

Intake, digestibility, and rumen and metabolic characteristics of cattle fed low-quality tropical forage and supplemented with nitrogen and different levels of starch

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Objective: Effects of nitrogen supplementation associated with different levels of starch on voluntary intake, digestibility, and rumen and metabolic characteristics of cattle fed low-quality tropical forage (*Brachiaria decumbens* hay, 7.4% crude protein, CP) were evaluated using ruminal and abomasal cannulated steers.

Methods: Five European×Zebu young bulls (186 kg body weight, BW) were distributed according to a 5×5 Latin square. The following treatments were evaluated: control, supplementation with 300 g CP/d (0:1), supplementation with 300 g starch/d and 300 g CP/d (1:1), supplementation with 600 g starch/d and 300 g CP/d (2:1), and supplementation with 900 g starch/d and 300 g CP/d (3:1). A mixture of nitrogenous compounds provided 1/3 from true protein (casein) and 2/3 from non-protein nitrogen (mixture of urea and ammonium sulphate, 9:1) was used as the nitrogen supplement. In order to supply energy a unique source of corn starch was used.

Results: Supplements increased ($p < 0.05$) dry matter intake, but did not affect ($p > 0.05$) forage intake. There was a cubic effect ($p < 0.05$) of starch on voluntary intake. This was attributed to the highest forage intake (g/kg BW) when using the 2:1 starch:CP ratio. Supplements increased ($p < 0.05$) organic matter (OM) digestibility, but did not affect ($p > 0.05$) neutral detergent fibre corrected for ash and protein (NDFap) digestibility. There was a positive linear effect ($p < 0.05$) of the amount of starch supplemented on OM digestibility. Total NDFap digestibility was not affected ($p > 0.05$) by the amount of supplemental starch. Ruminal ammonia nitrogen concentrations were higher ($p < 0.05$) in supplemented animals, however, a negative linear effect ($p < 0.05$) of amount of starch was observed. Supplements increased ($p < 0.05$) the nitrogen balance (NB) and efficiency of nitrogen utilization. These effects were attributed to increased body anabolism, supported by higher ($p < 0.05$) serum concentration of insulin-like growth factor 1. Increasing the amount of starch tended ($p < 0.06$) to linearly increase the NB. In spite of this, there was a highest NB value for the 2:1 starch:CP ratio amongst the treatments with supplementation.

Conclusion: Nitrogen supplementation in cattle fed low-quality tropical forage increases nitrogen retention in the animal's body. An additional supply of starch increases nitrogen retention by increasing energy availability for both rumen and animal metabolism.

Keywords: *Brachiaria decumbens*; Digestibility; Fibre; Nitrogen Balance; Zebu

INTRODUCTION

The low-quality of tropical forages during the dry season of the year is a result of reduced crude protein (CP) contents and higher lignification of insoluble fibre. The CP contents are usually below the critical values for the rumen microorganisms present full capacity for fibre degradation (<70 to 80 g CP/kg of dry matter [DM]) [1,2].

In tropical conditions, supplementation with nitrogenous compounds is the most important in

improving the use of low-quality forages [3,4]. On the other hand, negative effects can be observed on low-quality forage intake and utilisation in animals supplemented with non-fibrous carbohydrates [4,5]. Under these conditions, a decreased fibre utilisation could occur because of an increase in the competition for essential nutrients between fibrolytic and non-fibrolytic microbial species [6].

Despite the fact, studies conducted in the tropics have reported that the association between energy and nitrogen supplements may involve interactive effects on the metabolism of nitrogenous compounds that could override the deleterious effects on ruminal degradation of fibre when only energy supplementation is used. This may increase the production of rumen microbial nitrogen and dietary energy, possibly by avoiding the effects of competition between fibrolytic and non-fibrolytic species [3,7]. In these conditions, effects that maximize nitrogen retention in the animal's body can also be observed, which implies increased weight gain [7].

However, research illustrating the relationship between energy and nitrogen in supplements for animals fed low-quality tropical forages, and its effects on the metabolic and nutritional efficiencies are still scarce in the tropics.

