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ORIGINAL RESEARCH

Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds

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Abstract The effects of supplementation with nitrogenous compounds on intake, digestibility, and microbial protein synthesis in cattle fed low-quality tropical forage were assessed. Five rumen fistulated crossbred Holstein × Gir heifers were used, with initial average live weight of 180± 21 kg. Signal grass (*Brachiaria decumbens*) hay (48.6 g kg^{-1} of crude protein (CP), on a dry matter (DM) basis) was used as roughage. Five treatments were defined according to nitrogen supplementation level (0, 20, 40, 60, and 80 g of CP kg^{-1} above the CP level of the hay). A mixture of urea, ammonium sulfate, and albumin at the ratios of 4.5:0.5:1.0, respectively, was used as nitrogen source. The experiment consisted of five experimental periods, according to a 5×5 Latin square design. The average CP contents in the diets were 51.9, 71.1, 86.0, 116.7, and 130.2 g kg^{-1}, on a DM basis. A quadratic effect was detected ($P<0.10$) of the CP levels in the diets on DM and neutral detergent fiber intake (kg/day), with maximum response at the levels of 102.4 and 100.5 g CP kg^{-1} DM, respectively. The average daily concentration of rumen ammonia nitrogen showed increasing linear pattern ($P<0.01$) as function of CP levels in the diet, with estimated value of 9.64 mg dL^{-1} equivalent to the maximum DM intake. Microbial nitrogen flow in the intestine was linearly and positively related ($P<0.01$) with the CP levels in the diet.

Keywords Rumen ammonia nitrogen · Signal grass · Supplementation · Urea

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Introduction

Beef cattle production in tropical regions such as Brazil is based on pastures, which represent the lowest cost food resource for ruminant feeding. However, the largest pasture area in Brazil is located in the central region, where the climatic characteristics consist of an uneven rainfall distribution that leads to occurrence of two distinguished seasons of the year, rainy and dry, which result in qualitative and quantitative fluctuations in the offer of forage (Paulino et al. 2008).

During the dry season, tropical forages present high contents of cell wall and low quantities of crude protein (CP). Therefore, tropical pastures in this season rarely represent a balanced diet as their organic and inorganic constituents are not present in concentrations and proportions sufficient to meet animal requirements.

Normally, during dry season, tropical forages have CP levels lower than 70 g kg^{-1} CP, which is a value considered limiting for adequate ruminal fibrolytic activity (Sampaio et al. 2009; Lazzarini et al. 2009a), implying sub-optimal conditions in the rumen with low microbial growth and reduced fiber degradation.

Additional supply of nitrogenous compounds to animals fed low-quality forage favors the growth of fibrolytic bacteria, increases digestion rates and microbial protein synthesis and, thus, results in higher voluntary forage intake and energy extraction from the forage fiber, resulting in greater flow of nutrients to the intestine and volatile fatty acids production (Detmann et al. 2004).

Under these circumstances, strategic supplementation with nitrogenous compounds should be focused on increasing the CP content of the diet, supplying essential substrates to sustain rumen microbial activity, which, in turn, would increase forage intake and digestibility. In that way, the

nutritional requirements of the animals would be met, so they would not only maintain the live weight, but also could obtain satisfactory weight gains during the dry season.

Several studies have reported positive relationships between supplemental nitrogenous compounds and voluntary intake and digestibility of low-quality forage (i.e., Delcurto et al. 1990a; Mathis et al. 2000). However, the quantitative characterization of such associations is still scarce in the tropics, therefore justifying complementary studies to generate specific responses regarding this issue.

Thus, the objective of this study was to assess the effect of supplementation with nitrogenous compounds on intake, digestibility, rumen ammonia nitrogen (RAN) concentration, and microbial protein synthesis in cattle fed low-quality tropical forage.

Materials and methods

Location and animals

The experiment was carried out from December 2005 to February 2006 in Viçosa, MG, Brazil. Five crossbred heifers (Holstein × Gir) averaging 180±21 kg of live weight (LW) were surgically fitted with ruminal cannulae and kept in individual stalls of approximately 10 m^2. Water and mineral mixture were available to heifers at all time.

Experimental diets and feeding

The forage fed to the animals consisted of low-quality signal grass (*Brachiaria decumbens* Stapf.) hay, with average 48.6 g CP kg^{-1} dry matter (DM).

