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# Reproductive response in supplemented heifers in the humid tropics of Costa Rica

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**Abstract**

To evaluate the effect of two supplementary diets to determine the consequence on productive and reproductive performance in heifers (*Bos indicus* × *Bos taurus*) averaging between 24 and 36 months of age and grazing tropical pastures, two trials were conducted. Thirty animals (initial BW 325.1 ± 33.6 kg) were divided in two groups in the initial study: supplemented (SG) and control (CG); SG received a concentrate (5.5% CP and 2.85 Mcal/kg of DE dry matter basis) at 1% of body weight (BW). In the second study, 45 heifers (initial BW 332.6 ± 29.3 kg) were assigned in two treatments, with the same amount of supplement (1% BW) but with a greater nutrient content (13% CP and 3.15 Mcal/kg of DE). The proportion of animals with a corpus luteum at the end of each study was greater in the supplemented groups ($P < 0.05$). Ovarian follicular dynamics was similar between groups in the first study, but in the second study there were more heifers in the SG group with follicles larger than 9 mm in diameter ($P < 0.05$). Pregnancy rate was similar for SG and CG ($P > 0.05$). The response to a regimen of estrous synchronization in both trials was numerically superior in the SG group. No differences were observed in the length of estrus. Daily gain and body condition score were similar for supplemented and control groups (0.27 compared with −0.06 in the first study and 0.90 kg compared with 0.60 in the second study, respectively). Dietary supplementation improved the number of animals

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initiating estrous cycles and the expression of estrus when compared with unsupplemented control heifers. The dietary regimens imposed in these studies appear to be an adequate for the management of growing heifers destined to a reproductive program.


*Keywords:* Cattle–heifers; Supplementation; Estrous cycles; Weight gain; Condition score

## 1. Introduction

The selection and management of replacement females in cattle raised under tropical conditions have been some of the most important constraints in the development of livestock in this area. Selection of females for breeding purposes can be affected by nutritional and reproductive constraints associated with lesser weight gains in animals after weaning than what occurs in more temperate conditions. A lesser rate of growth is associated with a delay in the onset of puberty and, therefore, establishment of reproductive cycles (Plasse, 1978, 1979; Mukassa-Mugerwa, 1989).

To help alleviate these problems, nutritional programs providing limiting nutrients under grazing conditions have been implemented with various degrees of success (Boland et al., 2001). Responses have been variable because of the differences in forage availability, quality and quantity of the supplement, genetic characteristics and climatic conditions (Stonaker, 1975). Soto et al. (1997) evaluated the effect of dietary supplementation on productive and reproductive performance of grazing Brahman heifers, and found that dietary supplementation improved daily gain and body condition score (BCS), but not ovarian activity, estrous synchronization response, pregnancy rate and services per conception. In contrast, Cavalieri and Fitzpatrick (1995) observed that heifers with the greater rate of growth and BCS, had an enhanced probability for initiating estrous cycles and becoming pregnant during the breeding season. It is well known that nutritional status affects growth and development as well as reproductive performance in cows. Murphy et al. (1991) associated a reduction in the dominant ovarian follicle diameter and growth with amount of dietary intake. Stevenson et al. (1987) found there was a lesser estrous behavior response and poor reproductive success in herds where there was retarded body growth and ovarian follicle maturation of cattle experiencing a negative energy balance.

Two trials were conducted to evaluate the effect of supplementary diets to determine the consequence on productive and reproductive performance in heifers (*Bos indicus* × *Bos taurus*) averaging 24–36 months of age and grazing tropical pastures.

## 2. Materials and methods

The present study was conducted at the experimental station of the Technological Institute from Costa Rica, located in San Ramón, Alajuela (10°25′N and 84°32′O) at 172 m above sea level. The region is classified as humid tropical, with a mean temperature of 27.3 °C and rainfall 3062 mm, with a relative humidity of 85.3%.

Heifers (*Bos Taurus* × *Bos indicus*, $n = 75$) ranging from 24 to 36 months were used in the experiments. Each experiment was conducted in different years. In both experiments, heifers were grazing pastures of African stargrass (*Cynodon nlemfuensis*), Ratana grass (*Ischaemum indicum*) and Candelario grass (*Pennisetum purpureum*) with free access to mineral salts (Ganafos Plus® Piensos S.A., Costa Rica). Thirty days before the initiation of dietary supplementation, ultrasonographic evaluations of ovarian structures were performed twice per week. Heifers were weighed every 15 days and BCS was recorded using a scale from 1 to 5, where 1, emaciated and 5, obese (Edmonson et al., 1989).

