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ORIGINAL RESEARCH

Intake, digestibility and rumen dynamics of neutral detergent fibre in cattle fed low-quality tropical forage and supplemented with nitrogen and/or starch

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Abstract The objective of this work was to evaluate the effects of nitrogenous compounds and/or starch supplementation on the intake, digestibility and rumen dynamics of neutral detergent fibre (NDF) in cattle fed low-quality tropical forage. Four crossbred heifers (Holstein×Zebu) with a body weight 231.9±15.5 kg and fitted with ruminal cannulae were used. The forage fed to the animals consisted of low-quality signal grass (*Brachiaria decumbens* Stapf.) hay, with an average crude protein (CP) level of 51.6 g/kg, on a dry matter (DM) basis. Four treatments were evaluated: control, without supplementation; supplementation with nitrogenous compounds (CP of the roughage was raised to 100 g/kg), on a DM basis; supplementation with starch at a ratio of 200 g/kg DM of roughage; and supplementation with nitrogenous compounds and starch as described above. A mixture of urea, ammonium sulphate and albumin was used as a source of nitrogenous compounds at a ratio of 4.5:0.5:1.0. The experiment was carried out according to a 4×4 Latin square design in a 2×2 factorial arrangement. There was a positive effect of the nitrogenous compound supplementation on the DM and NDF intake ($P<0.01$). In contrast, starch supplementation decreased forage intake ($P<0.10$). Nitrogen supplementation increased the digestibility coefficient of DM and NDF ($P<0.05$). Supplementation with nitrogen and starch together increased the microbial assimilation of nitrogenous compounds in the rumen ($P<0.05$). We observed that nitrogen supplementation increased the estimated weighted degradation rate of NDF by 14.8%, whilst starch supplementation decreased this rate by 32.5%.

Keywords Non-fibrous carbohydrates · Rumen degradation · Rumen fill · Signal grass · Supplementation · Urea

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Introduction

Tropical grass forages are the primary nutrient resource for cattle production at pasture in the tropics, providing low-cost energy compounds from neutral detergent fibre (NDF) and supplying a large proportion of nutrients required for the animals.

However, in the tropical regions, there is an uneven distribution of rainfall throughout the year. This drastically reduces the forage available in the pastures during the low-rainfall season (dry season). During the dry season, researchers have observed increased lignin content in cell wall and decreased levels of nitrogenous compounds in the plant. These characteristics compromise the quality of forage available for grazing.

Low-quality tropical forages normally present crude protein (CP) contents lower than 70 g/kg of dry matter (DM), which is considered to be the critical threshold for adequate microbial growth on fibrous carbohydrates of basal forage (Lazzarini et al. 2009a). This deficiency implies poor utilisation of potentially degradable cell wall by microorganisms and decreases both intake and animal performance (Paulino et al. 2008).

In cattle fed low-quality forage, supplementation with nitrogenous compounds stimulates microbial activity in the rumen and improves NDF degradation, forage intake and

animal production (Paulino et al. 2008). According to results obtained by Lazzarini et al. (2009b) and Sampaio et al. (2010), nitrogen supplementation should be performed in the tropics under a point of view of two phases. In the first one, the practice will supply nitrogenous compounds to maximally stimulate microbial growth on the fibrous carbohydrates constituting low-quality forage. After that, the rumen should be exposed to CP so that the supplement's carbohydrates can be efficiently utilised by microorganisms and/or so that nutritional requirements of the animal can be directly supplied to achieve the expected increase in production level.

Some results obtained under tropical conditions demonstrate that the nitrogen supply sufficient to raise the CP of the diet to 100 g/kg DM is enough to obtain the maximum utilisation of fibre from low-quality tropical forage by cattle (Lazzarini et al. 2009b; Sampaio et al. 2010).

On the other hand, reductions in forage utilisation can be observed following supplementation with non-fibrous carbohydrates (NFC) such as starch (Mlay et al. 2007). Under these circumstances, the decrease in fibre degradation in the rumen is normally attributed to the carbohydrate effect (Costa et al. 2008), which is associated with competition for essential nutrients between fibrolytic and non-fibrolytic microbial species (Mould et al. 1983). This dynamic is more prominent under conditions of low-nitrogen availability (El-Shazly et al. 1961).

However, there are a few works examining the interaction between NFC and nitrogen supplements on low-quality forage utilisation by cattle in the tropics. Therefore, the objective of this work was to evaluate the effects of supplementation with nitrogenous compounds and/or starch on intake, digestibility and rumen dynamics of NDF in cattle fed low-quality tropical forage.

Material and methods

Location and animals

The experiment was carried out in the Animal Laboratory at the Department of Animal Science of the Federal University of Viçosa (UFV), Viçosa, Brazil.

Four crossbred heifers (Holstein×Zebu), averaging 18 months old and 231.9±15.5 kg of body weight, were surgically fitted with ruminal cannulae approximately 60 days prior to the beginning of the experiment. Ruminal fistulae and their surrounding areas were cleaned routinely during the experiment. The animals were treated for endo- and ectoparasites at the beginning of the experiment and kept in individual stalls of approximately 10 m^2 each (which were cleaned daily). Water and mineral mixtures were available for animals at all times.

Experimental diets and feeding

The forage fed to the animals consisted of low-quality signal grass (*Brachiaria decumbens* Stapf.) hay, with an average CP level of 51.6 g/kg, on a DM basis. The hay was produced from a dry season cutting (August 2005) of the forage available in a signal grass pasture located in the midwest region of Brazil.

Four treatments were evaluated: control, without supplementation; supplementation with nitrogenous compounds (CP of the roughage was raised to 100 g/kg, on a DM basis); supplementation with cornstarch (*Amisol 3408*®, CornProducts Co.) at a ratio of 200 g/kg DM of forage; and supplementation with nitrogenous compounds and cornstarch as described above.