The hay was produced from a dry season cutting (August 2005) of the forage available in a signal grass pasture located in the mid-west region of Brazil (latitude 18°41′ S, longitude 49°34′ W, average altitude 620 m). The climate is classified as Aw type, hot and humid, with coldest monthly temperatures above 18°C, annual average rainfall between 1,400 and 1,600 mm, rainy season from November to March, and dry season from April to October.

The five treatments assessed were set in order to raise the CP level of the diet to 0, 20, 40, 60, and 80 g kg^{-1}, on DM basis, above the CP level of the roughage. A mixture of urea, ammonium sulfate, and albumin was used as a source of nitrogenous compounds, at the ratios of 4.5:0.5:1.0, respectively. These supplements were calculated based on the DM intake computed on the previous day and placed directly in the rumen of the animals.

The supplement ingredients were chosen based on their carbohydrate absence, so supplementation effects with nitrogenous compounds could be evaluated without any supplementary source of fiber or energy interfering in the measurements. Albumin was included in the supplement to meet the microbial requirements of true degradable protein, allowing the supply of essential substrates, such as branched chain volatile fatty acids.

The forage was supplied ad libitum, allowing approximately 100 g kg^{-1} in orts, being fed twice a day, in equal portions, at 8 a.m. and 4 p.m. The supplements, in two portions of equal weight, were placed in the rumen of the animals when the forage was offered. The forage offered and the orts were quantified daily.

Handling, measurements, and samples

The experiment consisted of five 16-day experimental periods. The first 5 days of each experimental period were used to the adaptation of the animals to the supplementation levels.

For the quantification of voluntary intake, foodstuffs supplied between the sixth and the ninth days of each experimental period were considered and the orts were considered between the seventh and tenth day. Forage and orts samples were processed in a Wiley mill (1 mm) and stored for later analysis.

The digestibility coefficients were estimated from fecal samples taken directly from the rectum of the animals from the seventh to the tenth day in each experimental period according to distribution: seventh day—6 a.m. and 2 p.m.; eighth day—8 a.m. and 4 p.m.; ninth day—10 a.m. and 6 p.m.; and tenth day—12 p.m. and 8 p.m. The feces samples were oven dried (60°C/72 h) and processed in a Wiley mill (1 mm). Composite samples were elaborated per animal and experimental period.

To evaluate the rumen pH and RAN concentration, samples of rumen fluid were taken on the sixth day of each experimental period at 4 a.m., 8 a.m., 12 p.m., 4 p.m., 8 p.m., and 12 a.m. 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 by using a digital potentiometer (TEC-3P-MP, Tecnal®). A 40-mL aliquot was then separated, fixed with 1 mL H_2SO_4 (1:1), and frozen (−20°C) for later analysis.

Rumen microorganisms were isolated on the tenth day of each experimental period immediately before and 6 h after morning feeding according to Cecava et al. (1990).

On the 12th, 14th, and 16th days of each experimental period urine samples were obtained, approximately 4 h after morning feeding. The samples were filtered through cheesecloth and a 10-mL aliquot was separated and diluted with 40 mL H_2SO_4 (0.036 N).

Blood was collected from the jugular vein on the 16th day, 4 h after morning feeding, using test tubes with separator gel and coagulation accelerator (BD Vacuntainer®

SST II Advance). The samples were centrifuged at 2,700×*g*, for 20 min, to obtain the serum that was frozen (−20°C).

Chemical analysis

Samples of feeds, orts, and feces were analyzed regarding DM, organic matter (OM), CP, ether extract (EE), and acid detergent fiber (ADF) contents according to the methods of AOAC (Association of Official Analytical Chemists—AOAC 1990). In the neutral detergent fiber (NDF) analysis, the samples were treated with a heat-stable alpha amylase, without using sodium sulfite and corrected for residual ash (Mertens 2002). The NDF was also corrected for nitrogenous compounds content as described by Licitra et al. (1996). Lignin content was obtained by solubilization of cellulose by sulfuric acid. Supplement samples were analyzed regarding DM, OM, and CP as described above (Table 1).

Fecal excretion was estimated by using indigestible NDF (iNDF) as an internal marker, which was obtained by a 144 h in situ rumen incubation procedure. Potentially digestible NDF (pdNDF) was defined as the difference between NDF treated with a heat-stable alpha amylase and corrected for ash and nitrogenous compounds [aNDFom(n)] and iNDF.

