

Body Temperature and Reproductive Performance of Beef Heifers Supplemented With Rumen-Protected Methionine

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Abstract

This study aimed to assess the reproductive performance and body temperature of beef heifers supplemented with rumen-protected methionine (RPM) in an extensive grazing system and mated during the summer. One hundred 5/8 Angus 3/8 Brahman heifers were homogeneously divided into the control group (n=48, CG) supplemented with mineral salt ad libitum and methionine group (n=52, MG) who received the same supplementation plus 4 g of methionine per 100 g of mineral salt. The groups were fixed-time artificial inseminated (FTAI) 45 days after starting the supplementation. On day 0 of the FTAI protocol, ten animals per group received data-logger thermometers attached in progesterone implants recording the body temperature of the animals every 30 minutes. Pregnancy diagnosis was performed on days 30 and 60 after the artificial insemination to determine the rates of conception, pregnancy, and pregnancy loss. At the beginning of the supplementation, both groups had similar weights (MG was 304.6 kg and CG 304.4 kg), while on days 0 and 30 about the FTAI protocol, the MG was higher weight (365.9 vs. 346.7 and 384.8 vs. 371.5 Kg). During the FTAI protocol, the MG had lower body temperatures (38.77 vs. 38.99 °C) and a tendency to

have larger dominant follicles than the CG (11.76 mm x 10.8 mm). Although it did not impact reproductive performance, the observed higher weight, dominant follicle sizes, and lower body temperatures during FTAI protocols show the potential benefits of methionine supplementation in beef heifers mated during the summer.

Keywords: amino acids, beef cattle, breeding, fixed timed artificial insemination, thermoregulation

1. Introduction

The major meat and milk producing regions of the world are found in tropical and subtropical climates characterized by large thermal amplitudes, which is a constant challenge for the thermoregulation of the animals to increase productivity. The damage caused by heat stress to the animals' reproductive and productive abilities has been widely discussed (Arias et al., 2008; Key et al., 2014; Wiltbank et al., 2017). Heat stress can have direct effects on tissues, and can indirectly cause systemic alterations due to a reduced feed intake, which then affects several metabolic processes that lead to decreases in production and performance (Baumgard et al., 2014).

Heat stress in cattle leads to changes in thermoregulatory mechanisms that promote body heat loss, combined with metabolic and hormonal responses that alter the use of substrates (Faylon et al., 2015). The circulating concentrations of non-esterified fatty acids (NEFA) are lower during heat stress, even with high levels of stress hormones, such as epinephrine, glucagon, and cortisol, which are potent lipolytic agents (Zhao et al., 2018). Pathways such as proteolysis are prioritized for energy production, an observation that continues to generate discussions on the mechanisms controlling nutrient partitioning during stress (Rhoads et al., 2009; Baumgard et al., 2013).

Although the ideal time for beef cattle breeding season in beef cattle happens when there is a more significant pasture offer (Pessoa et al., 2018), this generally corresponds to periods of higher temperatures and humidity. The increase of external temperature can induce the increasing the body temperature, that in turn affect oocyte quality and follicle size (Castaño et al., 2014; Clements et al., 2017) as well to increase the pregnancy losses (Schüller et al., 2014; Schüller et al., 2017). Despite this, very few studies propose the use of additives in beef cows to attenuate the effects of environmental temperature on the reproductive performance of the herds.

On the other hand, in dairy cows nutritional strategies to attenuate the effects of heat stress have been constantly reviewed and improved (Ayasan and Karakozak, 2010; Conte et al., 2018). It recently has been showed that methionine and arginine supplementation could help maintain protein production in mammary gland cells exposed to high temperatures (Salama et al., 2019). As in mammary gland cells, methionine supplementation could also be beneficial for other cells during periods of high temperature and humidity. In mice, methionine restriction led to increases in body temperature and energy expenditure, which suggests that this amino acid has an important thermoregulatory function (Hasek et al., 2010). All studies in dairy cows, with findings on energetic gluconeogenic pathways supported by amino acids