A mixture of urea, ammonium sulphate and albumin (*Maximus*®, Arve Alimentos Co.) was used as the source of nitrogenous compounds at a ratio of 4.5:0.5:1.0, respectively. The supplement amount was based on the DM intake computed on the previous day and placed directly in the rumen of the animals.

The nitrogenous compound supplement ingredients were selected based on their lack of carbohydrate so that the supplementation effects of nitrogenous compounds could be evaluated without any supplementary source of fibre or energy interfering in the measurements. Albumin was included in the supplement to meet the microbial requirements for true degradable protein, which supplies essential substrates, such as branched chain volatile fatty acids.

The forage was supplied ad libitum, allowing approximately 100 g/kg in orts. The animals were 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 offered forage and the orts were quantified daily.

Handling, measurements and samples

The experiment consisted of four 18-day experimental periods. The first 5 days were allocated to the adaptation of animals to the supplements, followed by 13 days of sample collection.

To quantify voluntary intake, foodstuffs supplied between the sixth and the ninth days of each experimental period were considered, and the orts were calculated between the seventh and tenth days.

The forage and ort samples were processed in a Wiley mill (1 mm) and stored for later analysis.

The digestibility coefficients were estimated from faecal collections made directly from the rectum of the animals from the seventh to the tenth days of each experimental period according to the following 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 faeces samples were oven-dried (60°C) and processed in a Willey mill (1 mm). Composite samples were elaborated per animal and experimental period.

To evaluate the rumen pH and the rumen ammonia nitrogen (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 cheesecloth layer and submitted to pH assessment. A 40 mL aliquot was then separated, fixed with 1 mL of H_2SO_4 (1:1) and frozen at −20°C.

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

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

Blood was collected from the jugular vein on the 16th day, 4 h after the 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 serum that was frozen at −20°C.

Evaluation of rumen dynamics

The evaluation of fibrous particle transit kinetics was performed between the 11th and 18th days of each experimental period using a pulse dose of fibre mordent chromium (Udén et al. 1980), which was produced from hay samples.

On the 11th day of each experimental period, approximately 100 g of mordent fibre was placed in the rumens of the animals at 8 A.M. Faecal samples were taken from the rectum of the animals at 0, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60, 72, 84, 96, 120, 144, 168 and 192 h after marker administration. The samples were oven-dried (60°C/72 h) and processed in a Wiley mill (1 mm).

At the same time as the transit evaluation, we performed in situ incubation to estimate the rumen degradation parameters of NDF. Hay samples were processed in a Willey mill (2 mm) and placed in non-woven textile (100 g/m^2) bags, with a ratio of 20 mg DM/cm^2 of bag surface (Casali et al. 2008). The bags, in duplicate for each incubation time, were placed in the rumens of the animals. The following incubation times were used: 0, 3, 6, 9, 12, 18, 24, 36, 48, 72, 96 and 120 h. After incubation, the bags were cleaned with tap water and oven-dried (60°C).

Laboratory analysis

Feed, ort and faeces samples were analysed with regard to DM, organic matter (OM), CP and ether extract (EE) contents according to the methods of the AOAC (1990). In the NDF analysis, the samples were treated with a heat-stable alpha amylase, without using sodium sulphite, and corrected for residual ash (Mertens 2002). NDF was also corrected for the nitrogenous compounds as described by Licitra et al. (1996). The lignin content was obtained by cellulose solubilisation with sulphuric acid (Van Soest and Robertson 1985). The supplements were analysed with regard to DM, OM, EE and CP, as described above (Table 1).

Faecal excretion was estimated using the indigestible NDF (iNDF) as an internal marker. The iNDF contents in forage, orts and faeces were obtained by a 240 h in situ rumen incubation procedure (Casali et al. 2008). Potentially digestible NDF was defined as the difference between NDF corrected for ash and nitrogenous compounds [NDFom(n)] and iNDF.

The NFC intake was estimated according to Hall (2000):

$$\mathrm{INFC} = \mathrm{IOM} - (\mathrm{IEE} + \mathrm{IFDNom}(n) + (ICP - ICPu + Iu)), \quad (1)$$

where INFC, IOM, IEE, INDFom(n), ICP, ICPu and Iu are the intakes of NFC, OM, EE, NDFom(n), CP, CP from urea and urea, respectively (kg/day).

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

Table 1 Chemical composition of forage, protein supplement and starch

Nutrient	Hay	Nitrogen supplement	Starch
DM[a]	892.5	966.7	880.6
OM[b]	946.9	986.8	998.4
CP[b]	51.6	2508.4	0.0
EE[b]	7.6	–	5.0
NDF[b]	864.1	–	–
NDFom(n)[b]	828.9	–	–
NFC[b,c]	58.8	–	993.4
Lignin[b]	86.3	–	–

DM dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *NDF* neutral detergent fibre, *NDFom(n)* neutral detergent fibre corrected for ash and nitrogenous compounds, *NFC* non-fibrous carbohydrates

[a] g/kg as fed

[b] g/kg DM

[c] NFC = OM − [CP + EE + NDFom(n)]

[e] g/kg CP

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. The rumen pH values were combined in a similar way.

The rumen microorganism samples were assessed for DM, CP (AOAC 1990) and purine bases (Ushida et al. 1985) contents.

The urine samples, after thawing, were composed for each 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 urinary contents of allantoin and uric acid were estimated using colorimetric methods as reported by Chen and Gomes (1992). Total nitrogen content was estimated by the Kjeldahl method (AOAC 1990).

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

$$\mathrm{CE} = 32.27 - 0.01093 \times \mathrm{BW} \tag{2}$$

where CE is the daily creatinine excretion (mg/kg of LW) and BW is the body weight (kg).

