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ANIMAL SCIENCE

Nutritional performance and metabolic characteristics of cattle fed tropical forages with protein and starch supplementation

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Abstract: Effects of protein supplementation, with and without starch supplementation, on nutritional performance and metabolic characteristics of cattle fed low- and medium-quality tropical forages were evaluated using 4 cannulated steers distributed according to a 4 × 4 Latin square. Experimental periods were divided into two subperiods. In the first subperiod, two animals received low-quality hay and two animals received medium-quality. Supplementation schemes were evaluated in the second subperiod: low-quality hay with protein (300 g of crude protein – CP/d); low-quality hay with protein (300 g CP/d) and starch (225 g/d); medium-quality hay with protein (300 g CP/d); and medium-quality hay with protein (300 g CP/d) and starch (225 g/d) supplementation. Without supplementation, medium-quality forage provided higher intake, digestibility, nitrogen balance (NB) and efficiency of nitrogen utilization (EFNU). Comparing subperiods, supply of supplements depressed medium-quality forage intake, but did not affect low-quality forage intake. Supplementation increased NB, EFNU and serum concentration of IGF1 in animals fed low-quality forage. Protein supplementation increases nitrogen retention in animals, an effect attributed mainly to anabolic stimuli. However, this effect is more prominent when animals are fed low-quality forages. No positive impact on animal metabolism was obtained with combination of supplemental protein and starch.

Key words: *Brachiaria decumbens*, digestibility, fiber, IGF1, low-quality hay, nitrogen balance.

INTRODUCTION

The low quality of tropical forages during the dry season of the year is due to the low concentration of crude protein (CP) and high lignification of insoluble fiber. In these cases, the protein contents are lower than the minimal required for the rumen microorganisms present full capacity for degradation of fiber (<70-80 g CP · kg^{-1} of dry matter), which constrains the bacterial growth in the rumen (Lazzarini et al. 2009, Figueiras et al. 2010). Thus, in these circumstances, dietary protein deficiency is the main nutritional constraint of forage, making a supplementation with nitrogenous compounds the primary tool to improve the use of low-quality forage (Detmann et al. 2010, Souza et al. 2010). On the other hand, during the rainy season, the tropical forages can be considered an unbalanced diet, mainly with regards to protein-to-energy ratio, presenting a relative excess of energy (Detmann et al. 2010). Thus, supplementation programs used during the rainy season should focus on dietary balance that involves the increase of dietary protein concentration, so that the surplus of energetic substrates can be turned into animal products (Detmann et al. 2014a).

From these arguments, it can be stated that energy supplementation in both dry and rainy seasons is not able to provide substrates for utilization of forage nutrients, because it may imply a decreased fiber degradation (Costa et al. 2008, Mlay et al. 2008, Souza et al. 2010), a reduction of voluntary forage intake through metabolic imbalances (Costa et al. 2011a), or not cause any effect on production and metabolic efficiency of the animals (Lazzarini et al. 2013, Figueiras et al. 2016). However, the protein accretion in the animal body depends on the efficiency of metabolizable energy and protein utilization, since these are interrelated. Thus, protein deposition efficiency depends on the energy availability as well as the efficiency of energy utilization depends on the availability of amino acids in the metabolism (Schroeder & Titgemeyer 2008). Studies performed under tropical conditions in different seasons and with fed animals or grazing animals provide evidence that the association between energy and nitrogen supplements may involve interaction effects on the nitrogen metabolism, reducing the deleterious effects of exclusive energy supplementation as well as increasing nitrogen assimilation by rumen microorganisms and nitrogen retention in animal (Souza et al. 2010, Valente et al. 2011a, Lazzarini et al. 2013).

For cattle grazing production to be considered optimized, it must be based on the maximization of the positive interaction and/or minimization of the negative interaction between basal forage and supplements (Detmann et al. 2010). Thus, when considering the combination of supplemental nitrogen and energy compounds, maximizing the positive interaction between forage and supplement components should be based on increasing the retention of nitrogenous compounds in the animal body, which will be reflected in greater weight gain in the dry season (low-quality forage) and also in the rainy season (medium- to high-quality forage).

Therefore, the objective of this work was to evaluate the effects of exclusive nitrogen supplementation or, along with starch supplementation on nutritional performance and metabolic characteristics of cattle fed low- or medium-quality tropical forages.

MATERIALS AND METHODS

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

Four crossbred young bulls (European × Zebu) with an initial average body weight (BW) of 381±19 kg were used. The animals were surgically fitted with ruminal and abomasal cannulae 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 (protocol number 112/2014).

The experiment was conducted according to a 4 × 4 Latin square design balanced for residual effects. The animals were fed *ad libitum* with two different signal grass hays (*Brachiaria decumbens* Stapf.). The forages used were low- and medium-quality with an average crude protein (CP) of 81.4 and 96.2 g · kg^{-1} of dry matter (DM), and 334.7 and 290.1 g of indigestible neutral detergent fiber (iNDF) · kg^{-1} DM, respectively (Table I).

The animals were kept in individual 10-m^2 stalls, provided with rubber flooring, feeders and water dispensers, with unrestricted access to water and complete mineral mixture. Prior the experiment, the animals were adapted during 15 days to handling, facilities and forage allowance.

The experiment consisted of four 28-d experimental periods. Each experimental period

Table I. Chemical composition of forages, casein, and starch.

Nutrient	LQ hay[4]	MQ hay[4]	Casein	Starch
DM[1]	894.1	896.5	877.1	887.3
OM[2]	938.4	939.2	976.9	998.1
CP[2]	81.4	96.2	879.9	4.9
EE[2]	6.1	7.9	4.6	2.6
NDFap[2]	791.6	762.3	-	-
NDIP[2]	48.0	63.0	-	-
NFC[2,3]	59.3	72.8	92.4	990.6
Lignin[2]	72.1	58.2	-	-
iNDF[2]	334.7	290.1	-	-

***DM* dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *NDFap* neutral detergent fiber corrected for ash and nitrogenous compounds, *NDIP* neutral detergent insoluble protein, *NFC* non-fibrous carbohydrates, *iNDF* indigestible neutral detergent fiber[1] g · kg^{-1} as fed; [2] g · kg^{-1} DM; [3] Calculated according to Detmann & Valadares Filho (2010); [4] LQ, low-quality hay; MQ, medium-quality hay.**

was divided into two subperiods of 14 d. In the first subperiod there were no supplementation and only the effect of basal forage was evaluated (two animals per forage). In the second subperiod of each experimental period, it was performed the same measurements of the first subperiod; however, the animals were supplemented. A 5-d interval was used between periods to improve the control on the carry-over effects of the treatments. The forage was *ad libitum* fed to the animals at 6h00 and 18h00, allowing approximately 100 g · kg^{-1} in orts. Supplements were fed in the same time as forage and the total amount was fractionated into two parts of equal weight packed in paper bags, which were placed in the rumen of animals.