In the first experiment, heifers ($n = 30$) with an initial average weight of 325 ± 33.6 kg were randomly assigned to one of two treatments: dietary supplemented group (SG) and a grazing control group (CG). Supplement was provided in amounts that were determined based on 1% of BW per day of a commercial concentrate (5.5% CP and 2.85 Mcal/kg of DE dry matter basis) produced by Citrocom® Casa Dos Pinos, Costa Rica. In the second experiment, animals ($n = 45$) with an initial average weight of 332.69 ± 29.36 kg were randomly assigned to the same treatments, 22 heifers with the same amount of supplement (1% BW) but with a greater nutrient content in the concentrate (13% CP and 3.15 Mcal/kg of DE; Citrocarne® Casa Dos Pinos, Costa Rica), and the remaining 23 heifers to the control group. In both experiments, heifers were adapted to the concentrate over a 15-day period because these animals were not adapted to this type of dietary concentrate, and the supplemental period of 30 days followed. The animals were fed in group in a confined area. Live weight and BCS were measured every 15 days.

After the dietary supplementation period, estrous synchrony regimens were employed using a progesterone implant (norgestomet, Crestar, Lab. Intervet, México), placed in the ear for 9 days, followed by an intramuscular injection of estradiol valerate at the time of implant insertion. After implant withdrawal, estrus was detected continuously for 56 h and sexual behavior activities were classified according to the procedure described by Orihuela et al. (1988). Heifers were inseminated 12 h after the onset of estrus.

Ovarian activity was evaluated by ultrasonography twice a week starting 2 weeks prior to the time of initiation of the experiments using an Aloka SSD-500 machine with a 5 MHz linear transducer. The diameter of the dominant ovarian follicle was measured and blood samples were taken at the same time of reproductive evaluations by venipuncture of the coccygeal vein or artery. Samples were centrifuged at 7000 rpm, and serum progesterone was analyzed by a solid phase radioimmunoassay (Pulido et al., 1991). Heifers were classified according to their ovarian follicular characteristics in four categories: (1) animals without changes in the follicular population throughout the study with follicles under 3 mm of diameter; (2) animals with follicles between 3 and 9 mm on at least two consecutive evaluations; (3) animals with follicles larger than 9 mm on at least two consecutive evaluations; (4) heifers with a corpus luteum (CL) at the time of ultrasonographic examination and corroborated by serum progesterone (values of progesterone above 1 ng/ml; Zalesky et al., 1984).

The proportion of heifers initiating estrous cycles according to assessments of ovarian follicular diameter during the study was compared with a $Z$ test for two independent proportions (Dawson-Saunders and Trapp, 1997). Pregnancy rate was analyzed by Chi-square test. Estrous length and BCS were compared with a student "$t$" test. Average daily weight gain was analyzed as a completely randomized design using initial weight as a co-variable (Kuehl, 2001)

$$Y_{ij} = \mu_i + \tau_i + \beta(X_{ij} - x..) + e_{ij}$$

where $\mu_I$, general average (ADG); $\tau_i$, treatment effect; $\beta$, variable regression coefficient and $e_{ij}$, error.

## 3. Results

### 3.1. Experiment 1

Statistical differences were not detected in ADG and BCS, even when heifers in the control group had a slight negative energy balance. Percentage of heifers in estrus, length of estrus and number with CL were not affected by the addition of the dietary supplement. Onset of estrus after implant removal was similar for dietary supplemented and control groups (28.3 and 29.3, respectively). Sexual activity and intensity of sexual behavior were not affected by treatment (Table 1). The intensity of estrus (average of mounts and attempts to mount/heifer) were similar for both groups, SG was 22.7 and CG was 24.8. Data for the ovarian follicular dynamics of the heifers throughout the study are presented in Table 2, as can be observed after the estrous synchronization regimen a greater proportion of heifers were detected with a CL in the group which received the nutrient supplement.

After the dietary supplementation period, percentage of heifers initiating estrous cycles was greater ($P < 0.05$) in the supplemented (52.3%) than control (41.4%) group. Final pregnancy rate was similar for both groups ($P > 0.05$) being 60% in the SG (9/15) and 47% (7/15) in the control group.

