



In vitro degradation of neutral detergent fiber of high-quality tropical forage according to supplementation with different nitrogenous compounds¹

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ABSTRACT - The objective of this work was to evaluate the *in vitro* degradation dynamics of neutral detergent fiber (NDF) of high-quality tropical forage according to supplementation with different nitrogenous compounds sources. A signal grass sample harvested during rainy season was used as basal forage. Casein was used as true protein source and urea:ammonium sulfate (U:AS, 9:1) mixture (urea) as non-protein nitrogen source. The first three supplements were the addition of 0.5, 1.0 and 2.0 mg of casein per mL of final incubation solution. The urea was added to the same amount of protein supplied by equivalent levels 0.5, 1.0 and 2.0 mg/mL of casein. In total, seven treatments were evaluated, including a control (only grass). The supplements were evaluated in a simulated rumen environment by *in vitro* incubation, and submitted to different incubation times: 0, 3, 6, 9, 12, 24, 36, 48, 72, and 96 hours. The procedure was repeated four times, making a total of four evaluations per incubation time for each treatment. The incubation residues were evaluated for NDF contents and interpreted through a non-linear logistic model. Casein supplementation of 0.5 mg/mL increased the degradation rate of potentially digestible NDF (kpdNDF) by 1.1%. The addition of 1.0 and 2.0 mg/mL of casein provided an inhibitory effect on the estimates of this parameter in comparison to the control (-6.4 and -9.1% respectively). Moreover, the addition of urea, regardless the supplementation level, increased kpdNDF around 7.6%. The ammonia nitrogen (AN) concentration in the medium was increased by supplementation. However, supplementation with urea, even based on the same crude protein levels used with the supplementation with casein, has given higher ammonia nitrogen values in the incubation medium.

Key Words: ammonia nitrogen, casein, signal grass, supplement, urea

Degradação *in vitro* da fibra em detergente neutro de forragem tropical de alta qualidade em função de suplementação com diferentes fontes de compostos nitrogenados

RESUMO - Objetivou-se avaliar a dinâmica de degradação *in vitro* da fibra em detergente neutro (FDN) de forragem tropical de alta qualidade em função de suplementação com compostos nitrogenados. Como forragem basal, utilizou-se amostra de capim-braquiária colhida durante a estação chuvosa; como fonte de proteína verdadeira, caseína (0,5; 1,0 e 2,0 mg/mL de solução final de incubação); e, como fonte de nitrogênio não-protéico, mistura uréia:sulfato de amônia (U:SA; 9:1) (uréia). A uréia foi adicionada considerando a mesma quantidade de equivalentes protéicos fornecidos pelos níveis 0,5; 1,0 e 2,0 mg/mL de caseína. No total, incluindo o controle (somente forragem), obtiveram-se sete tratamentos, que foram avaliados em ambiente ruminal simulado por incubação *in vitro*, durante 0, 3, 6, 9, 12, 24, 36, 48, 72 e 96 horas. O procedimento foi repetido quatro vezes, perfazendo quatro avaliações por tempo de incubação para cada tratamento. Os resíduos de incubação foram avaliados quanto ao teor de FDN e interpretados por modelo logístico não-linear. A suplementação com caseína no nível 0,5 mg/mL aumentou em 1,1% a taxa de degradação da FDN potencialmente degradável (kFDNpd), enquanto, nos níveis 1,0 e 2,0 mg/mL, observou-se efeito inibitório sobre as estimativas deste parâmetro (-6,4 e -9,1%, respectivamente). Por outro lado, a adição isolada de uréia ao meio, independentemente do nível, ampliou em média 7,6% a kFDNpd. A concentração de nitrogênio amoniacal (NA) no meio aumentou com a suplementação, contudo, considerando-se os mesmos níveis de proteína bruta obtidos com a suplementação com caseína, a suplementação com uréia conferiu valores superiores de nitrogênio amoniacal ao meio de incubação.

