



Nutritional Performance of Cattle Grazing during Rainy Season with Nitrogen and Starch Supplementation

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ABSTRACT: The objective of this work was to evaluate the effects of supplementation with nitrogen and starch on the nutritional performance of grazing cattle during the rainy season. Five rumen cannulated Nellore steers, averaging 211 kg of body weight (BW), were used. Animals grazed on five signal grass paddocks. Five treatments were evaluated: control (forage only), ruminal supplementation with nitrogen at 1 g of crude protein (CP)/kg BW, ruminal supplementation with starch at 2.5 g/kg BW, supplementation with nitrogen (1 g CP/kg BW) and starch (2.5 g/kg BW), and supplementation with nitrogen (1 g CP/kg BW) and a mixture of corn starch and nitrogenous compounds (2.5 g/kg BW), thereby resulting in an energy part of the supplement with 150 g CP/kg of dry matter (DM). This last treatment was considered an additional treatment. The experiment was carried out according to a 5 × 5 Latin square design following a 2×2+1 factorial arrangement (with or without nitrogen, with or without starch, and the additional treatment). Nitrogen supplementation did not affect ($p>0.10$) forage intake. Starch supplementation increased ($p<0.10$) total intake but did not affect ($p<0.10$) forage intake. There was an interaction between nitrogen and starch ($p<0.10$) for organic matter digestibility. Organic matter digestibility was increased only by supplying starch and nitrogen together. Nitrogen balance (NB) was increased ($p<0.10$) by the nitrogen supplementation as well as by starch supplementation. Despite this, even though a significant interaction was not observed ($p>0.10$), NB obtained with nitrogen plus starch supplementation was greater than NB obtained with either nitrogen or starch exclusive supplementation. Supplementation with starch and nitrogen to beef cattle grazing during the rainy season can possibly improve digestion and nitrogen retention in the animal.. (**Key Words:** Beef Cattle, Digestibility, Intake, Metabolism, Nellore Steers, Nitrogen Retention)

INTRODUCTION

In the rainy season, tropical forages are not considered deficient in crude protein (CP). However, benefits of supplementation of grazing cattle with nitrogenous compounds in the rainy season have been reported (Zervoudakis et al., 2008; Costa et al., 2011a; Figueiras et al., 2015). However, the effects of supplemental nitrogen in the rainy season have been considered to be mainly metabolic when contrasted to the dry season, when the nitrogen has the main role of improving the microbial

activity in the rumen (Detmann et al., 2014a).

Recent studies conducted in the tropics have showed that nitrogen supplementation can improve the concentration of ruminal ammonia nitrogen (RAN) (Costa et al., 2011a) and decrease the participation of recycled nitrogen on total nitrogen assimilated by ruminal microorganisms (Batista et al., 2016). Additionally, nitrogen supplementation can improve the total availability of nitrogen for anabolic purposes by direct supply or by decreasing the muscle protein breakdown (Detmann et al., 2014a). Thus, the use of metabolizable energy from the forage could be increased by supplementation with nitrogen.

However, the inclusion of additional energy resources, mainly in the form of non-fibrous carbohydrates (NFC), could adversely affect the performance of ruminant animals

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in the rainy season because it could negatively affect the energy-protein balance in the diet, generating dietary and metabolic constraints that would lead to a decrease in nitrogen retention in the animal (Costa et al., 2011b; Detmann et al., 2014a).

Nevertheless, information related to the study of the inclusion of additional nitrogen and energy resources in the use of basal forage for beef cattle grazing during the rainy season is still scarce in tropical conditions. Thus, the objective of this work was to evaluate the effects of supplementation with nitrogen and starch on the nutritional performance of grazing cattle during the rainy season.

MATERIAL AND METHODS

The experiment was performed from January to March 2009 during the rainy season in Viçosa, Minas Gerais, Brazil (20°45' S, 42°52' W). The experimental period presented a total rainfall and average temperature of 677 mm and 22.5°C, respectively. Five Nellore steers, averaging 211±17 kg of body weight (BW), were surgically fitted with ruminal cannulae and kept under continuous grazing in individual signal grass (*Brachiaria decumbens*) paddocks of approximately 0.34 ha. Put-and-take animals were not used in this experiment. All surgical and animal care procedures were approved by the University Animal Care Committee. Ruminal fistulae and their surrounding areas were cleaned routinely during the experiment. Water and a mineral mixture were available to the steers at all times. A corral annexed to the paddocks was used for management of animals and sampling. The distribution of animals to the paddocks was randomly performed at the beginning of each experimental period.

