PASTURE AND FORAGE UTILIZATION

Energy supplementation as strategy of pasture management

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ABSTRACT. This study evaluated the effect of increased energy via supplementation on the performance, ingestive behavior, nutrient digestibility, and nitrogen metabolism of grazing heifers fed tropical forage in the rainy-dry transition season. Treatments consisted of mineral supplementation *ad libitum* (control) and multiple supplements formulated to provide different energy levels and the same amount of protein (300 g CP animal d-1) and were denominated as low (LE; 340 g TDN animal d^{-1}), medium (ME; 780 g TDN animal d^{-1}) and high (HE; 1220 g TDN animal d^{-1}) energy. Animals supplemented with ME, and HE had a greater average daily gain in relation to the control treatment, with an increase of 41 and 46%, respectively. Greater values for total apparent digestibility of neutral detergent fiber were observed for the treatment HE. Lesser values of urinary urea N were observed for the control and HE treatments. Our results define the use of energy levels in the supplement as a tool for pasture management. If the purpose of the production system is to enhance forage intake, the option is to supply supplements with less energy levels. In contrast, if the purpose is to increase the stocking rate, supplements with greater energy levels should be used.

Keywords: beef cattle; multiple supplementation; nutritional parameters; tropical pastures.

Introduction

Tropical pastures are the basis of Brazilian beef cattle feed. But even pastures fertilized during a period of high rainfall does not constitute a complete food for the herd, and therefore, its association with correct mineral supplementation is the premise for adequate animal development. In these production systems, the insertion of protein and energy via supplement is performed to overcome the nutritional deficits that depend on the forage characteristics, animal category, and desired productive performance.

The use of supplementation for grazing animals also constitutes a pasture management strategy, with changes in the stocking rate (Barbero et al., 2015; Fajardo et al., 2015), due to the associative effect of the supplement with the forage that modifies the ruminal metabolic condition (Dixon & Stockdale, 1999). Additionally, as there is a reduction in the amount of rainfall, a period recognized as a rainy-dry transition, there is a reduction in the quantity and quality of the forage produced, with emphasis on the beginning of the decrease in the nitrogen concentration.

In this situation, there is disagreement among some authors on the protein and energy levels that should be provided by the supplement to optimize the pasture use, since several works (Cabral, Paulino, Paula, Valadares, & Araújo, 2012; Cabral et al., 2014a; Cabral et al., 2014b) reported that increasing supplement supply without adequate protein and energy levels promotes an imbalance of nutrients and impairs animal performance.

Some authors claim that due to the still expressive protein concentration in this period, supplementation with a greater energy level should be promoted (Sales et al., 2008). In opposition, Porto et al. (2011) and Santos et al. (2019) did not observe an increase in weight gain with the increase of energy insertion in the supplement. Complementarily, Detmann, Paulino, Valadares Filho, and Huhtanen (2014)analyzed forage harvested from tropical pastures under continuous management and observed that most of the samples presented an energy and protein ratio above those demanded by the animals. In this situation, animals seek to reduce discomfort due to excess energy in the diet, alter the use of fiber, and thus reduce consumption (Forbes, 2003).

Based on this, we hypothesize that the greatest impact of supplementation with greater energy levels will be on weight gain per area and, therefore, the objective wasto evaluate the effect of increased energy via supplementation on the performance, ingestive behavior, nutrient digestibility, and nitrogen metabolism of grazing heifers fed tropical forage.

Material and methods

Experimental site, animals, and management

The experiment was carried out in Rondonópolis-MT, Brazil (16° 28'S, 54°31' O), during the rainy-dry transition season, from 11 March to 31 May 2015. Air temperature data (°C) during the experimental period was obtained from a recording station of the Federal University of Mato Grosso– Rondonópolis Campus and the monthly precipitation was measured with a rain gauge located in the experimental area (Figure 1).