The RAN content in rumen fluid samples was determined by the micro-Kjeldahl system, without acid digestion and after distillation with potassium hydroxide (2 N), after previous centrifugation of the sample to 1,000×*g*, for 15 min. The concentrations obtained at the different sampling times were combined by animal and period in order to obtain a single value that represented the average daily RAN concentration. Rumen pH values were combined in a similar way.

Table 1 Chemical composition of forage and supplement

Item	Forage	Supplement
DM[a]	899.6	896.4
OM[b]	942.6	989.1
CP[b]	48.6	2,512.0
EE[b]	15.3	–
aNDFom(n)[b]	769.8	–
NFC[b, c]	108.9	–
ADFom(n)[b]	531.1	–
Lignin(sa)[b]	79.6	–
ADIP[d]	324.5	–

DM dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *aNDFom(n)* neutral detergent fiber assayed with a heat-stable amylase and corrected for ash and nitrogenous compounds, *NFC* non-fibrous carbohydrates, *ADFom(n)* acid detergent fiber corrected for ash and nitrogenous compounds, *Lignin (sa)* lignin determined by solubilization of cellulose with sulphuric acid, *ADIP* acid detergent insoluble protein

[a] g kg^{-1} as fed

[b] g kg^{-1} of DM

[c] NFC=OM−[CP+EE+aNDFom(n)]

[d] g kg^{-1} of CP

The rumen microorganism samples were oven dried (60°C) and assessed for DM, CP (Association of Official Analytical Chemists—AOAC 1990), and purine bases (Ushida et al. 1985) contents.

The urine samples, after thawing, were composed per animal and experimental period. The urea concentrations in the blood serum and creatinine in the urine were obtained by colorimetric enzymatic (Bioclin K047) and modified Jaffé (Bioclin K016-1) methods, respectively. The contents of allantoin and uric acid in the urine were estimated using colorimetric methods, as reported by Chen and Gomes (1992), and the total nitrogen content was estimated by the Kjeldhal method (Association of Official Analytical Chemists—AOAC 1990).

The total urinary volume was estimated by the ratio of creatinine concentration in the urine upon excretion per unit of live weight (Chizzotti et al 2006):

$$\mathrm{CE} = 32.27 - 0.01093 \times \mathrm{LW} \quad (1)$$

where CE is the daily creatinine excretion (mg kg^{-1} of LW) and LW the live weight (kg).

Purine derivatives excretion was calculated by the sum of the quantities of allantoin and uric acid excreted in the urine. From this, the absorbed purines were calculated by the equation (Verbic et al. 1990):

$$\mathrm{AP} = \frac{\mathrm{PD} - 0.385 \times \mathrm{LW}^{0.75}}{0.85} \quad (2)$$

where AP is the absorbed purines (mmol day^{-1}); PD the purine derivatives excretion (mmol day^{-1}); 0.85 the recovery of absorbed purines as purine derivatives in the urine (mmol mmol^{-1}); and 0.385 the endogenous purine derivatives excretion in the urine per unit of metabolic size (mmol).

Microbial synthesis of nitrogenous compounds in the rumen was estimated as function of the absorbed purines and the N_{RNA}:N_{TOTAL} ratio in the microorganisms (Chen and Gomes 1992):

$$\mathrm{NMIC} = \frac{70 \times \mathrm{AP}}{0.83 \times R \times 1,000} \quad (3)$$

where NMIC is the microbial nitrogen flow in the small intestine (g day^{-1}); R the N_{RNA}:N_{TOTAL} ratio in the microorganisms (mg mg^{-1}); 70 the nitrogen contents in purines (mg mol^{-1}); and 0.83 the intestinal digestibility of the microbial purines (mg mg^{-1}).

Statistical analysis

The experiment design was a 5×5 Latin square with five animals and five experimental periods. The average diet content of CP in each level of supplementations was used as independent variable for interpretation of the treatments effects. An orthogonal partition of the sum of squares of treatments into linear, quadratic, cubic, and fourth degree effects was obtained following the analysis of variance. A linear regression model was then fitted (Littell et al. 1991). All the statistical procedures were carried out using SAS (α=0.10).

Results

The average CP levels in the diets, which were calculated from the ratio between the total CP intake (forage and supplement) and the total DM intake, were 51.9, 71.1, 86.0, 116.7, and 130.2 g kg^{-1}, on DM basis, for the supplementation levels of 0, 20, 40, 60, and 80 g kg^{-1}, respectively.

