Development of feed supplementation strategies for improving ruminant productivity on small-holder farms in Latin America through the use of immunoassay techniques

Proceedings of the final Research Co-ordination Meeting of a Co-ordinated Research Programme organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Piracicaba, Brazil, 27 September–1 October 1993
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DEVELOPMENT OF FEED SUPPLEMENTATION STRATEGIES FOR
IMPROVING RUMINANT PRODUCTIVITY
ON SMALL-HOLDER FARMS IN LATIN AMERICA
THROUGH THE USE OF IMMUNOASSAY TECHNIQUES
IAEA, VIENNA, 1996
IAEA-TECDOC-877
ISSN 1011-4289
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Printed by the IAEA in Austria
May 1996
FOREWORD

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture through its Coordinated Research Programme (CRP) supports studies aimed at improving livestock productivity in developing countries through the application of nuclear and related techniques.

The results of a CRP completed in 1989 and entitled "Regional Network for Improving the Reproductive Management of Milk, Meat and Fibre Producing Livestock with the Use of Radioimmunoassay Techniques" clearly indicated that nutritional inadequacies and livestock management deficiencies were the major factors affecting livestock productivity in Latin America. Based on these conclusions a CRP entitled "Development of Feed Supplementation Strategies for Improving Ruminant Productivity on Small-holder Farms in Latin America through the Use of Immunoassay Techniques" was initiated late in the same year. The primary aim of the Programme was to improve the productivity of indigenous ruminant livestock species maintained on typical small-holder farms in the region. Central to the approach was to first identify the nutritional and management constraints which affect reproductive and productive efficiency, and subsequently to devise and test corrective measures which would be practical, sustainable and economically viable. Important related goals of the Programme were to enhance the level of expertise and the educational quality within animal production research institutes in the region, to encourage close contact between scientists and institutions in developing and developed countries and to promote scientific information exchange on a regional basis.

Through this Programme substantial progress was made in understanding the relationship between the input of nutrients and the productive and reproductive functions in domestic animals under indigenous conditions. In most participating countries the understanding of these relationships enabled feeding strategies to be developed which overcame many of the local constraints. Considerable encouragement was given to Chief Scientific Investigators to transfer such findings to the field and to assist the uptake of the developed strategies by farmers.

The present publication contains the final reports of the participants of this Programme which were presented at the Final Research Coordination meeting held at the Centro de Energia Nuclear Na Agricultura in Piracicaba, Brazil from 27 September to 1 October 1993.

FAO and IAEA wish to express their sincere appreciation to the Research Contracts holders of this Programme who have so successfully interacted with small-holder farmers in implementing field activities, and as a result, developed practical and sustainable feeding strategies for improving livestock productivity under local production systems. We would also like to thank the Research Agreement Holders who have contributed enormously through their knowledge, expertise and advice and so effectively assisted counterpart scientists with their research work and even provided on-site training through IAEA expert missions. Particular thanks are due to B. Murphy and to R. Thummler for their great personal commitment in editing and revising the manuscripts.
EDITORIAL NOTE

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CONTENTS

CO-ORDINATED RESEARCH PROGRAMME ON DEVELOPMENT OF
FEED SUPPLEMENTATION STRATEGIES FOR IMPROVING RUMINANT
PRODUCTIVITY ON SMALL-HOLDER FARMS IN LATIN AMERICA
THROUGH THE USE OF IMMUNOASSAY TECHNIQUES ............................................ 7
M. García

1. Background ........................................................................................................... 7
2. Scientific and technical basis of the Programme ................................................. 8
3. Scope and objectives of the Programme .............................................................. 8
4. Implementation of the Programme ...................................................................... 9
   4.1. Research Contracts and Agreements .......................................................... 9
   4.2. Research Co-ordination Meetings .............................................................. 9
   4.3. Technical Assistance .................................................................................. 10
5. Conclusions and recommendations .................................................................... 11
   5.1. Conclusions ............................................................................................... 11
   5.2. Recommendations ...................................................................................... 12

COUNTRY REPORTS

Measuring reproductive performance in dairy cattle ............................................. 17
   J.C.B. Plaizier, G.J. King
Reproductive performance of dairy cows suckled or milked three or six times daily .... 25
   U. Bar- Peled, A.R. Lehrer, I. Bruckental, J. Kali, H. Gacitua, E. Maltz, H. Tagari,
   B. Robinzon, Y. Folman
Stress and reproduction in farm animals ................................................................. 31
   H. Dobson
Immunological strategies for increasing fecundity in domestic animals ...................... 35
   B.D. Murphy, C.E. Lindsell, V. Misra, M.J. Redmond
Diagnosis of post-partum anoestrus in dairy cattle ................................................ 41
   R. Gatica, A. López, C. Schuler, J.E. Correa
Use of inexpensive feed supplementation to improve reproductive efficiency of Pelibuey sheep
   in the tropics. Effect of pre- and post-partum supplementation .......................... 45
   J. Alvarez, L. Zarco, I. Rubio, C. Cruz
Effect of supplementation with agricultural by-products on onset of puberty and seasonality of
Corriedale-type ewes in the Bolivian highlands ...................................................... 53
   V.A. Choque, S.J. Mollo, C.R. Chiri, G.G. Sempéretégui
Minerals and non-conventional nitrogen sources as strategic supplements for
   dual purpose cattle ............................................................................................... 59
   C. Henríquez, R. Magaña, R. Araujo, M. Rodríguez
Performance of Hereford cows under conditions of varied forage availability during late gestation
   ................................................................................................................................. 69
The effect of feed supplementation on the onset of puberty in Brazilian dairy heifers .... 81
   C.F. Meireles, A.L. Abdalla, D.M.S.S. Vitti
Management and nutrition strategies to reduce the breeding season in beef cows .......... 89
   C.F. Meireles, D.M.S.S. Vitti, A.L. Abdalla
Effect of strategic feed supplementation with multinutrient blocks on productive and reproductive performance in dual-purpose cows ..................................................... 97
   C. Domínguez, N. Martínez, C. Labrador, J. Risso, S. López
Feed supplementation for improving reproductive efficiency of crossbred Zebu cattle in the
   Peruvian tropics ................................................................................................. 107
   L. Echevarría, M. De la Torre
Reduction of concentrate for bovine sires: Influence on metabolic status and semen quality under production conditions ................................................. 113
J.L., Alvarez, R. Faure, V. Zaldívar, A. Tamayo-Avilés, H. Pérez

Reproductive performance and metabolic status of crossbreed heifers fed sugarcane by-products in confinement ........................................................... 121

Pre- and post-partum feed supplementation to improve sheep productivity in small-holder farms in southern Chile ......................................................... 127
N. Sepúlveda, O. Balocchi, J. Oberg, I. Huaquimil, A. Neumann

Effect of strategic feed supplementation on productive and reproductive performance in dual-purpose cows ............................................................ 135
N. Martínez, A. Escobar, S. López, J. Combellas, L. Gabaldón

Effect of supplementation of concentrates or selenium on production and reproduction in cows grazing pastures of high protein degradability ................................... 145
M.E. Mongiardino, M. Humardn, C.N. Corbellini, A.M. Baldán, M. Cuneo, G. Balbiani

Factors affecting the reproductive performance in Nelore cattle raised in the humid tropical Amazon region ........................................................... 157

Effect of energy and protein levels on health, growth, puberty and semen quality of Holstein bull-calves undergoing progeny testing ........................................ 163
J.L. Alvarez, V. Zaldívar, A. Boado, M. Lannes, A. Tamayo-Avilés, R. Faure

Use of radioimmunoassay techniques to study the effects of nutritional status and breed on reproductive performance of goats ................................... 173

List of Participants ............................................................. 181
CO-ORDINATED RESEARCH PROGRAMME ON DEVELOPMENT OF FEED SUPPLEMENTATION STRATEGIES FOR IMPROVING RUMINANT PRODUCTIVITY ON SMALL-HOLDER FARMS IN LATIN AMERICA THROUGH THE USE OF IMMUNOASSAY TECHNIQUES

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1. BACKGROUND

The IAEA and FAO have run a joint programme for 30 years to assist the National Agricultural Research Systems (NARS) in Member States one aspect of which is to develop, test and apply nuclear and related techniques for improving the productivity of livestock. The programme is implemented through Co-ordinated Research Programmes (CRP), Technical Co-operation (TC) projects, training courses, symposia and workshops, with activities focused on studying and resolving constraints to the productive and reproductive performance of large and small ruminants reared on small-holder farms. In the research work, the use of radioimmunoassay (RIA) as a tool for monitoring the reproductive function of the females is generally used. The measurement of progesterone by RIA can be effectively used to determine the onset of puberty and oestrus, the duration of post-partum anoestrus or in general follow the onset of sexual functions after the birth of offspring.

CRPs are important vehicles for encouraging research in a wide variety of scientific disciplines based on the development and practical application of atomic energy for peaceful uses throughout the world. The IAEA awards research contracts and cost-free agreements with research centres, laboratories, universities and other institutions in Member States to conduct research in relation to its scientific programmes. The fields of research are precise for each CRP and all contracts and agreements follow the specific objectives of the particular programme. Once a CRP is formed, 12-15 institutes are selected to participate in the programme. CRP participants are invited to Research Co-ordination Meetings (RCM) at the beginning of the programme to prepare work plans and towards the middle and final part of the programme to present the results and conclusions. These programmes aim to generate technical information leading to better feed supplementation strategies, animal management practices, livestock breeding guidelines and animal disease diagnosis.

In 1984, a CRP entitled "Regional Network for Improving the Reproductive Management of Milk, Meat and Fibre Producing Livestock with the Use of Radioimmunoassay Techniques" was initiated. The Programme had 20 Research Contracts from 14 countries in the Latin American region and 4 institutes with Research Agreements, two from the region and two from outside. In 1985, that CRP and all ongoing national Technical Co-operation Projects were incorporated into the ARCAL Programme (Arreglos Regionales Cooperativos para la Promoción de la Ciencia y la Tecnología Nuclear en América Latina, or Regional Cooperation Arrangements for the Advancement of Nuclear Science and Technology in Latin America). There were several activities under the ARCAL programme and the animal science activities formed the ARCAL III project entitled "Radioimmunoassay in Animal Reproduction". The animal health component was added to the ARCAL III project in 1991 through a separate CRP and therefore, ARCAL III was renamed "Immunoassay in Animal Production and Health".

The CRP on "Development of Feed Supplementation Strategies for Improving Ruminant Productivity on Small-holder Farms in Latin America through the Use of Immunoassay Techniques" commenced in late 1989 as a second phase using funds available from the IAEA's Regular Budget. The major factors affecting livestock productivity previously identified were nutrition inadequacies, especially in the dry season, and lack of management expertise to enable livestock to maximize production utilizing existing resources. This present CRP was formulated around infrastructures already established and experience and knowledge gained from the previous Programme. The CRP aimed to define the nutritional factors, both macro- and micro-nutrients, which reduce the reproductive efficiency and productivity of ruminant livestock enterprises on typical small-holder farms; and thereafter to devise and test feed supplementation strategies taking into account the use of locally available feedstuffs.
2. SCIENTIFIC AND TECHNICAL BASIS OF THE PROGRAMME

The human population of the Latin American region is reportedly over 400 million and increasing at the rate of 2.5% per year (1985 FAO Handbook). Despite rapid urbanization and industrialization over the last 25 years, one-third of the population are rural dwellers dependent on agriculture for food and income; a high proportion of this population lives in extreme poverty and suffers the consequences of malnutrition and disease. Livestock products are the major components of the food consumed by the population; they also account for a significant proportion of the region's exports. However, as a result of the increasing domestic consumption related to the increased population over the last 15 years there has been a reduction in net exports and if this trend continues the region could become a net importer of livestock products in the very near future.

Latin America is a region with vast and varied animal resources which consist not only of the more conventional domesticated species (e.g. cattle, sheep and goats), but also of indigenous species such as the llama, alpaca and vicuña. The production of meat, milk, wool, fibre and hides from these animals is important to all countries of the region, providing food and clothing for the human population, valuable export earnings, and perhaps most important of all - employment and income for a high proportion of the poorer people living in the rural and highland areas.

The types of ecosystems under which domesticated animals are reared in Latin America also vary greatly - from tropical and temperate grasslands through semi-arid to desert scrubland, and often interspersed by areas of high fertility farming dependent upon irrigation water from mountain rivers. In most instances, however, animals are reared in areas where the climate is harsh and consequently they are subjected to a combination of stresses, e.g. extremes of heat, cold and high humidity; pastures of low nutritive value; and insufficient water supplies. Consequently, it is hardly surprising that the efficiency of livestock production in the region is low.

Nutritional limitations in livestock production, especially in the case of ruminant production in the Latin American region, are the most important factors preventing indigenous stock from achieving their potential. Pasture is the basic resource which sustains ruminant production in the region. Consequently development depends largely on the possibility of increasing its quality, quantity and year-long availability through modifications to existing pasture management and/or the provision of supplements to the animals in an attempt to balance the inadequacies of the pasture.

Improvements in grazing management and the utilization of better-adapted grasses and legumes as well as conservation of fodder have not been successfully adopted by small-holder farms within the region. This has been partly due to the technical limitations of this approach but mainly because economic factors have worked against the farmer's propensity to assume the investment risks involved in introducing technical change. It should be remembered that most technological advances in animal nutrition and feeding originate from developed countries and their adoption tends to be governed by a very different relative set of labour, land, input and capital goods costs than those prevailing in the Latin American region. Thus, the use of biotechnology at basic and adaptive research levels to improve forage crop performance has been rather poor; however, this technology may be a way to improve productivity at the animal level.

Crop residues and by-products and animal waste contribute important yet under-utilized sources of animal feed. One way to correct the seasonal deficiency of animal fodder would be to incorporate these residues in the diet and therefore improve the digestibility, intake and protein content of the ration as a whole. Mineral deficiencies or imbalances in soils and forages (and ultimately in animals) have long been held responsible for low production and for reproductive problems among grazing animals in the tropics. In the region, grazing cattle commonly suffer deficiencies of phosphorus and sometimes of cobalt copper and iodine.

3. SCOPE AND OBJECTIVES OF THE PROGRAMME

The primary aim of the Programme was to improve the productivity of indigenous ruminant livestock species maintained on typical small-holder farms in Latin America through the identification of nutritional and management constraints which affect their reproductive and productive efficiency, and subsequently to devise and test suitable corrective measures which are within the practical and economic capabilities of the farmer. To achieve these goals it was planned to provide technical support
(equipment, expert advice and training) and back-stopping to research institutions already conducting problem-oriented investigational work in livestock production in the region.

Different strategies involving manipulation of available feed resources to provide livestock with an optimum diet for production during the wet season as well as generating sufficient body reserves (internal fat stores) for meeting production requirements during times of nutritional inadequacy (dry season) were designed for evaluation. In this respect, the provision of locally available resources such as legume trees in El Salvador and Venezuela, carnation flowers in Colombia, lipids in Venezuela, farm-made concentrates in Bolivia, Cuba, El Salvador and Mexico, minerals in Brazil, agricultural by-products in Bolivia, Cuba, Mexico and Venezuela, silage in Chile, undegradable protein in Argentina, El Salvador and Peru, multinutrient urea-molasses-blocks in Colombia, Chile and Cuba, and grassland management in Uruguay played a major role in the research conducted under this CRP. The monitoring of the effect of nutrition on reproductive efficiency was conducted through measurement of the blood or milk levels of metabolic, gonadotrophic and steroid hormones using RIA and related procedures. This complemented other production and economic data. Progesterone measurement was widely used under this Programme in conjunction with clinical data (e.g. sexual behaviour) to get a good understanding of the ovarian activity, and thus, to properly evaluate the animal response to different diets.

It was anticipated that through the information generated by the Programme, practical advice would become available to small-scale farmers on how to increase livestock productivity and thereby improve the standard of living of the rural community as a whole. Other important goals of the project were to enhance the level of expertise and educational quality of animal production research institutes in the region; to encourage close contact between scientists and institutions in developing and developed countries; and to promote information exchange on a regional basis.

4. IMPLEMENTATION OF THE PROGRAMME

4.1. Research Contracts and Agreements

Many applications from scientists in Latin American research institutes and universities were received immediately after the announcement of the CRP in January 1989 and during the first year of the Programme. However, due to the scope of the project and limitation of available funds 13 Research Contracts were awarded at the commencement of the Programme and further 7 at a later stage. Similarly, five Research Agreements were awarded to technically support the research work. The 20 Research Contract holders were from 13 Latin American countries (Figure 1) and received US $2,000-5000 per year, partly for the purchase of minor equipment and consumables, and partly for local expenses. The Research Agreements were awarded to institutes in Canada, Chile, Israel and United Kingdom.

During the five year course of the Programme one contract was withdrawn by the recipient institute, and two contracts were not renewed as the studies under these contracts were not contributing effectively to the objectives of the Programme. In addition, two final reports were not accepted due to technical reasons and one Chief Scientific Investigator failed to participate at the final Research Coordination Meeting and a final report from him was never received.

4.2. Research Co-ordination Meetings (RCM)

The first RCM was held in Santiago de Chile from 14-18 May 1990. Thirteen Research Contract holders and 3 Agreement holders attended the meeting. In addition, representatives from Bolivia, Uruguay, Spain and FAO attended as observers. The venues for the event were the Faculty of Veterinary Medicine, Universidad Central de Chile and the Chilean Atomic Energy Commission. The work plans of each project was discussed, modified were appropriate and time frames for activities devised and agreed upon.

The Second RCM was held at the Universidad Nacional Autónoma de México in Mexico City from 4-8 November 1991. The meeting was attended by 19 Research Contract holders, 4 Agreement holders, 2 consultants (Australia and Mexico) and 1 observer (Spain). The research progress and research plans
for the second phase of the Programme were discussed. Approximately half of the participants had finished the experiments and therefore proposed new experiments based on previous results. Many of the papers were of a high scientific quality. The RCM was followed by a three-week Regional Training Course on the relevance of nutrition × reproduction interactions in livestock productivity. Several Research Contract holders participated.

The Third and Final RCM was held at the Centro de Energia Nuclear Na Agricultura in Piracicaba, Brazil from 27 September-1 October 1993. The meeting was attended by 15 Research Contract holders, 4 Agreement holders, 1 invited lecturer and 1 FAO staff member. Each participant submitted a final technical report in advance which was discussed during the course of the meeting. The country reports are included later in this technical document. Finally, the group formulated conclusions and recommendations on the basis of studies undertaken over the 5-year Programme (See Section 5).

![Countries that were awarded Research Contracts under the FAO/IAEA Coordinated Research Programme on "Development of Feed Supplementation Strategies for Improving Ruminant Productivity on Small-holder Farms in Latin America through the Use of Immunoassay Techniques"

4.3. Technical Assistance

Under the FAO/IAEA Coordinated Research Programme recipient institutes can receive cash installments to meet part of the local expenses such as fuel for transport to field study sites, animal feeds, medicines, consumables, labour and other needs related to the research activity. The approved fund can also be used as a whole or in part to provide equipment relevant to the contract such as manual gamma counters and micropipettes required for the radioimmunoassay technique, centrifuges, refrigerators, computers, glassware, material for blood and milk collection, and disposable material such as pipette tips.

An essential item, the standardized FAO/IAEA Progesterone RIA kit for measuring progesterone was provided every three months in quantities sufficient to meet the needs of each study. Kits were procured through the Regional Technical Co-operation Projects RLA/5/019 and RLA/5/028 (ARCAL III). All kit-users participated in the External Quality Control Service (EQC) which is operated by the Animal Production Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf.
Over the years, problems related to the technique itself, the calibration and functioning of pipettes and gamma counters and the ability of technicians have been highlighted and corrective measures taken in order to further develop expertise in the laboratories concerned.

IAEA Regional Technical Co-operation Projects for the Latin American region under the ARCAL III Programme have effectively and substantially contributed towards the success of the Programme. Two Regional Training Courses were conducted during the lifetime of this Programme to strengthen the capabilities of scientists within the Latin American region in the radioimmunoassay technique and its application for livestock field studies on improving animal productivity. The first course was held at the Centro Nacional de Sanidad Agropecuaria (CENSA), Havana, Cuba for three weeks in November 1990 and attended by 15 scientists from 9 countries. The monitoring of reproductive hormones by RIA, the analysis of nutritional metabolites for evaluating the nutritional status of the animal and the use of ELISA for disease diagnosis were the major topics of the course. The second course was held at the Universidad Nacional Autónoma de México in Mexico City for three weeks in November 1991 and attended by 27 participants representing 15 Latin American countries. The participants were trained on the use of the FAO/IAEA metabolic and progesterone RIA kits, and on the value of the laboratory information in relation to clinical findings and field data. Lectures on scientific writing were also provided. In addition to this, and through the RLA TC Projects several IAEA experts and the FAO/IAEA technical officer assigned to the Programme assisted many of the participating institutes.

Several institutes participating in the Programme also received assistance through national IAEA TC Projects. This enabled them to obtain larger items of equipment, e.g. multi-well gamma counters, refrigerated centrifuges to facilitate the work. These institutes also benefitted through fellowships awarded to their staff for training in selected laboratories abroad and through short-term expert missions who provided on-site training, advice and assistance on technical matters related to the research work.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

5.1.1. Feed supplementation with traditional feedstuffs

Results indicate that sexual maturity and age at first mating-calving of heifers can be accelerated by simple management improvement that should be cost-effective in Brazilian or other tropical environments. Nevertheless, the use of supplements, including sources of undegraded proteins, did not always improve cattle productivity. Basal diets may have adequate levels of protein, leading to unnecessary wastage of concentrates if supplements are not provided on a rational basis as occurred in some Venezuelan and Argentinean farms.

5.1.2. Non-traditional sources of protein

Non-traditional sources of protein such as *Gliricidia sepium*, poultry manure and urea increased milk production, shortened calving intervals and reduced production costs, thereby improving the economic efficiency of farms in several countries. Also, improving the level of feeding in crossbred cows with high Holstein inheritance increased milk production, reproductive performance and overall productivity of the herd.

5.1.3. Urea-molasses blocks

Supplementing dual-purpose cows with multinutrient blocks improved milk production through the dry season in Venezuela. There was a high correlation between protein quality of the forage and block intake, indicating adjustment of the animals in the nitrogen balance under grazed pasture conditions. This may explain the high variations in block consumption between weeks and seasons of the year observed in various locations.

The reproductive performance was also improved. The interval from calving to the resumption of ovarian cyclicity was shorter in supplemented cows compared with non-supplemented animals during the dry season.
5.1.4. Mineral mixtures

Use of mineral mixtures in supplements increased body weight gain, reduced age at first service, and improved pregnancy rates in dairy cows in El Salvador. Restricted suckling prior to the breeding season in Brazilian Nelore cows had an inconsistent effect on pregnancy rates; however, ovarian function was enhanced in cows that were supplemented with phosphorus.

5.1.5. Body condition score

Body condition at calving and during the early post-partum period was highly correlated with the reproductive performance of beef and dual-purpose cows under grazing conditions in Peru, Uruguay and Venezuela. Cows having better body condition at calving were able to produce more milk and to conceive in a shorter interval. This clearly indicates the importance of nutritional status of cows at calving on post-partum reproductive activity. Strategic supplementation in the pre-partum period reduced the use of body reserves in the final foetal growth, so supplemented animals calved in better condition with the resultant reduction in the calving to first post-partum ovulation interval. The studies carried out identified the smallest body condition score to allow the cow adequate reproductive performance in the following mating period.

Scoring the body condition is an easy and free tool that can be accessed by any farmer, technician or livestock professionals with minimum need for training.

5.1.6. Pasture management

Forage availabilities under native pastures during the last trimester of gestation had an important effect on productive and reproductive performance of beef cows. Body condition and liveweight at calving and at the beginning of the mating period were positively affected by increased forage availability during late gestation in Hereford animals in Uruguay. These findings lead to the conclusion that cow performance at calving can be managed in a predictable manner by controlling forage on offer during late gestation.

Improved pastures in the Amazon valley of Brazil shortened the onset of puberty in Zebu animals.

5.1.7. Nutritional requirements in bulls

The internationally recognized nutritional requirements for Holstein Friesian bulls in artificial insemination centres may not apply under tropical conditions. Studies in Cuba indicated that bulls fed reduced amounts of concentrate had better production and quality of semen than bulls fed with the whole ration.

5.1.8. Feeding trials in sheep

Feed supplementation of Corriedale-type ewes in the highlands of Bolivia after weaning does not advance puberty in female lambs. However, the higher weight gains recorded during the supplementation period translated into a higher body weight at puberty. Undoubtedly, this would result in improved reproductive performance.

Multinutrient urea-molasses blocks gave similar results to winter silage supplementation in southern Chile for improving offspring weight gains and improved reproductive performance during the first breeding season of ewe-lambs, even in sheep farms with scarce resources and poor quality animals. However, the blocks are much cheaper and indigenous farmers can prepare them using local resources (molasses, rapeseed, fish meal, urea, minerals, etc).

Supplementation of Pelibuey ewes in the Mexican tropics during lactation increased the growth rate of lambs and reduced the weight and body condition loss of dams during lactation. However, pre-partum supplementation during the rainy season did not provide any additional advantage if the ewes were supplemented during lactation.

5.2. Recommendations

Participating institutes and Member States should encourage the use of alternative diets identified in the present Programme and other related research programmes by their farming communities. Future research should continue the approach adopted in this Programme of first identifying the limiting factors...
to animal productivity and then developing practical solutions for its improvement. To reduce problems associated with experiments carried out on commercial farms, national research laboratories should have access to experimental animals and appropriate husbandry units.

5.2.1. Selection of feed resources
Crop residues, agricultural by-products and animal wastes are important sources of animal feed. They should continue to be evaluated and if appropriate, incorporated into diets. Changes in grazing management and the utilization of alternative grasses and legumes as well as conservation of fodder have to be further evaluated as cheap, simple alternatives for grazing cattle, especially under Latin American conditions.

Information relating to deficiencies or imbalances of nutrients in soils, plants and animals should be obtained before developing feeding strategies.

5.2.2. Feed supplementation strategies
It is necessary to develop nutritional strategies using local sources of supplements (commercial or farm-made concentrates, urea-molasses blocks, treated straw, etc.) or through adequate pasture management and proper stocking rates to ensure adequate body condition at calving. These strategies should consider the relationship between quality and availability of existing forage and quality and quantity of the supplement offered. Great care should be taken to avoid technical conclusions which cannot be derived from the limited biological data generated from specific studies.

5.2.3. Body condition score
Body condition score is a simple field evaluation method which correlated well with the nutritional status of animals and should continue to be used in experiments relating to productive and reproductive performance.

5.2.4. Monitoring of ovarian activity
The progesterone profile is an excellent tool for evaluation of reproductive functions. Radioimmunoassay is a well-established technique in many developing countries and its application should be continued (but with proper quality control).

The FAO/IAEA solid phase coated-tube assay for progesterone determinations in milk and plasma is highly convenient for use under decentralized laboratories. However, researchers should migrate towards the use of the newly available FAO/IAEA solid-phase self-coating RIA technique.

5.2.5. Economics of improved diets and feeding practices
The evaluation of field results should not only consider biological data to identify the best options but also the value of cost-benefit analysis of the various strategies. The capacity of local farmers to invest in new technologies should also be taken into consideration. Research on feed supplementation strategies dealing with temporary low-cost feedstuffs are useful but efforts should be focused on long-term sustainable solutions.

5.2.6. Integrated approach
The improvement of animal reproduction through better feeding practices requires the participation of a multi-disciplinary team, sound background information and adequate knowledge of farmer's perceptions.

It is essential that the knowledge gained from this Programme is disseminated throughout the research and farmer community in each location though one or several of the following mechanisms:
- Use of demonstration sites, particularly at the farm or village level;
- Seminars, workshops and short training courses addressed to professionals and technicians in the livestock field;
- Advisory leaflets and broadcasts (i.e. video, radio, TV);
- Publication of the results in local agricultural journals and newspapers and in international refereed journals for distribution amongst relevant scientific bodies.
COUNTRY REPORTS
MEASURING REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE

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Abstract - Resumen

MEASURING REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE.

Dairy herd profitability is closely related to reproductive performance, which is, in turn, strongly influenced by management. A regular monitoring of reproductive efficiency is essential to assess management and to avoid financial losses due poor performance. The measures for this efficiency commonly used are either not based on all animals in the herd, only reflect part of the reproductive process or influence each other. Thus, obtaining an overall picture of the herd's reproductive performance requires combination of various individual components into an integrated index. The minimum measures that should be included in an integrated index for herd fertility are: average calving to pregnancy interval, culling rate, services per conception, age at first calving and percentage of stillborn calves. Ideally, the resulting calculation should emphasize the estimated financial losses or gains due to deviations from the targets set for these measures. Any herd fertility summary or projection might indicate reproductive performance but not their causes. For the identification of these causes, the length of the voluntary waiting period, the efficiency of heat detection, the services per conception, the culling rate, the age at first calving and the percentage abortions and stillbirths need to be evaluated. An additional problem with the measures of herd reproductive performance is that they indicate past reproductive performance, rather than reflect current changes or future expectations. The "Projected Minimum Average Calving-to-Pregnancy Interval" is the best prediction for future reproductive performance of a herd, but must be combined with the "Integrated Fertility Index" to provide a complete picture.

MEDICION DEL COMPORTAMIENTO REPRODUCTIVO DEL GANADO LECHERO.

La rentabilidad del hato lechero está bastante ligada al comportamiento reproductivo; lo que a su vez, se encuentra fuertemente influenciado por el manejo. Un seguimiento regular de la eficiencia reproductiva es de suma importancia a fin de evaluar el manejo y poder evitar pérdidas económicas debido a un rendimiento animal pobre. Por lo general, las medidas más comunes para medir la eficiencia no consideran a todos los animales del rebaño, solo refleja una parte del proceso reproductivo o los parámetros se afectan entre sí. Debido a eso, a fin de obtener una imagen objetiva del comportamiento reproductivo del rebaño se requiere una combinación de varios componentes individuales dentro de un Índice Integrado. Las medidas mínimas que deben estar incluidas dentro del índice integrado para fertilidad del hato son: el promedio del intervalo entre el parto y la preñez, el porcentaje de descarte, el número de servicios por concepción, la edad al primer parto y el porcentaje de nonatos. En una forma ideal, el resultado de los cálculos debe hacer énfasis en las pérdidas o ganancias económicas estimadas como resultado de las desviaciones pre-establecidas de estas medidas. Cualquier resumen o proyección de la fertilidad del hato puede indicar el comportamiento reproductivo pero no las causas que lo condicionan. Para la identificación de estas causas se requiere evaluar el largo del período voluntario de descanso post-parto, la eficiencia de la detección del estro, los servicios por concepción, el porcentaje de descarte, la edad al primer parto y los porcentajes de abortos y de nonatos. Un problema adicional que se tiene con las mediciones del comportamiento reproductivo del hato es que estas mayormente indican el comportamiento reproductivo histórico, en lugar de reflejar los cambios actuales o las proyecciones futuras. El "Promedio Mínimo Proyectado del Intervalo Parto-Preñez" es la mejor predicción del comportamiento reproductivo del hato, pero debe ser combinado con el "Índice Integral de Fertilidad" a fin de obtener una imagen completa.

1. INTRODUCTION

The best dairy cows produce substantial quantities of milk during annual lactations for a considerable number of years and at reasonable input costs. Thus, productivity and profitability are closely related to reproductive performance since lactation is initiated by parturition and also through economic importance of calves both as heifer replacements and for veal or beef production.

A multitude of measures and procedures exist to describe the reproductive efficiency of dairy herds [1, 2, 3, 4]. A few provide information on overall herd performance, but most in routine use concentrate on one particular aspect and several are necessary to provide a comprehensive picture. An additional problem is that all indicate past reproductive performance rather than reflect current changes. Unfortunately, by the time poor reproductive management is reflected in these measures, financial loss may be substantial.

Herd fertility is to a large extend determined by the management practices adopted by the operator and assistants, with routine performance monitoring essential for assessing competence. Appropriate measures of herd fertility should indicate problems and, when evaluated in comparison with reasonable
production goals, assist in the diagnosis of underlying causes and finding solutions before their consequences become too severe. In addition, the ideal measures indicate the financial consequences of less than optimum reproductive performance to insure that the farmer is aware of this reduction in herd profitability.

This paper reviews commonly used measures for dairy herd reproductive performance, discusses the need for an integrated index for this performance, proposes which individual parameters should be included in this integrated index and speculates on the possibility of being able to predict a herd's future reproductive performance.

2. MEASURES FOR HERD FERTILITY

2.1. Measures for the success of inseminations

2.1.1. First service pregnancy rate

The first service pregnancy rate is the percentage of cows that become pregnant after the first post-partum insemination. Upham [3] advocated calculating this measure by shifting first services back 60 days to allow for pregnancy determination using the non-return rate, and dividing the number of successful first services. First service fertility is affected by the duration of the voluntary waiting period; first services occurring between 40 and 50 days post-partum are usually 5 to 10% less likely to result in a pregnancy than first services occurring between 60 to 70 days post-partum. The same author [3] further suggests that first service pregnancy rate has the advantage over total services per pregnancy since it is not influenced by a disproportionally large number of services on infertile cows. However, the particular sample used in calculating any statistic is important in interpretation and application. If the statistic is based on the cows that are currently pregnant, then the value will be higher compared to one based on all cows in the herd [4]. Also, if the measure is based only on currently pregnant cows, then culled and open cows are excluded.

2.1.2. Services per pregnancy

This measure is calculated as the number of AI breedings shifted back 60 days to allow for pregnancy determination, divided by the number of breedings that resulted in confirmed pregnancy [3, 5], or the total artificial insemination services to pregnant cows divided by the number of pregnant cows on the shifted period [1, 4, 5]. If the statistic is based on the services to pregnant cows, it excludes infertile cows and fertile cows that have not yet been serviced or confirmed pregnant. A second limitation is that less fertile cows that require several services to become pregnant contribute more than cows conceiving after fewer services.

The accuracy of the measure depends on the accuracy of pregnancy diagnosis. Etherington et al. [5] recommended calculating services per conception by diagnosing pregnancy through rectal palpation or by using the 65 day non-return rate. Users must be aware that results based on non-return rates usually appear better than those based on rectal palpation, but may be misleading.

The success of any service is affected by a multitude of factors including semen quality, semen handling, semen deposition, skill of the inseminator, nutritional and health status of the cow and timing of the service, so it is difficult to determine specific causes for low first service pregnancy rate or high services per pregnancy. Also, services per pregnancy is influenced by the culling rate. When animals are culled for failure to conceive after very few inseminations, then the services per conception will be low. In such situations, concluding that herd fertility is high would be incorrect.

2.2 Indices for overall herd performance

2.2.1. Calving interval

The calving interval is the measure most commonly used to describe the overall reproductive performance in dairy herds [1, 2, 3, 6].

\[ \text{Calving interval} = \sum_{\text{months previous to current calving for all parturient cows}} \frac{\text{number of parturient cows}}{\text{total number of parturient cows}} \]

18
This period, calculated as the average time between the two most recent consecutive calvings for all parturient cows in a herd, has several inherent limitations. One major deficiency is that first lactation cows are excluded entirely as they have not had two calvings. Secondly, the measure is based on the performance of parturient cows in the herd at the time of calculation, so cows culled for failure to conceive do not contribute to it. Thus, an apparently acceptable calving interval might misrepresent actual herd performance, since infertile cows with prolonged or no calving intervals are often culled from the herd [3, 4, 7, 8]. A high culling rate might result in a low average calving interval even in herds with serious problems. Therefore, results on calving intervals are only meaningful when considered in combination with culling rates.

Calving intervals are based on historic events (i.e. past calvings) so do not reflect recent changes in the reproductive performance. This is a serious disadvantage, since for proactive assessment and planning of reproductive management, current information on the reproductive performance is essential. Alternately, one advantage of this statistic is that short temporary fluctuations in herd reproductive performance are not reflected.

The calving interval consists of the period from one calving to conception and initiation of the next pregnancy plus the gestation length. The latter shows little variation and is not influenced by management so any prolongation of this interval results from delayed conception.

2.2.2. Calving to pregnancy interval

The use of calving to pregnancy interval, also described as the calving to conception interval, has distinct advantages over the calving interval as a measure for herd reproductive performance. Calculation involves summing days from the most recent date of calving to conception for all parturient animals in the herd, providing more recent information on reproductive performance than the calving interval. Also, two successive calvings are not necessary before the measure can be calculated, allowing inclusion of first calf heifers.

Calving to pregnancy interval should be used rather than calving to conception interval, since actual time of conception cannot be assessed in commercial herds and could be followed by early embryonic mortality, even if determination was possible. This statistic, when calculated in conjunction with early diagnosis of pregnancy and continual updating records, provides a useful indication of recent performance. Dairy herd operators must be cautioned that using the non-return rate is not sufficiently accurate for pregnancy diagnosis, since non-return rates overestimate true calving rates, especially if the heat detection efficiency is poor [9].

Cows with prolonged intervals are less profitable and should be removed whenever practical. However, a high culling rate for this problem results in a shorter calving to pregnancy interval. Thus, this interval, like calving intervals, must be interpreted in comparison with the culling rate.

2.2.3. Days Open

\[
Days\ Open = \frac{Total\ days\ calving\ to\ conception\ for\ pregnant\ cows + calving\ to\ sale\ days\ for\ barren\ cows}{Number\ of\ cows\ in\ the\ herd}
\]

Some operators only include pregnant cows when calculating "days open". However, like pregnancy rate, days open based only on pregnant cows conveys no information on the as yet not successfully re-mated or infertile proportions of the herd. This deficiency can be circumvented by calculating the days open as indicated in the above formula.

Even this result must be interpreted with caution since some animals might already have conceived by the time of calculation but have not yet been diagnosed pregnant or others might conceive within a few days. One suggestion to obtain a more accurate estimation of the projected calving to pregnancy interval was proposed by the American Association of Bovine Practitioners Ad Hoc Committee to Define Reproductive Performance. They modified the days open calculation somewhat to produce the 'Projected Minimum Average Calving-to-Pregnancy Interval' and suggest it is the best estimate of the minimum average days open that will be experienced in the herd's future [3, 5]. Three groups of cows contribute to this statistic:
Cows known to be pregnant. For these animals the days from calving to last service will be calculated.

- Cows that have been bred but that have not yet been confirmed pregnant, or non-pregnant. It will be assumed that these animals conceived at the last service.
- Cows past the voluntary waiting period but not yet bred, and cows previously bred but known to be open. It will be assumed that these cows will be detected in oestrus, bred and have conceived 10 days after the day of calculation.

One potential major disadvantage of this approach is that cows in the voluntary waiting period, i.e. the period after calving during which the farmer will not service a cow, are excluded. It could be assumed that these cows will conceive during the first 21 days after the voluntary waiting period. However, this situation is unlikely to occur in most dairy units, so this estimation will be somewhat biased. Also, a high percentage of infertile cows exist in many herds, reducing the accuracy of this measure as a prediction of future reproductive performance.

Despite these disadvantages, the 'Projected Minimum Average Calving-to-Pregnancy Interval' could be a useful estimate of present and prediction of the future herd reproductive performance, since it utilizes the most recent performance information. Users should always be aware, however, that this measure is a "best estimate" and that the true herd reproductive performance will be worse.

The duration of the calving to pregnancy interval, days open or any related measure is determined by the length of the voluntary waiting period, the efficiency of heat detection, the services per pregnancy, the conception/fertilization rate and embryonic mortality and the chance of a cow having a healthy genital tract accompanied by a normal ovarian activity [1, 4, 7, 10].

An optimal calving to pregnancy interval is around 85 days, so animals must conceive during the first 2 services following a voluntary waiting period 50 days to achieve this. If it is assumed that the fertilization rate is 90%, the rate of early embryonic death and abortion is 25 %, the chance of a cow having a healthy genital tract accompanied by a normal ovarian activity 90% and the chance of heat detection and mating at the proper time of 90% [1], then 80 % of the herd will conceive after 2 services. However, if the efficiency of heat detection and mating at the proper time is reduced to 80 or 50%, then the percentage of the herd conceiving after 2 services is reduced to 73 and 52% respectively. Poor heat detection is undoubtedly the major cause of reproductive inefficiency in most dairy herds. This problem contributes directly to long calving to pregnancy intervals, increased culling for failure to conceive and associated economic loss.

Prolonged calving to pregnancy intervals necessitate extended lactations, so infertile cows may yield more than cows with shorter calving to pregnancy intervals. The lifetime milk production of the latter cows will, however, be higher provided both stay in the herd for the same time [10]. Thus, costs associated with calving to pregnancy intervals longer than the target depends mainly on the related loss in lifetime milk production. It is difficult to obtain a specific cost for an extra day open, but a computer model could generate these precisely for any herd.

Dynamic stochastic model to simulate the reproductive process in a herd of dairy cattle and a model for lactation curves and the daily milk yield and total days in milk as functions of the days open have been developed [11, 12]. Applications compared the profitability of three heat detection rates and three conception rates. This should be a good tool for estimating cost of days open and the formulation of a management program to achieve optimal reproductive performance in a given environment. Other linear [13] and non-linear models using the dynamic programming technique [14] to estimate the financial consequences of the length of the calving interval are also available.

2.2.4. Culling rate

The rate at which cows are culled for reproductive reasons is a critical index of a herd's reproductive performance. This rate influences the values of other measures, particularly services per pregnancy, calving intervals and calving to pregnancy intervals. Many cows are not culled for one specific reason, but have primary secondary and tertiary reasons for removal [5], so failure to conceive might not be the only reason for culling. Also, a high-yielding cow that fails to conceive might not be culled as soon as a low-yielding, non-conceiving cow. It is usually impossible to distinguish between cows culled for reproductive reasons only and others with additional reasons also. All culled cows
influence herd reproductive performance, so the entire culling rate must be taken into account. However, targets for the percentage of cows culled for reproductive failure should still be set.

Economic factors provide the incentive for culling targets and decisions. Failure to conceive again within a certain period following parturition or a certain number of services should provide a basis for culling, as keeping deficient animals reduces the herd profitability [5]. Culling for infertility should not occur too soon after calving as the costs of extra days open can be met by the potential gain of allowing a cow a longer period to conceive. All culling decisions should be made on the basis of the cost of replacing that cow. Herdpersons must also realize that failure to conceive is not necessarily due to the cow, but can also be caused by poor management. In particular, poor heat detection delays successful re-mating and results in high culling rates for infertility. In addition to more efficient re-mating, another advantage of a low culling rate for reproductive failure is that a higher number of cows can be culled for other production related traits with a higher heritability.

There is a 'trade-off' between culling rate and the calving to pregnancy interval. The loss in profitability caused by a calving to pregnancy interval longer than the target can be compensated by a gain in profitability due to a reduced culling rate. The costs of an extra day open should be compared with the potential gain of allowing a cow an additional day to conceive and preventing the cow from being culled for failure to conceive. These comparisons and the studies on inseminations and replacement decisions can be made using computer simulation models. As mentioned previously, several of these models have already been developed [11, 12, 13, 14, 15].

2.2.5. Age at first calving

The age at first calving affects the productive and reproductive performance of cows in their first and subsequent lactations. The potential advantage of breeding heifers at an early age are reduced rearing costs and an earlier return of income from milk. However, many farmers do not breed heifers to calve by 24-25 months, fearing reproductive problems and low first lactation yields.

Data from the UK Milk Marketing Board show that the lifetime milk production of a heifer calving at 2 years is 6% higher than that of a heifer calving at 3 years, despite a lower first lactation yield of the younger animal [10]. Similarly, heifers calving at 24 months require less costs to rear from birth to first calving and become profitable 12 mo earlier than heifers calving at 36 months.

A comparison of milk production and the reproductive performance for a group of heifers bred at first oestrus after 350 days of age with those of a group of heifers that were bred after reaching 462 days of age showed a slightly better first lactation performance for the latter [16]. However, the average lifetime performance, calculated as the actual lifetime performance or the performance during a 61 month productive life, was 15% higher for the animals bred earlier. In contrast, there were no significant differences in average days open, first service conception rate, services per conception and gestation length between these groups, demonstrating that early breeding of heifers can improve profitability.

As the age at first calving or breeding influences the lifetime milk production of individuals and the profitability of the herd, it is an important factor in reproductive management. Thus, it should be included in any integrated index for assessing performance.

2.2.6. Calf survival

Beef herds exist to produce beef, so the percentage of all mature heifers and adult cows that wean calves in a particular year provides a comprehensive picture of the herd's reproductive performance [1]. In a dairy herd the principal objective is to produce milk, not offspring, but calves also contribute to profitability as they can be sold for meat production or reared as replacement heifers. Under intensive management dairy calves are normally separated from the cows soon after calving and the responsibility of the cow for her calf ends immediately after parturition. Calf growth and survival after birth, therefore, do not reflect on the reproductive performance of the dam. The percentage of calves born alive is, however, an indicator of this performance and should be included in an integrated index. Nearly all bull calves will be used for veal or beef production. Their value depends on bodyweight and breed but a generalized figure for the loss in profitability caused by a stillborn bull calf can be estimated. Female calves might be used for veal or beef production but are usually reared as replacements. Thus, the value of a female calf is usually higher than that of for a male but the actual amount depends on its intended use. For a full economic assessment, separate estimates covering losses for calves intended for replacements heifers and calves intended for beef/veal production are necessary.
2.3. Integrated indices for reproductive performance of dairy cattle

The Fertex, an integrated fertility index [2], estimates the financial losses or gains due to deviations from the target calving interval, high culling rate and services per conception. The index is calculated by a computerized data recording and analysis system for dairy herd reproductive performance (DAISY) developed by the University of Reading [17].

This integrated index has advantages over other measures since it includes both culling rate and calving interval. This is important as these two measures should always be interpreted together in assessing herd performance (see 2.2.1 and 2.2.4). The contribution that the value of each individual measure has to the profitability of the herd is considered, so a poor value of one can be compensated by a higher value of another measure. The Fertex also includes the services per conception. This is important since if the farmer has to pay for each service, then the number of services per pregnancy influence the costs of getting a cow pregnant again and herd profitability. The incorporation of several important components into an integrated index constitutes a substantial improvement in fertility evaluation but the resulting values still have some limitations.

The average calving interval is used to calculate the Fertex. As discussed in 2.2.2., the use of the calving to pregnancy interval instead of the calving interval as a measure of herd reproductive performance is advantageous, since it is based on more recent reproductive events and includes first calving cows. The calving to pregnancy interval, therefore, better reflects the current reproductive performance of a herd. Thus, an integrated index like the Fertex might even be better if it were based on calving to pregnancy interval instead of calving interval.

The average age at first calving or mating is another factor influencing herd profitability (see 2.2.5.). This measure is not included in the Fertex, and does not directly influence the values of the measures that are included in the index. Ideally, this should also be included in an integrated index for herd reproductive performance.

The Fertex is based on the average calving interval, services per pregnancy and culling rate of the herd. The cost of an extra day open or replacement of a cow will depend on the production level of this cow (see 2.2.2. and 2.2.3.). It should therefore be considered to use standards and costs or gain of deviation from these standards depending on the average production of the herd. This would improve the accuracy of the Fertex as an indicator for the herd profitability associated with the employed management.

The Fertex provides considerable information about the reproductive performance of mature animals but not their offspring viability. The financial loss due to a stillborn calf or abortion might be substantial in some instances and should be considered in any comprehensive fertility assessment.

3. DISCUSSION

Of the currently available procedures, the 'Projected Minimum Average Calving-to-Pregnancy Interval' is perhaps the most comprehensive and acceptable indicator for present and future overall herd reproductive performance. An accurate early pregnancy diagnosis is essential to confirm its validity and several other limitations were discussed earlier. Also, one must realize that this measure is a best estimate and actual herd reproductive performance, determined at a later date, will be worse.

Unfortunately, none of the currently available measures for herd reproductive efficiency give an overall picture of this performance for a variety of reasons. Some are not based on all the animals in the herd, while others yield values that cannot be interpreted in isolation. Effective operation of any dairy unit requires a concise comprehensive and current performance summary so that achievements can be compared regularly with management goals. This should combine the most important measures, namely the proportion of cows that re-calve successfully, the interval between successive calvings, or between calving and conception, the services per conception, the age at first calving and calf viability. Most of these components are inter-related and poor performance in one might be compensated by better performance in another. It is essential, therefore, to base any integrated index on the contribution that the value of each has to herd profitability. Such an index would be an excellent management tool, focusing attention on the costs of poor reproductive management which are often hidden. A comprehensive assessment of performance should create awareness of any financial loss resulting from
sub-optimal reproductive management. A combination of the 'Projected Minimum Average Calving-to-Pregnancy Interval' and the 'Integrated Fertility Index' might accomplish this task, but operation and interpretation would be challenging for most herd operators.

The Fertex, based on the financial loss or gain caused by a deviation from the targets of the average calving interval, the culling rate and the services per conception of the herd is a useful integrated index for assessing the reproductive performance of a dairy herd [2]. This comprehensive approach might be improved even further by substituting calving to pregnancy interval in place of calving interval and by incorporating age at first calving into the calculation, since, as currently determined, the index does not consider heifer performance. Some component to reflect calf viability, stillbirth or abortions should also be included as they influence profitability.

The calving to pregnancy interval (days open) is a useful measure since first calving cows can be included. Herd managers must always be aware that the most recent value still represents what occurred several months ago. A reasonable representative picture of the most recent happenings can only be obtained through regular updating of this statistic with all parturient cows included.

The objective of any fertility summary is to create a tool for the reproductive management of this herd. Well managed herds establish a series of reproductive goals or objectives and regularly assess performance against these to detect failure to meet targets. Whenever deficiencies occur, the management team should examine the entire procedure in an attempt to define specific problems and to devise practical solutions. Calculations such as the Fertex highlight presence of poor reproductive performance, but not the causes. A detailed examination of "individual" measures is necessary to determine the major problem areas. Also, dairy herd operators must never forget that the use of any one of them in isolation can be misleading.

REFERENCES


REPRODUCTIVE PERFORMANCE OF DAIRY COWS SUCKLED OR MILKED THREE OR SIX TIMES DAILY


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Abstract – Resumen

REPRODUCTIVE PERFORMANCE OF DAIRY COWS SUCKLED OR MILKED THREE OR SIX TIMES DAILY.

During 6 weeks after calving 29 dairy cows were kept in three treatment groups: M3 - milked three times daily, M6 - milked six times daily, and S - milked three times and suckled three times daily. Throughout the experiment a diet of 65% concentrate was fed ad-libitum. Visual observations of sexual behaviour were carried out four times daily. Cows were inseminated commencing 59 days post-partum. Blood was collected three times per week for progesterone, glucose and FFA determinations analysis.

Milk production during the first 6 weeks was 35.3, 42.6 and 50.0 kg/day (P <0.01) and during the whole 18 weeks 36.7, 42.5 and 41.7 kg/day (P <0.05) for cows of the M3, M6 and S groups respectively. Dry matter intake during the first six weeks was 16.8, 19.4 and 16.2 kg/day (P <0.05), and body weight loss during this period was 23.3, 31.4 and 59.1 kg (P <0.05) for cows of the M3, M6 and S groups respectively. In groups M3, M6 and S the first ovulation occurred on average 26, 37 and 48 days post-partum (P <0.01), conception rate was 27, 18 and 45% (P <0.01) and pregnancy rate at 150 days post-partum was 80, 33 and 80% (P <0.05) respectively.

COMPORTAMIENTO REPRODUCTIVO DE VACAS LECHERAS BAJO AMAMANTAMIENTO U ORDENO POR TRES O SEIS VECES AL DÍA.