derived from proteolysis, methionine supplementation with a protective effect against heat stress in mammary gland cells, and action of methionine on thermoregulation, demonstrate great potential for further studies on methionine as a nutritional alternative against heat stress during periods of high temperature and humidity. Based on these studies, we hypothesize that supplementation with rumen-protected methionine during periods of high temperature can modulate body temperature and improve the reproductive performance of heifers subjected to a fixed-time artificial insemination (FTAI) protocol. In this context, our study aimed to assess the body temperature, follicle development, conception rates, and embryo loss rates in beef heifers supplemented with rumen-protected methionine and subjected to FTAI during periods of high temperature and humidity.

The overall objective was to investigate the response of heifers supplemented with RPM in terms of their body temperature, as well as to evaluate methionine's influence on pregnancy rates on day 30 after the FTAI. In addition, the possible effects of this supplementation on embryo loss rates were analyzed. The effect of supplementation on the average daily gain (ADG) and the final weight (FW) of the heifers were also investigated.

2. Material and Methods

All procedures were approved by the Animal Experimentation Ethics Committee of the Federal University of Pelotas (UFPEL), under code 5069. The experiment was carried out in a commercial beef cattle farm in the municipality of São Gabriel (30°13'54"S - 54°37'29"W). In September 2017, 100 Brangus (5/8 Angus 3/8 Brahman) heifers aged 17 to 19 months were selected, weighed, and categorized according to their body condition scores (BCS), where 1=very lean and 5=very fat (Lowman et al., 1973). On the same day, blood samples were collected for bovine herpesvirus (BoHV) seroneutralization and to verify persistent infections with Bovine Viral Diarrhea Virus (BVDV) by direct ELISA (Idexx BVDV Ag/Serum Plus, Idexx Laboratories, Westbrook, USA). Animals identified as positive for these diseases were not included in the experiment.

On October 31 (D-56), the weight and BCS of the animals were measured again. In addition, heifers were subjected to a gynecological ultrasound examination. Only heifers that were cycling and had no genital tract pathologies remained in the study. On this day, the animals were divided into two groups: Control Group, which received only mineral supplementation with a commercial formulation (CG; n=48), and Methionine Group, which received the same mineral supplementation but with the inclusion of methionine at a proportion of 4 g for each 100 g of mineral salt (MG; n=52). The initial average weight of the groups was 304.4 kg and 304.6 kg, respectively. Both groups were allocated to two similar pickets regarding area and forage availability (CG=63.5 ha, MG=68.3 ha).

Cows were grazed on native pastures. Both groups were grazed in the same native pasture variety and, therefore, any difference of the cow performance would be assigned to the treatment effect.

To analyze the forage resources, forage was collected using the double sampling method according to Wilm et al. (1944) to calculate the available forage mass. In an area of 0.25 m²,

the forage was cut close to the soil and areas of root clumps were excluded. Forage samples were collected in paper bags, weighed to calculate forage availability, and stored. Subsequently, samples were dried in the laboratory in a forced air oven (Biopar, RS, Brazil, model S836SD) at 70 °C for 48 h to determine the amount of dry matter (DM), and then sent for bromatological analysis on nutrition lab at NUPEEC in Federal University of Pelotas. These analyses were realized at three different timepoints (D-45, D0, and D60, considering day 0 of the FTAI protocol the D0.) throughout the study. The farm is certified by the international organization Alianza del Pastizal® (BirdLife International, Cambridge, UK), which certifies that the cows are raised and maintained on native pasture. The native forage species with the highest prevalence in the area were *Andropogon lateralis*, *Aristida laevis*, *Axonopus affinis*, *Paspalum plicatulum*, and *Paspalum notatum*. The initial forage availability (842.6 and 728.3 kg DM/ha for the CG and MG, respectively) was considered sufficient for the animals according to the dry matter intake estimates proposed by Barioni et al. (2008) as shown in Table 1.