The treatments evaluated in the second subperiod were:

1) Low-quality hay with protein supplementation (300 g CP · d^{-1});
2) Low-quality hay with protein (300 g CP · d^{-1}) and starch (225 g · d^{-1}) supplementation;
3) Medium-quality hay with protein supplementation (300 g CP · d^{-1}); and
4) Medium-quality hay with protein (300 g CP · d^{-1}) and starch (225 g · d^{-1}) supplementation.

The amount of supplemental CP (300 g · d^{-1}) corresponded to 40% of the dietary CP requirements for a crossbred young bull (European × Zebu) with 350 kg BW and average daily gain of 0.5 kg (Marcondes et al. 2010). Casein (LabSynth, Diadema, São Paulo, Brazil) was used as the source of supplemental protein (Table I). The amount of supplemental starch (Amisol 3408®, João Pessoa, Paraíba, Brazil) was adopted such that the mixture with casein constituted a supplement with 500 g CP · kg^{-1} DM.

The voluntary intake and total digestibility were evaluated from 6th to 11th day and the 20th to 25th day of each period for evaluation without and with supplementation, respectively. In this sense, it was monitored the amount of supplied hay (6th to 10th and 20th to 24th day) and orts (7th to 11th and 21th to 25th day). Forage and orts samples were pooled per animal and experimental subperiod.

The total feces collection was performed on 7th, 9th and 11th; 21st, 23rd and 25th days of

each experimental period, starting at 6h00 and lasting for 24 hours, to evaluate without and with supplementation, respectively. At the end of each collection day, the feces were weighed, homogenized and an aliquot of approximately 50 g · kg^{-1} was taken and oven-dried (60°C).

The total urine collection was performed on the 13th and 27th day of each period, to evaluate without and with supplementation, respectively. This collection was performed using rubber funnels coupled in the penile region and fixed by elastic straps on the back of the animals. The funnels were equipped with hoses, which led the urine to polyethylene containers containing ice. After 24 hours, the total volume of urine was measured, and two 50-mL aliquots were obtained per animal and experimental subperiod. The first was immediately evaluated with regards total nitrogen (Kjeldahl method; Detmann et al. 2012) and urea (enzymatic colorimetric method, K056, Bioclin, Belo Horizonte, Minas Gerais, Brazil). The second aliquot was frozen (-80°C) and sent to a commercial laboratory for the quantification of 3-methylhistidine concentration by high-performance liquid chromatography (HPLC).

Samples of abomasal digesta were obtained from the 7th to 9th days and 21st to 23rd days of each experimental period, to evaluate the abomasal digesta flow without and with supplementation, respectively. Samples were taken every three hours, following the schedule: 7th and 21st days – 6h00 and 15h00; 8th and 22nd days – 9h00 and 18h00; 9th and 23rd days – 12h00 and 21h00.

Rumen fluid samples were taken on the 12th and 26th days of each experimental period to evaluate rumen pH, rumen ammonia nitrogen (RAN) and volatile fatty acids (VFA; acetate, propionate, butyrate, isobutyrate, valerate and isovalerate) and to isolate ruminal microorganisms. The samples were taken at 6h00, 12h00, 18h00 and 24h00; the microorganisms were isolated according to the technique described by Cecava et al. (1990). To evaluate pH, RAN and VFA, the samples were collected manually from the liquid/solid interface of the rumen mat, filtered through a triple layer of cheesecloth and submitted to pH assessment through digital potentiometer. A 40-mL aliquot was then separated, fixed with 1 mL of H_2SO_4 (500 mL · L^{-1}) and frozen (-20°C) for further analysis of RAN concentration. A second 10-mL aliquot was fixed with 1 mL of a metaphosphoric acid solution (200 g · L^{-1}) and frozen (-20°C) for later evaluation of VFA concentration.

Concurrently with the ruminal fluid, blood samples also were taken from the animals directly from the jugular vein using tubes with vacuum and gel coagulation accelerator (BD Vacutainer®, SST II Advance). The samples were immediately centrifuged (2700 × g, 20 minutes) to separate the serum and in the end of the day samples were pooled per animal (equal volumes for each collection) and analyzed with regards the concentrations of free amino acids (HPLC; quantitative chromatography of amino acid), glucose (enzyme method glucose oxidase-peroxidase, K082, Bioclin, Belo Horizonte, Minas Gerais, Brazil), insulin (Coat-a-count insulin, DPC-Medlab TKIN5®, Valença, Bahia, Brazil), IGF1 (immunoradiometric assay, DSL-5600 IRMA Active™, Santana do Parnaíba, São Paulo, Brazil) and urea (enzymatic colorimetric method, K056, Bioclin, Belo Horizonte, Minas Gerais, Brazil).

Ruminal evacuation procedure was performed to quantify the rumen pool and the rates of passage and degradation of fiber. Samples were collected at 12h00 (6 hours after the morning feeding, equidistant between meals) of the 14th and 28th days, and at 6h00 (before the morning forage supply) on the 15th day and in the morning after 28th day of each experimental period, to evaluate without and with supplementation, respectively. The collected

material was weighed and homogenized. An aliquot of approximately 50 g · kg^{-1} was taken and the remaining material was returned to the rumen of animals.

Samples of hay and orts were oven-dried (60°C) and ground in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, PA) to pass through a 2-mm screen. After that, half of each ground sample was ground again to pass through a 1-mm screen. Casein and starch samples were retained from each 25-kg package of product and pooled for subsequent analysis. Subsequently, samples were pooled per animal and experimental subperiod. Pooled samples ground to pass through 1-mm sieves were analyzed according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al. 2012) for DM (dried overnight at 105°C; method INCT-CA G-003/1), ash (complete combustion in a muffle furnace at 600°C for 4 h; method INCT-CA M-001/1), N (Kjeldahl procedure; method INCT-CA N-001/1), ether extract (EE; Randall procedure; method INCT-CA G-005/1), neutral detergent fiber corrected for ash and protein (NDFap; using a heat-stable α-amylase, omitting sodium sulfite and correcting for residual ash and protein; method INCT-CA F-002/1), and acid detergent lignin (ADL; method INCT-CA F-005/1). Casein and starch samples were analyzed only for DM, ash, CP, and EE. From samples of hay, orts, feces, abomasal digesta and ruminal contents processed through a 2-mm sieve, iNDF content was quantified as the residual NDF remaining after 288 h of ruminal in situ incubation using F57 filter bags (Ankom Technology Corp., Macedon, NY), according to Valente et al. (2011b). Potentially degradable NDF (pdNDF) was calculated as the difference between NFDap and iNDF.