Un total de 29 vacas fueron sometidas a tres diferentes tratamientos durante las seis semanas que siguieron al parto: M3 - ordenadas tres veces al día, M6 - ordenadas seis veces al día, y S - ordenadas tres veces y amamantadas tres veces al día. Todas las vacas fueron ordenadas tres veces al día durante las semanas 7 a 18. Una dieta consistente en 65% de concentrado fue ofrecida ad-libitum durante todo el experimento. Observaciones visuales de la conducta sexual se hicieron cuatro veces al día. Las vacas fueron inseminadas a partir del día 59 post-parto. Muestras de sangre para análisis de progesterona, glucosa y FFA fueron recolectadas tres veces por semana.

La producción de leche durante las primeras seis semanas fue de 35,3, 42,6 y 50,0 kg/día (P <0,01) y en las 18 semanas fue de 36,7, 42,5 y 41,7 kg/día (P <0,05) para las vacas de los grupos M3, M6 y S, respectivamente. El consumo de materia seca durante las primeras seis semanas fue de 16,8, 19,4 y 16,2 kg/día (P <0,05), y la pérdida de peso corporal en ese periodo fue de 23,3, 31,4 y 59,1 kg (P <0,05) para las vacas de los grupos M3, M6 y S, respectivamente. En los grupos M3, M6 y S, la primera ovulación ocurrió en promedio el día 26, 37 y 48 post-parto (P <0,01), el porcentaje de concepción fue de 27, 18 y 45% (P <0,01), y el porcentaje de preñez a los 150 días post-parto fue de 80, 33 y 80% (P <0,05), respectivamente.

1. INTRODUCTION

Profitability of farming has decreased considerably during the second part of the 20th century. Real prices of agricultural products are decreasing while prices of agriculture inputs are increasing. Farmers are therefore forced to improve the efficiency of agricultural production and to produce more per each unit of input of capital, labour and land.

Raising milk production per cow is one of the most important factors in increasing profitability on dairy farms. Higher milk production can be achieved in the high-yielding breeds mainly by adequate nutrition and increased milking frequency. Increasing milking frequency has been shown to elevate milk production significantly [1, 2, 3]. The future introduction of milking robots into dairy farms is bound to increase milking frequency considerably thus raising milk production. The increased milk production may adversely affect energy balance and thus reproductive performance.

The objective of the present experiment was to study the effect of increased milking and suckling frequency on milk yield, body weight loss and reproductive performance of high-yielding dairy cows.
2. MATERIALS AND METHODS

Twenty nine Israeli-Holstein cows, in their second lactation, were allotted according to their precalving body weight (BW) into three groups. During six weeks after calving cows of the control group were milked three times daily, cows of the second group were milked six times daily and cows of the third group were milked three times daily and suckled three times daily by two calves. During weeks 7-18 after calving all the cows were milked three times daily. The amount milked was recorded at each milking, and the amount suckled was recorded weekly by weighing the calves before and after suckling.

During the first 10 weeks after calving, cows were individually penned and fed. Throughout the experimental period of 18 weeks cows were fed ad libitum a total mixed ration of 650 g of concentrate and 350 g dry matter (DM) of roughage containing 170 g/kg of crude protein and 1.72 Mcal/kg of NE\textsubscript{r}. Calving weight and three day post-partum BW were averaged and defined as initial BW. Body condition score (BCS) was assessed on a scale of 1 to 5 every week.

Visual observations for signs of oestrus, 40 min each, were carried out four times daily. Blood for progesterone, glucose and FFA determinations was collected three times a week during four months after calving. Cows were artificially inseminated at the first oestrus commencing 59 days post-partum. Pregnancy was determined by rectal palpation of the uterus 45-50 days after artificial insemination. Conception rate was defined as the number of cows pregnant per number of inseminations. Pregnancy rate was defined as the number of cows pregnant per number of cows in the group.

3. RESULTS

Milk yield was significantly increased in the six milking and suckling groups (Table I, Fig.1). Milk production continued be significantly higher in the experimental groups after the termination of the experimental treatments, 6 weeks after calving. Milk production during the 18-week period was 36.7, 42.5 and 41.7 kg/day (P <0.05) for cows milked three times daily, six times daily and suckled, respectively.

| TABLE I. MILK PRODUCTION, DRY MATTER (DM) INTAKE AND BODY WEIGHT LOSS DURING 18 WEEKS POST-PARTUM IN ISRAELI-HOLSTEIN COWS MILKED THREE OR SIX TIMES, OR SUCKLED THREE TIMES AND MILKED THREE TIMES DAILY |
|-----------------------------------|-----------------|-----------------|------------------|
| N° of cows                        | Three milkings | Six milkings    | Three milkings and three sucklings |
| First 6 weeks post-partum        |                 |                 |                                |
| Milk yield (k/d)\textsuperscript{1} | 35.3\textsuperscript{a} | 42.6\textsuperscript{b} | 50.0\textsuperscript{c} |
| DM intake (kg/d)\textsuperscript{2} | 16.8\textsuperscript{a} | 19.4\textsuperscript{b} | 16.2\textsuperscript{c} |
| BW loss (kg/d)\textsuperscript{2}  | 0.6\textsuperscript{a} | 0.8\textsuperscript{b} | 1.4\textsuperscript{c} |
| Weeks 8-10 post-partum            |                 |                 |                                |
| Milk yield (k/d)\textsuperscript{1} | 39.3\textsuperscript{a} | 43.4\textsuperscript{b} | 36.7\textsuperscript{c} |
| DM intake (kg/d)\textsuperscript{2} | 20.2\textsuperscript{a} | 22.1\textsuperscript{b} | 18.2\textsuperscript{c} |
| Weeks 11-18 post-partum           |                 |                 |                                |
| Milk yield (k/d)\textsuperscript{1} | 36.3\textsuperscript{a} | 42.0\textsuperscript{b} | 39.2\textsuperscript{c} |

\textsuperscript{a,b,c} Values bearing different superscripts within a row are significantly different.

\textsuperscript{1} P <0.01.

\textsuperscript{2} P <0.05
FIG. 1. Milk production, body weight change and body condition score of 29 Israeli Holstein cows suckled or milked three or six times daily during the first 6 weeks after calving (all cows were milked three times daily during weeks 7-18).

DM intake was significantly higher in cows milked six times daily (Table 1). Nevertheless, body weight and body condition score loss were significantly greater in cows of this group than in cows of the three milking group (Table I, Fig. 1). Surprisingly, DM intake of cows in the suckling group was significantly lower than that of cows of the six-milking group. During the first six weeks after calving cows of the 3-milking, six-milking and suckling groups lost on average 23.3, 31.4 and 59.1 kg BW, respectively (P <0.05). However, whereas cows of the first two groups began gaining weight during the 7th and 8th weeks after calving, cows of the suckling group continued to lose weight during these two weeks (Fig. 1).

The interval from calving to the first post-partum ovulation was significantly longer in the suckling group (Table II). Nevertheless, conception and pregnancy rates were significantly higher in this group and 80% of the suckling cows conceived during the 3rd month after calving. Conception and pregnancy rates of cows milked six times daily were the lowest and only 5 out of 9 cows in the group were pregnant.
TABLE II. CALVING TO FIRST OVULATION INTERVAL, CONCEPTION RATE AND PREGNANCY RATE IN ISRAELI HOLSTEIN DAIRY COWS MILKED THREE OR SIX TIMES OR SUCKLED THREE TIMES AND MILKED THREE TIMES DAILY

<table>
<thead>
<tr>
<th></th>
<th>Three milkings</th>
<th>Six milkings</th>
<th>Three milkings and three sucklings</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° of cows</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Calving to 1st ovulation interval (d)</td>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td>27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Services per conception</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean progesterone during the first 25 d of pregnancy (mol/L)</td>
<td>9.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnancy rate (%) at 90 d</td>
<td>40&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>120 d</td>
<td>70&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>150 d</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>210 d</td>
<td>80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Open days</td>
<td>112&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b, c</sup> Values bearing different superscripts within a row are significantly different
<sup>1</sup> P <0.01.
<sup>2</sup> P <0.05

seven month after calving (Table II). Plasma progesterone concentrations before conception were similar in all groups. However during the first 25 days after conception plasma progesterone levels were lower in cows milked six times daily than in cows of the other two groups (Table II).

4. DISCUSSION

The increase in milk production as a result of more frequent milking or suckling has been previously reported [3, 4, 5]. This is however the first study in which cows were milked six times daily during a prolonged period. Perhaps the most interesting fact is that after the termination of the experimental period there was a clear after-effect, and cows that were milked six times daily during 6 weeks, continued to produce significantly more milk during the following 12 weeks.

Milking cows six times daily had a detrimental effect on reproductive performance despite the fact that the average body weight loss was only 8.1 kg greater than that of the control cows (three milkings). It is therefore of interest that cows of the suckling group that lost some 30 kg more BW had a good conception rate and 80% of them conceived within 90 days after calving. The mechanism for this difference between the two groups is at present unknown. It is however evident that under certain conditions a negative energy balance and a substantial loss of body weight are not necessarily detrimental to reproductive performance. There are other factors besides energy balance and body weight loss that determine the ability of cows to conceive [6].
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Stress and Reproduction in Farm Animals

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Abstract – Resumen

Stress and Reproduction in Farm Animals

Stress reduces reproductive efficiency in farm animals. Regulators of hypothalamus-pituitary-adrenal (HPA) activity such as CRV, AVP, ACTH and cortisol have deleterious effects on both GnRH and LH secretion. Chronic stressors, such as under-nutrition, can enhance HPA activity which probably results in a greater influence on the mechanisms controlling reproductive function.

Estres y reproduccion en animales de granja

El estres reduce la eficiencia reproductiva en los animales de granja. Los reguladores de la actividad del hipotalamo-pituitaria-adrenales (HPA) tales como el CRH, AVP, ACTH y el cortisol tienen efectos perjudiciales sobre la secrecion de GnRH y LH. Estres cronicos, tales como la sub-nutricion, pueden incrementar la actividad de la HPA lo que podria resultar en una mayor influencia sobre los mecanismos que controlan la actividad reproductiva.

1. Introduction

Stressful situations clearly have deleterious effects on reproductive efficiency in commercial farming environments [1, 2]. To investigate the mechanisms involved, it is necessary to consider control of the hypothalamus-pituitary-adrenal axis (HPA) and the hypothalamus-pituitary-ovarian axis (HPO).

2. Hypothalamus-Pituitary-Adrenal (HPA) Axis

A stressor such as transport for 30 min immediately elevated heart rate, respiration rate and cortisol secretion in goats, whereas it was only after transport for at least 40-50 min that glucose concentrations increased [3, 4]. Control of corticotrophin releasing hormone (CRH) release is mediated by effects in the higher brain and hypothalamus. Interpretation of results following administration of peripheral pharmacological agents must be made with caution; however, interesting guide-lines can be obtained.

The non-specific anaesthetic agent sodium pentobarbitone completely blocks both tonic and transport-stimulated cortisol secretion [3]. However, when another stimulation such as excessive blood carbon dioxide occurs during pentobarbitone anaesthesia, the suppression was overcome [5]. The use of more specific neurotransmitter agonists or antagonists have revealed that the β-adrenergic blocker, propranolol had no effect on either tonic or stimulated cortisol secretion. However, the α1-adrenergic antagonist, prazosin, stimulated tonic cortisol secretion, but was unable to affect transport-induced responses although clinical effects were observed, i.e. suppressed heart and respiration rates, respectively [4]. In contrast, the α2-adrenergic agonist, xylazine, decreased tonic cortisol secretion and blocked the initiation of a cortisol response as well as reducing already stimulated adrenal hyper-activity [6]. We have also shown that the resting HPA system appears to be under constant dopamine suppression because pimozide, a dopaminergic blocker, stimulated tonic cortisol secretion, whereas azaperone or bromocryptine (both agonists for the inhibitory DA2 receptor) also stimulated cortisol release [7].

Evidence from our work in cattle suggests that there is also tonic opioidergic suppression acting on the HPA axis. Treatment of cattle with morphine suppressed tonic cortisol secretion, whereas naloxone, an opiate antagonist, stimulated cortisol values with a greater effect as the time post-partum increased [8]. However, when measuring endogenous β-endorphin in peripheral plasma, although there was an initial increase for approximately 30 min, a marked decline followed, even though the stimulus of transport continued for another 3.5 hours [9].
Experiments in our laboratory on the interaction of stressors and energy metabolism have been carried out in the single-stomached animal, the donkey. In this species, cortisol secretion is pulsatile throughout the day with transport resulting in a maintenance of high cortisol values followed by a subsequent refractory period [10]. Unlike the goat, there is no increase in peripheral glucose concentrations in normal donkeys in response to 4 hours transport, but if food is limited for one to three days glucose responses to transport do occur. This has been clearly shown to be the result of increased insulin resistance [10]. Of great importance was the additional observation that there was an increased cortisol response to exogenous insulin in fasted animals, as well as an excessive cortisol response to transport. Clearly, prior nutritional experience can compromise activation of the HPA axis.

To gain a greater understanding of the higher centre control of cortisol release we have established a long-term cannulation method of the hypothalamus-pituitary-portal vessels [11]. CRH and ACTH increase after both transport and excessive insulin administration. It is possible that different stressors result in different ratios of CRH and arginine vasopressin release [11].

3. HYPOTHALAMUS-PITUITARY-OVARIAN (HPO) AXIS

As with the HPA axis, in the HPO axis there is evidence that there is neuronal control of the secretion of hypothalamic releasing factors, in this case GnRH. The existence of the pre-ovulatory gonadotrophin surge in the oestrous ewe is dependant on the activity of GnRH, unlike the second surge of FSH alone [12]. Measurement of catecholamines in hypothalamic tissue of anoestrous ewes treated with oestradiol revealed increases in nor-adrenaline concentrations from 4-12 hours after steroid injection in contrast to dopamine values which were reduced at 4-8 hours [13]. Treatment of oestrous ewes or cows with sodium pentobarbitone inhibited the onset of the pre-ovulatory LH and FSH surges but had no effect on the second FSH release [14, 15]. Similar results were obtained with the α-adrenergic blocker, phenoxybenzamine [16]. The use of the non-specific dopaminergic blocker, pimozide, suppressed the pre-ovulatory increase in gonadotrophins but only when given just before the onset of the surge. This compound was also, however, capable of suppressing the second surge of FSH [17]. Bromocryptine, the inhibitory DA₂ receptor agonist, caused in increase in FSH in oestrous ewes with or without prior oestradiol treatment.

The timing of the oestradiol-induced LH surge is also clearly under opioid control; treatment of cows with morphine suppressed LH secretion and the effects were reversed by simultaneous naloxone administration [18].

4. HPA AXIS INTERACTION WITH THE HPO AXIS

Attempts to inhibit an oestradiol-induced LH surge in anoestrous sheep were unsuccessful; the LH surge occurred at the correct time, in spite of four hours transport [9]. Repetition of the experiments in ewes in the breeding season suggested that transport could modify the timing of an LH surge, but the evidence is not yet conclusive [19].

Earlier experiments transporting cows in the post-partum period immediately prior to the expected time of an oestradiol-induced LH surge did result in a delay of gonadotrophin secretion [20]. It is possible that this was an opioid-mediated effect as, in some animals, naloxone was able to overcome the suppression induced by activation of the HPA [21]. In more recent work, using post-partum sheep this time, transport did significantly delay the oestradiol-induced LH surge, however, it was not possible to reverse the effects with simultaneous administration of naloxone in this model [22]. It is known that, during the first 3-4 weeks of the post-partum period, the HPO axis in the ruminant slowly overcomes the compromise exerted by the previous pregnancy [23, 24].

5. MECHANISM OF ACTION OF HPA HYPER-ACTIVITY ON THE HPO AXIS

Early work with cattle during transport or sheep during shearing revealed a reduced LH response to GnRH [25, 26]. Administration of ACTH also diminished the LH response to GnRH, but only when
GnRH was administered 3 hours after ACTH; not 0.5 hours [27]. This would suggest that some of the initial spontaneous responses which occurred after stressors (transport or shearing) were not mediated by ACTH exerted at the level of GnRH action on the pituitary. It is possible that the very early activation of the sympathetic nervous system was involved but this has not yet been tested.

When ACTH was administered immediately prior to the expected time of an oestradiol-induced LH surge, there was no elevated gonadotrophin secretion either in post-partum or anoestrous sheep [22, 27]. To investigate the mechanism of action further, the combined stressor of isolation and restraint was used. Two injections of GnRH at an interval of 2 hours result in a higher LH response to the second injection by the process of self-priming. This effect was not influenced by simultaneous isolation/restraint during GnRH administration. However, there was a significant delay in the appearance of the LH surge, especially when the animals had been previously treated with GnRH. We have interpreted these results to suggest that one of the mechanisms of HPA hyper-activity could be on the regeneration of GnRH receptors and/or on the replacement rate of releasable LH [28].

To confirm some of our hypotheses concerning actions of HPA compounds on the pituitary, we have examined their effects on GnRH-induced LH release by in vitro perfused ovine pituitaries. Both ACTH and CRH markedly suppressed the LH secreted in response to a second exposure to GnRH. This occurred with pituitaries obtained from anoestrous ewes irrespective of prior treatment with oestradiol suggesting that HPA compounds do not exert their effect on the oestradiol sensitising mechanisms on the pituitary [29, 30].

5. CONCLUSIONS

It appears that different stressors affect the hypothalamus-pituitary system in different ways. It is suggested that effects are mediated via neuro-transmitter actions within the higher brain/hypothalamus on the HPA axis in addition to interfering with efficient HPO activity. Stressors impair reproductive function more readily when the HPO axis is already compromised. Stressors impair pituitary LH release induced by GnRH. Although this effect can be mimicked by prolonged exposure to ACTH, both in vivo and in vitro, it appears that the initial suppression of GnRH action by spontaneous stressors is not under the influence of ACTH. This latter compound does, however, inhibit GnRH self-priming mechanisms.

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IMMUNOLOGICAL STRATEGIES FOR INCREASING FECUNDITY IN DOMESTIC ANIMALS

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Abstract — Resumen

IMMUNOLOGICAL STRATEGIES FOR INCREASING FECUNDITY IN DOMESTIC ANIMALS.

Single or few offspring are born per gestation in large domestic ungulates. The principal limitation to fecundity is the number of follicles which develop and subsequently ovulate during an oestrous cycle. The principal mechanism which controls folliculogenesis is the rate of secretion of follicle stimulating hormone (FSH) by the pituitary gland. One strategy by which FSH can be increased is by immunoneutralization of ovarian products which inhibit its secretion. Immunization against ovarian steroids has apparent side effects which limit its usefulness. Immunity against, inhibin, an ovarian peptide, increases FSH secretion and the frequency of multiple births in cattle and sheep. Inhibin cannot entirely account for the protein component of negative feedback on FSH secretion. Herein we describe the biotechnological means by which the recombinant form of another ovarian peptide follistatin has been produced. Immunization of cattle, sheep and goats against follistatin may be an effective means of increasing ovulatory rate.

ESTRATEGIAS INMUNOLOGICAS PARA INCREMENTAR LA FECUNDIDAD EN ANIMALES DOMESTICOS.

Los ungulates domésticos mayores tienen una o pocas crías por gestación. La principal limitante de la fecundidad es el número de foliculos que desarrollan y que llegan a ovular durante un ciclo estrual. El mecanismo principal que controla la foliculogénesis es la tasa de secreción de la hormona foliculo estimulante (FSH) por la glándula pituitaria. La immunoneutralización de los productos ováricos para inhibir su secreción es una estrategia que se puede utilizar para incrementar la secreción de FSH. La inmunización contra esteroides ováricos tiene efectos colaterales que limitan su utilidad. La inmunización contra la inhibina, un péptido ovárico, incrementa la secreción de FSH y la frecuencia de partos múltiples en ganado vacuno y ovino. Sin embargo, la inhibina no es el único componente protéico en la retroalimentación de la secreción de FSH. Aquí se describe los medios biotecnológicos por los cuales, la forma recombinante de otro péptido ovárico, la follistatina, ha sido producido. La inmunización de vacas, ovejas y cabras contra la follistatina puede ser un medio efectivo para incrementar la tasa de ovulación.

1. INTRODUCTION

In ungulates, the principal restraint on fecundity is the ovulation rate. The cow is usually monovular, sheep and goats produce offspring per gestation which average between one and two. Foliculogenesis is the process which regulates ovulation rate. It begins with the differentiation of oocytes in the mammalian fetal ovary and persists throughout the reproductive lifespan of mammals. The vast number of follicles which initiate development degenerate by a process known as atresia. A small portion of the follicles develop to the ovulatory stage, and the critical event which regulates the frequency of ovulation is the transition of follicles from the antral to the preovulatory state [reviewed in 1]. It has long been known that the follicle regulatory mechanisms can be overcome, and superovulation induced, by administration of gonadotropic hormones, in particular follicle stimulating...
hormone (FSH) [reviewed in 2]. In breeds of sheep such as the Boorola, which have been selected for fecundity, FSH secretion is elevated at virtually every stage of the life of the ewe [3].

The secretion of FSH is controlled by hypothalamic GnRH and is modulated by endocrine and paracrine feedback factors. Feedback elements which originate from the ovary are steroid and peptide in nature. Androgens and estrogens produced by the theca and granulosa cells of the follicle have a profound negative feedback effect on FSH secretion, which can be demonstrated both in vivo [4] and in vitro [5]. Bovine or porcine follicular fluid stripped of steroids will also suppress the synthesis and secretion of FSH, both in vivo [6] and in vitro [7]. At least two protein elements produced by follicles have been identified which can alter the circulating levels of FSH: inhibin and follistatin. These have been described in a recent review [8]. Inhibin is related to the transforming growth factor β family of growth factors, and is an heterodimer of α and β subunits. The homodimer of the β subunits is known as activin, and it enhances FSH secretion. Follistatin is a single chain glycoprotein which shows some heterogeneity in its protein and carbohydrate characteristics.

Recent studies have demonstrated that estrogen synthesis is greatest in growing and dominant follicles, while inhibin concentrations are maximal in follicles during the early stages of regression [9]. Gonadotropin regulation of estrogen synthesis in the follicle is well known, and the recent evidence is consistent with the view that gonadotropins regulate inhibin as well [8]. Follistatin gene expression increases as follicles develop, is clearly influenced in the short term by paracrine factors [10] and is regulated in longer term by gonadotropins [11].

Improvement in fecundity has been achieved by immunological neutralization of steroids [12] and inhibin [13]. No information is available on the use of follistatin as an immunogen. The purposes of this report are to describe results of experiments to produce follistatin as a recombinant protein for immunization, and to discuss the potential for use of follistatin and inhibin immunization as a strategy to increase ovulatory rates in domestic species.

2. MATERIALS AND METHODS

The entire coding sequence of the porcine follistatin gene [14] was amplified by the polymerase chain reaction (PCR) following reverse transcription of RNA isolated from 2 g porcine ovarian tissue. The primers employed amplified the region from the ATG initiation codon to nucleotide 1023. The product of 25 PCR cycles was evaluated by restriction enzyme mapping and by sequencing of the 275 bases originating at the 5' end of the gene. It was shown to be homologous with the proposed sequence of porcine follistatin [15].

Cells from the continuous insect line SF21 from Spodoptera frugiperda were infected with the baculovirus transfer vector containing the full length follistatin cDNA. The product was collected by lysing of the cells and follistatin production confirmed by Western blotting using a rabbit anti-porcine antiserum against follistatin. Preliminary trials demonstrated that follistatin accumulation was highest at 47-53 h cultures were therefore terminated at that time. Glycosylation was studied by culturing some cells with tunicamycin, which blocks the assembly of oligosaccharides [16] and by endoglycosidase analysis [17]. Affinity chromatography on heparin-sepharose was employed to purify the product whose biological activity was estimated in a bovine pituitary cell bioassay [7].

A second strategy for recombinant expression of follistatin employed a mammalian kidney cell line using a heat inducible promoter isolated from bovine cells [18]. The follistatin gene was cloned into a transfection vector behind the heat shock promoter and transfected into the cells. The cell system, when elevated to 43°C, produced 25-30 mg/L secreted protein, which was harvested and subjected to Western analysis and pituitary cell bioassay to determine the presence and bioactivity of the recombinant follistatin synthesized.

3. RESULTS

Western blotting of electrophoretically separated follistatin from the insect baculovirus system confirmed the presence of its existence in three forms, 35, 38 and 42 kD, respectively. Cultures treated
FIG. 1. Biological activity of heparin-sepharose purified fractions of cell lysate from insect cells producing recombinant follistatin. Fractions were administered to primary cultures of bovine pituitary cells and the consequent accumulation of follicle stimulating hormone (FSH) was monitored by ELISA. All calculations are presented as a percentage of control, designated 100%. Fractions 15-24 contained a product (recombinant follistatin) which profoundly interfered with the secretion of FSH.

with tunicamycin synthesized a protein which migrated as a single band and which reacted with the antibody at 35 kD, indicating that the insect system produces a single protein, the homologue of the human follistatin-315 [19]. This protein is differentially glycosylated by the insect cells to produce the 38 and 42 kD forms. Glycosidase analysis of insect cell lysates demonstrated that both the 38 and 42 kD forms were high in mannose containing carbohydrates. Heparin-sepharose purified recombinant follistatin proved to be biologically active in that it profoundly interfered with the secretion of FSH from bovine pituitary cells in vitro (Figure 1).

FIG. 2. The effects of supernatant from bovine kidney cell line cultures to which the complete coding region of follistatin DNA had been transfected behind a heat-induced gene promoter according to the methods of Kowalsky et al. [18]. Non-induced cells were maintained at 37°C while induced cells were subject to temperatures of 43°C to promote the transcription of the follistatin gene.
Transfection of mammalian kidney cells resulted in ten cell lines bearing the transgene in the appropriate orientation. When these were tested at 37° and 43°, an array of forms of follistatin were produced which appeared between 38 and 41 kD in Western blots. Comparison of electrophoretic patterns with others [19] suggested that the forms produced by the mammalian cell line correspond to glycosylated versions of human follistatin-315. As can be noted from Figure 2, the follistatin produced by both under heat-induced and non-induced conditions was biologically active as a potent suppressor of FSH secretion by bovine pituitary cells in vitro. Larger scale production of recombinant follistatin for immunization of cattle has been initiated.

4. DISCUSSION

4.1. Immunization against ovarian follicular components to increase FSH and ovulation rate

The use of androgens, estrogens and progestins as immunogens in sheep has been shown to increase ovulation rates by as much as 20 percent [12, 20]. Side effects, including persistent anoestrus in treated animals as well as increased embryonic loss and post-natal mortality [20], have limited the success of this treatment. Administration of antiserum against steroids, which offers the advantage of control over both dose and persistence of antibodies has been shown to increase ovulation rates in ewes.

Increasing endogenous FSH secretion by immunization against inhibin may be a more promising means of increasing ovulation rate [22]. Trials to date have focused on the use of the inhibin α chain as a vaccine to avoid induction of immunity against activin, which would be expected to have the opposite of the desired effect. Some success was also achieved with inhibin-enriched preparations of bovine follicular fluid [23]. Peptide fragment vaccines, comprising the first 26 amino acids of the α chain [24] or any of three other regions of this subunit [25] altered, for the better, the patterns of follicular development in cattle. In the latter study, the frequency of twinning was increased by immunization against any of the three peptides. Recombinant antigens, consisting of the entire α-subunit were equally effective in increasing ovulation rates in sheep [13, 26]. It was further shown that α-inhibin immunization was accompanied by an elevations in FSH levels. Passive immunization against inhibin using antiserum generated against the amino terminal region of the α-subunit increased the ovulation rate in rats [27]. Passive immunization of cattle with antiserum against recombinant bovine inhibin resulted in increases in circulating FSH accompanied by the development of multiple large follicles and supernumerary ovulations [28].

The results of our trials indicate that it is possible to produce recombinant forms of follistatin, and to manipulate their glycosylation in vitro. Further, the recombinant follistatin synthesized has been shown to retain its biological activity, i.e. the ability to suppress the secretion of FSH by pituitary cells. Ovarian proteins present in follicular fluid reduce FSH levels, and the effects cannot be entirely explained in terms of inhibin [29, 30]. It is highly likely that follistatin is also an important regulator of FSH secretion in vivo [29, 30]. Thus, follistatin will be a viable addition to, or alternative for inhibin in immunomodulation of FSH.

4.2. Formulation of a vaccine for use by small holders

A vaccine system by which cattle, sheep and goats could be routinely immunized against endogenous agents is a potentially useful tool to increase fecundity. Its application to small holders could be easily effected as part of a disease vaccination program. Research is yet necessary to increase the efficacy of fertility vaccines, both in terms of increasing the response in individual animals and in increasing the proportion of an animal population which responds. As the antigens to be employed are endogenous proteins, their presentation in a fashion which will allow recognition by the host immune system is of paramount importance. The molecular size of the antigen is an important aspect of this presentation, and the recognition of relatively small proteins such as α-inhibin and follistatin will be greatly enhanced by binding to a large foreign carrier protein [31]. Gradual release of the antigen is desirable to increase the response of the immune system, and oil emulsions, inert substances such as alum or microcapsules of biodegradable material will be required to maximize responses. As foreign proteins are taken up by antigen presenting cells such as macrophages and monocytes, addition of
substances to the vaccine which attract the relevant cells of the reticuloendothelial system will be necessary to improve the response [31].

A further problem to be addressed is the persistence of immunity in treated animals. Increased ovulatory rates are limited to two or three cycles in cattle immunized against inhibin [24, 25]. An effective and useful vaccine should produce a state of immunity which will neutralize the element of interest for a period of at least one year. Circulating antigen concentrations, in the nanomolar or picomolar range, fall within the zone of tolerance of the immune system, thus autoboosting of the immune response will not occur [32]. Microcapsular formulations which provide slow release of immunizing doses of antigen may provide the best solution to the problem of maintenance of immunity.

4.4. Potential hazards of immunization against ovarian proteins

A hazard to inducing immunity lies in the potential that the antigen of interest may be involved beyond the reproductive systems and may be essential to the function of the animal. Complete elimination of α-inhibin synthesis using transgenic technology demonstrated an apparent paracrine role for inhibin in suppression of ovarian tumors in mice [33]. Both inhibin and follistatin have been localized in tissues other than the ovary [8], and they may have functions in other sites. The potential for shared epitopes between proteins also represents a hazard to induction of immunity against endogenous proteins [32]. For inhibin, there is considerable preliminary evidence to suggest that immunization has no important side effects in livestock. Follistatin as an antigen remains untested, and it will be necessary to address the problems of autoimmunity before a vaccine can be marketed.

In summary, the immunomodulation of fecundity in domestic animals to produce multiple births is believed to be technically feasible and cost effective. Recombinant antigens have been produced and vaccine systems are under development.

ACKNOWLEDGEMENTS

The original work described herein was funded by Strategic Grant STRO101265 awarded to B.D. Murphy, V. Misra and C. Lindsell by the Natural Sciences and Engineering Research Council of Canada. We thank Wania Wagner and Colleen Christensen for technical assistance.

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DIAGNOSIS OF POST-PARTUM ANOESTRUS IN DAIRY CATTLE

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Abstract – Resumen

DIAGNOSIS OF POST-PARTUM ANOESTRUS IN DAIRY CATTLE.

A study was carried out to establish the incidence of anoestrus in dairy cattle in Southern Chile. Cows that had not been seen in oestrus up to 60 days after parturition were considered in anoestrus and were clinically examined. Cows without corpora lutea were designated clinically anoestrus and a milk sample was taken for progesterone radioimmunoassay. Cows with progesterone concentration below 9.5 nmol/L were considered to be in true anoestrus. A total of 1831 post-partum cows from 10 farms were studied. Based on the reproductive records, 208 cows were in anoestrus (11.3% with a range from 4.3 to 33.3%). The clinical examination revealed that only 66 out of the 208 cows were in clinical anoestrus reducing the anoestrus percentage to 3.6% (range 1.9 - 10.8%). The progesterone concentration in skim milk showed that only 41 cows had low values compatible with anoestrus. Thus the true incidence of anoestrus was 2.2% with a range of 0.8 to 7.0% between farms. In conclusion, the figures from the different methods of diagnosis of anoestrus (records, clinical and endocrine status) seem to be within the range of reported data in countries with high standards for livestock production. This study identified true reproductive problems, such as deficient oestrous detection and failures in clinical diagnosis of active CLs. Also, it confirmed that progesterone RIA is a valuable tool to monitor ovarian activity.

DIAGNOSTICO DE ANESTRO POST-PARTO EN GANADO LECHERO.

Se realizó un estudio para establecer la incidencia de anestros en ganado lecher del sur de Chile. Las vacas que no fueron observadas en oestrus hasta los 60 días posteriores al parto fueron consideradas en anestro y se les hizo un examen clínico. Las vacas sin cuerpo lúteo fueron consideradas en anestro clínico y se les colectó una muestra de leche para el análisis de progesterona a través del radioinmunoensayo. Las vacas que tuvieron niveles de progesterona inferiores a 9.5 nmol/L se les consideró como vacas en anestro verdadero. Se estudió un total de 1831 vacas durante el período post-parto procedentes de 10 fincas. En base a los registros se encontraron 208 vacas en anestro (11.3% con un rango entre 4.3 a 33.3%). El examen clínico reveló que solamente 66 de las 208 vacas presentaban un anestro clínico, lo cual redujo el porcentaje de anestro a 3.6% (rango 1.9 - 10.8%). La concentración de progesterona en leche descremada demostró que solamente 41 vacas tenían niveles bajos compatibles con anestro. La tasa de anestro verdadero fue de 2.2% con un rango de 0.8 a 7.0% entre las vacas. A pesar que las tasas de anestro encontradas bajo los diferentes métodos de diagnóstico (registros, exámenes clínicos y niveles de progesterona) se encuentran dentro de los rangos hallados en otros países con estándares altos de producción lechera, el estudio permitió identificar y cuantificar los problemas reproductivos existentes, tales como la deficiente detección de celos y fallas en el diagnóstico clínico de cuerpos lúteos activos. Además, permitió confirmar la utilidad del RIA de progesterona como indicador de la actividad ovárica.

1. INTRODUCTION

Milk in the South of Chile is mainly produced by European Friesian type cows (black and white, and red and white). In recent years, Holstein Friesian semen has been introduced to increase milk production; however the increment of milk yield usually negatively affects reproductive performance. Adequate reproductive management is based on good oestrous detection methods but oestrous behaviour in cows can differ in intensity and length becoming in some cases too short or weak (silent oestrus). Silent ovulation is most common during the early post partum period and in old cows [1].

After parturition there is a period of sexual rest and suppression of the completion of folliculogenesis until the resumption of follicular activity [2]. The length of this period is variable; however, for dairy cattle under appropriate feeding and management conditions, a period longer than 60 days is usually considered abnormal [3, 4, 5]. The absence of cyclicity in cattle is often related to sub-fertility and it might be considered an indicator of management problems [5]. The post-partum cow that has not shown oestrus after 60 days but bearing an active corpus luteum (CL) may be considered to be in sub-oestrus or false anoestrus [6]. Deficient oestrous detection has also been considered an important contributory factor to anoestrus in cycling cows [3]. Incorrect detection of oestrus decreases the conception rate to first artificial insemination (AI) [7].
In South America anoestrus is frequently related to insufficient resources that lead to poor pre-partum feeding and a low level of farm manpower. Thus, anoestrus is one of the most important problems in dairy cattle management in this region, as in others because its high incidence and detrimental economic effects [3, 8, 9, 10].

Progesterone measurements in milk and blood have been suggested as an efficient method to evaluate herd fertility, management practice and ovarian response to treatments. Progesterone concentration is a good indicator of CL function [2, 11] and, therefore, it is possible to monitor ovarian activity, correctness of oestrus detection, and occurrence of sub-oestrus and silent ovulations.

The objective of this paper was to establish the true incidence of anoestrus in dairy cattle in Southern Chile based on reproductive records, clinical examinations and milk progesterone concentrations.

2. MATERIALS AND METHODS

Reproductive records of dairy cattle in 10 farms of Southern Chile (40-42° South) were studied. Cows that had not been seen in oestrus 60 days after parturition were considered to be in anoestrus. These anoestrous cows were clinically examined, with emphasis on the uterus and the ovaries, by the bovine practitioner of the farm. A cow without a CL and follicles < 1 cm, accompanied by a flaccid uterus was considered to be in clinical anoestrus and, therefore a milk sample was collected equally from the four teats for progesterone analysis. Sodium azide was added to the milk samples as preservative at the time of collection. Samples were centrifuged to remove the fat and kept at 4° C until assayed.

The RIA progesterone was carried out using the FAO/IAEA kit previously validated in our laboratory [12]. The progesterone value of 9.5 nmol/L was established as the reference value for discrimination between animals bearing or not bearing a functional CL. Results on accuracy of clinical diagnosis were analyzed by Chi square test.

3. RESULTS

Based on the reproductive records, 208 cows out of a total of 1831 post-partum cows studied from 10 farms were considered to be in anoestrus. This figure represents 11.3% of the surveyed population with a range of 4.3 and 33.3% between farms as shown in Table I.

The clinical examination showed that 66 of the 208 anoestrus cows had non-palpable CLs, thus the incidence of clinical anoestrus was 3.6% of the total (range 1.9 - 10.8%, Table I). This means that only 31.7% of the animals presented as anoestrus from the records were in clinical anoestrus. The results indicated that 142 cows (68.3%) were erroneously classified as anoestrus; in fact they had a CL present.

The progesterone concentration in skim milk showed that 41 cows (2.2% of the surveyed cows) were acyclic (progesterone values lower than 9.5 nmol/L). This rate varied from 0.8 to 7.0% between farms as shown in Table I.

The results also indicate that the accuracy of field veterinarians in identifying an active CL on the ovaries in farms 1 to 5 was 87% and in farms 6 to 10 was only 48.8%; this difference, however, was not significant (P >0.05)

4. DISCUSSION

The percentage of cows in anoestrus was 11.3% according to the existing records (Table I) which is similar to figures reported elsewhere [3]. The percentage of anoestrus between farms were similar with the exception of Farm Six that showed a high incidence of anoestrous cows (33%); this is indicative of a serious management problem. The percentage of clinical anoestrus was much lower in all farms but, again, Farm Six showed the highest incidence. The difference between anoestrous cows based on records (cows not seen in oestrous up to 60 days after calving) and clinical anoestrus (the former cows without a palpable CL) indicates failures in management, especially in oestrus detection. The origin of this can be the ignorance of the signs of oestrus, lack of participation in farm work, errors in reproductive
TABLE I. POPULATION OF ANOESTRUS COWS BASED ON RECORDS, PALPABLE CORPORA LUTEA AND MILK PROGESTERONE CONCENTRATIONS ON 10 FARMS IN SOUTHERN CHILE

<table>
<thead>
<tr>
<th>Farm</th>
<th>Nº of cows</th>
<th>Anoestrus cows based on records</th>
<th>Palpable CL</th>
<th>Progesterone values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>220</td>
<td>20</td>
<td>8</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>10</td>
<td>6</td>
<td>8.4</td>
</tr>
<tr>
<td>3</td>
<td>208</td>
<td>9</td>
<td>4</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>146</td>
<td>13</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>92</td>
<td>10</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>6</td>
<td>120</td>
<td>40</td>
<td>13</td>
<td>10.8</td>
</tr>
<tr>
<td>7</td>
<td>132</td>
<td>17</td>
<td>4</td>
<td>3.0</td>
</tr>
<tr>
<td>8</td>
<td>192</td>
<td>25</td>
<td>10</td>
<td>5.2</td>
</tr>
<tr>
<td>9</td>
<td>370</td>
<td>30</td>
<td>7</td>
<td>1.9</td>
</tr>
<tr>
<td>10</td>
<td>280</td>
<td>34</td>
<td>9</td>
<td>3.2</td>
</tr>
<tr>
<td>Total</td>
<td>1,831</td>
<td>208</td>
<td>66</td>
<td>3.6</td>
</tr>
</tbody>
</table>

1 Cows that had not shown oestrus up to 60 days after parturition were considered to be in anoestrus.

2 9.5 nmol/L was the reference value for discrimination between cows bearing or not bearing a functional CL.

management, etc., as reported by others [3, 7]. Of course, silent ovulations may also be present, at an unknown frequency. The failure in oestrus detection increases the "apparent infertility" causing economic loss as the anoestrus rate affects calving intervals, pregnancy rates, milk and calf production [13, 14].

The progesterone values obtained by RIA showed that 41 out of the 66 cows considered in clinical anoestrus did not have a CL present. This indicated that the true anoestrous rate in these herds was 2.2%, which is less than other reported figures [3]. It is important to indicate that a single sample to diagnose anoestrus could be considered insufficient; however, when combining low progesterone values with the absence of follicles >1 cm and uterine tone, the diagnosis is more accurate. The difference between anoestrus confirmed by palpation per rectum and anoestrus confirmed by progesterone determinations was due to veterinarian inaccuracies in detecting ovarian structures. The 37.9% failure is high compared with 20-30% reported by others [15, 16]. The skill and ability of the clinician is very important. In the present study, cows from farms 1 to 5 were diagnosed by veterinarians with more than 10 years experience while cows from farms 6 to 10 were diagnosed by veterinarians with fewer than 5 years activity. This may explain the difference in accuracy between both groups of farms (87 vs 48.8%, respectively).

Diagnosis of ovarian cyclicity through progesterone concentrations in milk can induce error due to false high values [17]. Milk samples should be handled with care, and the fat portion must be completely removed in order to avoid this problem [18].

In conclusion, although the figures from the different methods of diagnosis of anoestrus (records, clinical and endocrine status) seem to be within the range of data reported in countries with efficient management of livestock production; this study identified true reproductive problems, such as deficient oestrus detection and failures in clinical diagnosis of active CLs. Also, it was confirmed that progesterone RIA is a valuable tool to monitor ovarian activity.
REFERENCES


USE OF INEXPENSIVE FEED SUPPLEMENTATION TO IMPROVE REPRODUCTIVE EFFICIENCY OF PELIBUEY SHEEP IN THE TROPICS. EFFECT OF PRE- AND POST-PARTUM SUPPLEMENTATION

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Abstract – Resumen

USE OF INEXPENSIVE FEED SUPPLEMENTATION TO IMPROVE REPRODUCTIVE EFFICIENCY OF PELIBUEY SHEEP IN THE TROPICS. EFFECT OF PRE- AND POST-PARTUM SUPPLEMENTATION.

The objectives of the present study were to determine the effects of pre- and post-partum supplementation of Pelibuey ewes on the nutritional status and resumption of ovarian activity of the dams, as well as on the performance of the lambs. Pregnant Pelibuey ewes (n = 109) were used. Two months before the expected lambing date, 52 of animals were randomly assigned to a supplemented group, while the other 57 remained non-supplemented. After lambing, half of the animals from each feeding group were assigned to the opposite treatment. Supplementation consisted of a concentrate containing 3000 kcal/kg and 16% crude protein, and was supplied daily in amounts equal to 2% body weight. The animals in all groups gained weight during the last 2 months of pregnancy. All groups lost weight during lactation, but the loss was larger in the groups that were not supplemented during lactation. Net weight loss from the beginning to the end of the experiment was significantly smaller (P <0.05) in the 2 groups that were supplemented during lactation than in the other 2 groups. There were no effects of supplementation on the intervals from lambing to first ovulation or to first oestrus. Pre-partum supplementation increased the birth weight of the lambs, and post-partum supplementation increased the weaning weight of the lambs. It is concluded that there was a beneficial effect of supplementation of Pelibuey ewes during lactation, since it caused a significant increase on the growth rate of the lambs and a reduction in the weight loss and body condition loss of the dams during lactation. Under the conditions of this trial, pre-partum supplementation did not provide an additional advantage if the ewes were supplemented during lactation.

1. INTRODUCTION

There is little information about nutrition-reproduction interactions in adult Pelibuey ewes. The effects of nutritional levels during gestation or lactation have been only partially evaluated. In Mexico [1] was reported that the interval from lambing to first oestrus was shortened by 24% when the ewes received a concentrate during late gestation and early lactation, while in other report [2] was stated that the first post-partum oestrus was delayed from 51 to 91 days when the ewes had lost 25% of their body...
weight at the time of parturition. However, oestrous behavior is not the best parameter to study post-
partum ovarian activity, because most animals have one or more post-partum ovulations without signs
of oestrus [2, 3]. Other authors have evaluated the effects of maternal supplementation on the
performance of the offspring without considering the effects on the dams. For example, supplementation
of Pelibuey ewes during late gestation increased the weaning weight of lambs, but the time of resumption
of post-partum ovarian activity in the dams was not reported [4]. In order to evaluate the full potential
economic benefit of strategic supplementation during critical periods of the reproductive cycle, it is
necessary to simultaneously measure the effects on the reproductive efficiency of the ewes and on lamb
performance.

The objectives of the present study were to determine the effects of pre- and post-partum
supplementation of Pelibuey ewes on birth weight of the offspring, neonatal mortality rate, weight gain
of lambs from birth to weaning, survival rate to weaning, and resumption of ovarian activity in the dams.
In addition, the effects of supplementation on the nutritional status of the dams were monitored through
changes in body weight, body condition, plasma concentrations of urea, β-hydroxybutyrate, albumin and
total protein.

2. MATERIALS AND METHODS

The experiment was carried out at the university tropical research station located in Martinez de
la Torre, in the Mexican State of Veracruz where the climate is sub-humid tropic. All the animals were
kept together and permitted to graze on African Star pasture.

One hundred and nine adult pregnant Pelibuey ewes were used. All the animals were bred during
the month of February and the mating date of each one was recorded. Two months before the expected
date of lambing, 52 ewes were randomly assigned to a supplemented group while the other 57 remained
as the control group. After lambing, half the animals from each feeding group were assigned to the
opposite treatment. Thus, the 4 experimental groups were: 1. Supplemented pre- and post-partum (S-S;

n = 28); 2. Supplemented pre-partum but not post-partum (S-NS; n = 24); 3. Supplemented post-partum
but not pre-partum (NS-S; n = 28); and 4. Not supplemented at any time (NS-NS; n = 29).

Ewes from all groups grazed together in 5 ha of Cynodon nlemfuensis grass. The 5 ha were
divided in 5 parcels, and the animals were rotated every week. The ewes had free access to mineral salts
and water. Forage availability was calculated comparing the biomass before and after each rotation;
crude protein was determined once a month in both leaves and stalks. Supplementation consisted of a
concentrate of regional agricultural by-products (molasses, poultry manure, corn- and corn cob meal, and
pulp residues from orange juice canneries). The concentrate, which was offered once a day in amounts
equal to 2% body weight, contained 3000 kcal/kg and 16% crude protein. The animals were weighed
every two weeks to adjust the amount of concentrate offered to each group [5]. The concentrate left by
each group was weighed daily in order to calculate real consumption.

To evaluate the resumption of ovarian activity, all animals were bled twice a week beginning 15
days post-partum and continuing until the second post-partum oestrus of each ewe. The blood samples
were centrifuged and the plasma was separated and kept frozen at -20°C until assayed for progesterone
by solid-phase radioimmunoassay using the FAO/IAEA kits. The sensitivity of the assay was 0.9
nmol/L, with intra-assay coefficient of variation of 8.9% and inter-assay coefficient of variation of
12.3%. The first ovulation was considered to have occurred when progesterone concentrations first
increased to more than 3 nmol/L. Oestrous detection was done twice a day using vasectomized rams.
The ewes were weighed every two weeks from 2 months before lambing through weaning of the lambs.
Body condition scores were recorded at the same time using a scale from one to five. Concentrations
of β-hydroxybutyrate, urea, albumin and total protein in plasma of the ewes were measured using the
FAO/IAEA colorimetric tests 15, 45 and 75 days after lambing in 10 animals from each group. The
lambs were weighed every week from birth to weaning, which was completed at 90 days of age.

The weight of the ewes at different stages of pregnancy and lactation, and the weight changes
during late pregnancy, delivery and lactation were compared by an analysis of variance that used the
effects of pre- and post-partum supplementation as independent variables and the initial weight and the
type of lambing (single or double) as covariables. The condition scores were evaluated using the same
model, except that the initial condition score was used as a covariable. The weight of the offspring at
birth and at 90 days, as well as the weight gains during lactation were compared by a three way analysis of variance, using pre-partum supplementation, post-partum supplementation and type of lambing as independent variables. The effects of pre- and post-partum supplementation on the intervals from lambing to first ovulation and from lambing to first oestrus were compared by a 2 way analysis of variance. This was also used to compare the effects of pre- and post-partum supplementation on the concentrations of urea, albumin, total proteins and β-hydroxybutyrate.

3. RESULTS AND DISCUSSION

The forage production during the period under study (June-October) was on average 31.4 kg of dry matter/day/hectare, with no significant differences between months (P >0.05). This resulted in a total dry matter production of 157 kg/day in the 5 hectares, or 1.44 kg/ewe/day. Table I shows the average crude protein content in leaves and stalks during each month. Although there were significant differences between months, the quality of the forage was fairly good throughout the duration of the

<table>
<thead>
<tr>
<th>Month</th>
<th>Crude protein in leaves</th>
<th>Crude protein in stalks</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>10.7 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 ± 0.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>July</td>
<td>12.0 ± 0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.7 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>August</td>
<td>13.9 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.8 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>September</td>
<td>12.7 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.3 ± 0.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>October</td>
<td>13.0 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.7 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Column values bearing different superscripts are significantly different (P <0.05).

TABLE II. AVERAGE WEIGHT (kg) AT DIFFERENT STAGES OF GESTATION AND LACTATION OF PELIBUEY EWES THAT WERE SUPPLEMENTED OR NOT SUPPLEMENTED BEFORE AND/OR AFTER LAMBING (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of gestation</th>
<th>Days of lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-S</td>
<td>S-NS</td>
</tr>
<tr>
<td>Days of gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 ± 7</td>
<td>40.2 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.6 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>114 ± 7</td>
<td>40.5 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.3 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>128 ± 7</td>
<td>43.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.8 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>142 ± 7</td>
<td>44.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.6 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lambing</td>
<td>40.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.3 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Days of lactation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 ± 7</td>
<td>41.0 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.3 ± 0.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>28 ± 7</td>
<td>39.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.6 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>42 ± 7</td>
<td>39.1 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.6 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>56 ± 7</td>
<td>39.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.5 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>70 ± 7</td>
<td>36.4 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.8 ± 0.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>84 ± 7</td>
<td>36.9 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.2 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> For a given day (row), values bearing different superscripts are significantly different (P <0.05).

S-S Supplemented before and after lambing.  NS-S Supplemented only after lambing.  S-NS Supplemented before but not after lambing.  NS-NS Not supplemented at any time.
study. As a result, Table II shows that the animals in all groups gained weight during the last two months of pregnancy. However, total weight gained during this period was associated with the conceptus, since immediately after lambing the weight of the ewes was similar or slightly lower than the weight they had 40 days before. All groups of ewes lost weight during lactation, but this loss was larger in the groups that were not supplemented during lactation. As a result, the body condition scores of the ewes that were not supplemented during lactation were lower at the end of the study than at the beginning, regardless of their nutritional level during late gestation (Figure 1). In contrast, the ewes that were supplemented during lactation finished the experiment at a condition score that was similar (group NS-S) or better (group S-S) than the score they had at the initial stage of the experiment.