Table 1. Values of dry matter (DM), crude ash (CA), ethereal extract (EE), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (FDA) at the three moments of pasture collection on the days -45 (D-45), 0 (D0), and 60 (D60) in relation to the FTAI protocol during all experimental period

	Control Group (CG)			Methionine Group (MG)		
	D-45	D0	D60	D-45	D0	D60
Dry matter kg/ha	916	832	780	710	780	695
Crude ash (%)	7,3	6,2	6,2	10,1	6,2	6,4
Ethereal extract	1,4	2,2	3,7	1,2	3,0	2,1
Crude protein (%)	8,6	8,3	7,5	10,9	8,0	8,4
NDF (%)	64,3	70,9	70,96	61,32	75,23	69,22
ADF (%)	32,8	31,7	33,34	31,19	41,3	34,2

NDF = Neutral detergent fiber; ADF = Acid detergent fiber

Both groups received the same mineral supplement of the control group during the 15-day adjustment period. The composition of the supplement is shown in Table 2.

Table 2. Minerals and ingredients of diets in the control group (CG) and methionine group (MG) during experimental period

Ingredient	Control Group (CG)	Methionine Group (MG)
Native pasture	<i>Ad libitum</i>	<i>Ad libitum</i>
Calcium	150 g/kg	150 g/kg
Phosphorus	55 g/kg	55 g/kg
Sodium	50 g/kg	50 g/kg
Sulfur	10.5 g/kg	10.5 g/kg
Zinc	2100 mg/kg	2100 mg/kg
Manganese	525 mg/kg	525 mg/kg

Copper	420 mg/kg	420 mg/kg
Iodine	31.5 mg/kg	31.5 mg/kg
Cobalt	15.8 mg/kg	15.8 mg/kg
Selenium	10.5 mg/kg	10.5 mg/kg
Lasalocid sodium	1800 mg/kg	1800 mg/kg
Methionine	–	40 g/kg

After this period, the MG received the rumen-protected methionine mixture (Smartamine® M, Adisseo, Antony, France) + mineral supplement. The supplement was provided ad libitum, and the intake was controlled weekly, in wooden troughs protected from rain. The supplement was provided for a period of 105 days until the end of the experiment.

After 30 days of supplementation, estrus synchronization was performed for fixed-time artificial insemination (FTAI). Before placing the progesterone intravaginal device (IVD), a new gynecological exam was performed in the heifers to assess the size of the follicles and/or the presence of corpus luteum. The synchronization protocol used is as follows: day 0 (D0), insertion of the progesterone IVD, 1.0 g monodose (Primer®) and 2.0 mg of estradiol benzoate (EB) (RIC-BE®) intramuscularly (IM); on D8, after removing the IVD, 0.15 mg of D-Cloprostenol (Prolise®) was administered IM. After 24 hours (D9), 1.00 mg of EB was administered IM, and FTAI was performed 28 hours after (D10). In 20 animals (10 in each group), randomly selected, the body temperature between D0 and D8 (during the period with the IVD) was monitored at 30 minute intervals using a data logger (Ibutton®, Thermochron, Whitewater, USA) coupled to the IVD according to the method proposed by Hansen (2012).

When the IVD was removed, the ovaries were analyzed to measure the size of the dominant follicle of a subset of the 20 animals whose temperatures were measured; this analysis was repeated on days 9 and 10 of the protocol. Transrectal ultrasonography (Mindray DP-2200, Shenzhen, China) with a 5 MHz probe was the method used. On D9 and D10, the body temperature was also measured by rectal examination using a digital thermometer. On D10, in the afternoon, starting at 2:00 p.m., FTAI was performed. All semen used belonged to the same bull and to the same freezing lot, previously analyzed for its physical, morphological, and thermoresistance properties. The semen was thawed in water at 37°C for 30 seconds with the aid of an automatic defroster.

