Multiple supplements containing spineless cactus enriched with urea forcattle

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ABSTRACT. This study was realized to evaluate the effect of the 'multiple supplements' containing spineless cactus enriched with urea (0, 1, 2 and 3% on dry matter basis - DM) as a replacement to a traditional supplement (control) on ruminal parameters and microbial protein synthesis in crossbred steers. Five steers(½ Holstein x Zebu) can nulated in the rumen, with an average initial body weight of 240 ± 22.1 kg were used in a 5×5 Latin square. A quadratic effect was observed for DM intake and N retention, with maximum values of 6.97 kg d^{-1} and 50.9 gd^{-1} with the inclusion of 1.8 and 2.1% urea, respectively. Maximum concentrations of 16.2, 23.2 and 24.3 mg dL^{-1}of N-NH$_3$were recorded in animals fed spineless cactus enriched with 1, 2, and 3% of urea. There was a quadratic effect on ruminal pH, with a value of 6.45 at 4.08 hours after feeding. Microbial synthesis efficiency of 103 g CPkg^{-1} TDN was obtained with the inclusion of 1.6% urea. Multiple supplements containing spineless cactus enriched with 1.6 up to 1.8% urea in replacement of a traditional supplement is recommended for cattle.

Keywords: intake, rumen pH, supplementation, volatile fatty acids.

Suplementos múltiplos contendo palma forrageira enriquecida com ureia para bovinos

RESUMO. Este trabalho foi realizado para avaliar o efeito de suplementos múltiplos contendo palma forrageira enriquecida com ureia (0, 1, 2 e 3% na matéria seca - MS) em substituição a um suplemento tradicional (controle) sobre os parâmetros ruminais e síntese de proteína microbiana em novilhos mestiços. Cinco novilhos canulados no rúmen (½ Holandês x Zebu), com peso corporal inicial de $240 \pm 22,1$ kg foram utilizados em um Quadrado Latino 5×5. Efeito quadrático foi observado para o consumo de MS e retenção de N, com valores máximos de 6,97 kg d^{-1}e 50,9 g d^{-1}com a inclusão de 1,8 e 2,1% de ureia, respectivamente. Concentrações máximas de 16,2; 23,2 e 24,3 mg dL^{-1} de N-NH$_3$ foram registradas nos animais alimentados com palma forrageira enriquecida com 1, 2 de 3% de ureia. Houve efeito quadrático sobre o pH ruminal, com valor de 6,45 às 4,08 horas após alimentação. Síntese de proteína microbiana de 103 g de proteína bruta kg^{-1}de nutrientes digestíveis totais foi obtida com a inclusão de 1,6% de ureia. Recomenda-se a utilização de suplementos múltiplos contendo palma forrageira enriquecida com 1,6 a 1,8% de ureia em substituição ao suplemento tradicional para bovinos.

Palavras-chave: consumo, pH ruminal, suplementação, ácidos graxos voláteis.

Introduction

The establishment of an efficient management of rearing females is the basis of any dairy production system, with significant participation in production costs (Queiroz, Berchielli, Signoretti, Ribeiro, & Morais, 2012). Tropical pastures are the main source of nutrients for this category of the animal due to the low cost and high convenience.

However, animals reared in tropical pastures may have multiple nutrient deficiencies, especially during the dormant season of grasses, induced by water deficit recorded during the dry season.In this case, the animal supplementation consists of providing an additional source of nutrients to the system to improve nutrient intake, increase the dietary energy concentration, enhance the biochemical precursors, and thereby promote greater precocity and animal performance (Zervoudakis et al., 2008; Villela et al., 2011).

Protein, followed by energy, is the most required nutrient by ruminants. The use of forage by cattle, especially about fibrous compounds, is directly related to ruminal microbial activity, which depends

on the level of nitrogen compounds in the rumen (Costa et al., 2011). In general, 50 to 70% of microbial nitrogen may be derived from ruminal ammonia, and the remaining peptides and amino acids from the diet (Santos et al., 2010).