Palavras-chave: capim-braquiária, caseína, nitrogênio amoniacal, suplemento, uréia

Introduction

During the rainy season, although the tropical grasses under grazing present adequate crude protein (CP) contents, the average weight gains obtained are below those observed under similar conditions in temperate regions. This discrepancy may be partly attributed to the high CP degradability of forage, which causes excessive loss of nitrogen compounds in the rumen as ammonia, causing protein deficiency in relation to the requirements for high weight gains (Poppi & McLennan, 1995)

During the rainy season, in function of the low nitrogen assimilation as microbial protein and the high digestibility of the energy components of the forage, an excess of ketogenic compounds can be found in animal metabolism (Leng, 1990; Detmann et al., 2005). Without an adequate supply of metabolizable protein, the excess of energy compounds must be eliminated, increasing the body heat production (Poppi & McLennan, 1995). This condition can, in many cases, cause reduction in food intake, a natural mechanism for adequacy of body heat release rate to a level close to comfort.

According to Detmann et al. (2005), although during the rainy season, the main nutritional deficiencies of pasture are related to protein, the improvement of the forage quality leads to change the nutritional focus of these deficiencies, going from diet during the dry season to metabolic in the rainy season. In this context, positive responses on the rumen microbial growth, and hence on the use of fibrous carbohydrates of basal grass and on animal performance would be obtained during the rainy season as a result of the supplementation with energy compounds or with degradable true protein. According to these assumptions, the supplementation with non-protein nitrogen would not lead to improvements in the use of fibrous substrates in the rumen and, in some cases, could lead to impairments on animal performance.

However, recent researches in tropical conditions bring in evidence some increments in animal production from the supplementation with non-protein nitrogen during the rainy season (Paulino et al., 2005; Porto, 2005), which contradicts the theoretical assumptions presented above.

Paez-Bernal (2007) and Costa et al. (2009) reported that the isolated supplementation with true protein cause deleterious effects on the use of neutral detergent fiber (NDF), which was possibly attributed to negative interactions between microbial fibrolytic and non-fibrolytic species.

However, further studies should be conducted to extend the understanding between supplementation with

different protein sources and the use of fibrous carbohydrates from high-quality forage, such as those observed during rainy season in the tropics.

Thus, the objective was to evaluate the *in vitro* degradation dynamics of NDF from high-quality tropical forage according to supplementation with different sources and levels of nitrogenous compounds.

Material and Methods

The experiment was carried out at the Animal Nutrition Laboratory, Department of Animal Science, Universidade Federal de Viçosa (UFV), Brazil. The forage used in the *in vitro* procedures was taken from signal grass samples (*Brachiaria decumbens* Stapf.) harvested at the beginning of the rainy season (December) through hand plucking method in a paddock of the Beef Cattle sector of UFV. The samples were oven-dried (60°C/72 hours) and processed in a Wiley mill (1 mm). Subsequently, the dry matter (DM), organic matter (OM), CP, ether extract (EE), acid detergent fiber (ADF) and 72% H₂SO₄ lignin (Silva & Queiroz, 2002), and NDF (Mertens, 2002) contents were evaluated. The NDF and ADF contents were corrected for ash and nitrogen compounds according to suggestions of Mertens (2002) and Licitra et al. (1996), respectively (Table 1).

The non-protein nitrogenous compounds contents were estimated by using the trichloroacetic acid method (Licitra et al., 1996).

The supplements were composed of sources and levels of nitrogen compounds, using casein as true protein source and the urea: ammonium sulfate (U: AS, 9:1) mixture (urea) as non-protein nitrogen.

The first three supplements were the addition of 0.5, 1.0 and 2.0 mg of casein per mL of final solution incubation. For the addition of urea, the same amount of equivalent protein supplied by levels 0.5, 1.0 and 2.0 mg/mL of casein was used. Thus, the supplements containing the urea was denominated "equivalent casein". In total, seven treatments were evaluated, including a control, without the addition of nitrogenous compounds (only forage).

The following sources were used in the composition of the supplements: casein from bovine milk (purified powder, Sigma C-5890), urea PA (Merck 108487) and ammonium sulfate PA (Merck 101217).