Five treatments were evaluated: control (without supplementation), supplementation with nitrogen at 1 g of crude protein (CP)/kg BW, supplementation with 2.5 g of starch (Amisol 3408, CornProducts Co., Santana do Parnaíba, SP, Brazil)/kg BW, supplementation with nitrogen (1 g CP/kg BW) and starch (2.5 g/kg BW), and supplementation with nitrogen (1 g CP/kg BW) and a mixture of corn starch and nitrogenous compounds (2.5 g/kg BW), thereby resulting in an energy part of the supplement with 150 g CP/kg of dry matter (DM). This last treatment was considered an additional treatment where it was objectified to evaluate if an additional nitrogen supply could be case any benefit on animal performance.

A mixture of urea, ammonium sulfate, and albumin (Maximus, Arve Alimentos Co., Viçosa, MG, Brazil) was used as a source of nitrogenous compounds at a ratio of 4.5:0.5:1.0. The supplement amount was calculated based on BW at the beginning of each experimental period and placed in two portions of equal weight in the rumen of the

animals daily at 0600 h and 1800 h during the experimental period.

The nitrogen supplement lacked carbohydrate content, thereby allowing the supplementation effects with nitrogen to be evaluated without any supplementary source of fiber or energy interfering with the measurements. Albumin was included in the supplement to meet the microbial requirements for true degradable protein and to supply essential substrates, such as branched chain volatile fatty acids.

The experiment consisted of five 15-day experimental periods. The first five days of each experimental period were used to adapt the animals to the supplements. In order to minimize the possible effects of paddocks on experimental treatments, the animals were rotated among the five paddocks every experimental period.

Available forage was estimated by cutting five square areas (0.5×0.5 m) in each paddock that were randomly chosen on the first day of each experimental period. Samples were oven-dried (60°C), processed in a knife mill (1-mm), and analyzed for DM content (method INCT-CA G-003-1; Detmann et al., 2012). Average forage availability during the experiment was 14.8±1.39 ton DM/ha.

Evaluation of the consumed forage was performed by hand-plucked sampling on the first, fourth, and seventh days of each experimental period. Samples were oven-dried (60°C) and processed in a knife mill (1- and 2-mm). Pooled samples were produced for each paddock and experimental period.

Fecal excretion was estimated using titanium dioxide as an external marker. The marker was infused (20 g/d) into the rumen of each animal at 1200 h from the first to the eighth day of each experimental period. Fecal sampling started on sixth day (Titgemeyer et al., 2001) and samples were taken from the rectum of each animal according to the following schedule: sixth day—0800 h and 1400 h, seventh day—1000 h and 1600 h, and eighth day—1200 h and 1800 h. The fecal samples were oven-dried (60°C) and processed in a knife mill (1- and 2-mm). Next, pooled samples were produced for each animal and experimental period.

To evaluate the RAN concentration and rumen pH, samples of ruminal fluid were taken on the ninth day of each experimental period at 0600 h, 1200 h, 1800 h, and 2400 h. Samples were collected manually from the liquid-solid interface of the rumen mat, filtered through a triple layer of cheesecloth, and submitted for a pH assessment using a digital potentiometer (TEC-3P-MP, Tecnal, Piracicaba, SP, Brazil). An aliquot of 40-mL was subsequently separated, fixed with 1 mL of H₂SO₄ (1:1), and frozen (-20°C) for a posterior RAN analysis.

On the 15th day of each experimental period, urine spot samples were obtained before (0600 h) and approximately

six hours (1200 h) after the morning supplementation. Samples were filtered through cheesecloth, and a 10-mL aliquot was separated, diluted with 40 mL H₂SO₄ (0.036 N), and frozen (-20°C). Concomitantly to the urine samples, blood was collected from the jugular vein of each animal using test tubes containing separator gel and a coagulation accelerator (BD Vacutainer SST II Advance, São Paulo, SP, Brazil). Samples were centrifuged at 2,700×g for 20 minutes to obtain the serum, which was frozen (-20°C).

