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ORIGINAL PAPER

Effect of ruminally protected Methionine on the productive and reproductive performance of grazing *Bos indicus* heifers raised in the humid tropics of Costa Rica

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Abstract With the objective of evaluating the effect of methionine supplementation prior to a breeding program, thirty one heifers (*Bos taurus x Bos indicus*) were used averaging 386 ± 29 days of age and a mean body weight of 402.6 ± 28 kg. Fifteen of the animals received a supplement (SG) during 45 days with molasses-urea mixture (2 kg molasses + 407 g urea/head/day), plus 10 g of ruminally protected methionine. The other sixteen heifers did not receive supplement (CG). Fecal and pastures samples were collected to assess dry herbage intake and digestibility. Serial ultrasound measurements from the ovary were performed in both groups to evaluate follicular dynamics. The heifers were categorized according to their follicular size and presence of a CL. Forage intake and dry matter digestibility were reduced ($P < 0.05$) and body condition tended to improve ($P = 0.07$) in the supplemented heifers, however, total intake, final weight, daily gain and dorsal back fat were not affected. After the supplementation period, the percentage of females in the categories <3 mm and 3 to <6 mm, was greater ($P < 0.05$) in CG (25% and 43.7%) that in SG (0% and 26.6%) but in the follicle category of ≥9 mm, the percentage of animals was 60% in SG and 18.8% in CG ($P < 0.05$). The percentage of ovulation for the SG and CG was 86.7% and 62.5%, respectively ($P < 0.05$). The combination of supplementation with methionine-urea and molasses at the end of the dry season and the anticipated onset of the rainy season favored the establishment of ovarian activity and follicular dynamics.

Keywords Methionine · Supplementation · Heifers · Tropics

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Introduction

The profitability and productivity life of beef cattle in the tropics is based on particular physiological events such as the onset of puberty, establishment of pregnancy and lastly the postpartum period (Galina and Arthur 1989; Bagley 1993). However, one of the main concerns under tropical conditions is the poor productive performance of growing animals due to the harsh environmental conditions present in this geographical region of the world (Stonaker 1975). This situation is reflected in deprivation of reproductive functions such as the onset of puberty and establishment of pregnancy. Sánchez and Soto (1999) in a study undertaken in the humid tropical region of Costa Rica, concluded that beef and dairy enterprises are limited due to the instability in the production of forage available mostly afforded through pastoral systems. Generally beef production under tropical conditions is based mostly by availability of grass and supplementation of bio products such as citrus pulp, molasses and coconut oil among others. In order to overcome these constraints, several experiments tested the effect of short term supplementation in heifers particularly in the stage prior to breeding and before calving (Peiris et al. 1995) with the aim to overcome nutritional deficiencies, unfortunately with conflicting results due to a variety of environmental and animal factors (Soto et al. 1997; Aranda et al. 2001).

It has been demonstrated that energy and protein are nutritional constrains in cattle raised under tropical conditions (Cabrera et al. 2000; Aranda et al. 2001) and also, limiting amino acids such as methionine and lysine (Richardson and Hatfield 1978). methionine in particular, has been related with many functions in the body, in addition with its role in protein synthesis. Studies conducted in Holstein steers demonstrated that supplementation of ruminally protected methionine improve ADG and protein efficiency, moreover, the use of ruminal protected amino acids in lactating dairy cows increased milk protein output about 5% (Robinson et al. 1995).

Waterman et al. (2007) tested the effect of methionine supplementation in late-gestation beef cows consuming low quality forages, suggesting that methionine was a limiting factor aminoacid on this particular physiological stage and also the supplementation of 5 g/d of rumen-protected methionine in combination with urea improved nitrogen retention and promoted protein accretion during late pregnancy, however, utilization of methionine in tropical conditions has not been investigated and the quality of the forage available is different than cattle raised under more temperate conditions. Therefore, the objective of the present study was to evaluate the effect of strategic supplementation, using a mixture of molasses and urea, with the addition of ruminally protected methionine on the productive and reproductive performance of heifers prepared for a breeding program.

Materials and methods

Location

The present experiment was conducted at the experimental station belonging to the Technological Institute of Costa Rica located in San Ramón Alajuela province (San Carlos region; latitude: 10°N, longitude: 84°W). This area is classified as humid tropical with a mean temperature of 27.3°C, annual precipitation of 3062 mm and a relative humidity of 85.3%.