Table 1
Effect of a lesser nutrient supplementation (5.5% CP, 2.85 Mcal/kg DE) on productive and reproductive performance of grazing heifers in humid tropics—Experiment 1

| | Supplement 1% BW | Control |
|---|---|---|
| Initial weight (kg) | 365.3 ± 24.4 | 367.4 ± 31.6 |
| Final weight (kg) | 376.7 ± 22.4 | 365.2 ± 31.7 |
| ADG (kg) | 0.270 ± 0.26 | −0.06 ± 0.25 |
| Initial BCS | 2.8 ± 0.31 | 2.6 ± 0.20 |
| Final BCS | 2.9 ± 0.40 | 3.0 ± 0.29 |
| Heifers in estrus (%)[a,b] | 100 (15/15) | 73.2 (11/15) |
| Estrus with ovulation (%)[a,b] | 66.6 (10/15) | 36.6 (4/11) |
| Estrous presentation after implant withdrawal (h)[a] | 28.3 ± 3.06 | 29.3 ± 5.26 |
| Estrous length[a] | 17.53 ± 7.28 | 17.09 ± 5.25 |
| Intensity of estrous activity (mounts and attempt to mount/heifer) | 22.76 ± 4.28 | 24.86 ± 4.81 |

[a] Recorded after implant withdrawal.
[b] $P < 0.05$.

Table 2
Proportion of heifers with different ovarian follicular dynamics in relationship to the dietary supplementation—Experiment 1

| | Supplement 1% BW | Control |
|---|---|---|
| Pre-supplementation | | |
| <3 mm | 41.2 | 30.3 |
| 3–9 mm | 26.7 | 37.0 |
| >9 mm | 8.5 | 4.2 |
| Corpus luteum | 23.0 | 28.5 |
| Supplementation | | |
| <3 mm | 6.7 | 8.7 |
| 3–9 mm | 35.9 | 27.7 |
| >9 mm | 20.0 | 38.5 |
| Corpus luteum | 36.9 | 25.1 |
| Estrous synchronization period | | |
| <3 mm | 6.7 | 13.3 |
| 3–9 mm | 20.0 | 33.3 |
| >9 mm[a] | 6.7 | 26.7 |
| Corpus luteum[a] | 66.7 | 26.7 |

[a] Differ $P < 0.05$.

### 3.2. Experiment 2

A positive energy balance was observed in both groups, without statistical differences in ADG and BCS. Onset of estrus after implant removal was different for dietary supplemented and control groups (27.3 and 30.8, respectively; $P < 0.10$; Table 3). The percentage of heifers in estrus and number of heifers that had CL were not affected by nutrient supplementation, however, the duration of estrus was longer (8.5 and 6.6 h for SG and CG, respectively; $P < 0.10$). The intensity of estrus (average number of mounts and attempts to mount/heifer)

Table 3
Effect of greater nutrient supplementation (13% CP, 3.15 Mcal/kg DE) on productive and reproductive performance of grazing heifers in humid tropics—Experiment 2

| | Supplement 1% BW | Control |
|---|---|---|
| Initial weight (kg) | 342.2 ± 36.0 | 326.3 ± 31.5 |
| Final weight (kg)[a] | 380.6 ± 31.7 | 351.8 ± 65.5 |
| ADG (kg) | 0.900 ± 0.40 | 0.610 ± 1.52 |
| Initial BCS | 2.9 ± 0.22 | 2.8 ± 0.23 |
| Final BCS | 3.0 ± 0.21 | 3.1 ± 0.20 |
| Heifers in estrus (%)[b] | 95.4 (21/22) | 91.3 (21/23) |
| Estrus with ovulation (%)[b] | 52.4 (11/21) | 42.8 (9/21) |
| Estrous presentation after implant withdrawal (h)[b,c] | 27.3 ± 7.04 | 30.8 ± 5.8 |
| Estrous length[b,d] | 8.47 ± 4.27 | 6.64 ± 4.05 |
| Intensity of estrous activity (mounts and attempt to mount/heifer) | 13.4 ± 2.10 | 11.0 ± 0.70 |

[a] $P < 0.05$.
[b] Recorded after implant withdrawal.
[c] $P < 0.10$.
[d] $P < 0.08$.