Forage and fecal samples (processed to pass through a 1-mm screen sieve) were analyzed for DM, (method INCT-CA G-003-1) organic matter (OM; method INCT-CA M-001/1), CP (method INCT-CA N-001/1), and neutral detergent fiber corrected for ash and protein (NDFap; using thermostable α-amylase and omitting the use of sodium sulfite; methods INCT-CA F-002/1, INCT-CA M-002/1 and INCT-CA N-004/1) contents, according to the standard methods for feed analysis of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2012). Supplement samples were analyzed for DM, OM, and CP contents (Table 1).

The fecal samples were evaluated for titanium dioxide content according to a colorimetric method (method INCT-CA M-007/1; Detmann et al., 2012). The fecal excretion of DM was obtained as the ratio of the daily dose to the fecal content of the marker. The estimates of forage intake were obtained using indigestible NDF (iNDF) as an internal marker. The iNDF contents in feces and forage were estimated in the samples processed to pass a 2-mm screen sieve using a 288-hours *in situ* incubation procedure (method INCT-CA F-008/1; Detmann et al., 2012).

The RAN content in the ruminal fluid samples was evaluated using an indophenol colorimetric method (method INCT-CA N-006/1; Detmann et al., 2012). The concentrations obtained at the different sampling times were pooled by animal and period to obtain a single value that represented the average daily RAN concentration. Ruminal pH values were combined in a similar manner.

Blood serum samples, after thawing, were analyzed for

urea concentration (enzyme-colorimetric method, Bioclin K047, Belo Horizonte, MG, Brazil).

After thawing, urine samples were pooled by animal and experimental period and then analyzed for creatinine contents estimated by the modified Jaffé method (Bioclin K016-1), total nitrogen content (method INCT-CA N-001/1; Detmann et al., 2012), uric acid (LCF enzymatic-colorimetric method, Human 10687, Itabira, MG, Brazil), and allantoin (colorimetric method; Chen and Gomes, 1992).

Total urinary volume was estimated using the ratio of creatinine excretion per unit of BW to its concentration in the urine (Chizzotti et al., 2006). Excretion of purine derivatives was calculated from the sum of the amounts of allantoin and uric acid excreted in the urine. From this finding, the absorbed purines were calculated by the following equation (Verbic et al., 1990):

$$AP = \frac{PD - 0.385 \times BW^{0.75}}{0.85} \quad (1)$$

where AP is the amount of absorbed purines (mmol/d), PD is the amount of excreted purine derivatives (mmol/d), 0.85 is the recovery of absorbed purines as purine derivatives in the urine (mmol/mmol), and 0.385 is the excretion of endogenous purine derivatives in the urine per unit of metabolic size (mmol).

The microbial synthesis of nitrogenous compounds in the rumen (NMIC, g/d) was estimated according to Chen and Gomes (1992) as follows:

$$NMIC = \frac{70 \times AP}{0.83 \times R \times 1,000} \quad (2)$$

where R is the N_{RNA}:N_{TOTAL} ratio in the microorganisms (mg/mg), 70 is the nitrogen content in purines (mg/mol), and 0.83 is the intestinal digestibility of the microbial purines (mg/mg). It was adopted R = 0.176 (Valadares Filho, 1995).

The experiment was analyzed according to a 5×5 Latin square design following a 2×2+1 factorial arrangement (with or without nitrogenous compounds and with or without starch, plus an additional treatment). After the analysis of variance, the treatments were compared using contrasts (Table 2). All statistical procedures were performed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.2) (α = 0.10). One animal developed problems unrelated to the experimental treatments during one experimental period; therefore, there was a loss of one experimental unit with respect to the variables associated with the voluntary intake and digestibility.

