixation and immunodiffusion; those who had converted to the use of RIA; and those who believed the ELISA to be the universal panacea.
Later, scientists gradually realized that each of these techniques had a role to play in disease diagnosis and that the choice of ‘appropriate’ technology was vital when introducing diagnostic techniques into developing countries. This paper looks at the advantages and disadvantages of radiolabelled and enzyme labelled techniques and, using rinderpest diagnosis as an example, considers current and future trends in diagnostic technology.

2. RADIOIMMUNOASSAY

Although widely applied in many laboratories in developed countries, RIA technology has not been transferred to many countries in the Third World. The short shelf life of RIA kit reagents is a major problem when bearing in mind the cost of regularly supplying kits overseas. Licensing laboratories to handle radiochemicals and the subsequent disposal of radioactive waste is another major problem. Although single channel readers are relatively inexpensive, the time taken to read large numbers of samples precludes their use for large scale epidemiological surveys. The larger automated gamma counters are prohibitively expensive and difficult to maintain under local conditions. The health hazard of inexperienced staff handling radiochemicals must also not be overlooked.

3. ELISA

The ELISA, which in recent years has largely replaced RIA for routine disease diagnosis, offers many advantages. The reagents are extremely stable and usually freeze-dried to give long shelf life. Commercial enzyme conjugates for many animal species are widely available, offering the opportunity for bulk purchase of quality controlled reagents, thus ensuring continuity of supply. The recent introduction of enzyme substrates, buffers and hydrogen peroxide in tablet form has greatly eased kit formulation and test standardization. Over the last five years ELISA plate technology has greatly improved, which has resulted in higher protein binding capacity, lower well to well variation and the absence of ‘edge-well effect’. ELISA readers have also improved beyond recognition from the early flow-through cell models. Modern readers have integral computers capable of data analysis and can read a 96 well plate in 5 s. The ease with which large numbers of samples may be tested has allowed large scale epidemiological surveys which would have been impossible using ‘traditional’ assays. Although some enzyme substrates are reported to have mutagenic properties tablet formulation greatly reduces the health hazard and alternative substrates are available which are non-mutagenic. Although spectrophotometric reading of ELISA results allows statistical analysis of data, the test may be specifically designed for eye reading if required at the local level (e.g. in field stations).
Owing to all the factors mentioned above, the ELISA has largely replaced RIA for routine disease diagnosis. When the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture decided to introduce a disease diagnosis component into its projects in Africa, the rinderpest ELISA was chosen as appropriate for its first diagnostic kit. Rather than listing the numerous ELISAs developed against various infectious agents we shall use the diagnosis of rinderpest to exemplify current and future trends in diagnostic technology.

4. RINDERPEST DIAGNOSIS (ANTIBODY DETECTION)

The detection of antibodies against rinderpest virus is important when evaluating the immune response following vaccination. Although rinderpest vaccine (tissue culture attenuated) induces life-long immunity following a single vaccination, the reconstituted freeze-dried vaccine is highly thermolabile. This necessitates the use of a cold-chain to ensure viability of the vaccine in the field. Seromonitoring following vaccination gives an indication of the vaccination team’s performance, the integrity of the cold-chain and the immune status of the cattle population.

In the past the virus neutralization test was used for seromonitoring. This requires the use of tissue culture and the provision of sterile serum samples. Both of these requirements are difficult to meet at present in developing countries. The test itself is labour intensive, requires 5-7 days’ incubation and is read microscopically. It would be virtually impossible to test the required number of sera (approximately 20,000 per year) using this method.

A simple indirect ELISA for the detection of antibodies against rinderpest virus was developed in 1983 [1]. Rinderpest antigen was pelleted from infected tissue culture supernatant (secondary bovine kidney cultures) by ultracentrifugation. Blocking buffer was phosphate buffer saline (PBS) supplemented with 3% bovine serum albumin and 0.1% Tween-20. Sera were tested at a dilution of 1/4 and detected using rabbit anti-bovine IgG horseradish peroxidase (HRPO) conjugate. The substrate-chromogen combination used was hydrogen peroxide and ortho-phenylenediamine. Comparative testing against the virus neutralization test gave a 95% correlation.

In 1985 it was decided to use the indirect ELISA for seromonitoring during the rinderpest eradication campaign in Tanzania. As large quantities of antigen were required, antigen preparation was modified to a crude infected cell sonicate (this would also allow antigen preparation in Tanzania without recourse to ultracentrifugation). The Madin Derby bovine kidney cell line was used for antigen production as this was more convenient than primary and secondary cell cultures and gave a more reproducible product. Sera were tested at a dilution of 1/4 (compared to the previous 1/2) as this resulted in a better binding ratio. Owing to the expense of bovine serum albumin, alternative blocking agents were examined and dried milk powder was shown to be optimal. The test performed extremely well under local conditions and 80,000 sera were tested over a period of four years.
Following validation under field conditions in Tanzania a diagnostic kit was developed in collaboration with the Joint FAO/IAEA Division. Over a period of five years this has been successfully introduced into 21 countries in Africa. Technology transfer has been achieved by the use of training courses followed by assistance in establishing the assay in each individual laboratory. There has been technical back-stopping whenever needed and continuous updating of the kit as improvements were evolved. The provision of substrate and chromogen in tablet form has solved the stability problems of these reagents. The major problem has been in the quality of the local distilled water. This may drastically affect the test and result in reduced binding ratios and hence a poor separation of negative and positive populations. This has been partly solved by the supply of sterile distilled water, sufficient to reconstitute the freeze-dried kit reagents, and also by the provision of modern distillation and deionizing apparatus.

The indirect ELISA has proved to be suitable for evaluating the immune response to rinderpest vaccination in cattle. However, as the Pan-African Rinderpest Campaign moves into the second phase, and most of the cattle population have been vaccinated and become antibody positive, serosurveillance of other animal species will become increasingly important. This will involve the testing of sera from sheep, goats and game animals, all of which could act as reservoirs of infection. Difficulties were experienced in the development of an indirect ELISA for sheep and goat sera owing to the high non-specific reactivity of such sera. A further complication was the presence in some countries of a closely related virus, peste des petits ruminants (PPR). Antibodies to PPR cross-react with rinderpest virus in the rinderpest ELISA. The lack of commercially available HRPO conjugates suitable for the various species of game animals also posed problems.

The solution to all of these problems lay in the development of a competitive ELISA using a rinderpest specific monoclonal antibody (MAb).

5. MONOCLONAL ANTIBODIES AGAINST RINDERPEST VIRUS

Before producing MAbs it was important to consider the structure and function of the various rinderpest viral proteins and compare their specificity and cross-reactivity with other related viruses, particularly PPR virus. Amino acid sequencing data have shown the H protein to be the most variable viral protein, with little sequence homology between rinderpest and PPR virus isolates [2]. Radioimmune precipitation data showed little cross-reaction between the H proteins of the two viruses, compared to the F and N proteins which had high levels of cross-reactivity [3]. DNA probes using the H gene and the N gene were both shown to be rinderpest specific [4]. The H protein is involved in host cell attachment and antibodies to H protein have a major role in virus neutralization [5]. From the above evidence it was decided that MAbs against the virus haemagglutinin (H protein) would be most suita-
ble for use in a rinderpest specific assay. Antisera from cattle vaccinated with an experimental vaccinia rinderpest-F recombinant vaccine were found to be virus neutralization positive but ELISA negative (using the standard ELISA antigen). When purified antigen was used in the ELISA instead of crude sonicated infected cell extract, high levels of anti-F antibody were detected in these sera. This indicated that some epitopes were not expressed on the crude ELISA antigen, probably owing to masking by host cell proteins, and highlighted the need for purified antigen preparations for immunization and screening procedures during the production of MAbs.

MAbs were produced against purified rinderpest vaccine strain virus and screened by indirect ELISA for specificity against rinderpest and PPR viruses. Those MAbs which were rinderpest specific were characterized by immunoprecipitation and Western blotting. All the MAbs were also evaluated for use in a competitive ELISA. One rinderpest specific MAb against rinderpest haemagglutinin (designated C1) was found to be suitable for use in a competitive ELISA. Using a similar procedure, MAbs against PPR virus were also produced and a PPR specific MAb against PPR haemagglutinin (designated C77) was found to be suitable for use in a competitive ELISA.

6. COMPETITIVE ELISA

6.1. Rinderpest

The rinderpest competitive ELISA has been shown to detect antibodies to the mild, virulent and vaccine strains of rinderpest virus and, unlike the indirect ELISA and the virus neutralization test, produced no cross-reactions with antibodies to PPR [6]. Also, compared to the indirect ELISA, there was a greater separation of positive and negative populations when sera were evaluated by frequency distribution analysis. As virus neutralizing antibody levels have been shown to correlate with protection, it was imperative that any new assay should correlate with the virus neutralization test. In comparative studies using field sera from Yemen, there was a 98% agreement between the competitive ELISA and the virus neutralization test. The major advantage of the competitive ELISA is the ability to test sera from any species whilst using a single enzyme conjugate. This will allow epidemiological studies on the role of sheep and goats and wildlife in the epidemiology of the disease. In the past this has been difficult owing to high levels of cytotoxicity in the sera of these species and the lack of species specific enzyme conjugates.

6.2. PPR

The PPR competitive ELISA has been shown to detect antibodies to all strains of PPR, and, like the rinderpest competitive assay, gives a good separation of nega-
tive and positive populations, and enables sera from all animal species to be tested using a single enzyme conjugate [7]. Although the MAb does not react with rinderpest antigen, some antibodies to rinderpest virus do compete in the assay. However, a combination of the two competitive assays allows differential diagnosis of the two related diseases. This has been confirmed by the examination of sheep and goat sera following vaccination with rinderpest vaccine in the Gambia. Using the two competitive assays it was possible to differentiate between animals which had responded to vaccination and those which had failed to respond to vaccination, owing to the presence of antibodies to PPR virus before vaccination. Antibodies to the two viruses are cross-protective; therefore, prior infection with PPR virus prevented replication of the attenuated rinderpest vaccine.

Both of these MAb based assays offer major advantages in standardization, which is an important consideration in kit development, especially for use in international eradication campaigns. MAbs, which are homogeneous in nature and available in unlimited amounts, offer the opportunity for all laboratories to have identical diagnostic reagents.

7. FUTURE DEVELOPMENTS

Research is under way to produce rinderpest antigens in expression vector systems such as baculovirus, yeast and *Escherichia coli*. The use of synthetic peptides is also being evaluated. These may be selected either in the 'classical' manner by producing overlapping polypeptides based on sequence data, or by the use of the mimotope-pepskan strategy [8]. In the latter system, all possible combinations of 20 × 20 amino acids are tested for their reactivity with a suitable polyclonal or preferably monoclonal antibody. Optimally reactive dimers are then increased in size by sequential addition of amino acids to the left and right of the original dimer. In this manner a synthetic hexapeptide may be generated with optimal reactivity with the desired diagnostic reagent. Vector expressed proteins and synthetic polypeptides offer the opportunity for producing highly defined, non-infectious antigens for use in diagnostic assays.

The rapidly developing techniques of amplified ELISA, bioluminescence, chemiluminescence, fluoroorlumunoassay and the use of biosensors may well have a role to play in disease diagnosis in the future. This will be dependent on the development of appropriate, economically priced readers and the supply of commercially produced reagents. At present the increased sensitivity offered by these techniques is neither necessary nor appropriate for antibody detection, especially in developing countries.
8. RINDERPEST ANTIGEN DETECTION

Although presumptive diagnosis may be based upon seroconversion of non-vaccinated animals, definitive diagnosis has always been based upon isolation of the infectious agent in tissue culture followed by neutralization of infectivity by virus specific antiserum. Rinderpest antigen was also detected by agar gel immunodiffusion and counter immunoelectrophoresis.

More recently, competitive ELISA using affinity purified antibody has been used for the detection of rinderpest antigen from eye swabs [9]. This has not been brought into routine use owing to the difficulty in affinity purifying large amounts of antibody. Identification of suitable MAbs for use in antigen competition ELISAs may allow more widespread application of this technique.

Radioactively labelled DNA probes have been used for a number of years for differentiating rinderpest from PPR virus strains [4]. More recently, they were used to diagnose for the first time the presence of PPR virus in India. They also demonstrated the relationship between 'phocine distemper' (seal morbillivirus) and rinderpest, PPR and canine distemper viruses. The technique has not been widely transferred to developing countries owing to the cost of reagents and the problems associated with the use of radioactivity. The technique may be more widely applied once enzyme labelled probes have been fully evaluated.

The polymerase chain reaction (PCR) now allows the possibility of rapidly amplifying a single copy of DNA to detectable levels. Although at present mainly a research tool, the advent of microplate technology for PCR and enzyme labelled probes may encourage more widespread application of the technique.

As mentioned previously, the techniques of amplified ELISA, bioluminescence, chemiluminescence, fluoroimmunoassay and the use of biosensors reportedly offer increased sensitivity over existing assays. However, they will not be widely applied until efficient, reliable, economically priced readers and reagents become commercially available.

The ELISA, DNA probes and PCR are primarily laboratory based techniques but there is a requirement for a 'pen-side' diagnostic test which field veterinarians could employ when presented with suspect cases in outlying areas. At present, the only technique available is latex agglutination. Once again the identification of suitable specific MAbs with which to label the latex particles would improve both the specificity and standardization of the assay.

9. CONCLUSIONS

In recent years, the use of nuclear and related techniques has had a major impact on disease diagnosis. Diagnostic methods are now being developed using an interdisciplinary approach involving molecular biology, immunology and epidemio-
Molecular biological studies have led to a better understanding of protein structure and function. Immunological research has led to an appreciation of the role and relative importance of individual proteins in the immune response to infection and possible mechanisms of protection. The requirement for highly sensitive and specific assays, necessary for epidemiological studies, has led to an amalgamation of these three sciences resulting in today's increasingly sophisticated assay systems. Although at present radiolabelled assays may not have a role to play in disease diagnosis in developing countries, the use of nuclear techniques is essential to the development of most enzyme labelled assays.

Although diagnostic assays may function perfectly in a European laboratory, there have been problems when the technology has been transferred to laboratories in developing countries. These may be problems related to the stability of the reagents, the quality of the local water, the high ambient temperature in the laboratory or even the buildup of static electricity in the plates.

The Joint FAO/IAEA Division has overcome most of the problems inherent in technology transfer by the use of kits containing standardized, quality controlled reagents which have undergone extensive field trials. Intensive training courses combined with technical assistance in setting up the assay in each individual laboratory and immediate technical backstopping when problems arose have given the Joint FAO/IAEA Division an impressive record in technology transfer.

REFERENCES


THE ROLE OF THE ELISA TEST IN THE CONTROL OF BOVINE BRUCELLOSIS IN DEVELOPING COUNTRIES

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Abstract

THE ROLE OF THE ELISA TEST IN THE CONTROL OF BOVINE BRUCELLOSIS IN DEVELOPING COUNTRIES.

An FAO/IAEA enzyme immunoassay (EIA) kit for the diagnosis of bovine brucellosis was compared in Argentina with two screening tests, the rose bengal (RB) and buffered plate antigen (BPA) agglutination tests, and two confirmatory tests, the 2-mercaptoethanol (2-ME) agglutination test and the complement fixation (CF) test. In the testing of *Brucella abortus* strain 19 (S19) vaccinated cattle from *Brucella* free dairy herds, the diagnostic specificity estimate of the EIA test (99.7%) was shown to be comparable to those of the RB (99.7%), 2-ME (99.8%) and CF (99.9%) tests and greater than that of the BPA test (90.6%). In the testing of S19 vaccinated cattle from infected herds, the sensitivity estimates of the BPA test (99.5, 99.6 and 98.6% respectively) relative to CF, 2-ME and EIA test positive reactors were comparable and high. The relative sensitivity estimates of the RB test (86.3, 81.4 and 79.1%) in the same comparison were disparate and lower. The EIA test demonstrated the highest relative sensitivity estimates (97.1 and 95.2% respectively) in a three way comparison between EIA, CF and 2-ME test reactors from these herds. Relative to BPA test reactors from the same infected herds, the sensitivity estimate of the EIA test (57.0%) was comparable to that of the 2-ME test (56.2%) and higher than that of the CF test (51.8%). These results would suggest that the overall diagnostic specificity and sensitivity of the EIA test are comparable, if not superior, to those of the tests used to confirm BPA test reactor status.

1. INTRODUCTION

Although bovine brucellosis has been successfully eradicated in some countries, it is still endemic in vast regions of the world. Brucellosis is responsible for considerable economic loss in cattle producing areas and is a severe human health hazard. In Argentina, for example, with more than 10% prevalence of bovine brucel-
losis, the estimated annual production losses are US $125 million. At a rate of 20,000 new cases of human brucellosis per year, annual medical costs are estimated at US $66 million. Brucellosis is also of relevance when considering restrictions on the international movement and marketing of animals or products of animal origin.

There are approximately 240 million cattle in South America and estimates of disease prevalence range from 0.5 to 10% depending on the country [1]. Given the economic and zoonotic impact of this disease, there is a definite need to establish programmes of control and eradication.

Serological detection of antibody is the mainstay of bovine brucellosis control and eradication programmes. Principally two types of conventional assay have been applied in serial or parallel testing strategies: agglutination and complement fixation. Of these, the buffered plate antigen (BPA) [2] and rose bengal (RB) [3] agglutination tests are most commonly used as laboratory based screening assays and the 2-mercaptoethanol (2-ME) tube agglutination test [4] and the complement fixation (CF) test [5] are commonly used as confirmatory tests.

In Argentina, where vaccination of calves with Brucella abortus strain 19 is extensively practised, the following testing strategy has been adopted nationally. All cattle to be used reproductively are first tested at the age of 6 months (non-vaccinated bulls) and 18 months (vaccinated heifers). Sera are initially screened using the BPA test and seropositive samples are then tested using the 2-ME test. All BPA test negative and 2-ME test negative cattle are allowed to remain in the herd. If the sample is 2-ME test positive, it is further tested using the CF test. Cattle which are 2-ME test positive but CF test negative are placed in isolation and retested after three months. If they revert to 2-ME test negative status, they are returned to the herd. If they become CF test positive, they are removed for slaughter, as are CF test positive cattle identified at the time of initial testing. All herds in which CF test positive cattle have been identified are tested at three month intervals until all CF test positive reactors have been eliminated. At this point, the herds are tested on an annual basis to maintain Brucella free status.

While these conventional assays have proven successful in this testing strategy, they do suffer to various degrees from inherent drawbacks related to assay and reagent standardization, sample pretreatment, diagnostic performance and cost efficiency. The enzyme immunoassay (EIA), on the other hand, is capable of overcoming these drawbacks and has potential application in all countries where brucellosis is a problem.

The study described here was undertaken to investigate the diagnostic potential of the EIA within the framework of serological testing in current use in Argentina. The materials, equipment and EIA kit used in this study were supplied by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture as part of an International Atomic Energy Agency co-ordinated research programme, entitled Regional Network for Latin America on the Use of Immunoassay and Labelled DNA Probe Methods for the Diagnosis of Livestock Disease.
2. MATERIALS AND METHODS

2.1. Test samples

Two herd types were selected on the basis of their serological reactor status in the current national testing programme described in the introduction. 

*Brucella* free herds were defined as those herds in which no CF test positive reactors had been identified in the previous two herd bleedings which had been conducted at three month intervals. A total of 1082 blood samples were collected from ten herds, which ranged in size from 40 to 270 cattle.

*Brucella* infected herds were defined as those herds in which CF test positive reactors were still being identified and culled on the basis of a three month bleeding schedule. A total of 4765 blood samples were collected from 30 herds ranging in size from 43 to 770 cattle.

2.2. Test procedures

The conventional screening assays (BPA and RB) and confirmatory assays (2-ME and CF) were prepared and conducted as described in the literature cited in the introduction. The EIA was conducted as described in the manual of the FAO/IAEA kit. The basic reagents and protocol for this kit have been adapted from Nielsen et al. [6].