Animals and treatment

Thirty one heifers (*Bos taurus x Bos indicus*) were used, averaging 386 ± 29 days of age, and a mean body weight of 402.6 ± 28 kg. Animals were divided randomly in two groups, one with 15 heifers that received a supplement (SG) during 45 days with molasses-urea mixture (2 kg molasses + 40 g urea/head/day), plus 10 g of ruminally protected DL-methionine (Mepron® M-85 DEGUSSA México), which was individually administered with a device for intra-ruminal capsules, and the control group (CG n=16) which was deprived from receiving any supplement. Heifers were raised in pastures composed of African star (*Cynodon nlemfuensis*), Mombaza (*Panicum maximum*) and Ratana (*Ischaemum indicum*) with free access to mineral salts (Ganafos® Plus Piensos S.A., Costa Rica).

Experimental phases

The study started in April (close to the end of the dry season) and ended in July (first pick of the rainy season) and was divided in four phases: pre-supplementation in which animals were selected and adapted to the experimental procedures; supplementation (45 days), synchronization period (last nine days of supplementation) and post-supplementation phase (7 days). Every 15 days, heifers were weighted and body condition score was recorded using a scale from 1 to 5 (Edmonson et al. 1989) where 1 was emaciated and 5 was obese. Additionally, dorsal back fat was measured with ultrasonographic equipment (Aloka SSD-500) using a sectorial transducer of 7.5 MHz according to the methodology published by Silva et al. (2005).

Digestibility and dry matter intake

Chromium oxide was used as an external marker and acid insoluble ash as internal indicator to estimate digestibility and intake (Owens and Hanson 1992). In both groups during 9 days, a dose of 3 g of chromic oxide/head/day was administered with a device for intra-ruminal capsules. In the last 5 days of this period, fecal grab samples were collected, oven dried at 60°C for 48 hours and stored until their later analysis. Samples from pastures were collected to determine the internal marker (Keulen and Young 1977). Dry herbage intake and digestibility were calculated as described by Geerken et al. (1987) and Aranda et al. (2001).

Reproductive evaluations

Ovarian activity was evaluated by ultrasonography twice a week prior to the supplementation regimen. When supplementation started, ultrasounds were performed daily using an Aloka SSD-500 with a 7.5 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 for 15 minutes. Samples were analyzed to detect blood progesterone values by a solid phase radioimmunoassay (Pulido et al. 1991). Heifers were classified according to their ovarian follicular characteristics in five categories:

(1) Animals without changes in the follicular population throughout the study with follicles under 3 mm of diameter,
(2) Animals with follicles between than 3 and 6 mm,
(3) Animals with follicles between than 6 and 9 mm,
(4) Animals with follicles larger than 9 mm,
(5) Heifers with a *corpus luteum* (CL) corroborated by serum progesterone (values of progesterone above 1 ng/ml; Zalesky et al. 1984).

Estrous synchronization was performed in all animals, using a progesterone implant device (Norgestomet, Crestar, Lab. Intervet, México), placed in the ear for 9 days previous to the end of supplementation plus an intramuscular injection of estradiol valerate at the time of implant insertion. After removal, ultrasonographic evaluations were performed during two weeks (two times per week) to determine the ovulation rate and follicular dynamics.

Statistical analyses

The proportion of heifers with different follicular diameters was compared with a Z test for two independent proportions (Dawson-Saunders and Trapp 1997).

The variables digestibility, forage intake, dry matter intake, and dorsal fat, were analyzed by ANOVA using PROC GLM procedure in SAS (2004) using initial weight as covariate:

$$Yij = \mu i + \tau i + \beta(Xij - x..) + eij$$

Where: μi, general average (ADG, digestibility, forage intake, dry matter intake, and dorsal back fat); τi, treatment effect; β, variable regression coefficient and eij, error.

Results from body condition were analyzed with the Wilcoxon test for non-parametrical data (Freund and Simon 1992).

Results

Forage intake and dry matter digestibility were reduced ($P<0.05$) in the SG, however total intake was not affected. Body condition score tended to improve ($P=0.07$) in supplemented heifers, although final weight, average daily gain and dorsal back fat were not affected (Table 1).