Table III shows the average weight changes of the ewes during late gestation (from 60 to 7 days pre-partum), during parturition (from 7 days pre-partum to 1 day post-partum) and during lactation (from lambing to weaning). As expected, the two groups that were supplemented pre-partum gained significantly more weight during late gestation than did the non-supplemented ewes, and the animals that were supplemented during lactation lost significantly less weight from lambing to weaning than the non-supplemented animals. The net weight loss from the beginning to the end of the experiment was significantly smaller (P <0.05) in the two groups that were supplemented during lactation than in the groups that were not supplemented during that period, regardless of pre-partum supplementation. These results indicate that, from the viewpoint of maternal well-being, post-partum supplementation is more effective than pre-partum supplementation, provided that the quality and quantity of forage is sufficient to provide basic maintenance and gestation requirements.

Plasma β-hydroxybutyrate and urea (Table IV) were not useful indicators of the metabolic status of the animals, since their concentrations were not different between groups in spite of the clear differences in body weight and condition scores between groups. In contrast, total protein concentrations appeared to be related to the metabolic status of the animals since concentrations (Table IV) decreased significantly (P <0.01) in all groups during peak lactation (d 45). The decrease was less marked in animals that had received supplementation either pre- or post-partum, and even less evident in the group that was supplemented both before and after lambing. Changes in protein concentrations were mainly due to changes in globulins, since the concentrations of albumin remained constant throughout lactation (Table IV).
TABLE III. EFFECTS OF PRE-AND POST-PARTUM SUPPLEMENTATION OF PELIBUEY EWES ON WEIGHT CHANGES (kg) DURING LATE GESTATION (day 60 to day 7 pre-partum), DURING LAMBING (day 7 pre-partum to day 1 post-partum) AND DURING LACTATION (day 1 to 90 post-partum) (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>S-S</th>
<th>S-NS</th>
<th>NS-S</th>
<th>NS-NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight changes during:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late gestation</td>
<td>-4.3 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-4.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-4.4 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lambing</td>
<td>-3.6 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-6.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.6 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactation</td>
<td>-3.4 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.9 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-3.4 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-7.1 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cumulative</td>
<td>4.5 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> For a given day (row), values with different superscripts are significantly different (P <0.05). Abbreviations as in Table II.

TABLE IV. EFFECTS OF PRE-AND POST-PARTUM SUPPLEMENTATION OF PELIBUEY EWES ON PLASMA CONCENTRATIONS OF 6-HYDROXYBUTYRATE, UREA, TOTAL PROTEIN AND ALBUMIN ON DIFFERENT DAYS POST-PARTUM

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Days post-partum</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S-S</td>
</tr>
<tr>
<td>6-hydroxybutyrate</td>
<td>15</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>45</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td>Urea</td>
<td>45</td>
<td>3.90±0.19</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>45</td>
<td>3.91±0.20</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>3.67±0.17</td>
</tr>
<tr>
<td>Albumin</td>
<td>45</td>
<td>39.5±0.27</td>
</tr>
<tr>
<td>(g/L)</td>
<td>45</td>
<td>39.3±0.32</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>38.5±0.36</td>
</tr>
<tr>
<td>Total protein</td>
<td>15</td>
<td>71.7±0.69&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>(g/L)</td>
<td>45</td>
<td>67.1±0.93&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>67.6±0.60&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

There were no significant differences between groups for the first three metabolites (P >0.05). Regarding total protein, different superscripts indicate significant differences (P <0.05). Abbreviations as in Table II.

The effects of supplementation on body weight and condition score were not reflected in earlier resumption of ovarian activity, as there were no differences (P >0.05) between groups in the intervals from lambing to first ovulation and from lambing to first oestrus (Table V). The average intervals from lambing to first ovulation and to first oestrus were 27.3 and 43.1 days, respectively. It should be taken into account, that the ewes used in this experiment lambed in the middle of the rainy season (July). It is probable that the good availability and quality of the forage were adequate to satisfy basic requirements for maintenance and gestation, so that the extra nutrients provided by supplementation were not needed for reproduction. Several authors working with Pelibuey ewes have found that the intervals from lambing to first oestrus [2, 6, 7], from lambing to first service [8] and from lambing to conception [8], are reduced during the rainy season compared to other seasons of the year. However, it is also unlikely that supplementation would affect the length of the post-partum period at other times of the
year, since reproductive seasonality prevents a rapid resumption of ovarian activity in Pelibuey ewes that lamb during the fall, winter or spring [9], even if they have had adequate nutrition [10]. So, effects of supplementation on ovarian activity would have been masked by seasonality.

TABLE V. EFFECTS OF PRE- AND POST-PARTUM SUPPLEMENTATION OF PELIBUEY EWES ON THE INTERVALS (days) FROM LAMBING TO FIRST OVULATION OR TO FIRST OESTRUS (mean ± SEM)

<table>
<thead>
<tr>
<th>Interval from lambing to:</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-S</td>
</tr>
<tr>
<td>First ovulation</td>
<td>26.0 ± 1.3</td>
</tr>
<tr>
<td>First oestrus</td>
<td>41.0 ± 1.7</td>
</tr>
</tbody>
</table>

There are not significant differences between groups (P <0.05). Abbreviations as in Table II.

Although there were marked differences in the performance of lambs depending on status (single or twins), the effects of supplementation were similar for single and twin lambs. The birth weights of the lambs from ewes that were supplemented during late gestation were significantly higher than those of lambs from ewes that were not supplemented pre-partum (Table VI). The most important effect on weaning weights was post-partum supplementation. The weaning weight of lambs from ewes that were supplemented during lactation were significantly higher than those of lambs from ewes that were not supplemented during lactation, regardless of the level of pre-partum nutrition (Table VI). However, differences in weaning weight were due to differences in weight gain during lactation. Figure 2 shows the average weight of the 4 groups of lambs at different ages. There were no effects of pre- or post-partum supplementation on lamb mortality. The number of lambs that died from birth to weaning was 3, 4, 3 and 3 for groups S-S, S-NS, NS-S and NS-NS, respectively. Both the birth weight and the weaning weight found in the different groups of this experiment are similar to those that have been reported by other authors [8, 11].

TABLE VI. BODY WEIGHT (kg) AT BIRTH AND AT WEANING, AND WEIGHT GAIN (kg) DURING LACTATION (mean ± SEM) OF SINGLE AND DOUBLE LITTERS OF PELIBUEY EWES THAT WERE SUPPLEMENTED OR NOT SUPPLEMENTED BEFORE AND/OR AFTER LAMBING

<table>
<thead>
<tr>
<th>Type of Lambing</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-S</td>
</tr>
<tr>
<td>Weight at birth Singletons</td>
<td>3.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Twin litters</td>
<td>5.7 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight at weaning Singletons</td>
<td>17.0 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Twin litters</td>
<td>21.6 ± 0.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight gain during lactation Singletons</td>
<td>13.7 ± 0.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Twin litters</td>
<td>18.9 ± 0.8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup> Different superscripts indicate significant differences (P <0.05). Abbreviations as in Table II.
FIG. 2. Body weights of Pelibuey lambs born from ewes maintained on different nutritional levels before and after lambing.

It is concluded that there was a beneficial effect of supplementation of Pelibuey ewes during lactation, because it caused a significant increase on the growth rate of the lambs and a reduction in the weight loss and body condition loss of the dams during lactation. Pre-partum supplementation during the rainy season did not provide an additional advantage if the ewes were supplemented during lactation. Post-partum ovarian activity of Pelibuey ewes lambing during the rainy season was not affected by either pre- or post-partum supplementation.

REFERENCES


EFFECT OF SUPPLEMENTATION WITH AGRICULTURAL BY-PRODUCTS ON ONSET OF PUBERTY AND SEASONALITY OF CORRIEDALE-TYPE EWES IN THE BOLIVIAN HIGHLANDS

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Abstract – Resumen

EFFECT OF SUPPLEMENTATION WITH AGRICULTURAL BY-PRODUCTS ON ONSET OF PUBERTY AND SEASONALITY OF CORRIEDALE-TYPE EWES IN THE BOLIVIAN HIGHLANDS.

Groups of Corriedale-type ewes born in May 1991 were supplemented (S, n = 30) with an agricultural by-product concentrate for three months after weaning, or not supplemented (N-S, n = 30). Animals were weighed every two weeks during the first five months after weaning, and monthly thereafter. Fifteen animals from each group were bled for progesterone determinations twice a week, from 6 to 9 months of age, and weekly thereafter. Sequential progesterone profiles were evaluated to determine time of first ovulation. Males were introduced for 45 days on January 15th, 1993, when ewes were 20 months old. S ewes gained more weight than the N-S ewes during the three months of supplementation (P <0.05). Difference in body weight accumulated during this period remained for the rest of the study, even though supplementation ceased. Supplementation did not advance puberty. However, it resulted in a significant (P <0.05) weight advantage in favor of S ewes at time of first ovulation (24.4 ± 0.5 kg in S ewes vs 16.7 ± 0.4 kg in N-S). A period of seasonal acyclicity occurred from January to August, so that ewe lambs that did not start cycling before January had to wait until the following August to reach puberty. The response to male introduction during the non-breeding season was better in S than in N-S ewes. Supplementation early in life produced a permanent increase in body weight, and this was reflected in better reproductive responses for at least one year after supplementation had been ceased.

EFECTO DE LA SUPLEMENTACION CON SUBPRODUCTOS AGRICOLAS EN EL INICIO DE LA PUBERTAD Y LA ESTACIONALIDAD EN OVEJAS TIPO CORRIEDALE EN LAS ZONAS ALTAS DE BOLIVIA.

Un grupo de ovejas tipo Corriedale nacidas en mayo de 1991 fue suplementado (S, n = 30) con subproductos agrícolas por tres meses después del destete. Otro grupo no fue suplementado (N-S, n = 30). Los animales se pesaron cada dos semanas durante los primeros cinco meses a partir del destete, y de allí en adelante en forma mensual. Se obtuvieron muestras de sangre para determinaciones de progesterona dos veces por semana a 15 animales por grupo desde los 6 a los 9 meses de edad, y en forma semanal de allí en adelante. Los perfiles de progesterona se evaluaron para determinar el momento de la primera ovulación. Los machos fueron colocados por 45 días a partir del 15 de enero de 1993 cuando las ovejas tenían 20 meses de edad. Las ovejas S ganaron más peso que las ovejas N-S durante los tres meses de la suplementación. La diferencia en peso corporal acumulado durante este período se mantuvo por el resto del estudio, aún a pesar de que la suplementación fue suspendida. La suplementación no adelantó la aparición de la pubertad; sin embargo, resultó en un mayor peso (P <0.05) en favor de las ovejas S en el momento de la ovulación (24.4 ± 0.5 kg en ovejas S vs 16.7 ± 0.4 kg en N-S). Un período de aciclicidad estacional se presentó entre enero a agosto, de modo que las ovejas que no comenzaron a ciclar antes de enero tuvieron que esperar hasta el siguiente agosto para alcanzar la pubertad. La respuesta a la introducción del macho fuera de la estación de monta fue mejor en ovejas S que en las N-S. La suplementación en una etapa temprana de la vida produjo un mayor peso corporal que se mantuvo en forma permanente, lo que resultó en una mejor respuesta reproductiva, aún después de un año de haberse descontinuado la suplementación.

1. INTRODUCTION

The central highlands of Bolivia provide appropriate conditions for sheep production. Therefore, husbandry of the species is common among peasants in the region [1]. Feed availability is one of the main constraints to sheep production in the region, since the traditional production system is based on native pastures of low nutritional quality, which fail to fulfill the requirements for both wool and meat production [2]. Supplementation with agricultural by-products at strategic times in the life of the animal could prove a cost-effective means to improve productivity. One such strategic periods is the time from weaning to onset of puberty, since increased growth rates will result in advanced puberty [3, 4] and/or a better body condition at the time of first ovulation [3] which should lead to higher conception rate. Furthermore, it is possible that the advantage in body weight and development obtained early in life could be maintained after supplementation is suspended, thus constituting a long term benefit for the animal [3].
The objectives of this experiment were: a) To evaluate the effects of supplementation with local agricultural by-products during the three months following weaning on body weight gain, age and weight at puberty, reproductive seasonality and reproductive efficiency of Corriedale-type ewes grazing in the highlands of Bolivia; b) To evaluate through serial progesterone determinations, the characteristics of ovarian activity during the peripuberal period of supplemented and non-supplemented ewes; c) To evaluate the characteristics of ovarian activity during the first year after onset of puberty; and d) To compare the response of previously supplemented or non-supplemented ewes to male introduction during the season when acyclicity prevails.

2. MATERIALS AND METHODS

The experiment was conducted at the experimental station "Fundo Condoriri", located at 3830 metres above sea level, 17° 31' South and 67° 14' West. The mean temperature is 8.9 °C and the annual rainfall is 360 mm, distributed mainly from February through June.

Sixty Corriedale-type ewe lambs born in May 1991 were weaned at the age of 4.8 months and were randomly divided into two groups. The animals in the supplemented (S) group received 350 g/head/day of a concentrate with 12 % crude protein. The concentrate contained broad bean stalks, "quinoa" (Chenopodium quinoa) stalks, barley grains, urea and mineral salts [5]. Quinoa is a pseudo cereal grain that is part of the basic diet of the human population in the highlands of Bolivia. The supplement was given daily for three months after weaning. The other group was not-supplemented (N-S). Animals from both groups grazed together on native pastures and were only separated at night. Groups were kept on separate pens and the concentrate was offered to the S group. The animals were weighed every two weeks during the first five months after weaning, and monthly thereafter.

Fifteen animals from each group were bled for progesterone determinations twice a week since 6 to 9 months of age. Heparinized blood samples were centrifuged and the plasma was separated and kept frozen at -20 °C until assayed for progesterone by solid-phase radioimmunoassay. Onset of puberty was assumed when progesterone concentrations were above 2.9 nmol/L for at least 2 consecutive bleedings, which presumably indicated ovulation followed by the formation of normal corpus luteum [3]. Transient progesterone increases (TPIs) were defined as an increase in progesterone values above 2.9 nmol/L in a single isolated sample, and were not considered indicative of true ovarian cyclicity [3]. Bleedings continued once a week from the age of 9 months until animals were 22 months old (March, 1993) in order to monitor changes in ovarian activity. No sampling was done during May of 1992 due to material shortages.

Three fertile males were introduced into the flock on January 15th, 1993 when the ewes were 20 months old, and remained with the flock for 45 days. Conception rate and dates of conception were calculated retrospectively from subsequent lambing dates.

The weight of the animals at different ages was compared by a two-way analysis of variance, using group and age as independent variables. The age at first TPI, number of TPIs previous to the first ovulation, interval from first TPI to first ovulation and age and weight at first ovulation were compared by the Student's "t" test. The proportion of ewes ovulating in each month were compared by X-square test.

3. RESULTS AND DISCUSSION

Figure 1 illustrates the growth pattern and average weight of the two groups at different ages and at different dates. It can be seen that the average weight of both groups was identical before starting the supplementation period (i.e. weaning on October 1st). The S ewes gained weight during the supplementation period (October-December), while the N-S ewes lost weight during the same months. As a result, after November 1st the body weights of animals in the S group were always significantly greater (P <0.05) than those in the N-S animals. Supplementation was ceased on December 31st, but both groups of animals gained weight continuously from January to July, which are the months with good forage availability due to the rainy season. Finally, animals from both groups lost weight gradually from August to November 1992. However, the difference in body weight which originated during the
FIG. 1. Average body weight at different ages and at different months in ewes supplemented and not supplemented during three months after weaning.

supplementation period was maintained throughout the study, even though the supplement was no longer given to the S group during 1992.

Figure 2 shows that there was seasonality in the ovarian activity of both groups. Some ewe lambs began cycling during December of 1991, when they were seven months old, but became acyclic from February to July and resumed ovarian activity in August. The proportion of cycling animals in both groups reached a peak in November and declined again in January. As a result of this inherent seasonality, not all ewe lambs reached puberty during their first year of life. A complete functional hypophyseal-hypothalamic-gonadal relationship was not established in some females within both groups before January 1992 and, since appropriate external stimuli were no longer present, puberty in these individuals was delayed until the onset of the following reproductive season (August 1992). This interaction between season and onset of puberty has been previously described [3, 4, 6].

Only 11 ewe lambs from the N-S group and 7 from the S group ovulated and formed a normal corpus luteum during their first year of life (Table I). However, all sampled animals showed transient progesterone increases (TPI) during the same period. TPIs have been described before as attempts to initiate cyclic ovarian activity by animals that are still partially inhibited due to insufficient body size or weight, or to the absence of appropriate external stimuli [3]. These transient increases in progesterone
TABLE I. CHARACTERISTICS OF OVARIAN ACTIVITY DURING THE FIRST YEAR OF LIFE OF CORRIEDALE-TYPE EWES THAT WERE SUPPLEMENTED OR NOT SUPPLEMENTED DURING THE FIRST THREE MONTHS POST-WEANING

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplemented</th>
<th>Non-Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ewes with TPIs</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age at first TPI (days)</td>
<td>239 ± 2\textsuperscript{a}</td>
<td>220 ± 2\textsuperscript{b}</td>
</tr>
<tr>
<td>Number of ewes ovulating</td>
<td>7\textsuperscript{a}</td>
<td>11\textsuperscript{a}</td>
</tr>
<tr>
<td>Interval from first TPI to first ovulation (days)</td>
<td>9.0 ± 2.3\textsuperscript{a}</td>
<td>16.4 ± 1.8\textsuperscript{b}</td>
</tr>
<tr>
<td>Age at first ovulation (days)</td>
<td>243 ± 2\textsuperscript{a}</td>
<td>234 ± 2\textsuperscript{b}</td>
</tr>
<tr>
<td>Weight at first ovulation (kg)</td>
<td>24.4 ± 0.5\textsuperscript{a}</td>
<td>16.7 ± 0.4\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} Means of variables marked with an asterisk were significantly different between groups (P < 0.05).

Values are expressed as mean ± SEM.

TPI Transient progesterone increase.

probably result from follicular luteinization [3]. Table I also shows the age at first TPI and the age and body weight at first ovulation in each group. These results demonstrate that under the conditions of this experiment, the onset of puberty was not advanced by supplementation. The first TPI and ovulation occurred at significantly lower age and weight (P < 0.05) in the N-S than in the S group. However, it should be pointed out that these parameters were calculated only from the data of those animals that cycled during the first year of life. Although age at first ovulation was only marginally different between groups, body weight at first ovulation was far higher in S than in N-S animals. This would be advantageous if mating occurs during their first reproductive season.

It is interesting that ovarian activity occurred mainly during winter and spring when the length of the photoperiod is increasing, and ceased during summer and fall when photoperiod is decreasing. This disagrees with the classical concept of photoperiodic control of reproduction in the ewe [6]. Furthermore, the onset of ovarian activity was not related to a seasonal increase in food availability, since the ewes in this experiment started cycling during the most critical part of the dry season (Aug-Dec), and stopped cycling in the months with best forage availability (Feb-May). This is clearly shown in Figure 3, which illustrates the inverse relationship between changes in body weight (Figure 3a) and number of ewes cycling in the second breeding season (Figure 3b).

This finding suggests that a selection mechanism operated during the 50 years in which Corriedale sheep have been present in the highlands of Bolivia, so that the persisting genotypes in the population represent those from animals that survived. This is because their dams could go through late gestation and lactation during the short period of good forage availability (Feb-June). These animals were the ewes that were able to reproduce between September and December even though forage was deficient during those months, and in spite of the fact, that photoperiod is increasing during this period in the Southern hemisphere.

The mechanisms controlling this unique reproductive strategy are still unknown. Photoperiod remains the most likely regulator, since it has been shown that light itself is neither a stimulator nor an inhibitor of ovarian activity, and that the animals learn to use the photoperiod as a signal to reproduce when it is convenient for their survival. For example, some ovine breeds or strains can use strategies such as photo-refractoriness to initiate and/or finish the breeding season at times when the direction of photoperiodic change would cause the opposite effect in most sheep [7, 8]. Although the changes in photoperiodic length are relatively small at the latitude where this study was carried out, other authors have demonstrated the existence of true seasonality in both goats [9] and sheep [3, 4] in similar latitudes in the Northern hemisphere.
FIG. 3. Weight changes (a) and percentage of ewes cycling (b) in supplemented (S) and not-supplemented (N-S) groups during the three months following weaning.

TABLE II. RESPONSE TO THE INTRODUCTION OF MALES DURING THE EARLY ANOESTROUS SEASON IN 20 MONTH-OLD EWES THAT WERE SUPPLEMENTED OR NOT SUPPLEMENTED DURING THE FIRST THREE MONTHS POST-WEANING

<table>
<thead>
<tr>
<th></th>
<th>Supplemented</th>
<th>Not-Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>% cycling in early January (before exposure to males)</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>% cycling in February (after exposure to males)</td>
<td>87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean interval between male exposure and ovulation (days)</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>Pregnancy rate in 45 days</td>
<td>55.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Row values bearing different superscripts are significantly different (P<0.05).
When the males were introduced to the flock on January 15th, 1993, most of the ewes were already acyclic (Figure 2). However a "male effect" [10] occurred, since several animals ovulated and formed a functional corpus luteum within three weeks after ram exposure (Table 2). This effect was more marked in the S group, probably due to the larger body weight. The number of ewes that became pregnant during the 30 days of exposure to the male was also higher in the S group (Table 2).

It is concluded that supplementation of Corriedale-type ewes during a period of three months following weaning does not advance puberty in May-born animals. However, the higher weight gains during the supplementation period translate into a sustained higher body weight. This results in higher body weight at puberty, which could translate in better reproductive performance if the animals are breed during their first breeding season [3]. The higher body weight that was still present during the second breeding season of S animals resulted in a better response to male exposure and a higher pregnancy rate.

REFERENCES

MINERALS AND NON-CONVENTIONAL NITROGEN SOURCES AS STRATEGIC SUPPLEMENTS FOR DUAL PURPOSE CATTLE

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Abstract – Resumen

MINERALS AND NON-CONVENTIONAL NITROGEN SOURCES AS STRATEGIC SUPPLEMENTS FOR DUAL PURPOSE CATTLE.

Three experiments were carried out to evaluate the influence of minerals and non-conventional sources of nitrogen (N) as strategic supplements for dual purpose cattle. Experiment I was carried out with 26 heifers supplemented with 2.0 kg/day concentrate per heifer with or without a 4.0% addition of a mineral mixture. Heifers receiving the mineral supplement showed higher body weight gains and all got pregnant, whereas in the non-supplemented group only 61.5% conceived. In Experiment II, 32 lactating cows were supplemented with 1.82 kg/day/cow of concentrate with one of four different N sources: Soybean meal (SBM), poultry manure (PM), Gliricidia sepium (Gs) or PM plus Gs. Milk yields, adjusted to 305 days of lactation, were 1509.8, 1656.2, 1628.7 and 1683.6 kg for treatments 1, 2, 3 and 4, respectively (P >0.05). The supplement with PM and Gs was significantly less expensive than the one with SBM. Experiment III was also carried out with lactating cows with higher milk yields. Twenty four animals were supplemented with 3.2 kg/day/cow of a control supplement © or 5.5 kg/day/cow of concentrates with SBM, PM or urea (U) as sources of N. The three latter treatments had similar milk yields and these were significantly higher (P <0.05) than those with the C supplement. It is concluded that under Salvadorean conditions the use of concentrates with PM and urea were more cost effective.

MINERALES Y FUENTES NITROGENADAS NO-CONVENCIONALES COMO SUPLEMENTO ESTRATEGICO PARA GANADO VACUNO DE DOBLE PROPOSITO.

Se llevaron a cabo tres experimentos con el fin de evaluar el efecto de minerales y fuentes nitrogenadas no-convenionales como suplementos alimenticios estratégicos para ganado vacuno de doble-propósito. El Experimento I se hizo con 26 vaquillas suplementadas con 2.0 kg/día/animal de un concentrado que contenía el 4.0% de una mezcla mineral. Las vaquillas que recibieron la mezcla mineral obtuvieron mayores ganancias de peso y todas quedaron gestantes en comparación con el grupo no-suplementado en el que solo se llegó a concebir el 61.5%. En el Experimento II, 32 vacas lactantes fueron suplementadas con 1.82 kg/día/vaca con concentrados que contenían cuatro diferentes fuentes de N: Harina de soya (SBM), gallinaza (PM), Gliricidia sepium (Gs) o PM más Gs. Las lactancias corregidas a 305 días fueron 1509.8, 1656.2, 1628.7 y 1683.6 kg para los tratamientos 1, 2, 3 y 4, respectivamente (P >0.05). Estos valores no fueron estadísticamente diferentes pero la suplementación con PM y Gs fue menos costosa que aquella con SBM. El experimento III se llevó a cabo con vacas lactantes pero de mayor producción lechera. Venticuatro animales fueron suplementados con 3.2 kg/día/vaca de un suplemento testigo (C) o con 5.5 kg/día/vaca de un suplemento con SBM, PM o urea (U) como fuentes de N. Los animales de los tres últimos tratamientos obtuvieron una producción de leche similar pero significativamente mayor que la obtenida por el grupo con el suplemento C (P <0.05). Bajo las condiciones salvadoreñas, el uso de concentrados con PM y urea mostraron ser más efectivos al considerar los costos.

1. INTRODUCTION

El Salvador is a tropical country with a high population density and an insufficient level of protein sources for human consumption. The dual-purpose cattle population is approximately one million with an average milk yield of 4.0 kg/day/cow and calf crop of one per two years per cow [1]. This low productivity is influenced by a series of factors. Among which nutritional constraints are believed to be the most important [2, 3, 4].

Two groups of nutrients frequently deficient in the diet are minerals [5] and nitrogen (N) [3, 6]. Mineral deficiencies can be resolved with the addition of a good quality mineral mixture. On the other hand, N deficiencies are occasionally ameliorated by means of expensive imported protein sources such as soybean meal. Supplementation with non-conventional N sources has been employed in recent years [3, 4], for example, the foliage of legume trees has been shown to be a good source of protein for ruminants [3, 7]. Legumes provide other advantages such as agricultural N fixation and contribute to soil preservation [8]. Poultry manure is another source of non-protein N [9, 10, 11, 12]. It is a by-product of the poultry industry that is abundant, cheap and supplies other nutrients including minerals [13, 14].
Three experiments were carried out in herds of dual-purpose cattle to evaluate the effects of supplementation with minerals and non-conventional sources of N on production and reproductive parameters.

2. MATERIALS AND METHODS

2.1. Experiment I Effect of mineral supplementation on body weight gain and reproductive efficiency in growing heifers

2.1.1. Location

The trial was carried out on the Monte Rico Farm, 75 km from San Salvador, at 50 m.a.s.l. Climatic conditions were: Mean temperature, 26.9°C, maximum temperature, 31.8°C, minimum temperature, 22.9°C, mean relative humidity, 80% and mean annual rainfall, 1695 mm [15].

2.1.2. Experimental design and treatments

Twenty-six heifers were randomly assigned to two treatments. The diet of forage was supplemented in the first group with a mineral mixture plus 2 kg concentrate/day/heifer. The other group remained as control with forage supplementation and concentrate alone. The observations continued until all animals receiving the mineral supplementation became pregnant. Statistical comparison was accomplished by means of the Student "t" test.

2.1.3. Animals and feed

Animals were crossbred type (European x Zebu). They were 16 months old with mean body weight of 222 kg at the beginning of the trial. Artificial insemination was performed at observed oestrus when the heifers reached 340 kg of body weight. Animals were allowed to graze on pastures which were predominantly Star grass (Cynodon plectostachius). They were divided into two groups every day to provide the supplement to the treated group. The composition of the concentrate and the mineral mixture is shown in Table I. Supplements were analysed for crude protein, ash, crude fiber [16], calcium [17] and phosphorus [18]. Energy content was estimated according to the NRC tables [19].

<table>
<thead>
<tr>
<th>TABLE I. COMPOSITION OF CONCENTRATE AND MINERAL MIXTURE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>With minerals</td>
</tr>
<tr>
<td>Without minerals</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cotton seed</td>
</tr>
<tr>
<td>Cotton seed meal</td>
</tr>
<tr>
<td>Poultry manure</td>
</tr>
<tr>
<td>Corn grain + cob</td>
</tr>
<tr>
<td>Rice polishing</td>
</tr>
<tr>
<td>Molasses</td>
</tr>
<tr>
<td>Urea</td>
</tr>
<tr>
<td>Mineral mixture(^1)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

\(^1\) Mineral mixture composition (prepared on farm).

<table>
<thead>
<tr>
<th>Components (ppm)</th>
<th>With minerals</th>
<th>Without minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>13.271 %</td>
<td>13.020 %</td>
</tr>
<tr>
<td>P</td>
<td>3.820 %</td>
<td>3.820 %</td>
</tr>
<tr>
<td>S</td>
<td>0.744 %</td>
<td>0.744 %</td>
</tr>
<tr>
<td>Mg</td>
<td>0.413 %</td>
<td>0.413 %</td>
</tr>
<tr>
<td>K</td>
<td>0.062 %</td>
<td>0.062 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>With minerals</th>
<th>Without minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit A</td>
<td>4 x 10^4 IU/kg</td>
<td></td>
</tr>
<tr>
<td>Vit D</td>
<td>4 x 10^6 IU/kg</td>
<td></td>
</tr>
<tr>
<td>Vit E</td>
<td>400 IU/kg</td>
<td></td>
</tr>
</tbody>
</table>

60
2.1.4. Measurements
Animals were weighed monthly. Pregnancy was confirmed by rectal palpation at 60 days after service. Weekly blood samples were collected and progesterone was determined by using the FAO/IAEA solid-phase RIA kits.

2.2. Experiment II  Effect of soybean meal, *Gliricidia sepium* and poultry manure on productive and reproductive performance of dual-purpose lactating cows

2.2.1. Location
The trial was carried out on the Calamar Farm, 85 km from San Salvador, at 10 m.a.s.l. The climatic conditions were: Mean temperature, 26.9°C, maximum temperature, 31.8°C, minimum temperature, 22.9°C, mean relative humidity, 80% and mean annual rainfall, 1754 mm [15].

2.2.2. Experimental design and treatments
A completely randomized design with four treatments and eight replicates per treatment was used to compare four N sources. The concentrates are described in Table II. The dependent variables were milk production, number of non-pregnant days, body condition and body weight. The N sources were:

- Control with soybean meal
- Poultry floor manure
- *Gliricidia sepium* (gliricidia) leaves and sprigs (6 mm diameter)
- Poultry manure + gliricidia (1kg:1kg)

**TABLE II. COMPOSITION (%) AND COST OF THE CONCENTRATE**

<table>
<thead>
<tr>
<th>Components (%)</th>
<th>Treatments</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
</tr>
<tr>
<td>Corn grain</td>
<td>48.5</td>
<td>48.8</td>
<td>49.4</td>
<td>46.2</td>
</tr>
<tr>
<td>Molasses</td>
<td>19.7</td>
<td>19.7</td>
<td>19.6</td>
<td>19.6</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>11.0</td>
<td>3.2</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Ground hay (<em>Cynodon plectostachius</em>)</td>
<td>4.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Poultry manure</td>
<td>--</td>
<td>24.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><em>Gliricidia sepium</em></td>
<td>--</td>
<td>--</td>
<td>24.6</td>
<td>--</td>
</tr>
<tr>
<td>Poultry manure + <em>Gliricidia sepium</em>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>29.3</td>
</tr>
<tr>
<td>Urea</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Biofos</td>
<td>1.7</td>
<td>0.1</td>
<td>1.7</td>
<td>0.7</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.8</td>
<td>1.1</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Salt (ClNa)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Cost of 100 kg (US$)</strong></td>
<td>15.64</td>
<td>12.97</td>
<td>13.98</td>
<td>12.55</td>
</tr>
</tbody>
</table>

<sup>1</sup> Ratio 1:1
T1 Control with soybean meal.
T2 Concentrate with poultry manure.
T3 Concentrate with *Gliricidia sepium*.
T4 Concentrate with poultry manure plus *Gliricidia*.

All concentrates had on average 18% crude protein. Ground hay was included in treatment 1 to balance the high crude protein content and the low fiber content of soybean meal. A fixed level of 1.82
kg/day/animal of concentrate was offered to all animals (1.58 kg/day/animal of dry matter). All animals grazed on pastures which were predominantly Star grass.

2.2.3. Animals and measurements

Thirty two dual-purpose crossbred Brahman × Holstein and Brown Swiss cows expected to calve over a 60 day interval. The animals were in their second or third pregnancy and had a mean body weight of 406 kg. The supplement was offered 15 days before expected calving in amounts equal to 1.82 kg/day/cow. After calving animals were milked once daily and suckling was allowed at 06:00 and at 16:00. Blood samples were taken weekly and analysed for progesterone using the FAO/IAEA RIA kits for pregnancy diagnosis. Body weight was estimated by measuring the chest girth every month. Body condition score was evaluated every month using a scale from 1 to 5. Natural breeding was used in the trial with 16 cows per bull.

2.3. Experiment III Effect of soybean meal, poultry manure and urea on productive and reproductive parameters in dual-purpose lactating cows

2.3.1. Location

The trial was carried out on the farm El Aguila, 70 km from San Salvador, at 1200 m.a.s.l. Climatic conditions were: Mean temperature, 16.8°C, maximum temperature, 24.6°C, minimum temperature, 12.4°C, mean relative humidity, 76% and mean annual rainfall, 2116 mm [16].

2.3.2. Experimental design and treatments

A completely randomized design with four treatments and six replications per treatment was used to compare three supplements with different N sources. The supplement offered to the control group was the same as that used by the farmers in the region. The treatments included soybean meal, poultry manure and urea as N sources and were as follows (see Table III):

- T1 - 3.2 kg/day farmer concentrate
- T2 - 5.5 kg/day concentrate containing soybean meal
- T3 - 5.5 kg/day concentrate containing poultry manure
- T4 - 5.5 kg/day concentrate containing urea

TABLE III. COMPOSITION (%) AND COST OF THE CONCENTRATE

<table>
<thead>
<tr>
<th>Components (%)</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>----------------</td>
<td>----</td>
</tr>
<tr>
<td>Corn meal</td>
<td>50</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>25</td>
</tr>
<tr>
<td>Poultry manure</td>
<td>--</td>
</tr>
<tr>
<td>Urea</td>
<td>--</td>
</tr>
<tr>
<td>Molasses</td>
<td>4</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>Crude protein</td>
<td>17.25</td>
</tr>
<tr>
<td>Cost 100 kg (US $)</td>
<td>19.87</td>
</tr>
</tbody>
</table>

1 Mineral mixture as in experiment I.
T1 Control.
T2 Concentrate containing soybean meal.
T3 Concentrate containing poultry manure.
T4 Concentrate containing urea.
2.3.4. Animals and measurements

A group of 24 crossbred dual-purpose cows (3/4 Holstein × 1/4 Santa Gertrudis) were used. Milking was performed twice a day. Measurements were the same as those described in Experiment 2. Artificial insemination was used in this herd.

3. RESULTS AND DISCUSSION

3.1 Experiment 1

The chemical composition of supplements used in this trial is shown in Table IV. Crude protein and energy contents were similar, but large differences were observed regarding the phosphorus content (0.34 vs 0.86%) and to a lesser extent the calcium concentration (0.30 vs 0.83%) as expected.

The interval between the initiation of the experiment and the time when all animals receiving mineral supplementation became pregnant was 385 days. The resulting pregnancy rate in the control group was 61.5% (P <0.05) at the end of the experiment (Table V). Body weight was higher in heifers receiving the mineral mixture (352 kg) as compared with heifers in the control group (333 kg).

<table>
<thead>
<tr>
<th>TABLE IV. CHEMICAL COMPOSITION OF THE CONCENTRATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
</tr>
<tr>
<td>Dry matter (%)</td>
</tr>
<tr>
<td>Crude protein (%)</td>
</tr>
<tr>
<td>NEm (Mcal/kg)</td>
</tr>
<tr>
<td>N Eg (Mcal/kg)</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
</tr>
<tr>
<td>TDN3</td>
</tr>
<tr>
<td>Calcium (%)</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
</tr>
</tbody>
</table>

1 Net energy maintenance.
2 Net energy gain.
3 Total digestible nutrients.

The cost of the two supplements is shown in Table V. The total cost of supplementation per animal was about 30% higher when the mineral mixture was included in the concentrate. However, the difference was much smaller if considered the additional cost of the concentrate without minerals which would have be necessary to feed the control animals so that they would reach the same body weight of the treated animals. The additional cost of supplements to increase body weight from 333 to 352, with a body weight gain of 0.291 kg/day has been estimated (Table V), and it can be seen that costs are comparable to mineral supplementation. Moreover, the shorter calving intervals and the increase in milk production in the treated group easily compensate for the extra cost of supplement with minerals [18].

Malnutrition is commonly accepted as the limiting factor for cattle production in the tropics. Major deficiencies are total energy and proteins [20], as well as minerals [5]. Other studies have shown that mineral imbalances in soils, forages and supplements resulted in lower feed consumption, lower body weight gain and reduced fertility under grazing conditions [2, 5, 21]. Other consequences include increased incidence of diseases [22]. Mineral requirements vary with growth rate and milk yield [23].
TABLE V. PRODUCTION CHARACTERISTICS OF HEIFERS AND ESTIMATED COST OF THE SUPPLEMENT

<table>
<thead>
<tr>
<th></th>
<th>Concentrate</th>
<th>With minerals</th>
<th>Without minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° of animals</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Initial age (months)</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Initial body weight (kg)</td>
<td>223</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>352</td>
<td>333</td>
<td></td>
</tr>
<tr>
<td>Body weight gain (kg/day)</td>
<td>0.34</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Pregnancy at 385 days (%)</td>
<td>100</td>
<td>61.54</td>
<td></td>
</tr>
<tr>
<td>Supplement (kg dry matter/day)</td>
<td>1.75</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>Cost of supplement (US$/day)</td>
<td>0.28</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Accumulated cost (385 days)</td>
<td>107.80</td>
<td>79.00</td>
<td></td>
</tr>
<tr>
<td>Additional days(^1)</td>
<td>--</td>
<td>--</td>
<td>65.50</td>
</tr>
<tr>
<td>Extra cost of supplement (US$)</td>
<td>--</td>
<td>--</td>
<td>13.40</td>
</tr>
<tr>
<td>Total costs (US$)</td>
<td>107.80</td>
<td>92.40</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Estimated additional days that control animals would require to reach the final body weight of animals that have received the concentrate with minerals.

The growth rate of the group of heifers supplemented with minerals in this study was better, perhaps due to an increased efficiency of rumen metabolism and skeletal development \cite{5, 21}. Ca and P are the macro-minerals considered most limiting and believed to be essential for puberty \cite{5}. Other macro- and micro-minerals required for ruminant development can be supplied by means of fertilization of pastures or, addition of cereals, meals or oil components. Other approaches include preparation of macro- and micro-mineral compounds to be added to the concentrate \cite{4, 5, 20}.

3.2. Experiment II

The estimated chemical composition and energy content of supplements used in this trial are shown in Table VI. Protein, energy, Ca and P contents were very similar between treatments.

Production and reproductive parameters are shown in Table VII. Body weight and body condition changes from calving to 105 days post parturition and from 106 to 210 days post parturition were similar in all treatments (P >0.05). Adjusted milk yield at 305 days of lactation was slightly lower in cows receiving the control diet with soybean meal, but the differences were not significant (P >0.05). Average milk yields of cows receiving poultry manure and Gliricidia varied from 5.3 to 5.5 kg/day/cow.

Supplementation with concentrates which fulfill nutritional requirements to maximize cattle reproductive performance is not be economically feasible for farmers in countries where raw materials must be imported \cite{24}. For this reason, non traditional sources available on site or in neighboring regions are being employed \cite{13, 14, 20, 24}. The present experiment, as well as others has demonstrated that bypass protein is not necessary for feeding native cattle with low level of milk production \cite{6, 24}. Adequate milk production and reproduction can be maintained through the use of concentrates based on legumes, poultry manure and urea. Milk yields and body weights of animals in this trial were lower to those reported for cattle fed with Gliricidia \cite{24} or with poultry manure \cite{25}. However, Table VIII shows that Gliricidia as a supplement to the diet of native cattle has given satisfactory results and has shown to be more economically feasible for increasing milk yield from 0.4 to 1.7 kg/cow/day \cite{6}.
### TABLE VI. CHEMICAL COMPOSITION OF THE CONCENTRATE AS DRY MATTER

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>87.02</td>
<td>86.66</td>
<td>86.59</td>
<td>86.57</td>
</tr>
<tr>
<td>NE (Mcal/kg)(^1)</td>
<td>2.96</td>
<td>2.99</td>
<td>2.88</td>
<td>2.90</td>
</tr>
<tr>
<td>NEm (Mcal/kg)(^2)</td>
<td>1.80</td>
<td>1.80</td>
<td>1.71</td>
<td>1.73</td>
</tr>
<tr>
<td>NEg (Mcal/kg)(^3)</td>
<td>1.16</td>
<td>1.16</td>
<td>1.08</td>
<td>1.10</td>
</tr>
<tr>
<td>NEI (Mcal/kg)(^4)</td>
<td>1.69</td>
<td>1.69</td>
<td>1.66</td>
<td>1.65</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>18.03</td>
<td>18.09</td>
<td>17.95</td>
<td>18.01</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>6.87</td>
<td>6.80</td>
<td>6.66</td>
<td>7.67</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>1.37</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Treatments as in Table II.

\(^1\) Net energy.
\(^2\) Net energy maintenance.
\(^3\) Net energy gain weight.
\(^4\) Net energy production milk.

### TABLE VII. PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF DUAL PURPOSE LACTATING COWS

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>403</td>
<td>412</td>
<td>408</td>
<td>410</td>
</tr>
<tr>
<td>Body weight 0-105 d (kg/day)</td>
<td>-0.2</td>
<td>-0.2</td>
<td>-0.2</td>
<td>-0.2</td>
</tr>
<tr>
<td>Body weight 106-210 d (kg/day)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Milk yield (kg)(^1)</td>
<td>1,509.8</td>
<td>1,656.2</td>
<td>1,628.7</td>
<td>1,683.6</td>
</tr>
<tr>
<td>Calving to conception interval (d)</td>
<td>147</td>
<td>141</td>
<td>133</td>
<td>151</td>
</tr>
<tr>
<td>Body condition score (1-5)</td>
<td>3.25</td>
<td>3.5</td>
<td>3.25</td>
<td>3.25</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Treatments as in Table II.

\(^1\) Adjusted milk yield to 305 days of lactation.

This study agrees with others that have shown that under the conditions of grazing dual-purpose cattle, non traditional protein sources can substitute soybean meal and other expensive proteins [3, 4, 6, 8, 11, 13, 14, 20, 24]. Results have shown that any of the supplements evaluated can be used to satisfy the N requirements of these low producing animals. Poultry manure is an abundant and inexpensive by-product. However, some processing is required by sieve and silage to avoid disease transmission and to remove metal objects. *Gliricidia* has a great value as a supplement, because it is produced in the farm and thus contributes to the sustainability of the system.

If costs are considered, the concentrate with soybean meal is the most expensive, (Table II) and consequently, the options using one of the other N sources are more readily justified.
TABLE VIII. FEEDING COSTS AND NET GAIN (US $) WHEN SUPPLEMENTING DUAL PURPOSE LACTATING COWS WITH POULTRY MANURE AND *Gliricidia sepium*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of supplement (US $/day)</td>
<td>0.28</td>
<td>0.24</td>
<td>0.25</td>
<td>0.23</td>
</tr>
<tr>
<td>Milk price/kg (US $)</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>Milk (L) required to cover costs</td>
<td>0.88</td>
<td>0.75</td>
<td>0.78</td>
<td>0.72</td>
</tr>
<tr>
<td>Basal diet, pasture (US $/day)</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Total feeding cost (US $)</td>
<td>1.11</td>
<td>0.98</td>
<td>1.01</td>
<td>0.95</td>
</tr>
<tr>
<td>Milk production (L/cow/day)</td>
<td>4.95</td>
<td>5.43</td>
<td>5.34</td>
<td>5.52</td>
</tr>
<tr>
<td>Milk production (US $/cow/day)</td>
<td>1.58</td>
<td>1.74</td>
<td>1.71</td>
<td>1.77</td>
</tr>
<tr>
<td>Net gain (US$/cow/day)</td>
<td>0.47</td>
<td>0.76</td>
<td>0.70</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Treatments as in Table II.

3.3. Experiment III

Higher milk yields were obtained in the three groups receiving 5.5 kg/day/cow of concentrate (Table IX) as compared with the control group receiving 3.2 kg/day/cow (P <0.05). However, due to the experimental design, it was not possible to separate the effect of the concentrate composition from the level of the concentrate.

There were no differences in milk yield, body condition score or body weight change among treatments 2, 3 and 4. All treatments increased milk yield from 16 to 25% and reduced the interval from calving to conception from 22 to 31% in relation to the control. The cost of concentrates that included poultry manure (T3) and urea (T4) was lower compared to the concentrate in treatment 2, which included soybean meal.

Treatments with poultry manure (T3) and urea (T4) also resulted in slightly higher body condition scores and lower body weight loss than treatment 2 with soybean meal (Table IX). Good body condition was related to lower calving to conception intervals in these treatments. It is concluded that poultry manure or urea could be used in place of soybean meal as a source of N in concentrates, maintaining animal response but reducing costs.

TABLE IX. PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF LACTATING COWS SUPPLEMENTED WITH 3.2 kg OR 5.5 kg OF CONCENTRATE CONTAINING DIFFERENT SOURCES OF N

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>470</td>
<td>478</td>
<td>455</td>
<td>394</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>390</td>
<td>410</td>
<td>400</td>
<td>355</td>
</tr>
<tr>
<td>Body weight loss (kg/day)</td>
<td>0.44</td>
<td>0.38</td>
<td>0.31</td>
<td>0.22</td>
</tr>
<tr>
<td>Milk yield (kg) (^1)</td>
<td>2,130(^a)</td>
<td>2,580(^b)</td>
<td>2,662(^b)</td>
<td>2,485(^b)</td>
</tr>
<tr>
<td>Calving to conception interval (d)</td>
<td>171.2(^a)</td>
<td>133.5(^b)</td>
<td>121.3(^b)</td>
<td>117.2(^b)</td>
</tr>
<tr>
<td>Body condition score (1-5)</td>
<td>2.75</td>
<td>3.00</td>
<td>3.25</td>
<td>3.25</td>
</tr>
<tr>
<td>Supplementation (kg/cow/day)</td>
<td>3.2</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
</tr>
</tbody>
</table>

\(^1\) Adjusted milk yield to 305 days of lactation.

\(^a,b\) For a given row, values that do not share a superscript indicate significant differences (P <0.05).
4. CONCLUSIONS

a. Use of mineral mixtures in supplements increased body weight gain, reduced age at first service, and improved rate of pregnancy of native heifers.

b. Non-traditional sources of protein such as *Gliricidia sepium*, poultry manure and urea increased milk production, shortened calving intervals and reduced production costs, thereby improving the economic efficiency of the farm.

c. Improving the level of feeding in crossbred cows with high Holstein inheritance increased milk production, reproductive performance and overall productivity of the herd.

REFERENCES


PERFORMANCE OF HEREFORD COWS UNDER CONDITIONS OF VARIED FORAGE AVAILABILITY DURING LATE GESTATION

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Abstract – Resumen

PERFORMANCE OF HEREFORD COWS UNDER CONDITIONS OF VARIED FORAGE AVAILABILITY DURING LATE GESTATION.

The objective of the experiment conducted in Uruguay, Paysandú (32° S; 58° W), was to study the effect of forage availabilities under native pastures during the last 73 ± 22 and 93 ± 24 days of gestation in 1991 and in 1992, respectively, on productive and reproductive performance of beef cows. Each year, one hundred Hereford cows were stratified by age, gestation month and body condition and randomly allotted to one of the following grazing treatments: a) low, b) low-medium, c) medium d) medium-high, or e) high forage availability, expressed as pasture height. Pasture height ranged from 2.0 to 5.5 cm in 1991, and from 1.9 to 4.2 cm in 1992, and it was adjusted by the "put and take" technique. Body condition and liveweight at calving and at the beginning of the mating period were positively affected by increased forage availability during late gestation (P ≤0.01). In 1992, forage availability affected the proportion of non-cycling cows at the end of the mating period and the percentage of pregnant cows (42 vs 6% and 42 vs 82%, for the low and high forage availability groups, respectively (P ≤0.01). No effect of treatment was observed neither on calf birth weight nor in weaning weight. A quadratic and linear model explained the variation in body condition and liveweight at calving, as related to pasture height and available forage during late gestation in 1991 and 1992, respectively. Calving to conception interval was reduced by 15 days (P ≤0.01) when the pasture height increased between 2 and 5.5 cm, and in 15 days per each unit of increase in body condition at calving (P ≤0.01). Cows should calve in body condition ≥4.0 (in an 8 point scale) in order to get a good reproductive performance in the subsequent mating period. Cow performance at calving can be managed in a predictable manner by controlling forage availability, expressed as pasture height, during late gestation.

COMPORTAMIENTO DE VACAS HEREFORD SOMETIDAS A DISTINTAS DISPONIBILIDADES DE PASTIZAL NATIVO DURANTE LA FASE FINAL DE LA GESTACION.

El objetivo del presente experimento llevado a cabo en Paisandú, Uruguay (32° S; 58° O) fue estudiar el efecto de la disponibilidad de forraje de pastizal nativo durante los últimos 73 ± 22 y 93 ± 24 días de gestación en 1991 y en 1992, respectivamente, sobre el comportamiento productivo y reproductivo de vacas de cría. Cada año, 100 vacas Hereford fueron estratificadas por edad, mes de gestación y condición corporal y asignadas al azar a uno de los siguientes tratamientos: a) baja b) bajo-medio, c) medio, d) medio-alto, y e) alto disponibilidad de forraje, expresada como altura de la pastura. La altura del pastizal varió entre 2.0 a 5.5 cm en 1991 y entre 1.9 a 4.2 cm en 1992, y fue controlada mediante la técnica del "put and take". La condición corporal, el peso vivo al parto y al inicio del entorno estuvieron positivamente relacionados con aumentos en la disponibilidad de forraje durante la fase final de la gestación (P ≤0.01). En 1992, la disponibilidad del forraje afectó la proporción de vacas que no ciclaban al final del período de entorno y el porcentaje de vacas gestantes (42 vs 6% y 42 vs 82%, en los grupos de baja y alta disponibilidad de forraje, respectivamente, P ≤0.01). Los pesos de los terneros al nacer y al destete no fueron afectados por el tratamiento. La condición corporal y peso vivo al parto variaron con la altura y disponibilidad del forraje durante la fase final de la gestación según un modelo cuadrático y lineal en 1991 y 1992, respectivamente. El intervalo parto-concepción disminuyó en 15 días (P ≤0.01) por cada unidad de incremento de la condición corporal al parto (P ≤0.01). Vacas de cría Hereford deben parir con una condición corporal de 4.0 (en una escala de 8 puntos) a fin de asegurar un adecuado comportamiento reproductivo en el siguiente entorno. El comportamiento reproductivo de la vaca en el parto puede ser manejado a través del control de la altura y disponibilidad de forraje de los potrores durante la fase final de la gestación.

1. INTRODUCTION

Beef production in Uruguay is carried out under grazing on native pastures. Reproductive efficiency is usually low (e.g. 60 - 65 calves are weaned per year per 100 cows exposed to a bull [1]). The nutritional status of the cow is the main factor that limits reproductive efficiency. Results obtained so far indicate that, although poor mineral nutrition may have some effect, the energy status (as estimated by liveweight and body condition) at calving and at mating, is of much greater importance to reproductive performance of cows in most herds in Uruguay [1]. Fertility of Hereford cows sharply decreases when body condition at calving and at mating descends below 4.0 (in an 8 point scale, where 1 is the emaciated animal and 8 is the obese one [2].
Body condition at calving, which depends on the level of nutrition in late gestation, markedly affects the length of the post-partum anoestrous period, and therefore cows with higher body condition have shorter anoestrous periods [3, 4, 5].