Forty crossbred heifers (Nelore breed predominance), with average initial ages and weights of 17 months and 229 kg, respectively, were assigned to an experimental area of 7 ha, consisting of four 1.75-ha paddocks uniformly covered with Marandu palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster]. The animals were managed and cared for according to the guidelines and recommendations of the International Guiding Principles for Biomedical Research Involving Animals (CIOMS) - Geneva, 1985. All procedures were approved by the Federal University of Mato Grosso (register no. 63/2014), Rondonópolis, Brazil.
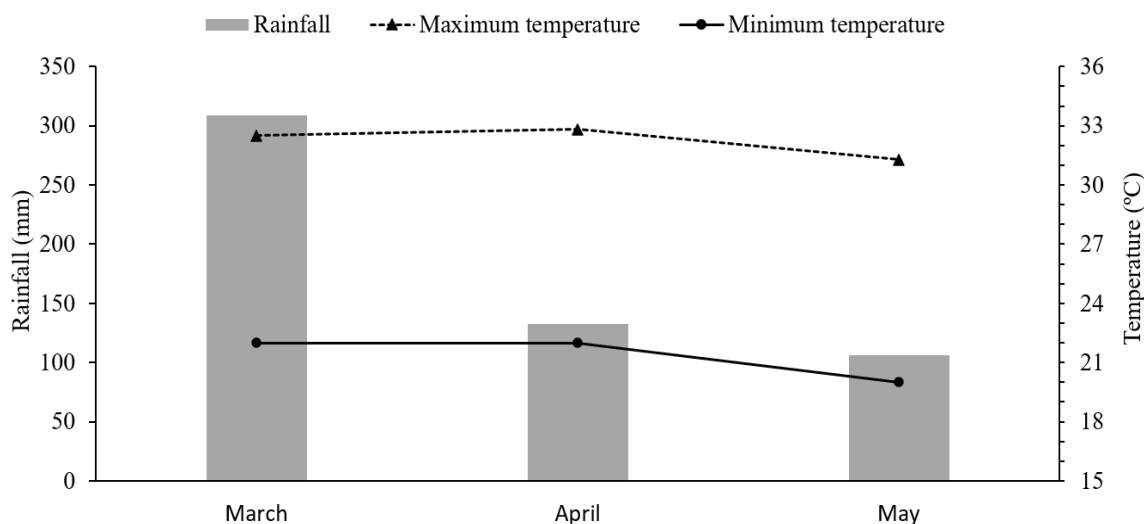

Figure 1. Rainfall and temperature during the experimental period.

Based on soil analyses and as recommended by Martha Júnior et al. (2007), fertilization with urea (100 kg ha^{-1}) and potassium chloride (50 kg ha^{-1}), was applied to all paddocks, not requiring liming and phosphate fertilization. Fertilizers were applied on 12 December 2014, 2 January 2015, and 20 March 2015.

Treatments (Table 1) consisted of mineral supplementation *ad libitum* (control) and multiple supplements formulated to provide different energy levels and the same amount of protein (300 g CP animal d^{-1}) and were denominated as low (LE; 340 g TDN animal d^{-1}), medium (ME; 780 g TDN animal d^{-1}) and high (HE; 1220 g TDN animal d^{-1}) energy supplements. Treatments LE, ME, and HE were provided in the amount of 0.5, 1.0, and 1.5 kg animal d^{-1}, respectively, daily between 10h00 and 12h00, in covered troughs, allowing access by the animals on both sides. Animals had unrestricted access to water throughout the experiment.

Productive performance

Heifers were weighed at the beginning and end of the experiment, after a 14-h fasting period. Total weight gain (TWG) was determined as the difference between the final and initial fasting body weight, and the average daily gain (ADG) was quantified as the ratio between TWG and the number of experimental days (84 days).

Table 1. Proportion of ingredients (based on natural matter) and chemical composition (based on dry matter) of supplements and ingredients amount supply.