The abomasal flow of DM was estimated by using the iNDF as internal marker taking into account its ingested proportion in ratio to its flow in the abomasum.

The RAN concentrations were quantified using the colorimetric technique described by Detmann et al. (2012; method INCT-CA N-006/1). The concentrations obtained at different times were combined by animal and time to produce only one value that represented the average daily RAN concentration. Similar combination was conducted to rumen pH values.

For VFA analysis, rumen fluid samples collected over time were pooled (equal volume for each time) per animal and subperiod, centrifuged (12,000 × g, 10 min, 4°C), and supernatants were treated as described by Siegfried et al. (1984). Ruminal VFA were analyzed by HPLC in a Dionex Ultimate 3000 Dual detector HPLC (Dionex Corporation, Sunnyvale, CA, USA) coupled to a refractive index (RI) Shodex RI-101 maintained at 40°C using a ion exchange column Phenomenex Rezex ROA, 300 × 7.8 mm maintained at 45°C. Mobile phase was prepared with 5 mmol · L^{-1} sulfuric acid (H_2SO_4) and the flow was 0.7 mL · min^{-1}.

Samples of ruminal microorganisms and abomasal digesta were analyzed for CP (Method INCT-CA N-001/1; Detmann et al. 2012) and purine bases (Ushida et al. 1985) contents. The rumen production of microbial nitrogen compounds was quantified through the product between concentration in abomasal digesta and abomasal DM flow. The purine bases were used as marker to evaluate microbial concentration in abomasal digesta, considering the N_{RNA}:N_{total} ratio in the rumen microorganisms.

Rates of intake and passage of pdNDF and iNDF were estimated through the ratios of daily intake and abomasal flow on ruminal pools. The degradation rate of pdNDF was calculated as the difference between the rates of intake and passage.

The experiment was carried out according to Latin square design 4 × 4 balanced for residual effects including the effects of treatment (fixed), animal (random), and experimental period (random). In the first subperiod it was evaluated the effect of forage quality without supplementation. Comparison between treatments in the second subperiod (with supplementation) was performed according to a 2 × 2 factorial arrangement (low- or medium-quality forage and presence or absence of starch supplementation). The comparison between the subperiods with or without supplementation was performed by using repeated measures techniques (Kaps & Lamberson 2004). The best structure of the (co)variance matrix was chosen according to the corrected Akaike information criterion. All statistical procedures were conducted through the MIXED procedure of SAS (version 9.4) adopting $\alpha = 0.05$.

RESULTS

Effect of forage quality without supplementation

There was a greater voluntary intake (P<0.05) of the medium-quality forage compared to low-quality forage (Table II). However, the CP-to-digested organic matter (DOM) ratio was not affected (P>0.05) by the forage quality.

The total digestibility coefficients of organic matter (OM), CP, NDF and dietary DOM content were greater (P<0.05) for the medium-quality forage (Table III).

With regards ruminal kinetics, there was an effect (P<0.05) of forage quality only on the iNDF ruminal pool, which was greater for animals fed low-quality forage (Table IV). Ruminal pools of NDF and pdNDF were not affected (P>0.05) by forage quality. Forage quality affected (P<0.05) the intake rate of NDF and the passage rate of iNDF, being greater in animals fed medium-quality forage. The intake, degradation and passage rates of pdNDF were not affected (P>0.05) by the forage quality without supplementation.

The supply of medium-quality forage decreased (P<0.05) the molar proportion of acetate and increased (P<0.05) the molar proportions of propionate, valerate and isobutyrate (Table V). As a result, a lower (P<0.05) acetate-to-propionate ratio was verified when medium-quality forage was fed. There was no effect (P>0.05) of the forage quality on RAN and VFA concentrations, ruminal pH and butyrate and isovalerate molar proportions.

The nitrogen intake showed similar pattern to the CP intake (Table VI). There was an effect (P<0.05) of forage quality on fecal nitrogen excretion (FNE), nitrogen balance (NB), efficiency of nitrogen utilization (EFNU) and rumen microbial nitrogen production (NMIC), whose greater values were obtained in animals that received medium-quality forage. The ruminal nitrogen balance (RNB) and efficiency of microbial synthesis were greater (P<0.05) when animals were fed low-quality forage. It is noteworthy that, in both forages, negative values of RNB were found when animals did not receive supplements. There was no effect (P>0.05) of forage quality on urinary nitrogen excretion (UNE), urinary excretion of urea nitrogen (UEUN), serum urea nitrogen (SUN), total blood amino acids concentration and urinary excretion of 3-methyl-histidine.

Only the serum concentration of IGF1 was affected (P<0.05) by forage quality, which was greater for animals fed medium-quality forage (Table VII). The glucose, triglycerides and insulin concentrations showed no effect (P>0.05) of forage quality.

Table II. Least squares means for average daily intake of dry matter (DM), forage DM (FDM), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ash and protein (NDFap), digested OM (DOM), digested NDFap (DNDFap), and the relationship between CP and DOM intakes (g CP · kg^{-1} DOM) according to the treatments.