Thus, the objective of this study was to evaluate the effects of supplementation with nitrogenous compounds associated with different levels of starch on voluntary intake, digestibility, and rumen and metabolic characteristics of cattle fed low-quality tropical forage.

MATERIAL AND METHODS

The experiment was carried out at the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais state, Brazil.

Five 10 months old crossbred bulls (1/2 European × 1/2 Zebu; averaging 186±7 kg of initial body weight [BW] – 209±12 kg of final BW, and 198±10 kg of average BW) were used. The animals were surgically fitted with ruminal and abomasal cannulas approximately 30 days prior the experiment. All surgical and animal care procedures were approved by the Animal Ethics Committee of the Universidade Federal de Viçosa, that was registered under the protocol number 112/2014.

The experiment was performed according to a 5×5 Latin square design, with five experimental periods lasting 15 days each. The first seven days of each period were used for adaptation of the animal to experimental diets. The animals were fed *ad libitum* with low-quality tropical forage (*Brachiaria decumbens* hay) with an average CP level of 7.4%, on DM basis (Table 1). The animals were kept in individual pens, with approximately 10 m², with unrestricted access to water and mineral mixture. Prior to the beginning of the experiment, the animals were adapted during 15 days to handling, facilities and forage allowance.

The animals were fed forage *ad libitum* at 0600 h and 1800 h,

Table 1. Chemical composition of tropical hay, and two supplements (protein supplement and starch)

Nutrient	Hay	Protein supplement	Starch
DM (% as fed)	92.6	91.8	88.8
OM (% DM)	95.3	98.6	99.8
CP (% DM)	7.4	114.0	0.5
EE (% DM)	0.5	0.3	0.3
NDFap (% DM)	80.9	-	-
NDIP (% DM)	4.3	-	-
Lignin (% DM)	7.3	-	-
iNDF (% DM)	38.1	-	-

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDFap, neutral detergent fibre corrected for ash and protein; NDIP, neutral detergent insoluble protein; iNDF, indigestible neutral detergent fibre.

allowing approximately 10% in orts. The total amount of supplement was divided into two parts of equal weight packed in paper bags, which were placed in the rumen of animals at the same time of forage allowance.

The following treatments were evaluated: control (only forage), supplementation with 300 g CP/d (0:1), supplementation with 300 g starch/d and 300 g CP/d (1:1), supplementation with 600 g starch/d and 300 g CP/d (2:1), and supplementation with 900 g starch/d and 300 g CP/d (3:1).

The amount of nitrogen supplement (300 g CP/d) corresponded to approximately 55% of the dietary requirements of CP for crossbred young bulls (European×Zebu) with BW of 200 kg and expected gain of 0.5 kg/d [8]. A mixture of nitrogenous compounds provided 1/3 from true protein (casein) and 2/3 from non-protein nitrogen (mixture of urea and ammonium sulphate, 9:1) was used as the nitrogen supplement [9]. In order to supply energy, a unique source of corn starch was used. The energy supplementation was added to a ratio of 0, 1, 2, and 3 times the equivalent weight of CP.

The voluntary intake and total digestibility were evaluated from the 8th to 14th day. In this sense, the amounts of supplied hay (8th to 13th d) and orts (9th to 14th d) were taken into account. Forage and orts samples were pooled per animal and experimental period.

Total collection of faeces was held on 10th, 12th, and 14th days of each experimental period, starting at 0600 h. At the end of each collection day, the faeces were weighed, homogenized and an aliquot of approximately 50 g/kg was taken and oven-dried (60°C).

Total collection of urine was performed on the 17th d of each period using funnel collectors coupled in the penile region and secured by elastic straps on the back of the animals. The funnels were equipped with hoses, which led the urine to polyethylene containers. The containers were maintained inside a Styrofoam box with ice that was replaced constantly. Urine collections began at 0600 hours and lasted 24 h, which is enough to represent an entire period and to evaluate urinary excretion [10]. After 24 hours, the total volume of urine was measured, and two aliquots

per animal and experimental period were obtained. The first one was used for evaluation of total nitrogen (Kjeldahl procedure) and urea (enzymatic colorimetric method with clearance factor of lipids; Human 10505, Wiesbaden, Hessen, Germany) contents. The second aliquot was frozen (-80°C) and sent to a commercial laboratory for the quantification of 3-methylhistidine by using high-performance liquid chromatography (HPLC). The data for 3-methylhistidine was presented in relation to urinary creatinine excretion.