Aliquots of forage (350 mg DM) were packed in glass bottles with 50 mL of total volume. Seven aliquots of 400 mL of McDougall solution (McDougall, 1949) were prepared in Erlenmeyer flasks, and the pH adjusted to 6.8 by spraying with carbon dioxide. Casein or urea was added to each bottle in order to provide the levels of nitrogen compounds for

Table 1 - Chemical composition of forage and supplements

Item ¹	Forage	Casein	Urea:SA(9:1)
DM ²	213.4	901.3	982.1
OM ³	907.4	978.7	997.6
CP ³	146.2	878.9	2610.0
CP _{NPN} ⁴	746.1	-	-
EE ³	23.1	5.6	-
TC ³	738.1	94.2	-
NDF ³	586.7	-	-
omNDF(n) ³	497.5	-	-
NDIP ⁴	204.7	-	-
NFC ³	240.6	94.2	-
ADF ³	240.2	-	-
omADF(n) ³	208.1	-	-
ADIP ⁴	22.7	-	-
Lignin	19.9	-	-

¹ DM = dry matter; OM = organic matter; CP = crude protein; CP_{NPN} = CP from non-protein nitrogenous compounds; EE = ether extract; NDF = neutral detergent fiber; omNDF(n) = neutral detergent fiber corrected for ash and nitrogenous compounds. NDIP = neutral detergent insoluble protein; NFC = non-fiber carbohydrates; ADF = acid detergent fiber; omADF(n) = acid detergent fiber corrected for ash and nitrogenous compounds; ADIP = acid detergent insoluble protein.

² g/kg as fed.

³ g/kg of DM.

⁴ g/kg of CP.

each supplement, as described above. Then, the McDougall solution (28 mL) was transferred to each glass bottle containing forage, according to the treatments, which were kept in warm room (39°C) for hydration of the samples.

During the hydration process, rumen fluid from one rumen fistulated steer was collected. The fluid donor was fed *ad libitum* with elephant grass (*Pennisetum purpureum* Schum.) silage and supplemented with 1 kg/day of soybean meal, with no fasting prior to collection. The basal diet of the donor animal and the absence of fasting were aimed to obtain a rumen fluid with similar characteristics of the animals kept in tropical forage pastures during the rainy season, without supplementation. The animal had unrestricted access to water and mineral mixture (6% of phosphorus).

The fluid was collected from the fluid:solid interface of the rumen mat, filtered through a triple layer of cheese cloth, conditioned in thermal container and immediately transported to the incubation room.

It was added 7 mL of rumen fluid per glass bottle, immediately followed by saturation with carbon dioxide and sealing of the glass bottles. The final statement for the treatments was 100 mg of forage DM/10 mL of final solution and 1 mL of rumen fluid/4 mL of buffer solution (Tilley & Terry, 1963). The glass bottles were kept at 39°C, under orbital agitation (40 rpm). The extraction of gases from the bottles was performed every three hours using needles.

The evaluation times were 0, 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours of incubation. The incubation process was replicated four times, making up a total of four evaluations per time of incubation for each treatment.

At the end of each incubation period, the glass bottles were removed from the warm room, opened and submitted to pH evaluation, and the content was vacuum filtered in a gooch crucible (gross porosity). The liquid fraction was reserved in plastic containers with the addition of 1 mL of H₂SO₄ (1:1) and stored at -20°C for later analysis of ammonia nitrogen concentration (AN).

The gooch crucibles were conditioned in polyethylene bottles (120 mL) with the addition of 50 mL of neutral detergent solution (Mertens, 2002). After sealed, the polyethylene bottles were autoclaved (105°C/1 hour) in order to extract all neutral detergent soluble components (micro-extraction method; Pell & Schofield, 1993). After this procedure, the gooch crucibles were again vacuum filtrated with sequential washing of the residue with hot distilled water and acetone. The NDF residue was obtained after oven drying the material (105°C/16 hours).

NDF residues of different times for each supplement were submitted, using Gauss-Newton algorithm (Souza, 1998), to a non-linear adjustment of the logistic model described by Van Milgen et al. (1991):

$$Rt = U \times \frac{[c \times \exp(-p \times t) - p \times \exp(-c \times t)]}{(c - p)} + I \quad (1)$$

where Rt is the non-degraded NDF residue at time "t" (%), U the potentially degradable NDF fraction (pdNDF) (%), I the undegradable NDF fraction (%), c the fractional degradation rate of the potentially degradable NDF fraction (h⁻¹), p the fractional lag rate (h⁻¹); and t the incubation time (h).

The function described in (1) is symmetrical in relation to fractional rates c and p, being commonly assumed that the lowest values are associated with c (Vieira et al., 1997). However, for cases where c and p tend to the same estimate, mathematical indetermination will be observed. Thus, for cases where it was observed, the model should be re-parameterized according to the L'Hospital rule (Van Milgen et al., 1991):

$$Rt = U \times (1 + \lambda \times t) \times \exp(-\lambda \times t) + I \quad (2)$$

where: λ is the combined rate of lag and degradation (h⁻¹).