Item	Treatments			
	Control	LE	ME	HE
	Proportion of ingredient (g kg^{-1})			
Mineral supplement[1]	1,000	107	57	38
Urea / ammonium sulphate (9:1)	—	69	36	25
Soybean meal	—	784	280	99
Ground corn	—	40	627	838
	Composition of supplement (g kg^{-1})			
DM	—	882	888	890
NDF	—	144	211	235
CP	—	580	295	195
TDN	—	674	775	811
	Amount supply (kg d^{-1})			
Supplement	—	0.5	1.0	1.5
CP	—	0.30	0.30	0.30
TDN	—	0.34	0.78	1.22

[1]Percent composition = dicalcium phosphate. 40.00; sodium chloride: 35.00; calcium carbonate: 14.83; sulphur: 4.00; calcium sulphate: 4.283; silicates: 1.00; copper sulphate: 0.48; zinc oxide: 0.3533; cobalt sulphate: 0.0367; calcium iodate: 0.00833; sodium selenite: 0.002. DM: dry matter; NDF: neutral detergent fiber; CP: crude protein; TDN: total digestible nutrients.

Ingestive behavior

Ingestive behavior was determined on days 31 and 52 of the experiment for 12 consecutive hours, from 06h00 to 18h00. Grazing time, rumination time, and idle time were evaluated every 15 minutes. Time spent in forage selection, apprehension, and semi-digested food manipulation, which included the short time intervals used for the forage selection, was denominated grazing time. For rumination time, we considered the time spent in regurgitation and semi-digested food remastication, and the time elapsed between swallowing and regurgitation. When the animals showed no locomotive activity and an absence of mandibular movements, this was considered idle time (Cabral, Bauer, Cabral, Souza, & Benez, 2011).

Feed efficiency for DMI (FE_{DM}) and NDF intake (FE_{NDF}); and rumination efficiency for DMI (RE_{DM}) and NDF intake (RE_{NDF}) were obtained by the following equations: $FE_{DM} = DMI/GT$; $FE_{NDF} = NDFI/GT$; $RE_{DM} = DMI/RT$; $RE_{NDF} = NDFI/RT$.

Where: DMI, dry matter intake; NDFI, neutral detergent fiber intake; GT, grazing time; RT, rumination time.

Experimental procedures and sampling

Forage samples were collected monthly at representative points of the mean canopy height with the use of a metal frame (0.25 m^2). Average canopy height was obtained from 100 points per paddock. All forage samples were collected at ground level. After collection, a sub-sample was taken to the laboratory for manual separation of the morphological components: leaf blade, stem + sheath, and dead material. These samples were oven-dried at 55°C for 72 hours and subsequently weighed to obtain dry weights. Also, forage samples for chemical analysis were collected monthly using the hand-plucked technique (Sollenberger & Cherney, 1995) and oven-dried at 55°C for 72 hours and ground to 1 and 2 mm in a knife mill.

To evaluate the intake and digestibility of the diet components, a digestibility trial was conducted over nine days, starting on the 35th and finishing on the 43rd day of the productive performance evaluation. The first six days were used for the adaptation of animals to the markers and the last three days were used for the collection of feces and urine.

Chromium oxide (Cr_2O_3) was used as an external maker to estimate fecal excretion and was supplied daily at 09h00 to each animal, at a dose of 15 g, introduced orally directly into the esophagus. Individual intake of supplement was estimated using titanium dioxide (TiO_2), being 15 g of TiO_2 per animal added to the supplement.

During the last three days of the trial, fecal samples were collected at different times for each collection day: 15h00, 10h00, and 7h00 hours, respectively. Approximately 200 g of feces were collected per animal immediately after defecation or directly from the rectum. The samples were packed in plastic bags, individually labelled, and oven-dried dried at 55°C. After drying, the samples were ground in a knife mill (1-mm sieve); samples were consolidated per animal, referring to the 3 days of collection (Table 2).

Table 2. Morphological and chemical composition of Marandu palisadegrass.