A quadratic effect of the CP levels in the diet on the DM (P<0.09), NDF (P<0.09), and iNDF (P<0.05) intakes (kg/day) was detected, with critical points (maximum response) at the CP levels of 102.4; 100.5, and 96.4 g kg^{-1} of DM, respectively. A similar pattern was also observed for DM (P<0.03) and NDF (P<0.04) intakes, when expressed in grams per kilogram of LW, with critical points (maximum response) at CP levels of 99.5 and 98.2 g kg DM^{-1}, respectively (Table 2). On the other hand, CP intake increased linearly (P<0.01) as the CP levels in the diet increased (Table 2).

DM and NDF digestibility coefficients were not affected by CP levels in the diet (P>0.10), while the CP digestibility coefficients were associated quadratically (P<0.10) with the CP levels in the diet (Table 3). In addition, the digestibility coefficient of OM (P<0.03) and of pdNDF (P<0.05) increased linearly with the CP levels in the diet (Table 3).

Rumen pH values were not influenced by the CP levels in the diet (P>0.10), and remained over 6.5 for all treatments. The mean daily RAN concentration had an increasingly linear profile (P<0.01) in function of the CP levels in the diet (Table 4).

The NMIC was linearly and positively (P<0.01) related to the CP levels in the diet (Table 4).

The serum urea nitrogen concentration (SUN) increased linearly (P<0.01) with the increase in the CP levels in the diet, thus indicating an increasing loss of nitrogenous compounds (Table 4).

Table 2 Least squares means and standard error of mean (SEM) for average daily intake of DM, OM, CP, NDF, digested OM (DOM), digested NDF (DNDF), and indigestible NDF (iNDF) according to CP levels in the diet

Intake	CP level in diet (gkg^{-1} of DM)						Effect degree			
	51.9	71.1	86.0	116.7	130.2	SEM	L	Q	C	F
kg										
DM[a]	2.43	2.67	3.07	3.23	3.07	0.21	0.079	0.088	0.235	0.857
OM	2.28	2.53	2.91	3.06	2.93	0.20	0.068	0.082	0.245	0.848
CP[b]	0.123	0.188	0.262	0.365	0.391	0.032	0.001	0.460	0.480	0.919
NDF[c]	1.88	2.06	2.36	2.44	2.33	0.17	0.130	0.081	0.265	0.823
DOM[d]	0.655	0.716	0.935	0.979	0.935	0.072	0.004	0.174	0.392	0.317
DNDF[e]	0.712	0.782	0.861	0.984	0.977	0.074	0.008	0.697	0.659	0.996
iNDF[f]	1.09	1.21	1.39	1.46	1.37	0.10	0.130	0.044	0.156	0.958
g kg^{-1} LW										
DM[g]	12.8	15.0	16.6	17.0	15.3	1.0	0.078	0.027	0.419	0.963
NDF[h]	10.0	11.7	12.8	12.8	11.8	0.8	0.167	0.040	0.555	0.977

L linear degree, *Q* quadratic degree, *C* cubic degree, *F* fourth degree

[a] $\widehat{Y} = -0.06215 + 0.062252X - 0.0003039X^2 (R^2 = 0.8184)$

[b] $\widehat{Y} = -0.04331 + 0.003353X (r^2 = 0.9735)$

[c] $\widehat{Y} = -0.0323 + 0.04811X - 0.0002394X^2 (R^2 = 0.7198)$

[d] $\widehat{Y} = 0.48096 + 0.03976X (r^2 = 0.7576)$

[e] $\widehat{Y} = 0.53016 + 0.003648X (r^2 = 0.9656)$

[f] $\widehat{Y} = -0.73210 + 0.046525X - 0.0002414X^2 (R^2 = 0.5071)$

[g] $\widehat{Y} = -2.21406 + 0.387231X - 0.0019455X^2 (R^2 = 0.9354)$

[h] $\widehat{Y} = -1.0817 + 0.28672X - 0.001460X^2 (R^2 = 0.9533)$

Table 3 Least square means and standard error of mean (SEM) for digestibility coefficients (g g^{-1}) of DM, OM, CP, NDF, and potentially digestible NDF (PDNDF) according to CP levels in the diet

Item	CP level in diet (gkg^{-1} of DM)						Effect degree			
	51.9	71.1	86.0	116.7	130.2	SEM	L	Q	C	F
DM	0.273	0.252	0.291	0.289	0.275	0.015	0.136	0.811	0.463	0.174
OM[a]	0.292	0.284	0.318	0.321	0.318	0.014	0.021	0.794	0.803	0.204
CP[b]	0.097	0.268	0.446	0.548	0.609	0.043	0.001	0.092	0.707	0.260
NDF	0.368	0.367	0.374	0.377	0.362	0.015	0.559	0.984	0.909	0.870
PDNDF[c]	0.750	0.777	0.780	0.848	0.828	0.039	0.049	0.872	0.857	0.705