2.3. Test interpretation

For the purpose of seroepidemiological analysis, cattle were classified as seronegative or seropositive according to the following seropositive reactor criteria:

<table>
<thead>
<tr>
<th>Test</th>
<th>Reactor Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>Agglutination of 1+ or greater</td>
</tr>
<tr>
<td>RB</td>
<td>Agglutination of 1+ or greater</td>
</tr>
<tr>
<td>2-ME</td>
<td>Agglutination of 3+ or greater at a serum dilution of 1/25</td>
</tr>
<tr>
<td>CF</td>
<td>Haemolysis of 50% or greater at a serum dilution of 1/10</td>
</tr>
<tr>
<td>EIA</td>
<td>Optical density value of 0.325 or greater at a serum dilution of 1/200</td>
</tr>
</tbody>
</table>

In the case of the EIA test, the threshold was calculated as the mean optical density value of the 100th percentile of 2300 samples collected from *Brucella* free herds.

2.4. Estimates of diagnostic performance

Diagnostic specificity was calculated according to the following formula:

\[
\text{specificity} = \frac{\text{total number of negatives}}{\text{total number of cattle}} \times 100
\]
Relative specificity of a particular test was calculated according to the following formula:

\[
\frac{\text{number of test negatives}}{\text{total number of screening/confirmatory test negatives}} \times 100
\]

Relative sensitivity of a particular test was calculated according to the following formula:

\[
\frac{\text{number of test positives}}{\text{total number of screening/confirmatory test positives}} \times 100
\]

3. RESULTS

3.1. Diagnostic specificity

Estimates of diagnostic specificity based on the testing of 1082 cattle from Brucella free herds are shown in Table I for all tests. The RB, 2-ME, CF and EIA tests all demonstrated high and comparable specificity estimates in the range of 99.7-99.9%, whereas the BPA test demonstrated a lower specificity estimate (90.6%).

<table>
<thead>
<tr>
<th>Test</th>
<th>Negative</th>
<th>Positive</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>980</td>
<td>102</td>
<td>90.6</td>
</tr>
<tr>
<td>RB</td>
<td>1079</td>
<td>3</td>
<td>99.7</td>
</tr>
<tr>
<td>2-ME</td>
<td>1080</td>
<td>2</td>
<td>99.8</td>
</tr>
<tr>
<td>CF</td>
<td>1081</td>
<td>1</td>
<td>99.9</td>
</tr>
<tr>
<td>EIA</td>
<td>1079</td>
<td>3</td>
<td>99.7</td>
</tr>
</tbody>
</table>
TABLE II. ESTIMATES OF TEST SPECIFICITY (%) RELATIVE TO BPA AND RB SCREENING TEST NEGATIVES IN Brucella INFECTED DAIRY HERDS

<table>
<thead>
<tr>
<th>Test</th>
<th>BPA test negatives (2692)</th>
<th>RB test negatives (3744)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>—</td>
<td>71.8</td>
</tr>
<tr>
<td>RB</td>
<td>99.8</td>
<td>—</td>
</tr>
<tr>
<td>2-ME</td>
<td>99.8</td>
<td>94.2</td>
</tr>
<tr>
<td>CF</td>
<td>99.8</td>
<td>96.0</td>
</tr>
<tr>
<td>EIA</td>
<td>99.4</td>
<td>93.3</td>
</tr>
</tbody>
</table>

* Number of negative reactors out of 4765 cattle tested.

3.2. Relative specificity

Estimates of specificity relative to the two screening tests (BPA and RB) were based on the testing of screening test negative cattle from Brucella infected herds. As shown in Table II, estimates of specificity for the RB, 2-ME, CF and EIA tests relative to 2692 BPA test negative cattle were high and comparable, ranging from 99.4 to 99.8%. Lower and disparate estimates of specificity relative to 3744 RB test negative cattle were observed for the BPA (71.8%), 2-ME (94.2%), CF (96.0%) and EIA (93.3%) tests.

3.3. Relative sensitivity

All tests are compared with respect to estimates of relative sensitivity in Table III. Relative to the 2-ME, CF and EIA test positive cattle from Brucella infected herds, the estimates of sensitivity for the BPA test were high and comparable, ranging from 98.6 to 99.6%. In the same comparison, the relative sensitivity estimates for the RB test were lower and disparate, ranging from 79.1 to 86.3%.

When BPA test positive cattle were considered, the relative sensitivity estimates of the EIA (57.0%) and 2-ME (56.2%) tests were comparable and higher than the estimate for the CF (51.8%) test. The RB test demonstrated the lowest relative sensitivity estimate (49.0%) in this comparison.
TABLE III. ESTIMATES OF TEST SENSITIVITY (%) RELATIVE TO BOTH SCREENING AND CONFIRMATORY TEST POSITIVE REACTORS IN *Brucella* INFECTED DAIRY HERDS

<table>
<thead>
<tr>
<th>Test</th>
<th>BPA (2073(^a))</th>
<th>RB (1021)</th>
<th>2-ME (1169)</th>
<th>CF (1074)</th>
<th>EIA (1199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>—</td>
<td>99.5</td>
<td>99.6</td>
<td>99.5</td>
<td>98.6</td>
</tr>
<tr>
<td>RB</td>
<td>49.0</td>
<td>—</td>
<td>81.4</td>
<td>86.3</td>
<td>79.1</td>
</tr>
<tr>
<td>2-ME</td>
<td>56.2</td>
<td>93.2</td>
<td>—</td>
<td>96.8</td>
<td>92.8</td>
</tr>
<tr>
<td>CF</td>
<td>51.8</td>
<td>91.2</td>
<td>89.4</td>
<td>—</td>
<td>87.4</td>
</tr>
<tr>
<td>EIA</td>
<td>57.0</td>
<td>92.9</td>
<td>95.2</td>
<td>97.1</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\) Number of positive reactors out of 4765 cattle tested.

In a three way comparison amongst the confirmatory tests, the EIA (97.1%) and 2-ME (96.8%) tests demonstrated comparable sensitivity estimates relative to the CF test. The sensitivity estimate of the EIA test (95.2%) was higher than the estimate for the CF test (89.4%) relative to the 2-ME test. Similarly, the sensitivity estimate of the 2-ME test (92.8%) was higher than that of the CF test (87.4%) relative to the EIA test. Relative to each other, the EIA test (95.2%) demonstrated a higher sensitivity estimate than the 2-ME test (92.8%).

4. DISCUSSION

The purpose of this study was to compare the EIA test with two screening assays, the BPA and RB agglutination tests, and two confirmatory assays, the 2-ME and CF tests, in order to determine the potential application of the EIA test in brucellosis control and eradication in Argentina.

One of the principal requirements of any screening assay is that it must be as diagnostically sensitive as possible in order to ensure that all true serological reactors are detected. However, this also means that a fairly high rate of false positive reactions may be expected. Hence there is a need to use one or more confirmatory tests to reduce the number of false positive reactors. Confirmatory tests must therefore demonstrate a high level of diagnostic specificity and yet maintain an effective diagnostic sensitivity.
By definition, an estimate of diagnostic sensitivity requires that the true disease status of the animal be known. In the case of brucellosis, this would require the recovery of the *Brucella* organism from infected tissues or from milk by bacteriological means. Because this was not feasible, estimates of relative sensitivity were used in this comparison to make inferences concerning diagnostic sensitivity.

The results of this study clearly demonstrate that the BPA test is superior to the RB test as a screening assay. The relative sensitivity estimates of the BPA test were the highest in all comparisons, which would suggest that this test has the highest diagnostic sensitivity of the five assays being compared. In contrast, the relative sensitivity of the RB test was the lowest in any comparison.

Given the high diagnostic sensitivity of the BPA test, it is perhaps not surprising that its diagnostic specificity is somewhat lower. A false positive rate of approximately 10% was observed in the testing of *Brucella* free herds. Because of this false positive rate, it is evident that confirmatory testing would be required in a testing strategy involving the BPA test. Again in contrast, the RB test demonstrated a high diagnostic specificity. This, in combination with its low diagnostic sensitivity, precludes its use in the present control and eradication programme.

The BPA test, in addition to having satisfactory diagnostic attributes, fulfils the requirements of a screening assay in that it is technically simple, rapidly performed and inexpensive and has a high sample throughput.

The diagnostic specificity of the EIA test compared favourably with both the 2-ME and CF tests in spite of the fact that the latter two tests had been used to cull reactors from these herds prior to application of the EIA test. In addition, in the testing of BPA negative cattle from infected herds, the EIA test demonstrated a relative specificity which was comparable to those of the two conventional confirmatory assays.

The relative sensitivity of the EIA test was comparable to, if not slightly higher than, that of the 2-ME test and was higher than that of the CF test in all comparisons. This, in combination with its high diagnostic specificity, suggests that the EIA test could effectively replace both the 2-ME and CF tests in this control and eradication programme.

The EIA test, in addition to demonstrating good diagnostic performance, offers some distinct advantages over the 2-ME and CF tests. No pretreatment of sera is required, eliminating the use of 2-mercaptoethanol for destroying IgM in the 2-ME test and the need for heat inactivation of complement in the CF test. The EIA test is performed at a single serum dilution rather than in a titration series, as is done for the 2-ME and CF tests. One major drawback to the CF test is the requirement for a steady supply of sheep red blood cells and guinea pig complement.

The results of both the 2-ME and CF tests are subjectively read by eye, whereas the optical density values of the EIA test are measured photometrically. This eliminates reader error and bias and also lends itself to laboratory automation and computerization. The EIA test is capable of a higher sample throughput and the
results are achieved in a single day. Finally, the EIA test reagents are highly stan-
dardized, have a long shelf life and are cost effective at the low concentrations used in this assay.

This study demonstrates the potential diagnostic benefit of the EIA test as a confirmatory assay in the brucellosis control and eradication programme in Argen-
tina. In combination with a screening assay such as the BPA test, the EIA test would be cost effective and would introduce a high degree of standardization to brucellosis diagnosis in Latin America.

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VACCINATION AND ELISA: A PARTNERSHIP AGAINST AUJESZKY’S DISEASE

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Abstract

VACCINATION AND ELISA: A PARTNERSHIP AGAINST AUJESZKY’S DISEASE.

Aujeszky’s disease is an important disease of swine. Vaccination is widely practised to control this disease. Although most vaccines offer clinical protection against the disease, they only reduce but do not prevent virus excretion or the establishment of latency after infection. Therefore vaccination alone will not lead to eradication of the disease. For this reason several serological test and elimination programmes have been developed. These programmes require either that pigs are not vaccinated or that they are vaccinated with vaccines that evoke an antibody response in serum that can be distinguished from the antibody response after infection. Differentiation between vaccinated and infected pigs is possible with the use of vaccine strains that lack one of the non-essential but highly immunogenic viral glycoproteins in combination with a glycoprotein specific antibody assay. Three such assays have been reported, based respectively on the demonstration of antibodies against the viral glycoproteins gl, gX and gIII. Because all field strains express gl and because most vaccines are gl negative, the presence of antibodies to gl seems the most logical indicator for infection with field virus. Several enzyme linked immunosorbent assay (ELISA) techniques have been developed to detect antibodies to gl, based either on the principle of a blocking ELISA or on the principle of an indirect ELISA, using affinity purified gl as antigen coated to the wells of microtitre plates. The antibody response against gl after infection of vaccinated and non-vaccinated pigs has been examined in detail. Infected pigs not vaccinated or vaccinated with a gl negative vaccine develop antibodies against gl which appear to persist for more than two years. Even pigs with maternal antibodies deficient in antibodies to gl that become infected with low doses of mildly virulent Aujeszky’s disease virus develop antibodies to gl, albeit at low titres.

1. INTRODUCTION

Aujeszky’s disease (AD) is caused by suid herpes virus type 1, which is usually called Aujeszky’s disease virus (ADV) or pseudorabies virus. ADV can cause disease in many animal species, including swine, cattle, sheep, goats, rabbits, mink, dogs, cats, monkeys and horses. Swine are the natural hosts for ADV and the sole
source of virus transmission. The other animal species are dead-end hosts, possibly with the exception of horses. Horses, however, do not excrete virus after infection [1].

After infection, young pigs in unvaccinated populations usually die from a severe meningoencephalitis. In fattening pigs, respiratory signs are the most prominent symptom, while mortality is usually low. Pregnant sows may abort or give rise to stillbirth. Major steps in the pathogenesis of AD in pigs are initial virus multiplication in the oropharyngeal and nasal mucosa, uptake and transport of the virus by axons, and multiplication and spread of the virus in the central nervous system (CNS). In the CNS, the virus may cause neuron necrosis and inflammation. The virus may also disseminate by viraemia, but this route seems less important [2]. The virus further gives rise to latent infection of neurons, which is probably lifelong. After reactivation of latent ADV, infectious virus may again be excreted and transmitted to other pigs.

Vaccination is widely practised to reduce clinical signs. Live attenuated, killed and subunit vaccines are applied [3]. Piglets of vaccinated sows are protected by maternal antibodies. Although most vaccines offer clinical protection against the disease, they only reduce but do not prevent virus excretion or the establishment of latency after infection [4, 5]. As a result, vaccination of swine can limit the amount of virus circulating in vaccinated swine populations, but it will usually not lead to eradication of the virus.

2. ERADICATION

Various strategies have been developed to eradicate AD from pig populations [6]. Strategies include: (1) serological surveys to identify infected herds, slaughter of the infected herds and repopulation — England and Denmark, which have never permitted vaccination against ADV, have followed this strategy, and have now nearly eliminated ADV; (2) offspring segregation to produce ADV seronegative replacement gilts; and (3) serological testing and slaughter of seropositive animals. The last strategy has been recommended in herds in which the proportion of seropositive animals is low and when there was no evidence that virus was circulating between the sows of the breeding herd and their offspring [6].

All test and removal strategies, whether applied on a herd or country-wide basis, require that serological tests can identify pigs that have undergone infection, and that are probably latently infected, from uninfected pigs. Therefore, either vaccination must not be permitted, or only those vaccines must be permitted that evoke an antibody response that can be discriminated from the antibody response after infection. This review will concentrate on the use of such marker vaccines and the enzyme linked immunosorbent assay (ELISA) techniques that can discriminate pigs vaccinated with marker vaccines from infected pigs.
3. THE GENOME OF ADV AND ADV VACCINE STRAINS

The genome of ADV is a linear dsDNA molecule of 150 kbp and consists of a unique short (Us) region flanked by inverted repeat (Ir) regions and a unique long (Ul) region [7]. A physical map of the genome is depicted in Fig. 1. It encodes at least fifty proteins. The Us region is the best characterized part of the genome and has been completely sequenced. It contains the genes encoding a protein kinase (PK), the glycoproteins gX, gp50, gp63 and gI, and genes encoding an 11K and 28K protein [9-13]. The Ul region contains genes encoding the thymidine kinase (TK), and the glycoproteins gII, gIII and gH [11, 14-17]. Of these genes, only gp50, gII and gH are essential for virus replication in cell culture [18, 19]. Both Ir regions contain a gene encoding the immediate early (IE) protein [7]. Comparison with herpes simplex virus type 1 (HSV-1) has revealed homology between ADV gp50 and HSV-1 gD, between ADV gp63 and HSV-2 gI, between ADV gI and HSV-1 gE, between ADV 11K and HSV-1 US9, and between ADV PK and HSV-1 US3 [9-11, 13]. Several functions have been ascribed to these genes: gp50 probably plays a role in penetration of the virus into cells [19]; TK, PK, gp63, gI and gIII are important for

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**FIG. 1.** Physical map of the genome of ADV strain NIA-3. The genome can be divided into a unique long (Ul) region (at the left) and a unique short (Us) region bracketed by inverted repeats (Ir) (open rectangles). The lower line shows an enlargement of the BamHI-7 fragment containing the major part of the Us region. Cleavage sites for HindIII and BamHI are depicted, as well as the location of the genes encoding gII, gIII, TK, gH (Ul region), gX, gp50, gp63, gI and 11K (Us region), and the location and size of deletions in the vaccine strain 783 [8].
the expression of virulence of the virus, in contrast to gX, and the 28K protein [20–24]; gl appears to affect tissue tropism of ADV for pigs, presumably by facilitating the spread of virus through the CNS [24]. Inactivation of gl strongly reduces virulence for pigs, as evidenced by strongly reduced mortality, fever and growth retardation. However, a gl negative strain of ADV still had the ability to cause some pneumonic lesions in pigs [24]. Both gl and gIII are involved in virus release from infected cells [25, 26], and gIII further functions in adsorption of the virus to cells and in giving thermostability to the virus [25].

Several conventionally attenuated live ADV vaccines were obtained by culturing the virus in heterologous bovine or chicken cells. These vaccine strains harbour several mutations in different parts of the genome. A common change is a deletion of 3.6–4.4 kbp in the Us region which usually renders these vaccine strains a gl negative phenotype [6]. Because gl is involved in neurovirulence of ADV for pigs (see above), these vaccines are in general safe for swine but not necessarily safe for other animal species. Examples of these conventional vaccine strains are the Bucharest (BUK), Bartha and NIA-4 strains. In addition to the change in the Us region, these vaccine strains contain several characterized and uncharacterized mutations in the Ul region [6]. Because such mutations may reduce the immunogenicity of these vaccine strains and because it cannot be excluded that they may revert to virulence, several groups developed genetically engineered vaccine strains with deletions in the genes encoding gl, gIII or gX [8, 20, 27]. To further enhance their safety these vaccines have also been rendered TK negative.

Quint et al. [28] and Moormann et al. [8] constructed the TK and gl negative vaccine strain 783 by subsequently excising 2055 bp from the Us region and 19 bp from the TK region. They used the virulent and immunogenic NIA-3 strain as parent strain. Strain 783 also harbours a deletion of about 100 bp at the HindIII sites in both Ir regions. The deletion in the Us region contains the gl gene and part of the gene encoding the 11K protein. The size and location of the deletions in strain 783 are depicted in Fig. 1. Marchioli et al. [20] developed a gX deletion mutant from a TK negative parent strain. The parent strain was originally selected by growing the virus in the presence of bromodeoxyuridine. Kit et al. [27] excised 148 bp from the TK gene and 1100 bp from the gIII gene of a plaque purified clone of the BUK vaccine strain. The parent strain for the genetic engineering had a 3.6 kbp deletion in the Us region but had a gl+ phenotype [6]. The gl gene in this strain encodes a truncated polypeptide which apparently contains the major antigenic and immunogenic sites which have been identified in the N-terminal part of gl [29, 30]. Thus the phenotype of the final vaccine strain is TK−, gIII−, gl+.

4. IMMUNOGENICITY OF VIRAL PROTEINS

Knowledge of the role of ADV proteins in inducing immune responses and protection is gradually emerging but far from complete. Also, the mechanisms that
are responsible for protective immunity against ADV are unknown. They likely consist of both antibodies and cytotoxic T cells [24]. Major targets for antibodies are the viral glycoproteins. The glycoproteins gp50, gl, gII and gIII are expressed on the viral envelope and on the membranes of infected cells [31-33]. In contrast, gX is made as a cell bound precursor that is processed by proteolytic cleavage to an extracellular protein [34]. In convalescent pig sera, gIII has been identified as a major target for neutralizing antibodies, whereas no or low neutralizing antibody was directed against gX and gI [35, 36]. In addition, gIII has been identified as a target antigen for cytotoxic T cells in pigs and mice [36]. Results of antibody binding assays showed that pigs also raise antibodies against gI, gII, gX and the IE protein [37-39].