Purine derivative excretion was calculated by adding the quantities of allantoin and uric acid excreted in the urine.

The absorbed purines were calculated from purine derivative excretion (Verbic et al. 1990):

$$\mathrm{AP} = \frac{\mathrm{PD} - 0.385 \times \mathrm{BW}^{0.75}}{0.85} \tag{3}$$

where AP is the absorbed purines (mmol/day) and PD is the purine derivative excretion (mmol/day).

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

$$\mathrm{NMIC} = \frac{70 \times AP}{0.83 \times R \times 1,000} \tag{4}$$

where NMIC is the microbial nitrogen flow in the small intestine (g/day) and R is the N_{RNA}/N_{TOTAL} ratio in the microorganisms (mg/mg).

Rumen dynamics modelling

The faecal samples for evaluating transit kinetics parameters were analysed with regard to DM (AOAC 1990) and chromium (Willians et al. 1962).

The degradation residues were analysed for NDF content using a heat-stable alpha amylase, but not sodium sulphite (Mertens 2002).

The faecal chromium contents obtained in all experimental periods for each treatment were evaluated as one dataset. The same procedure was performed for NDF degradation residues. Thus, for transit and degradation kinetics, one curve adjustment was done for each treatment.

The parameters of transit kinetics were estimated through an adjustment of a gamma-2 time-dependent model (Ellis et al. 1994):

$$C_t = Z \times (t - \tau) \times L \times \exp[-L \times (t - \tau)] \tag{5}$$

where C_t is the faecal concentration of chromium at time "t" (ppm), t is the time after marker administration (h), L is the time-dependent rate parameter related to rumen flow of fibrous particles (h^{-1}), Z is a parameter without biological meaning (ppm×h) and τ is the time of intestinal transit (h).

The graphical evaluation of non-degraded NDF residues as a function of incubation time indicated a heterogeneous degradation pattern for potentially degradable NDF (pdNDF; Fig. 1). Thus, the degradation profiles were interpreted considering two sub-compartments of pdNDF according to the model:

$$R_t = B_1 \times \exp(-\mathrm{kd}_1 \times t) + B_2 \times \exp(-\mathrm{kd}_2 \times t) + U, \tag{6}$$

where R_t is the non-degraded residue of NDF at time "t" (g/g), B_1 is the sub-compartment of pdNDF with a higher degradation rate (g/g), B_2 is the sub-compartment of pdNDF with a lower degradation rate (g/g), U is the undegradable fraction of NDF (g/g) and kd_1 and kd_2 are the fractional degradation rates (h^{-1}) of sub-compartments B_1 and B_2, respectively.

The nonlinear adjustments of models described in 5 and 6 followed the Gauss–Newton iterative algorithm which is implemented in the NLIN procedure of SAS (SAS Institute 1989).

The effective degradability of NDF was estimated by adapting the procedures of Ørskov and McDonald (1979) according to the equation:

$$\mathrm{ED} = \lim_{t\to\infty} \int_0^t \left[f(t) \times \left(-\frac{\mathrm{d}R_1 t}{\mathrm{d}t} - \frac{\mathrm{d}R_2 t}{\mathrm{d}t} \right) \right] \mathrm{d}t \tag{7}$$

where ED is the effective degradability of NDF (g/g), $f(t)$ is the mathematical function that describes particle flow in the rumen and R_1 and R_2 are the degradation residues of sub-compartments B_1 and B_2, respectively (Eq. 6).

The function $f(t)$ was obtained by the reparameterisation of 5 where a rumen resident particle profile was assumed (Ellis et al. 1994):

$$f(t) = (1 + L \times t) \times \exp(-L \times t). \tag{8}$$

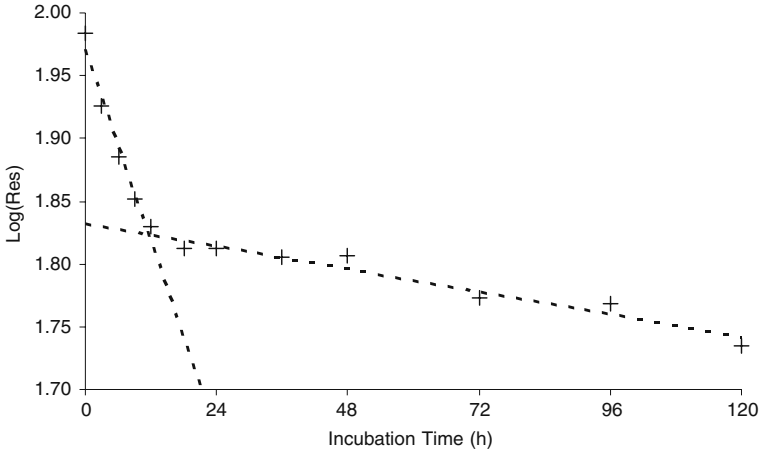

Fig. 1 Example for a pattern of decimal logarithm of non-degraded NDF residue [Log (Res)] as a function of rumen incubation time

The estimates of the NDF rumen fill effect were obtained by the adaptation of the method of Waldo et al. (1972):

$$\mathrm{RFpd} = \lim_{t\to\infty} \int_0^t \{[B_1p \times \exp(-\mathrm{kd}_1 \times t) + B_2p \times \exp(-\mathrm{kd}_2 \times t)] \times (1 + L \times t) \times \exp(-L \times t)\}\mathrm{d}t \quad (9)$$

$$\mathrm{RFu} = \lim_{t\to\infty} \int_0^t [\mathrm{Up} \times (1 + L \times t) \times \exp(-L \times t)]\mathrm{d}t \quad (10)$$

$$\mathrm{RFt} = \mathrm{RFpd} + \mathrm{RFu} \quad (11)$$

where RFt is the total rumen fill effect of NDF (h), RFpd is the rumen fill effect of potentially degradable NDF (h), RFu is the rumen fill effect of undegradable NDF (h) and B_1p, B_2p and Up are the standard fractions of NDF (g/g) obtained following the suggestions of Waldo et al. (1972).