L linear degree, *Q* quadratic degree, *C* cubic degree, *F* fourth degree

[a] $\widehat{Y} = 0.2552 + 0.006076X (r^2 = 0.7867)$

[b] $\widehat{Y} = -0.6777 + 0.0181592X - 0.000063673X^2 (R^2 = 0.9834)$

[c] $\widehat{Y} = 0.6729 + 0.0014151X (r^2 = 0.9583)$

The N_{RNA}:N_{TOTAL} ratio in the rumen microorganisms was not affected by the CP levels in the diet ($P>0.10$), with average value of 0.142 g g^{-1}. On the other hand, the CP contents in the microorganisms were affected quadratically by the CP levels in the diet ($P<0.05$), with lower contents observed at lower levels of CP in the diet.

Discussion

The increments in DM and NDF intakes observed up to limits close to 100 g CP kg^{-1} DM in the diet supports the importance of nitrogen in a supplementation program for animals fed low-quality tropical forage (Leng 1990; Paulino et al. 2008).

Increase in intake could be a reflection of increases in the digestibility of the fibrous compounds (Lazzarini et al. 2009b), which exert high rumen fill effect. Increases in the voluntary low-quality forage intake, as a result of supplementation with nitrogenous compounds, is frequently associated with higher forage passage and digestion rates, which accelerate the removal of the indigestible fiber compounds from the rumen, resulting in a higher rumen turnover (Paulino et al. 2008).

Low-quality tropical forages are deficient not only in nutrients for animal performance, but also in substrates for

Table 4 Least square means and standard error of mean (SEM) for rumen ammonia nitrogen concentration (RAN—mg dL^{-1}), rumen pH, intake (NI), nitrogen balance (NB—g day^{-1}), serum urea nitrogen (SUN—mg dL^{-1}), microbial nitrogen flow in small intestine (NMIC—g day^{-1}), and nitrogen content in rumen microorganisms (NBAC—g kg^{-1} of DM) according to CP levels in the diet

Item	CP level in diet (gkg^{-1} of DM)						Effect degree			
	51.9	71.1	86.0	116.7	130.2	SEM	L	Q	C	F
RAN[a]	3.82	6.32	9.62	8.72	14.24	1.41	0.008	0.835	0.114	0.137
pH	6.67	6.58	6.67	6.75	6.79	0.06	0.353	0.195	0.197	0.446
NI[b]	19.77	30.14	41.87	58.47	62.53	5.15	0.001	0.462	0.480	0.916
NB	−20.41	−17.69	−19.88	−14.66	−28.85	5.05	0.686	0.354	0.300	0.378
SUN[c]	10.97	12.17	16.18	20.91	23.52	1.12	0.001	0.540	0.535	0.343
SUN/NI[d]	0.676	0.417	0.448	0.403	0.494	0.073	0.115	0.046	0.708	0.330
NMIC[e]	21.11	29.40	34.15	34.97	40.23	3.25	0.005	0.149	0.614	0.728
NBAC[f]	51.0	55.4	63.5	62.4	61.0	2.6	0.010	0.048	0.663	0.268

L linear degree, *Q* quadratic degree, *C* cubic degree, *F* fourth degree

[a] $\widehat{Y} = -1.12 + 0.10511X (r^2 = 0.7921)$

[b] $\widehat{Y} = -6.92 + 0.53658X (r^2 = 0.9735)$

[c] $\widehat{Y} = 2.02 + 0.16001X (r^2 = 0.9788)$

[d] $\widehat{Y} = 2.109 - 0.03499X + 0.0001742X^2 (R^2 = 0.8635)$

[e] $\widehat{Y} = 15.00 + 0.17928X (r^2 = 0.8057)$

[f] $\widehat{Y} = 13.4 + 0.9385X - -0.004415X^2 (R^2 = 0.9043)$

microbial metabolism, mainly nitrogenous compounds (Detmann et al. 2004; 2009). Thus, the inclusion of nitrogen supplements in the diet would be beneficial to the rumen environment (Detmann et al. 2009) and would increase the microbial growth on the fibrous carbohydrates (Costa et al. 2008).