The role of different glycoproteins in inducing protection has been examined by active immunization with purified proteins, by active immunization with virus strains with a single inactivated gene, and by passive immunization with monoclonal antibodies (MAbs) directed against a viral protein. Immunization of pigs and mice with purified gp50 or gIII protected them against a challenge with virulent ADV, in contrast to immunization with gX [3, 23, 40]. Also, a fusion protein containing the N terminus of gl expressed by *Escherichia coli* has been reported to confer protection to mice against a lethal challenge of virus [30]. When pigs were immunized with ADV mutants that functionally lacked one of the non-essential genes in the Us region, the mutants that lacked gl or gX induced complete protection against a challenge with virulent ADV, in contrast to the mutants that lacked PK or gp63 [24]. Because PK and gp63 are reportedly not targets for neutralizing antibody and cytotoxic T cells, these data suggest that PK and gp63 are required for effective replication of the virus or for effective presentation of other viral antigens to the immune system. An important role for gIII in inducing protection is likely, because a gIII negative mutant virus was less effective in protecting mice against challenge with virulent ADV than gl or gX negative mutants [36]. Again, these differences may in part be explained by differences in virus replication in vivo, or by the different capacities of glycoproteins in inducing protection. Also, gIII has been identified as a target antigen for porcine and murine cytotoxic T cells [36]. Finally, MAbs directed against gII, gIII and gp50 passively protected mice and swine against a lethal ADV infection, whereas MAbs to gI protected only mice [3, 31, 41, 42].

5. **DISCRIMINATING ELISAs**

The use of live or killed vaccine strains that lack one or more of the non-essential but strongly immunogenic glycoproteins in conjunction with a serological test that detects antibodies against the protein which is not expressed by the vaccine strain allows the identification of infected pigs within vaccinated populations. So far, three such marker vaccine–test combinations have been reported, based respectively on gl, gX and gIII [37, 39, 43]. Antibodies against these proteins are usually measured by ELISA.
The use of a marker vaccine and its accompanying protein specific antibody assay should ideally fulfil the following requirements:

1. All field strains must express the gene that is deleted from the vaccine strain.
2. All field strains must evoke an antibody response to the gene product that is deleted from the vaccine strain. Also, partially immune animals that become infected with a low dose infection of a mildly virulent strain should evoke an antibody response to the marker protein and such antibodies should be present lifelong.
3. The diagnostic assay must be reproducible, sensitive and specific. The test must score positive with sera that contain low concentrations of the marker antibodies and negative with sera of uninfected pigs that have repeatedly been vaccinated with the marker vaccine.
4. The vaccine must be safe and highly efficacious.

In conclusion, the marker vaccine-test combination should allow monitoring of the spread of infection within infected herds and the identification of latently infected pigs within vaccinated pig herds. So far, most information has been collected for the gI marker and most of the above mentioned requirements seem to be fulfilled when a gI negative marker vaccine and a gI antibody test are used [3, 29, 30, 37, 44, 45]. Although the amount of expression of gI may vary in single plaque isolates of the field virus [46], all 300 field isolates of ADV examined so far expressed gI [3]. Because most vaccines harbour the gI deletion, the presence of antibodies to gI in pig sera would appear to be the most logical indicator of infection with field virus.

6. PRINCIPLES OF THE PROTEIN SPECIFIC ASSAYS

Most protein specific assays use the principle of a blocking ELISA with one or two MAbs directed against the viral protein to which antibody activity is measured. Van Oirschot et al. [37] developed a blocking ELISA that uses two MAbs directed against two separate discontinuous epitopes on the N-terminal part of gI. The antigen is a lysate of an infected cell culture treated with Nonidet P40. Sera containing antibodies to gI block the binding of gI antigen to one or both of the MAbs. Using this ELISA the antibody response to gI has been examined in detail [3, 37, 44, 47]. After infection, antibodies against gI can first be detected after two weeks and they remain detectable for 1.5 to 2 years. Two other blocking assays have been developed to detect antibodies against gI in which the antigen is directly coated to the wells of the microtitre plate [3, 48]. Mellencamp et al. [49] developed an indirect ELISA using affinity purified gI coated to the wells of the microtitre plates. In this ELISA exposed animals were detected as early as day 7 after field virus exposure.
McGinley and Platt [39] developed an indirect ELISA and a radio-immunoprecipitation assay to investigate the antibody response to gX. These authors used serum free tissue culture supernatant from cells collected 6 h after ADV inoculation as antigen directly coated to the wells of microtitre plates. This supernatant had been shown to contain a viral glycoprotein with the properties of gX. Antibodies that were gX specific appeared from 7 to 21 d after experimental infection. However, the ELISA failed to consistently detect antibody to gX in latently infected pigs [39].

Antibody against gIII has been measured in blocking ELISAs which utilize either a cloned gIII antigen [43] or a crude viral antigen and anti-gIII MAbs [48]. In the first test, gIII antigen was prepared from Triton X-100 extracts of tissue culture cells infected with a bovine herpes virus type 1 (BHV-1) vector, which harbours an insert of the ADV gIII gene in the BHV-1 genome. The test has been reported to detect gIII antibodies in pigs latently infected for six months or more. The test detects seroconversion to gIII by 7 d after primary infection and by 14 d after vaccinated animals are challenged with low doses (10^3 plaque forming units) of virulent ADV [6].

7. MONITORING INFECTIONS WITH PROTEIN SPECIFIC ANTIBODY ASSAYS

In laboratory experiments, Van Oirschot et al. [37] detected gl antibodies 2 weeks after experimental infection, whereas the virus neutralization test detected antibodies as early as 7 to 10 d after experimental infection with field virus. This discrepancy may result from the failure of the gl blocking ELISA to detect low affinity IgM antibodies [38]. Antibody titres to gl peak at 4 to 5 weeks after an experimental infection. They can be detected for up to 2 years after a natural infection. Vaccination with a gl negative vaccine before experimental infection reduced the gl antibody titre but did not seem to have an influence on the persistence of the gl antibody response [37]. The same was true when the animals were challenged with low doses of a mildly virulent strain of ADV [44]. Low titres of antibodies to gl were raised when pigs with high levels of maternally derived antibodies (deficient in gl antibodies) were infected with low doses of mildly virulent virus [47].

In field studies, Van Oirschot et al. [45] and Engel and Wierup [50] monitored the elimination of ADV from large swine herds. In the first study 10% and in the second study 96% of the breeding animals were gl seropositive at the start of the elimination programmes. In these herds a programme consisting of vaccination with gl negative vaccine, testing for gl antibodies and culling of gl positive pigs (not necessarily immediately after a positive test result) had been executed. Only gI seronegative replacement gilts were admitted to these herds. In another herd vaccinated with gl negative vaccine, a very limited spread of field virus was monitored although infected animals were not removed and 30% of the sows were gl seroposi-
tive at the start of the study [45]. As mentioned earlier, the presently available vaccines cannot completely prevent the transmission of virus and disappointing results have therefore also been reported. Kersten [51] monitored the spread of field virus in a large swine herd which had been thoroughly vaccinated with a gl negative vaccine. Within 9 months the percentage of gl seropositive sows rose from 4 to 97%, illustrating the failure of the vaccine and the success of the test.

Field studies with the use of gIII- and gX-specific antibody assays have not been reported so far.

8. CONCLUDING REMARKS

The development of gene deleted vaccines together with new detection methods has provided the opportunity of carrying out combined vaccination and monitoring programmes, either with or without removal of infected pigs. The practicability of eradication of ADV using strict and intensive vaccination of pigs with a gl negative vaccine and the identification of infected pigs using a gl ELISA is presently being examined on a large scale in several countries of the European Community.

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USE OF DNA PROBES IN ANIMAL DISEASE DIAGNOSIS

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Abstract

USE OF DNA PROBES IN ANIMAL DISEASE DIAGNOSIS.

Conventional approaches for detecting aetiological agents of infectious diseases include the isolation of microorganisms and their direct detection in pathological samples by microscopy and immunoassays. Although the availability of monoclonal antibodies has improved some of these techniques, none of them alone is completely reliable. The advent of genetic engineering has opened a new approach to the diagnosis of infectious diseases by permitting detection of the genetic blueprint of the causal agent. Since each pathogen has its own unique genetic material and because nucleic acid hybridization is based on the ability of DNA or RNA probes to hybridize to their complementary sequences, genomic DNA or RNA is an ideal target for specific diagnostic tests. This new technique will allow the detection of some organisms which are difficult to culture and can broaden the spectrum of diseases which can be diagnosed. In veterinary medicine, many infectious agents are now identified by nucleic acid hybridization and some examples are given in the review. However, the use of this technique is still limited to research laboratories. Indeed there are some drawbacks to the routine use of nucleic acid technology for diagnostic tests. Firstly, radiolabelled probes have a short half-life; they are hazardous and their handling requires special equipment. Secondly, non-radioactive probes are less sensitive than radiolabelled ones. Their use on crude samples can give rise to significant non-specific background reactions, which makes them less attractive for routine diagnosis. Improvements in their sensitivity and in the signal/background ratio may allow their wider application in the future.

1. INTRODUCTION

For a long time, the diagnosis of diseases caused by infectious agents was based mainly on:

— Direct microscopic observation of pathological specimens or body fluids,
— Reproduction of the disease in experimental animals,
— Cultivation of the causative agent in an in vitro system,
— Measurement of some biological constants,
— Titration of specific antibodies produced in an organism as a result of infection.
In most cases, none of these tests alone is satisfactory, for the following reasons:

— Microscopic examination is insensitive and sometimes very laborious; it does not allow a distinction to be made between pathogenic and non-pathogenic strains of the same species or sometimes between two closely related microorganisms.

— Variation of biological constants is not generally specific for a given disease and may be only an indication as to the causative organism.

— For cultivation of the agent in vitro and experimental reproduction of the disease, pathological specimens to be sampled must be kept in very good condition.

— Some microorganisms do not grow in vitro or, if they do, are difficult to culture. It will therefore take time to get a result.

— Laboratory animal experiments are expensive and time consuming.

— For some serological diagnoses, there was sometimes a need for paired sera: acute phase and convalescent phase sera.

Therefore, for the prevention and control of infectious diseases, it is necessary to have available sensitive, specific, rapid and practical diagnostic tests. In order to achieve this, a great effort has been put into improving immunoassays to increase:

— Their specificity by the use of monoclonal antibodies,

— Their sensitivity and rapidity by the introduction of radioimmunoassay and enzyme linked immunosorbent assay (ELISA).

Each technique has its advantages and disadvantages. Despite the fact that immunological tests are easy to perform, there are some limitations to their use. Indeed, the sensitivity of immunoassays is limited by the kinetics of the antibody-antigen reaction, a first order reaction whose kinetics cannot be influenced. Polyclonal sera sometimes lead to non-specific cross-reactions and the danger in the use of monoclonal antibodies is that the specific epitopes they recognize may be hidden by their environment, for example if the antigen is already in an immunocomplex. Finally, in the case of a latent disease, antigens may not be expressed and the immunoassay will not allow detection of the agent. There is still a need, therefore, for very sensitive, specific, rapid and practical diagnostic tests.

In this regard, nucleic acid hybridization techniques are very promising. This new approach to disease diagnosis is unique because it focuses on the genome of the organism and not its products, i.e. the proteins. The main feature of nucleic acids is the ability of two complementary single strands to associate into a duplex with efficient kinetics owing to the large number of hydrogen bonds involved in the reaction [1]. The forces which maintain this duplex are very strong and it is assumed that a nucleic acid hybrid is about $10^9$ times more stable than an antibody-antigen complex [2]. Under favourable conditions, the avidity of two complementary single
strands of 273 nucleotides in length has a $K_d$ of $10^{-23}$, as compared with only $10^{-5}$ – $10^{-9}$ for antibody–antigen complexes [3]. So the high affinity of two complementary nucleic acid strands for each other and the high specificity of their association make hybridization an ideal technique for the diagnosis of diseases. To achieve this, a suitable, easily detectable, label molecule should be attached to the nucleic acid probe.

2. DETECTION SYSTEM

The most common label used in DNA probe technology is a radioactive one ($^{32}$P, $^{35}$S, $^{125}$I) incorporated directly into the nucleic acid by one of the several techniques available. After hybridization, the duplex (probe–target) is detected by liquid scintillation counting or by autoradiography. This method is very sensitive and allows the detection of $\sim 10^4$ to $10^5$ molecules of target. The major drawbacks in using radioactive probes are:

- The short half-lives of the radioisotopes (14 d for $^{32}$P), requiring frequent preparation of a new probe;
- The requirement for special equipment for handling and storage of radioactive products;
- Safety procedures to prevent the health risks inherent in the use of radioisotopes.

The disadvantages of radioactive probes have provided the impetus to develop other systems for labelling nucleic acids. In 1981, Langer et al. [4] introduced a new system. Their method exploits the high avidity of biotin for avidin or streptavidin ($K_d = 10^{-15}$). The probe is labelled with biotin. After hybridization, it is detected by avidin (or streptavidin) to which is attached an enzyme able to transform a substrate into a coloured or chemiluminescent product [4–6]. In other non-radioactive detection methods, some nucleotides of the probe are modified and these modifications are recognized by antibodies coupled to an enzyme [7, 8]. In another system, anti-hybrid-DNA–RNA antibodies are used [9]. A direct detection technique is possible using a specific synthetic nucleic acid (an oligonucleotide) to which an enzyme is attached through a linker [10, 11]. This allows rapid diagnosis although it can be less sensitive than an indirect detection system.

The advantages of non-radioactive probes over radioactive ones are as follows:

- No special detection equipment required,
- Safety in handling,
- Long life (at least two years at $-20^\circ$C),
- Rapid detection (a few hours instead of one or two days for autoradiography).
3. TYPES OF PROBES

For a long time, probes used in nucleic acid detection have been mostly double stranded DNA (dsDNA) cloned from a genomic DNA or a cDNA bank derived from mRNA. The reporter molecule is introduced into dsDNA by nick translation or random priming. These two procedures yield a probe with high activity ($\geq 10^8$ counts $\cdot$ min$^{-1}$$ \cdot$ $\mu$g$^{-1}$ DNA if an isotope is used). Before hybridization, the dsDNA has to be denatured into single strands. During the hybridization procedure, two competing reactions occur:

- Hybridization of labelled nucleic acid to the target,
- Self-annealing of the probe.

The latter, unwanted reaction lowers the concentration of the probe available for hybridization to the target and therefore reduces the sensitivity of the reaction as well as its kinetics.

To avoid these disadvantages using dsDNA, single strands of RNA can be used as probes. DNA is cloned into a vector next to an SP$_6$ or T$_7$ RNA polymerase promoter. Then, using one of these polymerases, a complementary RNA (cRNA or riboprobe) is transcribed from one strand of the DNA [12]. During transcription, a reporter is introduced into the cRNA. By this procedure, it is possible to get a probe with high specific activity ($10^9$ counts $\cdot$ min$^{-1}$$ \cdot$ $\mu$g$^{-1}$ RNA with radioactive label). Such a probe is ten times more sensitive than dsDNA [13, 1]. One of the advantages of using an RNA probe is the possibility of reducing the background by digestion of the residual probe with RNase after hybridization.

The last type of nucleic acid probe in use is the labelled oligonucleotide. Many interesting features make this more attractive than the other types:

- No self-hybridization, in contrast to dsDNA.
- High specificity: an oligonucleotide of 20–30 nucleotides in length can differentiate two targets which differ by only one or two nucleotides.
- Rapid hybridization reaction.
- Inexpensive production.
- Excellent stability.

However, with oligonucleotides, hybridization and washing procedures should be carried out in a very precise way to ensure reliable results.

4. TECHNIQUES OF HYBRIDIZATION

Hybridization reactions are performed in three main formats: on a solid support, in solution and in situ.
Hybridization on a solid support is the most widely used technique. The target is immobilized, using various methods, on a membrane. To obtain the best results, the sample to be analysed is processed to purify the nucleic acid. It is then fixed onto a nitrocellulose or nylon membrane by slot blot or dot blot. Another method is to separate different fragments of nucleic acid, either complete or after digestion with suitable enzymes, by gel electrophoresis and to transfer them onto the membrane (Southern and Northern blot for DNA and RNA respectively). Immobilization of nucleic acid on the membrane facilitates the washing and the detection steps in the procedure.

Hybridization in solution is more efficient than solid support hybridization. The former goes to completion 5 to 10 times faster than the latter [14]. Indeed, all nucleic acids are free in solution so the chance that complementary sequences bind together is maximized [14]. However, after hybridization, the duplex must be removed. This can be done by hydroxyapatite beads which selectively bind nucleic acid double strands or by acid precipitation after digestion of excess probe. Sandwich nucleic acid hybridization is a technique which combines the advantages of both solid support and solution hybridization. A labelled nucleic acid hybridizes to the target in solution. Another probe, with a sequence complementary to the target next to the labelled one, is bound to a solid support and used to capture the hybrid. This technique is sensitive and can detect around 1 pg of nucleic acid in a crude material.

The last format used for the hybridization technique is in situ hybridization. The reaction is performed on a tissue section or a cytological smear fixed on a slide or a membrane. The sample is treated in a way which keeps the natural configuration of cells intact while allowing the penetration of the probe. This technique can be carried out after the traditional staining methods used in pathology. It provides information about the localization of an infectious organism.

5. THE NUCLEIC ACID DETECTION TECHNIQUE IN ANIMAL DISEASE DIAGNOSIS

To illustrate the applicability of nucleic acid probes in animal disease diagnosis, some examples are discussed below. For further examples, the reader is referred to previous reviews [15, 16].

5.1. Ruminant paratuberculosis or Johne’s disease

Johne’s disease is a chronic disease of ruminants caused by *Mycobacterium paratuberculosis*. Clinically, it is characterized mainly by wasting and by chronic and unresponsive diarrhoea. The control and management of this disease are difficult and complicated because clinical signs occur three or four years after an infectious contact but the infected animal can shed the bacteria long before the onset.
Moreover, there is no rapid and sensitive diagnostic method for identification of asymptomatic but infectious animals. Diagnosis of Johne’s disease relies mainly on the culture of the agent from faecal samples of suspected carrier animals. However, *M. paratuberculosis* grows slowly in vitro and it takes two to four months to get a complete result. There are other tests such as the intradermal johnin test, complement fixation and ELISA but they lack sensitivity and specificity [17]. A fragment of genomic DNA of *M. paratuberculosis* has been cloned into the bacteriophage M13 and used as a probe labelled with $^{32}$P. It detected as few as $10^5$ bacteria in faecal specimens from infected animals. The test detected 34.4% more positive samples than the culture method, whose efficacy is usually about 40% [17]. The new test takes only 72 h to complete.

5.2. Mycoplasmosis

Mycoplasma infections are common in all animal species. Clinically, they may be overt diseases or asymptomatic. Infected animals in the subclinical state are very dangerous because they may be one of the major sources of contamination. Depending on the causal mycoplasma, overt disease is characterized by respiratory symptoms, arthritis or mastitis.

Detection and identification of mycoplasmas are based on their cultivation from pathological specimens in association with biochemical and serological characterization. Because many of them are very difficult to culture, it takes two to four weeks, or even longer, to complete the identification. Therefore, many mycoplasmologists are working to improve the specificity and rapidity of tests for the diagnosis of mycoplasmas. Santa et al. [18] and Hyman et al. [19] have isolated, from genomic libraries, DNA clones that could be used for specific identification of *Mycoplasma gallisepticum*, one of the mycoplasmas causing respiratory disease in poultry. In small ruminant populations, contagious agalactia syndrome is characterized by mastitis, arthritis and keratitis. It is caused by *M. agalactiae* but two other mycoplasma species provoke the same syndrome although they have a pronounced pneumotropism: *M. mycoides* subsp. *mycoides* (large colony) and *M. capricolum*. Dedieu [20] has isolated a DNA clone which can permit the specific detection of *M. capricolum* when labelled with $^{32}$P or digoxigenin and used in DNA dot hybridization. However, this probe is yet to be tested on pathological samples.