Intake	Without supplementation[1]		SEM	With supplementation[2,3]				SEM	Effect[4]			
	LQ	MQ		LQ	LQ + S	MQ	MQ + S		Qws	Qs	S	Qs × S
kg · d^{-1}												
DM	5.92	7.14	0.315	6.09	6.37*	6.92	6.92	0.445	<0.001	<0.001	0.398	0.404
FDM	5.92	7.14	0.315	5.83	5.90	6.65*	6.46*	0.445	<0.001	<0.001	0.709	0.404
OM	5.56	6.71	0.296	5.73	6.01	6.51	6.53	0.418	<0.001	<0.001	0.302	0.374
CP	0.48	0.69	0.027	0.72*	0.72*	0.86*	0.84*	0.039	<0.001	<0.001	0.768	0.615
NDFap	4.68	5.44	0.266	4.62	4.67	5.07*	4.92*	0.377	<0.001	0.025	0.757	0.423
DOM	3.18	4.12	0.195	3.44	3.23	4.05	3.96	0.276	<0.001	0.001	0.419	0.756
DNDFap	2.76	3.42	0.182	2.85	2.50	3.14*	2.91*	0.257	<0.001	0.029	0.064	0.676
CP:DOM	151	167	10.6	209*	223*	212*	212*	15.0	0.069	0.766	0.656	0.340
g · kg^{-1} BW												
DM	14.7	17.6	0.53	15.1	15.8	16.9	17.1	0.75	<0.001	0.003	0.329	0.573
FDM	14.7	17.6	0.53	14.4	14.6	16.2*	15.9*	0.74	<0.001	0.003	0.920	0.563
OM	13.8	16.5	0.50	14.2	14.9	15.9	16.1	0.70	<0.001	0.002	0.252	0.571
NDFap	11.5	13.3	0.46	11.6	11.8	12.4*	12.2*	0.65	<0.001	0.104	0.948	0.554

[1] Subperiod without supplementation: LQ, low-quality forage; MQ, medium-quality forage; [2] Subperiod with supplementation: LQ, low-quality forage and supplementation with protein; LQ + S, low-quality forage and supplementation with protein and starch; MQ, medium-quality forage and supplementation with protein; MQ + S, medium-quality forage supplementation with protein and starch; [3] Means followed by (*) differ from the obtained with exclusive forage feeding in the first subperiod (P<0.05); [4] Qws, effect of forage quality without supplementation; Qs, effect of forage quality with supplementation; S, effect of starch supplementation; Qs × S, interaction between forage quality and starch supplementation in the second subperiod.
SEM Standard Error of the Mean.

Supplementation effect (comparison between subperiods)

Supplements supply increased CP intake (P<0.05; Table II). Overall, supplementation decreased (P<0.05) the medium-quality forage intake but did not affect (P>0.05) the low-quality forage intake. As a result, there was a decrease (P<0.05) in NDF and digested NDF (DNDF) intakes of medium-quality forage. The supplements supply did not affect (P>0.05) DOM intake. However, the CP:DOM ratio was increased (P<0.05) by the supplements supply in comparison with the exclusive forage feeding.

The total CP digestibility was increased (P<0.05) when supplements were supplied (Table III). However, the comparison between subperiods showed no effects (P>0.05) of the supplements on OM and NDF digestibilities, as well as on dietary DOM content.

The protein supplement (without starch) decreased (P<0.05) the NDF ruminal pool in both forages when compared to the subperiod without supplementation (Table IV). On the other hand, the iNDF ruminal pool was decreased (P<0.05) by both types of supplements when medium-quality forage was provided to the animals.

The pdNDF pool was not affected (P>0.05) by the supplementation. No effect was observed (P>0.05) on intake, degradation and passage rates when the two subperiods (without and with supplementation) were contrasted.

The comparison between the two subperiods indicated that supplementation increased (P<0.05) RAN concentration and molar proportions of butyrate, valerate and isovalerate for all treatments (Table V). The molar proportion of acetate was decreased (P<0.05) by supplementation, excepting (P>0.05) when the supplementation was exclusively with protein on medium-quality forage. The molar proportion of propionate was increased (P<0.05) and the acetate-to-propionate ratio decreased (P<0.05) only for exclusive protein supplementation when animals were fed low-quality forage. Supplementation did not affect (P>0.05) pH, total VFA concentration and isobutyrate molar proportion.

The nitrogen intake showed similar pattern to the CP intake (Table VI). The comparison between the subperiods indicated that supplements increased (P<0.05) UEUN, SUN and NMIC. The UNE was greater (P<0.05) only when exclusive nitrogen supplement and medium-quality forage were used. The supplements supply increased (P<0.05) RNB in animals fed low-quality forage only when starch was added and in a medium-quality forage with both supplements. Comparing the subperiods, the NB and EFNU were increased (P<0.05) by both supplements only for animals fed low-quality forage. There was no effect (P>0.05) of supplementation on FNE, blood amino acid concentration, urinary excretion of 3-methylhistidine and efficiency of microbial synthesis (EMS).

The comparison between subperiods indicated that supplement supply increased (P<0.05) serum glucose concentrations in animals fed medium-quality forage only when associated with starch (Table VII). Insulin concentrations were greater (P<0.05) for animals fed medium-quality forage with both supplements, whereas the IGF1 concentrations were increased (P<0.05) for animals fed low-quality forage with both

Table III. Least squares means for digestibility coefficients (g · g^{-1}) of OM, CP and NDFap, and concentration of digested organic matter in the diet (DOM, g · kg^{-1}) according to the treatments.

Item	Without supplementation[1]		SEM	With supplementation[2,3]				SEM	Effect[4]			
	LQ	MQ		LQ	LQ + S	MQ	MQ + S		Qws	Qs	S	Qs × S
OM	0.571	0.614	0.0132	0.601	0.537	0.622	0.607	0.0187	0.028	0.021	0.039	0.198
CP	0.566	0.644	0.0179	0.711*	0.659*	0.724*	0.709*	0.0253	0.003	0.184	0.156	0.421
NDFap	0.589	0.629	0.0135	0.616	0.536	0.620	0.592	0.0191	0.048	0.128	0.010	0.185
DOM	539	576	12.5	566	507	585	573	17.7	0.039	0.021	0.049	0.194

[1] Subperiod without supplementation: LQ, low-quality forage; MQ, medium-quality forage; [2] Subperiod with supplementation: LQ, low-quality forage and supplementation with protein; LQ + S, low-quality forage and supplementation with protein and starch; MQ, medium-quality forage and supplementation with protein; MQ + S, medium-quality forage supplementation with protein and starch; [3] Means followed by (*) differ from the obtained with exclusive forage feeding in the first subperiod (P<0.05); [4] Qws, effect of forage quality without supplementation; Qs, effect of forage quality with supplementation; S, effect of starch supplementation; Qs × S, interaction between forage quality and starch supplementation in the second subperiod.
SEM Standard Error of the Mean.

supplements. There was no effect (P>0.05) of supplementation on triglyceride concentration.