The weighted rates of NDF degradation and rumen dynamics were estimated as:

$$\delta = (B_1p \times \mathrm{kd}_1) + (B_2p \times \mathrm{kd}_2) + (\mathrm{Ip} \times 0) \quad (12)$$

$$\Delta = [B_1p \times (\mathrm{kd}_1 + 0.59635 \times L)] + [(B_2p \times (\mathrm{kd}_2 + 0.59635 \times L)] + (\mathrm{Up} \times 0.59635 \times L) \quad (13)$$

where δ is the weighted rate of NDF degradation (h^{-1}), Δ is the weighted rate of NDF rumen dynamics (h^{-1}) and 0.59635 is the factor applied for transforming the rate parameter L into a first-order fractional rate (Ellis et al. 1994).

Statistical analysis

Intake, digestibility and nitrogen metabolism

The experiment was analysed according to a 4×4 Latin square design, with four treatments, four animals and four experimental periods. The treatment sum of squares was interpreted according to a 2×2 factorial arrangement (with or without nitrogenous compounds and with or without starch) according to the model:

$$Y_{ijkl} = \mu + N_i + S_j + NS_{ij} + A_k + P_l + \varepsilon_{ijkl} \quad (14)$$

where μ is the general constant, N_i is the effect of nitrogenous compounds supplementation, S_j is the effect of starch supplementation, NS_{ij} is the interaction effect between nitrogenous compounds and starch supplementation, A_k is the effect of animal, P_l is the effect of experimental period and ε_{ijkl} is the random error.

All statistical procedures were performed using the GLM procedure of SAS (α=0.10).

Rumen dynamics

After adjusting the models for each treatment (Eqs. 5 and 6), they were compared using the nonlinear identity test described by Regazzi (2003) under the hypothesis:

$$H_0 : \beta_C = \beta_N = \beta_S = \beta_{NS} \quad (15)$$

where β is the evaluated parameter. The subscribed codes C, N, S and NS represent the treatments (control, nitrogen

supplementation, starch supplementation and nitrogen plus starch supplementation, respectively).

The treatments were considered to be similar when the null hypothesis was not rejected. The test described above was performed on the parameters B_1, B_2, U, kd_1, kd_2, L and τ (Eqs. 5 and 6) using the NLIN procedure of SAS (α=0.10).

Results

Intake, digestibility and nitrogen metabolism

There was no interaction between starch and nitrogenous compounds with regard to intake (P<0.10, Table 2).

We observed a positive effect of nitrogenous compounds supplementation on DM, OM and NDF intake (P<0.01). Starch supplementation did not influence these variables (P>0.10), but decreased the forage intake (P<0.10, Table 2).

The digestibility coefficient of DM was affected by both supplementation types (P<0.01). On the other hand, the digestibility coefficient of NDF (P<0.05) and the diet content of TDN (P<0.01) were only influenced by nitrogenous compounds supplementation (Table 3).

Starch supplementation increased the NFC digestibility coefficient (P<0.01). In this way, the digestibility coefficients of NFC were negative without starch supplementation (Table 3). This pattern indicates that NFC intake was lower than metabolic faecal NFC. Thus, starch supplementation increased NFC intake (Table 2), implicating positive NFC digestibility coefficients (Table 3).

We observed an interaction effect (P<0.10) between nitrogenous compounds and starch supplementation on the digestibility of OM and CP (Table 3). The digestibility coefficient of OM was only increased when nitrogenous compounds were provided without starch (P<0.10). In the presence of starch supplementation, the benefit of nitrogenous compounds was less than that observed in the absence of starch. The latter conditions did not result in a significant effect on OM digestibility (Table 3).

Nitrogenous compound supplementation increased the digestibility coefficient of CP at both levels of starch supplementation (P<0.01), but the increase was more prominent without starch supplementation (Table 3).

Rumen pH was not affected by starch supplementation (P>0.10), but it was decreased by nitrogenous compound supplementation (P<0.01, Table 4). This pattern seems to be a direct effect of the nitrogen-mediated increase in rumen microbial growth and digestibility (Table 3).

We observed an interaction effect between nitrogenous compounds and starch (P<0.10) on the urinary excretion of nitrogen (UN) and on levels of serum urea nitrogen (SUN) and RAN (Table 4). The evaluation of this effect indicated that nitrogenous compound supplementation increased UN, SUN and RAN estimates at both levels of starch supplementation (P<0.05). However, the presence of starch in the diet reduced these values (P<0.05) as compared to a diet without starch.

Table 2 Least squares means for average daily intake of DM, DM from forage (DMF), CP, NFC, EE, NDF, digested DM (DDM), digested NDF (DNDF) and TDN according to the treatments