Rumen fluid samples were taken on the 16th day at 0600 h, 1200 h, 1800 h, and 2400 h to evaluate pH and ruminal ammonia nitrogen (RAN) concentrations. The samples were collected manually from the liquid/solid interface of the rumen mat, filtered through a triple cheesecloth layer and submitted to pH assessment through digital potentiometer. A 40 mL aliquot was then separated, fixed with 1 mL of a H_2SO_4 solution (500 mL/L) and frozen (-20°C) for further analysis of RAN.

Concurrently with the ruminal fluid, blood samples were taken from the animals directly from the jugular vein using tubes with vacuum and gel coagulation accelerator (BD Vacutainer, SST II Advance, São Paulo, SP, Brazil). The samples were immediately centrifuged ($2,700\times g$; 20 minutes) to separate the serum. At the end of the day, samples were pooled per animal (equal volumes for each collection) that were sent to a commercial laboratory to be analysed for the concentration of free amino acids (HPLC; chromatography quantitative of amino acid), glucose (enzyme method glucose oxidase-peroxidase), insulin-like growth factor 1 (IGF1) (immunoradiometric assay; DSL-5600 IRMA Active), triglycerides (enzymatic-colorimetric method), and urea (as described for urine samples).

Hay, orts and faeces samples were processed in a Willey mill (1- and 2-mm). Subsequently hay, orts and faeces samples were compiled per animal and experimental period.

The samples processed to pass through the 1-mm screen sieve were evaluated for DM (method INCT-CA G-003/1), organic matter (OM; method INCT-CA M-001/1), CP (method INCT-CA N-001/1), ether extract (EE; method INCT-CA G-005/1), neutral detergent fibre corrected for ash and protein (NDFap; methods INCT-CA F-002/1, INCT-CA M-002/1 and INCT-CA N-004/1), neutral detergent insoluble protein (NDIP; method INCT-CA N-004/1) and lignin (H_2SO_4 720 g/kg; INCT-CA F-005/1 method) according to the standard techniques of Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; [11]).

Hay and orts samples processed to pass through the 2-mm screen sieve were evaluated for the indigestible NDF (iNDF; method INCT-CA 009/1) contents by using a 288-hours *in situ* incubation procedure.

The RAN contents were quantified by colorimetric technique adopted by the INCT-CA (method INCT-CA N-006/1). The concentrations obtained at different times were combined by animal and time to produce a single value that represented the

average daily RAN concentration. Similar combination was conducted on rumen pH values.

The experiment was analysed according to a 5×5 Latin square design including the effects of treatment (fixed), animal (random), and experimental period (random). Comparisons between treatments were conducted through the decomposition of the sum of squares in orthogonal contrasts, which were responsible for the comparison between control treatment and treatments involving the supplementation and evaluation of the linear, quadratic and cubic effects of the ratios of starch to CP in the supplements. All statistical procedures were performed using the MIXED procedure of SAS 9.4 ($\alpha = 0.05$).

RESULTS

Supplements increased ($p<0.05$) the DM, OM (in kg/d and g/kg BW), CP, and digested OM (DOM) intakes, and the CP:DOM ratio in the diet. Supplements did not affect ($p>0.05$) the intake of forage, NDFap (kg/d and g/kg BW) and digested NDFap (kg/d) (Table 2).

The inclusion of starch in the supplement linearly increased ($p<0.05$) the DM and OM intakes, when expressed in kg/d, and linearly decreased ($p<0.05$) the CP:DOM ratio in the diet. There were no effects ($p>0.05$) of the proportion of starch in the supplement on forage, CP and NDFap intake, when expressed in kg/d (Table 2).

On the other hand, a cubic effect ($p<0.05$) of the amount of starch in the supplement was found on DOM and digested NDFap intakes (kg/d) and on intakes of DM, forage, OM and NDFap when expressed in g/kg BW. This pattern was attributed to higher intakes observed when adopted the starch:CP ratio of 2:1 (Table 2).