Ovarian dynamics during the evaluation period are shown in Fig. 1, As can be seen, before the supplementation phase, the follicular dynamic was similar among the groups and the proportion of animals with corpus luteum was not different ($P>0.05$). Throughout the supplementation period, the percentage of follicles classified as >9 mm in the SG increased when compared to the CG 26.7% (4/15) vs 6.4% (1/16) respectively ($P<0.05$). During the synchronization

Table 1 Effect of supplementation with molasses, urea and methionine* on productive performance of beef heifers in humid tropic

Variable	Control (n=16)	Supplementation (n=15)
Dry matter digestibility (%)[a]	65.63±0.60	63.72±0.62
Forage DM intake (kg/day)[a]	11.69±0.47	9.85±0.48
Total DM intake (kg/day)	11.69±0.47	11.30±0.48
Initial weight (kg)	411.62±6.92	413.8±7.15
Final weight (kg)	423.75±7.53	423.80±7.78
Average daily gain (kg/day)	0.269±0.05	0.222±0.06
Body condition score[b]	3.44±0.10	3.73±0.11
Dorsal back fat (mm)	0.422±0.02	0.453±0.02

*Supplementation: molasses-urea mixture (2 kg molasses + 40 g urea/head/day), plus 10 g of ruminally protected DL-methionine (Mepron® M-85 DEGUSSA)

[a] Differences between groups P<0.05

[b] Differences between groups P=0.075

period, a higher percentage of animals in the CG showed follicles <6 mm of diameter (68.7%, 11/16) in comparison with the animals in the supplemented group (46.7%, 7/15). Additionally, the percentage of heifers with a corpus luteum was superior (P < 0.05) in SG (40% (6/15)) in comparison with CG (18.7% (3/16)). When the supplementation finished and the implant was withdrawn, the percentage of females in the categories < 3 mm and 3 to <6 mm, were greater (P < 0.05) in CG (25% (4/16) and 43.7% (7/16), respectively) that in SG (0% and 26.6% (4/15), respectively). For the category of ≥9 mm, the percentage of animals was 60% (9/15) in SG and 18.8% (3/16) in CG (P < 0.05). The percentage of ovulation after the period of estrous synchronization for the SG and the CG was 86.7% (13/15) and 62.5% (10/16), respectively (P < 0.05).