Intake	No nitrogen		Nitrogen		SEM	Effect[a]		
	No starch	Starch	No starch	Starch		*N*	*S*	*N*×*S*
kg/d								
DM	3.457	3.494	4.479	4.938	0.182	0.001	0.223	0.291
DMF	3.457	2.968	4.235	4.036	0.171	0.002	0.090	0.429
ΔDMF[b]	–	–0.93	+3.19	+0.64	–	–	–	–
OM	3.270	3.341	4.250	4.717	0.171	0.001	0.168	0.293
CP	0.182	0.156	0.841	0.774	0.039	<0.001	0.276	0.616
NFC	0.193	0.691	0.222	0.886	0.038	0.025	<0.001	0.169
EE	0.027	0.023	0.033	0.031	0.008	<0.001	0.023	0.430
NDF	2.868	2.470	3.512	3.353	0.153	0.002	0.108	0.450
ΔNDF[b]	–	–0.76	+2.64	+0.54	–	–	–	–
DDM	1.077	1.477	1.979	2.357	0.109	<0.001	0.012	0.920
DNDF	1.156	0.973	1.764	1.508	0.092	0.001	0.054	0.708
TDN	1.120	1.516	2.338	2.713	0.122	<0.001	0.020	0.935
g/kg of body weight								
DM	15.2	15.2	19.0	22.0	0.9	0.001	0.164	0.145
OM	14.4	14.5	18.1	21.0	0.9	0.001	0.128	0.143
NDF	12.7	10.7	14.9	15.0	0.7	0.003	0.216	0.198
TDN	5.0	6.6	9.9	12.0	0.6	<0.001	0.019	0.669

[a] *N*, *S*, *N*×*S*=effects of nitrogenous compounds and starch supplementation and their interaction effect, respectively

[b] Variation on intake of DM from forage and NDF according to different supplements (g/g)

Table 3 Least square means for digestibility coefficients (g/g) of DM, OM, CP, EE, NFC, NDF and potentially digestible NDF (pdNDF) and TDN levels in the diet (g/kg DM) according to the treatments

Item	No nitrogen		Nitrogen		SEM	Effect[a]		
	No starch	Starch	No starch	Starch		*N*	*S*	*N*×*S*
DM	0.320	0.430	0.441	0.483	0.018	0.003	0.005	0.103
OM	0.347	0.457	0.461	0.501	0.016	0.002	0.003	0.097
CP	0.166	0.204	0.798	0.754	0.018	<0.001	0.803	0.070
EE	0.294	0.391	0.362	0.355	0.054	0.796	0.426	0.370
NDF	0.397	0.388	0.482	0.438	0.019	0.011	0.205	0.376
NFC	−0.638	0.646	−0.755	0.597	0.110	0.480	<0.001	0.771
pdNDF	0.640	0.613	0.768	0.701	0.028	0.008	0.139	0.505
TDN	332	340	520	483	28	<0.001	0.366	0.175

[a] *N*, *S*, *N*×*S*=effects of nitrogenous compounds and starch supplementation and their interaction effect, respectively

Interaction effect was also observed ($P<0.07$) on the intestinal flow of microbial nitrogenous compounds (NMIC). The estimates of this variable were not affected by isolated supplementation with nitrogenous compounds or starch ($P>0.10$) despite the effects of nitrogen supplementation on RAN content (Table 4). NMIC improvements were only observed when nitrogenous compounds and starch were supplied together ($P<0.05$).

The N_{RNA}/N_{TOTAL} ratio in microorganisms was not affected by supplementation ($P>0.10$), averaging 0.146. However, we verified an interaction between nitrogen and starch on microorganism nitrogenous compounds content (NBAC, $P<0.06$). In this case, the NBAC estimates were only decreased by starch supplementation when nitrogen was not supplemented ($P<0.05$).

There was interaction ($P<0.05$) on efficiency of microbial synthesis (EFM) in the rumen (Table 4). In the absence of nitrogenous compounds, starch supplementation decreased EFM ($P<0.05$); in the presence of nitrogenous compounds, EFM was higher without starch supplementation ($P<0.05$).

Rumen dynamics

Supplementation did not alter estimates of the non-degradable NDF fraction ($P>0.10$). However, we observed an effect on the partition of pdNDF sub-compartments ($P<0.05$, Eq. 5). Nitrogenous compounds supplementation increased the estimate for the sub-compartment with a higher degradation rate (B_1); consequently, there was a decrease in the estimate for the sub-compartment with the lower degradation rate (B_2). Starch supplementation did not alter those estimates (Table 5).

We observed an effect of supplementation on B_2 degradation rate ($P<0.02$), but not on the degradation

Table 4 Least squares means for rumen pH, RAN concentration (mg/dL), nitrogen intake (NI, g/d), urinary nitrogen (UN, g/d), faecal nitrogen (FN, g/d), aparent nitrogen balance (NB, g/d), serum urea nitrogen (SUN, mg/dL), intestinal flow of microbial nitrogenous compounds (NMIC, g/d), N_{RNA}/N_{TOTAL} ratio in rumen microorganisms (g/g), nitrogenous compounds contents in rumen microorganisms (NBAC, g/kg DM), efficiency of microbial synthesis (EFM, grams microbial CP per kilogram NDT) according to the treatments

Item	No nitrogen		Nitrogen			Effect[a]		
	No starch	Starch	No starch	Starch	SEM	*N*	*S*	*N*×*S*
pH	6.76	6.72	6.48	6.20	0.10	0.007	0.151	0.270
RAN	2.74	2.53	9.72	6.24	0.78	0.001	0.056	0.080
NI	29.13	24.98	134.55	123.85	6.15	<0.001	0.273	0.614
UN	34.19	31.49	103.18	74.68	5.69	<0.001	0.034	0.064
FN	24.05	20.15	27.10	30.98	1.72	0.007	0.994	0.064
NB	−29.12	−26.68	4.25	18.21	6.32	0.001	0.242	0.398
SUN	6.01	4.30	16.76	11.03	0.91	<0.001	0.013	0.093
NMIC	40.74	40.61	41.14	67.01	4.27	0.063	0.070	0.068
N_{RNA}:NT	0.144	0.152	0.148	0.130	0.007	0.382	0.599	0.211
NBAC	57.5	49.5	54.2	55.8	2.0	0.470	0.168	0.057
EFM	152.8	122.3	90.1	129.2	13.1	0.026	0.625	0.013