Table 1. Chemical composition of forage and supplements

Item	Supplements				Forage ¹
	NIT	STA	NIT+STA	ADI	
DM (% as fed)	96.0	88.3	92.1	92.2	19.9±0.6
OM (% of DM)	98.7	99.9	98.7	98.9	89.7±1.3
CP (% of DM)	204.3	0.0	58.2	65.8	13.5±1.4
NDFap (% of DM)	-	-	-	-	55.0±2.7
NDIP % of CP	-	-	-	-	17.2±4.2
iNDF (% of DM)	-	-	-	-	17.7±1.8

NIT, nitrogen; STA, starch; CP, crude protein; ADI, nitrogen+starch mixture with 15% of CP; DM, dry matter; OM, organic matter; NDFap, neutral detergent fiber corrected for ash and protein; NDIP, neutral detergent insoluble protein; iNDF, indigestible NDF.

¹ Mean±standard error (hand-plucked samples).

Table 2. Distribution of coefficients employed in the contrasts among treatments

Contrast ²	Treatments ¹				
	CON	NIT	STA	NIT+STA	ADI
----- Orthogonal set -----					
N	+1	-1	+1	-1	0
S	+1	+1	-1	-1	0
N×S	+1	-1	-1	+1	0
----- Additional contrast -----					
A	0	0	0	+1	-1

¹ CON, control (without supplementation); NIT, supplementation with nitrogen; STA, supplementation with starch; NIT+STA, supplementation with nitrogen and starch; ADI, supplementation with nitrogen and a mixture of starch and nitrogen, which presented 150 CP/kg DM (additional treatment).

² N, effect of supplementation with nitrogen; S, effect of supplementation with starch; N×S, interaction between nitrogen and starch; A, additional comparison between treatments NIT+STA and ADI.

RESULTS

There was no ($p>0.10$) interaction between nitrogen and starch or the effect of the additional nitrogen supply to animals supplemented with nitrogen and starch in any of the variables associated with the voluntary intake (Table 3). Nitrogen supplementation increased ($p<0.01$) CP intake but did not affect ($p>0.10$) forage intake. Starch

supplementation increased ($p<0.02$) total DM intake but did not affect ($p>0.10$) forage intake. In addition, starch supplementation positively affected ($p<0.02$) CP and digestible OM (DOM) intake (Table 3).

There were interactions of supplementation with nitrogen and starch on OM ($p<0.02$) and NDFap ($p<0.07$) digestibilities (Table 4). The study of this effect indicated that OM digestibility was only increased ($p<0.10$) when nitrogen and starch were provided together. Evaluation of the interaction on NDFap digestibility indicated that solely starch supplementation depressed ($p<0.10$) fiber digestibility. However, this effect was avoided ($p<0.10$) when nitrogen was provided along with starch (Table 5).

Total CP digestibility increased ($p<0.01$) when nitrogen compounds were supplied; however, it was not affected ($p>0.10$) by starch supplementation (Table 5). Similar to voluntary intake, none of the variables associated with total digestibility demonstrated an effect ($p>0.10$) of the additional nitrogen supply to animals receiving nitrogen and starch supplementation (Table 4).

There was no effect of treatment ($p>0.10$) on ruminal pH, which had an average value of 6.53 (Table 6). Additionally, there was an interaction ($p<0.02$) between nitrogen and starch on the RAN concentration (Table 6). In this sense, nitrogen supplementation increased ($p<0.10$) RAN concentration either with or without starch. However,

Table 3. Least squares means of intake (kg/d) of dry matter (DM), DM from forage (DMF), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ash and protein (NDFap), digested OM (DOM), digested NDFap (DNDF), and indigestible neutral detergent fiber (iNDF) according to the treatments

Item	Treatments ¹					SEM	p-value ²			
	CON	NIT	STA	NIT+STA	ADI		N	S	N×S	A
DM	4.39	4.02	5.20	5.34	5.38	0.35	0.750	0.013	0.507	0.930
DMF	4.39	3.93	4.69	4.61	4.66	0.35	0.479	0.207	0.611	0.924
OM	3.98	3.59	4.69	4.87	4.87	0.32	0.747	0.012	0.414	0.984
CP	0.56	0.72	0.65	1.03	1.13	0.06	0.001	0.010	0.140	0.266
NDFap	2.46	2.13	2.59	2.60	2.48	0.20	0.477	0.178	0.444	0.664
DOM	2.15	1.88	2.41	2.96	2.99	0.22	0.556	0.015	0.109	0.919
DNDF	1.55	1.30	1.47	1.68	1.52	0.16	0.896	0.387	0.200	0.485
iNDF	0.74	0.71	0.82	0.77	0.75	0.06	0.507	0.284	0.921	0.760

¹ CON, control; NIT, nitrogen; STA, starch; NIT+STA, nitrogen+starch; ADI, nitrogen+starch mixture with 15% of CP.