It should be emphasized that for all evaluated supplements it was necessary to re-parameterize equation (1). Thus, only equation (2) was used for describing the degradation profiles.

In this case, since parameter λ represents the combination of degradation and lag rates, the fractional degradation rate was estimated from λ using the gamma-2 distribution properties (Ellis et al., 1994):

$$c' = 0,59635\lambda \quad (3),$$

where is the first order fractional degradation rate of pdNDF (h^{-1}).

Estimates of discrete lag were obtained according to derivations of Vieira et al. (1997):

$$L = \frac{R(0) - R(t_i)}{R'(t_i)} + t_i \quad (4),$$

where L is the discrete lag (h), R (0) the non-degraded NDF residue at $t = 0$ (%), R (t_i) the non-degraded NDF residue obtained at the inflection point of the degradation profile (%), R' (t_i) the mathematical derivative of the degradation curve at the inflection point (maximum degradation rate) (h^{-1}), t_i the time equivalent to the inflection point of the degradation curve (h).

The t_i values were obtained according to Van Milgen et al. (1991):

$$t_i = \frac{1}{\lambda} \quad (5).$$

The specific rate of microbial growth on the pdNDF was estimated according to Beuvinck & Kogut (1993):

$$Sgr = \frac{R'(t_i)}{U} \quad (6),$$

where SGR is the specific rate of microbial growth (h^{-1}).

From SGR estimates, the microbial growth efficiencies on the pdNDF were obtained according to Pirt (1965):

$$\frac{1}{Y} = \frac{m}{Sgr} + \frac{1}{Ym} \quad (7),$$

where Y is the microbial efficiency (g cells g^{-1} degraded carbohydrate), m the maintenance requirement of bacteria ($\text{g carbohydrates g}^{-1} \text{ cells h}^{-1}$), and Ym the theoretical maximum microbial efficiency on the substrate (g cells g^{-1} cells degraded carbohydrate).

The estimates of $0.4 \text{ g cells g}^{-1}$ degraded carbohydrates and $0.05 \text{ g carbohydrate g}^{-1} \text{ cells h}^{-1}$ were adopted as reference for the Ym and m parameters, according to specifications of Russell et al. (1992).

It should be emphasized that the estimates for the Sgr parameter were obtained based on the average values of

fractions U (77.92 ± 1.22) and I (22.08 ± 1.69) under the assumption that they are unique and exclusive characteristics of the substrate (forage) (Ørskov, 2000).

Samples of the fluid fraction, after thawing, were centrifuged at $1500 \times g$ for 10 minutes, and the supernatant was examined for AN contents according to colorimetric method of Chaney & Marbach (1962).

The adjusted degradation profiles of the different treatments were compared in a descriptive way. In turn, pH and AN concentration obtained for different incubation times were evaluated according to a complete randomized block design, considering each incubation replications as a block in a 7×10 factorial arrangement (7 supplements and 10 incubation times). All statistical procedures, both linear and nonlinear, were performed using SAS (*Statistical Analysis System*), assuming 0.05 as critical level of probability for type I error.

Results and Discussion

It should be emphasized that the slight differences in discrete lag were considered of minor magnitude, being thus omitted from the discussion (Table 2).

It was observed that supplementation with casein at the level of 0.5 mg/mL increased in 1.1% the pdNDF degradation rate. However the supplementation with this compound at the levels of 1.0 and 2.0 mg/mL produced inhibitory effect on the estimates of this parameter in comparison to the control (forage), where reductions of 6.4 and 9.1% were observed (Table 2, Figure 1).

Moreover, the isolated addition of urea in the medium, regardless the supplementation level, increased the pdNDF degradation rate, where an average increment of 7.6% was observed (Table 2, Figure 1).