5.3. Infection by *Escherichia coli*

Enterotoxin produced by some *Escherichia coli* is the cause of many cases of diarrhoea in young animals. There are different types of toxin and their identification is based on serological tests and on their toxicity on some cells in culture or in suckling mice. The genes encoding these toxins are carried by plasmids. They have been cloned and sequenced. From the sequence of the porcine heat stable enterotoxin,
oligonucleotides have been synthesized, coupled to alkaline phosphatase and used in dot blot hybridization for the identification of enterotoxigenic *E. coli*. The test starts with the growth of *E. coli* colonies on filter overnight at 37°C. After lysis treatment and the fixation of DNA onto the filter, the prehybridization and hybridization are carried out in 1.5 h. Thereafter, the filter is washed to remove the residual probe and the enzymatic detection gives a result within one hour for strongly positive samples [21]. Although this kind of probe is less sensitive than radiolabelled ones, it can be used in laboratories for common diagnostics in place of bioassays.

5.4. Trypanosomiasis

Trypanosomiasis is still a constraint to improved animal production in nearly all sub-Saharan countries, an area covering about 10 million square kilometres. It is a parasitic disease caused by different species of the genus *Trypanosoma*. The main vector of the parasite is the tsetse fly. Each subgenus of trypanosome is characterized by its localization in the fly. For epidemiological surveys and the determination of *Trypanosoma* infection, the vector is caught and dissected and its different organs are observed under the microscope for the presence of the parasite. This method is laborious and has inherent limitations in the number of flies that can be examined and in the sensitivity and specificity of trypanosome species identification. *Trypanosoma* genome contains repeated DNA sequences whose functions are unknown. Some of these DNA sequences have been cloned and used as probes for the identification of *Trypanosoma* at the level of subgenus or species. Their repetitive nature offers the advantage of high signal and hence high sensitivity [22–24]. As few as 100 trypanosomes can be identified by dot blot hybridization. Even the identification of individual trypanosomes is possible by in situ hybridization when a $^{32}$P labelled probe is used. For field use of the new test, the touch blot method is a simple procedure for diagnosis of the disease organisms. It involves excision of the fly to expose its internal organs (gut, for example), then squeezing them and touch blotting the contents, up to eight times, onto dry nylon filter. The DNA is then denatured and fixed. The filter could be stored at this stage for subsequent hybridization. However, for widespread application in the field this technique simply needs to be adapted for use with non-radioactive probes.

5.5. Anaplasmosis

*Anaplasma marginale* and *A. centrale* are two rickettsial haemoparasites of cattle. They are transmitted by ticks of different genera. Both cross-react. In contrast to *A. centrale*, *A. marginale* can cause a serious disease characterized by severe anaemia, abortions, weight loss and death. Animals which recover from an acute disease may become persistently infected and may remain a major source of contamination. Diagnosis of anaplasmosis is carried out by observation of stained blood
smeared under the microscope. However, carrier animals sometimes have very low parasitaemia undetectable by light microscopy. Such persistently infected animals can be identified by inoculation of their blood samples into splenectomized, susceptible recipients. This procedure is too expensive for routine diagnosis. Fragments of DNA of A. marginale or A. centrale have been cloned and some have been used as nucleic acid probes, but they allow detection only in the range of 8 ng to 100 pg of genomic DNA [25, 26]. Using a riboprobe synthesized from a DNA clone, the sensitivity has been dramatically improved and is about 10 pg of genomic DNA [27]. It can detect a parasitaemia 12 days before a microscopic examination can give a positive result. It allows the identification of 500–1000 infected erythrocytes in 0.5 mL of blood, equivalent to a parasitaemia of 0.000 025%, i.e. a sensitivity 4000 times better than one can expect with microscopy. So this technique can be used in the detection of carriers whose parasitaemia varies from 0.0025 to 0.000 025%. Complement fixation and immunofluorescence tests are also used to estimate the prevalence of A. marginale infection in cattle but they are less sensitive than the DNA probe method [28].

5.6. Foot and mouth disease

Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. It causes important losses in livestock production, so strict importation and quarantine measures in animal exchanges are adopted by many countries to exclude importation of the causal virus. Many diagnostic tests are in use to support these measures. They include animal inoculation with suspected samples, isolation of the virus in cell culture, complement fixation, the serum neutralization test and finally ELISA tests. With the advent of DNA technology, another approach has been looked at. Using cDNA probes corresponding to the gene of VP1 protein, McFarlane et al. [29] have detected 1 pg of viral RNA, which is equivalent to about $2.3 \times 10^5$ virions. This test is more sensitive than complement fixation and ELISA tests. The cDNAs used can differentiate between different serotypes just like the serum neutralization test [29]. In contrast to virus isolation in cell culture, nucleic acid probes can detect the presence of non-infectious viral mutants in FMDV carrier cattle. Indeed, it was not possible to recover the virus from oesophagopharyngeal fluids of some cattle 150 and 560 d post-infection although viral RNA was still detectable by dot hybridization using a probe corresponding to the polymerase gene [30].

5.7. Aujeszky’s disease

Aujeszky’s disease or pseudorabies is a viral disease of swine widely distributed throughout the world. It causes great losses among swine populations. After recovering from an acute phase of the disease, animals may be latently infected. From time to time, after physical stress or for some unknown reason, such an animal
can undergo viral reactivation which may or may not result in clinical disease but, in any case, the animal sheds the virus and contributes to its spread. In order to control and eradicate pseudorabies, it is necessary to identify latently infected animals. Evidence of latent pseudorabies virus (PRV) infection is demonstrated by repeated administration of corticosteroids, followed by isolation of the virus in cell culture and its identification by classical techniques. However, this procedure is not sensitive enough to allow an accurate diagnosis [31]. Nucleic acid hybridization using cloned PRV DNA has been tested for the diagnosis of acute and latent PRV infection. Biotin or digoxigenin labelled probes allow the detection of 1–5 pg of PRV DNA blotted onto a membrane [32]. Using an in situ hybridization format, it is possible to detect latent virus in tissues within 2 days; this technique is more sensitive than the explantation/co-cultivation method of virus isolation [33, 34].

5.8. Avian infectious bronchitis

Avian infectious bronchitis is a highly contagious respiratory disease of chickens. It causes high mortality among young chicks and can provoke a complete cessation of egg production in laying flocks. The causal agent is a coronavirus. It is diagnosed by amplification of the virus in embryonating chicken egg, followed by classical identification procedures: serum neutralization and haemagglutination tests. The in situ hybridization technique has been used to detect the viral RNA in tracheal and lung cells of chickens as early as 2 days after infection [35].

5.9. Bovine viral diarrhoea

Bovine viral diarrhoea (BVD) is the mucosal disease caused by a pestivirus. The virus is foetopathic if infection occurs early in pregnancy. Often, following foetal contamination, a status of persistent immunotolerant infection in post-natal life may occur. In such a case, the animal appears to be healthy although it may develop a mucosal disease after superinfection with another strain of the virus or may die after a chronic illness. This seronegative, persistently infected animal may be the major source of BVDV in nature. So, to control BVD, measures should be taken to identify and eliminate all infected animals. The only method available for detection of persistently infected cattle is virus isolation. The dot blot hybridization technique, using cDNA specific to BVDV, has been used to screen sera from different animals. Out of 141 tested samples, 55 were positive for BVDV. By comparison, virus could only be isolated from 25% of samples positive in the hybridization assay [36]. This discrepancy may be explained by:

— The presence of anti-BVDV antibodies in cell culture medium,
— The presence of the virus in complexes with antibodies.
Although it is known that buffy coat is the best sample for BVDV isolation, this virus may be present in serum, and the nucleic acid hybridization assay is the most sensitive technique for screening of BVDV contaminated sera.

5.10. Bluetongue

Bluetongue is a disease of sheep and cattle caused by an orbivirus. It is a source of important economic losses in sheep production. Traditionally, identification of the virus involved its isolation in egg or in cell culture, followed by its characterization using immunoassays. It takes at least two to three weeks to complete the entire procedure. There are 24 serotypes of bluetongue virus (BTV). The viral genome is composed of ten double stranded RNA segments coding for at least ten proteins. Among them, VP2, one of the outer proteins, displays a great variability and has epitopes specific to each serotype. The group specific immunological determinants are associated with the inner core proteins (VP1, VP3, VP4, VP6 and VP7). Different genes of BTV have been cloned and used for identification by nucleic acid hybridization techniques [37-41]. As expected, cDNA of VP2 is specific for the serotype while cDNA of VP3 is serogroup specific. However, it seems that there are a lot of variations within RNA3 (the genome segment coding for VP3) of BTV from different geographical areas, which render this segment unsuitable for serogrouping orbiviruses when used under conditions of high stringency [42]. However, it enables the geographical origin of the isolates to be determined. For the purpose of rapid diagnosis, a liquid format hybridization technique has been developed and allows detection of BTV within 3 h [37].

5.11. Rinderpest and peste des petits ruminants

Rinderpest virus (RPV) and peste des petits ruminants virus (PPRV) are two closely related viruses which infect ruminants, with PPRV being more specific to small ruminants. They are endemic in Africa, the Middle East and India. The distinction between the two viruses was made by:

- Animal inoculation. RPV causes disease in all ruminants but infection of cattle by PPRV is asymptomatic. This test is expensive.
- Virus isolation in cell culture followed by its characterization by the serum neutralization test. This procedure takes at least two to three weeks to complete, with no guarantee of success.

cDNA specific for the N protein of each virus, used in a nucleic acid hybridization test, can clearly differentiate the two viruses [43]. This technique has been used to identify, for the first time, PPR in India [44].
Success in the prevention, control and management of infectious diseases relies on the availability of sensitive, specific, rapid and practical diagnostic tests. Because none of the classical techniques alone, including microscopic observation and immunoassays, can satisfy all these requirements, another approach is now being tested: nucleic acid hybridization. The ability of a piece of DNA or RNA to detect its complement in mixtures with other nucleic acids provides an ideal basis for the diagnosis of infectious and genetic diseases. Immunoassays are now widely used for diagnosis of infectious diseases. However, their sensitivity is limited by the kinetics of antibody–antigen reactions and the quantities of immunoreactants present in the reaction. Hybridization of complementary nucleic acid strands is an efficient kinetic reaction owing to the large number of hydrogen bonds involved in base pairing and the stabilizing effect of the co-operative ‘stacking free energy’ between bases in the double helical structure [1]. This favourable kinetics enables a high degree of sensitivity to be achieved in nucleic acid hybridization reactions. Other advantages also make this new technique more attractive:

— Its high specificity, due to the fact that its target is the genetic material which is unique for each organism;
— The ability to detect latent infections in which antigen is not expressed or is expressed at a level below the threshold of immunoassays;
— The ability to detect microorganisms complexed with antibodies;
— The possibility of producing large quantities of stable probes (oligonucleotides).

However, despite all these advantages, nucleic acid hybridization techniques are still mainly confined to research laboratories in veterinary medicine. The use of radioactive reporters in the detection system has been a major obstacle to their wider application in the field. The improvement of non-radioactive probe technology would circumvent this stumbling block.

Simplification of the protocol by limiting it to its essential steps (lysis of samples, hybridization and detection of hybrids) would facilitate its application in the field and in poorly equipped laboratories.

ACKNOWLEDGEMENTS

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REFERENCES


AMPLIFYING GENES USING THE POLYMERASE CHAIN REACTION
A promising diagnostic tool

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Abstract

AMPLIFYING GENES USING THE POLYMERASE CHAIN REACTION: A PROMISING DIAGNOSTIC TOOL.

The power to amplify genetic material several millionfold using the polymerase chain reaction (PCR) has greatly enhanced the ability of molecular biologists to examine and manipulate genes. At the Australian Animal Health Laboratory, the authors are currently using this methodology to improve diagnostic capabilities and to aid research efforts into vaccines and the understanding of viral replication and viral diseases. The authors have used the PCR reaction to detect bluetongue virus (BTV) in infected animals and are currently able to serogroup, serotype and determine the geographic origin of a BTV isolate. Similarly, using a combination of hybridization analyses and direct sequencing of the PCR products they are able to rapidly detect avian influenza virus, Newcastle disease virus and Mycoplasma and predict if they have nucleic acid sequences that are characteristic of a virulent or avirulent isolate. The authors have developed several methods using synthetic oligodeoxynucleotides and PCR to amplify and clone viral genes of interest into a number of expression systems. This has enabled them to analyse important viral epitopes for vaccine production and for the generation of polyclonal monospecific antisera for use in diagnostic systems based on enzyme linked immunosorbent assay (ELISA). The ability to easily manipulate genetic information has made it possible to generate proteins containing deletions or create chimeric proteins which contain additions in their sequences. Such studies are important for the understanding of immune responses to various protein epitopes. Besides its sensitivity, PCR has the advantage of speed over some other detection systems. A comprehensive detection and diagnosis can be done in a few hours compared with several weeks previously required for virus isolations. However, there are disadvantages to using PCR. Because of its ability to amplify a sequence several millionfold, contaminants other than the target species may be amplified and since the DNA polymerase used in PCR has no editing or proofreading functions, errors may be quickly incorporated into the final PCR product.
A new technique in molecular biology termed the polymerase chain reaction (PCR) has been developed which affords great sensitivity and versatility for the detection of viral gene sequences and the manipulation of those viral genes for the development of new and improved diagnostic reagents.

The new technique employs a pair of chemically synthesized oligodeoxynucleotides or primers which are complementary to a gene sequence of interest and which flank either the entire gene or only those sequences of interest within that gene. DNA synthesis of the intervening sequences is done with a thermostable DNA polymerase, Taq polymerase [1]. After an initial round of DNA synthesis initiated by one of the primer pair, the duplex DNA strands are separated by heating to 94°C and the synthetic oligodeoxynucleotides are allowed to anneal to the single stranded DNAs at a temperature usually between 37 and 55°C. These DNA primers then initiate a further round of DNA replication at the elevated temperature of 72°C, which is the optimal temperature for the Taq polymerase. A further round of strand separation, annealing and DNA synthesis is initiated by varying the temperature between 94, 37 and 72°C. This cyclic procedure is repeated 25 to 40 times and results in the amplification of the target DNA gene sequence bounded by the two DNA primers by approximately \((1-5) \times 10^6\) times within approximately 4 h. The PCR process of gene sequence amplification has enabled researchers to detect gene sequences at the single copy level from individual cells, thus allowing the detection of viral gene sequences at levels not possible before and within a time-scale of hours.

2. DETECTION OF BLUETONGUE VIRUS

The Reoviridae family of viruses is divided into six genera. The orbivirus genus contains 13 serogroups with each serogroup further subdivided into a number of serotypes based upon virus neutralization tests. Within the orbivirus genus there are 24 separate serotypes of which 8 have been isolated in Australia. The genome of bluetongue virus (BTV) consists of ten double stranded RNA species, each of which encodes a single protein species. The genome is encapsidated in a bi-shelled coat, the inner core of which is composed of two proteins (VP3 and VP7). The outer coat is likewise composed of two proteins, VP2 and VP5, of which the former is serotype specific because it is capable of inducing specific virus neutralizing antibodies in an infected animal [2]. Similarly, antibodies directed against either VP3 or VP7 are capable of serogrouping orbiviruses [3]. However, serological classification of an orbivirus isolate can sometimes be confused owing to cross-reactions.
occurring between various serogroups [4]. During our studies we have accumulated a great deal of sequence data on both VP2 and VP3 genes [5, 6], which has enabled us to determine the evolutionary or phylogenetic relationships that exist between the orbiviruses (Figs 1 and 2).

It became apparent that for gene sequences of serogroup proteins there existed distinct geographic groupings (or topotypes) superimposed upon the previously determined serogroupings within the orbiviruses; in particular, for BTV and epizootic haemorrhagic disease of deer virus (EHDV). Members of a topotype varied by approximately 5% whereas when comparisons were made between topotypes the homology dropped to approximately 80%. Sequence homologies between the other serogroups dropped further, to 55–65%. Thus it became a challenge to design oligodeoxynucleotides that were capable of detecting all BTVs irrespective of geographic origin without cross-reacting with other orbivirus serogroups. Not only were we able to do this for serogrouping (Fig. 3(a)) but we also designed primers which were capable of determining the serotype (Fig. 3(b)) as well as the topotype of an isolate (Fig. 3(c)). However, it was apparent that for the detection of BTV in an infected animal it was often necessary to supplement PCR with a hybridization analysis of the PCR products to be able to detect very low levels of virus (Fig. 4). A more complete characterization of an unknown viral isolate was possible by actually sequencing the PCR product to generate sequence data for a viral gene. This has been done to analyse genes for sequence changes that may have occurred during attenuation of a virulent virus [7] and for topotyping an unknown isolate [8].

3. RAPID PATHOTYPING OF VIRAL ISOLATES

We have also been interested in the use of PCR to obtain sequence data of viral genes either directly from an infected animal or from a viral isolate (see above). The ability to directly sequence PCR products has enabled us to predict the probable pathogenicity of avian influenza virus (AIV) and Newcastle disease virus (NDV) isolates. With both of these viruses the cleavage of certain proteins (either the fusion or haemagglutinin–neuraminidase proteins of NDV or the haemagglutinin protein of AIV) is a prerequisite for the virus to infect the host cell [9, 10]. If these proteins are not cleaved they tend to be of moderate to low pathogenicity. Since the signals for proteolytic cleavage of the proteins are known, it should be possible to sequence over these regions to determine the amino acid sequence of potential cleavage sites and predict the probable virulence of a particular isolate. This is illustrated in Fig. 5 for the cleavage sites of NDV and AIV. In both cases alterations at the potential cleavage sites of these proteins were directly correlated with the known virulence of the isolate.
FIG. 1. Phylogenetic relationships within the orbivirus genus as determined by computer analysis of nucleotide changes in the gene sequences coding for the inner capsid protein (VP3). IBA denotes Ibaraki; BTV denotes bluetongue virus; EHDV denotes epizootic haemorrhagic disease of deer virus.
FIG. 2. Phylogenic relationships within the bluetongue virus serogroup as determined by computer analysis of the nucleotide changes between outer capsid protein (VP2) gene sequences. AUS denotes an Australian BTV, SA a South African BTV, US a USA BTV, F a field isolate of a BTV serotype 1 and V a virulent isolate of BTV1.
FIG. 3. Serogrouping, serotyping and topotyping of BTV isolates using PCR. (a) Serogrouping using primers based on VP3 gene sequences. 1, BTV1 from Australia; 2, BTV9 from South Africa; 3, BTV15 from Australia; 4, Warrego; 5, Eubenangee; 6, EHDV serotype 2 from Australia; 7, Corriparta; 8, Wallal; 9, Palyam; 10, Ibaraki (EHDV serotype 2 from Japan). λ represents DNA molecular weight markers derived from HindIII digestion of λ DNA. (b) Serotyping of BTV using BTV21AUS VP2 specific primers. The numbers 1 to 23 represent the Australian BTV serotypes 1, 3, 9, 15, 16, 20, 21 and 23. (c) Topotyping of BTVs using BTV15AUS specific primers. 1 and 4, BTV1AUS; 2 and 5, BTV15AUS; 3 and 6, BTV9SA. PCR was done on samples 1–3 at low stringency (37°C) and samples 4–6 at high stringency (65°C) of primer annealing.
FIG. 4. PCR amplification of BTV1AUS RNA3 sequences from blood fractions collected from individual sheep (sheep 1, lanes 1–4; sheep 2, lanes 5–8) at 9 days post-inoculation with BTV1AUS. (a) Agarose gel electrophoresis of PCR products. (b) Southern hybridization of DNA fragments in (a) using a $^{32}$P probe generated from BTV1AUS RNA3 sequences. Lanes 1 and 5, whole blood; lanes 2 and 6, platelets; lanes 3 and 7, buffy coat layer; lanes 4 and 8, washed, packed red blood cells; lane 9, cDNA transcribed from BTV1AUS RNA3.
4. EXPRESSION OF IMPORTANT ANTIGENS FOR DIAGNOSTICS

The PCR can produce a gene or gene fragment exactly tailored for expression in a number of plasmids to generate viral antigens as well as monoclonal or monospecific polyclonal antibodies. These can then be used in serological diagnostic tests. The advantages of such a procedure are severalfold. In the case of producing viral antigen either to raise antisera or to use in a competitive or blocking enzyme linked immunosorbent assay (ELISA), the antigen produced by recombinant DNA technology can be produced cheaply and free of other viral contaminants. In some situations the associated containment hazards involved with growing large quantities of viral antigen from tissue culture are removed. Similarly antisera can be raised which are monospecific without the necessity of extensive protein purification prior to antibody production. We have expressed the major capsid protein of BTV (VP7) in yeast cells [11] and tested its ability to detect and serogroup BTV in a blocking
FIG. 5. Amplification of the cleavage regions of (a) Newcastle disease virus fusion gene and (b) avian influenza virus haemagglutinin gene by PCR, and (c) nucleotide and deduced amino acid sequence analyses of these regions by DNA sequencing. 1, λ DNA HindIII markers; 2, V4 NDV; 3, Albiston–Gorrie; 4, Eaves–Grimes; 5, NDV (field isolate 90-0028); 6, H5N8, avian influenza. In (c) the cleavage regions are indicated by an arrow. Only amino acid differences relative to V4 or H5N8 are shown.