Seventeen days after artificial insemination, the estrus status was analyzed by visual detection in both groups for a 7-day period. Animals in estrus were inseminated after 12 hours the signals' observation. At the end of this period, two bulls were included in each group, which were rotationally changed every 15 days. Thirty days after the artificial insemination, a pregnancy diagnosis was performed by ultrasonography to determine the pregnancy after FTAI. The test was repeated 30 days later to determine pregnancy rates and pregnancy loss rates after another estrus cycle.

The animals were weighed when the groups were taken to the management center to perform a procedure, so measurements for both groups were performed at irregular intervals (D-56, D-18, D0, D42, and D76). An EziWeigh 7i electronic scale (Tru-Test®, Auckland, New

Zealand) coupled with the containment unit was used to weigh the animals. After weighing, the BCS was assessed by visual observation, and were rated on a scale of 1-5 (1=very lean, 5=very fat) according to the scale described by Lowman et al. (1973).

The climate data were obtained with an interval of 1 hour using a mobile weather station (Instrutemp® ITWH-1080, São Paulo, Brazil) installed next to the farm management center. Based on this information, the temperature-humidity index (THI) was calculated. The formula used to calculate the THI was: $THI = T_s + 0.36 T_{dp} + 41.2$ where T_s = dry bulb thermometer temperature and T_{dp} = dew point temperature. During the experiment, mean temperatures of 24.93 °C, 25.42 °C, and 24.95 °C were observed for the months of December 2016, January 2017, and February 2017, respectively.

This study used a completely randomized design with two treatments (CG vs. MG). The experimental unit considered was each heifer. The statistical analyses for body weight and follicle diameter were performed using ProcMixed Models using the SAS Studio® software (SAS Institute Inc., Cary, USA). Analyses involving repeated measures over the time (e.g. weight (kg), follicle diameter (mm)) were compared between treatments using ANOVA considering individual heifer as the RANDOM effect. Additionally, the model used for analysis of time-course data (repeated measures) included time (experimental day) as well as treatment × time interactions.

The conception and pregnancy rate analyses were performed by the Chi-square test using SAS Studio® software (SAS Institute Inc., Cary, USA). For the statistical analysis of body temperature, the same repeated measurements model was used, with group and experimental day as fixed variables and body temperature (°C) as the response variable. The results are presented as means ± standard error of the mean.

3. Results

During the experimental period, the animals of both treatments had been daily mineral supplement intake corresponding to 100 g/animal/day of the mixture, which correspond that the MG had a consumption of 4g of methionine/day, and it is the only difference in the mineral supplementation.

Forage availability for the MG and CG pickets was 710, 780, and 695; and 916, 832, and 780 kg DM/ha, respectively, for samples collected on D-45, D0 and D60 of the experiment. The results of forage availability and bromatological analysis are shown in Table 1.

The ADG throughout the experimental period was 0.706 kg/day for the MG and 0.637 kg/day for CG, and the MG had higher weights on days D0 and D30 in relation to the FTAI protocol ($P < 0.03$) (Figure 1). These differences influenced the FW, which was higher for the MG (398.57 ± 28.47 kg vs. 389.27 ± 21.44 kg; $P \leq 0.01$).