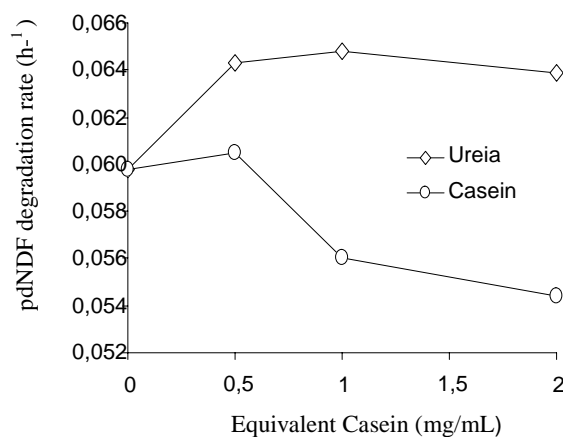


Figure 1 - Fractional pdNDF degradation rate according to supplementation with casein or urea.

Similar pattern was observed for the microbial growth efficiency on pdNDF (MEF) (Table 3). The addition of casein at levels of 0.5, 1.0 and 2.0 mg/mL implied in changes of +0.4, -2.4 and -3.4% in MEF in relation to control treatment. Moreover, positive changes in the order of 2.5, 2.8 and 2.3% were observed with the addition of urea, in the levels described above (Table 3).

The reduction in rumen degradation of fibrous carbohydrates with the addition of true protein has been reported by some authors. Cone & Van Gelder (1999) observed reduction in the *in vitro* gas production per unit of organic matter as casein was added to the medium. In similar *in vitro* study, Oliveira et al. (2005) observed reduction in the production of gas from cellulose as casein was added to the medium.

In addition, Paez-Bernal (2007) and Costa et al. (2009) observed reduction of 13.6 and 19.1% in the degradation rate of pdNDF from high-quality tropical grass forages with the addition of casein *in vitro*, respectively.

According to Paulino et al. (2006), microbial interactions, competitions or changes in priority of substrates utilization

seem to be the possible effects caused by exclusive protein supplementation on the use of pdNDF from high-quality tropical grass forages.

The negative effect of casein on the pdNDF degradation, as well as that observed with the supplementation levels of 1.0 and 2.0 mg/mL (Table 2, Figure 1), was called by Paez-Bernal (2007) and Costa et al. (2009) as "protein effect". Those authors argued that this effect could be attributed to the occurrence of amensal interactions between microbial species, that is, inhibition of growth of one or more species in relation to the production of inhibitory compounds by other species.

Examples of inhibitory compounds involved in amensal interactions are several bacteriocins, which may be defined as primary or modified products from extracellular release of ribosomal synthesis, which have narrow bactericidal activity spectrum, which, in turn, is characterized by the fact that the producer microorganism has some mechanism for self-protection (Parente & Ricciardi, 1999).

Results obtained *in vitro* indicated that the increment in the supply of amino acids could imply an increase in

Table 2 - Estimates of combined rate of lag and degradation (λ) fractional degradation rate obtained from the conversion of parameter λ (c') and discrete lag (L) for rumen degradation of potentially degradable NDF (pdNDF), relative value of degradation rate (RVDR) and asymptotic standard deviations (ASD) for of degradation profiles adjusted according to the treatments

Source	Level	Item					
		λ (h^{-1})	c' (h^{-1}) ¹	L (h)	RVDR (%) ²	ASD	n ³
Control		0.1003 ± 0.0089	0.0598 ± 0.0053	2.81	100.0	9.68	40
Casein	0.5	0.1014 ± 0.0067	0.0605 ± 0.0040	2.78	101.1	7.22	40
	1.0	0.0939 ± 0.0062	0.0560 ± 0.0037	3.00	93.6	7.36	39
	2.0	0.0912 ± 0.0056	0.0544 ± 0.0033	3.09	90.9	6.56	40
Urea	0.5	0.1079 ± 0.0073	0.0643 ± 0.0044	2.61	107.6	7.44	39
	1.0	0.1087 ± 0.0059	0.0648 ± 0.0035	2.59	108.4	5.73	38
	2.0	0.1071 ± 0.0068	0.0639 ± 0.0041	2.63	106.8	6.78	39

¹ Estimated according to the Gamma-2 distribution properties ($c' = 0.59635\lambda$).

² Estimated in relation to control.

³ The different number of replications for each supplement are due to the exclusion of outliers observed in the degradation profiles.