² N, effect of nitrogen supplementation; S, effect of starch supplementation; N×S, interaction between nitrogen and starch supplementation; A, additional contrast to verify the effect of additional supply of nitrogen in the supplementation of nitrogen plus starch.

Table 4. Least squares means (%) of total digestibility of organic matter (OM), crude protein (CP), and neutral detergent fiber corrected for ash and protein (NDFap) according to the treatments

Item	Treatments ¹					SEM	p-value ²			
	CON	NIT	STA	NIT+STA	ADI		N	S	N×S	A
OM	53.5	52.4	51.4	60.4	61.5	1.60	0.040	0.109	0.013	0.640
CP	52.6	67.6	48.0	72.1	75.6	3.11	<0.001	0.984	0.196	0.445
NDFap	63.0	61.1	56.9	63.7	61.2	2.02	0.267	0.416	0.065	0.396

¹ CON, control; NIT, nitrogen; STA, starch; NIT+STA, nitrogen+starch; ADI, nitrogen+starch mixture with 15% of CP.

² N, effect of nitrogen supplementation; S, effect of starch supplementation; N×S, interaction between nitrogen and starch supplementation; A, additional contrast to verify the effect of additional supply of nitrogen in the supplementation of nitrogen plus starch.

Table 5. Interaction between nitrogen and starch supplementation on total digestibilities of organic matter (OM) and neutral detergent fiber corrected for ash and protein (NDFap), and the concentration of ruminal ammonia nitrogen (RAN)

Nitrogen	Starch	
	Without	With
OM (%)		
Without	53.5 ^{Aa}	51.4 ^{Ba}
With	52.4 ^{Ab}	60.4 ^{Aa}
NDFap (%)		
Without	63.0 ^{Aa}	56.9 ^{Bb}
With	61.1 ^{Aa}	63.7 ^{Aa}
RAN (mg/dL)		
Without	5.57 ^{Ba}	5.61 ^{Ba}
With	17.20 ^{Aa}	11.14 ^{Ab}

^{A,B,a,b} Means followed by different capital letters within a column or different lowercase letters within a row are different at $p < 0.1$.

starch decreased RAN concentration ($p < 0.10$) when it was provided along with nitrogen compared to the nitrogen supply only (Table 5). For any of the other variables shown in Table 6, there was no interaction ($p > 0.10$) between nitrogen compounds and starch.

Fecal nitrogen excretion was increased ($p < 0.01$) with starch supplementation; however, it was not affected ($p > 0.10$) by nitrogen supplementation. Conversely, urinary nitrogen excretion was increased ($p < 0.01$) by supplementation with nitrogen, but it was not affected ($p > 0.10$) by supplementation with starch (Table 6). Nitrogen balance (NB; g/d) increased with nitrogen supplementation ($p < 0.01$) as well as with starch supplementation ($p < 0.05$). However, the apparent efficiency of nitrogen utilization in the animals' body (ENU; g of nitrogen retained/g of nitrogen intake) was increased only ($p < 0.01$) when nitrogen was provided (Table 6).