Performance of grazing animals is affected by quantity and quality of available forage. The performance of breeding herds has been related to the height of cultivated pastures [5]. However, the relationship between native pasture height and beef cow performance has not yet been studied.

An experiment was therefore conducted to study the effect of forage availability of native temperate pastures during late gestation on reproductive performance of beef cows. Liveweight and body condition at calving, at the beginning and at the end of mating period, percentage of non-cycling cows at the beginning and at the end of the mating period, calving to conception interval, pregnancy rate, birth weight and weaning weight of calves, were used to estimate the performance of cows.

2. MATERIALS AND METHODS

The experiment was conducted in 1991 and 1992 in the Experimental Station of Paysandú (33° S, 58° W), on a natural grassland with temperate grass species (Botriochloa sp., Paspalum sp., Stipa sp., and Sporobolus sp.) showing a spring - summer predominant production.

One hundred pregnant Hereford cows were stratified by age, gestation month and body condition in 1991 and 1992. The animals were randomly assigned to one of the following grazing treatments during the last 73 ± 22 and 93 ± 24 days of gestation in both years, respectively: a) low (L), b) low-medium (LM), c) medium (M), d) medium-high (MH) or e) high (H) forage availability, expressed as pasture height.

Pasture height was determined at 25-day intervals in 5 points of one diagonal of 25 x 50 cm² quadrant, which was thrown randomly in each paddock. Thirty measurements within each soil type and topographic position were taken in each paddock. Average pasture height during the experimental period ranged from 2.0 (L) to 5.5 (H) and from 1.9 (L) to 4.2 (H) cm in 1991 and 1992, respectively. Simultaneously to pasture height measurements, available forage was estimated by double sampling [6]. Allocation of cows in 1992 did not take into account treatments in 1991.

Each group of cows continuously grazed a 20 ha paddock during the experimental period. Forage availability was adjusted by the "put and take" technique [7]. Immediately after calving, the cows were taken out from their treatments and put into another native pasture where, thereafter, they grazed together.

The mating period began on December 16th and lasted for 75 days. Four fertile Hereford bulls per 100 cows were used. The intervals from calving to the beginning of the mating period were 44 ± 22 and 61 ± 24 days in 1991 and 1992, respectively.

At the beginning of the mating period an 11-day temporary weaning was applied in a factorial treatment design to 50% of calves that were older than 40 days of age. The other 50% remained suckling until weaning at 192 ± 24 days of age. Results of this treatment are not reported in this paper but were taken into account in the statistical analysis.

Liveweight (without previous fasting) and body condition of cows were recorded at the beginning of the experiment, at calving and at the beginning and end of the mating period. Birth date, sex, birth weight and weaning weight of calves were also recorded. Conception dates were estimated (calving date minus 287 days) [8]. Pregnancy diagnosis was carried out by rectal palpation 65 to 70 days after the end of the mating period. Four blood samples by jugular venipuncture were obtained from each cow: two at the beginning and two at the end of the mating period with 10 day interval between each par. Blood samples were centrifuged within 2 hours of collection and harvested plasma was frozen until assayed for progesterone. Plasma progesterone concentration was determined by radioimmunoassay using solid-phase RIA kits provided by the International Atomic Energy Agency [9]. Intra- and inter-assay coefficients of variation were 6% and 6.9% in 1991, and 9% and 12% in 1992. Cows with progesterone concentrations < 3.0 nmol/L in two samples 10 days apart were regarded as non-cycling.

Treatment effects on pasture height and pasture availability were submitted to analysis of variance in a completely randomized design [10]. The number of data in the analysis differed from the initial due to missing values. The effect of forage availability during late gestation on body condition and liveweight at calving, at the beginning and at the end of mating period and calving to conception interval.
were analyzed by the GLM procedure of the Statistical Analysis System [11]. The same GLM procedure was used to analyze the effect of treatments on birth and weaning weight of calves. Treatment effect on the number of non-cycling cows at the beginning and at the end of mating period and on the number of pregnant cows was analyzed by Fisher's Exact Test [10].

3. RESULTS AND DISCUSSION

Pasture characteristics, body condition and liveweight of cows at calving, at the beginning and at the end of mating period, reproductive performance of cows and the performance of calves in the two years of the study, are presented in Table I.

Five different pasture heights (P ≤0.10) were obtained as planned in 1991. However, only four levels were obtained in 1992 since pasture heights in treatments L and LM did not differ (P >0.10). Experimental conditions were different between years. Average pasture height ranged from 2.0 to 5.5 cm and from 1.9 to 4.2 cm in 1991 and 1992, respectively. Forage availability ranged from 900 to 1858 and from 1358 to 2986 kg dry matter/ha in 1991 and 1992, respectively.

Increased pasture height improved body condition and increased liveweight of cows at calving and at the beginning of the mating period (Table I). Similar data was reported in New Zealand [12, 13] with beef cows and heifers grazing different forage allowances in hill country pastures.

After adjusting for body condition at the beginning of the experiment and for length of the experimental period, 29% and 32% of the total variation in body condition at calving was accounted by pre-partum nutritional levels in 1991 and 1992, respectively. At the beginning of the mating period, 25% and 26% of body condition variation was accounted for forage availability in late gestation, respectively. However, at the end of the mating season, body condition was not related (P = 0.14) to the feeding regime during late gestation.

Forage availability neither affected the proportion of cycling cows nor the percentage of pregnant cows in 1991. However, in 1992, the proportion of non-cycling cows at the end of the mating period and the percentage of pregnant cows were affected by forage availability in late gestation.

Climatic conditions previous to the beginning of mating periods were similar in the two experimental years. However, in one hand there were 40 rainy days during the first mating period (from December 1990 through February 1991), while only 29 rainy days were registered during the same period in the second year of the experiment. This condition could favour forage production during the mating period in 1991 which, in turn, could have led to an improvement in nutritional status of cows in the poorest body condition at calving (L = 3.0), to begin cycling and to become pregnant. On the other hand, reproductive performance of cows in 1992 was more dependent on their body condition at calving since, apparently, weather conditions restricted forage production and the nutritional status of cows during the post-calving period.

Contradictory results are common in studies conducted on the effect of pre- and post-calving nutrition on reproductive performance of grazing cows. Cows fed under a high plane of nutrition before calving have been reported to come into oestrus earlier than those fed medium or low planes, nevertheless conception and pregnancy rates did not differ [14]. Also, shorter intervals from calving to oestrus have been reported in studies where pre-calving forage allowance increased [12]. In contrast, it is also reported that pre-calving pasture allowance affected neither calving-oestrous interval nor pregnancy rate, but both variables were affected by post-calving forage allowance [15].

No effect of treatment was observed on calf birth weight nor on weaning weight within each year, although differences among years were apparent. Other authors have also reported no effect of dam's pre-calving plane of nutrition on calf birth weight and subsequent growth rate [12, 15]. Another report [16] has indicated that a reduction in calf birth weight resulted only from severe under-nutrition of the dam, particularly near calving.

Calf weight at birth and at weaning in 1991 was higher than in 1992 (31.7 vs 29.3 and 147 vs 125 kg respectively, P <0.01). It does not appear to be an effect of nutrition of cows during late gestation, as expressed by their body condition and liveweight at calving, on birth weight of calves. However, the higher level of precipitation in 1991 could have affected the quantity and quality of available forage during the suckling period, improving milk production, and therefore, calf weaning weight. Also, a sire effect could be involved, since different bulls were used each year.
<table>
<thead>
<tr>
<th>Pasture height (cm)</th>
<th>1991</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>2.9</td>
<td>3.1</td>
<td>5.3</td>
<td>5.5</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>80</td>
<td>1245</td>
<td>1150</td>
<td>1787</td>
<td>1858</td>
<td>5685</td>
</tr>
<tr>
<td>Body condition score at</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calving</td>
<td>84</td>
<td>3.0</td>
<td>4.2</td>
<td>3.9</td>
<td>4.2</td>
<td>4.4</td>
<td>0.41</td>
</tr>
<tr>
<td>beginning of mating period</td>
<td>86</td>
<td>3.1</td>
<td>4.1</td>
<td>3.8</td>
<td>4.3</td>
<td>4.1</td>
<td>0.46</td>
</tr>
<tr>
<td>end of mating period</td>
<td>60</td>
<td>3.5</td>
<td>4.2</td>
<td>3.9</td>
<td>4.1</td>
<td>4.4</td>
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</tr>
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<td>Liveweight (kg) at</td>
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<td></td>
<td></td>
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<tr>
<td>calving</td>
<td>84</td>
<td>314</td>
<td>374</td>
<td>357</td>
<td>370</td>
<td>376</td>
<td>744</td>
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<tr>
<td>beginning of mating period</td>
<td>66</td>
<td>356</td>
<td>378</td>
<td>374</td>
<td>385</td>
<td>389</td>
<td>525</td>
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<tr>
<td>end of mating period</td>
<td>61</td>
<td>369</td>
<td>385</td>
<td>385</td>
<td>388</td>
<td>405</td>
<td>645</td>
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<tr>
<td>Cycling cows (%) at</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>beginning of mating period</td>
<td>18.8</td>
<td>45.5</td>
<td>22.2</td>
<td>23.1</td>
<td>31.6</td>
<td>43.8</td>
<td>16</td>
</tr>
<tr>
<td>end of mating period</td>
<td>93.9</td>
<td>100.0</td>
<td>87.5</td>
<td>100.0</td>
<td>93.8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Pregnant cows (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Calf liveweight (kg) at</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>birth</td>
<td>565</td>
<td>317</td>
<td>313</td>
<td>310</td>
<td>304</td>
<td>337</td>
<td>12.0</td>
</tr>
<tr>
<td>weaning</td>
<td>53</td>
<td>153</td>
<td>150</td>
<td>145</td>
<td>138</td>
<td>151</td>
<td>552</td>
</tr>
<tr>
<td>1992</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasture height (cm)</td>
<td>89</td>
<td>1.9</td>
<td>1.8</td>
<td>2.3</td>
<td>3.3</td>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>Pasture availability (kg DM/ha)</td>
<td>89</td>
<td>1,358</td>
<td>1,547</td>
<td>1,930</td>
<td>1,754</td>
<td>2,986</td>
<td>8,652</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calving</td>
<td>89</td>
<td>3.1</td>
<td>3.1</td>
<td>3.2</td>
<td>3.7</td>
<td>4.2</td>
<td>0.33</td>
</tr>
<tr>
<td>beginning of mating period</td>
<td>74</td>
<td>3.6</td>
<td>3.7</td>
<td>3.4</td>
<td>3.7</td>
<td>4.4</td>
<td>0.28</td>
</tr>
<tr>
<td>end of mating</td>
<td>77</td>
<td>3.7</td>
<td>3.9</td>
<td>3.7</td>
<td>3.9</td>
<td>4.0</td>
<td>0.24</td>
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<td>Liveweight (kg) at2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>calving</td>
<td>77</td>
<td>305</td>
<td>305</td>
<td>326</td>
<td>331</td>
<td>355</td>
<td>449</td>
</tr>
<tr>
<td>end of mating</td>
<td>77</td>
<td>363</td>
<td>375</td>
<td>369</td>
<td>369</td>
<td>381</td>
<td>417</td>
</tr>
<tr>
<td>Cycling cows (%) at</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>beginning of mating period</td>
<td>5.3</td>
<td>27.8</td>
<td>17.7</td>
<td>16.7</td>
<td>16.9</td>
<td>10.5</td>
<td>19</td>
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<tr>
<td>end of mating</td>
<td>57.9</td>
<td>83.3</td>
<td>70.6</td>
<td>83.3</td>
<td>84.1</td>
<td>94.5</td>
<td>18</td>
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<tr>
<td>Pregnant cows (%)</td>
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<td>Calf liveweight (kg) at</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>birth</td>
<td>565</td>
<td>28.0</td>
<td>28.0</td>
<td>29.5</td>
<td>29.3</td>
<td>29.1</td>
<td>11.8</td>
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<tr>
<td>weaning</td>
<td>56</td>
<td>124</td>
<td>114</td>
<td>125</td>
<td>136</td>
<td>126</td>
<td>619</td>
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</tbody>
</table>

Means with different superscripts within rows are significantly different (P < 0.10).
Values between brackets are number of cows.

1 Mean square error.
2 LS means adjusted by body condition and liveweight at beginning of the experiment and experiment length.
3 Model includes condition at the beginning of the experiment, date of birth and sex.
4 Model includes condition at the beginning of the experiment, date and weight of birth, sex and temporary weaning.
TABLE II. MATHEMATICAL MODELS DESCRIBING THE RELATIONSHIP BETWEEN HEREFORD COW PERFORMANCE AND PASTURE HEIGHT

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>df</th>
<th>Intercept</th>
<th>Beginning of Exp</th>
<th>Experiment length</th>
<th>Pasture height</th>
<th>Means</th>
<th>MSE a</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beginning of Exp</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Condition score</td>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Linear (cm)</td>
<td>Quadratic (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1991</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body condition at</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calving</td>
<td>59</td>
<td>-1.16</td>
<td>0.44</td>
<td>0.011</td>
<td>1.19</td>
<td>-0.117</td>
<td>3.77</td>
<td>0.324</td>
</tr>
<tr>
<td>beginning of mating</td>
<td>60</td>
<td>-0.33</td>
<td>0.41</td>
<td>-0.002</td>
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<td>-0.148</td>
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<td>0.492</td>
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<td>55</td>
<td>0.76</td>
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<td>0.86</td>
<td>-0.088</td>
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<td>0.865</td>
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<td>Liveweight at</td>
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<tr>
<td>calving</td>
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<td>-139.60</td>
<td>0.73</td>
<td>1.096</td>
<td>70.8</td>
<td>-7.56</td>
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<td>514</td>
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<td>beginning of mating</td>
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<td>-3.67</td>
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<td>Body condition at</td>
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<tr>
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<td>0.35</td>
<td>0.012</td>
<td>0.50</td>
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<td>3.47</td>
<td>0.321</td>
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<tr>
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<td>0.92</td>
<td>0.48</td>
<td>0.002</td>
<td>0.32</td>
<td>ns</td>
<td>3.78</td>
<td>0.308</td>
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<td>1.90</td>
<td>0.38</td>
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<td>0.13</td>
<td>ns</td>
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<tr>
<td>calving</td>
<td>75</td>
<td>-65.0</td>
<td>0.71</td>
<td>0.882</td>
<td>19.20</td>
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<td>462</td>
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<tr>
<td>beginning of mating</td>
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<td></td>
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<tr>
<td>end of mating</td>
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<td>0.93</td>
<td>0.470</td>
<td>4.45</td>
<td>ns</td>
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<td>430</td>
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a Means square error.
n.a. no data available.
<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>df</th>
<th>Intercept</th>
<th>Condition score</th>
<th>Experiment length</th>
<th>Pasture availability⁴</th>
<th>Means</th>
<th>MSE⁵</th>
<th>R²</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Liveweight at</td>
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<tr>
<td></td>
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<td></td>
<td></td>
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<td>0.50</td>
<td>-0.0001</td>
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<td>0.26</td>
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<td>0.50</td>
<td>-0.0002</td>
<td>4.04</td>
<td>0.845</td>
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<td>0.79</td>
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<td>-0.005</td>
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<td>473</td>
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<td></td>
<td>87</td>
<td>-0.54</td>
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<td>0.376</td>
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<td>0.65</td>
<td>0.49</td>
<td>0.003</td>
<td>0.05</td>
<td>ns</td>
<td>3.78</td>
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<td>1.84</td>
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<td>0.0025</td>
<td>0.02</td>
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<td>3.86</td>
<td>0.242</td>
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<td></td>
<td>75</td>
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<td>0.97</td>
<td>2.80</td>
<td>ns</td>
<td>324</td>
<td>510</td>
</tr>
<tr>
<td>Liveweight at</td>
<td>n.a.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>beginning of mating</td>
<td>n.a.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>end of mating</td>
<td>75</td>
<td>-28.7</td>
<td>0.94</td>
<td>0.49</td>
<td>0.84</td>
<td>ns</td>
<td>370</td>
<td>422</td>
</tr>
</tbody>
</table>

⁴ (kg DM/ha)²10E-2.
⁵ Means square error.
⁶ n.a. no data available.
Mathematical models showing the relationship between pasture height and cow performance in 1991 and 1992 are presented in Table II. Table III presents the relationship found between available forage and cow performance. A quadratic model explained the variation in body condition as related to pasture height (Table II) and available forage (Table III) during late gestation in 1991. However, in 1992 the linear, but not the quadratic effect, was significant (P < 0.001).

Figure I shows the variation in body condition at calving as pasture height increased in 1991 and 1992 for mean values of body condition at the beginning of the experiment (3.7) and the length of the feeding period (73 days). Highest body condition at calving (4.3) and at the beginning of mating (4.2) occurred at mean pasture heights, during late gestation in 1991, of 5.1 and 4.6 cm, respectively; with mean values for body condition at the beginning of the experiment and for length of the experimental feeding period.

![FIG. 1. Body condition at calving as affected by pasture height during late gestation of cows. (BBC: body condition at calving, PH: pasture height. Intercept corresponds to a 3.7 body condition at the beginning of the experiment and to 76 days of the feeding period).](image)

Quadratic relationships between these variables were reported elsewhere [7, 17] with cattle grazing cultivated pastures dominated by perennial ryegrass (*Lolium perenne*). They found that maximum liveweight gain and body condition of cows after calving were obtained at 8 to 10 cm of sward height. Under continuous stocking management, the reduction in animal performance at low sward heights is caused by a reduction in herbage intake [7] and/or by an increase in energy expenditure due to a greater grazing activity [18]. The reduction in cow performance at high mean sward heights was reported to be due to the presence of patches frequently and patches infrequently grazed, where dead material is accumulated [17]. Under these conditions, cattle rejects the forage from infrequently grazed areas and may be overgrazing the frequently grazed patches. This is a situation commonly observed in natural grasslands in Uruguay and may explain the results of cow performance obtained in 1991.

A linear relationship between animal performance and pasture characteristics was found in 1992. The lack of significance of the quadratic effect could be explained by the lower range of pasture heights in the experiment during this year. Within the same range of values of pasture heights, in years 1991 and 1992, there appears to be a similar relationship between cow performance at calving and mean pasture height during late gestation.

From the analysis of data obtained during the first year of the experiment, it was found that calving to conception interval (CCI, days) was negatively related to pasture height during late gestation (PH, cm) and body condition at calving (BCC) according to the following functions:

\[
CCI = 185.66 - 11.71 \text{BC1} - 0.85 \text{D} - 4.27 \text{PH} \quad R^2 = 0.40 \quad (n = 37)
\]

\[
CCI = 150.96 + 2.12 \text{BC1} - 0.43 \text{D} - 14.7 \text{BCC} \quad R^2 = 0.54 \quad (n = 44),
\]
where BC1 is body condition at the beginning of the experiment and D are days from the beginning of the experiment until calving.

Similar results were obtained by other authors [19]. They found that per each unit increase in body condition at calving (in a 6 point scale), there was a 9.5 day decrease in the beginning of the mating period to pregnancy interval.

In spite of differences in environmental conditions between years, it can be assumed that reproductive performance of cows in 1991 and 1992 depended mainly on their energy status at critical reproductive events (e.g. at calving and during mating period). Body condition and liveweight of cows are indicators of the energy status of cows. As a consequence, cows in similar body condition and liveweight, at the time these critical reproductive events are occurring, could be expected to have similar probabilities to be in oestrus and become pregnant in different years.

Data from non-cycling, pregnant and barren cows from 1991 and 1992 were pooled and analyzed together, in order to study possible relationships between reproductive performance and body condition and liveweight at calving and at the beginning of the mating period. Table IV shows the body condition and liveweight at calving and at the beginning of the mating period of non-cycling and cycling (or pregnant) cows at the beginning and at the end of the mating period. As expected, body condition and liveweight of cycling or pregnant was always higher (P <0.001) than non-cycling cows. Only 35 from 162 cows were cycling at the beginning of mating; their mean body condition at calving was 4.1 while mean body condition at calving of non-cycling cows was 3.5. Cows in lower body condition at calving begin to cycle later [20]. This explains the fact that cycling or pregnant cows at the end of mating had a lower mean body condition at calving (3.3) than those cycling at the beginning of the mating period (4.1). Twenty-three from 157 cows with a mean body condition at calving of 2.9 remained in anestrous at the end of mating period.

Figure 2 shows the percentage of non-cycling cows at the beginning and at the end of mating that calve in body condition that ranged from less than 3.25 through more or equal than 5. Figure 3 presents the pregnancy rate of cows as related to body condition at calving. There is a sharp decrease in the percentage of non-cycling cows and an increase in pregnancy rate, as body condition at calving increases from 3.25 or less up to ≥5 (P ≤0.05). This finding agrees with previous results obtained in Uruguay with Hereford cattle [2]. A prolonged anestrous of cows that calve and begin the mating period in poor body condition explains these results. Accordingly, other authors [4, 21] conclude that a body condition of 5 (in a 9 point scale) should be the target at calving to assure a high reproductive performance in the following mating period.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beginning of mating</th>
<th>End of mating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-cycling</td>
<td>Cycling</td>
</tr>
<tr>
<td>Body condition at calving</td>
<td>3.53 (127)\textsuperscript{a}</td>
<td>4.08 (35)\textsuperscript{b}</td>
</tr>
<tr>
<td>beginning of mating period</td>
<td>3.71 (116)\textsuperscript{a}</td>
<td>4.32 (33)\textsuperscript{b}</td>
</tr>
<tr>
<td>Liveweight (kg) at\textsuperscript{1}</td>
<td>328 (118)\textsuperscript{a}</td>
<td>366 (33)\textsuperscript{b}</td>
</tr>
<tr>
<td>calving</td>
<td>369 (52)\textsuperscript{b}</td>
<td>396 (21)\textsuperscript{b}</td>
</tr>
<tr>
<td>beginning of mating period</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1} Least square means adjusted for calving date. Values with different superscripts within period of mating and row differ (P <0.01). Number of cows is between brackets.
4. CONCLUSIONS

Cows should calve in body condition ≥ 4.0 (on an 8 point scale) in order to get a high reproductive performance in the following mating period.

Cow performance at calving can be managed in a predictable manner by controlling forage on offer, expressed as pasture height, during late gestation.
Pasture height of forage on offer during late gestation to get the target body condition of 4.0 at calving, depends on the initial body condition and on the length of the feeding period until calving. Preliminary results suggest that Hereford cows with an initial body condition of 3.5 have to graze a natural grasslands with a mean pasture height of at least 4-5 cm during the last 75-80 days of gestation to calve with a body condition of 4.0.

ACKNOWLEDGEMENTS

We wish to thank O. Bentancour, S. Chappuis, P. Souto, P. Amarante, J.L. López, J. Terra, C. Caillabet, J. Rodríguez and O. Fed, who were directly involved in data and sample collection and statistical analysis. Deep appreciation also goes to the personnel from "M. A. Cassinoni" Experimental Station who contributed to collect field records from cattle herd.

REFERENCES


THE EFFECT OF FEED SUPPLEMENTATION ON THE ONSET OF PUBERTY IN BRAZILIAN DAIRY HEIFERS

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Centro de Energia Nuclear na Agricultura,
Universidade de São Paulo,
Piracicaba, Brazil

Abstract – Resumen

THE EFFECT OF FEED SUPPLEMENTATION ON THE ONSET OF PUBERTY IN BRAZILIAN DAIRY HEIFERS

Most Brazilian dairy production is conducted by small holders whose general management skills and feeding programmes are often deficient. One common problem directly attributed to underfeeding is that heifers rarely reach sexual maturity before 15 months of age. Two experiments were carried out using growing heifers to determine the effect of protein supplementation (0.3 kg/heifer/day of a mixture of commercial concentrate with 18.59% crude protein (CP) and cottonseed meal with 28.43% CP) on sexual maturity (Experiment I); and protein supplementation plus anthelmintic treatment (benzimidazole, 10 mg/kg body weight) on sexual maturity (Experiment II). All but one of the 23 females in the supplemented group (96%), and only 32 of the 23 heifers in the control group (52%) reached sexual maturity before 18 months of age (P <0.01) in Experiment I. The first ovulation occurred at 513 ± 44 and 573 ± 36 days (x ± SE, P <0.01) in supplemented and control groups, respectively. Daily body weight gains from beginning of the trial to the first ovulation were 378 ± 0.02 and 331 ± 0.04 g for supplemented and control groups. Height at withers and body condition score did not differ between the two groups. In Experiment II, only 52.2% of the heifers in the not-supplemented groups (11 of 23), but 95.7% in supplemented groups (22 of 23), reached sexual maturity before the age of 18 months (P <0.01). Groups that had received supplementation presented higher concentrations of haemoglobin and elevated hematocrit throughout the year than the controls (P <0.01). Animals with anthelmintic treatment had better growth performance than the controls (P <0.01).

EL EFECTO DE LA SUPLEMENTACION ALIMENTICIA SOBRE EL INICIO DE LA PUBERTAD EN VAQUILLAS LECHERAS EN BRAZIL

La mayor parte de la industria lechera del Brasil está a cargo de pequeños productores, en los que su capacidad para el manejo del rebaño y conocimientos sobre esquemas de alimentación son a menudo deficientes. Un problema que es común y que se le puede atribuir directamente a una alimentación deficiente, es que las vaquillas raramente alcanzan la madurez sexual antes de los 15 meses de edad. Se llevaron a cabo dos experimentos con vaquillas en crecimiento a fin de determinar el efecto de la suplementación proteica (0.3 kg/vaquilla/día de una mezcla de concentrado comercial con 18.59% proteína cruda (CP) y harina de semilla de algodón con 28.43% CP) sobre la madurez sexual (Experimento I); y la suplementación proteica más el tratamiento antihelmíntico (benzimidazoles, 10 mg/kg peso vivo) sobre la madurez sexual (Experimento II). El 96 % de las vaquillas (22 de 23) del grupo suplementado y el 52% (12 de 23) en el grupo control alcanzaron la madurez sexual antes de los 18 meses de edad (P <0.01) en el Experimento I. La primera ovulación ocurrió a los 513 ± 44 y 573 ± 36 días (x ± SE, P <0.01) en el grupo suplementado y control, respectivamente. La ganancia de peso desde el inicio del experimento hasta la primera ovulación fue de 378 ± 0.02 y 331 ± 0.04 g para los grupos suplementado y control, respectivamente. No se encontraron diferencias en la altura a la cruz y la condición corporal entre ambos grupos. En el Experimento II, el 95.7% de los animales en el grupo suplementado (22 de 23) y el 52.2% de las vaquillas en el grupo control (11 de 23) alcanzaron la madurez sexual antes de los 18 meses de edad (P <0.01). Los grupos que recibieron suplementación proteica presentaron durante el año mayores concentraciones de hemoglobina y un elevado hematocrito con relación a los grupos control (P <0.01). Los animales con tratamiento antihelmíntico obtuvieron un mejor crecimiento corporal que los animales no dosificados.

1. INTRODUCTION

Economic analysis in industrialized countries with specialized dairy farming points out that dairy heifers should calve by 24 to 30 months of age to provide reasonable opportunity for lifetime profit. In Brazil, heifers rarely show oestrus before 36 months of age, so they cannot be mated earlier. In addition, in tropical areas, malnutrition and diseases are predominant factors contributing to poor reproductive efficiency [1]. Brazilian studies, based on statistic data and practical observations, lead to the conclusion that infertility or reduced fertility, due to poor nutrition, is the most important problem which contributes to low productivity. This is especially true in tropical areas, where the natural pasture is deficient in energy, protein and essential minerals during a good period of the year [2, 3, 4, 5, 6]. Both the quality and quantity of feed available influence the growth rate and time required to achieve the body weight that the animal must attain to reach sexual maturity.

Research conducted in Brazil to study competition between heifers and cows under range conditions, indicated that growth rate from birth to calving varied between 0.26 to 0.46 kg/day for
Jerseys and 0.36 to 0.50 kg/day for Holsteins, and slower growth did not delay the first calving, nor affected milk production [7]. A three year study on reproductive efficiency in a Brazilian herd indicated that 80% of first calving cows had body condition scores that were below the optimum or desirable levels for their age [8]. Visual examination of most of the calves suggested that many animals suffered from nutritional deficiencies, often compounded by parasitism. These factors would undoubtedly delay sexual maturity.

The objective of the present investigation was to establish the effect of dietary protein supplementation and anthelmintic treatment on body weight, body measurements and onset of ovarian activity in Brazilian Holstein heifers.

2. MATERIALS AND METHODS

2.1. Experiment I: Metabolic profile, growth rate and time to first ovulation

This work was carried out in a private farm located in Porto Feliz, São Paulo. The herd included 50 Holstein cows in lactation, 24 dry cows and 2 bulls. Replacement heifers were being reared in a conventional system with creep feeding. They received milk until 2 months of age plus a commercial concentrate (crude protein (CP) = 18.59%) and cottonseed meal (CP = 28.43%) blended by the farmer. Growing animals had continuous daily access to sugarcane tops, a mineral mixture, grass pasture and water. There was no control regarding quality and consumption of feed. Anthelmintic treatment was administered sporadically.

All heifers born prior to March 1990 (n = 25) and born from that date until August 1991 (n = 24) were identified with ear tags, arranged by month of birth and allotted into control or supplemented groups, according to their age, so that two groups of equal age were formed.

The control group received only the roughage, pasture, farm-blended concentrate and mineral as described above. The supplemented group received an additional daily ration of cottonseed meal as a protein source (0.3 kg/heifer/day). Data collection started in March 1990 and ended in December 1992.

Growth rate was followed monthly by measuring heart girth, height at withers, body weight (BW) and by evaluating the body condition score (BCS) [9]. Digestibility of roughage and pasture was determined monthly by the nylon bag technique [10]. At the same time, blood samples were collected for packed cell volume (PCV), haemoglobin [11], inorganic phosphorus [12] and glucose analysis [13]. Faeces were also sampled monthly for nematode egg count [14].

Heifers were treated with the anthelmintic benzimidazole (10 mg/kg BW) when the eggs per gram (epg) of faeces exceeded 300. Blood samples were also taken weekly to measure progesterone concentrations by a solid phase radioimmunoassay using the FAO/IAEA RIA kit [15]. Sexual maturity was assumed when progesterone showed cyclic patterns of high (> 3 nmol/L) and low concentrations.

Animals, in the control and supplemented groups that reached or failed to reach sexual maturity before 18 months, were organized into a 2 x 2 contingency table and these results were analyzed by Chi square. Repeated-measures analysis of variance and least square significant differences were used to test differences between metabolic profile data and growth traits of heifers [16].

2.2. Experiment II: Effects of strategic nematode control and protein supplementation on development of replacement heifers

Forty-eight 10 month old heifers were randomly assigned into four groups: I) AP = anthelmintic plus protein supplementation; ii) AC = anthelmintic without protein supplementation; iii) HP = untreated anthelmintic plus protein supplementation and iv) HC = untreated anthelmintic without protein supplementation. The animals were observed from January to December, 1992. Supplement was similar to Experiment I. Heifers undergoing regular parasitic control were dosed in February, April, August and November with benzimidazole (10 mg/kg BW). The growth rate was followed monthly by recording BW, height at hips and BCS. Blood samples were collected monthly for PCV, hemoglobin, glucose and phosphorus analysis and weekly to measure plasma progesterone concentrations. Incidence of parasitic infection was followed by monthly egg counting.
Statistical analysis was performed on data using the repeated-measure analysis of variance design with a factorial treatment structure. Data for egg count showed a normal distribution after a logarithmic transformation.

3. RESULTS AND DISCUSSION

3.1. Experiment I

During three years of sample collection, dry matter digestibility (DM) of sugarcane tops and pasture did not show significant variation with mean ± SE values of 53.74 ± 0.27% and 53.52 ± 2.82%, respectively. Crude protein of roughage and pasture was 8.22 ± 6.94 and 5.32 ± 6.59%, respectively. Assuming that each heifer in the supplemented group consumed 0.3 kg/day of cottonseed meal, this provides an estimated intake of 85.29 g of crude protein intake. All except one of the 23 females in the supplemented group (96%), and only 12 of the 23 heifers in the control group (52%) reached sexual maturity before 18 months of age ($\chi^2 = 11.27, P < 0.01$). These results indicate that supplementation was beneficial in reducing the age at which heifers could be bred. The first ovulation occurred at 513 ± 44 and 573 ± 36 days ($P < 0.01$) in supplemented and control groups, respectively. The mean age at the end of the trial (Dec 92) was 669.78 ± 31.02 for control and 633.35 ± 29.67 d for supplemented groups. Although puberty at 17 months is late when compared with developed countries where breeding is recommended between 11 and 15 months, the results are comparable to those for most commercial Holstein-Friesian heifers raised in São Paulo State, Brazil [17]. Results for other measurements performed on these experimental animals are presented in Table I.

<table>
<thead>
<tr>
<th>TABLE I. BODY CONDITION SCORE (BCS), HEIGHT AT WITHERS, BODY WEIGHT AND BODY WEIGHT GAIN (BWG) OF HOLSTEIN HEIFERS AT FIRST OVULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Supplemented</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

1 From beginning the trial to first ovulation

The present findings agree with other reports [3] showing that weight is more important than age in initiating events associated with puberty and culminating in sexual maturity. They also show that Brazilian heifers will grow quickly and can mate earlier when properly fed.

Heifers receiving protein supplementation were slightly heavier than the control group throughout the experimental period. However, height and body condition score did not differ between the two groups ($P > 0.01$) as shown in Figure 1. Cottonseed meal supplementation, did not only increase the protein content of the diet, but it also increased the energy intake thus enabling a higher growth rate. It must be acknowledged that forage quality may have produced an energy deficiency for at least some portion of the growing period. If this were the case, any effect of protein supplementation might be through gluconeogenesis to overcome an energy shortage.

Multivariate analyses of results for inorganic phosphorus, PCV, haemoglobin and glucose beginning at 3 months of age and continuing until 15 months did not reveal significant differences among C and S groups (Table II). The conclusion from this trial is that a substantial number of control heifers reached puberty later than those fed with the protein supplement.
FIG. 1. Height at withers and body condition score (BCS) of heifers during the observation period.

**TABLE II. LEAST SQUARE MEAN CONCENTRATION OF BLOOD CONSTITUENTS FOR THE SUPPLEMENTED AND NOT-SUPPLEMENTED HEIFERS AT FIRST OVULATION**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Supplemented</th>
<th>Not-supplemented</th>
<th>Probability level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic phosphorus (mg/100 ml)</td>
<td>7.41 ± 0.21</td>
<td>7.88 ± 0.09</td>
<td>0.88</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>25.72 ± 0.16</td>
<td>23.87 ± 0.17</td>
<td>0.43</td>
</tr>
<tr>
<td>Haemoglobin (g/100 ml)</td>
<td>8.83 ± 0.12</td>
<td>9.66 ± 0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>111.48 ± 1.97</td>
<td>109.53 ± 1.59</td>
<td>0.54</td>
</tr>
</tbody>
</table>

3.2. Experiment II

Figure 2 illustrates monthly rainfall and temperatures in Porto Feliz, SP, during the year of the experiment. Figure 3 shows the eggs per gram of faeces for anthelmintic treated and control heifers grazing the same pasture over one year. Consistent rainfall during the year and the absence of rotational grazing resulted in heavily contaminated pastures. The treatment did not eliminate the worms totally, suggesting an anthelmintic resistance or inappropriate dosage administration. However, the animals without protein supplementation were less able to withstand the effects of worm infection.

The groups that had received supplementation presented higher concentrations of haemoglobin and PCV throughout the year than the controls (P <0.01). Another report also indicates that cows fed with low amounts of protein show a decreased haemoglobin concentration [18].

No differences were detected with respect to percentages of heifers that presented the first ovulation by the end of the trial: HC = 70%, HP = 66.67%, AC = 50% and AP = 66.67% ($X^2 = 1.04$, P = 0.79). However, it should be mentioned that the number of heifers in each experimental group was small.

Growth performance is shown in Table III. Heifers that received protein supplementation and anthelmintic treatment grew faster than those in the control group.

Blood glucose and inorganic phosphorus are shown in Table IV. Although, groups without anthelmintic differed in phosphorus concentration (P <0.01), parameters studied were within normal ranges [19].
FIG. 2. Total monthly rainfall (mm) and monthly means of daily maximum and minimum temperature (°C) during the trial.

FIG. 3. Eggs per gram of faeces (EPG) during the trial. Arrows show the dates of anthelmintic treatment (1) = HC, (2) = HT, (3) = AC, (4) = AT

Improved feeding and management are essential for better growth and health status, so that heifers can reach sexual maturity in a reasonably short time. Once they begin to cycle, the farmer can decide whether and when to mate. In contrast, animals that do not receive adequate diets and do not cycle, impede owners to exercise any real control over calving. Results indicate that heifers' sexual maturity and age at first mating-calving can be accelerated by simple management improvement that should be cost-effective in Brazilian or other tropical environments.
TABLE III. LEAST SQUARE MEAN OF HEIGHT AT WITHERS, BODY WEIGHT, BODY WEIGHT GAIN, 
BODY CONDITION SCORE (BCS) AMONG SUPPLEMENTED AND NOT-SUPPLEMENTED HEIFERS AT 
THE END OF THE TRIAL (DEC 92)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Height (cm)</th>
<th>Body weight (kg)</th>
<th>Body weight gain(^1) (kg/day)</th>
<th>BCS (1 - 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>HC</td>
<td>10</td>
<td>109.7 ± 0.2(^a)</td>
<td>185.3 ± 2.5(^a)</td>
<td>0.30 ± 0.03</td>
<td>2.4 ± 0.03</td>
</tr>
<tr>
<td>HP</td>
<td>10</td>
<td>116.6 ± 0.3(^b)</td>
<td>244.8 ± 2.6(^b)</td>
<td>0.37 ± 0.03</td>
<td>2.5 ± 0.03</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>119.7 ± 0.2(^c)</td>
<td>236.2 ± 2.7(^c)</td>
<td>0.29 ± 0.05</td>
<td>2.4 ± 0.03</td>
</tr>
<tr>
<td>AP</td>
<td>9</td>
<td>121.2 ± 0.2(^d)</td>
<td>273.3 ± 2.7(^d)</td>
<td>0.34 ± 0.02</td>
<td>2.7 ± 0.03</td>
</tr>
</tbody>
</table>

1 From the beginning of the trial to first ovulation

Means within columns with different superscripts are significantly different (P <0.01)

HC = Untreated anthelmintic without protein supplementation

HP = Untreated anthelmintic plus protein supplementation

AC = Anthelmintic without protein supplementation

AP = Anthelmintic plus protein supplementation

TABLE IV. LEAST SQUARE MEAN OF PHOSPHORUS AND GLUCOSE IN THE SUPPLEMENTED AND 
NOT-SUPPLEMENTED HEIFERS AT THE END OF THE TRIAL (DEC 92)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Phosphorus (mg/100 ml)</th>
<th>Glucose (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>HC</td>
<td>10</td>
<td>6.92 ± 0.09(^a)</td>
<td>112.23 ± 6.49</td>
</tr>
<tr>
<td>HP</td>
<td>10</td>
<td>7.46 ± 0.09(^b)</td>
<td>110.58 ± 7.89</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>7.01 ± 0.15(^a)</td>
<td>114.56 ± 7.13</td>
</tr>
<tr>
<td>AP</td>
<td>9</td>
<td>7.28 ± 0.10(^a)</td>
<td>116.85 ± 8.82</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different (P <0.01)

HC = Untreated anthelmintic without protein supplementation

HP = Untreated anthelmintic plus protein supplementation

AC = Anthelmintic without protein supplementation

AP = Anthelmintic plus protein supplementation

ACKNOWLEDGEMENTS

The authors are grateful to the International Atomic Energy Agency (IAEA Research Contract 5843/RB) and Centro de Energia Nuclear na Agricultura (CENA) for the financial support. They are also grateful to the farm owner and to the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) for providing grants to the students (N° 800.430/87-5 RN). The authors wish to thank the trainees, students and personal from the Animal Science Section of CENA/USP who helped with data collection, blood samples and chemical analyses.

REFERENCES

MANAGEMENT AND NUTRITION STRATEGIES TO REDUCE THE BREEDING SEASON IN BEEF COWS

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Abstract – Resumen

Effect of temporary calf removal and phosphorus supplementation on conception rate in a 60 or 120 day breeding season period was observed in several trials conducted over three years on Nellore cows. Experiment I involved 47 acyclic Nellore cows with suckling calves ranging in age from 55 to 70 days. Calves were temporarily removed from their dams for 48 h at the beginning of the trial or stayed with the dams throughout the trial. The proportion of cows that cycled during the breeding season was 5 out of 25 (20%) in the control group and 15 out of 22 (68%) in the group whose calves had been removed (P <0.05). In Experiment II, 66 acyclic Nellore cows, averaging 60 ± 0.57 (± ± SE) days post-partum were allocated at random in three groups as follows: A, calves temporarily removed from their dams for 48 h on the first day of the breeding season; B, similar to Group A, except that calves were removed for 72 h and C, control group (no calf removal). The percentage of pregnant cows at the end of the breeding season was 54.6 in control cows compared with 50.0 in the 48 h removal group and 63.6 in the 72 h removal group. In Experiment 3, 75 pregnant cows were selected in the final trimester of gestation. Cows were allocated at random in four groups: Group A, the cows received free choice mineral mixture with 12% P during three months before and three months after the calving season, furthermore, calves were temporarily removed from their dams on first day of breeding season for a period of 96 h; B, same as Group A, except that the calves were not removed; C, same as Group A, except that the mineral mixture had 8.8% P; and D: same as Group C, except no calf removal. More cows receiving 12% P were cycling (P <0.05) at 30, 60 and 90 days into the breeding season. Under conditions in these trials, restricted suckling for 48, 72 or 96 h prior to the breeding season caused inconsistent results on pregnancy rates. However, the restricted suckling of cows with marginal range plasma phosphorus (<4.0 mg/100 ml) enhanced ovarian function.

1. INTRODUCTION

The cattle population of Brazil exceeds 145 x 10^6 [1]. Most of these animals are maintained under extensive management and graze native pastures without additional supplemental feeding, so that many show energy, protein and mineral deficiencies [2, 3, 4]. The current production efficiency is around 50-55% annual calf crop. If this could be increased to 70%, an additional 6 or 7 x 10^6 calves would be born each year.
Many beef in São Paulo State and throughout the country are Nellore; a breed that is adapted to survive in tropical climates, but demonstrates late maturity, short oestrous periods and long intervals of post-partum acyclicity [5]. Calving and weaning results collected over a prolonged period indicate, that this genotype is more productive than several other locally popular breeds [6].

Beef calves running with their dams contribute to delayed post-partum ovarian activity and reduce the reproductive efficiency. A number of reviews emphasize the serious extent of this factor affecting post-partum acyclicity in suckled cattle. Weaning the calf would be an obvious technique to hasten oestrus [7, 8, 9, 10]. Restricting suckling during a 48, 72 or 96 h period, in the second month post-partum has been reported to provide stimulation of ovarian activity [11]. However, 72 h calf removal does not always improve post-partum reproductive performance [12].

The aims of this study were: i) To observe the effects of temporary calf removal from acyclic cows on the onset of ovarian activity and subsequent conception rate; ii) To observe the effects of pre- and post-partum phosphorus (P) supplementation on the onset of ovarian activity and conception rate, and iii) to monitor body condition score and body weight changes during the post-partum and re-breeding period.

2. MATERIALS AND METHODS

2.1. Trial I

This study was carried out during the period from November 1991 to March 1992 at a private 600 ha ranch located in the central area of the São Paulo State. Sequential plasma progesterone profiles, compiled during ten weeks immediately after parturition, were used to select 47 acyclic Nellore cows with suckling calves ranging from 55 to 70 days of age. Cows were divided into two groups:

- T Treated group (n = 22). Calves were temporarily removed from their dams for 48 h at the beginning of the trial.
- C Control group (n = 25). Calves stayed with the dams throughout the trial.

Cows in both groups were exposed continuously over a 60 day period to bulls, previously evaluated for breeding soundness (♂♀ = 1/15). Animals grazed an 80 ha grass pasture (Brachiaria decumbens) with continuous access to water and mineral supplement in feeders without rain protection.

Animals were evaluated over 4 months for live weight change, plasma inorganic P [13] and glucose [14]. Ovarian activity was monitored by determining plasma progesterone concentration in blood samples collected twice per week using the FAO/IAEA solid phase radioimmunoassay kit [15]. Blood sampling continued throughout the breeding season. Pregnancy diagnosis was confirmed by rectal palpation 60 days after the end of the breeding period.

The chemical composition of the pasture [16] and its digestibility [17] were evaluated monthly, and P content in mineral salt was also analyzed.

Continuous data was analyzed by analysis of variance and multivariate analysis of variance. Chi square analysis was used to evaluate the conception rate [18].

2.2. Trial II

In the same private ranch, 66 Nellore cows, averaging 60 ± 0.57 days post-partum and diagnosed as acyclic by sequential plasma progesterone concentrations, were selected from a group of 100 animals. Blood samples were collected twice a week through the breeding season for determination of plasma progesterone concentration using the FAO/IAEA RIA kit [15]. Resulting values were plotted to provide sequential progesterone profiles, which indicated the presence or absence of ovarian cyclicity. Only cows with critical plasma phosphorus concentrations (<4.0 mg/100 ml %) were included in this trial.

The cows were allocated at random in three groups as follow:

- A Calves temporarily removed from their dams for 48 h on the first day of the breeding season (n = 22).
- B Similar to Group A, except that the calves were removed for 72 h (n = 22).
- C Control Group, no calf removal (n = 22).
The three groups were maintained on the same grass pasture (*Brachiaria humidicula*) separated by fencing. Glucose [14] and P [13] were determined on days 60, 90, 120 and 150 post-partum. Body condition score (BCS) in a 1 to 5 scale and live weight (BW) were measured the same days post-partum.

2.3. Trial III

In the same ranch, a total of 75 pregnant cows were selected in their final trimester of gestation. Cows were allocated at random in four groups.

- **A** Cows (n = 19) received a free choice mineral mixture with 12% P during three months before and three months after the calving season. Calves were temporarily removed from their dams on the first day of the breeding season for a period of 96 h.
- **B** Like Group A (n = 20), except that the calves were not removed.
- **C** Like Group A (n = 17), except that the mineral mixture had 8.8% P.
- **D** Like Group C (n = 19), except there was not calf removal.

Cows in all treatments were bled two consecutive weeks before and after the breeding season (4 months) to determine plasma progesterone concentration using the FAO/IAEA RIA kit [15]. Blood samples were collected monthly for glucose [14], P [13] and total plasma protein [19] analyses.

Chemical composition of mineral salt and digestibility were determined by the nylon bag technique, and crude protein content of pasture was evaluated at the beginning and end of the breeding season. Dams were weighed and body conditions were scored (scale 1 to 5) at monthly intervals during the study.

Multivariate repeated-measures analysis of variance was used to test the differences between reproductive and metabolic parameters. The differences between cyclic and acyclic cows were analyzed by Chi square test.

3. RESULTS AND DISCUSSION

3.1. Trial I

Mean dry matter, mineral matter, crude protein (CD) and P content of the pasture during the breeding season were: 97.03 ± 0.64%, 7.47 ± 0.27%, 6.04 ± 0.99%, 0.12 ± 0.03%, respectively. CP and P content declined about 50% and digestibility went down about 10 units suggesting that the pasture quality decreased as the trial progressed.

A cow with a 450 kg BW requires 0.23% P or 18 g P/day. Both mineral mixture and pasture showed low P level. In this study P level in the pasture was about 0.12%, which represents only 67% of P requirement. Considering an intake of 10 kg DM/day, the animal would be supplied with only 12 g P/day. Since the mineral P content was only 1.53%, it is unlikely that any of the cows would consume sufficient to satisfy their requirements. This is a common situation in many areas of Brazil, where producers often fail to provide an adequate mineral supplement [2, 3]. P concentration in plasma of both groups was 3.82 mg/100 ml in December at the beginning of the observation period, but the calf removal group showed higher mean values at the end (3.99 ± 0.2 vs. 3.11 ± 0.2 mg/100 ml, P <0.05, Table I).

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.82 ± 0.18</td>
<td>3.11 ± 0.21*</td>
</tr>
<tr>
<td>Treated</td>
<td>3.82 ± 0.25</td>
<td>3.99 ± 0.19b</td>
</tr>
</tbody>
</table>

* Different superscripts within a column mean statistical differences in Tukey's Test (P <0.05)
However, values in both groups were in the marginal range [20]. This may be reflecting the low P content in the pasture during the last two months. In cattle grazing low quality forage, supplementation with P did not improve reproductive performance or weight gains [21, 22, 23] unless cattle received sufficient protein [24]. However, in a borderline P deficient area in Australia, P supplementation improved reproductive performance [25].

Data for blood glucose did not show significant difference between the treatments, however, the concentration was low in both groups at 60 days breeding season (58.9 ± 1.1 mg/100 ml) but increased to 103 ± 7.2 mg/100 ml during the next three months (Table II). The absence of feed supplementation probably increased the period of negative balance of energy as revealed by the glucose profile. However, live weight changes during the 4 months breeding period were 11.6 and 10 kg for the control and the treated group, respectively.

The proportion of cows that cycled during the breeding season was 5 out of 25 (20%) in the control and 15 out of 22 (68%) in the treated groups ($X^2 = 11.1, P < 0.001$). True pregnancy rate was 20% in control and 55% in calf removal groups ($X^2 = 4.66, P < 0.05$). Calf removal substantially increased the reproductive performance in this trial, but results must be interpreted cautiously, because the number of cows per treatment is small.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days post-partum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>59.0 ± 1.1</td>
</tr>
<tr>
<td>Treated</td>
<td>58.8 ± 1.1</td>
</tr>
</tbody>
</table>

### TABLE II. LEAST SQUARE MEANS AND STANDARD ERROR FOR GLUCOSE (100 mg/ml) IN PLASMA OF COWS (n = 25 per group) WHICH CALVES WERE OR WERE NOT REMOVED FOR 48 HOURS AT THE BEGINNING OF THE TRIAL

3.2. Trial II

Body weight and condition changed somewhat during the observation period. However, analysis of variance using square root transformation of BW and BCS showed differences between 90 and 60 days as well as 150 and 120 days, and this indicated that the results were not different ($P > 0.05$).

In this trial, calf removal for 48 or 72 h did not improve the post-partum reproductive performance. The percentage of pregnant cows at the end of the breeding season was 54.6 in the control cows compared to 50.0 in the 48 h removal group and 63.6 in the 72 h removal group. The relatively small number of cows per treatment group may have prevented the demonstration that the pregnancy rate recorded after 72 h was actually higher than the control value.

3.3. Trial III

The chemical composition and digestibility of pasture presented in Table III show that P was low and digestibility declined throughout the experimental period. A treatment effect on BCS was observed between groups supplemented with mineral containing 12 or 8.8% P (Table IV), but the metabolic profile was not affected and plasma inorganic P in all groups was within the normal range [20]. Although there was no significant difference in plasma P values detected in this trial, animals with more P in their diets showed a trend toward higher plasma concentrations. In non-lactating, feed-restricted beef cows, oestrus cycles ceased when body condition fell below 3.5 on a 1 to 9 assessment scale [26]. In other trials similar severe reduction in BW in cattle causes quiescent ovaries and cessation of estrous cycles [27].