Effects of starch supplementation and forage quality with protein supplementation

During the subperiod with supplementation, higher intakes (P<0.05) were observed, excepting (P>0.05) for NDF intake (g · kg^{-1} BW) and iNDF (kg · d^{-1} and g · kg^{-1} BW) in medium-quality forage (Table II). There was no effect (P>0.05) of starch supplementation or interaction between quality forage and starch supplementation on the voluntary intake. However, it is noteworthy that there was a decreasing trend (P<0.07) in the DNDF intake when starch was supplied. The CP:DOM ratio was not affected (P>0.05) by the forage quality. Additionally, there were no effects of starch supplementation and interaction between starch supplementation and forage quality (P>0.05) on this variable.

There was an effect (P<0.05) of forage quality on OM digestibility, which was greater (P<0.05) in animals fed medium-quality forage (Table III). This fact resulted in a greater dietary DOM content (P<0.05) for animals fed medium-quality forage. Starch supplementation decreased (P<0.05) OM and NDF digestibilities. This pattern caused a decrease (P<0.05) in dietary DOM content in animals supplemented with starch. There was no interaction (P>0.05) between forage quality and starch supplementation on total digestibility.

The NDF and pdNDF ruminal pools were not affected (P>0.05) by the forage quality during the subperiod with supplementation (Table IV). In this case, only the iNDF ruminal pool was affected (P<0.05) by the forage quality, which was greater in animals receiving low-quality forage. An effect (P<0.05) of forage quality was observed on the NDF intake rate and pdNDF passage rate during the subperiod with supplementation, which were greater in animals fed medium-quality forage. Additionally, there was no effect (P>0.05) of starch supplementation or interaction between starch supplementation and forage quality on intake, degradation and passage rates.

In the presence of supplementation, forage quality exerted effect (P<0.05) on acetate and isobutyrate molar proportions (Table V). The acetate molar proportion was greater for animals fed low-quality forage, while isobutyrate presented a greater molar proportion in animals receiving medium-quality forage. Effects (P>0.05) of starch supplementation or interaction between starch supplementation and forage quality were not verified on ruminal fermentation characteristics (Table V).

The nitrogen intake showed similar pattern to the CP intake (Table VI). The NMIC was affected (P<0.05) by the forage quality, which was greater in animals fed medium-quality forage. The EMS was greater (P<0.05) in animals fed low-quality forage. There was a positive effect (P<0.05) of starch supply on NMIC, regardless of forage quality. There was no effect (P>0.05) of forage quality on UNE, FNE, NB, EFNU, RNB, UEUN, SUN, blood amino acids concentration and urinary excretion of 3-methylhistidine. Variables related to the metabolism of nitrogenous compounds were not affected (P>0.05) by the interaction between forage quality and starch supplementation.

There was no effect (P>0.05) of forage quality on serum components, except for glucose, which was greater (P<0.05) in animals with diets based on medium-quality forage (Table VII). Effects of starch and interaction between starch supplementation and forage quality were not observed (P>0.05) on the serum components (Table VII).

Table IV. Least squares means for ruminal pool of neutral detergent fiber (NDF), potentially digestible neutral detergent fiber (pdNDF) and indigestible neutral detergent fiber (iNDF), and ingestion (ki), degradation (kd) and passage (kp) rates of NDF, pdNDF and iNDF according to the treatments.

Item	Without supplementation[1]		SEM	With supplementation[2,3]				SEM	Effect[4]			
	LQ	MQ		LQ	LQ + S	MQ	MQ + S		Qws	Qs	S	Qs × S
Ruminal pool (g · kg^{-1} BW)												
NDF	16.0	15.4	0.63	14.6*	14.8	13.8*	14.1	0.89	0.232	0.099	0.544	0.979
pdNDF	3.5	4.1	0.33	3.0	3.5	3.7	3.9	0.47	0.083	0.164	0.298	0.757
iNDF	12.5	11.3	0.51	11.6	11.3	10.1*	10.2*	0.726	0.011	0.008	0.776	0.771
Rates (h^{-1})												
NDFki	0.030	0.036	0.0017	0.033	0.034	0.037	0.036	0.0024	<0.001	0.017	0.812	0.572
pdNDFki	0.083	0.089	0.0134	0.111	0.104	0.088	0.081	0.0189	0.706	0.203	0.684	0.995
pdNDFkd	0.076	0.079	0.0136	0.106	0.099	0.079	0.072	0.0192	0.864	0.124	0.674	0.990
pdNDFkp	0.007	0.010	0.0015	0.005	0.005	0.009	0.010	0.0021	0.065	0.022	0.928	0.877
iNDFkp	0.016	0.019	0.0012	0.018	0.018	0.019	0.019	0.0017	0.008	0.270	0.847	0.616

[1] Subperiod without supplementation: LQ, low-quality forage; MQ, medium-quality forage; [2] Subperiod with supplementation: LQ, low-quality forage and supplementation with protein; LQ + S, low-quality forage and supplementation with protein and starch; MQ, medium-quality forage and supplementation with protein; MQ + S, medium-quality forage supplementation with protein and starch; [3] Means followed by (*) differ from the obtained with exclusive forage feeding in the first subperiod (P<0.05); [4] Qws, effect of forage quality without supplementation; Qs, effect of forage quality with supplementation; S, effect of starch supplementation; Qs × S, interaction between forage quality and starch supplementation in the second subperiod. SEM Standard Error of the Mean.

DISCUSSION

In general, the forage quality influences intake during both subperiods (Table II), where lower intakes were recorded in animals fed low-quality forage. This pattern can be attributed to the lower NDF quality with higher iNDF concentration (Table I) and, consequently, low NDF digestibility of this forage (Table III). The iNDF presents more prominent ruminal fill effect compared to pdNDF, once its ruminal disappearance occurs only by passage (Detmann et al. 2008). On the other hand, it has been found that, in general, lower ruminal passage rates for low-quality forage (Table IV) confirms the greater fill effect associated with this forage.

Variations in the rumen-reticulum fill have been reported as possible adjustments concerning the balance between nutritional requirements and diet quality (Forbes 2007). This fact indicates that animals can increase the rumen-reticulum capacity due to decreases in the diet quality (Weston 1996, Schettini et al. 1999, Rinne et al. 2002). As seen in this study, the use of low-quality forage implied expansion of the iNDF ruminal pool, indicating an attempt by the animals to adapt to a lower-quality diet (Table IV). However, this adaptation was not fully effective in preventing decrease in voluntary intake (Table II).