Table 4
Proportion of heifers with different ovarian follicular dynamics in relation to dietary supplementation period—Experiment 2

| | Supplement 1% BW | Control |
|---|---|---|
| Pre-supplementation | | |
| <3 mm | 7.3 | 7.0 |
| 3–9 mm[a] | 28.2 | 13.1 |
| >9 mm | 51.8 | 42.6 |
| Corpus luteum | 12.7 | 4.3 |
| Supplementation | | |
| <3 mm | 5.6 | 5.9 |
| 3–9 mm | 21.4 | 34.2 |
| >9 mm[a] | 55.2 | 39.8 |
| Corpus luteum | 17.9 | 20.2 |
| Estrous synchronization period | | |
| <3 mm | 2.3 | 2.2 |
| 3–9 mm[a] | 13.6 | 30.4 |
| >9 mm | 31.8 | 26.1 |
| Corpus luteum[a] | 52.3 | 41.3 |

[a] Differ $P < 0.05$.

were similar for both groups, with SG being 13.4 and CG 11.0 ($P > 0.05$). Data related to ovarian follicular dynamics after supplementation during the estrous synchronization period are presented in Table 4. There was a greater proportion of heifers with a CL in the group that received the greater amount of nutrient supplementation.

After the supplementation period, percentage of heifers that had initiated estrous cycles was greater ($P < 0.05$) in the dietary supplemented (66.7%) than control (26.7%) group.

## 4. Discussion

Cavalieri and Fitzpatrick (1995) reported that animals with greater ADG and BCS had a greater probability of expressing normal estrous cycles and becoming pregnant than heifers with lesser weight gains and body condition scores. Consistent with the present study, that was a greater proportion of heifers that were fed dietary supplements that initiated estrous cycles regardless of the nutrient concentration that was supplied and the better ADG response showed by the supplemented group. Albeit, in the second experiment, the diet used had a more evident effect on body weight gain and BCS probably as a consequence of the greater nutrient content. It remains to be evaluated whether the combined effect of supplementation in conjunction with amount of forage availability, both in quantity and quality, as influenced by amount of rainfall can affect the latter. In effect, supplementation can be more significant if the previous year was poor in rainfall (Holroyd et al., 1983). Moreover, in one of the few long term studies, Holroyd et al. (1977) found that cows grazing fertilized legumes had a greater conception rate in only 1 of 5 years the study was conducted.

Several studies have demonstrated a great variability in response to a dietary supplementation program. Khireddine et al. (1998) working with feeds dense in energy content plus implementation of estrous synchronization programs suggested that the use of a supplement improved ovarian follicular development and pregnancy rate, but response was affected by the energy concentration in the diet. In addition Grings et al. (1998) suggested that supplemental feeding of pre-pubertal animals tended to increase fertility and advance puberty. In the present study, no differences were observed in fertility, but this may be attributed to the small number of animals in each study, even though two different amounts of nutrients were supplemented. When the data were pooled for the 2 years, the final pregnancy rate was different favoring the supplemented groups.

Ovarian follicular dynamics were similar between treatments in the first year but not in the second where the supplemented group had more animals with follicles greater than 9 mm. This was reflected in a greater proportion of animals with a CL. Diskin et al. (2003) suggested that animal nutritional status affects ovarian follicular growth, maturation and capacity to ovulate from a follicle, although the variability in the response can be related to the individual. Changes promoting a follicular development may trigger a more desirable ovulation rate. In addition, Rhodes et al. (1995) suggested that there was a linear reduction in persistence and maximum size of dominant and ovulatory follicles with decreasing body weight and condition score in feed-restricted beef heifers, whereas a linear increase in persistence, growth rate and maximum size of dominant follicles was observed during realimentation of nutritionally anestrous beef heifers.

Soto et al. (1997) have reported that dietary supplementation of animals does not enhance reproductive performance even in response to estrous synchronization. Similarly, in the initial study of the present research, a greater proportion of animals in estrus was observed in the supplemented group. In contrast, however, in the second study only estrous length and time of onset of estrus were different in the heifers fed the diet with greater nutrient content and the control group.

Proper use and management of dietary supplements can influence profit and loss status for small farmers (Aranda et al., 2001). It is, therefore, necessary to evaluate the cost-effective benefit of this practice as animals fed with a diet of lesser nutrient content had similar reproductive performance compared with those fed the nutrient supplemented diet in the present study. Further experiments should discern the economical impact of feeding diets with a greater nutrient content that results in enhanced weight gain and body condition. Evaluation of diets of this nature over short periods of supplementation warrant further study as to cost effectiveness with implementation under tropical scenarios. Nonetheless, supplementation improved the number of animals initiating estrous cycles and the response to estrous synchronization regimen when compared with unsupplemented control animals and appears to be an adequate strategy for the management of growing heifers destined for reproductive programs.

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