Significant differences were detected between Groups A, B, C and D for the percentage of cows cycling by 30, 60 and 90 days in the breeding season and between groups that received 8.8% or 12% P. There were no differences in pregnancy rate among the Groups A, B, C, and D after the 120 d of the

92
breeding season (Table V). It is concluded that a higher level of P in the mineral mixture increases the pregnancy rate and plasma P values, showing the importance of this element [28].

### TABLE III. DIGESTIBILITY, DRY MATTER AND PHOSPHORUS CONTENT OF THE PASTURE (x ± se)

<table>
<thead>
<tr>
<th>Period of collection</th>
<th>Dry matter (%)</th>
<th>Phosphorus (%)</th>
<th>Dry matter digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before calving</td>
<td>96.7 ± 1.8</td>
<td>0.1 ± 0.1</td>
<td>43.1 ± 3.7</td>
</tr>
<tr>
<td>After calving</td>
<td>97.3 ± 0.8</td>
<td>0.1 ± 0.1</td>
<td>32.9 ± 3.0</td>
</tr>
</tbody>
</table>

### TABLE IV. LEAST SQUARE MEANS AND STANDARD ERROR FOR PLASMA PROTEIN, PLASMA P AND BODY CONDITION SCORE (BCS) AT THE END OF THE BREEDING SEASON AND BODY WEIGHTS (BW) ON DAYS 0 AND 120 OF THE BREEDING SEASON

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Calf removal</th>
<th>No calf removal</th>
<th>Calf removal</th>
<th>No calf removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12% P</td>
<td>12% P</td>
<td>8.8% P</td>
<td>8.8% P</td>
</tr>
<tr>
<td>Plasma protein (g/100 ml)</td>
<td></td>
<td>7.55 ± 0.27</td>
<td>7.27 ± 0.23</td>
<td>7.65 ± 0.27</td>
<td>7.41 ± 0.29</td>
</tr>
<tr>
<td>Plasma P (mg/100 ml)</td>
<td></td>
<td>5.13 ± 0.14</td>
<td>5.04 ± 0.20</td>
<td>4.64 ± 0.23</td>
<td>4.94 ± 0.16</td>
</tr>
<tr>
<td>BCS (1 to 5)</td>
<td></td>
<td>2.73 ± 0.05a</td>
<td>2.73 ± 0.05a</td>
<td>2.68 ± 0.05ab</td>
<td>2.57 ± 0.05b</td>
</tr>
<tr>
<td>BW at day 0 (kg)</td>
<td></td>
<td>376.5 ± 12.3</td>
<td>387.5 ± 12.7</td>
<td>380.6 ± 15.3</td>
<td>361.2 ± 10.7</td>
</tr>
<tr>
<td>BW at day 120 (kg)</td>
<td></td>
<td>377.9 ± 10.7</td>
<td>379.3 ± 9.8</td>
<td>374.5 ± 12.6</td>
<td>368.1 ± 8.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Calf removal</th>
<th>No calf removal</th>
<th>Calf removal</th>
<th>No calf removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12% P</td>
<td>12% P</td>
<td>8.8% P</td>
<td>8.8% P</td>
</tr>
<tr>
<td>Acyclic cows</td>
<td></td>
<td>42 (n = 8)</td>
<td>50 (n = 10)</td>
<td>76 (n = 13)</td>
<td>79 (n = 15)</td>
</tr>
<tr>
<td>Cyclic cows</td>
<td></td>
<td>58 (n = 11)</td>
<td>50 (n = 10)</td>
<td>24 (n = 4)</td>
<td>21 (n = 4)</td>
</tr>
<tr>
<td>Actual pregnancy rate</td>
<td></td>
<td>84 (n = 16)</td>
<td>70 (n = 14)</td>
<td>59 (n = 10)</td>
<td>79 (n = 15)</td>
</tr>
</tbody>
</table>

\[ X^2 = 8.173, \quad P = 0.046 \]

\[ X^2 = 2.592, \quad P = 0.4589 \]
ACKNOWLEDGEMENTS

The authors are grateful to the International Atomic Energy Agency (IAEA Research Contract 5843/RB) and Centro de Energia Nuclear na Agricultura (CENA) for the financial support. They wish to thank the farm owner and to the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) for providing grants to the students (N° 800633/88-INV-Fellowship student). The authors are also grateful to the students and personal from the Animal Science Section of CENA/USP who helped with collection of data, blood samples and chemical analyses.

REFERENCES


EFFECT OF STRATEGIC FEED SUPPLEMENTATION WITH MULTINUTRIENT BLOCKS ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE IN DUAL-PURPOSE COWS

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Abstract – Resumen

EFFECT OF STRATEGIC FEED SUPPLEMENTATION WITH MULTINUTRIENT BLOCKS ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE IN DUAL-PURPOSE COWS

The study was carried out on four commercial farms in Guarico state, Venezuela to evaluate the effectiveness of multinutrient blocks (UMB) on the body condition score (BCS), milk production and reproductive performance of dual-purpose cows. Forty crossbred animals (Bos taurus × Bos indicus) in each farm were randomly allocated to supplemented and not-supplemented groups throughout a period of 105 days. The studies on Farms 1, 2, and 3 were done during the dry season, and on Farm 4 during the rainy season. The UMB composition was 46% sugar-cane molasses, 10% urea, 10% Glycrrhiza sepium, 5% mineral mixture, 11% calcium hydroxide, 5% triple superphosphate, 10% maize crop residue and 3% animal fat. UMB intake was recorded every two days and individual milk production was registered daily or twice a week. Body weight (by scale or estimated by chest girth measurement) and BCS (0 to 5) were measured every 15 or 21 days. Clinical examination of the ovaries via rectal palpation was done every 15 or 21 days from parturition until day 105 post-partum. Ovarian activity was monitored through progesterone concentrations in milk samples collected twice a week.

Block consumption was higher during the dry season than in the rainy season (0.27 ± 0.21 vs 0.085 ± 0.069 kg/cow/day respectively, P >0.05). There were no significant differences in total milk production during the first 15 weeks of lactation between treatments in Farms 1 and 3 (418.8 ± 402.8 kg and 387.9 ± 378 kg for treated and control groups in each farm, respectively). However, significant differences were found in Farm 2 (194.0 vs 116.7 kg in the treated and control group, respectively, P <0.05). Milk production was higher during the rainy season in the control group in Farm 4 (491.8 vs 413.3 kg). Overall data of Farms 1, 2, and 3 through the dry season showed a shorter interval from calving to resumption of ovarian activity in treated compared to control animals (60.2 ± 26 vs 73.5 ± 32 days, P <0.05). Body condition at calving and during the early post-partum period may explain some of the improvements on reproductive performance which occurred during the dry season. This indicates the importance of nutritional status of cows before calving on post-partum reproductive activity.

EFECTO DE LA SUPLEMENTACIÓN ESTRATÉGICA CON BLOQUES MULTINUTRIENTES SOBRE EL COMPORTAMIENTO PRODUCTIVO Y REPRODUCTIVO EN VACAS DE DOBLE PROPÓSITO

El estudio se llevó a cabo en cuatro fincas comerciales del estado de Guarico, Venezuela para evaluar la efectividad de los bloques multinutrientes (UMB) sobre la condición corporal (BCS), producción de leche y comportamiento reproductivo de vacas de doble propósito. Cuarenta vacas cruzadas (Bos taurus × Bos indicus) por finca fueron distribuidas al azar entre el grupo suplementado y el no-suplementado por un período de 105 días. El ensayo en las fincas 1, 2 y 3 se hizo durante la época seca y en la Finca 4 durante la época lluviosa. La composición del bloque fue: 46% melaza, 10% urea, 10% Glycrrhiza sepium, 5% mezcla mineral, 11% hidróxido de calcio, 5% superfosfato triple, 10% residuos de cosecha de maíz y 3% grasa animal. El consumo de UMB se registró cada dos días y la producción de leche por vaca se anotó en forma diaria o dos veces por semana. El peso corporal (en balanza o estimado en base al perímetro torácico) y la condición corporal (0 a 5) se tomaron cada 15 o 21 días. El examen clínico de los ovarios se hizo cada 15 o 21 días desde el parto hasta los 105 días post-parto. El perfil de la actividad ovárica se hizo mediante los valores de progesterona en muestras de leche recolectadas dos veces por semana.

El consumo del bloque fue mayor durante la época seca que durante la época de lluvias (0.27 ± 0.21 vs 0.085 ± 0.069 kg/vaca/día, respectivamente). No se encontraron diferencias significativas entre tratamientos en las fincas 1 y 3 con respecto a la producción de leche durante las primeras 15 semanas de lactación (418.8 ± 402.8 kg y 387.9 ± 378 kg en los grupos tratado y control de cada finca, respectivamente, P >0.05). Sin embargo, se encontraron diferencias significativas en la Finca 2 (194.0 ± 116.7 kg entre el grupo tratado y control, respectivamente, P <0.05). Hubo mayor producción de leche durante la época de lluvias en el grupo control de la Finca 4 con relación al grupo tratado (491.8 vs 413.3 kg). En general, durante la época seca en las fincas 1, 2 y 3 se encontró un menor intervalo entre el parto y el remicio de la actividad ovárica en los animales del grupo tratado en comparación con los animales control (60.2 ± 26 vs 73.5 ± 32 d, P <0.05). La condición corporal al parto y durante la primera etapa del periodo post-parto permiten explicar las mejoras obtenidas en el comportamiento reproductivo observado durante la época seca. Esto es una indicación de la importancia del estado nutricional de las vacas al parto sobre la actividad reproductiva en el periodo post-parto.
1. INTRODUCTION

Dual purpose cattle contribute around 90% of total milk production in Venezuela. Their productive performance is low (1000-2000 kg/cow/year and 400-500 kg/ha/year), due to several factors, including incorrect management of forage resources and unsuitable animal husbandry practices [1].

There is a vast amount of information about the evaluation of reproductive performance of crossbred cows [2]. However, there are only few reports on the effect of strategic supplementation with multinutrient blocks on the productive and reproductive performance of crossbred cows. Experimental responses on forage intake, body weight gain, survival of calves, milk production and reproductive performance following the use of urea-molasses blocks (UMB) have been reported in cattle, buffaloes and sheep in Ethiopia [3], Indonesia [4], Pakistan [5], Colombia and Venezuela [6].

Evidence indicates that the appetite of ruminants is depressed when crude protein falls below 6.8% [7]. Unfortunately, a large proportion of tropical grasses, particularly native pastures fall below this value. Actually, the use of tree legumes and UMB is becoming a practical solution to the necessary nitrogen supply to grazing animals. They provide energy and also they are a continuous source of degradable nitrogen to the rumen, even if consumption of the supplement is sporadic.

Cows with long post-partum anoestrous periods are common under tropical grazing conditions [8]. The re-establishment of ovarian cyclicity after parturition is influenced by body condition, suckling, milk yield and health disturbances which are the major factors affecting the reproductive physiology during the post-partum period.

The present research work was conducted on four commercial farms in Guarico State (Venezuela) under grazing conditions during the dry and rainy season. The objective of the study was to evaluate the effectiveness of multinutrient blocks on the onset of post-partum ovarian activity, milk production and body condition in dual purpose cows.

2. MATERIALS AND METHODS

2.1. Location

The present study was conducted from November 1991 to August, 1993, in four commercial farms of low milk production (3-6 L/cow/day) located in Guarico state. The range of mean daily temperature in the region is 24 - 30°C, mean rainfall is 1060 - 1200 mm/year and relative humidity is 60 - 70%. The dry season is from October to March and the rainy season from May to September; whereas April is a transitional month between the dry and rainy seasons.

2.2. Animals and feeds

Forty multiparous cows were selected in each farm and randomly allocated to two groups, taking into account breed and body condition. Twenty cows were supplemented with multinutrient blocks (urea-molasses blocks - UMB) and 20 cows were not-supplemented. The animals were mainly F, and F2 crossbreds (Zebu x Holstein and Brown Swiss). Both groups in each farm grazed the same pasture and had free access to a mineral salt supplement and water. Cows grazed crop residues of corn (Zea mays) and sorghum in Farm 1; Hyparrhenia rufa and native species in Farm 2; Brachiaria sp in Farm 3; and Cynodon plectostachyum and Hyparrhenia rufa in Farm 4. Paddock rotation was carried out every 15-20 days in Farms 1, 2 and 3, and once a month in Farm 4.

Cows were milked once a day and restricted suckling was applied. Cows were artificially inseminated in Farm 1, whereas natural mating was used in the other three farms. Farms 1 and 3 had a genetic improvement programme and productive and reproductive records of animals were kept. Record keeping in Farms 2 and 4 was limited.

The experimental period varied according to farms. It started in the dry season of 1991 (Nov-Mar) in Farms 1 and 2, in the dry period of 1992 (Oct-Feb) in Farm 3 and in the rainy season of 1992 (June-Aug) in Farm 4.
The composition of the UMB was: 46% sugar-cane molasses, 10% urea, 10% \textit{Glyricidia sepium}, 5% mineral mixture, 11% calcium hydroxide, 5% triple superphosphate, 10% maize crop residue and 3% animal fat.

2.3. Measurements

Samples of forage and UMB were collected in each farm to estimate the quality and availability (kg Dry matter/ha and block intake kg/cow/day). Forage samples were collected monthly and UMB at the time of making. Samples of grasses and UMBs were analyzed for crude protein [9], calcium [10], phosphorus [11], crude fiber and fat [9].

The UMBs were weighed every two days and the difference in weight was recorded as the consumption of the block in that period. Daily or twice a week milk production records were kept in the four farms.

Body weight of cows was measured every 15 or 21 days. Body weight was estimated by chest girth in Farms 2, 3 and 4. Calves were weighed once at the beginning and once at the end of the experiment. Body condition score was recorded every 15 or 21 days [12] with a score from 0 (emaciated) to 5 (fat).

Uterine involution and ovarian activity was monitored by rectal palpation on days 15 and 30 post-partum, and thereafter every 15 or 21 days until 105 days of the experiment. Ovarian activity was also monitored through progesterone concentration. Milk samples were collected twice weekly and progesterone concentrations subsequently determined using the solid-phase FAO/IAEA radioimmunoassay kits [13]. Progesterone profiles were used to calculate the intervals from calving to the resumption of ovarian activity and conception. Luteal activity was considered to have occurred if progesterone values were consistently elevated (≥3.18 nmol/L) during two consecutive weeks [14].

2.4. Statistical analysis

Data was analyzed by the method of least squares of analysis of variance to fit general linear models [15]. Differences between means of groups were tested using the Duncan’s multiple range test. The following linear models were used for luteal ovarian activity (OA) and milk production (MP):

\[
\text{OA} = \text{treatment, body condition, and its interaction} \quad (\text{MP was used as covariate}).
\]
\[
\text{MP} = \text{treatment, body condition, and its interaction} \quad (\text{OA was used as covariate}).
\]

The intervals from calving to the resumption of ovarian activity, first oestrus, first service and conception were analyzed by analysis of variance and T-test. Differences between means were tested using the Duncan, Scheffe and Tukey methods. The Chi-square method was used in the analysis of pregnancy rates.

3. RESULTS AND DISCUSSION

3.1. Feeds

Table 1 shows the chemical composition of the basic diet (forage or crop residues) and the UMBs used in each farm. In general, the basal diet was of poor quality during the dry season (Farms 1, 2 and 3) but was better during the rainy season (Farm 4) as expected.

Block consumption was variable among seasons: In the dry season trials carried out in Farms 1, 2 and 3 consumption was 0.27 ± 0.21 kg/cow/day; whereas in the rainy season (Farm 4) the block consumption was much lower (0.085 ± 0.069 kg/cow/day). Similar block intakes have been reported in cows grazing low quality roughages [3, 5, 16, 17]. The causes of the variability in block intake observed under grazing condition have been attributed to changes in the selection ability of animals while grazing and therefore guaranteeing a high intake of protein or other critical nutrients to maintain a higher rumen...
TABLE I. CHEMICAL COMPOSITION (%) OF THE BASAL DIET AND UREA-MOLASSES BLOCKS (UMB) FED TO DUAL-PURPOSE COWS IN FOUR FARMS (DM = dry matter; CP = crude protein; CF = crude fibre; EE = Ether extract) IN GUARICO STATE, VENEZUELA

<table>
<thead>
<tr>
<th></th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>93.7 ± 1.3</td>
<td>92.4 ± 1.4</td>
<td>90.5 ± 0.7</td>
<td>94.7 ± 0.8</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.4 ± 1.0</td>
<td>37.2 ± 1.6</td>
<td>35.4 ± 2.2</td>
<td>13.7 ± 2.0</td>
</tr>
<tr>
<td>CP (%)</td>
<td>2.9 ± 1.1</td>
<td>30.5 ± 0.8</td>
<td>4.9 ± 3.0</td>
<td>11.4 ± 3.0</td>
</tr>
<tr>
<td>CF (%)</td>
<td>41.3 ± 3.1</td>
<td>34.0 ± 3.1</td>
<td>7.0 ± 3.1</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>EE (%)</td>
<td>0.38 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.6 ± 0.4</td>
<td>4.5 ± 2.7</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.14 ± 0.04</td>
<td>7.0 ± 0.07</td>
<td>10.2 ± 0.4</td>
<td>16.2 ± 0.3</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.06 ± 0.05</td>
<td>1.3 ± 0.06</td>
<td>1.5 ± 0.2</td>
<td>3.9 ± 0.03</td>
</tr>
</tbody>
</table>

ammonia concentration through the recycling of nitrogen (e.g. urea) in the rumen. Hence ruminants supplemented with UMB ad libitum control the intake of urea adjusting it to the N content of the basal diet [18]. In the rainy season the consumption of UMBs was lower than in the dry season. There was a significant correlation (r = -0.92) between quality of the pasture (crude protein) and UMB consumption in all farms.

3.2. Milk production

Milk production data is shown in Figure 1. Block consumption did not significantly influence total milk production during the first 15 weeks of lactation in Farms 1 and 3 (418.8 vs 402.8 kg, and 387.9 vs 378 kg in treated and control groups in each farm, respectively, P >0.05). However, significant differences were found in Farm 2 (194.0 vs 116.7 kg in treated and control groups, respectively. P <0.05).

Surprisingly, milk production was higher in the control group in Farm 4 (491.8 vs 413.3 kg, P <0.05). Factors such as hoof injuries and vesicular stomatitis lesions on the udder in some cows of the supplemented group could have influenced low milk production levels in the group through a reduction in feed intake.

There is little information in the literature on the effect of UMBs on milk production in cows grazing pastures of low quality. However, most reports have described moderate responses in milk production [4, 5, 19, 20]. Information regarding responses to UMB on cultivated pastures is even more scarce. Available data also confirms low increments of milk production in dual-purpose cows supplemented with UMB during the rainy season [17, 21].

3.3. Body condition

Although animals in Farms 1, 2 and 3 had calved with a mean of BCS below 2 (poor condition), supplemented cows showed a slight improvement in body condition during the first 60 days post-partum (Figure 2). Animals in the control group in Farm 2 lost body condition during the first 60 days post-partum, which may explain the longer interval to onset of cyclicity of this group compared to the treated group (P <0.01). The literature also indicates that losses in body condition at calving affects post-partum ovarian activity [22]. The response was different in cows that have calved during the rainy season. The treated group lost body condition by day 60 post-partum (Figure 2) which can be attributed to health disturbances as indicated earlier.

Analysis of the data per farm and per post-partum week in Farm 1 showed that body condition and treatment significantly affected ovarian activity (P <0.01 and P <0.05, respectively). The interaction between BCS and treatment was also important. Supplemented cows with BCS ≥2.5 showed shorter
FIG. 1. Urea-molasses blocks (UMB) consumption (kg/cow/day) and milk production (kg/cow/day) in supplemented and not-supplemented dual-purpose cows during the dry (Farms 1, 2 and 3) and rainy season (Farm 4).

FIG. 2. Body condition (BCS) changes during the post-partum period in supplemented and not-supplemented dual-purpose cows during the dry (Farms 1, 2 and 3) and rainy season (Farm 4).
intervals to the resumption of ovarian cyclicity (P >0.05). The probability and r² of the model were P <0.004 and 0.34 respectively. Similar results were observed in Farm 3; however, in Farm 2 neither BCS nor the interaction affected ovarian activity.

A number of reports have pointed out the importance of BCS at calving [14, 22], and in general consider the score of 2.5 - 3.0 as adequate [23]. In fact, adequate BCS at calving in cows have been related to shorter intervals from calving to the resumption of ovarian activity and to conception in crossbred cows [20, 24]. There is also evidence that positive body condition changes during the post-partum period did not affect reproductive performance [23].

BCS affected milk production at different stages of the lactation during the study in Farms 1, 2 and 3 (P >0.05) but not in Farm 4. Figure 2 shows that supplemented cows tend to reduce their condition (Farm 2), because they had a better level of milk production than the control cows, probably due to the rate of mobilization of body reserves which contributes to fix those differences in milk production. This also could have affected the conception rate [25].

3.4. Body weight gain

Body weight gain in supplemented cows did not significantly differ from the controls with the exception of Farm 3 (Table II). These changes in body weight were in closely related to changes in body condition.

The average body weight gain in calves (Table II) was not different between treatments. However in Farm 1, calves from supplemented cows had better weight gains than the controls (P <0.10).

<table>
<thead>
<tr>
<th>Farm</th>
<th>Season</th>
<th>Animal</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>UMB</td>
</tr>
<tr>
<td>1</td>
<td>Dry</td>
<td>Calves</td>
<td>0.390 ± 0.13a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cows</td>
<td>0.390 ± 0.13a</td>
</tr>
<tr>
<td>2</td>
<td>Dry</td>
<td>Calves</td>
<td>0.260 ± 0.14a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cows</td>
<td>0.310 ± 0.460a</td>
</tr>
<tr>
<td>3</td>
<td>Dry</td>
<td>Calves</td>
<td>0.101 ± 0.14a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cows</td>
<td>0.097 ± 0.33a</td>
</tr>
<tr>
<td>4</td>
<td>Rainy</td>
<td>Calves</td>
<td>0.307 ± 0.12a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cows</td>
<td>0.311 ± 0.46a</td>
</tr>
</tbody>
</table>

Body weight was estimated through chest girth measurements in Farms 2, 3 and 4. Values with different superscripts within a row are significantly different (P <0.10).

3.5. Post-partum reproductive activity

Overall data in Farms 1, 2 and 3 over 120 animals (Table III) through the dry season showed significant differences between the treated and control group (P <0.05) on the timing resumption of ovarian activity (interval from calving to the first sustained high concentration of progesterone; 60.2 ± 26 vs 73.5 ± 32 d in the treated and control group, respectively). Additionally, there was a shorter interval from calving to conception (102 ± 29 vs 111 ± 23 d) and better pregnancy rates (50 vs 43%) in the treated group compared to the controls; however, these differences were not significant (P >0.05).
TABLE III. OVERALL POST-PARTUM REPRODUCTIVE PERFORMANCE IN GRAZING DUAL-PURPOSE COWS OF THREE FARMS FED LOW QUALITY FORAGES OR CROP RESIDUES AND SUPPLEMENTED WITH UREA-MOLASSES BLOCKS (UMB) DURING THE DRY SEASON IN GUARICO STATE, VENEZUELA

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Animals (n)</th>
<th>Calving to (d)</th>
<th>Conception rate at 150 days (%)</th>
<th>Pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMB</td>
<td>60</td>
<td>60.2 ± 26(^a) (58)</td>
<td>102 ± 29(^a) (32)</td>
<td>46 (28)</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>73.5 ± 32(^b) (55)</td>
<td>111 ± 23(^b) (28)</td>
<td>53 (32)</td>
</tr>
</tbody>
</table>

() Number of observations
a, b Mean values with different superscript are significantly different (P <0.05)

TABLE IV. POST-PARTUM REPRODUCTIVE PERFORMANCE (at 105 days) IN GRAZING DUAL-PURPOSE COWS FED LOW QUALITY FORAGES OR CROP RESIDUES AND SUPPLEMENTED WITH UREA-MOLASSES (UMB) BLOCKS DURING THE DRY AND RAINY SEASON

<table>
<thead>
<tr>
<th>Treatments</th>
<th>UMB</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval from calving to:</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>first ovulation (d)</td>
<td>59.5</td>
<td>16.4</td>
</tr>
<tr>
<td>first oestrus (d)</td>
<td>75.0</td>
<td>22.2</td>
</tr>
<tr>
<td>first service (d)</td>
<td>79.0</td>
<td>30.3</td>
</tr>
<tr>
<td>conception</td>
<td>112.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Fertility at first service (%)</td>
<td>80.0</td>
<td>66.6</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>45.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Services per conception (n)</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Anoestrous rate (%)</td>
<td>55.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Farm 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval from calving to:</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>first ovulation (d)</td>
<td>43.7(^a)</td>
<td>28.3</td>
</tr>
<tr>
<td>conception</td>
<td>90.0</td>
<td>27.5</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>55.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Farm 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval from calving to:</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>first ovulation (d)</td>
<td>74.9</td>
<td>23.7</td>
</tr>
<tr>
<td>conception</td>
<td>106.9</td>
<td>27.5</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>50.0(^a)</td>
<td>36.0(^b)</td>
</tr>
<tr>
<td>Farm 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval from calving to:</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>first ovulation (d)</td>
<td>90(^a)</td>
<td>26</td>
</tr>
<tr>
<td>conception</td>
<td>125(^a)</td>
<td>11.2</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>25(^a)</td>
<td>36.8(^b)</td>
</tr>
</tbody>
</table>

\(^a, b\) Means with different superscripts within rows are significantly different (P >0.05)
Reproductive parameters of the three farms during the dry season are shown in Table IV. No significant differences were found between treatments in Farm 1; however, the interval from calving to first service in the supplemented group was 13 days shorter than in the control group and there was a better fertility rate at first service (80 vs 67%). (The interval from calving to the resumption of ovarian activity was significantly shorter in the treated group in Farm 2 (43.7 ± 28.3 vs 66.0 d), and the pregnancy rate in Farm 3 was higher 50 vs 36% in the treated and control group, respectively, P <0.05)). These results are similar to those reported in South-east Asia using UMB in cows fed rice straw [8].

Supplementation with UMB during the rainy season did not improve reproductive performance of cows in Farm 4 (Table IV). The treated group showed 35 days longer interval from calving to the resumption of the ovarian cyclicity compared to the control group. Also, they needed 22 extra days to conceive and the conception rate was even lower (25 vs 36%) at 105 days post-partum in the treated and the control group, respectively.

4. CONCLUSIONS

Results of the present study in four farms indicate a variable block consumption between weeks and seasons of the year. There was a high correlation between protein quality of the forage and block intake (r = -0.92) which indicates a probable adjustment of the animals in the nitrogen balance under grazed pasture conditions in two seasons of the year. Supplemented cows with multinutrient blocks improved milk production through the dry season. In contrast, milk production was lower during the rainy season but this was probably due to extra-experimental factors.

The interval from calving to the resumption of ovarian cyclicity was shorter in supplemented cows compared with non-supplemented animals during the dry season (P <0.05). Body condition at calving and during the early post-partum period may explain some of the improvements on reproductive performance which occurred during the dry season. This indicates the importance of nutritional status of cows at calving on post-partum reproductive activity.

It is necessary to develop nutritional strategies using UMB to ensure adequate body condition at calving. These strategies should consider the relationship between quality and availability of forage and block intake.

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FEED SUPPLEMENTATION FOR IMPROVING REPRODUCTIVE EFFICIENCY OF CROSSBRED ZEBU CATTLE IN THE PERUVIAN TROPICS

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Abstract — Resumen

FEED SUPPLEMENTATION FOR IMPROVING REPRODUCTIVE EFFICIENCY OF CROSSBRED ZEBU CATTLE IN THE PERUVIAN TROPICS

Thirty three multiparous Holstein x Nelore cows were used to evaluate the effect of a pre-partum supplementation diet on calving to first ovulation and conception intervals, milk yield, cow body weight changes and newborn calf weight. Cows were randomly assigned, either to a supplemented group (n = 16), or to a non-supplemented group (n = 17). The supplemented animals received 4 kg of concentrate mixture (rice hull and fish meal) containing 20% total protein (CP) for approximately 90 days before parturition. Animals were milked once a day and grazed Brachiara decumbens grass (8-10% CP and 55-60% digestibility) with a stocking rate of 1.5 AU/ha (1 AU = 1 cow of 400 kg body weight). Milk samples were collected twice a week for progesterone analysis to monitor ovarian activity. Supplemented cows had greater average daily weight gain than control animals in the pre-partum period of the experiment (0.730 and 0.567 kg/cow/day, respectively, P <0.05). Supplemented animals had a significant shorter interval from calving to first ovulation (42.7 days) than the control animals (73.8 days, P <0.01), but the differences on the interval from calving to conception were not significant (149.6 and 163.8 days, respectively, P >0.05). No significant differences were found between groups with respect to milk production (480 ± 105 vs 477 ± 122 l/cow in the first 100 days of lactation), calving body weight (446 ± 52 vs 446 ± 44 kg) and newborn calf body weight (35.7 ± 4 vs 35.4 ± 6 kg). It is concluded that supplementation in the last trimester of gestation is a useful strategy to improve body condition; and thereby, to reduce calving to first ovulation interval.

SUPLEMENTACION ALIMENTICIA PARA LA MEJORA REPRODUCTIVA DEL CEBU CRUZADO EN EL TROPICO PERUANO

Treinta y tres vacas multiparas Holstein x Nelore fueron utilizadas para evaluar el efecto de la suplementación pre-parto sobre los intervalos del parto a la primera ovulación y a la concepción, la producción de leche, los cambios de peso corporal de la vaca y el peso al nacimiento del ternero. Las vacas fueron asignadas al azar al grupo suplementado (n = 16) o al grupo control (n = 17). Los animales suplementados recibieron 4 kg de concentrado (cáscara de arroz y harina de pescado) con un contenido de 20% de proteína total (CP). El concentrado fue suministrado por aproximadamente 90 días previos al parto. Los animales estuvieron sobre una pastura de Brachiarar decumbens (8-10% CP y 55-60% de digestibilidad) con una carga animal de 1.5 UA/ha (1 UA = 1 vaca de 400 kg de peso vivo), y se realizó un ordena al día. Se tomaron muestras de leche para análisis de progesterona a fin de hacer un seguimiento de la actividad ovárica. Las vacas suplementadas tuvieron una mejor ganancia diaria de peso que las vacas control durante el período de suplementación (0.730 y 0.567 kg/vaca/día, respectivamente, P <0.05). El intervalo del parto a la primera ovulación fue significativamente más corto en los animales suplementados (42.7 días), que en los animales del grupo control (73.8 días, P <0.01). Sin embargo, las diferencias en el intervalo del parto a la concepción no fueron significativas (149.6 y 163.8 días, respectivamente, P >0.05). No hubieron diferencias significativas entre grupos con respecto a la producción de leche (480 ± 105 vs 477 ± 122 l/vaca en los primeros 100 días de lactancia), peso vivo al parto (446 ± 52 vs 446 ± 44 kg) y peso al nacer del ternero (35.7 ± 4 vs 35.4 ± 6 kg). Se concluye que la suplementación en el último trimestre de la gestación es una adecuada estrategia para mejorar la condición corporal, y por lo tanto, reducir así el intervalo del parto a la primera ovulación.

1. INTRODUCTION

The Amazon region of Peru has more than 70 million ha of forest and represents 60% of the country's area. There has been an increased influx of people into this region during the last decades, and many of the new settlers are engaged in agricultural production. The Nelore has been the predominant breed of Zebu cattle in the area. However, its reproductive performance is fairly poor, as it shows a long post-partum anoestrous and a low conception rate. There have been attempts to improve the reproductive efficiency of Zebu cattle in the region. Crossbreeding of Zebu animals is a realistic option for improving reproductive efficiency [1]. Previous studies have shown that a better reproductive efficiency of crossbred animals can be easily attained by improving the quality of the diet and by restricting suckling [2, 3].
Nutrition plays an important role influencing productive and reproductive performance of pure-and crossbred Zebu cows. In field studies previously undertaken [3], body-weight at calving and subsequent weight changes during the post-partum period, influenced post-partum reproductive performance, especially the calving to the first ovulation interval. Low nutritional quality diets affected both the developmental rate of large follicles and the timing of first ovulation, and resulted in periods of acyclicity. Furthermore, the milk yield was also affected [3]. It has also been reported that beef cows, able to maintain their body condition through the post-partum period, have shorter intervals to first post-partum oestrus than cows which have lost body weight [4].

The hypothesis of the present study is that the addition of some nutrients in the diet during the last period of gestation and after parturition can maintain body condition during the lactational period; thereby shortening the interval to first ovulation and to conception. A further advantage of the treatment would be an increase in milk production.

The present study was designed to examine the effects of pre-partum supplementation on first post-partum ovulation, calving to conception interval and to correlate pre- and post-partum body weight changes with reproductive performance.

2. MATERIAL AND METHODS

Thirty three crossbred Holstein x Nelore multiparous cows were used. The animals were approximately six months pregnant and had 2 to 11 parturitions each.

These animals grazed *Brachiaria decumbens* (8-10% crude protein and 55-60% digestibility) with a stocking rate of 1.5 AU/Ha (1 AU = 1 cow of 400 kg body weight). Cows were randomly assigned either to a supplemented group (n = 16) or to a non-supplemented group (n = 17). The supplemented animals received 4 kg of concentrate mixture (rice hull and fish meal) containing 20% total protein (CP), for approximately 90 days before parturition. The supplement was offered individually to insure the consumption. All cows were kept together rotating in a group of paddocks. One fertile bull was permanently kept together with the experimental animals. Suckling was restricted from 07:00 to 15:00 h. Hand-milking was performed once a day at 06:00.

Cows were weighed every 15 days before parturition and throughout the post-partum period. In addition, animals were weighed the day of calving. Milk samples for progesterone determination were collected twice a week beginning on day 15 post-partum and ending when first ovulation was detected by consistently elevated progesterone concentration. An ovulation was considered to have occurred if there were consistently elevated levels of progesterone (≥3.5 nmol/L in skim milk) during two consecutive samplings. Milk samples were preserved with sodium azide. Samples were cooled (+4°C) immediately after collection, defatted by centrifugation and stored at -20°C until assayed. The progesterone content of milk samples was determined by radioimmunoassay using the solid-phase FAO/IAEA RIA kit [5]. The intra- and inter-assay coefficients of variation were 9.4 and 12.6%, respectively. The statistical analysis of the data was performed using analysis of variance.

3. RESULTS

Table I shows the body weight of the two experimental groups at various periods close to parturition. Body weight of the supplemented group at the beginning of the trial was approximately 20 kg lower in the supplemented group that the control group (P >0.05). Supplemented cows had greater average daily weight gain than control animals in the pre-partum period of the experiment (0.730 and 0.567 kg/cow/day, respectively, P <0.05).

Supplemented animals had a significant shorter interval from calving to first ovulation (42.7 days) than did the control animals (73.8 days, P <0.01), but the differences on the interval from calving to conception were not significant (149.6 and 163.8 days, respectively, P >0.05).

No differences were found between groups with respect to milk production (480 ± 105 vs 477 ± 122 l/cow in the first 100 days of lactation), calving body weight (445.9 ± 52 vs 446.4 ± 44 kg) and newborn calf body weight (35.7 ± 4 vs 35.4 ± 6 kg).
FIG. 1. Pre- and post partum body weight in Holstein × Nellore cows supplemented or not supplemented with a mixture of rice hull and fish meal during the last trimester of gestation.

TABLE I. Body weight pre- and post partum and pre-partum weight gain in supplemented or not supplemented dual-purpose cows.

<table>
<thead>
<tr>
<th></th>
<th>N° of cows</th>
<th>Body weight (x ± sd, kg)</th>
<th>Pre-partum body weight gain (mean, g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial¹</td>
<td>Pre-calving² At calving</td>
</tr>
<tr>
<td>Supplemented</td>
<td>16</td>
<td>446 ± 46</td>
<td>507 ± 54</td>
</tr>
<tr>
<td>Control</td>
<td>17</td>
<td>465 ± 57</td>
<td>509 ± 48</td>
</tr>
</tbody>
</table>

¹ 83 ± 24 days before parturition
² Within 10 days before parturition

⁴ Different superscripts within a column indicate significant differences (P > 0.01)

4. DISCUSSION

The key to early return to oestrus in beef cows is an adequate pre-partum programme of nutrition, which results in cows calving in moderate to good body condition. There must be adequate planning regarding condition for cows at parturition in order to be in oestrus within 80 days after calving [6]. However, determination of adequate levels of pre-partum nutrition is sometimes difficult, especially at small-holder farm level, since cows differ in their response to a given level of nutrition due to differences in age, size, milking ability, state of pregnancy, environment and body condition.

As the conceptus has high priority for the nutrients, the maternal system will sacrifice body reserves to meet this demand. Sometimes, due to various factors, large weight losses occur during the last trimester of gestation, causing cows to calve in poor body condition, this results in a decreased percentage of cows in oestrus early in the subsequent breeding season [6]. Cows in the supplemented group had lower but not significantly different body weight than did the control group at the commencement of the experiment. As expected, daily weight gain during the pre-partum period in the present study was greater in the supplemented group (Table I). The results suggest that supplementing cows in poor condition during the last trimester of gestation is a useful strategy to improve their physical condition. Supplementation of these animals will allow them to reach adequate body condition at parturition and have a similar or better post-partum reproductive performance than herdmates that had good body condition throughout the gestation period. In the present experiment, nutritional requirements were partially supplied by the supplement, allowing cows to calve in better condition than
control animals, and with approximately a similar body weight to that which they had 80 days prior to parturition.

Both groups had similar body weight at calving but control animals lost more weight than the supplemented animals. This fact may be due to a different energy balance. Control animals used body reserves to meet the nutritional requirements of late gestation, but supplemented animals used nutrients provided by the supplement; and therefore, were able to maintain better body condition.

The present study showed that supplemented animals had a reduced calving-first ovulation interval in contrast to the non-supplemented cows. The intervals were shorter than those reported in tropical areas of Latin America using animals with lower or similar calving weights [8, 9, 10]. The protein content of the concentrate (22.9% undegraded protein, fish meal) was sufficient to meet fetal growth needs. Undegradable protein supplementation in the pre-partum period may improve post-partum reproductive performance by minimizing mobilization of maternal labile protein pools to meet fetal and maternal growth requirements in late gestation [11]. In many cases, the major portion of fetal weight gain occurs at cost of maternal system [6]. However, it has also been reported that pre-partum nutrition at different levels of energy in the diet, did not significantly influence the interval to first post-partum in cows [7].

As the first ovulation is inhibited by suckling stimulus [12], poor nutrition exacerbates suckling-induced anoestrous. In this experiment the effect of suckling could be reduced by restricting the calf's access to the dam. Nevertheless, the inhibitory effect of suckling appeared to be secondary to nutritional status [14]. Suckling may have not influenced the calving-ovulation interval in the present study.

A negative energy balance can determine the timing of resumption of ovulatory ovarian cycles [12]. In the present study, control animals lost more weight by day 15 post-partum than did supplemented animals. In fact, the latter maintained their pre-partum body weight. This loss of weight in the control animals would indicate a negative energy balance in the first 20 days post-partum and is expected to have had an impact on longer intervals to the resumption of ovarian activity [15]. In this trial, the negative balance could have delayed resumption of LH secretion pattern, resulting in a longer post-partum interval. Supplemented animals had sufficient body reserves to keep body weight until approximately 30 days post-partum when is too late to affect the frequency of LH secretion.

The mean conceptus weight was 58 kg at birth. This means that they gained 0.45 kg/day during the last trimester of gestation [6]. Weight of the conceptus at term can be reduced somewhat under severe nutrient restriction [6] although there is evidence that pre-partum diet using undegraded protein did not influence calf birth weight [11]. Also, restricted feeding of cows (energy level) during the last 100 days of gestation caused a reduction in calf birth weight. No differences in the newborn calf weight were observed between groups in the present study.

No significant differences in body weights nor body weight changes at 15, 30 and 45 days post-partum, were found between groups. These results agree with earlier reports from the same region using crossbreed cows grazing cultivated pastures [3]. The results failed to show significant differences between groups with respect to the interval from calving to conception, probably due to the number of animals per group and the high coefficient of variation (56 and 65% in control and supplemented animals, respectively).

As there were no significant differences in milk production and newborn calf body weight between groups, Brachiaria pastures appear to provide sufficient nutrients to avoid the drastic body weight loss reported in deficient native pastures and in cows with calves at foot [3].

It is concluded that body weight changes during the pre-partum period influenced body weight and body condition at calving. Body weight gain during the pre-partum period affected the first ovulation interval. Strategic supplementation in the pre-partum reduced the use of body reserves in the final foetal growth, so supplemented animals calved in better condition with the resultant reduction in the calving to first post-partum ovulation interval.

REFERENCES


REDUCTION OF CONCENTRATE FOR BOVINE SIRES: INFLUENCE ON METABOLIC STATUS AND SEMEN QUALITY UNDER PRODUCTION CONDITIONS


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Abstract - Resumen

REDUCTION OF CONCENTRATE FOR BOVINE SIRES: INFLUENCE ON METABOLIC STATUS AND SEMEN QUALITY UNDER PRODUCTION CONDITIONS.

The effects of reduced concentrate fed in rations of Holstein Friesian bulls for artificial insemination was evaluated with respect to metabolic status, sexual behaviour, semen production and semen quality during one year. In the first of two studies, twenty bulls were fed diets based on hay, green forage and concentrate according to the standard nutrient requirements for dairy cattle in artificial insemination centres. Bulls were divided into two groups: Group 1 (n = 10, control, 5 kg concentrate) and Group 2 (n = 10, experimental, 1 kg concentrate). Feed, blood and semen samples were taken for bromatological analysis, metabolic profile and semen evaluation, respectively. Group 2 had lower plasma concentrations of urea (P <0.001), calcium (P <0.05) and phosphorous (P <0.01). Urea values were below the reference range. Season of the year affected lipid metabolite concentrations (P <0.001) and osteotrophic minerals (P <0.05 to P < 0.001). Group 2 had better production and quality of semen than did Group 1. In the second study, five bulls were fed as the experimental group in the first study. Diurnal hormone secretion values (testosterone, LH and cortisol) in the dry and rainy seasons were determined. Also, hormonal levels were correlated with sexual behaviour. Blood samples were taken at different periods of the mounting: in the stall before mating, when the bull saw the teaser bull in the mounting room, during the maximum excitation period, and at 10 and 30 minutes post-mounting. Erection and ejaculation reflexes were recorded, and production and semen quality indicators were determined. All animals were slaughtered at the end of the experiment and histopathological studies were performed. Time of sampling, season of the year and sire affected the hormonal secretion pattern (P <0.001). Average time for erection and ejaculation reflexes were 1.6 ± 0.5 and 2.9 ± 0.6 minutes, respectively. There were no differences in testosterone and LH plasma concentrations before and after mounting; however, cortisol concentrations showed a significant raise during the period of maximum excitation. Individual secretion patterns varied between bulls and were related to pathological morphology of reproductive and endocrine organs. The effect of sire was significant on all the indicators of the sperm production, except to percentage of live sperm. Season of the year significantly affected sperm concentration and number of doses of extended sperm produced.

It is concluded that a reduction of concentrate in the diet did not affect the metabolic status, sexual behaviour, semen production or sperm quality of sires.

REDUCCION DEL CONCENTRADO EN LA RACION DE TOROS REPRODUCTORES: INFLUENCIA EN EL ESTADO METABOLICO Y CALIDAD SEMINAL BAJO CONDICIONES DE PRODUCCION.

Se evaluó la reducción del concentrado en las raciones de toros reproductores Holstein Friesian usados en inseminación artificial sobre el estado metabólico, la conducta sexual, la producción de semen y la calidad seminal durante un año. En el primer estudio se utilizaron 20 toros de un centro de inseminación artificial alimentados con una dieta a base de heno, forraje verde y concentrado de acuerdo a requerimientos nutricionales estándares. Los toros fueron divididos en dos grupos: Grupo 1 (n = 10, control, 5 kg de concentrado) y Grupo 2 (n = 10, experimental, 1 kg de concentrado). Se tomaron muestras de alimento, sangre y semen para análisis bromatológico, perfiles metabólicos y evaluaciones espermáticas, respectivamente. El Grupo 2 tuvo concentraciones plasmáticas más bajas de urea (P <0.001), calcio (P <0.05) y fósforo (P <0.001). Los valores de urea estuvieron por debajo de los rangos referenciales. La época del año afectó la concentración de los metabolitos lípidos (P <0.001) y de los minerales osteotroficos (P <0.05 a P < 0.001). El Grupo 2 tuvo una mayor producción de semen y de mejor calidad que el Grupo 1.

En el segundo estudio se utilizaron cinco toros que se alimentaron al igual que el Grupo 2 del primer estudio. Se evaluó la secreción hormonal diurna (testosterona, LH y cortisol) durante la época lluviosa y seca. Asimismo, se correlacionaron los valores hormonales con la conducta sexual. Muestras de sangre se recolectaron en diferentes momentos de la monta: En el toril, en el momento de presentarse al toro receptor, al entrar a la sala de monta, en el momento de máxima excitación y a los 10 y 30 minutos post-monta. Se registraron los reflejos de erección y de ejaculación y se determinó la producción y calidad del semen.
Todos los animales fueron sacrificados al final del experimento para estudios histopatológicos. El momento del muestreo, la época del año y el toro afectaron significativamente el perfil secretorio de las hormonas en estudio (P < 0.001). El tiempo promedio de erección y eyaculación fueron de 1.6 ± 0.5 y 2.9 ± 0.6 minutos, respectivamente. No se encontraron diferencias en concentraciones plasmáticas de LH y testosterona antes y después de la monta; sin embargo, las concentraciones de cortisol tuvieron un incremento marcado en el periodo de máxima excitación. Los perfiles secretorios fueron diferentes entre toros y estuvieron correlacionados con estructuras patológicas en órganos reproductivos y endocrinos. El efecto del toro fue significativo en todos los indicadores de producción de semen, con excepción del porcentaje de espermatozoides vivos. El efecto de la época del año fue importante en la concentración de espermatozoides y en el número de dosis producidas.

Se concluye que la reducción del concentrado en la dieta no afectó el estado metabólico, la conducta sexual, la producción de dosis de semen, ni la calidad espermática de los toros reproductores.

1. INTRODUCTION

The causes of removal of 1377 bulls from artificial insemination centres in Cuba indicated that 36% were due to reproductive problems and within these, 22.4% of the bulls had spermatogenic alterations [1]. Excess fat deposition in testicles, degenerative processes and reduced testicular development, often associated with feeding problems were the main pathological findings in sub-fertile bulls in Cuba [2]. Attempts to alleviate the problem were initiated. Among them, protein levels of concentrate were decreased from 17 to 13% with satisfactory results on metabolism [3] and on sperm production [4]. Moreover, suitable protein sources [5] and frequency of feeding [6] were identified as potential sources of spermatogenic disturbances.

The aim of this study was to evaluate the effect of reduction of concentrates in feed of sires on the metabolic status, sexual behaviour and semen quality of the animal.

2. MATERIALS AND METHODS

2.1. Study 1

Twenty 3.5 year-old Holstein Friesian bulls from artificial insemination (AI) centres were evaluated over one year. Animals were fed diets based on hay, green forage and concentrate (13.4% crude protein) according to standard nutrient requirements for dairy cattle in AI Centres. The animals were divided into two groups: Group 1 (n = 10, control, standard diet) and Group 2 (n = 10, experimental, diet containing reduced concentrate). Concentrate composition, diet characteristics and amount of offered feed are shown in Table I.

Feed and blood samples were taken every month for bromatological analysis and metabolic profiles, respectively [7, 8]. Body weight was taken monthly to calculate the quantity of feed offered.

Semen samples were collected twice a week for volume, concentration and sperm abnormality determinations [8]. Statistical analysis of the results was performed by General Linear Model Test (GLM) [9] with treatment and season of year as factors.

2.2. Study 2

Five 3 year-old Holstein Friesian bulls were used. They were fed with the same diet as the experimental group in Study 1 for one year.

In order to determine diurnal LH, testosterone and cortisol secretion patterns, blood samples were taken from the jugular vein at 30 minute intervals from 9:00 am to 4:00 pm. The sampling was repeated three times in the dry season and three times in the rainy season.

To correlate hormone levels with sexual behaviour, blood samples were taken at different periods during mating: in the stall before mating, when the bull saw the teaser bull in the mounting room, during the maximum excitation period, and at 10 and 30 minutes post-mounting. The trial was performed twice in the rainy season. The interval between visualization of the teaser bull and penis erection, that included mounting preparation and two false mountings (erection reflex), and the interval between penis erection and complete ejaculation (ejaculation reflex), were recorded.

LH and cortisol were determined following standard procedures in Cuba [10, 11]. Testosterone was determined using the FAO/IAEA solid-phase RIA kit.
TABLE I. FEED COMPOSITION OF RATIONS FED TO HOLSTEIN FRIESIAN BULLS IN
AI STUDS TO DETERMINE THE EFFECTS OF FEED ON METABOLIC PARAMETERS
AND REPRODUCTIVE PERFORMANCE. Rations were fed over a period of one year in two
separate trials.

<table>
<thead>
<tr>
<th>Composition of concentrate feed</th>
<th>Nutritive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>Dry matter (%)</td>
</tr>
<tr>
<td></td>
<td>85</td>
</tr>
<tr>
<td>Barley</td>
<td>Crude protein (%)</td>
</tr>
<tr>
<td></td>
<td>13.40</td>
</tr>
<tr>
<td>Oats</td>
<td>Crude fiber (%)</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
</tr>
<tr>
<td>Fish meal</td>
<td>ME (Mj)</td>
</tr>
<tr>
<td></td>
<td>10.50</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>Ca (%)</td>
</tr>
<tr>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>P (%)</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td></td>
</tr>
<tr>
<td>Vitamin/Mineral mixture</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amount of offered feed per day (kg)

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Concentrate (kg)</th>
<th>Green forage (kg)</th>
<th>Hay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>5.0</td>
<td>1.0</td>
<td>30.0</td>
</tr>
<tr>
<td>900</td>
<td>5.0</td>
<td>1.0</td>
<td>30.0</td>
</tr>
<tr>
<td>1000</td>
<td>5.3</td>
<td>1.0</td>
<td>32.0</td>
</tr>
<tr>
<td>1100</td>
<td>6.0</td>
<td>1.0</td>
<td>35.0</td>
</tr>
</tbody>
</table>

Semen samples were obtained at the same intervals as in Study 1 and similar semen indicators were determined. All animals were slaughtered at the end of the experiment. Macroscopic and microscopic evaluation of internal organs, especially the reproductive organs were performed [8].

Hormonal results were statistically analyzed by the General Linear Model [9] taking into account sampling time, season of the year, and bull (with or without histological alterations) effects. The area below curve was calculated for LH and testosterone in two seasons of the year, as well as before and after mounting. Means were compared by Student's "t" test.

3. RESULTS AND DISCUSSION

3.1. Study 1

There was a treatment effect on plasma concentrations of urea, calcium and phosphorus (Table II). Urea values were lower in the treated group, below the previously established reference range [3]. This result could be due to the smaller concentrate intake, as it is known that there is a close relationship between protein intake and plasma urea values [12, 13]. In a similar manner, the highest values for Ca and P, found in the control group, could be due to the higher intake, particularly calcium carbonate and dicalcium phosphate from concentrate feed.