Table 6. Least squares means of ruminal pH, concentration of ruminal ammonia nitrogen (RAN; mg/dL), nitrogen intake (NI; g/d), fecal nitrogen (FN; g/d), urinary nitrogen (UN g/d), apparent balance of nitrogen compounds (NB; g/d), efficiency of nitrogen utilization (ENU; g/g nitrogen intake), serum concentrations of nitrogen urea before (NUSb; mg/dL) and after supplementation (NUSa; mg/dL) and production of microbial nitrogen in the rumen (NMIC g/d) according to the treatments

Item	Treatments ¹					SEM	p-value ²			
	COM	NIT	STA	NIT+STA	ADI		N	S	N×S	A
pH	6.53	6.54	6.53	6.53	6.52	0.10	0.967	>0.999	0.967	0.942
RAN	5.57	17.20	5.61	11.14	14.61	1.09	<0.001	0.017	0.016	0.043
NI	89.2	115.6	104.7	164.2	180.6	9.83	0.001	0.010	0.140	0.266
FN	40.8	37.4	52.8	45.9	43.8	2.86	0.112	0.005	0.564	0.594
UN	36.9	44.2	31.3	47.1	34.9	5.09	0.054	0.799	0.442	0.117
NB	11.5	34.0	20.6	71.2	102.0	9.20	0.003	0.040	0.187	0.042
ENU	0.11	0.29	0.16	0.43	0.56	0.06	0.006	0.163	0.514	0.161
SUNb	10.4	16.2	8.9	16.2	16.6	0.95	<0.001	0.472	0.406	0.805
SUNa	13.3	15.5	9.4	20.2	22.6	2.36	0.047	0.874	0.134	0.529
NMIC	57.5	57.2	66.8	76.7	73.4	5.13	0.367	0.015	0.332	0.651

¹ CON, control; NIT, nitrogen; STA, starch; NIT+STA, nitrogen+starch; ADI, nitrogen+starch mixture with 15% of CP.

² N, effect of nitrogen supplementation; S, effect of starch supplementation; N×S, interaction between nitrogen and starch supplementation; A, additional contrast to verify the effect of additional supply of nitrogen in the supplementation of nitrogen plus starch.

Concentrations of serum urea nitrogen, both before ($p < 0.01$) and after ($p < 0.05$) supplementation, were significantly increased by the nitrogen supplementation, but they were not affected ($p > 0.10$) by starch supplementation (Table 6).

Production of NMIC was not affected ($p > 0.10$) by supplementation with nitrogen, but it was increased ($p < 0.02$) by starch supplementation (Table 6).

Effects of the additional nitrogen supply to animals supplemented with both starch and nitrogen were only observed on RAN concentration ($p < 0.05$) and NB ($p < 0.05$). In both cases, the additional supply of nitrogen increased the estimates obtained (Table 6).

DISCUSSION

In this study, no effects of nitrogen supplementation were observed on forage voluntary intake (Table 3). This behavior confirms the results obtained by other authors in tropical conditions when protein supplements were provided to grazing cattle during the rainy season (Zervoudakis et al., 2008; Costa et al., 2011c).

Results obtained in Brazilian studies indicated that the inclusion of supplemental nitrogen in the diet of cattle that are fed tropical grasses can improve the forage intake up to protein levels near to 10% (Figueiras et al., 2010; Sampaio et al., 2010). From this dietary CP level, the microbial nitrogen requirements would be met and stimulation on forage digestion would no longer be observed (Detmann et al., 2014b). These assumptions would be applied to the conditions of this study, considering the actual CP content of the basal forage (Table 1) and the absence of effects of supplementation with nitrogen on the DOM and digested NDF intakes (Table 3).

Starch supplementation did not change forage intake. However, indirectly, small stimuli on forage intake were observed because the starch supplementation increased CP intake ($p < 0.02$; Table 3). Starch has no nitrogen in its composition (Table 1), thus this small stimulus could only result from the increase in forage intake, which was observed, although without significant effects ($p > 0.10$; Table 3).