[a] *N*, *S*, *N*×S=effects of nitrogenous compounds and starch supplementation and their interaction effect, respectively

Table 5 Estimates of standard fractions of pdNDF sub-fraction with higher degradation rate (B_1p, g/g), of pdNDF sub-fraction with lower degradation rate (B_2p, g/g), of undegradable NDF (Ip, g/g), of fractional degradation rates of B_1p (kd_1, h^{-1}) and B_2p (kd_2, h^{-1}), of time-dependent rate parameter of rumen flow of fibrous particles (L, h^{-1}) and time of intestinal transit (τ, h) according to different treatments

Item	No nitrogen		Nitrogen		*P* value
	No starch	Starch	No starch	Starch	
B_1p	0.2878	0.2704	0.4139	0.3712	0.046
B_2p	0.3331	0.3439	0.2030	0.2412	<0.001
Ip	0.3790	0.3857	0.3832	0.3876	0.987
Kd_1	0.2507	0.1795	0.2043	0.1595	0.574
Kd_2	0.0060	0.0044	0.0033	0.0032	0.013
L	0.0144	0.0170	0.0165	0.0218	0.003
τ	6.10	6.40	7.33	5.32	0.813

rate of B_1 ($P>0.10$, Table 5). Nevertheless, the simultaneous alteration of degradation rate and pdNDF sub-compartment dimensions prevents accurate interpretation of the effects of supplementation on rumen degradation dynamics.

These effects can be elucidated by evaluating the weighted rate of NDF degradation (δ, Eq. 12; Table 6) because this parameter considers both degradation rate and sub-compartment fractionation. The δ estimates were increased 14.8% and decreased by 32.5% according to individual supplementation of nitrogenous compounds and starch, respectively. In addition, simultaneous supplementation with nitrogenous compounds and starch decreased δ by 19.1% as compared to control treatment (Table 6).

Supplementation affected the passage rate of fibrous particles in the rumen ($P<0.01$, Table 5). In this way, both nitrogenous compounds and starch supplementation increased the estimates of this parameter.

Discussion

Intake, digestibility and nitrogen metabolism

In general, voluntary intake increased with nitrogenous compounds supplementation (Table 2), which supports the importance of nitrogen supplementation for animals fed low-quality tropical forage (Paulino et al. 2008; Wickersham et al. 2008).

Microbial growth, especially on fibrous carbohydrates, is dependent on nitrogen availability in the rumen (Detmann et al. 2009). Supplying additional nitrogenous compounds to animals fed low-quality forage stimulates the growth of fibrolytic bacteria (Russell 2002) and increases both the rate of passage and voluntary intake. These factors increase the energy supplied to the animal by fibrous carbohydrates in forage (Detmann et al. 2009).

Although there were no effects of starch supplementation on total DM intake ($P>0.10$), forage intake was negatively affected by starch supplementation ($P<0.10$, Table 2), representing a substitutive effect on forage intake. When ruminant animals are fed low-quality forage, the substitution of forage by grain (or NFC supplements) is typically reduced as compared to animals fed high-quality forage. However, the substitution can be sufficient to render the supplementation inefficient (Dixon and Stockdale 1999).

The substitutive effect caused by starch supplementation was close to unity (0.93 g of forage DM per gram of supplement), implying the lack of any alteration in total DM intake ($P>0.10$, Table 2).

When ruminant animals are feeding on low-quality tropical forage, the rumen fill effect is expected to be the main mechanism of intake control (Detmann et al. 2003). Supplementation with NFC can indirectly modulate the fill effect by altering the quantity of non-degraded residues in the rumen as a result of changes in fibre degradation and

Table 6 Estimates of weighted rate of NDF degradation (δ, h^{-1}), weighted rate of rumen dynamics of NDF (Δ, h^{-1}), effective degradability of NDF (ED, g/g), rumen fill effect of pdNDF (RFpd, h), rumen fill effect of undegradable NDF (RFu, h) and total rumen fill effect of NDF (RFt, h) according to the treatments

Item	No nitrogen		Nitrogen	
	No starch	Starch[a]	No starch[a]	Starch[a]
δ	0.0742	0.0501 (67.5)	0.0852 (114.8)	0.0600 (80.9)
Δ	0.0827	0.0602 (72.8)	0.0951 (115.0)	0.0730 (88.3)
ED	0.4181	0.3707 (88.7)	0.4322 (103.4)	0.3944 (94.3)
RFpd	28.79	30.14 (104.7)	20.55 (71.4)	20.16 (70.0)
RFu	51.59	44.97 (87.2)	45.95 (89.1)	35.48 (68.8)
RFt	80.38	75.11 (93.4)	66.50 (82.7)	55.64 (69.2)

[a] Values between parentheses represent the relative value (%) as basis of control treatment (no nitrogen/no starch)

passage (Dixon and Stockdale 1999). This process could reduce forage intake.

Exclusive supplementation with nitrogenous compounds exhibited an additive effect on forage intake >3 g/g (Table 2). According to the results expressed in Table 2, the absence of an interaction effect between nitrogenous compounds and starch on intake ($P>0.10$) indicates that the effects of these supplements are additive. The additive effect of nitrogen was reduced by the substitutive effect of starch, implicating a lower additive effect on forage intake when both supplements were supplied together (+0.64 g of forage DM per gram of supplement). A similar pattern was observed for NDF intake (Table 2).

The increase in NDF digestibility caused by nitrogen supplementation ($P<0.02$) implied improvement in diet TDN content (Table 3), which corroborates the positive effect of nitrogenous compounds on microbial activity in the rumen (Detmann et al. 2009; Sampaio et al. 2009; Lazzarini et al. 2009a).