Season of the year affected plasma concentrations of lipid metabolites (P <0.001). Higher total lipids and cholesterol concentrations were found in the dry season (2.7 ± 0.4 vs 1.8 ± 0.4 g/L; and 2.5 ± 0.6 vs 2.1 ± 0.4 nmol/L, respectively), corroborating the results obtained by other local researchers in dairy cows [14] and in bulls [3]. Higher Ca (P <0.001), P and Mg (P <0.05) concentrations were also found in the dry season, although they were not physiologically important. In general, values for the haematological indicators (except urea) in both groups were within the reference values previously established for Holstein sires [3, 15]. This suggests that a reduction of the concentrate in the diet does not affect metabolic status of sires.
TABLE II. HAEMATOLOGICAL PARAMETERS IN HOLSTEIN FRIESIAN SIRES FED CONTROL DIET OR A DIET CONTAINING REDUCED CONCENTRATE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Experimental group</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>2.81</td>
<td>2.93</td>
<td>ns</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.50</td>
<td>2.33</td>
<td>ns</td>
</tr>
<tr>
<td>Total lipids (g/L)</td>
<td>2.15</td>
<td>2.07</td>
<td>ns</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>85.10</td>
<td>82.30</td>
<td>ns</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>33.60</td>
<td>34.30</td>
<td>ns</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>51.50</td>
<td>48.00</td>
<td>ns</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>4.69</td>
<td>2.59</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>137.10</td>
<td>137.20</td>
<td>ns</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.22</td>
<td>4.20</td>
<td>ns</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.59</td>
<td>2.30</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>2.28</td>
<td>2.01</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>0.93</td>
<td>0.92</td>
<td>ns</td>
</tr>
<tr>
<td>Cu (µmol/L)</td>
<td>15.72</td>
<td>16.03</td>
<td>ns</td>
</tr>
<tr>
<td>Zn (µmol/L)</td>
<td>17.07</td>
<td>15.98</td>
<td>ns</td>
</tr>
</tbody>
</table>

The production and quality of semen was significantly better in the experimental group (Table III). These results agree with previous experiments in which protein metabolites, from protein excesses or from animal origin proteins, were toxic to sperm production. In those reports, neither metabolic alteration was found when the level of crude protein in the concentrate was reduced from 17 to 12% [12] nor was semen biochemistry altered [16]. Therefore, semen production was higher [4]. Alvarez et al., 1992 [8] found that proteins of plant origin were less toxic than those of animal origin. The latter affected post freezing behaviour of sperm cells. The same author [17] found an inverse relationship between amount of protein in feed and semen freezing capability.

TABLE III. SEMEN CHARACTERISTICS OF HOLSTEIN FRIESIAN BULLS FED CONTROL DIET OR A DIET CONTAINING REDUCED CONCENTRATE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Experimental group</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° of ejaculates</td>
<td>1,186</td>
<td>1,094</td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>5.97</td>
<td>6.21</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Density (%)</td>
<td>84.10</td>
<td>88.40</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>85.80</td>
<td>90.11</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Concentration (x10⁶)</td>
<td>1,426.31</td>
<td>1,993.26</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Total abnormalities</td>
<td>13.75</td>
<td>8.65</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Primary abnormalities</td>
<td>9.21</td>
<td>2.34</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Suitable ejaculate (%)</td>
<td>81.80</td>
<td>92.10</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Suitable ejaculate (%)</td>
<td>72.29</td>
<td>87.45</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>
3.2. Study 2

3.2.1. Andrological findings

Variation in reproductive function was observed in the five bulls fed reduced concentrate. Bull 4 had a subnormal development of gonads as well as alterations in testicular consistency, and bull 3 had fibrosis of the epididymal tail. Development and clinical status of the reproductive organs of the other animals were satisfactory and agree with normal parameters reported elsewhere for this breed and age [18].

3.2.2. Pattern of plasma hormones

Sire, time of sampling and season of the year affected the occurrence of the studied hormones (P <0.001). Extended profiles of LH, testosterone and cortisol showed that testosterone

![Graph showing mean diurnal variations in plasma concentrations of testosterone, LH and cortisol in 5 Holstein Friesian bulls during the rainy and the dry seasons. Significant mean diurnal variations (P < 0.05) occurred during the rainy season and are expressed by different superscripts.]
and cortisol were affected by sampling hour (Fig. 1). Variations in testosterone levels were not found in relation to season of the year \((Jd(T) = 14.22 \text{ vs } 9.72 \text{ in rainy and dry seasons, respectively})\). Testosterone values were similar to those found elsewhere [19, 20, 21]. The testosterone profile also corroborates the circadian behaviour of this hormone which declines around 4 p.m. in both seasons of the year [22, 23]. Plasma testosterone concentrations found in this study were similar to other values reported in the literature [24] (1978) and did not differ significantly between seasons.

LH did not show an effect of sampling hour but showed a season of the year effect \((P <0.05\), Fig. 1\). The areas below the curves for LH were 35.50 vs 24.76 for the rainy and dry seasons, respectively. These values were higher than those previously reported [24].

Cortisol levels showed significant fluctuations with higher peaks on the mounting hours and at 4 p.m. Season of the year was statistically important \((P <0.001, Jd(F) = 4.96 \text{ vs } 2.30 \text{ for the rainy and dry seasons, respectively; Fig. 1})\). These results agree with those previously reported in Cuba for heifers [25] and for sires [22]. In heifers, it was found that the initial raise at the beginning of the rainy season, was followed by a decrease in the plasma level that could have been due to an adrenal failure caused by a high relative humidity and temperature stress.

The sire effect was significant for all three hormones \((P <0.001)\). The hormone concentration in bulls with pathological lesions was different from those which were classified as normal. Testosterone concentration in animals 2 and 4 were lower and close to the detection limit of the assay. Animal 2 also presented the lowest LH concentrations (Fig. 2). These reduced hormone concentrations may have been due to a pituitary dysfunction. The pituitary alteration may have resulted from an acute vascular dysfunction or by Rathke's bursa abnormality, although its etiology and consequences are not clearly understood [26]. The hormonal aberration in bull 4 may be explained by bilateral testicular fibrosis with alterations in Leydig cells that reduced the capacity of the gonads to produce testosterone in the presence of normal levels of LH.

**FIG. 2.** Mean diurnal variations in plasma concentrations of testosterone (T), LH and cortisol in two Holstein Friesian bulls with pathological gonadal morphology and reproductive dysfunctions (Comparative values are presented for rainy and dry seasons).
All the animals displayed mounting reflexes. Average time for erection and ejaculation reflexes were $1.6 \pm 0.64$ and $2.9 \pm 0.6$ minutes, respectively. Longer reaction times corresponded to bulls presenting higher sexual excitation once they visualized the teaser bull and started the training for mounting (bulls 1, 3, 5, 4, and 2 in decreasing order). The intervals were shorter than previously reported. Some authors recommend 10 minutes, including false mountings, for sexual adjustment in order to achieve better expression of sexual reflexes [27] and sperm quality. Nevertheless, the results agree with those reported for Holstein bulls under similar conditions in Cuba, when animals received 40% of the total ration in the form of concentrate feed [28].

Neither the total area under the curve nor episodic testosterone and LH plasma concentration levels were statistically different before and after mounting. Cortisol concentration levels showed a significant rise ($P < 0.01$) during the maximum excitation and values returned to normal 30 minutes after mounting. A similar pattern for both testosterone and cortisol has been reported under similar experimental conditions [29].

The effect of sire was significant in all of the semen characteristics studied. Season of the year significantly affected sperm concentration and number of doses that could be produced (Table IV). In all cases, motility and sperm concentration were greater than requirements specified for freezing semen in Cuban AI Centres.

It is concluded that a reduction in concentrate quantities from the diet did not affect metabolic status but improved production and quality of sperm. The experimental diet fulfilled the research objectives and guaranteed better semen production and considerable savings in concentrate feeds.

### TABLE IV. VARIATION IN SEMEN CHARACTERISTICS IN FIVE 3-YEAR OLD HOLSTEIN FRIESIAN BULLS FED CONTROL DIET OR A DIET CONTAINING REDUCED CONCENTRATE

<table>
<thead>
<tr>
<th>Bull</th>
<th>Volume (ml)</th>
<th>Motility (%)</th>
<th>Density (%)</th>
<th>Live sperms (%)</th>
<th>Concentration ($\times 10^6$) (mean)</th>
<th>Doses/ ejaculate (%)</th>
<th>Suitable ejaculate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.0 ± 2.8</td>
<td>97 ± 2.9</td>
<td>95 ± 1.3</td>
<td>85 ± 0.1</td>
<td>2,493 ± 617</td>
<td>331 ± 74</td>
<td>98.3</td>
</tr>
<tr>
<td>2</td>
<td>5.8 ± 1.5</td>
<td>80 ± 10.0</td>
<td>80 ± 16.0</td>
<td>56 ± 0.1</td>
<td>1,296 ± 450</td>
<td>143 ± 77</td>
<td>73.0</td>
</tr>
<tr>
<td>3</td>
<td>4.4 ± 1.3</td>
<td>93 ± 2.9</td>
<td>92 ± 2.9</td>
<td>80 ± 4.9</td>
<td>2,497 ± 434</td>
<td>215 ± 109</td>
<td>95.0</td>
</tr>
<tr>
<td>4</td>
<td>5.0 ± 1.0</td>
<td>82 ± 5.8</td>
<td>82 ± 9.4</td>
<td>68 ± 6.3</td>
<td>1,293 ± 432</td>
<td>124 ± 21</td>
<td>66.0</td>
</tr>
<tr>
<td>5</td>
<td>6.0 ± 1.3</td>
<td>95 ± 2.3</td>
<td>92 ± 3.2</td>
<td>87 ± 0.8</td>
<td>2,379 ± 520</td>
<td>321 ± 89</td>
<td>97.3</td>
</tr>
</tbody>
</table>

Nº of ejaculates were 712, 704, 708, 711 and 701 for bull 1, 2, 3, 4 and 5, respectively.

### REFERENCES


REPRODUCTIVE PERFORMANCE AND METABOLIC STATUS OF CROSSBRED HEIFERS FED SUGARCANE BY-PRODUCTS IN CONFINEMENT

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Centro de Investigaciones para el Mejoramiento Animal, Havana, Cuba

Abstract – Resumen

Two experiments were conducted in a herd of 1276 crossbred heifers (% Holstein x % Zebu) fed sugarcane by-products in confinement for a period of 120 days. In Experiment I, the reproductive performance was evaluated by clinical and endocrinological procedures. In Experiment II, the metabolic status was evaluated through biochemical indicators in blood and urine, and levels of minerals in liver and bone. During the confinement period, 83.8% of the heifers were inseminated and 62.5% of the total number of heifers became pregnant. The principal reasons for infertility were anoestrus and irregular ovarian activity. The metabolic status indicated low levels of haematocrit, haemoglobin and serum total protein and β-carotene. The hepatic levels of Cu and Fe were low. Values of inorganic P in bone were also reduced. The urine pH and the renal output of acid suggested metabolic acidosis. Supplementation with Cu, Fe and inorganic P and the use of β-carotene associated with the addition of buffers should be evaluated to improve the reproductive performance under this system of management.

COMPORTAMIENTO REPRODUCTIVO Y ESTADO METABOLICO DE VAQUILLAS CRUZADAS, Y MANTENIDAS EN CONFINAMIENTO Y ALIMENTADAS CON SUBPRODUCTOS DE LA CANA DE AZUCAR.

Se llevaron a cabo dos experimentos en un rebaflo de 1276 vaquillas cruzadas ¾ Holstein × ¼ Cebu alimentadas con subproductos de la caña de azúcar, que se mantuvieron en confinamiento por un periodo de 120 días. En el Experimento I se evaluó el comportamiento reproductivo por métodos clínicos y análisis hormonales. En el Experimento II se evaluó el estado metabólico a través de indicadores bioquímicos en sangre y orina, además de niveles de minerales en hígado y hueso. Durante el periodo de confinamiento, 83.8% de las vaquillas fueron inseminadas y 62.5% del total quedaron preñadas. Las principales causas de infertilidad fueron el anestro y la actividad ovárica irregular. El estado metabólico indicó niveles bajos de hematocrito, hemoglobina, así como de β-caroteno y proteína total en suero. Los niveles hepáticos de Cu y Fe fueron bajos. Los valores de P inorgánico estuvieron igualmente reducidos. El pH de la orina y la eliminación de ácidos en la orina sugirieron la presencia de acidosis metabólica. Se debería evaluar el efecto de la suplementación con Cu, Fe y P inorgánico, y el uso de β-caroteno asociado a la adición de sustancias tampón, en el mejoramiento del comportamiento reproductivo de los animales mantenidos bajo este sistema.

1. INTRODUCTION

The major factor that limits cattle productivity in tropic climates is underfeeding, which results in acute and chronic malnutrition [1, 2, 3]. Underfeeding in general produces metabolic imbalance [4, 5]. Some authors have reported a relationship between metabolic disturbance and reproductive disorders [2, 6, 7].

During the dry season in Cuba (November to May), a large number of cattle are confined in corrals and fed sugarcane by-products. However, there is little information available on their metabolic status related to reproductive performance. The aim of this study was to evaluate the reproductive performance and to characterize the metabolic status of cattle confined to corrals.

2. MATERIALS AND METHODS

2.1. Animals

Two experiments were carried out over a period of 18 months, using a herd of 1276 crossbred heifers (¾ Holstein × ¼ Zebu) of 24 to 30 months of age and from 320 to 350 kg liveweight. The performance of the animals was studied during the grazing period (October) and over the confined period (November through May).
TABLE I. CHEMICAL COMPOSITION OF FEEDS SUPPLIED TO CROSSBRED HEIFERS DURING A PERIOD OF CONFINEMENT IN CORRALS

<table>
<thead>
<tr>
<th>Composition</th>
<th>Bagasse/molasses/urea</th>
<th>Cachasse</th>
<th>Sugar cane straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>57.0</td>
<td>69.7</td>
<td>74.0</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>8.0</td>
<td>9.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>29.0</td>
<td>2.5</td>
<td>44.6</td>
</tr>
<tr>
<td>EM (Mcal)</td>
<td>1.8</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.1</td>
<td>0.4</td>
<td>0.82</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.6</td>
<td>0.6</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Heifers were fed with 15 kg sugarcane straw, 3 kg cachasse and 3 kg bagasse/molasses/urea per animal during the confined period (Table I). During the grazing period, the nutritional base was grass, principally *Bermuda cruzada*. Animals were supplemented with 1 kg/animal/day of concentrate (13% total protein) and 80 g/animal/day of mineral salts in both periods. Animals had also free access to water throughout the experiment.

2.2. Experiment I. Evaluation of reproductive performance

All animals were subjected to clinical reproductive examination before being confined to corrals. However, a group of 46 heifers was selected to evaluate ovarian activity over 72 days by means of rectal palpation and progesterone levels in blood serum. The clinical examination was conducted, and blood samples were collected every 10 days. This group of animals were not inseminated. A regular cycle was defined to have occurred if progesterone values were >3 nmol/L and if a corpus luteum was palpated 10 days after progesterone values were <3 nmol/L. Anoestrous was defined to have occurred if progesterone values were <3 nmol/L and no palpable corpus luteum was found in the ovaries during the 72 days of the experimental period. Irregular cycles were defined to have occurred if progesterone values were >3 nmol/L and a palpable corpus luteum was found in the ovaries, and both indicators lasted for three ten-day periods (long luteal phase); or if progesterone values were <3 nmol/L and no palpable corpus luteum was found in the ovaries during two ten-day periods (long follicular phase).

The detection of oestrus was carried out from 07:00 to 11:00 am and from 15:00 to 18:00 by a farmer with the aid of a vasectomized teaser bull. The bull and cow ratio was 1:30. Artificial insemination was carried out by experienced technicians 14 and 24 h after the detection of oestrus. Pregnancy diagnosis was performed by rectal palpation after 70 to 90 days of the non-return service. Pregnancy rate was defined as the ratio of animal pregnant to the number of animals intended to be bred. Progesterone concentration was determined in serum using the FAO/IAEA solid-phase progesterone RIA kit [8]. Intra- and inter-assay coefficients of variance were 6.0 and 12.3%, respectively.

The frequency of heifers showing oestrus, been inseminated and becoming pregnant during the experiment was evaluated. Also, the frequency of repeat-breeders, heifers with more than three unsuccessful services, in which no clinical abnormalities in reproductive organs could be detected) during the period of confinement was determined.

2.3. Experiment II. Metabolic status

The evaluation of metabolic status was undertaken in 360 heifers during three periods: pre-confinement (7 days before confinement in corrals), and at 60 and 120 days after initiation of confinement to corrals. Body weight was estimated every 15 days by chest girth measurements.

Jugular blood samples were collected for determination of glucose, total protein, albumin, globulin, haematocrit (PCV), haemoglobin, and inorganic P, Na, K, Cu, Zn and β-carotene in serum. Urine samples were collected from the same animals for determination of pH and the presence of ketone
bodies. Samples of bone and liver were taken from 35 slaughtered heifers. Ten were slaughtered before confinement and 20 during confinement for determination of Cu, Zn, Fe and Mn in liver and Ca, P and Mg in bone. Samples were collected, stored and analyzed according to methods reported elsewhere [9].

2.4. Statistical analyses

Experimental responses were analyzed by least squares analysis of variance using the General Linear Model procedures of the Statistical Analysis System and by the comparative proportion test [10].

3. RESULTS AND DISCUSSION

3.1. Experiment I

A proportion consisting of 1070 of the 1276 heifers (83.8%) were detected in oestrus and 68.2% (730 heifers) were pregnant following the first service. One of the most interesting findings was the progressive and significant reduction of heifers in oestrus after 48 days of confinement (Figure 1, P <0.05). This indicates that this system of management may reduce the expression of oestrus behaviour or the efficiency of oestrous detection, as it was previously observed [11].

The overall pregnancy rate was 74.5% and the pregnancy rate during the confinement period was 62.5%. The lowest pregnancy rate occurred during the first 24 days of confinement (Figure 1, P <0.05). The frequency of repeat-breeders was high (21.3%, 102/478). In addition, 16.1% of the heifers did not show oestrus during the confinement period. The reproductive performance of the experimental animals was similar to that observed under grazing systems by other researchers in Cuba [12, 13].

![Fig. 1. Distribution of artificial insemination services and pregnancies of crossbred heifers (¼ Holstein x ¾ Zebu) during 120 days of confinement in corrals and fed sugar cane by-products.](image-url)
In the group in which ovarian activity was studied, 37% of heifers did not show ovarian activity over the 72 days of observation, while 48% had irregular oestrous cycles either long luteal phase (>24 days) or long follicular phase (>14 days). Animals in this group had periods of regular oestrous cycles but without manifestation of oestrous behaviour.

These results suggest that anoestrus and silent heat (or failures in oestrous detection) were the two major factors affecting oestrous presentation. It may be a useful management strategy to seek alternatives for adequate oestrous induction and oestrous synchronization in confined animals, as suggested by other researchers [1, 14].

3.2. Experiment II

Tables II, III and IV show the results of biochemical analysis of blood serum, liver, bone and urine samples. The principal differences associated with confinement were lower values for PCV, haemoglobin, total protein and β-carotene.

The hepatic results were more variable. The liver reserves of Cu and Fe were at a critical level. 60% of the samples had deficient quantity of copper (<60 ppm) as reported previously [13]. During confinement, the levels of Cu and Zn in liver decrease, while Fe and Mn are increased (Figure 2, P <0.05). These findings confirm the results of other studies in an identical management system [11, 15, 16]. Cu deficiency was more frequent when heifers were fed sugarcane by-products and Fe deficiencies were more often found in grazing heifers.

The content of inorganic P in bone was low, while levels of Ca and Mn were acceptable. The concentrations of the macro-elements did not change during confinement. It indicates that the P deficiencies may occur in both nutritional management systems. Results obtained in urine indicate acid-base disturbances as previously reported [17, 18, 19, 20]. The number of animals with acid-base disturbances increased over the period of confinement (Table IV). These results agree with others reports that have used similar nutritional management [15, 16].

The body weight gain was 358 g/animal/day. This value is judged to be low for expected body development [3]. The application of methods to prevent metabolic disturbance may improve the reproductive development of heifers confined in corrals and fed sugarcane by-products.

**TABLE II. SERUM LEVELS OF BIOCHEMICAL INDICATORS OF METABOLIC STATUS OF CROSSBRED HEIFERS (¾ HOLSTEIN × ¼ ZEBU) CONFINED IN CORRALS AND FED SUGAR CANE BY-PRODUCTS (n = 120, x ± s.e)**

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Pre-confinement</th>
<th>Confinement period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day -7</td>
<td>day 60</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>30.7 ± 0.82a</td>
<td>26.7 ± 0.07b</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>9.2 ± 0.06</td>
<td>9.0 ± 0.79</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.8 ± 0.01a</td>
<td>3.8 ± 0.01a</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>74.0 ± 0.01a</td>
<td>69.0 ± 0.28a</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>34.0 ± 0.09</td>
<td>33.0 ± 0.04</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>40.0 ± 0.07a</td>
<td>35.0 ± 0.03a</td>
</tr>
<tr>
<td>β-carotene (ug/dl)</td>
<td>950.0 ± 0.8a</td>
<td>377.0 ± 0.7b</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>141.0 ± 0.01</td>
<td>140.0 ± 0.03</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.4 ± 0.05</td>
<td>4.5 ± 0.04</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>1.9 ± 0.01a</td>
<td>2.0 ± 0.04b</td>
</tr>
<tr>
<td>Cu (µmol/L)</td>
<td>10.5 ± 0.03a</td>
<td>9.6 ± 0.04b</td>
</tr>
<tr>
<td>Zn (µmol/L)</td>
<td>15.2 ± 0.03a</td>
<td>16.2 ± 0.04b</td>
</tr>
</tbody>
</table>

a, b, c Different superscripts within a row indicate significant differences (P <0.05)
Fig. 2. Mineral content in liver of crossbred heifers (¾ Holstein × ¼ Zebu) before and during a confinement period while fed sugar cane by-products.

TABLE III. MINERAL CONTENT (x ± s.d., value in dry weight) IN LIVER AND BONE OF CROSSBRED HEIFERS (¾ HOLSTEIN × ¼ ZEBU) CONFINED IN CORRALS AND FED SUGAR CANE BY-PRODUCTS

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Cu</th>
<th>Zn</th>
<th>Fe</th>
<th>Mn</th>
<th>P</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (ppm)</td>
<td>73.3±40.9</td>
<td>268±56.5</td>
<td>44±14.4</td>
<td>10.1±1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone (g/100)</td>
<td>6.4±0.5</td>
<td>39.3±2.5</td>
<td>0.53±0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE IV. pH AND EXCRETION OF ACID-BASE (EBN, nmol/L) IN URINE OF 120 CROSSBRED HEIFERS (¾ HOLSTEIN × ¼ ZEBU) CONFINED IN CORRALS AND FED SUGAR CANE BY-PRODUCTS

<table>
<thead>
<tr>
<th>Groups</th>
<th>Indexes</th>
<th>Pre-confinement day 7</th>
<th>Confinement day 60</th>
<th>Confinement day 120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>pH</td>
<td>8.3</td>
<td>8.4</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>EBN</td>
<td>131.3</td>
<td>124.0</td>
<td>145.0</td>
</tr>
<tr>
<td>AC</td>
<td>pH</td>
<td>7.7</td>
<td>7.3</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>EBN</td>
<td>56.0</td>
<td>33.3</td>
<td>48.8</td>
</tr>
<tr>
<td>AD</td>
<td>pH</td>
<td>6.1</td>
<td>6.1</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>EBN</td>
<td>-49.0</td>
<td>-33.0</td>
<td>-39.0</td>
</tr>
<tr>
<td>Affected (%)</td>
<td>30.0^a</td>
<td>70.0^b</td>
<td>85.0^b</td>
<td></td>
</tr>
</tbody>
</table>

a, b Different superscripts within a row indicate significant differences (P <0.05)
AC Uncompensated acidosis
AD Compensated acidosis
4. CONCLUSIONS

Crossbred heifers confined in corrals and fed sugarcane by-products had lower frequency of oestrus after 48 days of confinement. The principal reproductive problems were anoestrus and silent heat (or failed oestrous detection). The use of adequate methods of oestrus induction and synchronization during the confinement period could improve the reproductive management. Some metabolic disturbances were associated with reduced body weight gain. The supplementation with Cu, Fe, inorganic P, and the use of β-carotene associated with the addition of buffer substances may improve the reproductive performance of heifers under this system of management.

REFERENCES


PRE- AND POST-PARTUM FEED SUPPLEMENTATION TO IMPROVE SHEEP PRODUCTIVITY IN SMALL-HOLDER FARMS IN SOUTHERN CHILE

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Abstract – Resumen

PRE- AND POST-PARTUM FEED SUPPLEMENTATION TO IMPROVE SHEEP PRODUCTIVITY IN SMALL-HOLDER FARMS IN SOUTHERN CHILE.

Two experiments were carried out to assess the effect of feed supplementation in ewes during the pre- and post-partum period and subsequent ewe-lamb growth rates and reproductive performance during their first breeding season. In the first experiment, 50 Romney Marsh ewes on one farm were used and half were supplemented for 50 days before and after lambing with 500 g dry matter of grassland silage/day, and half were not supplemented. In the second experiment, six small flocks of Criollo ewes belonging to Mapuche farmers were used. Three flocks (total 30 ewes) were supplemented with a commercial multinutrient block (molasses, urea and mineral salts) for 50 days before and after lambing. The other 3 flocks (total 45 ewes) were maintained without supplementation. In both experiments, ewe live weight, body condition score (BCS), lamb birth weight and the subsequent ewe-lamb growth rate and reproductive performance were evaluated.

The birth weight of ewe-lambs born to the supplemented dams in Experiment I was not statistically different (P >0.05) from the control group (3.9 kg vs. 4.2 kg). There was, however, a significant improvement (P <0.05) in weaning weight (31.4 vs. 27.6 kg), BCS, age at onset of ewe-lamb luteal activity assessed by progesterone measurements (171.7 vs. 194.9 days); and percentage of ewe-lambs showing oestrus activity at the end of the first breeding season (50 vs. 30%).

There were no significant differences between groups for lamb birth weight in Experiment II (4.5 vs. 4.3 kg; P >0.05). However, differences (P <0.05) were found between ewe-lambs born to the supplemented dams and non-supplemented dams on weaning weight (21.8 vs. 17.2 kg), BCS, the age at onset of luteal activity (269 vs. 290 days) and pregnancy rate at the end of the first breeding season (55.6 vs. 30%).

The results indicate that pre- and post-partum supplementation of ewes can improve ewe-lamb performance within their first reproductive season.

SUPLEMENTACION PRE- Y POST-PARTO PARA LA MEJORA DE LA PRODUCITIVIDAD DE LA OVEJA EN PEQUEÑAS GANADERIAS DEL SUR DE CHILE.

Se llevaron a cabo dos experimentos para evaluar la suplementación alimenticia en ovejas durante el periodo pre- y post-parto de la madre y la tasa de crecimiento y el comportamiento reproductivo del cordero hembra durante la primera estación de monta. En el primer experimento se utilizaron 50 ovejas Romney Marsh de una finca privada. La mitad de los animales fue suplementado durante 50 días previos al parto y 50 posteriores al mismo con 500 g de materia seca de ensilado de pasto por día; y la otra mitad no fue suplementado. En el segundo experimento se utilizaron seis rebaños de ovejas Criollas pertenecientes a los Mapuches. Tres rebaños (total 30 ovejas) fueron suplementados con bloques comerciales multinutrientes (melaza, urea y sales minerales) durante 50 días previos al parto y 50 posteriores al mismo. Los otros tres rebaños (total 45 ovejas) quedaron sin suplementar. En ambos experimentos se evaluó el peso vivo y la condición corporal (BCS) de la oveja, el peso del cordero al nacimiento, su tasa de crecimiento y su comportamiento reproductivo.

En el Experimento I, el peso al nacimiento de los corderos de las ovejas suplementadas no fue diferente (P=0.05) del grupo no suplementado (3.9 vs. 4.2 kg). Sin embargo, se encontró una mejora significativa (P <0.05) en el peso del destete (31.4 vs. 27.6 kg), BCS, edad al inicio de la actividad luteal en las corderas detectado a través de mediciones semanales de progesterona (171.7 vs. 194.9 días); y en el porcentaje de corderos que mostraron actividad luteal al final de la primera estación de monta (50 vs. 30%).

No se encontraron diferencias significativas en el peso al nacimiento de los corderos por efecto del tratamiento en el Experimento II (4.5 vs. 4.3 kg; P=0.05). Sin embargo, se encontraron diferencias estadísticas (P <0.05) entre los grupos de corderos de madres suplementadas y no suplementadas con relación al peso al destete (21.8 vs. 17.2 kg), BCS, la edad al inicio de la actividad luteal en las corderas (269 vs. 290 días) y en la tasa de preñez al final de la primera estación de monta (55.6 vs. 30%).

Los resultados indican que la suplementación pre- y post-parto en ovejas puede mejorar el comportamiento productivo y reproductivo del cordero en su primera estación de monta.

1.  INTRODUCTION

Sheep production in Southern Chile (IX and X Regions) is socially and economically important for small-holder farmers, mainly of indigenous origin. There are approximately 148,000 Mapuches in the IX Region (64% of the rural population) who live in farms of an average size of 8.4 ha [1]. These
farmers use part of the area for cereal crops (wheat and oat) and therefore can only maintain small flocks of sheep (8.2 ewes per flock) [1, 2]. Sheep are mainly Criollo descendants of European breeds. Animals are highly seasonal, mating from February to April and lambing from June to September [2]. Sheep are maintained all year on low productive pastures [3] which vary seasonally in the Mediterranean climate of the region. As a result, there is a marked annual variation in ewe bodyweight and body condition score (BCS) [3]. This effect produces a critical situation during the winter months. This scarce pasture coincides with the greatest requirements for ewe nutrients (last trimester of gestation and early lactation) and is reflected in the lamb birth weights, liveweight gains and in the low number of ewe-lambs which reproduce in their first year of life [2].

Reproduction in ewe-lambs allows for an increase in total reproductive life, a reduction in generation intervals and a greater availability of ewes for selection and sale, culminating in a higher income for the farmers [4].

An increase in feed supply at the end of pregnancy and in early lactation should secure adequate birthweight and may also allow the ewe to produce colostrum and milk to levels needed to achieve low perinatal lamb mortality and high lamb growth rates [5, 6]. Ewe-lambs with higher growth rates have their first oestrus and lamb at an earlier age than do ewe-lambs with low growth rates [7, 8, 9, 10].

The objective of the present experiments was to evaluate the effect of pre- and post-partum feed supplementation of ewes and subsequent effect on growth and reproductive activity of their offspring in their first year of life.

2. MATERIALS AND METHODS

Two experiments were carried out, the first in controlled conditions using purebred Romney Marsh ewes, which are well-adapted to the region. The second was a field experiment using native sheep owned by indigenous Mapuches. The supplementation source was silage from pasture, a common farm resource during winter season in this region and a non-common supplement resource, multinutrient blocks.

2.1. Experiment I. Effect of pre- and post-partum feed supplementation of Romney Marsh ewes on offspring growth and reproductive activity.

Fifty pregnant 6-tooth Romney Marsh ewes with body condition of 2.5 according to the scale of 1-5 were selected in March 1992. Ewes were maintained under continuous grazing on perennial Rye grass/white clover sward with nocturnal housing (18.00 to 08.00). High grazing pressure (15 to 30 ewes/ha) maintained low forage availability between March and September. Forage mass availability (kg/ha) was estimated by cutting at least 5 quadrants (0.5 x 1.0 m) per ha two times per month. Dry matter (DM) production (kg/ha/month) was assessed using exclusion cages. Chemical analysis of the pasture was performed by standard methods to determine crude protein (CP) [11] and metabolizable energy (ME) [12].

Twenty five ewes were maintained on pasture without supplementation © and 25 were supplemented (S) at night (500 g DM grassland silage/day, CP = 9.7%; ME = 2.3 Mcal/kg DM) for 50 days before and after lambing. Ewe liveweight and body condition [13] were measured monthly. Lamb birth weight was recorded at lambing. From January to April 1993, oestrus was detected in ewe-lambs using vasectomized rams and the presence of a corpus luteum was recorded through progesterone determinations in serum using solid phase RIA kits [14]. Blood samples were collected twice a week.

Progesterone data obtained at oestrus (n = 35) gave a mean progesterone concentration of 0.59 nmol/L. This basal progesterone concentration plus 2 standard deviations (sd = 0.35) was considered as the threshold concentration above which a ewe-lamb was considered to have a corpus luteum, and thus initiated ovarian activity.

A complete randomized design was used with two treatments and 25 replications. Data were analyzed using a computer program (Statgraphics) [15]. Student's t test was used to compare weights; Wilcoxon's test was used to compare BCS and Chi squared test was used to compare luteal activity.
2.2. **Experiment II.** Pre- and post-partum feed supplementation with nutritional blocks in sheep on small-holder farms.

The experiment began on March 1992 in the Rupahue sector of Nueva Imperial County (IX Region, Chile). Six Criollo ewe flocks belonging to Mapuche farmers were used (8-14 ewes per flock totaling 75 ewes). Each flock had a ram together with the females. The animals were maintained during the day on low quality indigenous species of pasture (predominantly Agrostis and Hypochoeris) with a DM production not higher than 2 t DM/ha/year [2]. Animals were maintained in a yard between 17.00 to 07.00. Three flocks received feed supplementation through commercial nutritional blocks (urea-molasses-mineral salts) for 50 days before and after lambing. The block composition per kg was: urea 100 g; Na 210 g; Cl 323 g; CaO 136 g; P₂O₅ 88 g; MgO 17 g; S 343 mg; Zn 180 mg; Fe 225 mg; Mn 150 mg; Cu 75 mg; Mo 67 mg; I 59 mg; Co 20 mg and 500 g molasses. One 11 kg-block was placed in each flock yard during the supplementation period. The other 3 flocks were maintained non-supplemented.

The ewes had a great variation in size and weight; therefore a relative live weight (weight/initial weight x 100) was used in the experiment.

Live weight, BCS and block intake were measured monthly. Ewe-lamb birth-weights, weaning weights and weights at 6 months of age were also recorded. At the end of the reproductive season (April and May), 3 ewe-lambs from each flock were blood sampled at weekly intervals to measure serum progesterone concentration by the solid phase RIA technique to detect luteal activity [14].

A complete randomized design with two treatment and 30 replications was used. Data were analyzed as in the former study.

3. RESULTS
3.1. **Experiment I**

Figs 1 and 2 show existing data with regards to rainfall, air temperature, bodyweight and BCS throughout the year in the region. Dry matter production during the winter months was insufficient to meet the ewes' DM requirements (Table I). Forage mass availability reached the lowest levels during these months.

Figure 3 shows the average of live weights and BCS. The body weights from lambing to 60 days post lambing were statistically different (P <0.05) between the control group and the supplemented animals. BCS (before and after lambing) was statistically different (P <0.05) through all the supplementation period.

![FIG. 1. Mean annual minimum and maximum temperature and rainfall in Temuco, Chile.](image-url)
FIG. 2. Live weight and body condition score in 83 Criollo ewes from 6 smallholder farms in Southern Chile.

FIG. 3. Live weight and body condition score in supplemented and non-supplemented Romney Marsh ewes (animals were supplemented with 500 g/day (DM) of grassland silage for 50 days before and after lambing).

TABLE I. DRY MATTER PRODUCTION, AVAILABILITY, CRUDE PROTEIN AND METABOLIZABLE ENERGY (ME) OF THE PASTURE EMPLOYED IN EXPERIMENT I TO TEST THE EFFECTS OF SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE IN SHEEP

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM production (kg/ha/month)</td>
<td>236</td>
<td>182</td>
<td>65</td>
<td>71</td>
<td>125</td>
<td>634</td>
<td>1,347</td>
</tr>
<tr>
<td>DM availability (kg/ha)</td>
<td>56</td>
<td>48</td>
<td>20</td>
<td>23</td>
<td>55</td>
<td>209</td>
<td>392</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>15.3</td>
<td>16.8</td>
<td>18.1</td>
<td>16.8</td>
<td>16.6</td>
<td>16.2</td>
<td>17.8</td>
</tr>
<tr>
<td>ME (Mcal/kg DM)</td>
<td>2.35</td>
<td>2.54</td>
<td>2.54</td>
<td>2.54</td>
<td>2.54</td>
<td>2.54</td>
<td>2.65</td>
</tr>
</tbody>
</table>
Table II shows that birth weights were not statistically different (P >0.05) between ewe-lambs born from supplemented or non-supplemented dams. Nevertheless, at 30, 60 and 90 days of age the offspring of supplemented ewes were heavier than the ewe-lambs from the control group (P <0.05).

Luteal activity and oestrus activity of ewe-lambs during their first breeding season were both improved (P <0.05) by supplementation of their dams. However, the weight of ewe-lambs at their first luteal activity (x = 35 kg; P >0.05) and occurrence of the first observed oestrus was not affected by the treatment. Ewe-lambs born to supplemented ewes showed their first luteal activity and oestrus activity 23 days and 20 days earlier than the control group (P <0.05).

### TABLE II. LIVE WEIGHT, GROWTH AND REPRODUCTIVE PERFORMANCE DURING THE FIRST BREEDING SEASON OF ROMNEY-MARSH EWE-LAMBS BORN TO DAMS SUPPLEMENTED OR NON-SUPPLEMENTED WITH PASTURE SILAGE

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Non-supplemented</th>
<th>Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth</td>
<td>3.9 ± 0.7a</td>
<td>4.2 ± 0.6a</td>
</tr>
<tr>
<td>At 30 days of age</td>
<td>11.9 ± 1.1a</td>
<td>14.6 ± 0.8b</td>
</tr>
<tr>
<td>At 60 days of age</td>
<td>20.6 ± 2.1a</td>
<td>24.4 ± 0.9b</td>
</tr>
<tr>
<td>At 90 days of age</td>
<td>27.6 ± 2.1a</td>
<td>31.4 ± 2.5b</td>
</tr>
</tbody>
</table>

| Luteal activity (%)   | 70a              | 90b           |
| Body weight (kg) at onset | 35.4 ± 6a       | 36.3 ± 6a    |
| Age (days) at onset    | 194.9 ± 13.5a    | 171.7 ± 23.7b|

| Oestrus activity (%)  | 30a              | 50b           |
| Body weight (kg) at onset | 39.5 ± 3.1a     | 38.8 ± 4.1a  |
| Age (days) at onset    | 230 ± 12.3a      | 210 ± 7.8b   |

Each treatment had a total of 10 ewe-lambs.

Means with different letters in the same row are significantly different (P <0.05).

### 3.2. Experiment II

The average intake of the nutritional blocks was of 45 g/ewe/day (range was 30-60 g). Figure 4 shows the variation in the index of relative liveweight and BCS of Criolla ewes in the control and block-supplemented group. Relative liveweight of supplemented dams was slightly higher than that in the non-supplemented dams, but this was not statistically different (P >0.05). However, BCS of the supplemented animals was higher (P <0.05) at the end of the supplementation period.

Table III shows that the birth weight of ewe-lambs was similar in both groups (P >0.05). However, ewe-lambs born to supplemented dams were heavier (P <0.05) 100 and 150 days after lambing.

In the first breeding season, more ewe-lambs initiated luteal activity in the supplemented group (P <0.05) and more conceived (P <0.05) albeit at a similar weight to the control ewe-lambs. In addition, the ewe-lambs born to supplemented dams were pregnant 21 days earlier than the control group (P <0.05).

Although the use of multinutrient blocks was unknown to Mapuche farmers prior to the initiation of the trial, they were well-accepted.
FIG. 4. Relative live weight and body condition score in supplemented and non-supplemented Criollo ewes from smallholder farms in Southern Chile (supplemented animals received urea-molasses-mineral salt blocks for 50 days before and after lambing. Relative live weight = weight / initial weight x 100).

TABLE III. LIVE WEIGHT, GROWTH AND REPRODUCTIVE PERFORMANCE DURING THE FIRST BREEDING SEASON OF CRIOLLO EWE-LAMBS BORN TO DAMS SUPPLEMENTED AND NON-SUPPLEMENTED WITH NUTRITIONAL BLOCKS

<table>
<thead>
<tr>
<th>Dam's treatment</th>
<th>Ewe-lambs</th>
<th>Non-supplemented</th>
<th>Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>At birth</td>
<td>4.3 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>At 100 days of age</td>
<td>17.2 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.8 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>At 150 days of age</td>
<td>27.5 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.2 ± 4.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Reproductive parameters</td>
<td>Luteal activity (%)</td>
<td>77.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Body weight (kg) at onset</td>
<td>41.1 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.5 ± 6.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Age (days) at onset</td>
<td>290.0 ± 10.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>269.0 ± 18.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pregnancy (%)</td>
<td>30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Each treatment had a total of 9 ewe-lambs.
<sup>2</sup> Onset of luteal activity.
<sup>a, b</sup> Means with different letters in the same row are significantly different (P < 0.05).

4. DISCUSSION

During pregnancy and lactation the development of the foetus and the provision of milk to the lamb require high levels of energy, essential amino acids, minerals and micronutrients which are often obtained from reserves within the ewe. The dam may respond to these increased nutrient demands by eating more, or by mobilizing fat tissue [16]. Under small-holder farm conditions in Southern Chile, increased forage intake is limited by low DM availability of pasture, and therefore, the ewes need to use fat reserves and consequently lose body condition.
Several authors from other parts of the world have reported that feed supplementation during the last 8 weeks of pregnancy increased both BCS in ewes before lambing, and lamb birth weights. However, this effect has been masked in cases of multiple birth [17, 18]. Pre-lambing supplementation in Experiment I increased the BCS and live weight of the dams, but had no effect on lamb birth weight. Silage supplementation increased ewe-lamb weight gain and allowed them to initiate luteal activity and show the first oestrus earlier than the controls.

Pre-lambing supplementation in Experiment II had no effect on dam BCS nor the liveweight of lambs at birth. Multinutrient block intake had no effect during the pre- and post-partum period. However, BCS showed a clear improvement after the supplementation period. It is possible that the nutritional blocks increased feed intake and enhanced the digestibility of natural pasture during the spring season (September to October) [18]. This would increase the ewe milk production and hence ewe-lamb growth rates allowing the offspring to initiate luteal activity, show oestrus and attain higher pregnancy rates during their first breeding season.

In the present study, silage supplementation for ewes in winter has proven to be a good alternative in Southern Chile. However, it is not possible for some farms in which sufficient pasture and fences are not available; in these cases the multinutrient block supplementation could be appropriate [19]. The commercial block used in the present experiment had a cost per kg equivalent to 0.64 kg of lamb. During 100 days of supplementation period, the intake per ewe was 4.5 kg, thus the total cost per animal was equivalent to 2.88 kg of lamb. The weaning weight of lambs born to supplemented dams was 4.6 kg higher than that of lambs born to non-supplemented dams, clearly indicating the advantage of using the multinutrient blocks. Indigenous farmers can prepare the multinutritional block themselves with local resources (molasses, rapeseed, urea and minerals).

It is concluded that pasture silage or nutritional block supplementation for 50 days before and after lambing in Romney Marsh or Criollo ewes increased offspring weight gains and improved reproductive performance during the first breeding season of ewe-lambs.

REFERENCES


EFFECT OF STRATEGIC FEED SUPPLEMENTATION ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE IN DUAL-PURPOSE COWS

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Abstract – Resumen

EFFECT OF STRATEGIC FEED SUPPLEMENTATION ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE IN DUAL-PURPOSE COWS

Six experiments were carried out to study the effect of strategic supplementation during the critical period of the dry season on milk production and reproductive performance in two locations (Tucupido and Tucacas) in Venezuela. Fish meal as a source of undegrade protein was used in two experiments without significant biological responses on production or reproductive performance. However, in two other experiments using either fish meal or cotton-seed meal, higher milk production and improved reproductive performance were found in supplemented animals that had higher body condition at calving (e.g. 15.3 vs 13.3 kg milk/cow/day and 136 vs 200 days interval from calving to conception for cotton-seed supplemented cows with ≥2.5 body condition -Nird and non-supplemented cows with < 2.5 body condition, P <0.05). In another experiment, cows were supplemented with multinutritient blocks (Urea-molasses blocks - UMB) in the pre-partum period and with undegrade protein during the post-partum period. There was an interaction between pre-partum supplementation and body condition. Cows with Nird <2.5 at calving and consuming UMB had an inferior reproductive response compared to those without blocks (days to resumption to ovarian cyclicity 90.2 vs 62.8, days open 140.4 vs 98.2, pregnancy rate at 200 days post-partum 60 vs 80, respectively, P <0.05). In the last experiment, lipid supplementation and calf rearing systems (restricted suckling and artificial rearing) were compared. Lipid supplementation did not affect production or reproductive performance whereas cows with restricted suckling had longer calving-conception intervals than cows without suckling (117.0 vs 93.0 days, P <0.05).

1. INTRODUCTION

The success of a dairy cattle production system depends greatly on the reproductive performance of the herd, since sustained levels of milk production will be obtained only if reproduction is adequate. Nutrition is considered to be one of the most important factors affecting cattle performance in the tropics, due to the low quality of tropical forages and seasonal variation in dry matter production. Cows can become undernourished, especially at the beginning of the lactation period, where there are critical nutritional requirements for body maintenance, milk production and the resumption of the post-partum reproductive activity.

During prolonged dry seasons, the low level of energy, protein, minerals and vitamins in the pasture can affect milk production and fertility [1]. Nutritional deficiencies at the beginning of the
lactation can induce mobilization of fat deposits affecting, in the long term, the productive life of the cow. Therefore, it is of vital importance to design and implement simple, cheap and sustainable feed supplementation practices to solve these problems.

There is vast information concerning production and reproductive performance of crossbred cattle under tropical conditions [2]. However, very little can be found in the literature on the use of feed supplementation strategies during critical periods nor on the use of undegraded protein in lactating dairy cows [3]. Most reports dealing with endocrinological aspects of the post-partum period in dairy and beef cows and in relation to energy protein balance in the cow come from research performed in temperate regions. Nevertheless, some information from tropical areas has recently become available [4].

The present study was carried out to evaluate the effect of feed supplementation strategy on body condition, productive and reproductive performance of cows during the dry season in two regions of Venezuela.

2. MATERIALS AND METHODS

2.1. Location

Six experiments were conducted in two commercial farms located in Tucupido (Guárico State) and Tucacas (Falcon State). Climatic conditions in Tucupido are: mean temperature, 30°C; mean relative humidity, 60%; mean annual rainfall, 1300 mm, and six months of rainy season. In Tucacas the conditions are: mean temperature, 28°C; mean relative humidity, 70%; mean annual rainfall, 1500 mm, and three months of dry season.

2.2. Animals and treatments

Multiparous crossbred Zebu cows were used in both locations. Animals from Tucupido had little Bos taurus inheritance, were hand-milked. Suckling was allowed for two hours/day and animals were naturally bred. In contrast, cows from Tucacas had more Bos taurus genotype, greater genetic aptitude for milk production, were mechanically milked and artificial inseminated.

The experimental period lasted for 90 days in all experiments. Experiments I, II and III were conducted in Tucupido and experiments IV, V and VI in Tucacas. Table I summarizes chemical composition of feeds.

2.2.1. Experiment I

a) Control (n = 15). Cows grazed Cuji negro (Mimosa termiflora) leaves and were fed sorghum crop residues in the dry season, and grazed German grass (Echinochloa polystachya) in the rainy season. In addition, animals received 2 kg/cow/day of commercial concentrate.

b) Supplemented (n = 14): Same basal diet as above plus 2 kg/cow/day of a concentrate containing urea (1.5%), molasses (20%), sorghum meal (39.5%), fish meal (10%), laying hen manure (25%) and a mixture of salt and minerals (4%).

2.2.2. Experiment II

All cows had free access to multinutrient blocks (urea-molasses blocks - UMB) during milking from 5 to 8 a.m. Mean consumption was 150 g/cow/day. UMB consisted of molasses (38%), urea (10%), calcium oxide (5%), Canavalia ensiformis (5%), corn meal (30%), gypsum (2%) and a mixture of salt and minerals (10%). The treatments were:

a) Control (n = 19). Cows were fed sorghum crop residues without additional supplementation during the dry season.

b) Supplemented (n = 19). Same basal diet as above plus 1 kg/cow/day of concentrate containing fish meal (28.5%), molasses (35.7%), sorghum meal (30%), urea (2.9%) and a mixture of salt and minerals (2.89%).
TABLE I. CHEMICAL COMPOSITION OF BASAL DIETS, CONCENTRATES AND UREA-MOLASSES BLOCKS (UMB) USED IN SIX EXPERIMENTS WITH CROSSBRED ZEBU COWS IN THE TUCUPIDO (Exp. I, II and III) AND TUCACAS (Exp. IV, V and VI) REGIONS (DM = dry matter; CP = crude protein; EE = ether extract; Ca = calcium; P = phosphorus. All expressed in %)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Basal diet</th>
<th>DM</th>
<th>Ash</th>
<th>CP</th>
<th>Cell wall</th>
<th>EE</th>
<th>Digestibility</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Basal diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>89.03</td>
<td>10.35</td>
<td>6.89</td>
<td>-</td>
<td>1.02</td>
<td>19.26</td>
<td>0.63</td>
<td>0.08</td>
<td></td>
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<tr>
<td>Rainy season</td>
<td>26.10</td>
<td>12.84</td>
<td>10.65</td>
<td>-</td>
<td>2.73</td>
<td>44.42</td>
<td>1.31</td>
<td>0.25</td>
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<tr>
<td>Commercial concentrate</td>
<td>87.04</td>
<td>26.50</td>
<td>20.40</td>
<td>-</td>
<td>2.66</td>
<td>73.99</td>
<td>3.46</td>
<td>1.18</td>
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<td>9.53</td>
<td>16.16</td>
<td>-</td>
<td>5.57</td>
<td>78.21</td>
<td>1.24</td>
<td>0.49</td>
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</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Basal diet</td>
<td>88.71</td>
<td>8.02</td>
<td>4.56</td>
<td>75.25</td>
<td>-</td>
<td>-</td>
<td>0.32</td>
<td>0.30</td>
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<tr>
<td>UMB</td>
<td>-</td>
<td>25.39</td>
<td>15.28</td>
<td>22.48</td>
<td>-</td>
<td>-</td>
<td>5.86</td>
<td>0.56</td>
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<tr>
<td>Concentrate</td>
<td>92.65</td>
<td>20.01</td>
<td>39.71</td>
<td>28.37</td>
<td>-</td>
<td>-</td>
<td>5.29</td>
<td>2.15</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pre-partum</td>
<td>89.20</td>
<td>7.75</td>
<td>4.80</td>
<td>80.08</td>
<td>-</td>
<td>-</td>
<td>0.66</td>
<td>0.09</td>
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</tr>
<tr>
<td>Post-partum</td>
<td>26.70</td>
<td>16.81</td>
<td>11.37</td>
<td>72.25</td>
<td>-</td>
<td>-</td>
<td>0.34</td>
<td>0.26</td>
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</tr>
<tr>
<td>UMB</td>
<td>-</td>
<td>32.82</td>
<td>29.78</td>
<td>-</td>
<td>3.65</td>
<td>-</td>
<td>9.17</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Concentrate</td>
<td>90.14</td>
<td>25.36</td>
<td>33.12</td>
<td>-</td>
<td>4.13</td>
<td>-</td>
<td>4.96</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment IV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>27.10</td>
<td>9.41</td>
<td>10.45</td>
<td>-</td>
<td>1.22</td>
<td>25.49</td>
<td>0.95</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Rainy season</td>
<td>28.20</td>
<td>9.99</td>
<td>11.05</td>
<td>-</td>
<td>1.20</td>
<td>41.46</td>
<td>0.19</td>
<td>0.26</td>
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</tr>
<tr>
<td>Commercial concentrate</td>
<td>96.77</td>
<td>9.16</td>
<td>27.06</td>
<td>-</td>
<td>7.56</td>
<td>76.87</td>
<td>2.89</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Experimental concentrate</td>
<td>98.08</td>
<td>12.84</td>
<td>26.67</td>
<td>-</td>
<td>9.54</td>
<td>75.27</td>
<td>1.90</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment V</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Basal diet</td>
<td>26.30</td>
<td>10.84</td>
<td>9.21</td>
<td>76.04</td>
<td>-</td>
<td>-</td>
<td>0.41</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Conc without cotton seed meal</td>
<td>90.17</td>
<td>8.44</td>
<td>27.16</td>
<td>39.24</td>
<td>5.88</td>
<td>-</td>
<td>1.17</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Conc with cotton seed meal</td>
<td>89.62</td>
<td>8.29</td>
<td>22.78</td>
<td>37.31</td>
<td>6.81</td>
<td>-</td>
<td>1.14</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment VI</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal diet</td>
<td>27.40</td>
<td>10.19</td>
<td>8.25</td>
<td>77.36</td>
<td>-</td>
<td>-</td>
<td>0.52</td>
<td>0.14</td>
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<tr>
<td>Barley</td>
<td>23.00</td>
<td>6.64</td>
<td>29.27</td>
<td>74.72</td>
<td>-</td>
<td>-</td>
<td>0.46</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Rice meal with lipids</td>
<td>92.18</td>
<td>8.03</td>
<td>12.77</td>
<td>29.78</td>
<td>30.59</td>
<td>-</td>
<td>0.16</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Rice meal without lipids</td>
<td>91.51</td>
<td>9.72</td>
<td>14.90</td>
<td>34.29</td>
<td>14.74</td>
<td>-</td>
<td>1.48</td>
<td>1.69</td>
<td></td>
</tr>
</tbody>
</table>

2.2.3. **Experiment III**

Cows were fed sorghum crop residues prior to calving and grazed *Digitaria sanguinales* after calving. The treatments were:

a) **Control (n = 12).** Free access to a mixture of salt and minerals without additional supplements during the pre- and post-partum period.

b) **Post-partum supplementation (n = 13).** No supplementation in the pre-partum but 1 kg/cow/day during the post-partum of a concentrate containing cotton-seed meal (50%), sorghum meal (30%), molasses (15%), urea (2.5%) and minerals (2.5%).