ELISA diagnostic test. This recombinant DNA antigen is quick to produce and when used in a blocking ELISA is able to detect and differentiate antibodies to BTV from those to EHDV, an orbivirus which in other serological tests can cross-react and confuse the diagnosis (Fig. 6). This methodology is easily adapted to other important epitopes or antigens.

5. DETECTION AND DIFFERENTIATION OF Mycoplasma SPECIES

Mycoplasma mycoides subsp. mycoides is the aetiologial agent of contagious bovine pleuropneumonia (CBPP). The disease is characteristic in cattle and easily
FIG. 6. Ability of yeast expressed BTV VP7 group-reactive antigen to discriminate between BTV and EHDV in a blocking or competitive ELISA. Columns represent sheep antisera to BTV serotypes 1–15 and antisera to EHDV serotypes 2, 5, 6, 7 and 8.

diagnosed once symptoms have developed. The organism can be isolated from nasal discharge, pleural fluid and in post-mortem from lung lesions, lymph nodes or bronchiopulmonary exudate. Identification of the organism is usually done by culture, biochemical characterization and serology, which can occupy some weeks of intensive effort. We have identified cloned DNA from *M. mycoides* subsp. *mycoides* which can reliably differentiate between the strains of this agent (Fig. 7(a)). From this clone we have developed a PCR based diagnostic procedure which can rapidly and sensitively identify this agent from the tissues of an infected animal (Fig. 7(b)). This procedure greatly decreases the time and effort needed to diagnose this disease.

6. MANIPULATION OF GENETIC MATERIAL

Since DNA synthesis in PCR is initiated with synthetic oligodeoxynucleotides, the possibility exists of manipulating the sequence of these primers to include either (1) deletions of genetic information or (2) additions to form chimeric molecules. This latter technique, called ‘splice overlap extension’ [12, 13], does not rely on the presence of restriction enzyme sites to ligate or join two DNA molecules but instead
FIG. 7. Detection and characterization of Mycoplasma mycoides subsp. mycoides using rDNA probes and PCR. (a) Southern blot of TaqI digested genomic DNAs from the Mycoplasma mycoides cluster using an rDNA probe derived from M. mycoides subsp. capri. 1, M. mycoides subsp. mycoides, large colony form; 2, M. mycoides subsp. mycoides, small colony form; 3, F38 mycoplasma; 4, M. mycoides subsp. capri; 5, M. capricolum; 6, bovine group 7 mycoplasma. (b) Detection of contagious bovine pleuropneumonia in bovine tissue samples using PCR and agarose gel electrophoresis. X, X DNA digested with HindIII restriction enzyme; 1, positive control M. mycoides subsp. mycoides (strain gladysdale); 2, total nucleic acid from lung tissue biopsy (animal 1); 3, total nucleic acid from lung tissue biopsy (animal 2); 4, sample as in 3 except diluted by factor of 10^2 prior to PCR; 5, sample as in 4 except diluted by factor of 10^2 prior to PCR; 6, tracheal swab from an experimentally infected animal; 7, synovial aspirate from an experimentally infected animal; 8, negative control bovine lung tissue.

relies upon the presence of overlapping homologous regions which are able to hybridize together during the PCR annealing step and serve as templates for DNA extension. This process is illustrated in Fig. 8, where a chimeric molecule consisting of four pieces was ligated using overlap extension and then trimmed using restriction endonuclease cleavage sites coded within the extension primers, to generate a molecule for insertion into an expression vector.
FIG. 8. Agarose gel electrophoretic analysis of PCR mediated DNA splicing to form genes coding for chimeric proteins. DNA fragments 1 and 2 were spliced to DNA fragments 3 and 5 to give fragments 4 and 6 respectively. Fragments 4 and 6 were then spliced to give fragment 8 by agarose gel electrophoresis. Fragment 8 was then digested with BamHI to give fragment 9, which was a chimeric, full length gene of fragment 2 (the native gene). M denotes λ DNA digested with HindIII restriction enzyme.

7. DISADVANTAGES OF PCR

During the development of the above procedures we have become aware of a number of limitations to the PCR procedure which may influence the outcome of the final result. Two of these are illustrated by attempts to amplify BTV gene sequences from the blood of infected sheep and also those of Mycoplasma from mucosal swabs of infected cattle. In the first case, washing or fractionation of whole blood (prior to PCR) removes an inhibitor, while simple dilution of the mucosal sample by two orders of magnitude decreases the concentration of an inhibitor in the mucosal sample so that PCR may give an unequivocal result for Mycoplasma detection. Also, each virus or agent to be detected by PCR has a different genome, i.e. double or single stranded DNA or RNA. In the case of BTV a double stranded RNA genome has first to be converted to a single stranded DNA copy (cDNA) prior to PCR. This step in the conversion of RNA to DNA is limited by the efficiency of the enzyme reverse transcriptase, which in turn lowers the ability to detect or even amplify a PCR product. Another problem is the actual number of PCR cycles which can be effectively done on a sample. In our hands (with certain primers) this cannot go past 35–40 cycles before spurious priming and DNA synthesis occur on either the cDNA
or the primers themselves; however, some samples can be taken to 60 cycles before this becomes a problem.

Lastly, the inherent inability of the thermostable Taq polymerase to edit or proofread its transcription product(s) can lead to the incorporation of errors into the final PCR product. If these errors occur early in the reaction then erroneous transcripts will accumulate such that they are a large percentage of the final product. This can decrease the ability to synthesize and express fully functional proteins or epitopes using the PCR approach.

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INTRODUCING DISEASE RESISTANT GENES INTO THE ANIMAL GENOME

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Abstract

INTRODUCING DISEASE RESISTANT GENES INTO THE ANIMAL GENOME.

Conventional breeding strategies for disease resistance in farm animals encounter many problems because disease susceptibilities often have low heritabilities and are not inherited in a simple manner in most cases (threshold characters), and there may also be negative correlations with other traits. Because of these principal disadvantages little progress has been achieved up to now. In the past few years techniques for gene transfer have been developed. These new breeding techniques allow modification of the genome by transferring gene constructs into somatic and germ cells of farm animals. Possible candidates for gene transfer of mammalian genes for improvement of resistance are discussed: MHC genes, T cell receptor genes, immunoglobulin genes, genes encoding lymphokines and specific disease resistance genes. Further experiments and studies are required before transgenic animals can be integrated in breeding programmes for improving disease resistance.

1. INTRODUCTION

The genetic complexity of the specific and unspecific humoral and cellular defence mechanisms against pathogens explains the variation in resistance to infectious agents between and within breeds. Indeed, there are many well documented examples of genetic variation in hosts correlated with susceptibility to pathogens. In farm animals genetic variation in resistance to nearly all kinds of infectious agents has been described for ecto- and endoparasites, bacteria, viruses and the unusual pathogen(s) causing scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle. Genetic variation in disease resistance of farm animals can be observed at all levels of defence against infectious agents.

Usually disease resistance has been and continues to be achieved in animals by vaccination and/or genetic improvement. Vaccination programmes have been used very successfully but there are also examples where they failed or could not be used because of non-availability of appropriate vaccines, logistic or management problems, especially in developing countries, immunosuppressive effects of vaccines or outbreaks of disease despite vaccination, or because vaccination programmes had to be stopped.
Vaccination and the use of drugs have failed to eradicate some of the major infectious diseases of livestock. Basic research into the mechanisms of disease defences will provide new approaches for eliminating severe diseases. Recently developed techniques in molecular biology have allowed the detailed study of the mechanisms of disease resistance and the linkage of known genetic loci with disease susceptibilities. Genetic engineering techniques can be used to clone specific disease resistance genes, which will be a prerequisite for the improvement of genetic disease resistance by employing new breeding strategies.

Conceptually, resistance to disease can be enhanced by at least four different genetic engineering approaches:

- Development of recombinant vaccines
- Development and production of recombinant therapeutic agents
- Development and usage of marker assisted selection strategies
- Transfer of recombinant genes into the animal genome.

After a short introduction to genetics and the problems with conventional disease resistance breeding programmes this paper will outline the principles of gene transfer in livestock and present an overview of possible attempts to improve disease resistance by the application of gene transfer techniques. A survey of methods allowing identification of disease resistance genes and their potential usage in farm animals are described elsewhere [1].

2. GENETICS AND EXAMPLES OF DISEASE RESISTANCE IN FARM ANIMALS

After the first studies at the beginning of this century [2] showing the existence of genetic variations in resistance or susceptibility to infections, in the 1930s evidence for specific disease resistance was deduced from findings that mice highly resistant to one infectious pathogen were highly susceptible to other infections.

Generalized disease resistance is relatively independent of the nature of the infectious agent. It is caused by the co-operative effects of many genes and strongly influenced by environmental factors. Antigenic drift of the pathogens usually has only minor or no effects on this polygenic type of defence mechanism. Such polygenic traits are found in trypanotolerant cattle breeds (e.g. N'Dama and West African Shorthorn), which, in addition to disease resistance, possess the capacity to tolerate heat, the ability to conserve water, and low maintenance requirements [3].

Disease resistance or susceptibility to a certain pathogen or disease is usually controlled by a major single locus but the defence mechanism may be modulated by unidentified loci, including genetic regulatory elements, and by environmental factors. The expression of the resistance locus may be a specific predisposing or conditioning factor among a series of factors. The mechanisms underlying resistance can
frequently be explained by the presence or absence of certain molecules in the host which are critical for infection or recognition or elimination of the pathogen. Thus, in contrast to general disease resistance, the trait depends more on genetic and antigenic drift of the pathogen (e.g. the major histocompatibility complex and disease associations [4], the resistance to neonatal diarrhoea due to *Escherichia coli* K88 in

**TABLE I. HOSTS FOR WHICH THERE IS EVIDENCE OF GENETIC VARIATION IN RESISTANCE TO A PARASITE [9]**

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<td><em>Hymenolepis citelli</em></td>
<td>Deer mouse</td>
<td><em>Boophilus microplus</em></td>
<td>Cattle</td>
</tr>
<tr>
<td><em>H. nana</em></td>
<td>Mouse</td>
<td><em>Ixodid spp. ticks</em></td>
<td>Cattle</td>
</tr>
<tr>
<td><em>Echinococcus multilocularis</em></td>
<td>Mouse</td>
<td><em>Ornithonyssus sylvarium</em></td>
<td>Fowl</td>
</tr>
<tr>
<td><em>Taenia taeniaeformis</em></td>
<td>Mouse, rat</td>
<td><em>Polyplax serrata</em></td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Melophagus ovinus</em></td>
<td>Sheep</td>
</tr>
<tr>
<td><em>Arthropoda</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIG. 1. Essential steps in the study of genetic resistance to disease and strategies for improvement of disease resistance [1].

pigs [5], and the susceptibility of mice to influenza viruses [6]). It is obvious that there are types of resistance or susceptibility which may not fit the two types defined above. Examples are non-specific defence mechanisms influenced by the expression of major genes such as the activity of lysozymes, interferons, phagocytes, etc., and also monogenic deficiencies causing general susceptibility.

Defence mechanisms against infectious diseases start at the sites of entry (physical–chemical and biological categories) and continue at the second level characterized by pathogen receptors. Defences preventing the establishment and reproduction of pathogens are produced mainly by general immune response mechanisms and the expression of the major histocompatibility complex (MHC). The resistance mechanisms at this level are very elaborate. After infection by a pathogen, macrophages activate diverse T and B lymphocytes which eventually initiate a
variety of humoral and cellular responses. A plethora of molecules that modulate immune reactions have been identified. They include interferons, neuropeptides, hormones and interleukins.

Other mechanisms provoking resistance to the establishment and reproduction of pathogens include natural killer cells that are present in the host regardless of its prior exposure to antigens against which they are active. Natural killer cells seem to play a role in resistance to Marek’s disease in poultry [7]. In cattle a major gene has been shown to regulate the lysozyme system, a general bacteriolytic mechanism [8].

Wakelin [9] has reviewed the genetic control of susceptibility and resistance to parasitic infections, examples of which are shown in Table I.

Examples of differential susceptibility to bacterial infections are resistance to mastitis in dairy cattle [10], resistance to neonatal scours in swine [5, 11, 12], resistance to pullorum disease in fowl [13] and resistance to fowl cholera [14]. The genetic control of resistance to Brucella spp. infections has been studied in swine [15] and cattle [16].

Genetic variations in protection against viruses have been observed for Marek’s disease [17], avian leukemia virus (ALV) and Rous sarcoma virus (RSV) in chickens [18–20], equine sarcoidosis [21], caprine arthritis encephalitis (CAE) [22], African swine fever [23], bovine leukosis virus (BLV) [24–26] and myxomatosis in rabbits [27]. Scrapie susceptibility in sheep also shows genetic variations [28, 29].

As shown in Fig. 1, several steps are necessary in the study of genetic resistance to disease [30]. For further explanations see Ref. [1].

3. PROBLEMS WITH CONVENTIONAL BREEDING PROGRAMMES FOR IMPROVEMENT OF DISEASE RESISTANCE

In many cases disease susceptibility varies discontinuously but is not inherited in a simple Mendelian manner. Animals may be afflicted or not afflicted, i.e. healthy or ill, and in spite of the existence of these two phenotypic classes in which continuous variation cannot be observed, any genetic analysis must be carried out quantitatively rather than qualitatively. These phenomena are interpreted by assuming the existence of unknown continuity causing factors which become noticeable above a certain threshold level. With endogenous variables somewhere below the threshold an individual will not appear to be affected, while values of the variables above the threshold will display the phenotype of an affected animal. The continuous variable in question has been termed liability. In contrast to the situation of a single individual, in a family, a group or a population of individuals a certain percentage of animals will be affected, depending on the incidence of the disease. For genetic analysis disease incidences must be converted to mean liabilities. Falconer [31] has
calculated the correlation of liability ($t$) between relatives of any specified sort and the heritability ($h^2$):

$$t = (x_p - x_R)/i; \quad h^2 = t/r$$

where $x$ is the normal deviation of the threshold from the mean, $P$ is population, $R$ is relatives, $i$ is the mean deviation of the number of affected individuals from the population mean, and $r$ is the coefficient of relationship.

As in conventional breeding programmes selection for threshold characters depends on the selection differential. Because there is no discrimination between animals of high and low liability within a phenotypic class the selected animals will be a random sample of the desired class. Selecting a smaller fraction than the incidence will therefore not provide an advantage. If the selected fraction is greater than the incidence, even animals from the undesired class will have to be used, and this will inevitably result in some negative selection. The success of selection for resistance could be improved if it were possible to change an 'all or nothing' disease trait into a trait with more classes or with an underlying continuous variation by discovering, for example, polygenic parameters which influence liability. If a trait like disease resistance must be included in a breeding programme animal breeders will have to consider the four criteria which they already use when dealing with productive or reproductive traits:

- Genetic variability and heritability
- Economic value
- Possibilities and costs of recording data
- Usage of marker traits and genes.

Shook [32] has discussed these four criteria with special attention given to disease resistance traits. Although disease trait heritabilities are normally low, the genetic variation of disease incidences is economically important and justifies the inclusion of disease traits in breeding programmes. Unfortunately standards for recording and accumulating field data for disease have not yet been set up [32]. However, several systems for collecting disease data in breeding populations have been developed and tested recently [33, 34]. If these systems were applied on a wider scale similar to those used for milk yield recording a plethora of data concerning disease and treatment of breeding animals would soon be available. These data could then be pooled with information about relationship and breeding values in traits under selection, data about fertility and productive traits, and data on characterization of management levels in herds and farms. Improvement of disease resistance in livestock by genetic means is a difficult and time consuming task requiring long term strategies. Progress in resistance breeding is limited and delayed by a number of factors:
— Lack of efficient tools and recording systems
— Inadvertent enhancement of susceptibility to a disease by selection for specific resistance to another disease
— Lack of strategies allowing selection for overall resistance.

Selection for disease resistance by employing conventional breeding strategies has at least two advantages:

— All genetic host factors influencing resistance or susceptibility are automatically included.
— Selection for the resistance trait is independent of shifts in environmental factors and disease profiles over time.

The principal disadvantages of direct breeding methods are introduced by the following factors:

— Low heritability of disease traits (especially when recorded as all or nothing responses), thus necessitating expensive progeny testing with prolonged generation intervals;
— Age and sex restriction of disease traits, which may also affect the generation interval;
— Heterogeneity of disease traits, which may also be moderately defined;
— Genotypes which result in high productive yield but which may increase susceptibility to severe diseases (e.g. slight correlation between milk yield and mastitis).

4. PRODUCTION OF TRANSGENIC ANIMALS

During the past few years the technique of gene transfer into mice has been developed to such a degree that the generation of transgenic mice is a routine experiment in many laboratories. However, for many reasons the production of transgenic farm animals is still a laborious project involving great expense. Firstly, there are the biologically determined differences between species which lead to various problems such as the isolation of sufficient numbers of oocytes, the injectability of the pronuclei, and the ability of the transferred embryos to undergo further development. In comparison with mice, experience in working with farm animal embryos is less developed, and there is a lack of suitable genetically characterized breeding lines. These facts reduce the success rates in programmes aimed at the generation of transgenic farm animals. Major obstacles for efficiently tackling the problem, however, are the enormous expenditure required for animals and personnel and the duration of the experiments caused by the very long generation intervals.

It is conceivable that animal breeders have tried to adopt this new technique quickly. The first successful generation of transgenic rabbits, pigs and sheep was
described as early as 1985 [35, 36]. Activities in this field have increased tremendously. It must be said, however, that so far there has been no direct impact on practical animal breeding. As a matter of fact this is not astonishing if one considers the time required to carry out these experiments [37]. It should be mentioned also that the spectacular successes achieved in gene transfer experiments in mice have no direct comparable counterpart with regard to farm animals. The more we learned about gene transfer in farm animals the more it became clear that there are still a number of unsolved problems.

The only successes in work concerned with experiments to generate transgenic rabbits, pigs, sheep, goats and cattle published so far were those in which gene transfer was achieved by DNA microinjection into pronuclei of zygotes.

In principle three different approaches for producing transgenic animals are available:

— Microinjection of DNA into pronuclei of zygotes
— DNA transfer by means of retroviral vectors
— Generation of transgenic chimeras by injection of genetically transformed embryonic stem cells into embryos.

Gene transfer is aimed at creating organisms in which as many cells as possible of an individual will carry the new gene. Therefore, the transfer of the new gene into the organisms must be undertaken very early in its development. Microinjection of DNA is virtually carried out either with fertilized oocytes (zygotes) or with two-cell stages.