This pattern apparently contradicts some results obtained in the tropics in which it was reported that NFC supplementation for grazing animals during the rainy season could lead to a high substitutive effect on forage intake (Costa et al., 2011b). Additionally, starch supplementation could decrease NDF utilization in the rumen, as seen through the reduction in NDF digestibility (Table 4). Theoretically, this decreased fiber degradation would increase the rumen fill effect of NDF and reduce the forage voluntary intake (Detmann et al., 2014b). However, at least one characteristic appears to be associated with the absence of negative effects of starch supplementation on forage intake. The forage grazed during the rainy season should be understood as a diet, in which one of the main nutritional characteristics is its capacity to supply energy and protein in accordance with the animal's requirements. Accordingly, dietary energy-to-protein ratio could be unbalanced by energy supplements as they may cause a relative excess of energy when the only source of dietary nitrogen is the forage CP (Detmann et al., 2014b). Such a dietary pattern would increase animal discomfort (Illius and Jessop, 1996) and hence decrease forage intake (Detmann et al., 2014a; b). However, the CP content of the forage in this study was above the levels usually seen in tropical pastures during the rainy season (average of 9.42% reported by Detmann et al., 2014b). Thus, under the conditions of this study, starch supplementation would cause less interference in the energy-to-protein ratio in the diet, leading to the absence of negative effects on forage intake. This is reinforced by the similarity in the CP levels in the diet for the control and animals supplemented only with starch (12.7% and 12.6%, on a DM basis, respectively).

In general, the effects of supplementation on the digestibility were characterized by an interaction between nitrogen and starch (Tables 4 and 5). Considering NDFap digestibility, it was found that nitrogen supplementation did not cause a positive effect, which reflects the fact that the basal forage has no deficiency of nitrogen compounds for growth of fibrolytic microorganisms. Moreover, starch supplementation depressed the NDFap digestibility (Table 5). This reflects the fact that the inclusion of readily fermentable carbohydrates favors the growth of NFC fermenting bacteria rather than fibrolytic bacteria. This phenomenon, known as the "carbohydrate effect", increases the competition for essential nutrients between groups of

microbial species, decreasing the utilization of insoluble fiber by fibrolytic microorganisms that have lower competitive capacity (El-Shazly et al., 1961; Arroquy et al., 2005; Carvalho et al., 2011). However, when starch was combined with nitrogen compounds, its deleterious effects were avoided, indicating that the nitrogen supply minimized the competition events between microbial species in the rumen, making NDFap digestibility similar to that found in animals without supplementation. Such a result agrees with Heldt et al. (1999) and Arroquy et al. (2004), who found that an adequate supplying of rumen degradable protein for cattle fed low-quality forage was able to overcome the negative effect of supplemental NFC on fiber digestion.

On the other hand, the increase of OM digestibility when starch and nitrogen supplementation was combined (Table 5) seems to indicate that both compounds interact with each other with respect to their ruminal utilization. Accordingly, starch would imply a better microbial assimilation of additional nitrogen, which is indirectly perceived by the decreased RAN concentration compared with animals supplemented only with nitrogen (Table 5). Additionally, although NMIC has been increased only by starch supplementation, it can be seen that the observed value for starch plus nitrogen supplementation was higher than those observed with the isolated supplementation of nitrogen or starch (Table 6). This reflects the fact of supplementation with nitrogen has allowed better use of energy from the NFC in the rumen, which has been reported by other authors in tropical conditions (Souza et al., 2010). Even without significant interaction ($p > 0.10$), DOM intake was higher when nitrogen and starch were offered together, which shows a positive effect on the total intake of digestible energy.

The NB has been improved with nitrogen supplementation as well as with starch supplementation. In the latter case, the increase of nitrogen retention in the animal agrees, at least partially, with the increased NMIC production obtained with starch supplementation (Table 6). Such increased NMIC is supposed to improve the metabolizable protein (MP) supply. However, the positive effects on NB were more prominent considering the supplementation with nitrogen, without effects on NMIC (Table 6).

Detmann et al. (2015) used a meta-analytical approach to quantify the impacts of the increase in microbial protein caused by supplemental nitrogen and the improvement in NB in the tropics. These authors found that, even using supplements based on non-protein nitrogen, the improvement in microbial nitrogen production responds only for 21% of the improvement on NB. Such a pattern indicates the occurrence of post-digestive and metabolic effects associated with supplemental CP as observed by other authors in the tropics (Costa et al., 2011a; Rufino,

2011; Detmann et al., 2014a).