Table 3 - Maximum degradation rate ($\mu - h^{-1}$), specific growth rate of microorganisms (Sgr - h^{-1}) and microbial growth efficiency on the pdNDF (MEF - g DM microbial/kg of degraded carbohydrate) according to the treatments

Source	Level	Item ¹		
		μ	Sgr	MEF
Control		3.7338 (100.0)	0.0369 (100.0)	259.4 (100.0)
Casein	0.5	3.7747 (101.1)	0.0373 (101.1)	260.4 (100.4)
	1.0	3.4955 (93.6)	0.0345 (93.5)	253.3 (97.6)
	2.0	3.3950 (90.9)	0.0336 (91.1)	250.6 (96.6)
Urea	0.5	4.0167 (107.6)	0.0397 (107.6)	266.0 (102.5)
	1.0	4.0465 (108.4)	0.0400 (108.4)	266.6 (102.8)
	2.0	3.9869 (106.8)	0.0394 (106.8)	265.3 (102.3)

¹ Values in brackets show values in relation to control treatment (forage).

the production of bacteriocins as a response of favoring the growth of some microbial species (eg *Lactococcus* sp.) (De Vuyst & Vandamme, 1993; Kim et al., 1997; Parente & Ricciardi, 1999; Aasen et al., 2000), which would support the results obtained in this study.

According to Wolin et al. (1997), the action of bacteriocins may involve reduction of the cellulolytic activity in the rumen, which is reinforced with estimates of the specific growth rate and microbial growth efficiency on pdNDF (Table 3).

Deleterious effects similar to those observed with the supplementation with casein would not be achieved with supplementation with urea due to the fact that amino acids were not added to the medium. Similar behavior was reported by Paez-Bernal (2007).

Moreover, it was found that for the lowest casein supplementation level (0.5 mg/mL), low stimulus on the use of pdNDF was verified (Tables 2 and 3; Figure 1), contradicting the results for higher supplementation levels.

According to Bryant (1973), the fibrolytic processes, and the growth of bacteria that do it, should be emphasized under the point of view of the importance of the interactions with other microbial species, which provide essential compounds such as B complex vitamins and branched chain volatile fatty acids, which act as precursors of essential amino acids, structural fatty acids and some aldehydes.

In this context, the addition of small amount of true degradable protein should be enough to cause a little growth stimulus through the increase in availability of precursors for microbial synthesis, which is concurrently observed without significant expression of the protein effect.

This behavior can be reinforced by the results obtained by Paez-Bernal (2007), which observed in an *in vitro* experiment that the replacement of part of the urea by casein led to stimuli on the pdNDF degradation rate of grass similar to that evaluated in this study.

The pH values were not affected by different treatments ($P>0.05$), remaining within the limits considered appropriate for the cellulolytic microbial activity (7.21 ± 0.19) (Mold et al., 1983).

In addition, the AN concentration was affected only by the main effects (supplement and time) ($P<0.05$), and interaction effects were not observed ($P>0.05$). The AN concentration increased with the incubation time ($P<0.05$), regardless the supplement (Figure 2).

The AN concentration has been frequently used as reference for qualifying rumen conditions for microbial

activity, mainly for microorganisms that degrade fibrous carbohydrates, which use AN as main nitrogen source for growth (Russell et al., 1992). The ammonia nitrogen concentration should be in adequate conditions for the optimization of the microbial growth and subsequent use of fibrous substrates from forage.

Sampaio (2007) suggested, in tropical conditions, values of 5.32 and 6.24 mg AN/dL to sustain stable microorganism growth maintenance in the rumen environment and to provide full conditions for the use of pdNDF of the basal forage, respectively.

Even the case of *in vitro* experiment, where AN recycling is not observed *in vivo*, it was observed that the absence of supplementation did not present an average AN level adequate for the use of pdNDF of forage, as proposed by Sampaio (2007) (Figure 3). In addition, it was observed that supplementation gave more appropriate NA levels in the incubation medium (Figures 2 and 3).