A gene transfer programme (Fig. 2) can be subdivided into six stages:

1. Preparation of suitable donor animals, collection of zygotes (fertilized oocytes) and visualization of pronuclei (particularly in cattle and pigs);
2. Preparation of the DNA solution to be injected;
3. Microinjection of DNA into the pronuclei of zygotes;
4. Transfer of injected zygotes into the oviducts of synchronized recipients;
5. Detection of integrated and expressed transgenes;
6. Generation of transgenic progeny by conventional breeding techniques and establishment of homozygous transgenic lines.

The isolation of fertilized oocytes is an extremely laborious process because no non-surgical techniques are available. It requires surgical opening of the abdominal cavity by making an incision in the median line, which allows flushing of the oviducts. Surgery is also associated with great expense in terms of technical supplies and personnel. An alternative is to obtain embryos from slaughtered superovulated animals. Reproductive organs may be obtained easily and quickly during the normal process of slaughtering. Oocytes are isolated by flushing the oviduct approximately 12–24 h after fertilization. They are washed several times and can be kept in standard culture medium.
In pigs and cattle the pronuclei in fertilized oocytes are invisible because they are covered by lipid-containing dark granulae. They can be visualized by centrifugation of the oocytes at 15 000g for 3 min. During centrifugation granulae will migrate to one pole of the oocyte cell. Separation of cellular components allows visualization of the pronuclei [38].

The gene constructs required for injection are cloned in plasmid or cosmid vectors. They can be purified from the vector component by cleavage with restriction endonucleases, followed by extraction, precipitation and dissolving in injection buffer. All solutions used for microinjections must be filtered sterile to remove particulate matter to avoid problems during the injection process. The concentrations
of the DNA solutions used for microinjection are adjusted to contain approximately 1000 copies of the gene construct per picolitre.

The most suitable set-up for carrying out microinjections consists of an inverted microscope and two Leitz micromanipulators. A microscope slide containing one drop of culture medium which is covered with paraffin oil is placed under the microscope. The manipulator at the left hand side carries the holding pipette, while the injection pipette is fastened to the other manipulator. The injection pipette is filled with DNA solution and then connected by a silicone rubber tube to the injection apparatus. The zygotes to be injected are placed in the drop of culture medium, held by the holding pipette and turned, if necessary, until the pronuclei are in the correct position. The injection pipette is then used to penetrate the zona pellucida, the cellular membrane and the membrane of one of the pronuclei and to inject the DNA. Successful injection is indicated by an increase in volume of the pronucleus. Following injection the oocytes are transferred to a culture dish, where they can be inspected after a short time to select degenerated zygotes.

As shown in Table II, there are many differences between species regarding the reproductive characteristics important for the generation of transgenic animals. The time required for the specific steps is also quite different. For example, in cattle it takes about 10 years before a transgene will be usable in the population.

Surviving zygotes are transferred into the oviducts of synchronized recipients, for which surgery is mandatory. A possible simplification of gene transfer programmes arises from the fact that injected oocytes may be cultivated in vivo for several days using the oviducts of pseudopregnant rabbits, sheep, pigs or cattle. Embryos can be recovered by flushing the uterus of these intermediate recipients and can be evaluated with respect to their development. Normally developed embryos (morulae–blastocysts) may then be transferred into synchronized recipients by conventional techniques.

Tissue samples are collected from animals that have developed from microinjected zygotes. They will be tested by genetic analysis for the presence of the injected DNA. Proof of the stable integration and expression in a host animal of a gene construct introduced by microinjection techniques is obtained in three steps:

— Detection of the microinjected gene construct
— Isolation of cytoplasmic RNA and Northern blot analysis
— Translation studies.

Integration of DNA into the host genome is a random process, i.e. the number of integrated copies and the integration sites cannot be determined before the experiment. The efficiency of gene transfer programmes is generally low. It may be assumed, however, that gene transfer programmes will be improved in future years.

Little is known about the processes taking place during the integration of foreign DNA into the host genome. It is also unknown when integration occurs and whether integration is stable during the first division of an embryo. It has been estab-
TABLE II. COMPARISON OF IMPORTANT REPRODUCTIVE DATA AND TIME SCHEDULES FOR GENERATION OF TRANSGENIC ANIMALS IN DIFFERENT MAMMALIAN SPECIES

<table>
<thead>
<tr>
<th></th>
<th>Mice</th>
<th>Rabbits</th>
<th>Pigs</th>
<th>Sheep</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of injectable eggs per superovulated donor</td>
<td>15</td>
<td>20</td>
<td>15</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Number of donors per recipient</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Animals born/injected embryos (%)</td>
<td>10-20</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Integration frequency (%)</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>5-10</td>
<td>5-10</td>
</tr>
<tr>
<td>Transgenic animals/injected eggs (%)</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Time schedule (months)

<table>
<thead>
<tr>
<th></th>
<th>Mice</th>
<th>Rabbits</th>
<th>Pigs</th>
<th>Sheep</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parturition of F0 animals</td>
<td>0.75</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Generation interval (F1 animals born)</td>
<td>2.5</td>
<td>6</td>
<td>12</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Homozygous F2 animals available for breeding</td>
<td>3.25</td>
<td>7</td>
<td>16</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>Usage of transgenic animals in population (F3)</td>
<td>10</td>
<td>23</td>
<td>50</td>
<td>70</td>
<td>130</td>
</tr>
</tbody>
</table>

lished, however, that mosaics may be obtained in spite of DNA being injected into the pronuclei of a zygote. Mosaics are defined as animals consisting of different cell lines, although all of them derived from the same zygote.

Transgenic mosaics are composed of cells which harbour the transgene and those that do not. If the cells of the gonads do not contain transgenes, they may not be passed on to the offspring.

Transgenic animals which harbour the gene construct in their germ cells transmit the transgene to their offspring. These genes are normally inherited as Mendelian genes. Normally integration occurs only at one site within a chromosome. These primary transgenic animals have therefore been termed hemizygous. By definition they are not heterozygous because the transgenic allele is missing from the homologous chromosome. Statistically approximately 50% of the offspring of a transgenic animal whose gonads are not mosaics will inherit the transgene.
If the gonads contain transgenic and non-transgenic cell lines the frequency of transgenic offspring may be between 0 and 50%, depending on the degree to which the transgenic cell lines contribute to the composition of the gonads. This problem is only encountered in the offspring derived from the primary transgenic animal. If a transgenic F1 generation has been obtained, the transgenic offspring will harbour the gene construct in all its somatic and germ cells. The only uncertain factor is introduced by the instability of the integrated transgene. Some cases have been described in which the transgene was lost. It has been assumed that the integrated foreign DNA may be removed from the genome by repair mechanisms. In some cases this may lead to offspring, none of which would be transgenic.

There are relatively few exceptions of animals containing more than one integration site for a transgene. If the distance between these sites is great enough to allow random recombination more transgenic offspring may be generated. A transgenic animal harbouring two independent integration sites would transmit the transgene to 75% of its offspring; 25% of these would inherit both transgenes, 25% would harbour one or other of the two integration sites and 25% of the offspring would not be transgenic.

By mating hemizygous transgenic animals one would normally obtain 25% homozygous transgenic, 50% hemizygous transgenic and 25% normal offspring. Since integration is a random event one might also expect cases in which the transgene has been positioned within a gene which is, for example, essential for development. No problems should be encountered as long as an intact allele is retained functional on the homologous chromosome. An exception would be additive genetic effects if integration occurred in one of these genes, thus leading to a reduced expression of its allele.

No transgenic offspring is to be expected in matings between hemizygous animals carrying an insertion mutation if the gene that has been affected by the integration event is essential for further development of the foetus. Hemizygous lines are able to be bred by mating with normal mice and have led to 50% healthy transgenic offspring.

The following is a brief summary of points that must be expected from a transgenic breeding line:

— Integration of the transgene in all cells
— Stable transmission of the transgene to offspring
— Expression of the transgene (mRNA; protein)
— Biological activity of the protein expressed from the transgene.

Taking into consideration the experience gathered so far it can be estimated that approximately five transgenic animals will have to be produced under normal conditions in order to guarantee with sufficient probability the generation of a stable homozygous transgenic line displaying good expression of the transgene and possessing the expected phenotype.
5. GENE TRANSFER APPROACHES FOR IMPROVEMENT OF DISEASE RESISTANCE IN ANIMALS

Five classes of mammalian genes are currently seen as possible candidates for gene transfer experiments because they have been implicated in regulating disease resistance [1]:

— MHC genes
— T cell receptor genes
— Immunoglobulin genes
— Genes encoding lymphokines
— Specific disease resistance genes.

Most gene transfer experiments with the listed mammalian genes have been done in mice and will not be reviewed here. Only some examples for using gene transfer in farm animals for improvement of disease resistance are mentioned below.

5.1. Intracellular immunization of mammals against virus infections

An interesting approach to establish the resistance to virus infections in mammals is known as 'intracellular immunization' [39]. Baltimore [39] has suggested applying the strategy of intracellular immunization for gene therapy in order to overcome viral diseases against which conventional immunization has proven to be difficult.

Studies involving the use of cultured cells infected with virus have shown that endogenously produced viral proteins, and also their mutated forms that predominantly interfere with the corresponding wild type virus proteins, protect against infections by the cognate virus. By using transgenic chicken that expresses the ALV envelope glycoprotein, Salter and Crittenden [40] have demonstrated the defence against ALV.

5.2. Monoclonal antibodies expressed in transgenic animals

A further possibility for protection of animals against infectious diseases could be a strategy called 'genetic immunization'. It is based on the expression of definitive antibody genes in transgenic livestock. As shown by many investigations, cloned genes of monoclonal antibodies can be expressed in large amounts in transgenic mice after transfer of suitable gene constructs into the germ line. These mice produce antibodies against specific antigens without any prior immunization or contact [41]. Most experiments in mice have focused attention only on genes for heavy or light chains of antibodies and the primary events induced at the genomic level rather than making a full study of the exact composition of secreted antibodies. In an attempt to extend these investigations to other animals, genes for the light and the heavy
chain of a mouse monoclonal antibody directed against 4-hydroxy-3-nitrophenyl-acetate were introduced not only into mice but also into the germ line of rabbits and pigs [42]. Serum antibody titres have been achieved in transgenic rabbits and pigs of 100 and 1000 µg/mL respectively [42]. Unfortunately, only a few bands observed in isoelectric focusing gels were identical to those of the purified mouse antibody. One possible explanation may be that the levels of κ chain expression were insufficient for complete allelic exclusion. The incorporation of additional sequences might be helpful in overcoming this problem. In principle it should be possible in the future to develop gene constructs allowing expression in transgenics sufficient to protect animals against severe attack by viruses or bacteria in a manner equivalent to immunization. This strategy would be particularly useful for protection against diseases where vaccination is not allowed, difficult or impossible.

5.3. Transgenic animals with antisense RNA genes directed against viruses

The use of antisense polynucleotides provides another powerful possibility for protection against viral infections [43, 44]. In prokaryotes a large number of natural antisense RNAs (asRNA; also complementary RNA, cRNA) have been found to regulate, among other things, the replication of plasmids, or the translation of mRNA of bacteria, bacteriophages and transposons [45, 46]. In eukaryotic cells asRNA molecules have been discovered in both nuclear and cytoplasmic extracts [45]. The functional importance of these RNAs is still unknown and genes encoding asRNA have not yet been identified. The modulation of gene activity in eukaryotic cells by antisense transcription is associated both with the inhibition of mRNA translation and with the disruption of splicing and transport of mRNA into the cytoplasm.

The ability of asRNAs and complementary oligodeoxynucleotides to inhibit replication of viral genomes and expression of their genes has been demonstrated for a number of animal, plant and bacterial viruses in cell culture experiments [47]. The attempt to create an antiviral state in animals based on asRNA transgenes seems to be a logical consequence of the cell culture experiments [47, 48].

Ernst et al. [48] have described the generation of transgenic rabbits expressing an asRNA gene directed against adenovirus h5 (Ad5). The asRNA gene was controlled by the murine metallothionein-1 (MT-1) promoter. Despite rearrangements, deletions, amplifications and a reduction of copy numbers observed during the generation of transgenic founder animals and transgenic offspring, some rabbits had integrated intact asRNA gene copies and stably transmitted the transgenes to their offspring. The resistance to Ad5 infections was tested in primary kidney cell cultures of transgenic rabbits and non-transgenic control animals. Cell lines correctly processing the asRNA transgene have been estimated to be 90–98% more resistant to Ad5 than normal kidney cell lines.

The gene constructs used so far have to be improved to guarantee their stability in the host genome. In addition the use of regulatory elements which are naturally
active during defence against infections (e.g. promoters of interferon (IFN) genes or IFN dependent genes) may be preferable to constitutive or housekeeping gene promoters such as the metallothionein promoter.

5.4. Transgenic animals with the murine influenza resistance gene Mx1

Orthomyxovirus infections are common and occasionally cause epidemic diseases, not only in man, but also in a number of domestic animals, such as chicken (fowl plague), horse (epizootic cough, equine influenza) and swine (hog flu, swine influenza) [5]. In the mid-1980s the cloning and functional characterization of the anti-influenza gene Mx1 in mice [49] and the progress made in the transfer of genes into farm animals [35, 36] presented the promising possibility of attempting to improve disease resistance characteristics in farm animals by gene transfer. In the first series of gene transfer experiments in pigs, the gene constructs consisted of the murine Mx1 cDNA placed behind the human metallothionein IIA promoter or the SV40 early enhancer/promoter (C. Weissmann and M. Noteborn, Institute of Molecular Biology, Zurich, personal communication, 1986). The results of these experiments indicated that high expression of the Mx1 protein during embryogenesis may be deleterious: the efficiency of gene transfer with the SV40 early enhancer/promoter Mx1 gene construct decreased dramatically in comparison with results obtained with other gene constructs [50]. All transgenic pigs harbouring the Mx1 constructs either had extensive rearrangements that abolished Mx1 protein expression or did not express Mx1 protein despite the fact that the insertion was intact and stable (M. Müller et al., unpublished data). Arnheiter et al. [51] have made similar observations in their attempts to generate transgenic mice constitutively expressing Mx1 protein.

The use of a virus-responsive regulatory element — the murine Mx1 promoter itself [52] — linked to the Mx1 cDNA for microinjection into pronuclei of fertilized porcine oocytes has yielded eight transgenic pigs. The efficiency of the gene transfer (transgenic piglets/embryos transferred) was 0.5% [50]. The correct integration of the gene construct and the stable transmission to progeny have been examined by differential restriction analysis, Southern blotting and the polymerase chain reaction (PCR). The transgenic founder animals contained 10 to 30 intact integrated copies and transmitted the transgenes stably to their offspring. The inducibility of the transgenes has been determined in vitro by culturing peripheral blood lymphocytes (PBLs) which have been treated with native porcine IFNs or double stranded RNA (poly[I]-poly[C]), and in vivo by intravenous application of porcine IFNs \((2-5) \times 10^5 \text{ U IFN/m}^2 \text{ body surface}\) (M. Müller et al., unpublished data). After IFN induction, total RNA of PBLs was prepared and subjected to Northern analysis using probes of the gene construct. Two founder animals and their offspring showed an IFN-inducible increase of transgene mRNA levels.
Protein analysis of Mx1 gene constructs has been carried out by methionine labelling of cultured blood cells, followed by immunoprecipitation of proteins with anti-mouseMx antibodies and by indirect immunofluorescence of tissue sections derived from piglets treated in vivo by IFN. However, no Mx1 protein was found in our transgenic pigs. Transgene expression and inducibility depend on the position of the transgene in the genome. What is important for the resistance to influenza virus in transgenics is the levels to which Mx1 protein can be induced. High responders are protected against virus infection, while low responders are not protected, or are protected only when infected with high virus doses. This indicates that only the efficient transgene induction is capable of producing the desired effect.

6. CONCLUSIONS

Up to now only a few attempts have been made to improve disease resistance by gene transfer techniques in farm animals. As shown in this review there are at least five different candidate systems for enhancing resistance. In spite of the possibilities many experiments still remain to be done in this area.

It will also be necessary to do more studies on the mechanisms of gene regulation and the interplay of the defence molecules, before such systems could be implemented routinely in breeding programmes.

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USE OF RECOMBINANT DNA IN THE DIAGNOSIS AND CONTROL OF BABESIOSIS

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Indooroopilly, Queensland,
Australia

Abstract

USE OF RECOMBINANT DNA IN THE DIAGNOSIS AND CONTROL OF BABESIOSIS.

The potential of a vaccine against babesiosis using antigens produced through recombinant DNA technology has been experimentally demonstrated in the control of Babesia bovis infections in beef cattle. In order to develop this vaccine, the antigens first had to be identified using the only secure method available: through fractionation of the antigen and vaccination trials using the normal host. In the penultimate step of purification, a series of monoclonal antibodies were generated with the protective fraction and used as affinity ligands to obtain homogeneous antigen. In this manner, several protective antigens have been identified and characterized. These monoclonal antibodies, and/or synthetic oligonucleotides based on amino acid sequence information, were used to isolate the appropriate genes from cDNA libraries prepared from B. bovis mRNA. Fusion proteins of the antigens produced by overexpression in Escherichia coli were shown to be effective in reducing the development of babesiosis in cattle as determined by a number of parameters. Notably, vaccination with each of the antigens led to at least a 10-fold decrease in the maximal parasitaemia developed. When these antigens were used together in a small field trial, only 10% of the animals required treatment to prevent severe illness, compared with 40% of control animals. Using DNA hybridization techniques, one of these antigens (the 12D3 antigen) has been found in other species of Babesia, including B. bigemina. Thus a candidate protective antigen for use in controlling B. bigemina infections has been identified without the large scale and costly purification procedures which were used to identify the B. bovis antigen. The ability to produce the protective antigens in quantity in bacteria provides the prospect for overcoming the limitations of the existing live vaccine. A recombinant vaccine is potentially available for countries where the use of a vaccine is presently restricted. Important and practical spin-offs from this work have resulted in the characterization of strains present in field populations of Babesia using the DNA probes obtained. This has made it possible for the first time to characterize the subpopulations in field isolates and how they could interact with the existing live and recombinant vaccines. Moreover, the antigens obtained through genetic engineering have provided the basis for a specific and sensitive enzyme linked immunosorbent assay for the detection of B. bovis. The potential exists for the extension of this work to the development of vaccines against other economically important Babesia spp., including B. divergens, B. canis and B. ovis.
1. INTRODUCTION

1.1. Present control of Babesia and limitations

Babesiosis of domestic animals is relatively uncontrolled throughout the world. Vector control rather than parasite control is the major constraint on the disease and is largely ineffective. In endemic areas, stability of the infection is achieved by exploiting the fact that the progeny of immune mothers receive protection via colostral milk. This protection persists for up to six months after birth. With medium tick burdens (> 50 ticks/d), it is expected that all young animals will be naturally infected before they are nine months old, mainly during the period of protection due to maternal antibodies. These animals undergo a mild or unapparent infection and are subsequently immune for life. In most instances, however, this procedure is not followed, with resultant heavy mortality upon exposure of susceptible animals to infected ticks.

In a limited number of countries, and particularly in Australia, a live attenuated vaccine is used. While this vaccine is extremely effective against Babesia bovis, its use is limited by a number of factors, including short shelf life, the potential for reversion to virulence followed by transmission by the tick vector, and the possibility of contamination with other pathogens [1]. In addition some vaccinated animals react severely to the live vaccine and require treatment. It is for all of these reasons that research aimed at developing a stable, defined vaccine utilizing recombinant DNA technologies has been undertaken both at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and at laboratories in the United States of America. The CSIRO vaccine is now undergoing registration trials and it is hoped to extend these trials to developing countries soon. Such a vaccine will enable a coordinated programme to be established for the control of babesiosis in those countries where there is currently no satisfactory management of this disease.