Conversely, reductions in DM and NDF intakes were observed at CP levels higher than 100 g kg^{-1} DM (Table 2). This fall may, at least in part, be justified by the possible occurrence of protein excess for the microbial/animal metabolism, which was verified by greater SUN observed as the CP levels in the diet increased (Table 4). This pattern agrees with Detmann et al. (2009), who affirmed that excess of nitrogen in the rumen decreased the NDF voluntary intake. According to these authors, when low-quality tropical forage was supplemented with nitrogenous compounds, the ammonia production in the rumen became more prominent at levels higher than 109 g kg^{-1} DM.

Protein in excess implies in greater urea synthesis in the liver. The urea cycle depends directly on the Krebs cycle for the supply of energy and oxaloacetate, which, in turn, is involved, together with glutamate, in the metabolic step responsible for introducing the second amine grouping to form urea. Ammonia in excess increases the formation of glutamate and partially inhibits the Krebs cycle by α-ketoglutarate depletion, thus damaging the energetic metabolism and ATP synthesis in the liver and other tissues. As an alternative to eliminate ammonia, the tissue can synthesize glutamine from glutamate, a reaction that involves ATP expenditure and drains energy. In this context, excess circulating ammonia can lead to a situation of poor brain tissue functioning due to energy deficit, causing discomfort to the animals and resulting in a reduction in voluntary intake as a mechanism to reduce the indisposition (Detmann et al. 2007). Thus, reductions in intake can be observed in animals supplemented with excessive CP (Detmann et al. 2009).

This assumption is supported by the results presented by DelCurto et al. (1990a, b), who found reduction in forage intake by heifers supplemented above the level of 4 and 5 g kg^{-1} live weight when the supplement protein level was raised from 280 to 410 g kg^{-1} DM and from 250 to 390 g kg^{-1} DM, respectively; although increases in forage intake had been observed by raising the CP level of the supplements from 120 to 280 g kg^{-1} DM and from 130 to 250 g kg^{-1} DM, respectively for supplementation levels of 4 and 5 g kg^{-1} of live weight.

The pattern observed for the CP digestibility coefficient (Table 3) seemed to be related to the increase in the nitrogenous compounds losses (Table 4), since the supplementary resources used had high rumen degradability and no readily degradable energy source was incorporated in the diet.

On the other hand, the absence of effects of the supplement levels on the NDF digestibility apparently contradicts the assumptions presented above, where the inclusion of nitrogen compounds in the diet would increase the digestibility of low-quality forage by stimulating rumen fibrolytic activity (Detmann et al. 2009; Souza et al. 2010). However, there is a negative correlation between NDF intake and digestibility (Van Soest 1994). On the other hand, as NDF intake increased there was a simultaneous increase in iNDF intake (Table 2). The indigestible fraction represented about 40–45% of NDF. So, the potentially digestible fraction of NDF was lower compared to high-quality forage or concentrate. This pattern can difficult the verification of significant effects on total NDF digestibility because pdNDF is diluted by high content of iNDF.

Under such circumstances, the evaluation of digestibility coefficient of pdNDF can lead to a more accurate evaluation of the microbial degradation of the fibrous carbohydrates, because the indigestible fraction is not considered, which, regardless of the feeding situation, cannot be used by the rumen microorganisms (Paulino et al. 2008). In this way, the digestibility coefficient of pdNDF increased in function of the CP levels in the diet ($P<0.05$; Table 3) which reinforced the benefits of nitrogen supplementation on microbial utilization of fiber.

The beneficial effects of the nitrogen supplementation on digestibility were also confirmed by the intakes of digested OM (DOM) and NDF (DFDN), which increased linearly ($P<0.01$) with the CP levels in the diet (Table 3).

The measurement of the intake of digested compounds (e.g., DOM, DNDF) permitted the integration of the effects of supplementation on intake and digestibility, supporting the arguments presented previously.

It should be emphasized that the intake of DNDF was superior to the intake of DOM (Table 2), which could be explained by the fact that the NDF digestibility coefficient did not involve fecal metabolic losses (true digestibility) as observed for OM digestibility (apparent digestibility).

The positive effects of nitrogen supplementation on the fibrous particle transit in the rumen can be indirectly identified by the iNDF intake, which performed quadratically ($P<0.05$), with maximum value estimated at the level of 96.4 g CP kg^{-1} DM (Table 2). The iNDF can be removed from the rumen only by transit (Paulino et al. 2008). Thus, assuming steady-state for iNDF mass in the rumen, the increase in iNDF intake indicated positive effects of nitrogen supplementation on the fibrous particle flow to the lower gastrointestinal tract, as also verified in a companion paper (Sampaio et al. 2009).