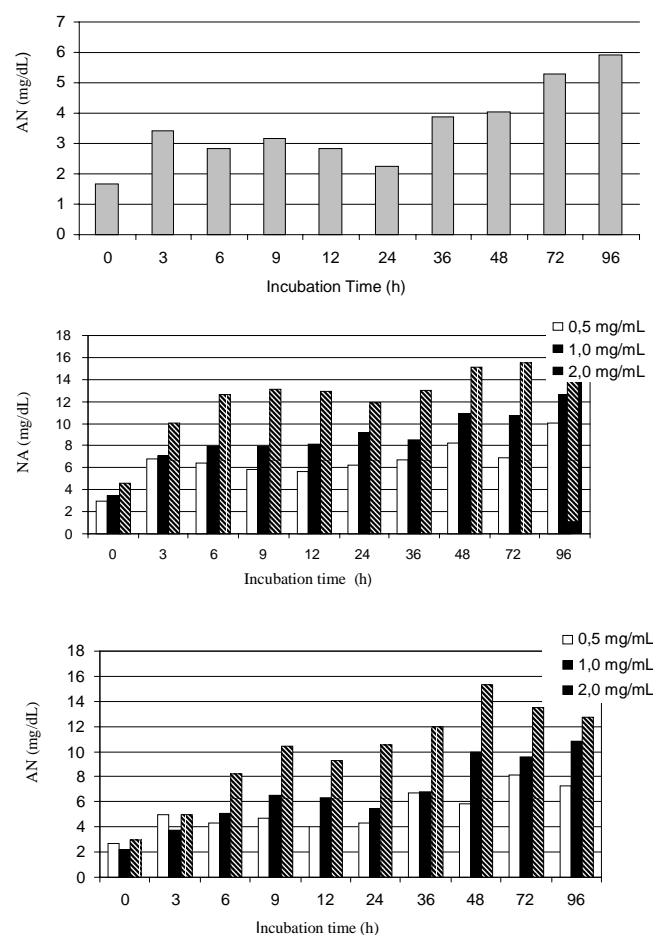


Figure 2 - Ammonia nitrogen (AN) concentration in the medium in function of the incubation time [(a): control (forage), (b): casein, and (c): equivalent casein obtained from urea].

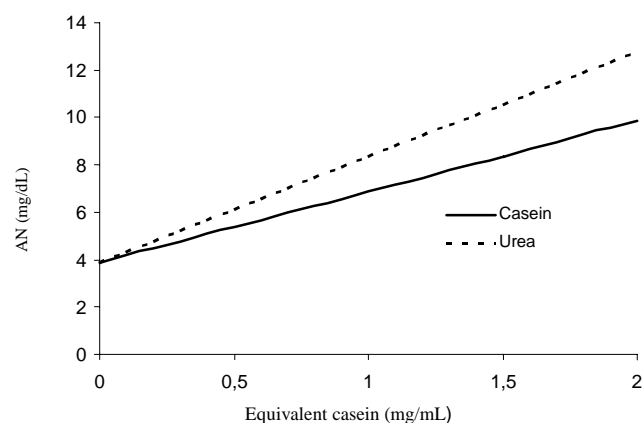


Figure 3 - Estimation of ammonia nitrogen (AN) concentration function of the supplementation level and source (mean value for all incubation time) [$\hat{Y} = 3.88014 + 2.99405 X_1 + 4.44645 X_2$, $R^2 = 0.9888$; where X_1 = casein level (mg/mL), and X_2 = level of equivalent in casein from urea (mg/mL)].

Dissimilarly, the supplementation with urea, even based on the same CP levels used with the supplementation with casein, provided higher AN values in the incubation medium (Figure 3). The higher efficiency for implementing AN concentration could contribute, in addition to the absence of the protein effect, for the best use of pdNDF observed with supplementation with urea (Tables 2 and 3).

Furthermore, it was observed that, regardless the supplementation level, the stimuli observed with the supplementation with urea tended to be stable (Figure 1). By means of the regression equation (Figure 3), it was observed that supplementation levels of 0.5 mg/mL provided approximately 6.10 AN mg/dL, value close to that suggested by Sampaio (2007) for the establishment of full condition for the use of fiber carbohydrates of basal forage, justifying the stability of the estimates of the pdNDF degradation rate in the higher supplementation levels (Figure 1).

Thus, these results indicate that implementation of appropriate AN levels in the rumen environment may be another factor of stimuli on the degradation rate of pdNDF from high-quality tropical forage with supplementation with urea.

Conclusions

The supplementation with casein caused reduction in the NDF degradation rate of high-quality tropical forage, which seems to reflect the occurrence of amensal interactions between microbial species.

Moreover, the supplementation with urea caused benefits on the use of fibrous compounds, possibly due to the absence of amensal interactions and to the higher efficiency for improving the availability of ammonia nitrogen for microbial growth.

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