The comparison between subperiods showed that supplementation decreased the voluntary intake of the medium-quality forage (Table II), which did not occur with low-quality forage. This fact indicates that there was a

Table V. Least squares means for ruminal ammonia nitrogen concentration (RAN, mg · dL^{-1}), ruminal pH, volatile fatty acids (VFA, mmol · dL^{-1}), molar proportion of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate (mmol · $100mmol^{-1}$) and acetate:propionate ratio (A:P) according to the treatments.

Item	Without supplementation[1]		SEM	With supplementation[2,3]				SEM	Effect[4]			
	LQ	MQ		LQ	LQ + S	MQ	MQ + S		Qws	Qs	S	Qs × S
RAN	6.01	7.55	1.018	13.40*	12.61*	12.50*	14.40*	1.439	0.194	0.701	0.633	0.253
pH	6.53	6.54	0.093	6.48	6.42	6.49	6.44	0.131	0.813	0.733	0.313	0.931
VFA	14.70	15.93	0.769	14.66	13.52	14.28	15.67	1.088	0.181	0.329	0.889	0.169
Acetate	86.13	84.66	0.310	84.15*	84.62*	83.49	83.00*	0.438	0.002	0.012	0.972	0.254
Propionate	8.48	8.91	0.134	9.23*	8.73	9.28	9.27	0.189	0.018	0.098	0.142	0.156
Butyrate	0.22	0.29	0.025	0.49*	0.44*	0.42*	0.48*	0.035	0.072	0.658	0.856	0.117
Isobutyrate	4.23	4.98	0.156	4.30	4.25	5.09	5.23	0.220	0.001	<0.001	0.817	0.625
Valerate	0.73	0.84	0.042	1.20*	1.17*	1.12*	1.26*	0.060	0.023	0.978	0.242	0.084
Isovalerate	0.21	0.32	0.075	0.63*	0.79*	0.60*	0.76*	0.106	0.299	0.828	0.133	>0.999
A:P	10.17	9.52	0.164	9.12*	9.71	9.00	9.00	0.232	0.006	0.064	0.177	0.176

[1] Subperiod without supplementation: LQ, low-quality forage; MQ, medium-quality forage; [2] Subperiod with supplementation: LQ, low-quality forage and supplementation with protein; LQ + S, low-quality forage and supplementation with protein and starch; MQ, medium-quality forage and supplementation with protein; MQ + S, medium-quality forage supplementation with protein and starch; [3] Means followed by (*) differ from the obtained with exclusive forage feeding in the first subperiod (P<0.05); [4] Qws, effect of forage quality without supplementation; Qs, effect of forage quality with supplementation; S, effect of starch supplementation; Qs × S, interaction between forage quality and starch supplementation in the second subperiod.
SEM Standard Error of the Mean.

substitutive effect of forage by supplement when a better-quality forage was supplied. The range of replacement supplements for forage intake is positively correlated with forage quality (Minson 1990).

Supplementation with nitrogen compounds to animals fed low-quality forage would have the potential to provide substrates to rumen and, thus, improve the growth of fibrolytic bacteria; as a consequence, there would be increased fiber degradation and improvement of voluntary forage intake (Leng 1990, Detmann et al. 2009, Souza et al. 2010). However, positive effects were not observed for the supply of nitrogenous compounds on forage intake (Table II), degradation rate of pdNDF (Table IV), and fiber digestibility (Table III) compared to the subperiod without supplementation. According to Van Soest (1994) and Lazzarini et al. (2009), responses to nitrogen supplementation for forage intake become less apparent when the CP content of basal forage is greater than 70-80 g · kg^{-1} DM, which is close to the low-quality forage used in this study (Table I).

Less digestibility of CP was observed in low-quality forage in the subperiod without supplementation (Table III). Considering that the apparent digestibility of non-fiber components, such as CP, is proportional to the component intake (Van Soest 1994), the pattern of the digestibility is justified in this case, given the lower CP intake with low-quality forage (Table II). The lower CP digestibility associated with lower NDF digestibility, as previously discussed, caused

Table VI. Nitrogen intake (NI, g · day^{-1}), urinary nitrogen excretion (UNE, g · day^{-1}), fecal nitrogen excretion (FNE, g · day^{-1}), nitrogen balance (NB, g · day^{-1}) efficiency of nitrogen utilization (EFNU, g of retained N · g^{-1} of ingested N), urinary excretion of urea nitrogen (UEUN, g · day^{-1}), serum urea nitrogen concentration (SUN, mg · dL^{-1}), blood amino acids concentration (AA, mol · L^{-1}) and urinary excretion of 3-methylhistidine (3MH, mmol · g^{-1} creatinine), intestinal flow of microbial nitrogen compounds (NMIC, g · day^{-1}) and efficiency of microbial synthesis (EMS, g microbial CP · kg^{-1} DOM) according to the treatments.

Item	Without supplementation[1]		SEM	With supplementation[2,3]				SEM	Effect[4]			
	LQ	MQ		LQ	LQ + S	MQ	MQ + S		Qws	Qs	S	Qs × S
NI	77.4	110.2	4.37	114.6*	115.3*	137.7*	134.7*	6.18	<0.001	<0.001	0.765	0.615
UNE	39.2	38.4	5.34	57.3	49.2	61.7*	59.0	7.56	0.896	0.277	0.407	0.675
FNE	33.3	39.1	2.21	33.2	39.1	38.3	39.1	3.12	0.007	0.194	0.094	0.194
NB	4.9	32.8	5.01	26.2*	27.0*	37.7	36.7	7.09	<0.001	0.116	0.985	0.887
EFNU	0.059	0.292	0.0404	0.220*	0.238*	0.263	0.283	0.0571	<0.001	0.409	0.719	0.985
RNB	-10.9	-31.4	6.94	-6.0	8.0*	13.5*	6.9*	9.81	0.004	0.155	0.560	0.116
UEUN	20.5	21.6	4.26	45.0*	36.0*	45.3*	45.7*	6.03	0.843	0.370	0.439	0.401
SUN	10.7	10.6	1.11	17.0*	16.5*	15.7*	17.7*	1.56	0.852	0.987	0.456	0.246
AA	1692	1624	301.6	2334	2330	2325	2097	426.5	0.828	0.697	0.709	0.717
3MH	116.4	149.4	22.49	99.3	93.5	169.3	140.3	31.80	0.297	0.074	0.579	0.710
NMIC	65.2	66.7	0.67	69.4*	71.1*	69.9*	74.0*	0.95	0.023	0.010	<0.001	0.066
EMS	129	103	6.4	128	140	112	118	9.0	<0.001	0.003	0.122	0.646

[1] Subperiod without supplementation: LQ, low-quality forage; MQ, medium-quality forage; [2] Subperiod with supplementation: LQ, low-quality forage and supplementation with protein; LQ + S, low-quality forage and supplementation with protein and starch; MQ, medium-quality forage and supplementation with protein; MQ + S, medium-quality forage supplementation with protein and starch; [3] Means followed by (*) differ from the obtained with exclusive forage feeding in the first subperiod (P<0.05); [4] Qws, effect of forage quality without supplementation; Qs, effect of forage quality with supplementation; S, effect of starch supplementation; Qs × S, interaction between forage quality and starch supplementation in the second subperiod. SEM Standard Error of the Mean.

lower OM digestibility and dietary DOM content in low-quality forage without supplementation (Table III).