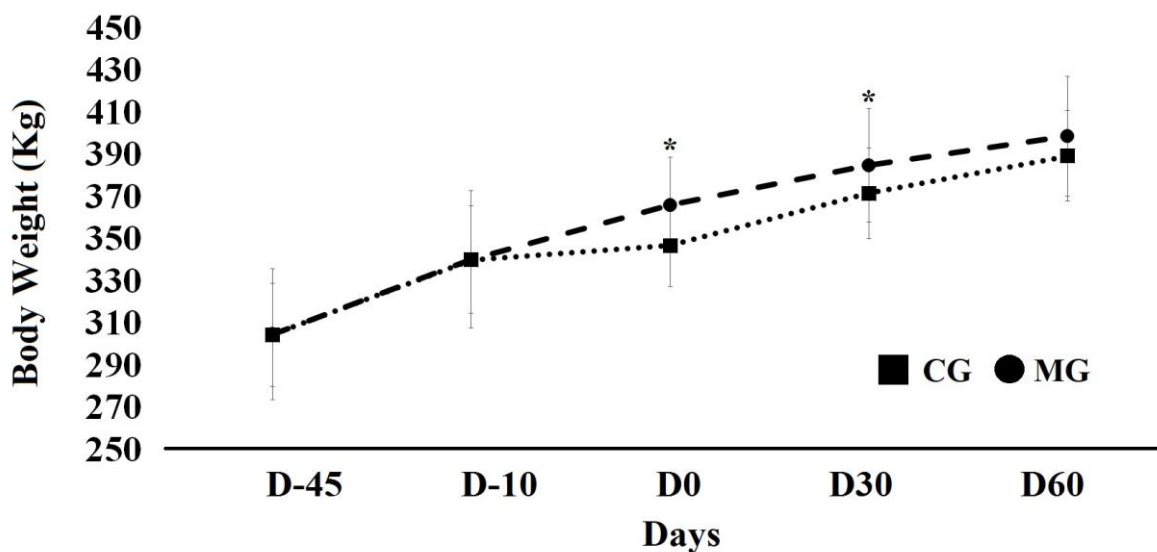


Figure 1. Evolution of body weight (Kg) in heifers of the control group (CG) and methionine group (MG) during all experimental period. * $P \leq 0.05$ (Tukey)

The minimum, mean, and maximum values of THI in the experimental period are shown in Table 3.

Table 3. Temperature-humidity index (THI) minimum, medium and maximum during the first nine days in the fixed-time artificial insemination protocol (FTAI)

THI	D0	D1	D2	D3	D4	D5	D6	D7	D8
Minimum	62,34	62,05	63,54	63,34	65,44	64,34	65,14	62,04	62,34
Medium	63,35	64,93	65,91	67,96	68,01	70,75	70,54	68,35	65,12
Maximum	66,75	67,95	70,45	73,95	73,65	79,15	78,55	74,15	65,55

Between D0 and D6, when the highest THI values were recorded, considering as heat stressed heifers when the THI is higher than 73, because it already shows effects on the reproduction (Schüller et al., 2014), the MG had lower mean body temperature values ($P \leq 0.01$), which was also observed at different timepoints during the implementation of the protocol ($P \leq 0.01$). However, on the days before the FTAI, there was a reduction in the THI and body temperatures. Those differences no longer existed during less challenging climate periods, THI lower than 73 (Figure 2).