This trend directly reflects the intake of fibrous components in forage which exert a substantial rumen fill effect (Table 2). Increased voluntary intake of low-quality forage is frequently associated with elevated passage and digestion rates (Paulino et al. 2008), which lead to higher removal of undigested and non-degradable fibre and reduce the NDF rumen turnover time (Allen 1996; Detmann et al. 2008). One would therefore expect to observe an increase in TDN intake (Lazzarini et al. 2009b; Sampaio et al. 2010), such as that reported here (Table 2).

Thus, the increase in DM intake with nitrogen supplementation was due to increased degradation of potentially degradable fibre (Lazzarini et al. 2009a), as demonstrated by the positive effects on pdNDF digestibility coefficient (Table 3).

The absence of any effect of starch supplementation on NDF digestibility ($P>0.10$) seems to contest some previous reports (Chase and Hibberd 1987; Simko et al. 2007) where lower fibre digestibility associated with increases in NFC supplementation was observed.

However, the correlation between NDF intake and digestibility is negative (Van Soest 1994). Therefore, the decrease in NDF intake caused by starch supplementation would result in the apparent absence of negative effects on NDF digestion (Table 3).

The measurement of ingested and effectively digested feed allows the simultaneous integration of the effects of supplementation on intake and digestibility (Sampaio et al. 2010). In this way, it was verified that starch supplementation decreased ($P<0.06$) the intake of digested NDF (Table 2), which indicates an indirect decrease in NDF digestibility.

The deleterious effect of starch on rumen degradation of NDF could be due to lower rumen pH or to the carbohydrate effect (Mould et al. 1983; Arroquy et al. 2005). Nevertheless, effects of starch on rumen pH were not observed (Table 4). Thus, the decrease in NDF digestion could only be due to the carbohydrate effect, defined by amensalistic relationships between fibrolytic and non-fibrolytic species in the rumen (El-Shazly et al. 1961; Costa et al. 2008).

The evaluation of RAN, SUN, UN and NMIC indicates that exclusive supplementation with nitrogenous compounds, although increasing the intake and NDF utilisation in the rumen, could not increase nitrogen assimilation by rumen microorganisms; this may be due to the inefficient coupling of energy from NDF. On the other hand, exclusive supplementation with starch did not allow increased microbial synthesis due to the severe deficiency in nitrogenous compounds.

Simultaneous supplementation with nitrogen and starch promotes higher nitrogen assimilation in the rumen (which is reinforced by decreases in RAN and SUN and by increased NMIC) and consequently reduces nitrogen loss in the urine (Table 4). So it brings into evidence that responses of microbial protein synthesis to protein supplementation differ according to the type of energy supply in the diet (Edwards et al. 2008).

This pattern reflects positively on apparent balance of nitrogenous compounds (NB, Table 4). Even with no interaction effect ($P>0.10$), it was verified that supplementation with nitrogen and starch elevated the NB estimate. This could indicate greater nitrogen retention in animals that received both nitrogen and starch and agrees with assumption that efficiency of protein utilization in the animal depends on energy supplementation (Schroeder and Titgemeyer 2008).

From the evaluation of these variables, it can be inferred that despite the additive effects of nitrogen and starch on intake, their effect is interactive with regard to nitrogen assimilation in the rumen.

Thus, supplementation with degradable nitrogenous compounds allows greater intake of TDN from low-quality forage (Table 2), which can result in improved animal performance. The association with highly degradable energy sources (such as starch), at levels where constraints on voluntary intake do not occur, could increase animal performance: there would be a greater quantity of metabolisable protein resulting from improved microbial assimilation of nitrogen in the rumen (Table 4).

When microbial activity is limited by low nitrogen availability and NFC are in excess, microbial protein synthesis can become deficient. In this situation, ATP can accumulate in microbial cells. This reduces the availability of ADP for phosphorylation reactions in monosaccharide oxidation (Russell 2002). This would result in the accumulation of carbohydrates in the cell (Russell et al. 2009) and

a proportional reduction in nitrogenous compound content, as observed in this work with starch supplementation (Table 4).

In the absence of nitrogenous compounds, starch supplementation decreased the EFM (Table 4). On the other hand, the average value observed for this variable in the absence of carbohydrate (152.8 g microbial CP per kilogram TDN) is higher than that reported by Valadares Filho et al. (2006) (120 g microbial CP per kilogram TDN) as a reference for tropical conditions. However, when diet is deficient in nitrogenous compounds, researchers can expect to observe a net gain of nitrogen in the rumen due to recycling (NRC 2001). This process could support the high estimate of EFM.

When there is a deficiency of nitrogenous compounds, the inclusion of highly degradable carbohydrates in the diet can increase microbial energy spilling. This behaviour is mediated by the futile cycling of protons through the cell membrane and is activated by ATP synthase. Due to high ATP hydrolysis, the microbial cells can increase the proton-motive force which decreases the membrane resistance to protons and thus increases futile cycling (Russell 2002). This would decrease the EFM, as observed with starch supplementation (Table 4).

In the presence of nitrogenous compounds, we observed lower EFM (90.1 g of microbial CP per kilogram NDT). According to NRC (2001), if the availability of nitrogen is higher than the fermentation rate of OM, the EFM will be reduced as a consequence of poor coupling during microbial synthesis. In this situation, the inclusion of starch increased the EFM (Table 4), supporting better nitrogen assimilation in the rumen. This pattern was previously indicated by the means of other variables (RAN, SUN and UN) and suggests again that nitrogenous compounds and starch exert an interactive effect on the assimilation of protein for animal metabolism.

Rumen dynamics

From a theoretical point of view, the dimensions of the NDF compartments (and possibly of its sub-compartments) are an inherent characteristic of the feed (Detmann et al. 2008). However, some alterations in compartment size have been reported for low-quality tropical forage as a response to nitrogenous compounds supplementation (Ortiz-Rubio et al. 2007; Lazzarini et al. 2009a; Sampaio et al. 2009).