137
c) Pre-partum supplementation (n = 13). Cows had free access to UMB in the pre-partum period but not after calving. These blocks contained fish meal (20%), sorghum meal (12.5%), molasses (35%), calcium oxide (10%), urea (10%), minerals (5%), salt (5%) and sorghum hay (2.5%).

d) Pre- and post-partum supplementation (n = 12). Cows had access to the above UMB before calving and to the same supplement as (b) above after calving.

2.2.4. Experiment IV

a) Control (n = 18). Cows grazed Star grass (Cynodon plectostachyus), Bermuda grass (Cynodon dactylon) and Pangola grass (Digitaria decumbens) and were fed 4 kg/animal/day of concentrate without fish meal.

b) Supplemented (n = 15). Same as above but the concentrate had 5% fish meal.

2.2.5. Experiment V

a) Control (n = 23). Basal diet was similar to Experiment IV. Cows were fed 6 kg/animal/day of commercial feed supplement.

b) Supplemented (n = 17). Same as above, but with the addition of 700 g/animal/day of cotton-seed meal.

2.2.6. Experiment VI

Basal diet was similar to that in Experiment IV. Besides, all animals had free access to barley distillers and UMB composed of rice meal (30%), calcium oxide (10%), molasses (30%), mineral mixture (6%), salt (4%), hay (10%) and urea (10%). The treatments were:

a) Calves were weaned on the third day of age and fed milk substitutes. Cows received 4 kg/animal/day rice meal (n = 15).

b) Calves were allowed to suckle twice daily for 30 minutes after milking during the first 75 days of age. Cows were fed similar to (a) above (n = 12).

c) Similar as (a) above but cows received 300 g/cow/day of crude palm oil (n = 15).

d) Suckling was similar to above (b) and feeding regime similar to © above (n = 14).

2.3. Measurements

Samples were collected at each grazing cycle from all kinds of feedstuffs to evaluate dry matter, crude protein [5], cell wall content [6], calcium [7] phosphorus [8], and in vitro digestibility [9]. Daily intake of supplements was recorded.

Body condition was scored by the number of visible ribs (VR) and by the Nird method [10], (where 1 = too thin and 5 = obese). Milk production was recorded every week from calving to day 90 of the lactation.

Clinical examination of the ovaries and reproductive tract was performed by rectal palpation at monthly intervals until first service. Milk samples were collected twice a week from calving to first observed oestrus and thereafter, on a weekly basis. Frequency of anoestrus was evaluated at 120 days post-partum and pregnancy rate at 200 days post-partum. Milk samples were skimmed and analyzed for progesterone concentration using the solid-phase progesterone FAO/IAEA RIA kits [11].

2.4. Statistical analysis

Factorial analyses were used to evaluate milk production, ovarian activity and days open. A 2 x 2 factorial was used in Experiments I, II, IV and V. Factor A was supplementation and factor B was body condition (VR: group 1 = >3, and group 2 = ≤3. Nird: group 1 = >2.5, and group 2 = ≤2.5).

A 2 x 2 x 2 factorial was used in Experiment III. Factor A was pre-partum supplementation, factor B was post-partum supplementation and factor C was body condition score (VR and Nird).

A 2 x 2 factorial was used in Experiment VI. Factor A was suckling and factor B was lipid supplementation. In all cases, the interaction between factors was also part of the model.

In the presence of a significant F value, individual means were compared by the Student 't' Test. Frequency of anoestrus and pregnancy were analyzed by Chi-square in all six experiments.
3. RESULTS AND DISCUSSION

3.1. Experiments conducted in Tucupido

Chemical composition of feedstuffs used in Experiments I, II and III are shown in Table 1. Basal diet during the dry season was sorghum crop residues of low protein content (4.56 - 6.89%) and high cell wall content (75.35 - 80.1%), similar to diets previously employed [12]. Basal diet during the rainy season (Experiment I and the post-partum period in Experiment III) had better quality in both cultivated and natural grasses. The nutritive values were similar to those previously reported [13].

Neither fish meal supplementation nor VR at calving in Experiment I affected milk production at day 90 of the lactation period (x = 5.1 kg/cow/day), resumption of ovarian cyclicity (x = 71.2 days), days open (x = 87.4 days), pregnancy rate (100%) and anoestrous rate (0%, P >0.05). These results agree with previous studies [3]. The lack of response to fish meal supplementation could be attributed to relative low protein and energy requirements of the animals used in the experiment.

On the contrary, animals receiving concentrate with fish meal in Experiment II produced more milk in the first 90 days of the lactation period than did controls (6.2 vs 5.5 kg/cow/days, respectively, P <0.05), in agreement with reports from other tropical regions [14]. Moreover, reproductive parameters were not affected, as previously reported [15]. Body condition (VR) was an important factor in reproductive performance. Cows with ≤3 vs >3 VRs had a shorter interval from calving to the resumption of ovarian activity (59 vs 80 d), less days open (70 vs 116 d), a lower anoestrous rate at 120 days post-partum (0 vs 28.6%) and a higher pregnancy rate at 200 days post-partum (100 vs 85.7%), as found in other trials [16].

The interaction between feed supplementation and body condition (VR) at calving proved to be significant (P <0.05) in productive and reproductive parameters (Table II). Cows having ≤3 VR and supplemented with fish meal-concentrate, had the highest milk production (P <0.01). Similar results have also been reported in the tropics [17] and in temperate areas [18, 19] as well.

Figure 1 shows the variation of VR during the first 120 post-partum days in Experiment II. Cows with ≤3 VR at calving and supplemented or not supplemented with fish meal-concentrate, lost body condition until day 90 post-partum. A similar response has also been reported elsewhere [14]. Milk production can be increased in cows consuming undegradable protein due to its nutrients, and mobilization of fat deposits may also occur.

<table>
<thead>
<tr>
<th>N° of visible ribs</th>
<th>&gt;3</th>
<th>≤ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows (n)</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>With fish meal</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Milk production (kg/day)</td>
<td>5.5^a</td>
<td>5.9^a</td>
</tr>
<tr>
<td>Resumption of ovarian cyclicity (d)</td>
<td>91.4^a</td>
<td>69.7^b</td>
</tr>
<tr>
<td>Days open (d)</td>
<td>134.5^a</td>
<td>99.5^b</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>84.6^a</td>
<td>86.7^a</td>
</tr>
<tr>
<td>Anoestrous rate (%)</td>
<td>38.5^a</td>
<td>20.0^b</td>
</tr>
</tbody>
</table>

1 First rise of progesterone in two consecutive samples
2 At day 200 post-partum
3 At day 120 post-partum

Mean values within a row with different superscripts are significantly different (P <0.05; Milk production P <0.01)
Pre-and post-calving supplementation in Experiment III did not affect performance. Body condition (Nird) at calving affected post-partum reproductive parameters. Cows with Nird $\geq$2.5 had shorter intervals to the resumption of ovarian activity ($P <0.01$) and to conception ($P <0.05$) than cows with Nird <2.5 (Table III). Similar findings have been reported, but using a 1-to-9 scoring method [20]. There were an unforeseen interaction between pre-partum supplementation and body condition. Cows with Nird <2.5 and consuming UMB had inferior reproductive performance relatively to those not fed blocks (resumption to ovarian cyclicity 90.2 vs 62.8 d, days open 140.4 vs 98.2, pregnancy rate at 200 days post-partum 60 vs 80, respectively, $P <0.05$). These could be due to low UMB intake (291 ± 25 g/cow/day) which may have caused a mineral deficiency, thereby affecting reproduction. Phosphorus deficiency in the soil and plants has been reported to cause fertility disturbances and anoestrus in the tropics [21, 22].

**TABLE III. MILK PRODUCTION AT 90 DAYS OF LACTATION AND REPRODUCTIVE PERFORMANCE IN DUAL PURPOSE COWS ACCORDING TO BODY CONDITION (NIRD) AT CALVING IN THE TUCUPIDO REGION (Experiment III)**

<table>
<thead>
<tr>
<th>Body condition (Nird)</th>
<th>&lt;2.5</th>
<th>$\geq$2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows (n)</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Milk production (kg/day)</td>
<td>3.7$^a$</td>
<td>3.9$^b$</td>
</tr>
<tr>
<td>Resumption of ovarian cyclicity (d)$^1$</td>
<td>76.5$^a$</td>
<td>47.7$^b$</td>
</tr>
<tr>
<td>Days open (d)</td>
<td>119.3$^a$</td>
<td>85.2$^b$</td>
</tr>
<tr>
<td>Pregnancy rate (%)$^2$</td>
<td>75.0$^a$</td>
<td>90.0$^b$</td>
</tr>
<tr>
<td>Anoestrous rate (%)$^2$</td>
<td>30.0$^a$</td>
<td>0$^b$</td>
</tr>
<tr>
<td>Visible ribs (n)</td>
<td>4.2$^a$</td>
<td>3.2$^b$</td>
</tr>
</tbody>
</table>

$^1$ First rise of progesterone in two consecutive samples

$^2$ At day 200 post-partum

$^3$ At day 120 post-partum

$^a$,$^b$ Mean values within a row with different superscripts are significantly different ($P <0.05$; Resumption of ovarian cyclicity, visible ribs, $P <0.01$)
3.2. Experiments conducted in Tucacas

Chemical composition of feedstuffs used in Experiments IV, V and VI are shown in Table 1. Basal diet consisted of improved grasses, and therefore was of better quality than in the Tucupido region. The chemical composition data agree with values previously reported for the region [13]. However, despite of the better quality of the forage, a major limitation during the trials was forage availability.

Neither fish meal supplementation nor VR at calving in Experiment IV affected milk production at day 90 of the lactation period (11.3 kg/cow/day), resumption of ovarian cyclicity (66.7 days), days open (93.9 days), pregnancy rate (100%) and anoestrus rate (0%). These results agree with previous studies using cultivated grasses in the tropics of Latin America [23], but differ from studies conducted in other tropical regions [14]. The lack of response could be attributed to the higher protein content and digestibility of improved grasses compared to native grasses and crop residues.

### TABLE IV. MILK PRODUCTION AT 90 DAYS OF LACTATION AND REPRODUCTIVE PERFORMANCE IN DUAL PURPOSE COWS WITH RELATION TO BODY CONDITION (Nird) AND BY-PASS PROTEIN CONCENTRATE IN THE TUCACAS REGION (Experiment V)

<table>
<thead>
<tr>
<th>Nird</th>
<th>Without cotton seed meal</th>
<th>With cotton seed meal</th>
<th>Without cotton seed meal</th>
<th>With cotton seed meal</th>
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</thead>
<tbody>
<tr>
<td>&lt;2.5</td>
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<td>7</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>≥2.5</td>
<td>13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>76.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>174.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>137.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>135.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>33.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1. First rise of progesterone in two consecutive samples
2. At day 200 post-partum
3. At day 120 post-partum

Mean values within a row with different superscripts are significantly different (P <0.01; Milk production, visible ribs, P <0.05)

There was no significant effect of cotton-seed meal supplementation on productive and reproductive performance in Experiment V (P >0.05). In general, on one hand, the results were similar to those observed in previous experiments despite the fact that the level of undegraded protein was higher (700 g) in this trial. On the other hand, body condition (Nird), as in Experiments II and III, significantly influenced the milk production and reproduction (P <0.05). The interaction between Nird and supplementation was significant for milk production (Table IV, P <0.05). Cows with Nird ≥ 2.5 and fed cotton-seed meal had the highest milk production in the group (15.3 kg/day). The interval from calving to the resumption of ovarian activity was significantly lower between cows with Nird <2.5 and ≥2.5 (P <0.05) whether they were or not supplemented. The Nird method as well as the number of visible ribs in Experiment II proved to be a useful tool to estimate body condition.

Table V shows the results from Experiment VI. Saleable milk production through day 100 of the lactation period, but not total milk production, was less in animals under restricted suckling compared to the group with artificial rearing (12.8 vs 15.7 kg/cow/day, P <0.05 and 15.5 vs 15.7 kg/day/cow, P >0.05). Similar results have been reported elsewhere [24, 25, 26, 27]. Lipid supplementation did not improve the criteria under evaluation. This could be due to the relatively good body condition of the
### TABLE V. MILK PRODUCTION AT 110 DAYS OF LACTATION AND REPRODUCTIVE PERFORMANCE IN DUAL PURPOSE COWS WITH RELATION TO LIPID SUPPLEMENTATION AND CALF REARING SYSTEMS IN THE TUCACAS REGION (Experiment VI)

<table>
<thead>
<tr>
<th>Supplementation</th>
<th>Calf rearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>With lipids</td>
<td>Without lipids</td>
</tr>
<tr>
<td>Cows (n)</td>
<td>27</td>
</tr>
<tr>
<td>Saleable milk (kg/day)</td>
<td>14.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk production (kg/day)</td>
<td>15.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Resumption of ovarian cyclicity (d)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>78.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Days open (d)</td>
<td>104.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Visible ribs</td>
<td>3.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nird</td>
<td>3.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> First rise of progesterone in two consecutive samples  
<sup>a, b</sup> Mean values within a row with different superscripts are significantly different (P < 0.05)

Cows at calving, which helped to alleviate the negative energy balance at the beginning of lactation. In addition, all cows were fed rice polishing, what is rich in lipids (17.8%).

Artificial calf rearing allowed cows to have better reproductive performance compared with cows under the restricted suckling system (P < 0.05). Other reports in the literature show similar effects on the interval from calving to conception [28].

Changes in body condition (Nird) during the first 110 days of lactation are presented in Figure 2. Independent of the method for calf rearing, cows with lipid supplementation had better body condition than cows not supplemented at the end of the trial. Previous studies [29] have shown an increase in body weight gain in steers consuming lipid-rich diets.

![Figure 2](image.png)

**FIG. 2. The effect of lipid supplementation on body condition (Nird) changes during the first 110 days of the post-partum period in dual-purpose cows with weaned or restricted suckled calves in the Tucacas region of Venezuela (Experiment VI).**
4. CONCLUSION

The use of fish meal as a source of undegraded protein did not affect the productive and reproductive performance of cows. The adequate level of protein content in the basal diet may have masked the potential benefit of this supplement (Experiment I and IV). Nevertheless, fish meal supplementation did improve the reproductive and productive performance of the cows supplemented with UMB (Experiment II). Body condition was an important factor in performance of animal in all experiments. As was shown in most of the experiments, cows having better body condition at calving and receiving a supplement were able to produce more milk and to conceive in a shorter interval than the other groups.

Supplementation through multinutrient blocks before calving negatively affected the reproductive performance of cows with low body condition score (Nird < 2.5). This could be attributed to the low intake of the blocks which could have caused a mineral deficiency in the cows.

Lipid supplementation improved neither milk production nor reproductive activity although there was an improvement in body condition of the cows. Restriction of suckling diminished the quantity of saleable milk but not the total milk production of the cows compared to artificial calf rearing system. Artificially rearing initiated ovarian cycles sooner after calving and reduced the number of days from calving until conception.

REFERENCES


143


EFFECT OF SUPPLEMENTATION OF CONCENTRATES OR SELENIUM ON PRODUCTION AND REPRODUCTION IN COWS GRAZING PASTURES OF HIGH PROTEIN DEGRADABILITY

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Abstract – Resumen

EFFECTS OF SUPPLEMENTATION OF CONCENTRATES OR SELENIUM ON PRODUCTION AND REPRODUCTION IN COWS GRAZING PASTURES OF HIGH PROTEIN DEGRADABILITY.

Two experiments were carried out to determine whether the deleterious effects of high amounts of degradable protein on reproduction and production of dairy cows could be minimized by a supplemental source of undegradable protein, or grain supplementation while grazing; and to study the effect of selenium supplementation before calving on the incidence of stillbirths, mastitis, puerperal and metabolic disorders.

In a first experiment, 24 Holstein cows fed on red and white clover pasture, paired by previous milk production, calving date and body condition, were supplemented with corn silage and one of two concentrates differing only in the proportion of degradable protein (Group H: 71.5% and Group L: 51.5%). The degradable protein intake from pasture supplied 93% of the requirements in both groups. The addition of undegradable protein in the concentrate of Group L did not improve reproductive performance nor milk, butterfat or solids not-fat production.

In a second experiment, 132 Holstein cows and heifers were paired likewise. Both animals in each pair were fed similar forage resources, but each one was supplemented with 2 kg/cow corn grain four times a day (Herd 1) or 4 kg/cow twice a day (Herd 2). One animal in each pair was randomly assigned to receive a barium selenate injection before calving. In this experiment, rumen ammonia was higher in Herd 1 in both sampling dates (17 vs 4.2 mg/100 ml and 12 vs 9 mg/100 ml), as well as serum urea up to 50 days post-partum (26 vs 19 mg/100 ml, P < 0.02). Body condition scores were similar at calving but significantly lower in Herd 1 during the lactation period (P < 0.05). Total milk and butterfat production were higher in Herd 2 (6406.2 vs 6893.8 kg and 190.4 vs 203.5 kg, respectively). Selenium treatment improved pregnancy rate to first artificial insemination in Herd 2 (71 vs 50%), and decreased the frequency of downer cows in both herds (5 vs 0%), but had no effect regarding the Wisconsin Mastitis Test results.

EFFECTOS DE LA SUPLEMENTACION DE CONCENTRADOS 0 SELENIO SOBRE LA PRODUCCION Y REPRODUCCION EN VACAS EN PASTURAS CON PROTEINAS DE ALTA DEGRADABILIDAD.

Se llevaron a cabo 2 experimentos para determinar si los efectos adversos de los altos niveles de proteína degradable en la dieta sobre la reproducción y la producción de vacas lecheras podían ser minimizados mediante una fuente de proteína sobrepasante, o con la suplementación con grano durante el parto; así como para estudiar los efectos de la suplementación pre-parto con selenio sobre la mortalidad perinatal, la incidencia de mastitis y los desórdenes puerpares y metabólicos.

En un primer experimento, 24 vacas Holstein que pastoreaban trébol rojo y blanco, fueron suplementadas con 2 kg/cow de grano de maíz cuatro veces al día (Herd 1) y 4 kg/cow dos veces al día (Herd 2). Una vaca de cada par fue asignada abarium selenite injection before calving. In this experiment, rumen ammonia was higher in Herd 1 in both sampling dates (17 vs 4.2 mg/100 ml and 12 vs 9 mg/100 ml), as well as serum urea up to 50 days post-partum (26 vs 19 mg/100 ml, P < 0.02). Body condition scores were similar at calving but significantly lower in Herd 1 during the lactation period (P < 0.05). Total milk and butterfat production were higher in Herd 2 (6406.2 vs 6893.8 kg and 190.4 vs 203.5 kg, respectively). Selenium treatment improved pregnancy rate to first artificial insemination in Herd 2 (71 vs 50%), and decreased the frequency of downer cows in both herds (5 vs 0%), but had no effect regarding the Wisconsin Mastitis Test results.

1. INTRODUCTION

Grazing systems for dairy herds in Argentina include grass, legumes and winter cereals, which are generally deficient in energy, but rich in highly degradable protein and non-protein nitrogen [1]. Excess degradable protein increases both the concentration of rumen ammonia and the ruminal efflux of
ammonia by absorption and passage [2]. Absorbed rumen ammonia is converted to urea by the liver, a process that places an additional energy drain on the cow in early lactation [3]. When large amounts of degradable protein are present, much of the absorbed ammonia by-passes the liver or enters the circulation by means other than the portal vein [4]. Cows with serum urea values greater than 20 mg/100 ml have been reported to have conception rates lower than 25% [5].

Subfertility could result from one or more of the following: direct toxic effects of nitrogenous compounds accumulated in reproductive tissues and fluids; imbalances in protein and energy supply that may affect the efficiency of metabolism and energy status; disturbances of the hypophyseal pituitary ovarian axis function due to N by-products or deficiencies in energy utilization [6].

Selenium is involved in glutathione peroxidase activity, which is an essential component of the intracellular antioxidant system, assisting the prevention of membrane damage [7]. A major sign of selenium deficiency is white muscle disease [8], but it has also been associated with retained placenta [9] and mastitis [10]. Injections of selenium 21 days pre-partum lowered the incidence of puerperal metritis [11] and the interval required for uterine involution after parturition [12]. A survey carried out in Argentina in 1981 revealed low glutathione peroxidase levels in blood of cows and sheep from different areas of the country, indicating that a subclinical deficiency of selenium may exists on these farms [13].

Two field experiments were designed to determine whether the detrimental effects of high amounts of degradable protein on reproduction and production could be minimized by a supplemental source of undegraded protein, or by grain supplementation while grazing.

In the second experiment, a selenium injection before calving on productive and reproductive traits was added as an experimental factor.

2. MATERIALS AND METHODS

2.1. Experimental animals

Each experiment was conducted under field conditions in commercial dairy herds. In Experiments I and II, 24 and 132 Holstein cows were selected respectively on a paired basis, so that both animals in each pair had the same lactation number; the previous lactation differing by no more than 550 kg of 4% fat corrected milk (FCM). Additionally, the expected dates of calving were no more than 15 days apart and body condition scores before calving were similar within experiments (1.75 - 2 in Experiment I and 3 - 3.5 Experiment II on a scale 1-5).

2.2. Nutritional management

2.2.1. Experiment I

The cows grazed a pasture of mixed species with a dominance of white and red clover for approximately 7 h per day, and were offered 25 kg/cow/day of corn silage with 26.3% dry matter (DM), 61.4% total digestible nutrients (TDN) and 2.28 Mcal/kg, pH 3.9. The protein content of the corn silage was 5.9% on DM basis. DM, neutral detergent fiber [14], digestibility [15], and crude protein [16] from grass samples were 19.5%, 23.9%, 83.9% and 24.1%, respectively. DM intake of the pasture was assumed to be 7.5 kg (1.5 kg/h) for a mean body weight of 500 kg (Table I), based on the pasture apparent digestibility and considering an effective grazing time of 5 h [17].

Each animal received 4, 6 or 8 kg of concentrate with low (L) or high (H) protein degradability, during milking and according to three levels of milk production (<15, 15 - 20, <20 kg FCM/day, respectively). Both concentrates were iso-caloric (2988 vs 3100 Kcal/kg), iso-nitrogenous (17 vs 16.6% protein), but of different protein fraction degradability (L: 51.9%; H: 71.5%). The concentration of nutrients in diets L and H was calculated according to animal requirements [18], and the individual chemical analyses of grass, silage and concentrates (Table II).
TABLE I. DAILY QUANTITIES OF DRY MATTER (DM), METABOLIC ENERGY (ME), CRUDE PROTEIN (CP), DEGRADABLE AND UNDEGRADABLE PROTEIN INTAKE (DP AND UP) FOR HIGH AND LOW DIETS FED TO 26 HOLSTEIN COWS IN EXPERIMENT I, IN COMPARISON WITH THE THEORETICAL NUTRITIONAL REQUIREMENTS. (Diets High and Low contain concentrates with high and low protein degradability, respectively).

<table>
<thead>
<tr>
<th>Feedstuffs</th>
<th>Quantity (kg/day)</th>
<th>DM (kg/day)</th>
<th>ME (MJ)</th>
<th>CP (g/day)</th>
<th>DP (g/day)</th>
<th>UP (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture</td>
<td>n/a</td>
<td>7.5</td>
<td>78.13</td>
<td>1,807.5</td>
<td>1,355.63</td>
<td>451.88</td>
</tr>
<tr>
<td>Silage</td>
<td>25</td>
<td>6.6</td>
<td>62.72</td>
<td>1,475.0</td>
<td>929.25</td>
<td>398.25</td>
</tr>
<tr>
<td>High concentrate</td>
<td>6</td>
<td>5.25</td>
<td>39.38</td>
<td>900.8</td>
<td>644.22</td>
<td>256.38</td>
</tr>
<tr>
<td>Low concentrate</td>
<td>6</td>
<td>5.27</td>
<td>42.33</td>
<td>876.37</td>
<td>455.52</td>
<td>420.84</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High diet</td>
<td>19.32</td>
<td>180.23</td>
<td>4,183.30</td>
<td>2,929.09</td>
<td>1,106.70</td>
<td></td>
</tr>
<tr>
<td>Low diet</td>
<td>19.34</td>
<td>183.19</td>
<td>4,158.87</td>
<td>2,740.40</td>
<td>1,270.97</td>
<td></td>
</tr>
<tr>
<td>Requirements†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 days post-partum</td>
<td>19.57</td>
<td>178.61</td>
<td>2,810</td>
<td>1,452</td>
<td>1,034</td>
<td></td>
</tr>
<tr>
<td>&gt;50 days post-partum</td>
<td>19.32</td>
<td>172.30</td>
<td>2,686</td>
<td>1,399</td>
<td>986</td>
<td></td>
</tr>
</tbody>
</table>

† A mean milk production of 25 L/day, 3.5% milk fat and no weight gain during the first 50 days of lactation was assumed; and 21 L/day milk production, 3.8% milk fat and a body weight gain of 0.25 kg/day after 50 days of lactation.

n/a Not available

TABLE II. COMPOSITION (%) OF ISO-CALORIC AND ISO-NITROGENOUS CONCENTRATES WITH LOW OR HIGH PROTEIN DEGRADABILITY GIVEN TO 26 HOLSTEIN COWS GRAZING WHITE AND RED CLOVER PASTURES IN EXPERIMENT I

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentrate type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Wheat middling</td>
<td>45.12</td>
</tr>
<tr>
<td>Corn</td>
<td>40</td>
</tr>
<tr>
<td>Meat meal</td>
<td>5</td>
</tr>
<tr>
<td>Sunflower meal†</td>
<td>5</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>3.36</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>0.52</td>
</tr>
<tr>
<td>Urea</td>
<td>--</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
</tr>
</tbody>
</table>

† solvent extracted

2.2.2. Experiment II

Both animals in each pair had access to similar forage but one set (x pairs) was supplemented with 2 kg/cow corn grain four times a day (Herd 1); and the other set (y pairs) received 4 kg/cow twice a day (Herd 2). In addition, one of each pair in Herds 1 and 2 received barium selenate supplementation [5 ml of Deposel (Rycovet Lab., Glasgow, Scotland), with 50 mg Se/ml, subcutaneously 17 days before calving on average], and the other animals remained as controls.

Before the experiment began, a rumen canula was fixed in two cows from each herd. Rumen ammonia was measured [19] before a grain feed, and subsequently at 60, 120 and 150/180 min on two occasions during the experiment.

Biochemical and chemical characteristics of feedstuffs were determined using the same methodology as in Experiment I.
2.3. Production Recording

Milk and butterfat production were recorded monthly during the first 7 months in Experiment I and during the whole lactation period in Experiment II. Total milk production was adjusted for age, butterfat, length of lactation and season of calving. In Experiment I, total solids non-fat (SNF) were estimated from the percentage of total solids and fat content during the first 5 months of lactation. In Experiment II, the Wisconsin Mastitis Test was performed twice to determine the percentage of cows with subclinical mastitis.

2.4. Body Condition Scoring and Metabolic Profiles

Body condition score [20] was recorded every two weeks in all animals from Experiment I and in 15 animals per group from Experiment II. On these days, blood samples were collected three times a day (before-, after 0.5 h and 2 h after concentrate supplementation) in Experiment I; and once a day in Experiment II to analyze serum urea [21]. Plasma glucose [22], serum albumin [23], serum total protein [24], calcium and magnesium [25] were also analyzed in samples taken every two weeks in both experiments.

In Experiment II, whole blood samples were taken before the selenium injection, and monthly afterwards to determine glutathione peroxidase activity [26].

2.5. Reproductive management

Uterine involution and resumption of ovarian activity were assessed weekly by palpation per rectum in Experiment I and at least twice in Experiment II. Heat detection was conducted twice a day for 40 min by the inseminator in the first experiment, and by the milkers in the second experiment. Artificial insemination was performed 12 h after oestrus was observed. In Experiment II, the first breeding date was May 1st for cows that calved before March 1st, and 60 d after calving for cows that calved after March 1st.

Pregnancy rate, metritis, number of services per pregnancy and intervals from calving (Experiment I) or from the beginning of the breeding period (Experiment II) to uterine involution (only Exp. I), first oestrus, first service and conception were recorded. The number of downer cows, assisted calvings, placental retention for >12 h and stillbirths were also recorded in Experiment II.

Milk samples were collected twice a week from the same animals sampled for metabolic profiles. Skim milk progesterone concentration was determined using the FAO/IAEA solid-phase RIA kit [27]. In Experiment I, the sensitivity of the assay was 1 nmol/L, and the intra- and inter-assay coefficients of variation were 5.6 and 3%, and 12 and 10% for low and high quality control samples, respectively. In Experiment II, the sensitivity of the assay was 0.6 nmol/L, and the intra- and inter-assay coefficients of variation were 15, 13 and 12%, and 20, 15 and 4% for low, medium and high quality control samples, respectively.

2.6. Statistical analysis

Production and metabolic variables were analyzed by the univariate approach for repeated measurement design. The least square analysis of variance [28] was used to evaluate differences between herds and selenium treatment within each sampling date. Type III Sum Square was used to avoid the effect of imbalance, and the level of significance was fixed at 2-5% to avoid the effects of possible departure from the assumption of compound symmetry.

Differences in reproductive intervals between groups (L and H, Experiment I) and between herds (1 and 2, Experiment II) were analyzed using the Mann-Whitney Wilcoxon test and the Kruskall-Wallis test, respectively. Frequencies of pregnancy and metritis were analyzed by Chi-squared test. The same analysis was applied to frequencies of anoestrus and ovarian dysfunction in groups L and H [28, 29].

Ammonia in ruminal liquor was used only as a descriptive measurement due to the small number of fistulated animals.
3. RESULTS

3.1. Experiment I

There was no effect of diet throughout the experimental period on milk production, body condition score, serum albumin (Figure 1), serum urea (Figure 2), SNF or butterfat production.

Body condition score tended to improve immediately after calving in group L and after 50 days post-partum in group H. There was a slight increment in milk production during the second month of lactation in group H. Milk production declined steadily in both groups during the 3rd month and remained stable after the fifth month. Though not statistically different, cows from group L had a

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**FIG. 1.** Milk production (kg/day), body condition score and serum albumin (g/100 ml) in 26 Holstein grazing dairy cows supplemented with concentrates containing high or low protein degradability over 150 days after calving in Experiment I.
tendency towards production of 0.5 - 2.5 kg/day more milk in the first 5 months of lactation than cows in group H. Butterfat production paralleled milk production. The percentage of SNF increased only after milk production declined, coinciding with an improvement in body condition score.

Serum albumin was lower in both groups between 30 and 50 days after calving coinciding with the period of expected low DM intake. Serum urea increased 30 minutes after concentrate consumption and tended to be lower 2 h after concentrate feeding, specially in group L. Urea values were consistently >20 mg/100 ml from 40 days post-partum.

The progesterone profiles revealed a high incidence (73.1%) of ovarian functional disorders and non-detected heats. There were low progesterone concentrations in 2 cows from group L, short cycles (<18 days) in 7 cows from group L and 1 from group H, non-detected heats in 4 cows from group L, and anoestrus in 4 cows from group H and 1 from group L. Regular profiles were obtained in 6 cows from group H and 1 cow from group L. However, reproductive parameters measured were not significantly different between groups H and L (Table III).

3.2. Experiment II

Mean concentration of serum albumin was 3.2 g/100 ml during the experiment and without differences between herds. Serum urea was significantly higher in Herd 1 than in Herd 2 during the first 50 days post-partum (P <0.05, Figure 3). Ammonia in ruminal liquor was higher in cannulated cows during grazing in Herd 1 compared to cows in Herd 2 (17 vs 4.2 mg/100 ml and 12 vs 8 mg/100 ml, 60 min after concentrate feeding, in the two sampling dates, respectively). Selenium treatment increased glutathione peroxidase activity in both herds (Figure 4).
TABLE III. MEAN VALUES OF REPRODUCTIVE PARAMETERS AND INTER-QUARTILE RANGES OF
HOLSTEIN GRAZING COWS SUPPLEMENTED WITH CONCENTRATES CONTAINING HIGH (H, n = 13)
OR LOW (L, n = 13) PROTEIN FRACTION DEGRADABILITY IN EXPERIMENT I

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Diet H</th>
<th>Diet L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calving to first AI (d)</td>
<td>Mean 74</td>
<td>Mean 78</td>
</tr>
<tr>
<td></td>
<td>Quartiles 54 - 82</td>
<td>Quartiles 66 - 101</td>
</tr>
<tr>
<td>Days open (d)</td>
<td>106</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>70 - 130</td>
<td>102 - 130</td>
</tr>
<tr>
<td>Calving to uterine involution (d)</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>20 - 45</td>
<td>22 - 44</td>
</tr>
<tr>
<td>Calving to first ovulation (d)</td>
<td>59</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>35 - 82</td>
<td>27 - 62</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>58.3</td>
<td>38.5</td>
</tr>
<tr>
<td>Services per pregnancy (n)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Endometritis (%)</td>
<td>23</td>
<td>47</td>
</tr>
</tbody>
</table>

FIG. 3. Serum urea (mg/100 ml) in Holstein grazing dairy cows supplemented with corn grain two (Herd 1) or four (Herd 2) times per day in Experiment II (Values bearing different letters are significantly different, P > 0.05).

Body condition score was 3 ± 0.25 in both herds at calving, and decreased to 1.75 ± 0.25 in Herd 1 and to 2.25 ± 0.25 in Herd 2, 45 days after calving. This level was maintained until the end of the experiment, possibly due to a higher energy demands for walking long distance to reach the pastures.

Total milk and butterfat production were lower in Herd 1 than in herd 2 (6406.2 ± 894.7 kg vs 6893.8 ± 822.6 kg and 190.4 ± 28.1 kg vs 203.5 ± 20.5 kg, respectively, P < 0.02).

Oestrous detection was more efficient in Herd 1 than in Herd 2 (59.3 vs 48.7%, P < 0.05). This is reflected in the shorter calving to first observed oestrous interval (44.7 ± 24.5 vs 61.4 ± 28.5 days, P < 0.05), and in the lower number of services per conception (1.7 vs 2.2, P < 0.05). The overall percentage of pregnancy was similar in both groups (92 vs 84%).

Selenium treated cows had an apparent lower incidence of puerperal metritis (6.6 vs 13.7%), assisted calvings (13.5 vs 17.5%) and stillbirths (3.9 vs 5%). However, neither of these differences were significant, nor was the difference in percentage of cows having at least one teat with a WMT score equivalent to 800,000 cells/mm³ (33 vs 42% and 33 vs 17%, in the treated and control groups, respectively regarding the two sampling dates).
The downer cow syndrome was present in 5% of the controls but none of the treated cows (P < 0.05). Calcium levels were within the expected physiological range around parturition and declined steadily to 6.7 mg/ml at 135 days after calving in treated and control cows in both herds.

4. DISCUSSION

The low milk, butterfat and SNF production in the first 2 months after calving can be attributed to the low body reserves at calving and to a deficit in DM intake. Though calculations indicated that energy requirements were met (Table II), we assumed that DM intake from the pasture was overestimated considering the low SNF levels, which are more related to energy than protein availability [32].

In the Experiment I, the whole dietary CP content was in excess compared with requirements both before and after the peak of lactation at 50 days post-calving (Table II). Due to the high percentage of CP in the pasture, and its high degradability, almost 93.5% of the DP requirement was provided from pasture intake (Table II). The DP would have been in excess even if the pasture intake had been lower than the estimated 7.5 kg DM/cow/day. The energy wastage for urea detoxification may have deepened the negative energy balance and worsened the metabolic situation. Under these conditions, the undegradable protein in concentrate L plus the high amounts of degradable protein from the other foodstuffs, had no effect on serum urea concentrations. Serum urea values below 20 g/100 ml were only found at calving, as a result of the low DM intake before calving.

Despite of undegradable protein supplementation, cows that produced more milk, did not resume normal reproductive activity. This finding supports the view that there was a lack of energy in the diet. Improvement in energy balance from the nadir during the first weeks after calving provides an important signal for the initiation of ovarian activity, with an increase in LH pulse frequency 11-16 days later, leading to the first ovulation [33]. The low reproductive performance in both groups seemed not to be due to the amount of degradable protein, but to the lack of energy in the diets. Results from this experiment do not support the hypothesis that an excess of degradable protein in the diet influences reproductive performance per se, but through an exacerbation of negative energy balance, when energy yielding nutrients are marginal. Reproductive parameters were not significantly different between groups H and L maybe because of the low number of animals, but the reproductive tract tended to recover from pregnancy sooner, and fertility seemed better in group H than in group L. Low degradable protein had an effect on energy balance because the amino acids supplied in excess in relation to energy yielding nutrients require deamination. There is also an energy waste involved in the oxidization of the keto acid and the conversion of the amino group in urea [6].
The higher BCS, milk and butterfat production in Experiment II suggests that there was a higher amount of energy available than in Experiment I. The lower BCS in Herd 1 may be explained due to the longer distances walked to pastures. The energy wastage seemed enough to produce a difference in body condition and in milk and butterfat production compared with Herd 2.

Higher rumen ammonia levels after grain intake in fistulated cows from Herd 1, suggest that 2 kg of corn grain did not provide enough carbohydrate in the rumen to increase bacterial protein synthesis (from the ammonia produced by protein degradation), or that cows fed 2 kg corn grain consumed more grass immediately after concentrate administration. Any of these reasons could have caused the higher serum urea values between 30 and 50 days after calving in cows from Herd 1, and hence could have deepened the energy deficit in those animals. Serum urea levels did not differ between Herds 1 and 2 during the breeding season, so, differences found in reproductive performance could not be attributed to a toxic effect of blood nitrogen compounds.

Increments in total milk and butterfat production following selenium treatment in goats have been reported [34]. However, in this study differences were evident only during some months under certain conditions (e.g., parity and month of calving). This was not related to the incidence of subclinical mastitis as no difference occurred between selenium-treated and control cows.

Prevention of the downer cow syndrome with selenium treatment could have been the result of a better magnesium absorption or due to a prevention of a subclinical myopathy [35].

Selenium injection 17 days before calving in Experiment II did not improve reproductive performance. The only exception was the pregnancy rate at first insemination in Herd 2, as described elsewhere [36]. This benefit was not seen in Herd 1, indicating the influence of other factors which cannot be identified in this experiment [9].

The lack of statistical differences in the percentages of stillbirths and metritis may have resulted from the proximity of selenium injection to the calving date.

5. CONCLUSIONS

Grazing dairy herds are subjected to variations in forage availability that can jeopardize efforts to maximize milk production and reproductive efficiency in spite of protein supplementation. Under these conditions, and considering the decrease in forage intake shortly after calving and certain limitations in the introduction of some components in the concentrate (economy, palatability and energy content), the increase in the proportion of undegraded protein in the concentrate which can be attained does not solve the problem of low percentages of SNF and milk protein, and produces no benefit on reproductive performance. The results of the first experiment supports the hypothesis that the addition of rumen-undegradable protein may deepen the negative energy balance when energy yielding nutrients are low or marginal in relation with the requirements.

Further efforts to decrease serum urea should be achieved by other means, rather than supplementing the concentrate several times a day while the animals are grazing.

Supplementation of selenium 15 to 30 days before calving in selenium-deficient herds decreased the risk of the clinical development of the downer cow syndrome and may improve pregnancy rate at first insemination.

ACKNOWLEDGEMENTS

The authors wish to thank Drs. Y. Folman and H. Dobson for the discussion and correction of the manuscript; to the owners and staff of La Corona in Carlos Casares, and Los Alamos farms in Alsina, Bs. As. Province for their invaluable help in the fulfillment of the experiences; to Drs. G. Ramos and L. Marangunic for their guidance in the statistic analysis of the data, and to Dr. B. Ruksan ans Messrs. A. Zimmerman, H. Huertas and A. Cerutti for the determination of metabolite concentrations in blood samples.
REFERENCES


FACTORS AFFECTING THE REPRODUCTIVE PERFORMANCE IN NELORE CATTLE RAISED IN THE HUMID TROPICAL AMAZON REGION

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Abstract - Resumen

FACTORS AFFECTING THE REPRODUCTIVE PERFORMANCE IN NELORE CATTLE RAISED IN THE HUMID TROPICAL AMAZON REGION.

Onset of puberty and postpartum ovarian activity were studied in Nelore cattle. A group of 10 heifers and 11 cows reared under extensive management, and 8 heifers reared under improved nutritional management in a private farm in the humid tropical Amazon region were used. Sequential rectal examinations were performed once or twice a week to assess morphological changes of the uterus and the development of ovarian structures. Visual observation of oestrous signs with the aid of a teaser bull was undertaken twice daily. Weekly blood samples were collected to monitor plasma progesterone profiles. The age and body weight at puberty were 646 ± 69 days and 364 ± 34 kg, and 760 ± 35 days and 316 ± 18 kg, for heifers under improved nutrition and extensive management, respectively (P <0.01). The age at first calving was 920 ± 68 and 1044 ± 34 days for heifers in the same groups mentioned above, respectively (P <0.01). The intervals from calving to a complete clinical uterine involution were 40 ± 45 days, to the first oestrus was 106 ± 45 days and to conception was 191 ± 83 days; and the calving interval was 478 ± 72 days. The results indicate that the reproductive performance of Nelore animals can be improved through management and nutrition.

FACTORES QUE AFECTAN EL COMPORTAMIENTO REPRODUCTIVO DEL GANADO NELORE CRIADO EN LAS CONDICIONES DE LA AMAZONIA TROPICAL HUMEDA.

Se estudió el inicio de la pubertad y la actividad ovárica post-parto del ganado Nelore. Se utilizó un grupo de 10 vaquillas y 11 vacas criadas en condiciones de manejo extensivo y un grupo de 8 vaquillas criadas en un sistema de alimentación mejorada. Los animales pertenecían a una finca comercial de la Amazonía tropical húmeda. Exámenes rectales periódicos se realizaron una o dos veces por semana para evaluar los cambios morfológicos del útero y el desarrollo de estructuras ováricas. La detección del estro se hizo dos veces por día mediante observación visual con ayuda de un toro celador. Se recolectaron muestras de sangre en forma semanal para hacer un seguimiento del perfil de progesterona plasmática. La edad y el peso corporal a la pubertad fueron de 646 ± 69 días y 364 ± 34 kg; y 760 ± 35 días y 316 ± 18 kg, en vaquillas criadas con una alimentación mejorada o en un sistema de manejo extensivo, respectivamente (P <0.01). La edad al primer parto fue de 920 ± 68 y 1044 ± 34 días para las vaquillas de los grupos arriba indicados, respectivamente (P <0.01). El intervalo entre el parto a la involución uterina fue de 40 ± 45 días, al primer estro fue de 106 ± 45 días y a la concepción fue de 191 ± 83 días. El intervalo entre partos fue de 478 ± 72 días. Los resultados indican que el comportamiento reproductivo de los animales Nelore puede ser mejorado a través de cambios en el manejo y la alimentación.

1. INTRODUCTION

Nelore (Bos indicus) is the most important Zebu breed in tropical areas of South America. This breed was introduced into America in 1878, imported from the zoological gardens of Hamburg and London by a farmer from Rio de Janeiro, Brazil [1].

Many other Zebu breeds have been introduced in South America, but the Nelore type is still the predominant most profitable breed. These animals have been disseminated through most of America and other continents [1]. Nowadays, Brazil has a cattle population of 145 million head, 70% of which are Nelore or other Zebu types [2].

The Amazon region is well-known for its high tropical temperature and humid environmental conditions. Bos indicus cattle have proved to be the most adapted and most used species for beef production in the region. However, its potential for meat and milk production is lower when compared...
to *Bos taurus* breeds reared in temperate areas. In addition, Zebu breeds mature late, produce less milk and often only let-down the milk in the presence of their calves.

Zebu breeds have been reported to show inherent poor reproductive performance, especially under extensive grazing conditions. However, it must be taking into account that environmental, nutritional and management conditions play important roles on the reproductive phenomena [3, 4].

An important requirement for a profitable beef farm is to shorten the period from birth to puberty and to decrease the period of post-partum anoestrous. Unfortunately, there are several factors affecting the onset of puberty and the post-partum reproductive performance of the Zebu. Among the most important factors, inadequate management practices, poor nutrition and health disturbances play a major role [3, 4, 5].

It is generally accepted that the age of puberty occurs 6 to 12 months later in *Bos indicus* than in *Bos taurus* breeds. Furthermore, grazing cattle are likely to suffer from nutritional deficiencies and to be more susceptible to loss of body weight and body condition during the dry season, therefore, resulting slower growth rates, extended age at puberty, delayed uterine involution, longer post-partum anoestrous and reduced fertility [3, 4, 5, 6, 7, 8].

The aim of this study was to establish basic parameters of the reproductive performance of young Nelore females reaching puberty under an intensive management rearing system and the post-partum period of cows under extensive farming conditions.

2. MATERIAL AND METHODS

2.1. Location

The farm was located in the Northern region of Para State, Brazil, situated at 1° 30' S, 48° 30' W and was representative of cattle farms in the region, established through deforestation of the native Amazon jungle and its replacement by native or cultivated grasses. The climate is characterized by two main seasons, rainy (December to June) and dry (July to November). Climatic conditions were: Mean temperature, 26.9 and 28.1°C; mean relative humidity, 86.8 and 76.3%; and mean seasonal rainfall, 2286 and 525 mm in the rainy and dry season, respectively.

2.2. Animals and feeds

Group 1 consisted of eight heifers which were reared under an improved nutritional management. These animals were given cut Napier grass (*Pennicetum purpureum*) ad libitum and 3 kg/animal/day of commercial supplement containing 22% crude protein. A second group of 10 Nelore heifers and 11 pluriparous cows ranging from 6 - 11 years of age was kept under extensive grazing conditions on *Brachiaria humidicola* grass. Both groups had free access to a mineral mixture.

2.3. Measurements

The experiment was carried out over a period of 630 days. The heifers were 12 - 15 months old at the commencement of the experiment. Cows entered in the experiment 10 days after parturition. Body weight and body condition score (scale 0 - 5 [9]) was performed either twice or four times a month. Calves were kept with their dams, and suckling was allowed without restriction for eight months after calving.

Visual observation for signs of oestrus was carried out twice a day from 6.00 - 8.00 and 17.00 - 19.00. Oestrus was considered to have occurred when a female accepted mounting by a teaser bull or another female. Weekly blood samples were collected from all animals for monitoring progesterone profiles until heifers became pregnant or cows reassumed ovarian activity and showed regular oestrous behaviour. Blood samples were analyzed for progesterone using the FAO/IAEA RIA kit [10]. Rectal examinations were performed once per week to assess morphological changes of the uterus and ovaries. The presence of ovarian follicles and corpora lutea were recorded. The animals were subjected to
artificial insemination with frozen semen for up to two heats, and thereafter, mated by a bull if conception did not occur. Heifers were served when the reach 290 kg body weight or were than 20 months of age. Pregnancy was determined by rectal palpation in cows that had not return to oestrus 40 days after mating, and confirmed by plasma progesterone values. It was considered that ovarian cyclic activity had begun when plasma progesterone values were above 3 nmol/L in at least two consecutive samples.

Animals were subjected to the health programme of the herd. They were vaccinated against foot and mouth disease, Brucellosis and dewormed three times a year.

2.3 Statistical procedure

Data were analyzed by the Student "t" test, regression and Pearson correlation analyses using a computer statistical package.

3. RESULTS AND DISCUSSION

3.1. Puberty

Progesterone peaks, usually lower than 3.0 nmol/L were frequently observed prior to first ovulation in both groups of heifers. Figure 1 illustrates the progesterone profile of four heifers from puberty to conception.

The age at puberty was 646.0 ± 69.7 and 760.2 ± 34.7 days for Groups 1 and 2, respectively (P <0.01). Heifers in the present study reached puberty at a younger age than those reported in Brazil for heifers of this breed that were raised on extensive grazing [11]. Reports from elsewhere indicate puberty occurs at a later age in Zebu cattle [3, 7, 11].

![FIG. 1. Progesterone profiles from before puberty until conception or regular oestrous cyclicity in Nelore heifers reared under improved nutrition (heifers A, B and C) and extensive management (heifer D) in the Amazon region.](image-url)
TABLE I. BODY WEIGHT, AGE AT PUBERTY AND AGE AT FIRST CALVING IN NELORE HEIFERS REARED UNDER IMPROVED NUTRITION AND EXTENSIVE MANAGEMENT CONDITIONS IN THE AMAZON REGION (x ± sd).

<table>
<thead>
<tr>
<th>Group</th>
<th>Heifers (n)</th>
<th>Body weight at puberty (kg)</th>
<th>Age at puberty (days)</th>
<th>Age at first calving (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved nutrition</td>
<td>8</td>
<td>364.0 ± 33.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>646.0 ± 69.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>920.4 ± 68.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extensive management</td>
<td>10</td>
<td>315.8 ± 17.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>760.2 ± 34.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,044.4 ± 34.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b</sup> Column values bearing different superscripts are significantly different (P <0.01).