In the comparison between subperiods, expansion of CP digestibility with supplements supply was observed (Table III), reflecting a high digestibility of protein supplement and a positive effect of increased intake of nitrogenous compounds (Table II) on apparent digestibility of CP, as previously discussed.

During the supplementation subperiod, higher OM digestibility was found for animals fed medium-quality forage (Table III). However, this effect should be analyzed together with all digestibility coefficients, since, unlike the absence of supplementation, there was no difference between forage on NDF digestibility. The average difference between forages regarding OM digestibility was similar for both subperiods, without and with supplementation (0.043 g · g^{-1} versus 0.046 g · g^{-1}). However, in the first subperiod (without supplementation), the largest contribution to the higher OM digestibility of medium-quality forage is attributed to the higher NDF digestibility, which accounted for more than 75% of the diet (Table I). In the second subperiod (with supplementation), concerning the inclusion of highly digestible components

Table VII. Least squares means for blood glucose concentration (mg · dL^{-1}), triglycerides (mg · dL^{-1}), insulin (µUI · mL^{-1}) and IGF1 (ng · mL^{-1}) according to the treatments.

Item	Without supplementation[1]		SEM	With supplementation[2,3]				SEM	Effect[4]			
	LQ	MQ		LQ	LQ + S	MQ	MQ + S		Qws	Qs	S	Qs × S
Glucose	64.5	65.9	3.45	67.8	69.5	74.0	85.8*	4.88	0.772	0.026	0.165	0.299
Triglycerides	21.4	22.8	1.81	21.0	20.8	18.5	25.5	2.56	0.569	0.640	0.171	0.143
Insulin	0.70	0.88	0.430	1.50	2.15	2.55*	2.58*	0.608	0.734	0.161	0.514	0.545
IGF1	207.5	285.0	28.27	248.5*	256.3*	287.0	311.5	39.97	0.003	0.053	0.485	0.715

[1] Subperiod without supplementation: LQ, low-quality forage; MQ, medium-quality forage; [2] Subperiod with supplementation: LQ, low-quality forage and supplementation with protein; LQ + S, low-quality forage and supplementation with protein and starch; MQ, medium-quality forage and supplementation with protein; MQ + S, medium-quality forage supplementation with protein and starch; [3] Means followed by (*) differ from the obtained with exclusive forage feeding in the first subperiod (P<0.05); [4] Qws, effect of forage quality without supplementation; Qs, effect of forage quality with supplementation; S, effect of starch supplementation; Qs × S, interaction between forage quality and starch supplementation in the second subperiod.
SEM Standard Error of the Mean.

(starch and casein), the difference between forage on OM digestibility remained stable on average, indicating the combined effect of depression of NDF digestibility caused by starch supplementation (Table III). This fact resulted in declines in OM digestibility, dietary DOM content (Table III) and DNDF intake (Table II) with starch supplementation in the second subperiod.

The inclusion of readily fermentable carbohydrates in the diet favors the bacteria that ferment non-fibrous carbohydrates instead of fibrolytic bacteria (Carvalho et al. 2011). This phenomenon, known as the "carbohydrate effect", increases the competition for essential substrates between groups of microbial species (El-Shazly et al. 1961, Mould et al. 1983), which disfavors the use of insoluble fiber by fibrolytic microorganisms (Costa et al. 2008, Huhtanen 1987, Rooke et al. 1987) which present lower growth rate and, consequently, lesser competitive capacity (Carvalho et al. 2011). In a study conducted in tropical conditions, Lazzarini et al. (2013) found that the combination of supplemental starch and nitrogen compounds eliminated the deleterious effects of exclusive starch supplementation, minimizing the competition events in the rumen. However, in this study, even adding starch together with nitrogenous compounds, the presence of the "carbohydrate effect" was verified on the digestion of fibrous components. It is noteworthy that, during the subperiod with supplementation, RAN levels were higher than those considered critical for ruminal fibrolytic activity (8 mg RAN · dL^{-1}; Detmann et al. 2009).

Prominent impacts of the supplements were observed on RAN concentration, while average values in unsupplemented and supplemented animals were 6.78 and 13.23 mg · dL^{-1} of rumen fluid, respectively (Table IV). This pattern confirms that the nitrogen supplements made the rumen environment better suited to fibrolytic activity (Detmann et al. 2009), despite the absence of positive effects on digestibility (Table III) and NDF degradation (Table IV).

Even with the provision of different supplements, no changes in pH and total VFA concentration were observed. The main effects were observed on ruminal fermentation pattern.

During the subperiod without supplementation, the supply of medium-quality forage decreased the molar proportion of acetate, increased butyrate molar proportion and decreased the acetate:propionate (A:P) ratio (Table V), which was a possible reflection of the higher digestibility of the medium-quality forage fiber (Table III). Besides, the supply of medium-quality forage increased molar proportions of isobutyrate and valerate (Table V), which can be associated with a high concentration of CP (Table I). It is noteworthy that, with supplements supply, the differences between forages became less evident, while maintaining the previously reported pattern only for the molar proportion of acetate and isobutyrate (Table V).

The supplementation increased the molar proportion of valerate and isovalerate, a direct effect of the true protein supply. Moreover, supplementation increased the molar proportion of butyrate and decreased the molar proportion of acetate in three of the four treatments with supplementation. This seems to indicate improvements in the ruminal fermentation pattern with supplementation, although consistent changes in the A:P ratio were not perceived (Table V).

Despite of the effects of forage quality and supplementation on voluntary intake, digestion and ruminal fermentation pattern, the most prominent effects seem to have occurred on the metabolism and efficiency of nitrogen utilization (Tables VI and VII), as shown by other authors in tropical conditions (Costa et al. 2011b, Lazzarini et al. 2013, Batista et al. 2016, Rufino et al. 2016).