The primary role of recombinant DNA technology, therefore, is to identify the genes encoding protective antigens, to produce these antigens in quantity for vaccination purposes and to utilize the genes and antigens as diagnostic tools in the identification and characterization of strains. However, it is essential not to look at recombinant DNA in isolation from all the other research that has led to the successful outcome. This review will cover recent research in the development of a B. bovis vaccine conducted at the CSIRO laboratories.

2. PRODUCTION OF PROTECTIVE ANTIGENS

2.1. Purification of protective antigens from Babesia infected erythrocytes

One of the major problems in developing a vaccine against any parasitic disease using recombinant DNA technology is the identification of the antigens that par-
ticipate in the protective immune response. In the two instances (described in this review) where successful protection has been achieved in experiments with a recombinant antigen, the native antigens were first identified solely on the basis of the ability of the native antigen to protect in vaccination trials with the normal host. With this approach it is not necessary either to use model animals or to guess the nature of the protective antigen.

The discovery that a crude extract of *B. bovis* would induce a level of protection in vaccinated cattle similar to that produced by natural infection [2] indicated that it should be possible also to induce protection with presumably the individual antigens.

In order to identify the protective antigen(s), our group has adopted a pragmatic approach whereby extracts are fractionated and then tested in vaccination and challenge experiments. The process is repeated until a pure protein is obtained. The purification includes various chromatographic and electrophoretic steps, among them affinity chromatography using polyvalent antisera, lectins, hydrophobic and hydrophilic ligands and proteinase inhibitors.

A number of protective fractions were identified in these initial studies. These were then subjected to further purification procedures by affinity chromatography with an array of monoclonal antibodies (MAbs) raised to the various fractions. Monoclonal specificity to the different components in the various fractions was determined by both Western blotting and immune fluorescent antibody test (IFAT) staining specificity.

The first protective antigen purified by this method was designated 15B1 [3, 4]. This antigen was subsequently redesignated 12D3 (after the more stable A12D3 IgG1 MAb) and was cloned and expressed using recombinant DNA technology [5, 6]. Another antigen, designated 11C5, was isolated from a heterogeneous protective fraction, designated ‘β’ [7]. The difficulty in targeting antigens by this approach is evidenced by the fact that eight separate antigens were extracted from the β fraction using eight distinct MAbs. Each antigen was then tested in cattle in vaccination–challenge trials before the actual protective antigen was identified. This antigen has now been cloned, expressed and optimized in vaccination trials [8, 9].

A third antigen, a protease, was identified by substrate electrophoresis whereby gelatin was immobilized in acrylamide and, after electrophoresis in the presence of sodium dodecyl sulphate (SDS) of a soluble *B. bovis* extract, the gel was washed in an alkaline buffer and incubated at room temperature before staining with Commassie Blue [10]. A clear band indicating protein digestion was evident. This was excised from the gel and used to vaccinate cattle which were subsequently shown to be protected upon challenge with virulent organisms. A series of MAbs were raised to this gel band material and used to affinity purify the antigen from the crude extract (M.A. Commins et al., CSIRO, unpublished data, 1991).

The pragmatic approach used to identify protective antigens has been both expensive and time consuming; it has also been highly successful. In all instances,
the protective antigens are present in minute amounts and can only be identified once considerable enrichment has already taken place. Further, their protective status can only be confirmed after vaccination-challenge trials. Of particular interest from these studies was the observation that immunodominant antigens generally were non-protective and/or immunosuppressive. Furthermore, the indications are that the protective antigens have discrete biochemical functions rather than being common structural proteins. These latter findings are contrary to the concept that only structural immunodominant antigens are likely to be protective [11]. The use of protein fractionation and subsequent animal trials has also proven to be the only successful method for the isolation of protective parasite antigens.

2.2. Cloning and analysis of genes encoding protective antigens

This section will describe the cloning and analysis of the primary structure of only the two lead antigens for a B. bovis vaccine.

A partial cDNA encoding the 11C5 antigen was obtained by immunoscreening an expression library of the B. bovis Samford attenuated line (Sa) in λgt11 using the MAb W11C5 [9, 10]. The DNA sequence showed that the fragment corresponded to the 3′ end of a possibly much longer cDNA and consisted of two structurally discrete parts. The 5′ half of the fragment consisted of multiple DNA repeat segments, while the 3′ part consisted of a non-repeat region. Accordingly, an open reading frame indicated a protein consisting of repeated amino acid sequences followed by a non-repeat region at the C terminal.

Hybridization of the cDNA to B. bovis (Sa) mRNA indicated that the 11C5 antigen was encoded by an mRNA of > 10 kbp and showed that the translation product would be about > 320 kdaltons. This is consistent with the estimated size of the 11C5 antigen in immunoblotting of native antigen with the MAb W11C5.

Hybridization studies of the genomic DNA and mRNA isolated from various strains of B. bovis indicated that the gene and the corresponding mRNA were present in all strains of B. bovis. However, no hybridization was detected in other species of Babesia, including B. bigemina. One consequence of this is that the antigen may be suitable for vaccination against strains of B. bovis but may not necessarily be of use in vaccination against other species of Babesia. The 11C5 cDNA may have some value as a B. bovis specific probe.

Hybridization of the cDNA to restriction digests of B. bovis genomic DNA indicated that the gene encoding 11C5 is correspondingly large. Two fragments are apparent in EcoRI digested DNA: a 15 kbp and a 1.2 kbp fragment (in the Sa line), and this is consistent with the large mRNA observed. A strategy has been designed to obtain the remainder of the cDNA in order to determine the primary structure of the N terminal part of the protein. Electron microscopic examination of parasite infected erythrocytes treated with immunogold labelled W11C5 MAb indicated specific localization of the 11C5 antigen to the erythrocyte membrane.
The other lead antigen is designated 12D3 [5, 6]. The affinity purified antigen consisted of two polypeptides of 38 and 22 kdaltons. The A12D3 MAb immunoblotted with the 38 kdalton protein after electrophoresis in SDS and 2-mercaptoethanol (2-ME). The N terminal sequence of the purified 38 kdalton antigen (hereinafter referred to as the 12D3 antigen) was obtained and an oligonucleotide synthesized using the least degenerate portion. This oligonucleotide was used to screen a λgt10 library of B. bovis Samford strain cDNA, which yielded several cDNAs whose authenticity was proven by DNA sequencing. One of the cDNAs (1250 bp) contained the complete open reading frame of the 38 kdalton protein and included the amino acid sequence determined by N terminal degradation of the purified native antigen. The cDNA hybridized to a single band in mRNA obtained from B. bovis (Sa) and to a single EcoRI fragment of B. bovis (Sa) genomic DNA. The translated amino acid sequence contained a hypothetical peptide for secretion. The N terminal region determined on the native antigen corresponded to the sequence of the mature protein (estimated 36.1 kdaltons) after signal peptide cleavage. Thus, it is likely that this protein is secreted from B. bovis.

2.3. Expression of recombinant antigens

The 11C5 antigen was expressed as a β-galactosidase fusion protein firstly from the λgt11 clone and thereafter from the plasmid pUR288 which produces the same fusion protein but in higher quantity; the fusion protein was purified for vaccination trials by preparative SDS electrophoresis. The same cDNA insert was also subcloned in the expression vector pGEX1, which produces a fusion protein with glutathione-S-transferase (GST) [12]. In this case, quantities of the protein could be produced for vaccination trials using affinity chromatography on glutathione-agarose beads.

In all cases fusion proteins produced in Escherichia coli blotted with the MAb W11C5 after transfer to membranes from denaturing and reducing gels. The fusion proteins were soluble and appeared to be contained in the cytoplasm of the bacteria. It is interesting to note that the proteins produced in this manner had the banding pattern characteristic of the native antigen (presumably caused by breakdown of the antigen at the repeats). Typically, milligrams of fusion protein could be obtained from several litres of E. coli bearing the recombinant plasmid.

The 12D3 antigen was expressed as a fusion with the β-galactosidase α-complementation factor of the pUC19 vector. This protein was very insoluble even after treatment with SDS, but could be partially solubilized with the inclusion of 2-ME. The protein appeared to exist as inclusion bodies in the cytoplasm of the E. coli. The partially solubilized material was used in vaccination trials. The cDNA was also cloned into the pGEX3X vector (as described for the 11C5 antigen), but the protein showed only a marginal improvement in solubility and once again SDS–2-ME was required to solubilize the protein. These solubility properties could
be used to advantage in the large scale purification of the fusion protein for testing in vaccination trials: thus most of the protein could be removed by treatment with SDS during sonication, and the fusion protein could be dissolved upon the addition of 2-ME. In all preparations of fusion protein, the MAb A12D3 blotted specifically to the fusion protein, even after the process of denaturation involved in solubilization. The amino acid sequence shows that 6% of the mature 12D3 antigen consists of cysteines which are likely to be involved in disulphide bonds and thus contribute to the difficulty experienced in expressing soluble protein.

2.4. Vaccination trials and assessment

Since the early work of Mahoney [13] and Mahoney and Wright [2], when high levels of immunity were induced in cattle with non-living Babesia material, our group has undertaken over eighty trials of various Babesia extracts in cattle. One of the critical observations made in the early work was that dominant antigens were generally non-protective and were often immunosuppressive. It was also recognized that antibody response per se was also not a good indicator of a protective immune response; protective antigens generally only induce a weak antibody response. A large number of pathophysiological parameters have been used to assess the protective status of animals upon challenge with virulent heterologous strains of B. bovis.

FIG. 1. Mean daily parasitaemias in controls and in animals vaccinated with two doses of 10 μg of 11C5 and 25 μg of 12D3 recombinant antigens after heterologous challenge with $1 \times 10^3$ B. bovis parasites. Figures in brackets indicate survivors (animals not treated) in each group.
The most important parameters are haematocrit fall, rectal temperature, cryofibrinogen formation and peak parasitaemias. In naturally immune animals and in those immunized with protective antigens, the parasitaemia is one to two orders of magnitude lower than in susceptible animals. Similar levels are induced in animals immunized by the live attenuated vaccine which is widely used in Australia.

The recombinant protective antigens have been subsequently tested in numerous vaccination-challenge trials in adult cattle. A number of variables have been studied. These include (i) dose, (ii) adjuvant, (iii) different fusion proteins, and (iv) deleted forms of the antigens.

In general, 10–25 μg of the recombinant antigens have been shown to induce levels of protection similar to that induced by the native antigen. We have concentrated on using the pGEX GST expression system which produces, in general, soluble fusion proteins of high abundance. The GST moiety induces insignificant levels of antibodies to itself, which is a useful property in its own right, not only in preventing autoimmune reactions, but also because the fusion protein itself can be used in an enzyme linked immunosorbent assay (ELISA) to monitor the serological response of the vaccinated cattle.

We have focused our efforts on two antigens, 12D3 and 11C5, although a number of other antigens have also been tested. Separately, each of these recombinant proteins reduces parasitaemias 10-fold or more, and when used in combination the reduction is even greater (95–99%) (Fig. 1).
A third protective antigen has been cloned from a fraction characterized by its proteolytic activity, using a monoclonal antibody (T21B4) raised with that fraction. One portion of the T21B4 antigen (consisting of 51 amino acids fused to GST, designated 309 antigen) reduces parasitaemias 5–10-fold.

In the initial field trial, two groups of 30 adult cattle were vaccinated with the 11C5 and 11C5–12D3 antigens respectively, while 40 cattle were unvaccinated controls. These animals were subjected to natural challenge with the tick vector. Ten per cent of cattle in the double antigen group, 12.5% in the single antigen group and 40% of the controls required treatment to prevent serious illness (Fig. 2).

These antigens have been further optimized with respect to adjuvant (Quil A), dose and solubility and, together with the 309 portion of T21B4, are now being tested in a second field trial. It is expected that a commercial vaccine will be available within 18 months, after a further series of longevity and strain evaluation trials have been completed.

3. APPLICATIONS TO DIAGNOSIS AND TO OTHER Babesia SPECIES

3.1. Cross-reactivity of Babesia species

During our search for protective B. bovis antigens, a large number of mono- and polyvalent antibodies to a small group of defined antigens were produced. These have been subsequently used to study their cross-reactivity with a number of other Babesia spp. of economic importance ([14]; B.V. Goodger, M.A. Commins and I.G. Wright, CSIRO, unpublished data).

We have observed that, in most instances, antigens that are present in one species are also present in others. However, while this holds true with IFAT utilizing polyvalent sera, the MAb to one species may not react with the other, indicating only partial conservation of the protein across the species. An example of this is the MAb A12D3 to the protective B. bovis 12D3 antigen. This MAb cross-reacts with B. bigemina but not with B. canis, nor with B. divergens, but the polyclonal antibody to the recombinant 12D3 B. bovis antigen cross-reacts with all four species. On the other hand, with the 11C5 antigen of B. bovis, both the MAb and the polyvalent sera to the native and/or recombinant antigen cross-react with B. ovis (I.G. Wright, CSIRO, unpublished data, 1989).

However, these antigens are not identical among species of Babesia, as has been shown in cross-protective vaccination trials. The B. bovis derived recombinant 12D3 antigen does not protect cattle, sheep or dogs against subsequent challenge with B. bigemina, B. ovis or B. canis respectively. Likewise, sheep and dogs vaccinated with the B. bovis derived recombinant 11C5 antigen are not protected against subsequent B. ovis or B. canis challenge respectively. As is to be expected, this observation is supported by sequence data of the 12D3 antigen homologues from
B. bigemina and B. canis. No 11C5 homologue has yet been identified in any other species of Babesia by DNA hybridization techniques. This has now led to the current position whereby, using DNA hybridization techniques, it is hoped that the species specific genes can be cloned, and expressed, to produce species specific protective antigens.

3.2. Cross-hybridization of protective antigens to other species

The best prospect for cross-hybridization to other species is the 12D3 antigen described above. The 12D3 gene itself cross-hybridizes with DNA taken from all strains of B. bovis and several species of Babesia looked at so far. The cDNA for the corresponding 12D3 antigen isolated from B. bigemina has been completely sequenced and shows conservation of essential features, including the signal peptide and the number and position of the cysteine residues. It is clear that this antigen is highly conserved between B. bovis and B. bigemina. It is likely that it will be found in other species of Babesia, but we have not been able to find hybridization with one species of Theileria (a haemoprotezoan of cattle which also infects lymphocytes).

Nevertheless, isolation of the 12D3 equivalent antigen from other species of Babesia does provide the prospect for the production of candidate protective antigens in quantity without having to resort to the lengthy process of vaccination trials and purification of the native material. Thus, a recombinant vaccine could be produced against B. bigemina, B. canis and B. divergens and other economically important species.

3.3. Serodiagnosis of Babesia infections

Serodiagnostic tests must meet a number of criteria to have practical value, including specificity, sensitivity and reproducibility. They must also be relatively quick and easy to perform. It is particularly essential that long term infections are accurately detected.

While a large number of tests have been developed for the diagnosis of babesiosis they all have shortcomings; notably they are generally not very sensitive, they use crude antigen preparations which have a negative impact on reproducibility and they are often difficult to perform. With the expression of specific Babesia antigens by recombinant DNA technologies, it is now possible to develop serodiagnostic tests which meet all of the above criteria.

The search for protective Babesia antigens has also led to the discovery of immunodominant antigens which are excellent serodiagnostic agents. Three of these have now been used in ELISA. The 11C5 antigen is a large molecule comprising an immunodominant portion which contains a number of repeating sequences and a smaller non-repeat region. The repeat sequence appears to be relatively well conserved in field isolates in Australia and is thus an ideal diagnostic component. An
ELISA was developed at CSIRO [15]. This test utilizes 20 ng of GST-11C5 fusion protein per well. In a comparative study with an ELISA which uses a crude *B. bovis* antigen [16], the recombinant antigen based ELISA was found to be as sensitive and specific for both recent and long term infections as well as for sera from multiple field infections. As the antigen can be produced in quantity in a highly reproducible state, this test is now used routinely at CSIRO.

A second test has also been developed at CSIRO, in conjunction with the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, which utilizes two non-protective recombinant antigens designated Z3A and C51A. The advantage of this test over the one based on the 11C5 antigen is that it can be used to monitor subsequent natural field challenge to animals vaccinated with the protective 11C5 antigen. The second test is now being evaluated under field conditions in Latin America by the Joint FAO/IAEA Division.

A further advantage of ELISA using recombinant antigens is the fact that some antigens, including the 11C5 *B. bovis* antigen, share some attributes with antigens in other *Babesia* species. The 11C5 antigen has recently been used in Turkey to evaluate the epidemiological status of *B. ovis* infections, in association with both the Joint FAO/IAEA Division and the Turkish Atomic Energy Agency [17]. In this instance, 95% of known *B. ovis* positive and 0% of negative sera gave positive reactions to the *B. bovis* derived recombinant 11C5 antigen. Thus, this test can be used for epidemiological studies for either *B. bovis* or *B. ovis* infections. In all instances optimal reactions are obtained with between 10 and 20 ng of the peptide, which makes this an extremely economical test to perform.

4. STRAIN VARIATION

In recent years, breakthrough of the live attenuated vaccine has become a major problem [18]. Although the reasons for this are unknown it is possible that selection of strains of *B. bovis* no longer susceptible to the immune response of animals vaccinated with the Ka line has occurred. Extensive use of a vaccine containing a few antigens may select for resistant populations, similarly to selection by drugs. The type of antigens in the vaccine and their importance to the cell, their variability and genetic linkage and the structure of the natural populations of *B. bovis* may all have to be considered in the future selection of resistant phenotypes. The function and hence importance to the cell of the 11C5 and 12D3 antigens are presently not known. In order to address the other issues we have embarked on a study of the extent of variation of the natural population of *B. bovis* with samples isolated at different times and from different locations in Australia. The 11C5 antigen from the Sa line has a highly conserved repeat sequence. The repeat sequence is present in all 13 genetically distinct subpopulations of *B. bovis* which have been examined, with a limited variation in the number of repeats. These observations sug-
FIG. 3. Composition of the Townsville isolate and some derivatives as determined by analysis using the C51A gene repeat region probe. Different subpopulations are represented by different shadings. (Ta: Townsville attenuated; Ttc: Townsville tissue culture.)

gest that the repeat sequence is not highly polymorphic. Using the 12D3 cDNA clone, six distinct groups of flanking restriction fragment length polymorphisms (RFLPs) have been defined for the 12D3 gene in the 13 subpopulations studied. This is the first step in assessing the extent of natural variation of the genes. The next step is to sequence representative genes from each of the RFLP groups.

The next major goal was to develop a system for the identification of different strains of *B. bovis* and for the analysis of potentially complex mixtures of subpopulations. We have isolated a cDNA clone, designated C51A, which contains a 219 nucleotide repeated DNA sequence. This repeat sequence is part of a single copy gene. The number of repeats of the sequence within the gene varies among the different alleles of the gene. Most isolates of *B. bovis* contained more than one allele of C51A, and hence more than one different subpopulation of parasites. The relative intensities of the hybridization signals can be used to determine the relative proportions of different subpopulations of parasites. Changes in these proportions can be followed after passage through splenectomized animals and propagation in tissue culture (Fig. 3).

*B. bovis* has an apparent genome size of 6–7 Mbp with three chromosomes separable by pulse field gel electrophoresis. We have assigned 15 genes to the chromosomes by hybridization. Most of the genes mapped, including the rRNA genes [19] and the genes for the protective antigens 12D3 and 11C5, are on the largest chromosome. Two high copy number sequences are predominantly on the smallest
chromosome, which is variable in length among the different isolates studied. The genes were hybridized to genomic DNA from the different isolates of *B. bovis* separately digested with five different restriction enzymes. For each probe, the subpopulations were assigned to a group with identical RFLP patterns. The complete genotypes of the subpopulations were compiled and compared, showing that only a few subpopulations had very similar genotypes.