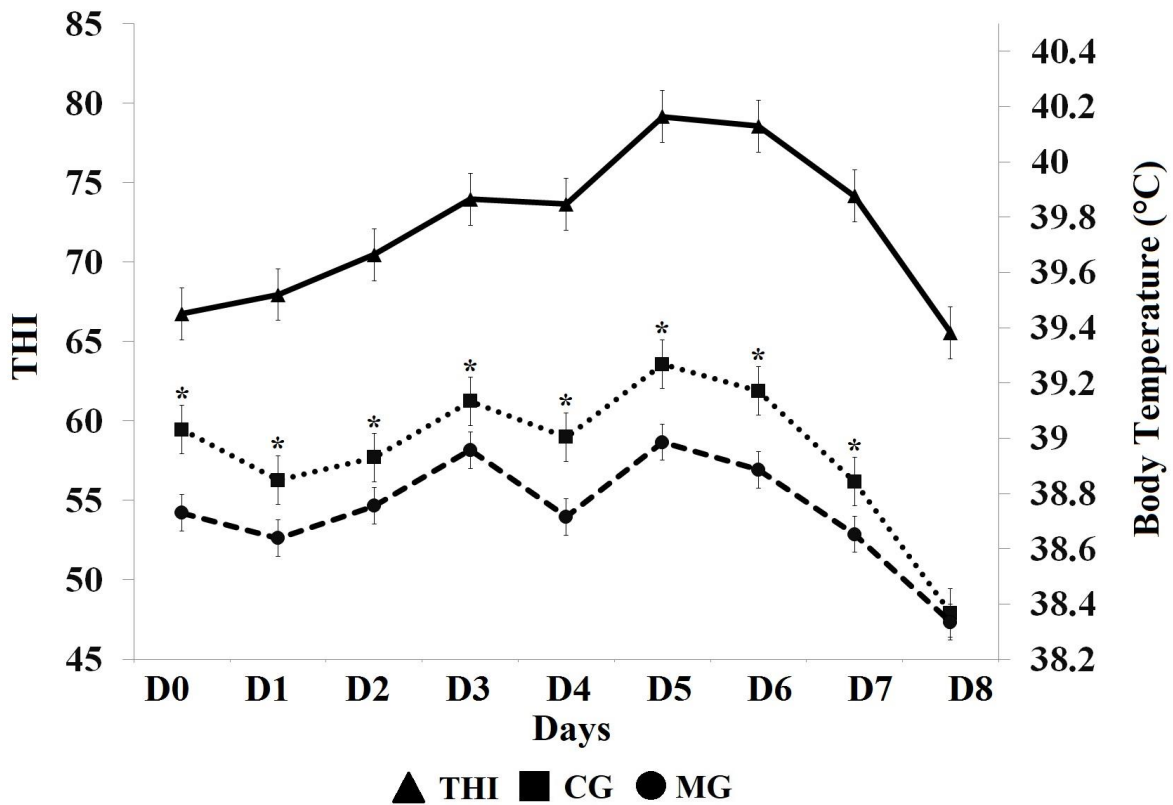


Figure 2. Relationship between body temperature (°C) in heifers of the control group (CG) and methionine group (MG) and the temperature-humidity index (THI) during the first nine days in the fixed-time artificial insemination protocol (FTAI). * $P \leq 0.05$ (Tukey)

MG heifers had a tendency to larger follicles ($11.76 \text{ mm} \pm 2.07 \times 10.86 \text{ mm} \pm 2.75$, $P=0.06$). This difference was mainly determined by differences on D8 and D9 ($P < 0.05$); however, these differences disappeared on D10, suggesting that the control group may have had a higher growth rate during this period, although no statistical difference was observed for this variable, that is probably because of the size of the subset (Figure 3).