The provision of supplements increased ($p<0.05$) the dietary DOM and total OM and CP digestibilities. There was no effect ($p>0.05$) when providing supplements on total NDFap digestibility (Table 3).

There was a linear positive effect ($p<0.05$) of the amount of starch in the supplement on the dietary DOM and total OM digestibility. There was a trend for a linear negative effect ($p<0.07$) on total CP digestibility according to the amount of starch in the supplement. The NDFap digestibility was not affected ($p>0.05$) by the amount of starch in the supplement (Table 3).

The RAN concentration was, on average, higher ($p<0.05$) in supplemented animals. There was no effect ($p>0.05$) of the supplementation on rumen pH (Table 4). Moreover, there was a negative linear effect ($p<0.05$) of the starch amount in the supplement on RAN concentration and ruminal pH.

The nitrogen intake showed a similar pattern to the CP intake (Table 4). There was an overall increasing effect ($p<0.05$) of the supplementation on the urinary nitrogen excretion (UNE), nitrogen balance (NB), efficiency of nitrogen utilization (ENU), urinary excretion of urea nitrogen (UEUN) and serum urea nitrogen

Table 2. Average nutrient intake of bulls fed low feed value tropical hay with nitrogen and energy supplements

Intake	Treatments ¹⁾					SEM	Effect ²⁾			
	C	Starch:N supplement					C vs S	L	Q	C
		0:1	1:1	2:1	3:1					
	----- kg/d -----									
DM	3.42	3.53	3.70	4.39	4.27	0.356	0.012	0.002	0.383	0.101
DMF	3.42	3.26	3.17	3.60	3.21	0.356	0.577	0.741	0.383	0.101
OM	3.26	3.37	3.54	4.23	4.11	0.340	0.009	0.001	0.391	0.094
CP	0.25	0.53	0.53	0.56	0.53	0.026	<0.001	0.613	0.453	0.073
NDFap	2.77	2.65	2.56	2.94	2.62	0.294	0.625	0.639	0.415	0.084
DOM	1.94	2.06	2.24	2.75	2.74	0.209	<0.001	<0.001	0.277	0.047
DNDFap	1.50	1.41	1.32	1.59	1.28	0.178	0.240	0.901	0.324	0.034
CP:DOM	126	267	240	203	197	11.9	<0.001	<0.001	0.352	0.390
	----- g/kg BW -----									
DM	16.2	17.5	18.2	22.0	21.4	1.21	0.001	<0.001	0.396	0.047
DMF	16.2	16.1	15.5	18.0	16.0	1.24	0.817	0.544	0.385	0.046
OM	15.5	16.7	17.4	21.1	20.6	1.16	<0.001	<0.001	0.403	0.043
NDFap	13.1	13.1	12.6	14.6	13.1	1.03	0.760	0.465	0.413	0.038

SEM, standard error of the mean; DM, dry matter; DMF, DM from forage; OM, organic matter; CP, crude protein; NDFap, neutral detergent fibre corrected for ash and protein; DOM, digested OM; DNDFap, digested NDFap; BW, body weight.

¹⁾ Control, no supplementation; 0:1, 1:1, 2:1, 3:1, starch:crude protein ratio in the supplement (g/g).

²⁾ C vs S, control versus supplementation; L, Q, C, linear, quadratic, and cubic effects of starch:crude protein ratio in the supplement.

concentration (SUN). There was no effect ($p > 0.05$) of supplementation on faecal nitrogen excretion (FNE), blood amino acids concentration and ratio of urinary 3-methylhistidine:creatinine (Table 4).

There was a negative linear effect ($p < 0.05$) of the starch amount in the supplement on UNE, UEUN, and SUN. Moreover, there was a trend of linear increase of NB ($p < 0.06$) and ENU ($p < 0.07$) with the increase in the amount of starch in the supplement. The variation in the amount of starch did not affect ($p > 0.05$) FNE, blood amino acids concentration and ratio of urinary 3-methylhistidine:creatinine (Table 4).

Only the serum IGF1 concentration was affected ($p < 0.05$) by the supplement supply, with higher values being verified in supplemented animals. Blood glucose and triglycerides were not affected ($p > 0.05$) on average, by supplementation (Table 4).