The rumen pH values were not influenced by the CP levels in the diet ($P>0.10$), and remained above 6.5 for all treatments (Table 4). These values were considered adequate for fibrolytic activity (Hoover 1986).

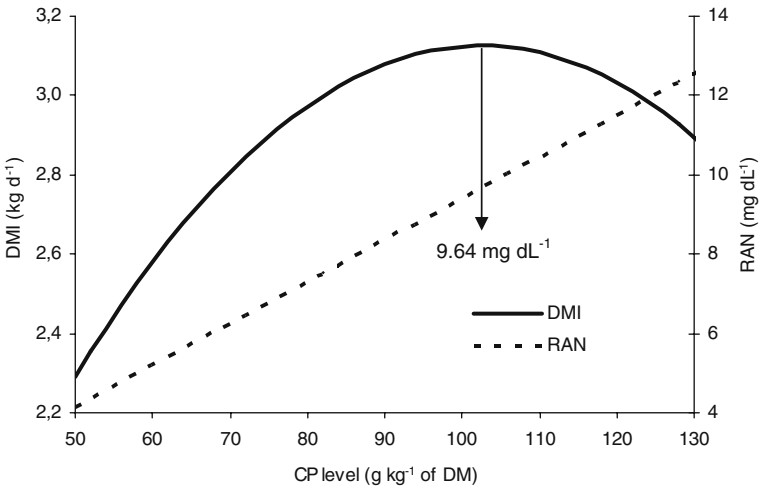

Fig. 1 Relationship between DM intake (*DMI*) and rumen ammonia nitrogen (*RAN*) concentration as a function of CP level in the diet

The supply of adequate RAN levels in the rumen fluid as such most of the requirements for microbial growth are met should be the first priority for optimizing the rumen fermentation process (Leng 1990). There are several reference estimates of RAN concentration in the literature (i.e., Satter and Slyter 1974; Leng 1990). However, variations in the microbial requirements according to substrate, rumen pH, and microbial species are impediments for a wide-ranging application of such references (McAllan and Smith 1983; Hoover 1986).

In the context of the experimental conditions of this study, the joint assessment of the pattern of the voluntary DM intake and the RAN concentration, described by their respective regression equations (Tables 2 and 4), showed that the voluntary DM intake was maximized at a RAN concentration of 9.64 mg dL^{-1} (approximately 10 mg dL^{-1}; Fig. 1).

Although this value was lower than that reported by Leng (1990), as necessary to optimize the voluntary intake of low-quality forage (20 mg dL^{-1}), higher RAN levels were associated with intake reductions (Fig. 1). Thus, increments of RAN in relation to the optimum point obtained in this study may not lead to productive benefits, as excess RAN could not be used due to the absence of high degradable carbohydrate sources in the rumen, causing excessive nitrogen losses. Thus, it can be pointed out that RAN levels close to 10 mg dL^{-1} could be considered adequate for use or intake of low-quality tropical forage.

This result was similar to that reported by Ortiz-Rubio et al. (2007), who observed optimization of intake and rumen degradation of sugarcane tops (54 g CP kg^{-1} DM) in cattle at RAN levels between 9 and 10 mg dL^{-1}. Actually, this RAN level seems to be associated with decreased in forage fill, which implicate maximum intake (Fig. 1).

The CP contents in the microorganisms were affected quadratically by the CP levels in the diet ($P<0.05$), with lower contents observed at lower levels of CP in the diet. This pattern may have reflected the effect of diluting the microbial nitrogenous compounds in function of the accumulation of intracellular carbohydrates, which is normally observed in nitrogen deficient mediums (Nocek and Russell 1988).

Nitrogen intake (NI) for the lowest CP level in the diet (51.9 g kg^{-1}) was lower than the NMIC (Table 4). Underfeeding nitrogen will not limit the rumen fermentation as long as urea recycling through saliva can satisfy the microbial requirements. However, there is a threshold level (approximately 60 to 80 g CP kg^{-1} DM in the diet) below which recycling does not satisfy the microorganism requirements, and either intake or digestibility falls off (Van Soest 1994).