	Months		
	March	April	May
Morphological composition (kg ha^{-1})			
Forage mass	1930	4280	4530
Leaf blade	630	1700	1300
Stem	310	1030	1650
Dead material	990	1550	1580
Leaf: stem ratio	0.63	1.09	0.78
Chemical composition (g kg^{-1})			
CP	120	122	114
NDF	567	570	574
ADF	240	230	227
ADIP	46	54	36

CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADIP: acid detergent insoluble protein.

Chemical analysis

Forage and supplement samples were analyzed for dry matter (DM), mineral matter (MM), crude protein (CP; Silva & Queiroz, 2002) and neutral detergent fiber (NDF; Mertens, 2002), using thermostable alpha-amylase and omitting the use of sodium sulfite; neutral detergent insoluble fiber (NDFI; Valente et al., 2011) were quantified by in situ incubation procedures with Ankon® bags (F57) for 288 hours in samples processed at 2-mm, and acid detergent insoluble protein (ADIP) was determined according to the methodology described by Licitra, Hernandez, and Van Soest (1996).

Fecal samples were evaluated for TiO$_2$ content, according to the colorimetric technique described by Titgemeyer, Armendariz, Bindel, Greenwood, and Löest (2001). Chromium oxide content was evaluated in an atomic absorption spectrophotometer, according to Williams, David, and Iismaa (1962). Fecal excretion was estimated by an intermediate of the ratio between the provided dose of the indicator and the fecal concentration of chromium oxide (Smith & Reid, 1955)

To estimate voluntary forage intake, the internal iNDF indicator was used, according to Detmann et al. (2001). An estimate of the individual supplement intake was obtained according to Santos et al. (2019). Total dry matter intake (kg d^{-1}) was estimated as the sum of individual forage dry matter intake and individual supplement intake.

Nitrogen metabolism

Spot urine samples from spontaneous urination were collected from all animals (Valadares et al., 1999) on the 9th day of the digestibility trial, 4h after feeding. One sample of 10 mL was diluted in 40 mL of H$_2$SO$_4$ (0.036 N) to reduce the pH to values below 3.0, avoiding nitrogen loss. The concentrations of creatinine, urea, and purine derivates were determined in the diluted sample.

The creatinine content was determined according to the modified Jaffé method, while uric acid was measured using the enzymatic-colorimetric method with a clear lipid factor. Allantoin concentrations were determined according to the colorimetric method described by Chen and Gomes (1992), and urea was measured via the urease method/glutamate dehydrogenase (GLDH). Total urinary volume was estimated based on the relationship between daily creatinine excretion as a function of body weight and the creatinine concentration in urine. Creatinine excretion per unit of body weight was obtained via the equation in Costa e Silva et al. (2012).

Urinary urea N (UreaN) was estimated as the product between the concentration in the urine spot samples and the estimated urinary volume. The excretion of total purine derivatives (mmol d^{-1}) was calculated as the sum of the amounts of allantoin, and uric acid excreted via the urine. The absorbed purines were calculated from the excretion of total purine derivatives, using the equation in Barbosa et al. (2011). Ruminal synthesis of microbial nitrogen (N$_{MIC}$) was estimated based on the absorbed purines, using the equation proposed by Chen and Gomes (1992) and N$_{TOTAL}$ in bacteria, which is 0.134 according to Valadares et al. (1999).

Blood samples were collected via jugular venipuncture, using commercial vacuum kits and coagulation accelerator gel, and were immediately centrifuged at 400 rpm for 15 minutes. The obtained serum was frozen at -20°C for the quantification of serum urea nitrogen (SUN). Blood serum samples were analyzed for urea levels via the urease/GLDH method.