The body weight at puberty was 364.0 ± 33.8 and 315.8 ± 17.6 kg in Group 1 and 2, respectively (Table I, P <0.01). These weights were lower than others reported for Nelore [6], Gyr and Indobrazil heifers [11, 12, 13]. Body condition at puberty varied between 3 - 4 in heifers on improved management and between 2 - 4 in heifers raised on extensive conditions. The major factor controlling the onset of puberty is the body development (body weight), and this is directly affected by nutrition. It is well documented that age at puberty is highly correlated with growth and body weight [3, 7, 13, 14].

The length of the first and subsequent oestrous cycles was 19.2 ± 2.8 (range: 17 - 21) days in Group 1 and 20.3 ± 1.8 (range: 17 - 21) days in Group 2. No significant statistical differences were found between groups (P >0.05), as it is reported in the literature [6, 7]. Progesterone levels varied from < 1 nmol/L during the follicular phase to 20 nmol/L during the luteal phase. Values were even higher during the early stages of pregnancy. These values were higher than previously reported [12, 15]. The number of inseminations per conception was 1.8.

The age at the first calving was 920.4 ± 68.1 and 1044.4 ± 34.4 days in Groups 1 and 2, respectively (P <0.01), and lower than those reported in other regions [12, 15]. A significant correlation was observed between body weight at puberty and age at puberty (r = 0.50, P <0.05) and body weight at puberty and age of first calving (r = 0.53, P <0.05). There was also a significant relationship (P <0.01) between body weight at puberty, age at puberty and age of first calving which could be described by the following equations:

\[
\text{Age at puberty: } y = 1402.58 + (-1.26.x) \\
\text{Age at first calving: } y = 1070.87 + (-1.11.x)
\]

where 'x' is body weight at puberty.

Figure 1 illustrates the progesterone pattern of four heifers between puberty and conception. A common situation was the occurrence of unobserved heats. There were also a few cases of anoestrous periods after puberty.

3.2. Post-partum period

Table II shows the intervals from calving to complete clinical uterine involution, to first observed oestrus, to conception and the interval between two calvings. Uterine involution occurred on average at 40 ± 45 (range: 21 - 83) days after calving. Although the importance of the uterine involution on the resumption of ovarian function after parturition has been suggested for many years, there are few data which demonstrate this phenomena in the post-partum Zebu cow [15]. The first oestrus occurred 106 ± 45 (range: 58 - 198) days after calving while conception occurred at 191 ± 83 (range: 115 - 276) days after calving.

The number of inseminations per conception was 1.6. The calving interval of 478 ± 72 days obtained in the present study is similar to other values reported for Zebu breeds in Latin America, which are also lower than in other tropical areas [5, 7, 11].

Figure 2 illustrates the progesterone pattern of three cows during the post-partum period. The length of the anoestrous period varied between the animals, and oestrus was not always well detected.

160
TABLE II. REPRODUCTIVE PARAMETERS IN NELORE COWS REARED UNDER EXTENSIVE MANAGEMENT CONDITIONS IN THE AMAZON REGION.

<table>
<thead>
<tr>
<th>Cow</th>
<th>Uterine involution (days)</th>
<th>Calving to first oestrus (days)</th>
<th>Calving to conception (days)</th>
<th>Calving interval (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>58</td>
<td>166</td>
<td>441</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>63</td>
<td>270</td>
<td>551</td>
</tr>
<tr>
<td>4</td>
<td>83</td>
<td>160</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>198</td>
<td>198</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>119</td>
<td>276</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>108</td>
<td>129</td>
<td>412</td>
</tr>
<tr>
<td>8</td>
<td>21</td>
<td>136</td>
<td>156</td>
<td>486</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>59</td>
<td>126</td>
<td>405</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>67</td>
<td>338</td>
<td>615</td>
</tr>
<tr>
<td>11</td>
<td>21</td>
<td>115</td>
<td>115</td>
<td>438</td>
</tr>
</tbody>
</table>

\[ \bar{x} \pm sd \quad 40.5 \pm 45 \quad 105.8 \pm 45 \quad 191.5 \pm 83 \quad 478.4 \pm 72 \]

**FIG. 2. Post-partum progesterone profiles in multiparous Nelore cows reared under extensive management conditions in the Amazon region.**

One animal developed a follicular cyst-like structure and was successfully treated with prostaglandins. Although more than half of the cows began cycling within 90 days after calving, it took nearly three further months on average for pregnancy to be achieved.

It is possible that the grazing pastures were too fibrous in the dry season, therefore with poor protein content. Inadequate supply of protein or roughage of poor quality adversely affects the
development of heifers and may result in late onset of puberty. In post-partum cows, the effects of inadequate protein are slower uterine involution and delayed post-partum ovarian activity [3, 7, 11, 14, 16].

Rectal palpation findings and progesterone values showed a reasonable correlation and an accuracy of nearly 75%. However, there was a large variation among individual palpators.

4. CONCLUSIONS

The results indicate that the reproductive performance of Nelore animals can be improved through management and nutrition.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Fazenda Itaqui Agropecuaria for providing the animals and facilities for this study. Thanks are also expressed to the Brazilian Council of Science and Technology and to the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for partial financial support of the study.

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EFFECT OF ENERGY AND PROTEIN LEVELS ON HEALTH, GROWTH, PUBERTY AND SEMEN QUALITY OF HOLSTEIN BULL-CALVES UNDERGOING PROGENY TESTING


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Abstract – Resumen

EFFECT OF ENERGY AND PROTEIN LEVELS ON HEALTH, GROWTH, PUBERTY AND SEMEN QUALITY OF HOLSTEIN BULL-CALVES UNDERGOING PROGENY TESTING.

Three energy and protein levels in the ration fed potential artificial insemination sires during the first year of age were evaluated with respect to health, body and testicular growth rate, puberty and sperm quality of the animals. Treatments were, Group I, NRC requirements for energy and protein for growing dairy bulls with daily weight gains from 0.4 to 0.9 kg/day; and Groups II and III which were fed 15 and 30% over the recommended energy and protein requirements, respectively. Metabolic weights \( W_{75} \) at one year of age were 81.2, 89.6, and 86.5 kg for groups I, II and III, respectively (\( P < 0.05 \)). Blood metabolites were found within the reference range. Plasma Cu and Zn were lower in Groups I and III as compared to Group II. Histological studies and gross weights of the thyroid gland were compatible with hypofunction of the glands in animals from Groups I and III.

In Group III, 60% of animals showed abundant fat deposition in testicles. Normal fibroelastic consistency of testicles was found in 30, 80, and 20% of animals in Groups I, II, and III, respectively, at 365 days of age. Libido was present at the end of the experiment in 50, 100 and 70% of animals in Groups I, II and III, respectively. Testosterone levels were influenced by age (\( P < 0.01 \)). In that levels were higher at 148 and 258 days of age (13.88 and 16.59 nmol/L). The amount of testosterone produced during the experimental period was significantly different for all groups (\( P < 0.001 \)). The area under the curve was 641.5, 982.9 and 462.2 for Groups I, II and III, respectively. Volume, motility and percentage of living spermatozoa increased with age (\( P < 0.05 \)) while total morphological alterations decreased significantly. Animals in Group II had the best sperm production. Histological evaluation revealed 80 and 100% of testicular degeneration in animals from Groups I and III, respectively. The application of diet in Group II is recommended to be used in specialized AI bull rearing units in Cuba according to the quantities and characteristics evaluated in this design.

EFECTO DE NIVELES DE PROTEINA Y ENERGÍA EN LA SALUD, CRECIMIENTO, PUBERTAD Y CALIDAD SEMINAL DE TOROS HOLSTEIN JOVENES SOMETIDOS A PRUEBA DE PROGENIE.

Se evaluaron los efectos de tres niveles de energía y proteína en las dietas de toros Holstein jóvenes para inseminación artificial durante su primer año de vida sobre salud, tasa de crecimiento corporal y testicular, pubertad y calidad espermática. Los tratamientos fueron: Grupo I, con los requerimientos de energía y proteína del NRC para toros de leche y en crecimiento, con ganancias de peso entre 0.4 a 0.9 kg/día; y los Grupos II y III que fueron alimentados por encima del 15 y el 30% de los requerimientos recomendados de energía y proteína. Los pesos metabólicos \( W_{75} \) al año de edad fueron de 81.2, 89.6 y 86.5 kg para los grupos I, II y III, respectivamente (\( P < 0.05 \)). Los niveles plasmáticos de Cu y Zn en los Grupos I y III fueron más bajos que aquellos en el Grupo II. Los resultados de la aplicación del diet en Group II is recommended to be used in specialized AI bull rearing units in Cuba according to the quantities and characteristics evaluated in this design.

Efectos de tres niveles de energía y proteína en las dietas de toros Holstein jóvenes para inseminación artificial durante su primer año de vida sobre salud, tasa de crecimiento corporal y testicular, pubertad y calidad espermática. Los tratamientos fueron: Grupo I, con los requerimientos de energía y proteína del NRC para toros de leche y en crecimiento, con ganancias de peso entre 0.4 a 0.9 kg/día; y los Grupos II y III que fueron alimentados por encima del 15 y el 30% de los requerimientos recomendados de energía y proteína. Los pesos metabólicos \( W_{75} \) al año de edad fueron de 81.2, 89.6 y 86.5 kg para los grupos I, II y III, respectivamente (\( P < 0.05 \)). Los metabolitos sanguíneos se encontraron dentro de los rangos referenciales. Los niveles plasmáticos de Cu y Zn en los Grupos I y III fueron más bajos que aquellos en el Grupo II. Los resultados de la evaluación histológica y de los pesos de la glándula tiroides fueron compatibles con una hipofunción de las glándulas en los Grupos I y III.

En el Grupo III el 60% de los animales tuvo un exceso de depósito graso en los testículos. Se encontró consistencia fibroelástica normal en el 30, 80 y 20% en los testículos de los animales en los Grupos I, II y III, respectivamente, al año de edad. La libido se presentó al final del experimento en el 50, 100 y 70% de los animales en los Grupos I, II y III, respectivamente. Los niveles de testosterona fueron afectados por la edad del animal (\( P < 0.01 \)). Los mayores niveles se encontraron a los 148 y 258 días de edad (13.88 y 16.59 nmol/L). La cantidad de testosterona producida durante todo el periodo experimental fue significativamente diferente entre los grupos (\( P < 0.001 \)). El área bajo la curva fue de 641.5, 982.9 y 462.2 para los Grupos I, II y III, respectivamente. El volumen, la motilidad y el porcentaje de espermatozoides vivos aumentó con la edad (\( P < 0.05 \)) en tanto que las alteraciones morfológicas disminuyeron. Los animales del Grupo II tuvieron la mejor calidad espermática. Las evaluaciones histológicas revelaron un 80 y 100% de degeneración testicular en animales de los Grupos I y III, respectivamente. Se recomienda la utilización de la dieta del Grupo II para la crianza de toros jóvenes a ser utilizados en Centros de Inseminación Artificial en Cuba, de acuerdo a las cantidades y las características del presente diseño.
1. INTRODUCTION

It has been reported that the principal causes of failure of acceptance of potential bulls in artificial insemination (AI) centres in Cuba are testicular alterations, many of them related to inadequate feeding system (1). Another study [2] reported that artificial insemination (AI) bull-calves that were fed excessively during the first year of life presented a number of metabolic alterations such as severe liver and thyroid gland damage, low plasma and hepatic levels of Cu, small testes, lack of testicular consistency, excessive fat deposition and testicular germinal epithelium degeneration.

The aim of the present study was to compare three energy and protein levels in the feed of potential AI sires during the first year of age and to identify a suitable levels of energy and protein to allow adequate body growth and sperm production.

2. MATERIALS AND METHODS

Thirty healthy Holstein Friesian male calves from birth to one year of age were used. They were divided into three groups: Group I, fed energy and protein requirements for growing dairy males with daily weight gains from 0.4 to 0.9 kg/day; and Groups II and III, were fed 15 and 30% respectively, over the recommended energy and protein requirements. The basal diet was formulated according to international recommendations [3]. The concentrate feed had 86% dry matter (DM), 19.23% crude protein (CP) and 11.46 Mj/kg DM. The quantity of feed offered throughout the experiment is shown in Table I.

Concentrate feed and weekly milk samples were collected for bromatological analysis and aflatoxin M1 (AfM1) determinations. Green forage and hay samples were also collected.

Na and K were determined by flame spectrophotometry (FLM-2 adjusted for milk); P was colorimetrically determined by the molybdate-manadate method with previous acid digestion; Ca, Mg, Cu and Zn were determined by atomic absorption spectrophotometry [4]. Crude protein (CP) and crude fiber (CF) were determined by the Kjeldahl and Weende methods [5].

Blood samples for haematological, chemical and testosterone determinations were collected in the morning hours before feeding on days 10, 28, 59, 90, 110, 148, 203, 258, 313 and 365 of age. Glucose, total lipids, albumin, globulins, urea, Na, K, Ca, Mg, Cu, and Zn were analyzed as reported elsewhere.

TABLE I. FEED COMPOSITION QUANTITIES OFFERED TO THREE GROUPS OF IMMATURE HOLSTEIN FRIESIAN BULL CALVES. Ration I met the energy and protein requirements for growth. Rations II and III exceeded 15 and 30% of the basal energy and protein requirements

<table>
<thead>
<tr>
<th>Group</th>
<th>Feed</th>
<th>Age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>11-39-81-101-121-176-231-286-341-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38 80 100 120 175 230 285 340 395</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Feed</th>
<th>Age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Milk (L)</td>
<td>5.00 5.0 4.0 4.0 2.0 -- -- --</td>
</tr>
<tr>
<td></td>
<td>Concentrate (kg)</td>
<td>0.05 0.4 0.7 1.2 1.0 2.5 2.8 3.0 3.8</td>
</tr>
<tr>
<td></td>
<td>Hay (kg)</td>
<td>0.05 0.5 1.0 1.0 1.5 1.5 1.5 1.5 2.0</td>
</tr>
<tr>
<td></td>
<td>Forage (kg)</td>
<td>0.10 0.5 1.0 1.0 1.0 3.0 5.0 10.0 12.0 15.0</td>
</tr>
<tr>
<td>II</td>
<td>Milk (L)</td>
<td>5.0 5.0 6.0 4.0 2.0 -- -- --</td>
</tr>
<tr>
<td></td>
<td>Concentrate (kg)</td>
<td>0.1 0.5 1.0 1.5 2.0 2.7 3.4 3.8 4.0</td>
</tr>
<tr>
<td></td>
<td>Hay (kg)</td>
<td>0.2 0.5 1.0 1.0 1.5 1.8 1.5 1.5 2.0</td>
</tr>
<tr>
<td></td>
<td>Forage (kg)</td>
<td>0.2 0.5 1.0 2.0 2.0 4.0 8.0 12.0 13.0 16.0</td>
</tr>
<tr>
<td>III</td>
<td>Milk (L)</td>
<td>6.5 6.5 7.0 6.0 2.0 -- -- --</td>
</tr>
<tr>
<td></td>
<td>Concentrate (kg)</td>
<td>0.1 0.5 1.2 1.5 2.3 3.0 4.0 4.5 5.0</td>
</tr>
<tr>
<td></td>
<td>Hay (kg)</td>
<td>0.1 0.5 1.0 1.0 1.5 1.5 1.5 2.0 2.0</td>
</tr>
<tr>
<td></td>
<td>Forage (kg)</td>
<td>0.1 0.5 0.5 2.0 6.0 10.0 12.0 12.0 14.0</td>
</tr>
</tbody>
</table>
testosterone concentrations were determined by radioimmunoassay (RIA) [6]; protein-bound iodine (PBI) by a colorimetric method [7]. Glutamate dehydrogenase activity levels (GLDH) and aspartate amino-transferase (ASAT) were also determined [8].

Body weight and testicular measurements were recorded the same days of blood sampling. Metabolic weight (body weight$^{0.75}$) was calculated for each animal. Sexual behaviour, onset of puberty and characteristics of reproductive accessory glands, were studied. Semen samples were weekly collected after puberty, and volume, density, motility, live sperm, concentration and sperm abnormalities were analyzed according to the methodology described elsewhere [9]. Clinical examinations of testes and epididymis were performed by rectal palpation beginning at five months of age.

Five bulls per treatment were randomly selected and slaughtered at 13 months of age. Macroscopic evaluations of organs were performed. Six samples were taken from each testicle and 100 seminiferous tubules per sample were studied. The diameter, membrane and cellular characteristics of spermatogenic layers were recorded. Samples of epididymis, ampulla, prostate, seminal vesicle, liver and thyroid gland were also collected and studied microscopically. Standard staining techniques (Best's Carmine for glycogen, Oil Red for lipids, Van Giesson's for collagen fibers and Gomori's for reticulin) were used.

Statistical analysis of the results were done by the Discriminant Test and General Linear Model (GLM) [10] using age, treatment and their interactions as effect factors, according to the model:

$$Y_{ijk} = \mu + A_i + T_j + (A \times T)_j + e_{ijk}$$

where $Y_{ijk}$ = nutrients (DM, ME, CP, CF......), haematological and chemical indicators (glucose, protein ..), body and testicular measurements, and sperm parameters.

$\mu$ = least squares mean

$A_{10}^{1365}$ = effect of the ith age (10, 28, 59......365 days)

$T_j$ = effect of the treatment (diet) jth diet ( I, II and II)

$(A \times T)_j$ = interactions

$e_{ijk}$ = a random error effect

Testosterone secreted during the entire period in each treatment was calculated as the area below curve, according to Simpson’s rule and mean value for each treatment group were compared by the Student’s “t” test.

3. RESULTS AND DISCUSSION

Nutrient content of the offered feed is shown in Table II. Protein, fat, Ca, P, Mg, Cu and Zn concentrations in milk were low and K was high as reported by other authors [11,12]. Zn and Cu contents varied in the concentrate, but energy and protein concentrations were more stable than previously found [13]. The nutritive value of green forage was satisfactory but showed low levels of Cu and Zn, as previously reported [14], while in hay, CP, CF and microelements were as stated in the literature [15].

Metabolic weights (W$^{0.75}$) at birth were similar between groups. However, animals in Group I showed lower weight gain than the other two groups throughout the experiment. After weaning, metabolic weight gain was higher in animals of Group II (Fig. 1). Similar performance has been observed in Cuba [16] in calves of the same breed under intensive feeding regimes. Metabolic weights at one year of age were 81.2, 89.6 and 86.51 kg$^{0.75}$ for groups I, II and III, respectively (P <0.05).

Feed consumption was influenced by interaction of age x treatment (P <0.001). The ratio of energy/ protein varied between 1:19 to 1:16 and was considered suitable for dairy sires. Maximum CF consumption occurred in Group II. The ratio between concentrated feed with green forage and hay at the end of the experiment was 56:44, 42:58, and 60:40 for animals in Groups I, II and III, respectively. This ratio in Group II was the most suitable for male Holstein sires under Cuban conditions. Mineral consumption covered most requirements, but the Cu and Zn intake was only adequate in Group II according to reported requirements for cattle [17].
TABLE II. PROXIMATE AND MINERAL ANALYSIS OF FEED COMPONENTS USED TO FEED HOLSTEIN FRIESIAN BULL CALVES. Green forage was composed of *P. purpureum* and *P. thyphoides*. Hay was composed of *C. nlemfluensis*. DM = dry matter, ME = metabolizable energy, CP = crude protein, CF = crude fibre

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Milk</th>
<th>Concentrate</th>
<th>Green forage</th>
<th>Hay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± s.e.</td>
<td>x ± s.e.</td>
<td>x ± s.e.</td>
<td>x ± s.e.</td>
</tr>
<tr>
<td>DM (%)</td>
<td>12.35 ± 0.82</td>
<td>88.40 ± 1.23</td>
<td>20.08 ± 5.36</td>
<td>90.63 ± 1.53</td>
</tr>
<tr>
<td>ME (Mj/kg DM)</td>
<td>18.98 ± 0.06</td>
<td>11.84 ± 0.21</td>
<td>9.53 ± 0.58</td>
<td>9.20 ± 0.50</td>
</tr>
<tr>
<td>CP (%)</td>
<td>28.00 ± 0.83</td>
<td>19.04 ± 0.98</td>
<td>8.48 ± 1.28</td>
<td>8.00 ± 1.28</td>
</tr>
<tr>
<td>CF (%)</td>
<td>---</td>
<td>2.88 ± 0.47</td>
<td>32.23 ± 2.57</td>
<td>31.55 ± 2.46</td>
</tr>
<tr>
<td>Na (%)</td>
<td>0.40 ± 0.13</td>
<td>0.31 ± 0.11</td>
<td>0.10 ± 0.04</td>
<td>0.22 ± 0.16</td>
</tr>
<tr>
<td>K (%)</td>
<td>1.37 ± 0.13</td>
<td>0.89 ± 0.11</td>
<td>0.79 ± 0.48</td>
<td>1.17 ± 0.53</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.87 ± 0.11</td>
<td>0.90 ± 0.41</td>
<td>0.50 ± 0.13</td>
<td>0.59 ± 0.17</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.88 ± 0.18</td>
<td>0.95 ± 0.18</td>
<td>0.25 ± 0.10</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.07 ± 0.01</td>
<td>0.24 ± 0.07</td>
<td>0.31 ± 0.11</td>
<td>0.29 ± 0.10</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>0.72 ± 0.01</td>
<td>19.29 ± 12.24</td>
<td>21.16 ± 10.31</td>
<td>10.54 ± 2.87</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>21.01 ± 0.09</td>
<td>52.01 ± 30.02</td>
<td>33.20 ± 7.22</td>
<td>35.45 ± 12.37</td>
</tr>
</tbody>
</table>

FIG. 1. Changes in body weights of three groups of 10 Holstein Friesian bull calves fed diets containing minimum energy and protein requirements (Group I) and 15 and 30% excess of energy and protein (Groups II and III respectively). Metabolic weight was calculated as body weight\(^{75}\).

Age and diet affected significantly blood glucose, plasma total lipids, albumin and urea concentrations (P <0.0001). The lowest glucose and total lipids concentrations were found in animals from Group I (P <0.005) and the highest in animals from Group III (P <0.005). Initial high blood glucose values (6.0 ± 0.4 mmol /L) lowered with age. Group II showed higher levels of albumins (P <0.05) but all groups had similar globulin values. Globulins were not a good indicator of nutritional status. Urea concentration was lower in Group II (3.4 ± 0.1 mmol/L), as compared with Groups I and III (4.2 ± 0.4 and 4.3 ± 0.2 mmol/L, respectively). Metabolite concentrations were in the range of values reported by other authors for glucose [18, 19, 20] and for urea [20, 21].

The concentration of some osteotrophic minerals were affected by diet and age. Mg was lower during the first months of age in animals of Group I relative to all other groups. This could be due to the
low concentration of Mg in milk. Nevertheless, there were no differences in Mg content in the bone between groups at twelve months of age. Means values were of 24 ± 1.7, 9.80 ± 0.71 and 0.54 ± 0.07% defatted dry matter for Ca, P and Mg, respectively. Blood concentrations and bone reserves were within reported range of normality for plasma concentrations [22, 23] and for bone reserves [24, 25, 26, 27]. Microelement levels were not affected by the factors studied, but their values were in the lowest reference range limit. Hepatic reserves in Group II were within the reference range, 119.0 ± 12.9 and 171 ± 14.3% defatted dry matter for Cu and Zn, respectively, while in Groups I and III, 66% of the animals were in the critical lower limit according to established values [28].

PBI was affected by diet (P <0.01) and age (P <0.001). Highest values were found during the first month of age probably due to intraplacental iodine transmission and colostrum feeding [29]. PBI decreased to 258 days of age (P <0.05) and to rose again at puberty [30]. Nevertheless, values were within the established reference range [27].

The weights of the thyroid gland in Group II were similar to those reported for animals under adequate nutritional plane [31, 32]. Thyroid gland weights in the other two groups were similar to those reported in animals subject to high nutrition planes [33] (Table III). These authors reported 93% goitre with gland weights twice as much as in animals fed control diets (22.9 ± 8 g vs. 19.9 ± 5 g). Microscopically, thyroid follicles in animals from Group II had uniform normal size cubic epithelium. In the other groups, small and medium size follicles, isolated cell groupings and hyperplastic foci nodules were indicative of gland hypofunction [34]. These findings suggest that the low and high concentrate feeding regimes (Groups I and III) in this study are in close relationship with the aforementioned alterations, although there is a possibility of a primary iodine deficiency.

Alkaline phosphatase (AP) and GLDH plasma activity levels were affected by diet and age (P <0.001). GLDH levels were within the reference range values [35] in animals from Group II (0.89 IU/L). High pathological values were found in calves from Group III (x = 3.02 IU/L), especially during the overfeeding periods. However, histopathological alterations were not found. Higher AP levels were found in Group II compared to the other two groups (58.0 ± 0.8; 73.3 ± 0.8 and 60.3 ± 0.6 IU/L for Groups I, II and III, respectively). High AP levels are indicators of liver damage [36] and high osteoblastic activity [37]. Liver function was considered satisfactory in the study, suggesting high AP activity found in Group II results from bone origin. There is a close relationship between AP levels and biometric values. Groups I and III had the lowest values. In this two groups, 60 and 80 % of the animals also had biometric values below those established as selection parameters for AI sires at 12 months of age in Cuba [38].

Abundant fat deposition was found in testicles of animals from Group III. Live weight values of animals in this group were influenced by fat deposition, and this, together with a possible decrease in protein synthesis and altered digestive alimentary efficiency are believed to have caused the obtained results. A significant interaction between treatment and age was found with respect to Testicular Volume Index (TVI) and biometric epididymal measurements. In both cases, the largest testes were found in calves from Group II. Normal fibroelastic consistency of testicles was found in 30, 80 and 20 % of the

---

**TABLE III. WEIGHT AND DIMENSIONS OF THYROID GLANDS OF HOLSTEIN FRIESIAN BULL CALVES FED DIETS CONTAINING MINIMUM ENERGY AND PROTEIN REQUIREMENTS (GROUP I) AND 15 AND 30% EXCESS OR ENERGY AND PROTEIN (GROUPS II AND III RESPECTIVELY).** Values on relative weights expressed the ratio of thyroid gland weight to body weight.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (g)</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>Relative</td>
<td>Right</td>
</tr>
<tr>
<td>I</td>
<td>27.6b</td>
<td>0.007b</td>
<td>16.7b</td>
</tr>
<tr>
<td>II</td>
<td>17.6a</td>
<td>0.003a</td>
<td>18.1a</td>
</tr>
<tr>
<td>II</td>
<td>26.8b</td>
<td>0.007b</td>
<td>18.6b</td>
</tr>
</tbody>
</table>

Different superscripts within columns are significantly different (P <0.05)
animals in Groups I, II and III, respectively, at 365 days of age. The rest of the animals in Groups I and III had testicles with either soft or hard consistency due to degenerative processes [39]. These conditions have unfavourable prognosis for normal sperm production [40, 41].

Testosterone production was influenced by age (P < 0.001). Secretion pulses up to 1.53 nmol/L were detected in the first months. They significantly increased in amplitude at 148 and 258 days of age (13.88 and 16.59 nmol/L). The amount of testosterone produced during the experimental period was significantly different for all groups (P < 0.001). Areas below the curve were (J(12 f(T) t = 641.5, 982.9 and 462.2 for Groups I, II and III, respectively. The results indicate the best genital development of animals in Group II (Fig. 2), including penis projection, completed glans keratinization and separation from the preputial sac at 226 ± 11 days of age. The first mounting reflexes occurred at 198 days of age. Libido was present by the end of the experimental period in 50, 100, and 70 % of the animals of Groups I, II, and III, respectively. This behaviour may be related to testosterone increases as this hormone plays a role on the growing of reproductive organs and libido [42]. Animals in Groups I and II, that never displayed libido, produced lower levels of testosterone.

It has been suggested [43, 44] that episodic testosterone production raises intratesticular concentration thereby promoting normal spermatogenesis. Table IV shows the differences between groups at the beginning of the production and the characteristics of semen. The best performance occurred in Group II, in agreement with results reported for Holstein males when energy levels were adequate [45].

Age and diet influenced ejaculate and sperm characteristics (Table V, P < 0.001). Volume, motility and percentage of living spermatozoa increased with age (P < 0.05), while total morphological alterations decreased significantly. Volume of ejaculate was not influenced by the diet in agreement with clinical findings on seminal vesicles and ampullae. Nevertheless, other reproductive parameters were better in animals from Group II (P < 0.05) which had fewer alterations in sperm morphology. The most frequent sperm abnormalities in animals of this group were the occurrence of a cytoplasmatic droplet. In the other two groups degenerative shapes including macrocephaly and alterations in the neck and tail were found. These results agree with those reported elsewhere [46, 47, 48, 49].

Unilateral degeneration was predominant in animals of Group I, while bilateral degeneration was more common in Group III (Fig. 3). No alterations were found in animals of Group II. Hyperplastic epithelium and edematization in epididymis with no subepithelial vacuoles were found in animals of Group III. Reduced energy and protein in the diet have been reported to cause a number of pathological alterations, including reduction in the number of ejaculates, testicular size, total sperm production and an increase in total and segmentary spermatozoa alterations [50]. In addition, a reduction in motility,
sperm concentration [51] and a delay in puberty [52] are results of insufficient diet. High energy levels, as in Group III, impaired body growth and productive performance, as reported elsewhere [53, 54, 55, 56].

Reproductive disturbances found in Group III may be due to the effect of insufficient feed on the central nervous system or perhaps a direct effect on the testis. In the first case, an inhibition of the hypothalamic pulse generator of GnRH and consequently, inhibition of LH and FSH release has been proposed [57, 58]. In the second case, two mechanisms have been proposed: An interference in the testicular thermoregulatory mechanisms due to excessive fat deposition [59] with the consequence of an endocrine disturbance produced by aromatization of testosterone to 17β-oestradiol [60]. Alternatively, a direct suppression of testosterone secretion by the Leydig cells [61] may occur. The final result is a lower plasma testosterone concentration, retarded onset of puberty, lack of libido and sperm alterations.

Based on the aforementioned factors, we recommend the application of diet II in specialized AI bull rearing units in Cuba according to the quantities and characteristics evaluated in this design.

TABLE IV. AGE OF ONSET OF PUBERTY, ASSESSED BY THE OCCURRENCE OF EITHER 5.0 x 10^2 SPERM/EJACULATE OR 8.0 x 10^4 SPERM/EJACULATE IN HOLSTEIN FRIESIAN BULL CALVES FED RATIONS AS DESCRIBED IN TABLE I.

<table>
<thead>
<tr>
<th>Groups</th>
<th>5.0 x 10^2 sperm (10% motility)</th>
<th>8.0 x 10^4 sperm (≥80% motility)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± sd</td>
<td>%</td>
</tr>
<tr>
<td>I</td>
<td>332 ± 21</td>
<td>40</td>
</tr>
<tr>
<td>II</td>
<td>283 ± 12</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>345 ± 26</td>
<td>40</td>
</tr>
</tbody>
</table>

TABLE V. CHARACTERISTICS OF SEMEN COLLECTED FROM HOLSTEIN FRIESIAN BULLS BETWEEN 8 AND 12 MONTHS OF AGE.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>8 months</th>
<th>9 months</th>
<th>10 months</th>
<th>11 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± se</td>
<td>x ± se</td>
<td>x ± se</td>
<td>x ± se</td>
<td>x ± se</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>3.1 0.33a</td>
<td>3.3 0.23a</td>
<td>3.7 0.14ab</td>
<td>4.0 0.14b</td>
<td>4.3 0.36b</td>
</tr>
<tr>
<td>Density (%)</td>
<td>18.3 0.03a</td>
<td>37.8 0.03b</td>
<td>48.7 0.04c</td>
<td>54.5 0.33c</td>
<td>57.5 0.03c</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>24.7 0.03a</td>
<td>31.5 0.02*</td>
<td>42.3 0.02b</td>
<td>49.2 0.00b</td>
<td>48.9 0.03c</td>
</tr>
<tr>
<td>Living sperm (%)</td>
<td>38.0 0.05*</td>
<td>50.0 0.03*</td>
<td>63.7 0.07b</td>
<td>68.5 0.02b</td>
<td>29.7 0.01b</td>
</tr>
<tr>
<td>Concent. (x 10^9)</td>
<td>226.4 0.30a</td>
<td>269.9 0.20a</td>
<td>413.7 0.30b</td>
<td>488.8 0.22c</td>
<td>492.0 0.30c</td>
</tr>
<tr>
<td>Total abnorm. (%)</td>
<td>40.5 0.07a</td>
<td>38.9 0.03*</td>
<td>28.3 0.02b</td>
<td>23.4 0.02bc</td>
<td>16.8 0.05c</td>
</tr>
<tr>
<td>Abnormal heads (%)</td>
<td>18.0 0.04a</td>
<td>18.4 0.02*</td>
<td>10.5 0.01b</td>
<td>9.4 0.01b</td>
<td>7.7 0.03b</td>
</tr>
<tr>
<td>Abnormal tails (%)</td>
<td>8.3 0.04a</td>
<td>10.1 0.02*</td>
<td>5.6 0.01b</td>
<td>4.8 0.01bc</td>
<td>2.7 0.03c</td>
</tr>
</tbody>
</table>

Different superscripts within rows are significantly different (P<0.005)
FIG. 3. Examples of testicular degeneration in bull calves fed diets containing 30% excess protein and energy. Panel A shows normal spermatogenic morphology with spermatocytes and spermatids present. Panel B shows tubules in which no development beyond the spermatocyte stage is present. (HE 125×).

REFERENCES


[57] THIBIER, M., ROLLAND, O., The effect of dexamethasone (DXM) on circulating testosterone (T) and luteinizing hormone (LH) in young postpuberal bulls, Theriogenology 5 (1976) 53.


USE OF RADIOIMMUNOASSAY TECHNIQUES TO STUDY THE EFFECTS OF NUTRITIONAL STATUS AND BREED ON REPRODUCTIVE PERFORMANCE OF GOATS

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Centro de Investigaciones para el Mejoramiento Animal, Havana, Cuba

Abstract -- Resumen

USE OF RADIOIMMUNOASSAY TECHNIQUES TO STUDY THE EFFECTS OF NUTRITIONAL STATUS AND BREED ON REPRODUCTIVE PERFORMANCE OF GOATS.

Goats from 2-5 years old in 15 herds of four breeds, (Saanen, Nubian, Toggenburg and Alpine), were used in three experiments to determine reproductive behaviour (Experiment I), metabolic profile (Experiment II) and fertility of induced and synchronized oestrus (Experiment III). Season, breed and physiological status were significant factors affecting reproductive behaviour and metabolic status (P <0.05). The principal causes of infertility in induced oestrus were failures in conception, early embryonic mortality and anovulatory oestrus. The herds had long intervals from birth to first insemination (306-458 days) and to first pregnancy (471-511 days), and low fertility (14-31%). The breeding pattern was similar to breeds in temperate zones. Energy and mineral imbalances were found. Further research is necessary to improve reproductive performance of these breeds under tropical conditions.

EL USO DE LA TECNICA DEL RADIOIMMUNOENSAYO PARA ESTUDIAR EL EFECTO DEL ESTADO NUTRICIONAL Y LA RAZA SOBRE EL COMPORTAMIENTO REPRODUCTIVO EN CABRAS.

Cabras Saanen, Nubian, Toggenburg y Alpina, entre 2 a 5 años de edad, y procedentes de 15 rebaños, fueron utilizadas en tres experimentos. Se estudió el comportamiento reproductivo (Experimento I), perfil metabólico (Experimento II) y la fertilidad en celos inducidos y sincronizados (Experimento III). Los factores época del año, raza y estado fisiológico del animal tuvieron un efecto significativo sobre el comportamiento reproductivo y el estado metabólico de la cabra (P <0.05). Las principales causas de infertilidad en celos inducidos fueron las faltas en la concepción, muerte embrionaria temprana y celos anovulatorios. El intervalo entre el nacimiento y el primer servicio (306-458 días) y la primera gestación (471-511 días) fueron bastante prolongados, y la fertilidad fue baja (14-31%). La estacionalidad reproductiva fue similar a lo observado en razas de zonas templadas. Se encontraron desbalances energéticos y minerales. Se requiere profundizar la investigación a fin de mejorar el comportamiento reproductivo de las cabras criadas bajo condiciones de clima tropical.

1. INTRODUCTION

Small ruminants are important sources of milk and beef in many Latin American countries due to their relative low cost and efficiency of conversion of feedstuffs. The productivity of goats, as well as other livestock species, is limited by inadequate reproductive management practices [1, 2, 3].

Metabolic profiles may be a useful tool to identify metabolic disorders in cattle herds, but unfortunately, this has not been well studied in goats under tropical conditions yet [4, 5]. Useful blood indicators of metabolic activity are currently being sought in goats [6, 7, 8, 9, 10].

The early pregnancy diagnosis in goats can be done by using progesterone assays [11, 12]. Low levels on day 20 after the service are indicative of absence of pregnancy [13], and therefore the technique can be employed to evaluate fertility problems in goats [11, 14, 15, 16].

The objective of the present studies in goats of different genotypes was to evaluate reproductive performance, determine metabolic status and progesterone levels during pregnancy, and evaluate the cause of infertility at synchronized and induced oestrus.

2. MATERIALS AND METHODS.

2.1. Experiment I. Evaluation of reproductive performance

The experiment was carried out in a total of 1992 goats of Saanen (n = 370), Nubian (n = 649), Toggenburg (n = 187) and Alpine (n = 786) breeds. All animals were under semi-intensive management and fed with pasture, supplementary feed (0.9 kg/lactating goat/day and 0.45 kg/dry female/day) and mineral salts with constant access to water over a period of three years.
Oestrus was detected by an observer and vasectomized male twice a day. Females in oestrus were artificially inseminated two times with fresh semen. In the non-breeding season (February-July), groups of animals were oestrus-synchronized at different intervals to maintain milk yield.

Reproductive performance was evaluated retrospectively by means of reproductive indices like age at first service, at first pregnancy, and at first parturition; number of services per pregnancy, distribution of artificial inseminations (AI), parturitions through the years and frequency of abortions.

2.2. Experiment II. Evaluation of metabolic status

The study was carried out in a herd of 1000 2-5 years old goats, 12 km east of Artemisa in La Habana. The herd was subdivided in flocks of 250 females according to breed (Saanen, Nubian, Toggenburg and Alpine) and stocked at 8 goats/ha. All animals were fed with green forage and concentrate. Soya meal and molasses were added during the dry season. Through the year the animals received a mineral supplementation ad libitum in the form of blocks (mixtures I and II, Table I). Another mineral mixture in powder form (mixture III) mixed with phenothiazine at a ratio 1:7, was supplied ad libitum to non-lactating goats more than two months of pregnancy. In addition, goats were allowed to graze approximately 8 h per day.

The study was carried out during the dry (March 1990) and rainy (October 1990) seasons. Blood samples were taken from 20% of the goats in lactation between 7-20 days, from goats in lactation for more than 60 days, and from non-lactating goats. Haematocrit (PCV), hemoglobin (Hb), glucose, total protein, albumin, globulins, Na, K, Ca, Mg, P, Cu, Zn and Fe were determined by visible spectrophotometry, flame photometry and atomic absorption spectrophotometry.

TABLE I. COMPOSITION OF THREE MINERAL MIXTURES SUPPLIED TO SAANEN, NUBIAN, TOGGENBURG AND ALPINE GOATS REARED UNDER SEMI-INTENSIVE MANAGEMENT.

<table>
<thead>
<tr>
<th></th>
<th>Mixture 1</th>
<th>Mixture 2</th>
<th>Mixture 3</th>
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<tbody>
<tr>
<td>Ca (%)</td>
<td>0.10</td>
<td>0.12</td>
<td>0.24</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>P (%)</td>
<td>5.01</td>
<td>2.28</td>
<td>8.47</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>18</td>
<td>353</td>
<td>435</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>31</td>
<td>284</td>
<td>883</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>8</td>
<td>295</td>
<td>314</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>13</td>
<td>3,179</td>
<td>5,010</td>
</tr>
</tbody>
</table>

2.3. Experiment III. Infertility in the synchronized and induced oestrus and the endocrinology of pregnancy in goats

A group of 151 goats of four breeds (Saanen, Nubian, Toggenburg and Alpine) were treated with progesterone (35 mg) on days 1, 4 and 7 and PMSG (300 UI) and PGF$_2$$_a$ on day 9 of the trial. The experiment was carried out during the breeding season (July-February, n = 73 goats) and the non-breeding season (March-June, n = 78 goats). Goats were equally distributed with respect to breed.

Oestrus was detected by observation and by vasectomized males. The animals were inseminated two times 24 and 60 h after PMSG and PGF$_2$$_a$ injection. Vaginal examination and progesterone levels indicated that all goats included in the study were in oestrus at the time of insemination.

Blood samples were taken from animals at AI and 8, 21, 30, 40, 50, 80, 110 days after AI for progesterone analysis. Progesterone was assayed using a solid-phase radioimmunoassay kit [17]. Pregnancy was considered to have occurred when progesterone values were higher than 4.67 nmol/L [11, 18].

The reproductive indices obtained were conception rate, kidding rate, fertilization failure rate, early embryonic death rate, frequency of anovulatory oestrus, frequency of embryonic loss at 21-30 and 30-40 days of AI, and abortion rate.
2.4. **Statistical analysis**

The indices of reproductive performance were analyzed by means of comparative proportion analysis and the results of metabolic profile were analyzed by a General Linear Model procedure (GLM) of the Statistical Analysis System [19]. Dependent variables were the metabolic indicators and the independent ones were the effects of season (rainy and dry), breed (Saanen, Nubian, Toggenburg and Alpine) and physiological status (early lactating, advanced lactating and non-lactating).

3. **RESULTS AND DISCUSSION**

3.1. **Experiment I**

The reproductive performance is shown in Table II. Data indicates a long interval from birth to first insemination and to first pregnancy, low fertility and high abortion rate. The analysis of comparative proportions showed statistical differences between breeds in some reproductive indices ($P < 0.05$). The reproductive performance recorded in the present study is very different to others previously reported which indicate shorter intervals from birth to puberty and pregnancy, and a higher level of fertility [1, 16, 20].

Figure 1 shows the distribution of AIs throughout the year. Most of the goats were inseminated during July-September or during January-February. Statistical differences between months ($P < 0.05$) and breeds ($P > 0.001$) were found. Despite of breed differences, all breeds followed a similar basic animal reproductive pattern in which there was a reduction in the proportion of cycling animals during the months of long photoperiod (March-June) and an increase in the proportion during the months of short photoperiod. This suggests that seasonality in all four breeds is subject to photoperiod similar to temperate regions [2, 3].

**TABLE II. REPRODUCTIVE PERFORMANCE OF SAANEN, NUBIAN, TOGENBURG AND ALPINE GOATS UNDER ARTIFICIAL INSEMINATION (AI) SCHEME IN BOTH BREEDING- AND NON-BREEDING SEASONS.**

<table>
<thead>
<tr>
<th>Breed</th>
<th>Saanen x ± sd</th>
<th>Nubian x ± sd</th>
<th>Toggenburg x ± sd</th>
<th>Alpine x ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>370</td>
<td>649</td>
<td>187</td>
<td>786</td>
</tr>
<tr>
<td>Age at first AI (d)</td>
<td>453 ±209$^a$</td>
<td>458 ±231$^a$</td>
<td>307 ±133$^b$</td>
<td>429 ±116$^a$</td>
</tr>
<tr>
<td>Age at first conception (d)</td>
<td>511 ±160</td>
<td>471 ±147</td>
<td>486 ±153</td>
<td>493 ±122</td>
</tr>
<tr>
<td>Age at first parturition (d)</td>
<td>598 ±212$^a$</td>
<td>654 ±148$^b$</td>
<td>578 ±218$^a$</td>
<td>637 ±190$^b$</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>24.8</td>
<td>14.7</td>
<td>16.0</td>
<td>31.0</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td>69.4$^a$</td>
<td>27.7$^c$</td>
<td>65.9$^{ab}$</td>
<td>62.5$^b$</td>
</tr>
<tr>
<td>Services / conception (n)</td>
<td>4.0$^{ab}$</td>
<td>6.0$^a$</td>
<td>5.9$^a$</td>
<td>3.2$^b$</td>
</tr>
<tr>
<td>Abortion (%)</td>
<td>12.7$^c$</td>
<td>13.2$^b$</td>
<td>8.0$^d$</td>
<td>11.9$^a$</td>
</tr>
</tbody>
</table>

$^a,b$ Different superscripts within a row indicate significant differences ($P < 0.05$)

Most goats resumed cyclic activity in July. Thus, the anoestrous season is limited to four months (March to June). This fact suggests that other factors such nutrition, management and social interactions may be influencing the length of anoestrus [21]. Future studies should be addressed to defining the nutritional-social-reproduction interactions to improve feed management.

The pattern of parturitions and abortion rates in the year was affected by the breed (Figure 2, $P < 0.001$). Also, the occurrence of parturitions increased during January and February and the abortion rate was higher in March-June ($P < 0.001$). The conception and pregnancy rates between breeds were also different (Figure 3, $P < 0.05$).
FIG. 1. Distribution of 1,153 first artificial insemination services and 3,499 total services throughout the year in goats of four breeds (Saanen, Nubian, Toggenburg and Alpine) under semi-intensive management.

FIG. 2. Distribution of 1,052 parturitions and 242 abortions throughout the year in goats of four breeds (Saanen, Nubian, Toggenburg and Alpine) under semi-intensive management.

3.2. Experiment II

Table III shows the mean values of the studied blood indicators. An iron deficiency was identified during all the experimental period in the whole herd. Although the levels of haematocrit and haemoglobin were similar to those reported elsewhere [8, 10, 22], there were occasional cases of anemia which were followed by death, mainly in the Nubian and Toggenburg breeds.
TABLE III. EFFECTS OF SEASON OF THE YEAR (DRY, RAINY), BREED (SAANEN, NUBIAN, TOGGENBURG, ALPINE) AND PHYSIOLOGICAL STATUS (EARLY LACTATION, LATE LACTATION, AND NON-LACTATING) ON METABOLIC INDICATORS OF THE NUTRITIONAL AND HEALTH STATUS IN GOATS REARED UNDER SEMI-INTENSIVE MANAGEMENT.

<table>
<thead>
<tr>
<th></th>
<th>Season</th>
<th>Breed</th>
<th>Physiological status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry (n = 182)</td>
<td>Rainy (n = 191)</td>
<td>Saanen (n = 110)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nubian (n = 83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Toggenburg (n = 71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpine (n = 109)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Early lactation (n = 56)</td>
</tr>
<tr>
<td>PCV</td>
<td>0.26</td>
<td>0.25</td>
<td>0.26</td>
</tr>
<tr>
<td>Hb (mmol/L)</td>
<td>5.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>2.86</td>
<td>2.76</td>
<td>2.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T. Protein (g/L)</td>
<td>74.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>27.94</td>
<td>26.82</td>
<td>28.69</td>
</tr>
<tr>
<td>Globulins (g/L)</td>
<td>46.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.18</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>132.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>133.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>2.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cu (μmol/L)</td>
<td>15.23</td>
<td>14.29</td>
<td>15.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zn (μmol/L)</td>
<td>12.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>I (μmol/L)</td>
<td>12.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Different superscripts within rows and groups (season, breed, physiological status) are statistically different (P <0.05)
The levels of calcium were low in the rainy season. The glucose level in Saanen goats were particularly low. Also, the interaction between the breed and physiological status showed that glucose levels decreased as the lactation progressed in Saanen and Toggenburg goats during the dry season. This may suggest that the energy balance was not adequate during the dry season, especially affecting higher milk producer breeds.

Na levels were lower in Saanen and Toggenburg than in the other breeds and the values were lower than described elsewhere [5, 6].

The haematocrit, globulins, Ca, Mg and Fe levels increased during the lactation period. Similar findings occurred with respect to haemoglobin and albumin but only in Saanen and Toggenburg goats.

3.3. Experiment III

Table IV shows that the conception rate as indicated by elevated progesterone on day 21 after AI was lower in the Toggenburg breed than in the others (P <0.05). Alpine females presented the highest kidding rate 67.7% (P <0.05) among all breeds while the Nubian and Toggenburg goats presented the lowest (25.6 and 24.4%, respectively).

The main causes of infertility in induced oestrus were fertilization failure and early embryonic mortality, especially in Toggenburg goats (39%). Embryonic losses between 21-40 days after service were similar between breeds.

Embryonic losses could involve both the interruption of pregnancy by embryo mortality at 21-40 days and the false pregnancy diagnosis at 21 days due to disturbances in the length of oestrous cycle, as described elsewhere [14, 15].

In general, the frequency of abortion was low and therefore, a non-significant cause of infertility in the experiment. The indeterminate losses represented an average of 9.3% for all four breeds. In these cases, the progesterone levels appeared to have falsely diagnosed pregnancy. Four Nubian females and one Alpine maintained progesterone levels higher than 4.67 nmol/L until expecting kidding date suggesting the possible occurrence of pseudopregnancy. This has been referred by other authors in this species [11, 23, 24].

The season effect regarding infertility causes affected only the frequency of anovulatory anoestrus. It was higher during the breeding season (P <0.05).

The progesterone profile during pregnancy was similar between breeds and has the same pattern as previously reported [13, 24, 25].
TABLE IV. REPRODUCTIVE PERFORMANCE AND FERTILITY DISTURBANCES IN INDUCED OESTRUS IN GOATS OF FOUR BREEDS (SAANEN, NUBIAN, TOGGENBURG AND ALPINE) UNDER ARTIFICIAL INSEMINATION.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Nº</th>
<th>Conception</th>
<th>Kidding</th>
<th>AO</th>
<th>CF &amp; EED</th>
<th>Losses 21-40 d</th>
<th>Abortion</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saanen</td>
<td>36</td>
<td>66.7a</td>
<td>44.4b</td>
<td>11.1ab</td>
<td>22.2</td>
<td>2.8</td>
<td>5.6ab</td>
<td>13.9</td>
</tr>
<tr>
<td>Nubian</td>
<td>43</td>
<td>58.1a</td>
<td>25.6c</td>
<td>18.6ab</td>
<td>23.2</td>
<td>9.3</td>
<td>7.0ab</td>
<td>16.3</td>
</tr>
<tr>
<td>Toggenburg</td>
<td>41</td>
<td>36.6b</td>
<td>24.4c</td>
<td>24.4a</td>
<td>39.0</td>
<td>7.3</td>
<td>0.6b</td>
<td>4.9</td>
</tr>
<tr>
<td>Alpine</td>
<td>31</td>
<td>77.4a</td>
<td>67.8a</td>
<td>9.7b</td>
<td>12.9</td>
<td>3.2</td>
<td>3.2ab</td>
<td>3.2</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>58.3</td>
<td>38.4</td>
<td>16.6</td>
<td>25.1</td>
<td>6.0</td>
<td>4.0</td>
<td>9.3</td>
</tr>
</tbody>
</table>

*ab Different superscripts within columns indicate statistical differences (P <0.05)
AO Anovulatory oestrus
CF Fertilization failure
EED Early embryonic death

4. CONCLUSIONS

The Saanen, Nubian, Toggenburg and Alpine goats have a period of anoestrus that is apparently driven by photoperiod. Under tropical management conditions these breeds have infertility problems associated with deficient nutrition, poor reproductive management and organic reproductive disturbances. Future studies should be addressed to better defining of the interactions among animal nutrition, reproductive behaviour and disease to improve management practices.

Assuming that the flock was deficient in Fe and had low levels of Ca during the rainy season, corrective mineral supplementation, including Fe throughout the year and Ca during the rainy season should be employed. Also, an improved diet to increase energy intake may help hypoglycemic Saanen goats, especially during the dry season.

The main causes of infertility of induced oestrus were fertilization failure and early embryonic death.

REFERENCES


<table>
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<tr>
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