There was a linear increase ($p < 0.05$) in the amount of starch in the supplement on blood concentration of IGF1. The serum glucose and triglyceride concentrations were unaffected ($p > 0.05$) by different amounts of starch in the supplement (Table 4).

DISCUSSION

In general, supplementation of animals consuming low-quality forage with nitrogenous compounds has the potential to provide substrates to the rumen environment, which would favour the growth of fibrolytic bacteria. As a consequence, fibre degradation and forage intake increased [4,12,13]. However, a positive effect of the nitrogen supplements on forage intake (Table 2) and fibre digestibility (Table 3) was not verified. According to Lazzarini et al [1] and Van Soest [14], responses to nitrogen supplementation on forage intake become less apparent when the CP content of the basal forage is greater than 7% to 8% DM, such as the forage used in this study (Table 1).

However, by considering that supplementation increased DM and OM intakes (Table 2), but did not affect forage intake (Table 2), it can be understood that supplementation with starch and nitrogenous compounds, in general, did not cause any substitutive effect on forage intake. The substitutive effect caused by supplements is positively correlated with forage quality [15]. Thus,

Table 3. Means of digestibilities of OM, CP, and NDFap (%) and dietary digested organic matter (DOM, g/kg) and treatment effects

Item	Treatments ¹⁾					SEM	Effect ²⁾			
	C	Starch:N supplement					C vs S	L	Q	C
		0:1	1:1	2:1	3:1					
OM	59.6	61.1	63.9	65.2	66.4	1.70	0.032	0.040	0.643	0.872
CP	46.5	75.2	74.4	71.5	71.0	1.68	<0.001	0.062	0.922	0.543
NDFap	58.8	57.6	57.2	57.5	55.1	2.27	0.407	0.444	0.617	0.703
DOM	569	584	611	627	640	16.2	0.024	0.025	0.668	0.933

SEM, standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; NDFap, neutral detergent fibre corrected for ash and protein; DOM, digested OM.

¹⁾ Control, no supplementation; 0:1, 1:1, 2:1, 3:1, starch:crude protein ratio in the supplement (g/g).

²⁾ C vs S: control versus supplementation; L, Q, C, linear, quadratic, and cubic effects of starch:crude protein ratio in the supplement.

Table 4. Means of the metabolic characteristics of bulls fed low feed value tropical hay with nitrogen and energy supplements

Item	Treatments ¹⁾					SEM	Effect ²⁾			
	C	Starch:N supplement					C vs S	L	Q	C
		0:1	1:1	2:1	3:1					
RAN (mg/dL)	3.59	29.95	20.77	18.28	10.26	3.521	<0.001	<0.001	0.852	0.387
pH	6.52	6.55	6.37	6.41	6.23	0.114	0.146	0.016	0.979	0.229
NI (g/d)	39.4	85.3	83.8	89.4	84.9	4.12	<0.001	0.612	0.456	0.072
UNE (g/d)	16.0	40.8	41.4	29.6	29.0	4.57	<0.001	0.018	0.874	0.200
FNE (g/d)	21.2	21.3	21.7	25.7	24.6	2.35	0.362	0.144	0.716	0.349
NB (g/d)	2.3	23.2	20.7	34.1	31.4	4.44	<0.001	0.058	0.980	0.104
ENU (g of retained N/g of ingested N)	0.041	0.272	0.246	0.377	0.372	0.0517	<0.001	0.069	0.828	0.198
UEUN (g/d)	10.0	38.3	37.1	29.4	24.5	4.60	<0.001	0.006	0.583	0.551
SUN (mg/dL)	9.2	27.8	23.5	19.8	16.3	2.12	<0.001	<0.001	0.793	0.938
3MH:cre (mmol/g creatinine)	98.0	77.3	80.9	91.6	97.4	19.21	0.316	0.151	0.912	0.787
AA (mol/L)	2238	2181	2172	2365	2268	226.3	0.936	0.497	0.872	0.564
Glucose (mg/dL)	54.4	57.4	59.3	56.9	57.8	5.20	0.083	0.872	0.765	0.319
Triglycerides (mg/dL)	19.7	21.4	24.0	21.2	21.6	3.07	0.193	0.752	0.484	0.231
IGF1 (ng/mL)	38.6	40.4	76.0	80.0	95.5	15.35	0.014	0.005	0.297	0.413