Experimental design and statistical analysis

The experimental design was a completely randomized design, with four treatments. Data were analyzed using the fixed-model method, with a parametric structure, using the SAS® statistical software. A multiple comparison of the means, associated with the fixed effect of supplementation, was performed with a Tukey test (p = 0.05). The mathematical model was as follows: $Y_{ij} = \mu + D_{i(j)} + e_{ij}$, where Y_{ij} was the dependent variable of the supplementation i, measured in animal j; μ was the overall mean; D_i was the fixed effect of supplementation i, measured in animal j; and e_{ij} was the random error of supplementation i, measured in animal j.

Results

There was an effect of supplementation on the animals' performance (p < 0.05; Table 3). Animals supplemented with ME, and HE had a greater ADG in relation to the control treatment, with an increase of 41 and 46%, respectively. However, there was no difference between supplemented animals.

Supplementation affects the ingestive behavior (p < 0.05; Table 3). Lesser grazing times were observed for animals supplemented with HE. Animals of LE treatments had lesser rumination times, however, animals supplemented with HE had longer idle time. Lesser RE_{DM} was observed for animals supplemented with LE, and greater FE_{NDF} was observed for animals supplemented with HE.

Table 3. Effect of energy levels in supplements on performance and ingestive behavior of grazing heifers.

	Treatments				SEM	p-value
	Control	LE	ME	HE		
Animal performance						
TWG (kg)	37[b]	42[ab]	52[a]	54[a]	3.44	0.0041
ADG (g d^{-1})	0.46[b]	0.53[ab]	0.65[a]	0.67[a]	0.04	0.0042
Ingestive behavior						
Grazing (min d^{-1})	494[b]	552[a]	487[b]	378[c]	10	< 0.0001
Rumination (min d^{-1})	85[b]	52[c]	68[bc]	142[a]	36	< 0.0001
Idle (min d^{-1})	142[bc]	116[c]	165[ab]	200[a]	32	< 0.0001
FE$_{DM}$ (kg DM h^{-1})	0.64	0.56	0.58	0.78	0.05	0.0736
FE$_{NDF}$ (kg DM h^{-1})	0.37[ab]	0.33[b]	0.35[b]	0.50[a]	0.03	0.0111
RE$_{DM}$ (kg DM h^{-1})	4.55[ab]	5.75[a]	4.45[ab]	2.35[b]	0.74	0.0407
RE$_{NDF}$ (kg DM h^{-1})	2.62	2.40	2.72	1.51	0.443	0.0668

LE: low energy supplement (340 g TDN animal d^{-1}); ME: medium energy supplement (780 g TDN animal d^{-1}); HE: high energy supplement (1220 g TDN animal d^{-1}). TWG: total weight gain; ADG: average daily gain; FE$_{DM}$: feed efficiency for dry matter intake; FE$_{NDF}$: feed efficiency for neutral detergent fiber intake; RE$_{DM}$: rumination efficiency for dry matter intake; RE$_{NDF}$: rumination efficiency for neutral detergent fiber intake. SEM: standard error of the mean. Means in the same column with different superscript letters differ (p < 0.05) by the Tukey–Kramer.

There was no supplementation effect on intakes of total DM, CP, NDF, and digested NDF (p > 0.05; Table 4). However, there was a reduction of 41% on forage DM intake of animals supplemented with HE in relation to the control (5.33 kg d^{-1}). In terms of nutrient digestibility, supplementation affects the total apparent digestibility of NDF, with greater values for animals supplemented with HE (Table 4).

Table 4. Effect of energy levels in supplements on intake and total apparent digestibility of nutrients of grazing heifers.

	Treatments				SEM	p-value
	Control	LE	ME	HE		
Intakes (kg d^{-1})						
Total DM	5.33	5.06	4.64	5.07	0.36	0.8199
Forage DM	5.33[a]	4.73[ab]	3.75[bc]	3.12[c]	0.34	0.0013
CP	0.64	0.63	0.63	0.67	0.06	0.4120
NDF	3.08	2.98	2.83	2.90	0.27	0.8752
Digested NDF	1.88	1.99	1.97	2.12	0.16	0.8062
Digestibility (g kg^{-1})						
DM	547	572	540	553	18	0.6499
CP	804	790	796	810	13	0.7224
NDF	611[c]	670[b]	693[ab]	735[a]	14	< 0.0001

LE: low energy supplement (340 g TDN animal d^{-1}); ME: medium energy supplement (780 g TDN animal d^{-1}); HE: high energy supplement (1220 g TDN animal d^{-1}). DM: dry matter; CP: crude protein; NDF: neutral detergent fiber. SEM: standard error of the mean. Means in the same column with different superscript letters differ (p < 0.05) by the Tukey–Kramer.