The observation that mixed infections of animals are genetically distinct lines may be frequent, and evidence of recombination between lines of parasites suggests that sexual reproduction may play a significant role in the population structure of *B. bovis*.

The approaches described above will enable us to estimate population diversity when more isolates have been examined. The possible linkage of 12D3 and 11C5 will be determined by the mapping of the genes to specific locations on the chromosomes. Examination of RFLPs should significantly reduce the amount of sequencing required to determine the extent of natural variation in the candidate vaccine antigens. Such information is required for the design of genuine heterologous challenge experiments and will also facilitate the analysis of parasites from unprotected animals in controlled trials. In the field, genetic analysis of breakthrough strains may significantly reduce the time required to develop refinements of the *B. bovis* recombinant vaccine.

5. CONCLUSIONS

This research on the development of a recombinant *B. bovis* vaccine has led to identification of a number of important concepts and development of practical applications.

The recombinant antigens are generally produced having a similar efficacy to the native antigens. However, immunity produced by vaccination with one antigen is not as high as that produced with the live vaccine or by natural infection. This level of immunity is only approached when two or more antigens are used as a multivalent vaccine or 'cocktail'. However, our work on strain variation cautions against the use of a vaccine containing several antigens in order to minimize the effect of possible variation of antigen among lines of *Babesia*. It is likely that vaccines against other economically important species of *Babesia* can be developed by targeting antigens using the antibody and DNA probes obtained during the *B. bovis* research. The recombinant antigens produced also find application as diagnostic agents in ELISA. The DNA probes developed during this work provide the basis for strain identification and monitoring. Future work will include identification of additional protective antigens to extend the usefulness of the recombinant vaccine, examination of the importance of different lines and strains of *Babesia* and how the use of vaccine technology should be modified as a result, and extension of the research to the develop-
ment of other Babesia vaccines. It is important not to underestimate the parasite’s ability to adapt to its changing environment, even as it is changed through vaccination with recombinant antigens.

ACKNOWLEDGEMENTS

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REVIEW OF THE ACTIVITIES OF THE INTERNATIONAL TRYPANOTOLERANCE CENTRE IN IMPROVING THE PRODUCTIVITY OF TRYPANOTOLERANT LIVESTOCK

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Banjul

Abstract

REVIEW OF THE ACTIVITIES OF THE INTERNATIONAL TRYPANOTOLERANCE CENTRE IN IMPROVING THE PRODUCTIVITY OF TRYPANOTOLERANT LIVESTOCK.

The International Trypanotolerance Centre (ITC) was established in the Gambia in 1982 to carry out research for the purpose of increasing the production and quality of trypanotolerant livestock. The ITC initially studied the performance of the N’Dama cattle kept under different traditional management systems and trypanosomosis risks. The constraints to higher productivity were identified as poor nutrition and disease (mainly helminthosis and trypanosomosis). The studies showed variation between individuals in growth rate, milk production and response to artificial disease challenge. The heritability of these traits is being elaborated and superior animals will be identified and selected to create an elite herd of Gambian N’Dama. This herd will be expanded by use of artificial insemination and embryo transfer.

1. INTRODUCTION

The International Trypanotolerance Centre (ITC) was established in 1982 by act of the Gambian parliament with the objective of promoting the use of the N’Dama cattle in tsetse infested tropical Africa. There are approximately 4.9 million N’Dama cattle (49% of all trypanotolerant cattle in West and Central Africa) of which 300,000 are found in the Gambia. The ITC study sites are located in three different tsetse challenge areas described as free, low-medium challenge and high challenge. This made it ideal for the ITC to study the N’Dama under village management practices and varying trypanosomosis risks. The ITC’s mandate is to “carry out research for the purpose of increasing the production and quality of trypanotolerant livestock in tropical Africa”. To fulfil this mandate the programmes are being implemented by four research teams, namely:

(a) Production
(b) Nutrition
(c) Disease and epidemiology
(d) Entomology.
2. ACTIVITIES

2.1. Production

The production team initially embarked on collection of baseline data on the productivity, health and reproductive performance of village raised cattle. Herds were selected from villages within the three study sites of varying trypanosomosis risk. All animals in each herd were initially identified by ear tag, aged and then weighed. A veterinary assistant was assigned to monitor 10-14 such herds on his weekly visits when he, in addition to clinical interventions, recorded all births, deaths and entries/exits. Blood and faecal samples were collected monthly to screen for trypanosomes and gastrointestinal helminth infections. Milk extracted from lactating dams for human consumption was also measured for the entire lactation. Aliquot samples of milk were obtained for laboratory analysis for milk fat and protein.

The team monitored about 5000 village and 2000 on-station cattle. The on-station cattle were managed in a manner similar to those kept in villages but received daily nutritional supplementation of 4 kg of a concentrate mixture, containing groundnut cake, rice bran and *Andropogon guyanus* hay.

Table I gives a comparison of the productivity of N'Dama village cattle kept in areas of low and high tsetse challenge and on-station in the Gambia during 1986-1987.

<table>
<thead>
<tr>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>Tsetse challenge</td>
<td>Low</td>
<td>High</td>
<td>Free</td>
</tr>
<tr>
<td>Trypanosome prevalence (%)</td>
<td>1</td>
<td>10</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cow viability (%)</td>
<td>98</td>
<td>84</td>
<td>98</td>
</tr>
<tr>
<td>Calf viability to 1 year (%)</td>
<td>63</td>
<td>43</td>
<td>90</td>
</tr>
<tr>
<td>Calf weight at 1 year (kg)</td>
<td>72</td>
<td>82</td>
<td>120</td>
</tr>
<tr>
<td>Animal’s milked out yield (L)</td>
<td>401</td>
<td>171</td>
<td>267</td>
</tr>
<tr>
<td>Calving (%)</td>
<td>56.6</td>
<td>50.9</td>
<td>85</td>
</tr>
</tbody>
</table>
TABLE II. SEMEN QUALITY OF N'DAMA BULLS KEPT ON-STATION DURING THE DRY AND WET SEASONS IN THE GAMBIA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dry season</th>
<th>Wet season</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>3.24 ± 0.18</td>
<td>3.01 ± 0.18</td>
<td>0.363</td>
</tr>
<tr>
<td>Motility score</td>
<td>3.27 ± 0.18</td>
<td>2.79 ± 0.17</td>
<td>0.059</td>
</tr>
<tr>
<td>% Live</td>
<td>66.07 ± 3.52</td>
<td>56.21 ± 3.46</td>
<td>0.50</td>
</tr>
<tr>
<td>Concentration (mm$^{-3}$)</td>
<td>886 428.57</td>
<td>74 068.66</td>
<td>0.142</td>
</tr>
<tr>
<td>Total abnormality (%)</td>
<td>7.09 ± 0.48</td>
<td>7.95 ± 0.48</td>
<td>0.211</td>
</tr>
</tbody>
</table>

The baseline information available on individual animals provided initial criteria for selection of superior animals. The ITC uses a composite record based on:

(a) Post-weaning growth rate  
(b) Milk production of dams  
(c) Trypanotolerance (packed cell volume (PCV) values after infection).

Young bulls selected from the record were tested for breeding soundness (fertility) by scrotal circumference measurement, semen evaluation and finally test mating. Semen samples were collected twice-weekly for a year and essential parameters recorded. The information provided data on semen quality characteristics of the N'Dama during the two seasons (wet and dry) in the Gambia (Table II).

A pilot artificial insemination (AI) trial was initiated to investigate its potential application in N'Dama cattle production. Thirty-two primiparous cows were kept in a fence during the dry season together with their calves. They had free access to water and were fed on groundnut hay plus a concentrate mixture (17% A. guyanus, 68% rice bran, 15% groundnut cake). The cows were synchronized into heat by PGF$_{2a}$ and inseminated at 72 and 96 h after the second synchronization dose.

Fresh (liquid) semen extended in milk extenders was used for the insemination. The results demonstrated that AI following heat synchronization can be carried out in suckling N'Dama cattle provided that feeding and watering are adequate. Compared with trials using natural service, the pregnancy rates were lower but AI had the advantage of identifying the exact parentage of each calf born and the breeding period was shortened.

It is proposed to use a station based open nucleus breeding scheme involving screening of animals exhibiting outstanding productivity characteristics. These animals will be bred by AI using liquid semen as facilities for deep freezing semen are not available.
TABLE III. MEAN DECLINE IN PCV OVER 24 d AFTER DETECTION OF PARASITAEMIA, LIVE WEIGHT GAINS AND BODY CONDITION SCORES DURING 9 WEEKS FOLLOWING *T. congolense* INFECTION OF N’DAMA BULLS IN LOW OR MEDIUM BODY CONDITION AND FED AT A LOW (LP) OR HIGH (HP) PLANE OF NUTRITION [3]

<table>
<thead>
<tr>
<th>Initial body condition score</th>
<th>PCV decline (units/d)</th>
<th>Live weight gain (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP</td>
<td>HP</td>
</tr>
<tr>
<td>Low (3.5)</td>
<td>0.223</td>
<td>0.173</td>
</tr>
<tr>
<td>Medium (4.5)</td>
<td>0.322</td>
<td>0.226</td>
</tr>
<tr>
<td>SEM/LSD:</td>
<td>0.0337/0.068</td>
<td></td>
</tr>
</tbody>
</table>

2.2. Nutrition

Under traditional management systems animals rely on grazing on native pasture throughout the year and during the dry periods forages become scarce. It has been demonstrated at the ITC that poor nutrition in the village conditions is one of the constraints to N’Dama productivity. Feeding trials were done in three villages to assess responses throughout the dry season to small inputs of a protein supplement in recently calved cows and suckling calves [2]. The results indicated that provision of groundnut meal to lactating cows during the dry season significantly reduced total weight loss. There was also an increase in milk output for human consumption. In the five month period calves of the supplemented females grew faster than those of the controls. Each kilogram of groundnut meal yielded a mean extra 350 g of milk offtake, prevented the loss of 150 g of maternal live weight and produced 80 g more calf growth.

Groups of N’Dama bulls were also fed on high and low planes of nutrition after infection with *Trypanosoma congolense*. The results show that the uninfected controls gained weight faster than the infected groups, indicating a deleterious effect of trypanosome infection on nutrient utilization [3].

Supplementation also had a significant effect on PCV values in that the value for supplemented cattle was 0.53 units higher than that of the uninfected cattle (Table III).

The nutrition team is actively involved in routine analysis of nutrient content and digestibility of available feedstuffs in the Gambia to expand the feed database.
2.3. Disease and epidemiology

The health of the animals was assessed as part of the investigation of the productivity of trypanotolerant animals kept under village management conditions. Blood samples are collected monthly for PCV and presence of haemoparasites. Faeces samples and gut specimens from necropsied animals were also collected for studies on the epidemiology of helminthosis. Other diseases encountered either as isolated cases or sporadic outbreaks included blackquarter, haemorrhagic septicaemia, anthrax and acarine infestations.

Alongside field investigation, on-station N'Dama bulls were artificially infected with *T. congolense* (strain IL1180) and their parasitaemia, PCV and weight values monitored. All the N'Dama bulls survived the entire experiment period without requiring treatment, whereas 30% of the Zebu had PCV values below 17 and had to be treated to save their lives [4]. All the N'Dama regained their original PCV values after treatment at the end of the experiment. Similar artificial infection experiments on the N'Dama indicate a high repeatability for PCV values within individual animals. A protocol to determine the heritability of PCV is now under study. This will form the basis for selection for trypanotolerance based on PCV values.

The helminthological data demonstrated the seasonal epidemiology of gastrointestinal nematode infection in the Gambia [5]. *Haemonchus contortus* was found in 67% of all animals examined and represented 99% of the total abomasal worm burden. High abomasal parasite recovery was at the beginning of the rains (May, June) and a second peak occurred in September. A steady decrease was observed in the dry season months of December–March. Low nematode burdens were found in suckling calves up to 10 months of age. The highest worm burdens were detected in 1.5–3 year old animals. The studies laid the foundation for further research and establishment of a control strategy for helminthosis in the Gambian herds.

The research work also revealed a variation in susceptibility of individual animals to helminthosis and that N'Dama cattle could be selected for resistance to helminth infections.

2.4. Entomology

The entomology unit focused its attention on assessing the risk of trypanosomosis infection to village cattle in the three districts monitored by the ITC.

Many traps were laid to monitor tsetse fly numbers, distribution and infection rates and these observations complement data obtained by the disease and production teams. The survey showed that only two species of tsetse exist in the Gambia, i.e. *Glossina palpalis* and *G. morsitans submorsitans*. 
3. CONCLUSION

The performance of the N'Dama cattle kept under different management systems and tsetse fly challenge were studied during the initial phases of the ITC's activities. The constraints to greater productivity were identified (as poor nutrition and health) by trials on animals kept on-station. Presently studies are geared towards identifying the nutritive values of locally available forages and agricultural by-products, and health measures to control losses due to diseases. Research work in the coming years will include the identification and selection of superior animals. An elite herd of Gambian N'Dama in terms of productivity and disease resistance will be established. Artificial insemination and embryo transfer techniques will be utilized to multiply the herds on-station.

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RABBIT HAEMORRHAGIC DISEASE: 
DIAGNOSIS BY HAEMAGGLUTINATION TESTS, ELISA AND 
IMMUNOPEROXIDASE TEST USING MONOCLONAL ANTIBODIES

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Rabbit haemorrhagic disease (RHD), which spread through European countries in 1987–1988, is associated with a 60–100% death rate in affected colonies. The causal agent is a virus (RHDV), classified variously as a picornavirus, a parvovirus or, most frequently, as a calicivirus. The last classification is supported by the ultrastructure of the virion, by the density of RHDV in CsCl and by the presence of the dominant protein which is typical of caliciviruses. On the other hand, RHDV differs from known caliciviruses in several respects, in particular its haemagglutinating activity and failure to propagate in vitro.

Although the results obtained hitherto suggest a fair specificity of haemagglutination (HT) and haemagglutination inhibition (HIT) tests, non-specific reactions were also observed occasionally. An accurate assessment of their reliability has not been possible owing to the lack of alternative diagnostic methods.

Enzyme linked immunosorbent assay (ELISA) techniques for the demonstration of antibodies to RHDV have been developed in our laboratory. The evaluation of results is based on optical absorbance of the substrate solution at the standard sample dilution of 1:100 in terms of the following scheme: \( A < 0.1 \), negative; \( 0.1 \leq A < 0.3 \), doubtful; \( A > 0.3 \), positive. This evaluation corresponded to results of Western blot analysis. Up to 19.4% of carriers of naturally acquired antibodies to RHDV were detected during examinations of 46 rabbit blood samples collected in colonies where clinical RHD had never occurred. The presence of antibodies has a decisive influence on the susceptibility of animals to experimental infection (Table I). Monoclonal antibodies were prepared and used for RHDV demonstration in infected cells by the indirect immunoperoxidase method. The specificity of this method was compared with that of the HT and HIT during our investigations of the possible role of the brown hare in the spread of RHD.

Positive HT titres of 1:20, 1:40 and 1:1280 were found in organ samples collected from 3 of the 33 examined dead hares, Pasteurella multocida sepsis being
TABLE I. INFLUENCE OF NATURALLY ACQUIRED RHDV ANTIBODIES ON MORTALITY IN EXPERIMENTALLY INFECTED RABBITS

<table>
<thead>
<tr>
<th>Absorbance values and serum classification</th>
<th>Negative</th>
<th>Doubtful</th>
<th>Weakly positive</th>
<th>Strongly positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A &lt; 0.1$</td>
<td>75/73</td>
<td>14/11</td>
<td>9/2</td>
<td>23/1</td>
</tr>
<tr>
<td>$0.1 \leq A &lt; 0.3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0.3 &lt; A \leq 0.7$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A &gt; 0.7$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mortality rate (%)  
97.3  76.6  22.2  4.3

demonstrated in the two with the higher titres. The absence of RHDV was demonstrated by the indirect immunoperoxidase technique using monoclonal antibodies in all of them. Similarly, a comparison of specificity of HIT and ELISA revealed an agreement between results of ELISA and Western blot analysis. Of the 24 hare sera, pretreated with China clay, 21 and 2 were positive by HIT and ELISA respectively. These results confirm the low specificity of haemagglutination tests for the diagnosis of RHD.

BIBLIOGRAPHY


EVALUATION OF AN ELISA TEST FOR SERODIAGNOSIS OF NATURAL AND EXPERIMENTAL FASCIOLIASIS IN SHEEP

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Although several investigations have established the high degree of sensitivity of the enzyme linked immunosorbent assay (ELISA) in animals experimentally and naturally infected with liver fluke [1-4] there have been very few reports regarding the evaluation of the test in field situations [5, 6]. Improvement of the specificity of the test has been a difficult task because the fluke was shown to be composed of a mosaic of antigens. Many fractions of these antigens were shared with other helminths [7-9]. Numerous studies have claimed to have isolated specific antigens from Fasciola hepatica [10-12]. However, there have been no reports on their application to the serodiagnosis of fascioliasis in field situations.

In the present study, the sensitivity and specificity of the ELISA were assessed using somatic, metabolic and tegument fluke extract and sera from naturally and experimentally infected sheep. Sera were collected, prior to necropsy, from sentinel sheep raised in an arid area free of fascioliasis and from sentinel sheep that were confirmed to be infected with F. hepatica at necropsy.

The antibody response of sheep to experimental infection is shown in Fig. 1. With somatic extracts, significant increases in ELISA optical densities (ODs) (P < 0.01) were observed from the third week of infection in sheep infected with a single dose of 100, 500 or 1000 metacercariae (mc). There was no significant difference between the three groups, even though 500 and 1000 mc levels elicited a somewhat higher OD than 100 mc. However, in sheep experimentally infected with 10 mc every two days for three months, positive ELISA ODs were obtained at the sixth week post-infection.
<table>
<thead>
<tr>
<th>Fluke burden</th>
<th>Number of sera</th>
<th>Somatic extract</th>
<th>Metabolic extract</th>
<th>Tegument extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean OD</td>
<td>% positive</td>
<td>Mean OD</td>
</tr>
<tr>
<td>1-10</td>
<td>83 (7)</td>
<td>0.582 ± 0.126</td>
<td>95.2</td>
<td>0.484 ± 0.058</td>
</tr>
<tr>
<td>11-20</td>
<td>31 (1)</td>
<td>0.681 ± 0.178</td>
<td>100</td>
<td>0.544 ± 0.045</td>
</tr>
<tr>
<td>21-48</td>
<td>41 (3)</td>
<td>0.744 ± 0.127</td>
<td>100</td>
<td>0.632 ± 0.065</td>
</tr>
<tr>
<td>≥52</td>
<td>24 (5)</td>
<td>0.992 ± 0.176</td>
<td>100</td>
<td>0.678 ± 0.050</td>
</tr>
<tr>
<td>Juvenile fluke</td>
<td>8</td>
<td>0.517 ± 0.142</td>
<td>75</td>
<td>0.535 ± 0.107</td>
</tr>
<tr>
<td>Control</td>
<td>75</td>
<td>0.443 ± 0.147</td>
<td>42.6</td>
<td>0.350 ± 0.075</td>
</tr>
</tbody>
</table>

* Number of ewes with juvenile flukes recovered is given in parentheses.
The results of examination of sera from naturally infected ewes showed that the sensitivity of the ELISA varied from 25% to 100% depending on the level of fluke infection and the antigen used (Table I). Somatic and metabolic antigens were the most sensitive in detecting light infections, while tegument antigen showed the least cross-reactivity of all the antigens (8% false positives). Additionally, somatic antigen was found to be a very sensitive antigen with almost any fluke burden, except when tested with sera from sheep harbouring only juvenile flukes (75% sensitivity). Sensitivity of the tegument extract was high in sera of sheep infected with a large fluke burden (more than 20 flukes per sheep), but was low in sheep with only juvenile flukes (25%).
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