SEM, standard error of the mean; RAN, ruminal ammonia nitrogen concentration; NI, nitrogen intake; UNE, urinary nitrogen excretion; FNE, faecal nitrogen excretion; NB, nitrogen balance; ENU, efficiency of nitrogen utilization; UEUN, urinary excretion of urea nitrogen; SUN, serum urea nitrogen concentration; 3MH:cre, ratio of urinary 3-methylhistidine:creatinine; AA, blood concentrations of amino acids; IGF1, insulin-like growth factor 1.

¹⁾ Control, no supplementation; 0:1, 1:1, 2:1, 3:1, starch:crude protein ratio in the supplement (g/g).

²⁾ C vs S, control versus supplementation; L, Q, C, linear, quadratic, and cubic effects of starch:crude protein ratio in the supplement.

even with the additional starch supply, the low-quality forage used in this experiment seems to be the main reason of the absence of substitutive effect.

The increased DOM intake observed with the supplement supply (Table 2) reflects only the addition of high digestibility components to the diet, since there were no effects of supplementation on digested NDFap intake (Table 2) and NDFap digestibility (Table 3).

The evaluation of intake pattern among supplements, especially with values expressed in relation to BW, indicate that the amount of starch produced a cubic effect on total and forage intake, which is justified by the most prominent intake observed when the starch:CP ratio used in supplement was 2:1 (Table 2). This behaviour contrasts with the linear increase of the starch amount on the diet energy content, supported by the linear effects on the dietary DOM (Table 3) and CP:DOM (Table 2).

The control of feed intake by ruminants is a complex multifactorial process in which animals aim at reaching the minimal total discomfort [16]. In this sense, even when provided low-quality forages, mechanisms related to physical gastrointestinal distension and metabolic regulation act simultaneously on the control of feed intake [17]. Among the metabolic mechanisms, the dietary energy-to-protein ratio plays a preponderant role, since excess of available energy in relation to protein availability may cause discomfort because of the increased body heat production. By contrast, excess of protein availability in relation to available energy can extend the discomfort by excessive nitrogenous compounds catabolism [18,19]. Thus, it may be understood that under the feed conditions of this study, the use of a starch:CP ratio of 2:1 promoted, amongst the evaluated supplements, a closer condition

of comfort (Table 2). It is noteworthy that, even without an overall effect of supplementation on intake, forage intake using the starch:CP ratio of 2:1 was approximately 11% higher (in g/kg BW) compared to the control.

Studies conducted in the tropical environment have reported decreases in NDFap digestibility with supplement starch [4,7]. It was reasoned with increasing dominance of non-fibrolytic bacteria species than fibrolytic bacteria in the rumen, which depresses the fibrolytic activity [20,21,22]. However, the increase starch supplement did not affect fibre digestion in this study (Table 3). Moreover, it must be noted that the starch supply was done together with nitrogenous compounds. In these situations, the increased availability of nitrogen can decrease the microbial competition for substrates, reducing or avoiding the deleterious effects on fibre degradation, as shown by other authors [4,7].

The most prominent effects of supplement supply consisted of increasing NB and ENU (Table 4). This effect must be attributed to an increase in nitrogen status in the animal organism [23,24]. The term "protein status" or "nitrogen status" defines the quantitative and qualitative availability of nitrogen supplements for all physiological functions in animal metabolism, including the functions associated with the metabolism of other compounds (e.g., energy) [25]. The nitrogen supplements available for the animal metabolism would be used for different metabolic functions in a priority order for the animal as follows: survival (e.g., nitrogen recycle), maintenance and production (e.g., growth, reproduction, etc.). Therefore, nitrogen deposition in body tissues or products will only occur after supplying the demands of nitrogen supplementation of higher priority [25]. Thus, the increase in NB and ENU reflects in an improved nitrogen availability for animal

growth and in a decrease in nitrogen proportion used for either maintenance or surviving functions caused by nitrogen supplementation (Table 4).