Regarding the nitrogen metabolism, a supplementation effect was observed for UreaN and SUN (p < 0.05; Table 5). Lesser values of UreaN were observed for the control and HE treatments. Supplemented animals presented greater SUN values.

Table 5. Effect of energy levels in supplements on nitrogen metabolism of grazing heifers

	Treatments				SEM	p-value
	Control	LE	ME	HE		
	g/d					
NI	158	167	148	137	18	0.6955
N_{MIC}	78	89	94	97	12	0.5459
UreaN	37[b]	66[ab]	77[a]	46[ab]	8	0.0137
UN	6.4	9.8	10.9	6.8	2.0	0.0860
FeN	46	47	53	74	9	0.1871
UN_{BW}, %	28	38	40	26	5	0.1963
SUN, mg dL^{-1}	11[b]	17[a]	17[a]	15[a]	1	0.0438
N_{MIC}: NI	55	50	63	71	10	0.4935

LE: low energy supplement (340 g TDN animal d^{-1}); ME: medium energy supplement (780 g TDN animal d^{-1}); HE: high energy supplement (1220 g TDN animal d^{-1}). NI: nitrogen intake; N_{MIC}: ruminal synthesis of microbial nitrogen; UreaN: urinary urea N; UN: urinary N; FeN: fecal N excretion; UN_{BW}: urinary nitrogen in percentage of body weight; SUN: serum urea nitrogen; N_{MIC}: NI: ruminal synthesis of microbial nitrogen and nitrogen intake ratio. SEM: standard error of the mean. Means in the same column with different superscript letters differ (p < 0.05) by the Tukey–Kramer.

Discussion

For better supplement use efficiency, it is necessary to know the plant and animal interface, which involves studies of how grazing conditions interfere with the ingestive behavior, intake, and performance, to identify the appropriate management conditions for the animal production system. According to previous studies, the responses related to animals raised in pastures are directly related to the nutritional levels of the diet (Mendes et al., 2015; Brandão et al., 2017).

With the additional supply of nutrients via supplementation, the daily metabolic requirements are met more quickly (Brandão et al., 2016b). Despite the difference in control treatment performance for the supplemented animals (Table 3), ADG of control treatment was satisfactory, with greater values than those previously found in similar studies (Brandão et al., 2016a; Brandão et al., 2017; Almeida et al., 2018). This occurred because the forage consumed had a relatively good nutritive value, with high CP concentration (> 120 g kg^{-1}; Table 2), above that recommended for ruminal microorganism maintenance (70 g kg^{-1}; Sampaio et al., 2010). Besides that, there was an increase in forage mass in April and May as a result of the fertilization carried out in March and the precipitation in the experimental period (Table 2; Figure 1). This increase in the forage mass provides two management strategies, which are the increase in the stocking rate during the period of greater precipitaion, or the possibility of starting the dry season with a greater forage mass since this is a bottleneck in forage-based systems.

Treatments ME and HE allowed greater ADG, however, there is a reduction in forage DM intake compared to control, that is, there was a substitution effect of pasture by supplement, having seen the equal DM intake among all treatments. This effect is characterized by the reduction in forage DM intake with an increase in supplement intake while maintaining the total DM intake (Minson, 1990). As forage is the most economical source of nutrient supply to animals, the adoption of supplements should optimize the use of fodder resources, promoting minimal substitution. However, ME and HE treatments become a viable alternative in systems where the objective is to increase the stocking rate. This is the reality in properties where the high value of the land requires the intensification of cattle raising, with an increase in the offtake rate and working capital invested.