During the subperiod without supplementation, negative RNB was observed for both types of forage, with the lowest value obtained for medium-quality forage (Table VI). This pattern reflects the higher dietary DOM content of medium-quality forage (Table III). According to Kennedy & Milligan (1978) and Kennedy et al. (1981), the amount of OM degraded in the rumen has a positive effect on the urea transfer from the bloodstream into the rumen. Thus, with greater transfer, it increases the abomasum flow of nitrogen compounds relative to nitrogen intake, in turn decreasing the RNB.

However, negative values of RNB are associated with low metabolic efficiency in cattle fed tropical forages (Detmann et al. 2014b). In this context, the supplementation increased RNB in three of the four treatments evaluated in the second subperiod (Table VI). However, it is emphasized that the RNB was negative in the absence of supplementation and zero ($P>0.05$) for the four treatments evaluated in the second subperiod. This pattern is attributed to the increased nitrogen supply through supplementation, which allows the establishment of equilibrium between input and output of nitrogen compounds in the rumen (Detmann et al. 2014b). No effect of supplementation with starch was found, which is in line with Detmann et al. (2014b), who stated that the NBR is dependent on the dietary nitrogen supply and is not influenced by supplemental energy.

The protein supply increased urinary losses of urea nitrogen. The increase occurred in only one of the treatments in compare to the subperiod without supplementation (Table VI), in which it was found that the average of UNE in the supplemented subperiod was approximately 46.5% higher than the average of UNE verified in the subperiod without supplementation. The provision of additional nitrogen increased RAN and SUN concentrations, which caused the greatest elimination of nitrogen compounds via urine.

The improved forage quality increased NB and EFNU during the subperiod without supplementation (Table VI). However, the evaluation of 3-methylhistidine excretion

showed no effect of the forage quality on muscle protein catabolism (Table VI). Thus, the effects in this subperiod appear to be predominantly anabolic, which is supported by the higher blood concentration of IGF1 in animals fed medium-quality forage (Table VII). The IGF1 is an endocrine regulator of muscle growth in cattle, which, in addition to its independent action, is an important link between growth hormone and the metabolic process of growth (Pell & Bates 1990, Drewnoski et al. 2014), particularly in skeletal muscle (Lobley 1992). The greater anabolism in animals fed medium-quality forage seems to reflect the higher CP and DOM intakes compared to low-quality forage (Table II).

The supplements supply affected NB and EFNU. However, these effects were more prominent when the animals were fed low-quality forage, such that, in the subperiod with supplementation, the responses obtained with both forages became similar (Table VI). According to Detmann et al. (2014b), animals respond in terms of weight gain with protein supplementation up to dietary CP content of 225 $g \cdot kg^{-1}$ DM. However, responses in the gain (which reflect the NB) decrease as the CP content in the forage increases. Thus, it seems appropriate not to observe responses to supplementation with higher quality forage. The increase in NB and EFNU with supplementation and low-quality forage was accompanied by an increase in IGF-1 concentrations compared to the subperiod without supplementation (Table VII).

It must be highlighted that, for most of the variables associated with the metabolism of nitrogen, providing supplements annulled the differences between low- and medium-quality forage (Table VI).

However, despite the lack of differences between forages on NB in the subperiod with supplementation and the lack of supplementation effect on the medium-quality forage (Table VI), the NB values were on average 40% higher for medium-quality forage compared to low-quality forage when the supplements were provided. Concomitantly, it was found that the medium-quality forage during the subperiod with supplementation tended to show ($P<0.06$) higher IGF1 concentration. Additionally, in this same situation, there was a higher glucose concentration and increasing in insulin levels concerning the subperiod without supplementation (Table VII). The highest glucose concentration may be associated with lower A:P ratio ($P<0.07$) observed with supplementation on the medium-quality forage (Table V), which may have increased the availability of gluconeogenic precursors. Insulin affects the uptake of glucose and amino acids by muscle tissue for anabolic purposes (Cunningham 1992, Davis et al. 2003, Rooyackers & Nair 1997). Thus, the body and muscle anabolism appears to have been stimulated by the supplementation of medium-quality forage, although less prominently when compared to low-quality forage.

The microbial nitrogen production in the rumen was superior for medium-quality forage in both subperiods (Table VI), a direct effect of the greater availability of nutrients for microbial growth. On the other hand, nitrogen availability through supplementation caused an increase in NMIC, which was even more pronounced when the starch was added to supplements. The additional supply of readily available energy in the rumen may increase the microbial production by increasing nitrogen assimilation by microorganisms (Souza et al. 2010, Lazzarini et al. 2013).

On the other hand, the increase in forage quality decreased the EMS in both subperiods (Table VI). According to Detmann et al. (2014b), there is a negative correlation between dietary DOM concentration and EMS in animals fed tropical forages. In this context, it is noteworthy that, in both subperiods, the dietary DOM

contents were higher for the medium-quality forage (Table III). Decreases in the EMS are associated with increases in dietary DOM concentration, which is possibly due to the asynchrony between the nitrogen and energy availability (Bach et al. 2005). It is noteworthy that the average value of EMS obtained in this study was 120 g microbial CP · kg^{-1} DOM, which approximates to the average value suggested by the CSIRO (2007) (130 g microbial CP · kg^{-1} MOD).

Except for the effect on the rumen microbial production, supply starch did not affect animal metabolism and the efficiency of nitrogen utilization (Tables VI and VII).

CONCLUSIONS

Protein supplementation increases nitrogen retention in the animal organism, an effect attributed primarily to anabolic stimuli. However, this effect is more prominent when the animals are fed low-quality forage. No positive impact on animal metabolism was obtained with the combination of supplemental protein and starch.

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Marcia de Oliveira Franco performed the experiment, analyzed samples, contributed to analyze the data, wrote the manuscript, reviewed and edited; Edenio Detmann acted as a PhD supervisor of Marcia de Oliveira Franco, acquired the financial resources of this research, designed the study, analyzed the data, supervised the project and revised the manuscript for intellectual content; Erick Darlisson Batista and Luana Marta de Almeida Rufino assisted in performing the experiment and revised the manuscript for intellectual content; Mario Fonseca Paulino and Sebastião de Campos Valadares Filho revised the manuscript for intellectual content. All authors discussed the results and approved the final version of the manuscript.