Increases in nutritional performance with the use of nitrogen supplements have been attributed to improvements in nitrogen status in the animal (Egan, 1965a; Egan and Moir, 1965). In general, the nitrogen status defines the quantitative and qualitative availability of nitrogen compounds for all metabolic and physiological functions, including functions associated with the metabolism of other compounds (e.g., energy). Based on this concept, it can be established that the nitrogen compounds available for animal metabolism would be used for different metabolic functions followed by an order of priority, namely: survival, maintenance, and production (Detmann et al., 2014a). Several reports in the tropics has confirmed that supplemental nitrogen impacts negatively on breakdown rate of myofibrillar protein and positively on the blood concentration of anabolic hormones (e.g., IGF1), even without a concurrent source of supplemental energy (Rufino, 2011; 2015; Franco, 2015; Batista et al., 2016). These both impacts implies in a net increment in nitrogen accretion in the animal.

Some values of NB were apparently too high and seemed above biological limits of protein deposition in the lean tissues (Table 6). Therefore, the absolute values of NB should be evaluated with caution because they likely overestimate protein accretion. Gerrits et al. (1996) compared nitrogen retention obtained in digestion trials with protein accretion obtained by serial slaughter, and they reported that nitrogen retention overestimated protein accretion of growing cattle. This seems to occur mainly due the underestimation of urine nitrogen (e.g., volatile nitrogen losses from containers), fecal nitrogen (e.g., incomplete collection, volatile losses during either collection or drying), or both (Spanghero and Kowalski, 1997). However, when experimental procedures are standardized among treatments and experimental periods within an experiment, as performed in this study, in spite of bias in the absolute values, the relative comparisons between treatments should be valid.

Moreover, one of the metabolic functions of higher priority is nitrogen recycling to the gastrointestinal tract because a continuous supply of nitrogen for microbial growth in the rumen should be seen as a survival strategy (Egan, 1965b; Van Soest, 1994). Considering a dietetic situation in which there is no prominent deficiency of nitrogen compounds, the amount of nitrogen recycled to the rumen remains relatively constant (Marini and Van Ambourgh, 2003). Therefore, there is less nitrogen for tissue deposition under low nitrogen status because a greater percentage of nitrogen intake is directed towards recycling and, as a result, a lower percentage of nitrogen will be available for anabolic purposes.

Therefore, considering that efficiency of nitrogen

utilization is more strongly associated with nitrogen supply than with energy supply (Detmann et al., 2014a), nitrogen retention in the body will then be improved by increasing the nitrogen status with the use of protein supplements (Table 6).

Moreover, even without an interaction effect ($p > 0.10$), it was found that NB and ENU estimates were optimized by the combined supplementation of nitrogen and starch (Table 6). In ruminants, protein deposition efficiency depends on energy availability, and energy utilization efficiency depends on amino acids availability (Schroeder and Titgemeyer, 2008). Thus, even with improvements in nitrogen status, MP would not be retained in the body due to a probable relative deficiency of metabolizable energy. Thus, the relative MP excess (which in turn is determined by the metabolizable energy availability) would be eliminated, which would increase urinary nitrogen excretion, as found in this study (Table 6). Such a pattern was also reported by Lazzarini et al. (2013). In those circumstances, the additional inclusion of starch would provide energy for better MP retention, increasing the NB (Table 6). This confirms that tissue deposition is defined by an interactive process in which the energy and MP utilization efficiencies are interrelated (Schroeder and Titgemeyer, 2008; Lazzarini et al., 2013).

The purpose of including an additional treatment was to determine whether an additional supply of nitrogen compounds could have beneficial nutritional effects on animals supplemented with nitrogen and starch (Table 2). In this context, an increase in NB was observed (Table 6). According to Detmann et al. (2014a), nitrogen supplementation during the rainy season would provide positive effects on ENU up to RAN concentrations of approximately 13 mg/dL, since RAN is assumed to be an indicator of nitrogen availability. This would result in a better balance of nitrogen compounds between rumen and the bloodstream, improving the availability of nitrogen compounds for anabolic purposes. The additional supply of nitrogen compounds for animals receiving nitrogen and starch supplementation increased RAN concentration to levels greater than that described above (11.14 to 14.61 mg/dL), justifying the results obtained in NB.

CONCLUSION

The supplementation with starch and nitrogen compounds for grazing cattle during the rainy season results in interactive effects by improving digestion and nitrogen retention in the animal.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any

financial organization regarding the material discussed in the manuscript.

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