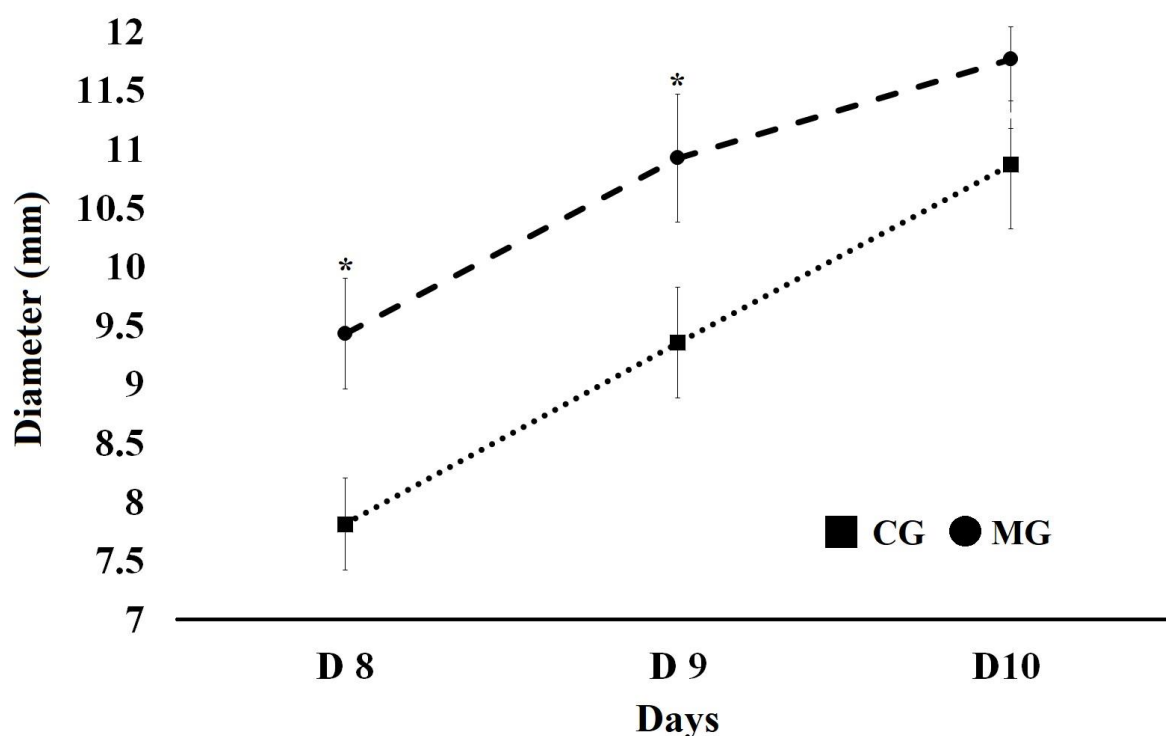


Figure 3. Follicle diameter (mm) in heifers of the control group (CG) and methionine group (MG) during the last three days in the fixed-time artificial insemination (FTAI). * $P \leq 0.05$ (Tukey)

There were no differences in conception and pregnancy rates 30 and 60 days after the FTAI, with values of 42.3% (22/52) and 76.9% (40/52) for the MG and 39.5% (19/48) and 79.1% (38/48) for the CG, as shown in Table 4.

Table 4. Pregnancy rates (%) at day 30 (D30) and day 60 (D60) in relation to the FTAI protocol in heifers of the control group (CG) and methionine group (MG)

Pregnancy rates (%)	Control Group (CG)	Methionine Group (MG)	P Value
D30	39,5 (19/48)	43,3 (22/52)	$P > 0,05$
D60	79,1 (38/48)	76,9 (40/52)	$P > 0,05$

There were no significant differences in BCS. The initial BCS of the heifers presented the following distribution in the CG: 6.25% score 2, 52.08% score 2.5 and 41.67% score 3 and at the end of the experiment had 41.67% score 3.5 and 58.33% score 4. In the GM they had the following distribution: 9.6% score 2, 44.2% score 2.5, 44.2% score 3 and 2% score 3.5. At the end of the experiment 2% score 3, 36.5% score 3.5, 59.5% score 4 and 2% score 4.5.

4. Discussion

Methionine supplementation has been shown to improve growth performance in cattle (Schroeder et al., 2006). This was further confirmed in our study as there was a greater weight gain in the MG group, especially in December and January, which registered higher environmental temperatures. Despite the differences in animal category and nutritional management, similar results were found in Nellore steers at 12 months of age in a grazing

system with *Brachiaria* and supplementation with essential fatty acids plus methionine (FEFA and EFA), demonstrating that methionine supplementation should be considered for animals at the end-stage (Fernandes et al., 2008), and can be used to reduce the incidence of metabolic disorders in ruminants (Gomez et al., 2011). The ideal supplementation of methionine is responsible for more retention of nitrogen (Schroeder et al., 2006) and it has been proved to rumen-protected methionine and lysine may enhance gain efficiency and dietary energetics in growing animals (Torretera et al., 2017).

In a forage-based diet, the fewer methionine levels can compromise the productivity of ruminants (Veira et al., 1991). We speculated that it could be related to the effects of the methionine restriction on biochemical and physiological responses that limit the accumulation of fat in tissues. It has been recently shown that this amino acid is directly related to the expression of the uncoupling protein-1 (UCP1) protein, which is responsible for the decoupling of cellular respiration in mitochondria, which in turn is directly related to increased energy expenditure, weight loss, and increased body temperature of the animals (Brondani et al., 2012). The restriction of this amino acid promotes the regulation of lipogenic genes in the liver, resulting in a lower hepatic capacity to synthesize and export lipids, in addition to altering the capacity of white adipose tissue to synthesize and oxidize fatty acids (Hasek et al., 2013).