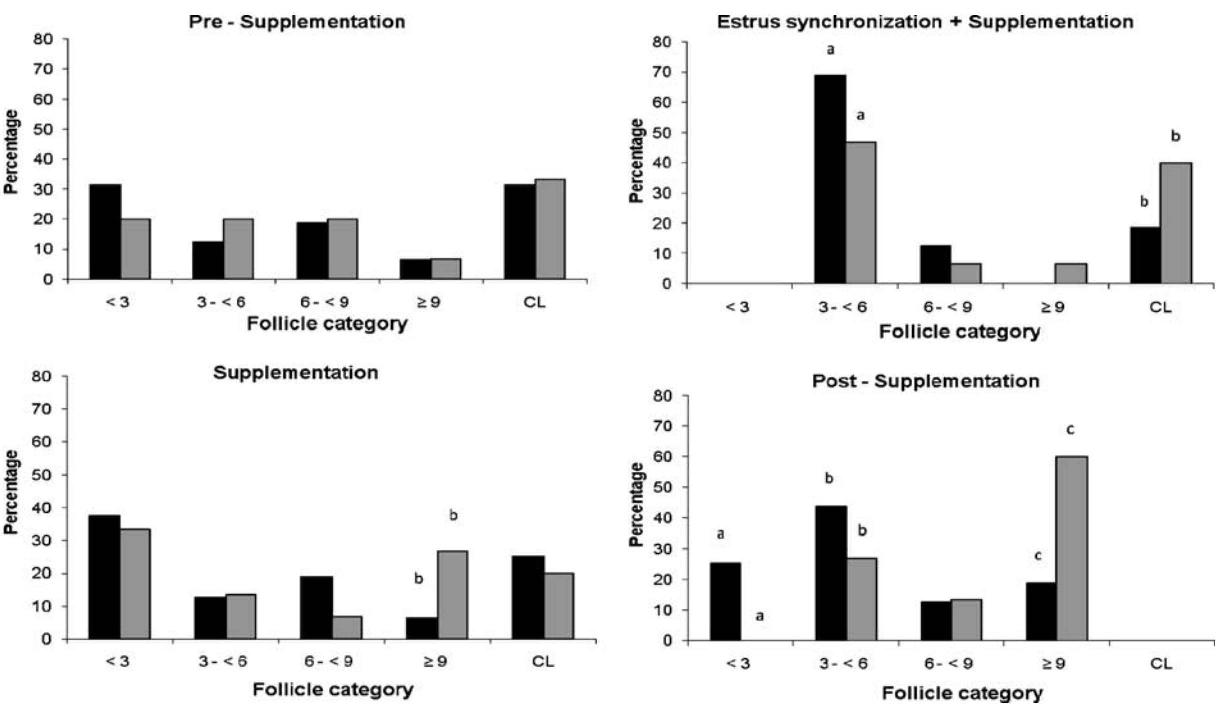

Fig. 1 Effect of supplementation with molasses, urea and methionine* on ovarian dynamic in heifers during the four experimental periods Supplemented group (SG grey bars) and control group (CG Black bars). [a, b, c] Different letter between follicle category show differences (P<0.05)

Discussion

The decision of supplementing during April stands in accord with the usual practices in the region. Supplementation with soluble carbohydrates usually reduces digestibility of forages because of the pH reduction in the rumen (Aranda et al. 2001). Forage intake is also diminished by substitution of the supplement as observed in grazing trials (Aranda et al. 2001; Cabrera et al. 2000), therefore, nutrient intake remains similar, and weight gain is not affected.

A clear explanation of the reason why molasses-urea with ruminally protected methionine improves the rate of ovulation to our knowledge does not exist, but it can be hypothesized that it is due to metabolic changes in energy utilization associated to an increase in the consumption from carbohydrates (molasses) and absorbed methionine. Canfield and Butler (1990) have shown a relation between energy status post-partum in the cow and the increasing in the LH secretion after the animals reached their NADIR. A similar mechanism can be the explanation in the heifers in the present study, as these animals are coming out of the dry season and thus it can be assumed, heifers have a feeding restriction during this period. It is also possible that the availability of methionine improved the energy efficiency at cellular level, since the methionine is transformed during its catabolism into methylmalonyl-CoA, and then in succinyl- CoA by methylmalonyl-CoA to enter to Krebs cycle (Le Grusse and Watier 1993), improving the energy status of heifers which received the supplement. However, this possible use of methionine as an efficient energy promoter in the animal was not reflected in our measurements of dorsal back fat, probably, as the effect of supplementation was too short to measure the effect of this parameter. Also, it may be due to the fact that these heifers are still in the phase of corporal development.

Golam et al. (2006) working with urea-molasses blocks in dairy cattle, found a reduction in the calving interval to first ovulation and estrus in relation to the control group. On the other hand, the use of urea plus ruminally protected methionine in pregnant beef cattle, did not affect the response in insulin, blood glucose and non esterified fatty acids (NEFAs), but showed reduction in the serum concentration of insulin like grown factor–I (IGF-I) with abomasal infusion of 5 or 10 g/day of methionine (Waterman et al. 2007). Nevertheless, results of our study, suggest that heifers could exhibit an improvement in the follicular dynamics and ovulation rate. However, further research is needed to determine how the use of molasses-urea in combination with the methionine increases LH surge and the potential effects in other hormones such as insulin, the IGF-I and leptin, to explain the variations in the pattern of follicular development observed.

The percentage of ovulation can be affected by environmental and nutritional factors, having the latter one a direct effect in the ovary (Hunter et al. 2004). On the other hand, in spite of speculating that a regime of supplementation could promote a direct effect on productive and reproductive parameters, several studies have shown this was not the case (Soto et al. 1997; Maquivar et al. 2006; Cooke et al. 2007). In this study, previous to the supplementation period, the follicular population was similar between groups, nonetheless, after 30 days of methionine-urea-molasses supplementation, the SG improved in the percentage of larger follicles ≥ 9 mm, which at the end of the supplementation period was reflected in a superior percentage of animals ovulating (SG and CG 86.67% y 62.5% respectively). In contrast, the CG exhibited a larger population of small follicles probably the consequence of impairment of follicular function. Possibly, if the study had continued with daily ultrasonography for a longer period of time, a more defined difference could have been observed between the SG y CG in follicular dynamics and ovulation as a response of the imposed treatments but this hypothesis will need further studies. Diskin et al. (2003) suggested that the nutritional status of the animal will certainly affect follicular growth, maturation and of course ovulation. Nevertheless, variations in the response can be the consequence of the animal's own metabolism.

In conclusion, the combination of supplementation with methionine-urea and molasses favored the establishment of ovarian activity measured as the development and increase in the number of intermediate follicles which in turn probably facilitated the presence of larger follicles capable of ovulating.

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