The increase in the sub-compartment with a lower degradation rate (B_2) in the absence of nitrogenous compound supplementation (Table 5) demonstrates the deficiency of some microbial enzymes with regard to fibre degradation (Detmann et al. 2009). It can be presumed that the estimate obtained in the presence of nitrogen supplementation reflects the true value of this sub-compartment. Thus, under situations where there is a deficiency of nitrogenous compounds, a portion of the sub-compartment with a higher degradation rate is apparently converted to pdNDF with a lower degradation rate.

This pattern suggests that the degradation dynamics of NDF is a second-order process (Detmann et al. 2008). In other words, when nitrogenous compounds in the diet are deficient, degradation is limited by both substrate and microbial enzyme characteristics (Detmann et al. 2009).

There were simultaneous alterations in the size and degradation rate of pdNDF sub-compartments. Thus, a better understanding of the effects of supplementation on these parameters can be obtained by analysing the weighted rate of NDF degradation (δ, Table 3). We observed that exclusive supplementation with nitrogenous compounds increases the δ estimates by 14.8%, whilst the starch supplementation decreases the δ estimates by 32.5%. On the other hand, simultaneous supplementation with nitrogenous compounds and starch decreases δ by 19.1% as compared to control treatment (without supplementation).

This pattern may indicate that the effects of nitrogenous compounds and carbohydrate on NDF degradation are additive, similar to the trend observed for voluntary intake (Table 2).

The stimulatory effect of nitrogen supplementation on NDF degradation is due, as discussed previously, to improved rumen conditions with respect to the availability of nitrogenous compounds for the growth of fibrolytic microorganisms (Detmann et al. 2009).

The decreased NDF degradation observed with starch supplementation has been attributed, at least in the tropics, to the carbohydrate effect (Costa et al. 2008). The carbohydrate effect seems to involve competition for essential nutrients among microbial species, which results in higher proliferation of starch-degrading microorganisms, once these species have attained a higher growing rate as compared to fibrolytic microorganisms (El-Shazly et al. 1961; Mould et al. 1983). Such competition would lead to an initial utilisation of starch as preferential energy substrate in the rumen, with fibrous carbohydrates becoming the predominant substrate as starch availability is reduced with time (El-Shazly et al. 1961), which can also involve the inhibition of fibrolytic activity (Arroquy et al. 2005).

It must be emphasised that even though nitrogenous compounds caused an increase in δ, the stimulus was not enough to completely neutralise the carbohydrate effect in the context of starch supplementation (Table 6).

In addition, the rate of passage indicates that nitrogenous compounds and starch effects can be assumed to be additive. Despite changes in δ, we observed an increase in passage with both starch and nitrogen supplementation as compared to control treatment (Table 5). Nevertheless, those stimuli seem to have different mechanisms of action.

The increase in passage caused by nitrogen supplementation is directly associated with a higher rate of NDF degradation (Paulino et al. 2008). As pdNDF is degraded, gas production is decreased and the relative concentration of iNDF in the particle is increased. These dynamics lead to a continuous migration of the particle feed to a more ventral position in the rumen, which enlarges the probability of particle escape (Allen 1996) and increases the rate of fibrous particle turnover in the rumen.

On the other hand, cattle supplementation with concentrate can increase the liquid transit, and this can indirectly increase the solid transit (Detmann et al. 2005). In this case, the increase in fibrous particle transit with starch supplementation would not reflect better rumen dynamics, but rather an indirect alteration in fibre transit as a consequence of non-fibrous transit.

The disappearance of NDF from the rumen is a time-dependent process which integrates the velocities of pdNDF degradation and of the transit of non-degraded NDF from the rumen (Ellis et al. 1994). These velocities, as well as the low density of NDF, are the main factors affecting the voluntary intake of high-forage diets (Detmann et al. 2003). The dynamics of NDF in the rumen can be correctly analysed by the rumen fill effect (Paulino et al. 2008).

Similar to the variables previously discussed, both starch and nitrogen supplementation decreased the rumen fill effect as related to pdNDF and iNDF (Table 6). This would indicate increased fibre intake with starch supplementation, which was not observed (Table 2).

The reduced rumen fill effect observed with starch supplementation seems to result from high levels of solid transit (Table 5) rather than improved conditions for NDF degradation in the rumen, as revealed by the starch supplement-mediated decrease in δ estimates (Table 6).

Although pdNDF could be considered as an energy resource for animal production, it must be emphasised that this fraction is an asymptotic concept. In other words, the supposition could be true at infinite time. However, from a practical point of view, the events involved in rumen dynamics occur at finite time. We must therefore consider a portion of pdNDF, which is represented by effective degradability (ED) of NDF. Thus, the rational exploration of an interaction between forage and supplements should seek maximum agreement between ED and pdNDF since there are no constraints on voluntary intake (Detmann et al. 2008).

In this way, it can be observed that nitrogen supplementation was the only measure that increased the ED estimate; starch supplementation reduced the estimate of this variable (Table 6). Notably, the evaluation of the weighted rate of rumen dynamics (Δ), which integrates the dynamics of transit and degradation, suggests that starch supplementation causes deleterious effects on the utilisation of forage NDF in the rumen despite positive effects on the rate of passage and rumen fill (Table 6).

Conclusions

Supplementation of cattle with nitrogenous compounds and starch results in positive and negative effects on low-quality forage intake, respectively. These effects are additive and reflect alterations in neutral detergent fibre digestibility. However, under these circumstances, the effects of nitrogenous compounds and starch are interactive with regard to nitrogenous compound metabolism because nitrogen assimilation in the rumen is optimised when supplements are supplied together.

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