Thus, while fertilization provides an increase in the stocking rate without changing the individual performance of the animals (Delevatti et al., 2019), energy supplementation increases the stocking rate and the average daily gain of the animals, boosting meat production per area.

The ingestion of forage and supplements causes modifications in the energy: protein ratio of the diet, one of the determinants of consumption (Illius & Jessop, 1996), and the animal can make adjustments in this ratio by increasing or reducing the use of forage. Excess energy in the diet causes a decrease in consumption (Forbes, 2003), while protein excess causes an increase in hepatic energy expenditure and, consequently, a reduction in productive performance (Detmann, Paulino, Valadares Filho, & Huhtanen, 2014).

Ingestive behavior variables confirm the explanations described above. The animals of LE treatment had the longest grazing times (Table 3) since depending on the amount of supplement included in the diet, the

increase in supplementation levels promotes a reduction in grazing time because of the substitutive effect of pasture intake per concentrate (Minson, 1990), which was observed in the present study.

Our rumination efficiency (RE) values are above what is in the literature because in this work the behavior was evaluated for 12 hours, while the works that evaluated these efficiencies did the behavior for 24 hours (Araújo et al., 2021; Silva et al., 2021). Thus, there is an increase in the RE values, due to the shorter times of rumination with emphasis on the fact that the animals ruminate with greater intensity at night (Kilgour, 2012).

Microbial growth is potentiated by the combination of the availability of fermentable energy and degradable nitrogen in the rumen (Russel, O'Connor, Fox, Van Soest, & Sniffen, 1992). With the increase in nitrogen and energy supply due to the multiple supplementation, ruminal cellulolytic bacterial activity also increases (Silva-Marques et al., 2015), which explains the greater NDF digestibility in the HE treatment. Besides that, the inclusion of supplement and the reduction in forage DM intake also contributes to the increase of the NDF digestibility, because the NDF of supplement is more digestible than the NDF of forage.

Supplemented animals received the same amount of protein via supplement, explaining the similar values for NI, UN, and FeN. However, a lesser NI was expected for the control treatment, which was not observed due to the pasture CP concentration (Table 2) and the reduction in forage DM intake in the supplemented treatments.

The lesser energy level of the supplement (LE treatment) and the difference in the speed of use of the protein and energy fractions of the supplement and the pasture increased UreaN. This can be observed by the non-difference in the intake of digested NDF, which consequently increased UreaN (Table 5).

The metabolic responses to protein supplementation in ruminants can be determined via the SUN concentration. The high concentration of SUN is related to the inefficient use of dietary CP (Broderick & Clayton, 1997). Valadares et al. (1997) have stated that SUN values of 14 to 16 mg dL-1 result in dietary protein loss. For all supplemented treatments, the SUN values were within this range, while for the control treatment, the value was below it.

Despite the high CP concentration, the forage presented a significant amount of ADIP (Table 2), compromising the nutritive value of the diet, as ADIP is the fraction of N which represents the unavailable protein contained in the cell walls, resistant to the action of ruminal microorganisms and non-digestible in the intestines of ruminants (Sniffen, O'Connor, Van Soest, Fox, & Russell, 1992).

Conclusion

For production systems that wish to maximize the use of tropical forages, with a minimum of substitute effect from the concentrate, the energy protein supplement indicated, when forage has a crude protein concentration of up to ~120 g kg-1, is the low energy supplement (340 g TDN animal d-1), resulting in satisfactory weight gain and a high nitrogen use efficiency.

Our results define the use of energy levels in the supplement as a tool for pasture management. If the purpose of the production system is to stimulate the forage intake, the option is to supply supplements with less energy levels. In contrast, if the purpose is to increase the stocking rate, supplements with greater energy levels should be used.

Acknowledgements

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