Some authors have found that the nitrogen supply can decrease the muscle protein catabolism for use in metabolic demands of higher priority [26,27]. However, the supplement supply did not affect the ratio of urinary 3-methylhistidine:creatinine (Table 4), which is a direct indicator of muscle protein catabolism.

However, the supplements enhanced the serum concentration of IGF1 (Table 4), indicating that the effects of increased nitrogen supply were essentially anabolic. The IGF1 is an endocrine regulator of muscle growth in cattle. In addition to their independent action, it promotes important links between growth hormone and the metabolic growth process [28,29], particularly in skeletal muscle [30]. The IGF1 concentrations are responsive to nutritional plane as well as the diet composition, with an apparently more prominent response to dietary protein concentration than energy concentration [29]. This justifies the increase of its serum concentration with the nitrogen supply. Similar results were obtained by Rufino [31], who supplemented cattle fed tropical forage and supplemented with nitrogenous compounds.

In addition, it was found that the supplementation tended ($p < 0.09$) to increase the blood glucose concentration (Table 4). This seems to support the idea of enhancing the nitrogen status in the animal organism with nitrogen supplementation. Part of glucose demanded by the ruminant can be synthesized from gluconeogenic amino acids [14]. However, low nitrogen availability could increase the demand of amino acids for hepatic urea synthesis [32]. Thus, the additional nitrogen supply could incur less use of gluconeogenic amino acids in the intermediary metabolism of nitrogen, enhancing as a consequence the availability of precursors for the glucose synthesis [31].

The increased levels of starch in the supplement lowered ruminal pH values, and this did not negatively affect the normal ruminal fermentation pattern. Comparing the results obtained between supplements, it was found that the increase in the starch amount tended to increase the NB ($p < 0.06$). Part of this effect appears to result from higher efficiency of microbial nitrogen utilization in the rumen. This argument is based on the linear decrease of RAN and SUN concentrations as starch supply increased. Additionally, the CP digestibility was decreased with the increase in starch supply (Table 3), which may reflect lesser loss of nitrogen as ammonia in the rumen. Whereas the nitrogen intake was similar for all supplements (Table 4), the decrease of those variables (i.e., RAN and SUN) could only be obtained through a better efficiency of microbial nitrogen utilization in the rumen. The increase in nitrogen assimilation by providing additional starch was reported under tropical grass feeding [4,7]. Greater microbial nitrogen assimilation implies an increase in metabolisable protein (MP) supply. This is supported by no significant changes in faecal nitrogen excretion (Table 4), indicating that any additional microbial nitrogen produced with the starch

supplementation was actually digested in the intestine and, therefore, converted to MP.

However, according to Detmann et al [27], increases in MP supply from microbial origin account for only 18.5% of the observed increase in NB in cattle fed tropical forages. This indicates that secondary or metabolic effects of supplementation also affect the efficiency of nitrogen utilization in the animal organism.

In ruminants, the protein deposition efficiency depends on the energy availability, additionally, the efficiency of energy utilization in the metabolism depends on the amino acids availability [33]. Thus, the starch supplement seems to have increased the availability of metabolisable energy, which resulted in an increase in nitrogen retention in the animal organism. This hypothesis is reinforced by the linear decrease in the nitrogen losses in the urine by increasing the starch amount in the supplement (Table 4). It is noteworthy that there has been a linear increase in the blood concentration of IGF1 with the increase in the starch supply (Table 4), confirming the increase in body anabolism with the increased starch supply.

In relation to increasing trend in the NB with the increasing starch supplement level, means indicated highest value for starch:CP ratio of 2:1 (Table 4). This pattern is associated with the arguments made on voluntary intake, indicating that higher metabolic comfort adaptation may have been obtained with this ratio.

IMPLICATIONS

Nitrogen supplementation in cattle fed low-quality tropical forage increases nitrogen retention in the animal mainly by raising the nitrogen status in the body. However, an additional starch supply is able to enhance nitrogen retention through increasing the energy availability in the rumen and animal metabolism. Among the starch:CP ratios, 2 to 1 is most recommendable.

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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