During the ovulation synchronization period of heifers, the measured THI reached values higher than 75, as shown in Table 4, and negative reproductive effects were already observed for THI close to 73 (Schüller et al., 2014). Analysis of the impact of climate factors from 7 days before the FTAI to 3 days after the protocol in Hereford and Hereford x Angus cows by Biggers et al. (1987) showed that THI increases lead to lower conception rates (23% with $\text{THI} \geq 80$). Heat stress also causes a decrease in the intensity of the estrus signs, coloration of the mucosa of the vagina, and size of the dominant follicle, after one-point increases in the THI on the day of estrus (Schüller et al., 2017). However, this study with heifers showed that the two groups had similar pregnancy rates. However, this study with heifers showed that the two groups had similar pregnancy rates. The whole protocol period was marked with high THI and a significant difference in the internal temperature between treatments, only the last protocol day marked with decreasing of THI and no differences groups in internal temperature. This fact could have contributed to no group differences in the pregnancy rates already that the critical phase of heat stress in the ruminants fertility is when the embryo has two to four cells corresponding to the day 2 - 3 after ovulation (Edwards and Hansen, 1996).

The lower body temperatures in heifers of the MG could be explained by an insufficient amount of methionine in the control group, thus affecting metabolic flexibility and thermoregulation as discussed previously (Hasek et al., 2010). UCP1 is a protein in the inner mitochondrial membrane that assists in the transfer of protons (H^+) to the interior of the mitochondria, but this movement only causes the release of heat and does not lead to ATP formation. It has recently been shown that methionine restriction increases the number and size of mitochondria in inguinal white adipose tissue cells, expression of UCP1, respiratory proteins, and citric acid cycle enzymes. This, in turn, increases the oxidative capacity of mitochondria (Patil et al., 2015). These mechanisms directly impact the generation of heat,

which suggests that supplementation with this amino acid could increase adaptability in highly-challenging thermoregulation situations. The amino acid profile of the pastures and the effective absorption of each amino acid is still poorly understood, and our study shows the requirement of further knowledge in this area. As previously mentioned, proteolysis in dairy cows exposed to high temperatures is an important pathway for energy production (Baumgard et al., 2013), which apparently can also be applied to heifers exposed to high temperatures, as these animals had higher weight gains and lower body temperatures when supplemented with amino acids. However, the effects of methionine on the microbiota of the small intestine could also directly interfere with the absorption of nutrients, leading to higher weight gains (Shoveller et al., 2005).

MG heifers had larger follicles at the time of insemination, as already demonstrated in *Bos taurus* x *Bos indicus* heifers supplemented with methionine and urea, which had a higher percentage of follicles larger than 9 mm (Alonso et al., 2008). Increases in body temperature can impact follicle stimulating hormone concentrations, thus compromising follicle growth, estrogen synthesis, estrus manifestations, and luteinizing hormone peaks (Nardone et al., 2010; Li et al., 2016), which then affects follicle size at the time of ovulation. This information is concordant with the results found in the experiment with heifers. Although pregnancy rates in heifers with larger follicles at the time of ovulation (≥ 12.8 mm) are expected to be higher than those in heifers with smaller follicles (≤ 10.7 mm) (Perry et al. 2007), we were unable to observe this in our study. Marked effects on weight gain, body temperature throughout the FTAI protocol, and increases in follicle size and pregnancy rates in this study were not influenced by these factors, which suggests that other factors, such as mild temperatures after the FTAI, a well-conducted transfer, and a correct bull-to-cow ratio, ensured good pregnancy rates for both groups.

5. Conclusion

In conclusion, the results of this study show that methionine supplementation in Brangus heifers in a native pasture leads to increases in weight gain and size of the dominant follicle and decreased the internal temperature during the periods with high THI.

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