Comparison between NI and NMIC by their respective regression equations (Table 4) indicated that their estimates became equivalent at the level of 61.3 g CP kg^{-1} DM (Fig. 2), which corresponded to 5.32 mg RAN dL^{-1} (approximately 5 mg dL^{-1}; Table 4). This RAN concentration was close to the value suggested by Satter and Slyter (1974) for maintaining microbial growth on carbohydrates (5 mg dL^{-1}).

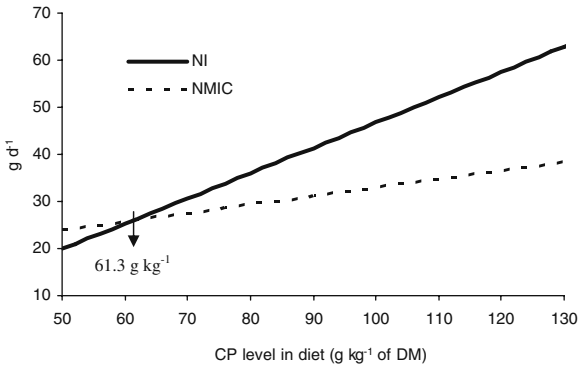

Fig. 2 Relationship between nitrogen intake (*NI*) and microbial nitrogen flow in small intestine (*NMIC*) as a function of CP level in the diet

Serum urea nitrogen estimates have been used to diagnose the suitability of the use of nitrogenous compounds in the rumen according to availability of degradable organic matter. Values between 5 and 8 mg dL^{-1} are considered an indicative of good synchronization between protein and energy in the rumen (Vasconcelos et al. 2004). Values higher than that, such as those reported in this study (Table 4), could indicate excessive dietary nitrogenous compounds compared to the energy availability in the rumen.

However, it should be emphasized that the feeding conditions in this study did not effectively represent field production situations, but rather a theoretical assessment of the use capacity of low-quality substrate in function of supplementation with nitrogenous compounds. Thus, absolute SUN values may not correspond to indications of energetic use of low-quality forage in function of the limited availability of energy from fiber (Table 3).

In this context, functional ratios between SUN and variables associated with availability of nitrogen compounds and energy in the rumen may provide information more relevant to interpret the theoretical condition established in the current experiment. The ratio between SUN concentration and NI permits the indirect expression of the relative loss of nitrogenous compounds in the rumen. Thus, lower ratios could indicate a greater proportion of ingested nitrogen assimilated as microbial protein.

A quadratic effect of CP levels in the diet ($P<0.05$) was detected for this ratio (SUN/NI), with a critical point (minimum response) estimated at the level of 100.4 g CP kg^{-1} DM. This indicated that there were increases in the nitrogen assimilation efficiency in the rumen and consequently smaller relative nitrogen losses when the protein level in the diet was increased until the level mentioned above (Table 4).

Signs of greater efficiency in nitrogen use at CP levels close to 100 g kg^{-1} DM were detected on the nitrogen balance (Table 4). Although significant effects were not detected for the CP levels in the diet on this variable ($P>0.10$), it was observed that greater values were attained for the intermediate CP levels (71.1 to 116.7 g kg^{-1}). On the other hand, the increase in the SUN/NI ratio observed for the higher CP levels indicated that the process of nitrogen compounds loss became more prominent above 100 g CP kg^{-1} DM, indicating the establishment of an effective limit of energy extraction from the fibrous carbohydrates of the forage.

Generally, integration among the variables analyzed in this study permitted the inference that benefits from the use of low-quality forage were obtained with supplements that raised the CP level in the diet to close to 100 g kg^{-1} DM. Thus, when defining the supplements to be offered to animals kept under similar conditions, the supplementary CP should be analyzed from the point of view of two different pools.

The first one should be planned to optimize the use of the energy substrates within the forage, providing sufficient CP to raise the protein content of the forage close to 100 g CP kg^{-1} DM. Thus a greater amount of low-cost energy is provided to the animal metabolism from fibrous carbohydrates of the forage. The second protein pool should, therefore, focus on the use of the carbohydrate sources present in the supplements and, when necessary, on the protein requirements of the animal so that the planned weight gain levels be attained.

Conclusions

Supplementation with nitrogen compounds in quantities that raise the crude protein content in the diet to levels close to 100 g kg^{-1} of dry matter optimize the use of low-quality tropical forage. Rumen ammonia nitrogen concentrations of 5 and 10 mg dL^{-1} are enough to supply nitrogen substrates necessary to maintain the microbial activity in the rumen and to maximize voluntary low-quality forage intake, respectively.

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