The spineless cactus, before considered a food alternative, has become important in dairy production systems, due to the high dry matter production per unit area. Also, it is an excellent source of energy from the non-fiber carbohydrates and total digestible nutrients (Ferreira, Bispo, Rocha, Urbano, & Costa, 2012). Thus, 'multiple supplements' are used in pasture-based systems to manage deficits in the forage, and they can be composed of a controller mixture (e.g. urea + mineral mixture) to regulate the intake of the animals (Valente et al., 2011). Considering this, we hypothesized that spineless cactus enriched with urea, in the form of a 'multiple supplements', could replace traditional supplements composed of corn, wheat bran, and soybean meal for cattle.

This study realisedto evaluate the effect of the 'multiple supplements' containing spineless cactus enriched with urea, as a replacement to a traditional supplement on the rumen fermentation and microbial protein synthesis in crossbred steers.

Material and methods

This study was carried out in the Department of Animal Science at the Federal Rural University of Pernambuco, located in Recife, Pernambuco State, Brazil.

The diets were formulated to meet dairy cattle requirements according to the National Research Council (NRC, 2001). The forage:concentrate ratio was 80:20 on a dry matter (DM) basis with Tifton-85 (*Cynodon* spp.) hay as the forage, which was used to simulate the pasture. The diets consisted of four levels of inclusion of urea/ammonium sulfate (0, 1, 2, and 3% on DM basis), and a control treatment consisting of a traditional 'multiple supplements' (composed by wheat bran, soybean meal, urea, and mineral). The urea + ammonium sulfate was used to correct the spineless cactus (SC) protein. The mixture of ingredients was performed manually in the feeders, highlighting that the SC mucilage allowed a uniform aggregation of urea.The proportions of ingredients and the chemical composition of the diets are shown in Table 1.

The management and care of animals were performedby the guidelines and recommendations of the Committee of Ethics on Animal Studies at the Federal Rural University of Pernambuco (License N°009/2015), Recife, Brazil.

Table 1. Ingredients proportion and chemical composition of experimental diets (g kg^{-1} on dry matter basis).

Item	Control	Urea levels, %			
		0	1	2	3
Ingredients, g kg^{-1}					
Tifton hay	800	800	800	800	800
Wheat bran	150	0	0	0	0
Soybean meal	30	0	0	0	0
Spineless cactus	0	190	180	170	160
Urea/AS[a]	10	0	10	20	30
Mineral mix[b]	10	10	10	10	10
Diet composition, g kg^{-1} of DM					
Dry matter, g kg^{-1}	893	355	366	378	392
Organic matter	913	903	894	885	877
Crude protein	115	60	87	115	143
apNDF	586	565	563	561	560
Non-fiber carbohydrates	212	255	249	243	237

DM, dry matter; apNDF, neutral detergent fiber corrected for ash and protein. [a]9 parts of urea and 1 part of ammonium sulphate (AS). [b]Ca (min.) – 98 g kg^{-1}, Ca (max.) – 113 g kg^{-1}, P – 45 g kg^{-1}, S – 40 g kg^{-1}, Mg – 44 g kg^{-1}, K – 61.5 g kg^{-1}, Na – 114.5 g kg^{-1}, Co – 48.5 mg kg^{-1}, Cu – 516 mg kg^{-1}, I – 30 mg kg^{-1}, Mn – 760 mg kg^{-1}, Se – 9 mg kg^{-1}, Zn - 2516 mg kg^{-1}, F – 450 mg kg^{-1}.

Five rumen fistulated steers (½ Holstein x Zebu) with an average initial body weight (BW) of 240 ± 22.1 kg weremaintained in individual stallsand usedin a 5 x 5 Latin square design. The trial lasted 80 days, with five consecutive 16-day periods, and was divided into a 7-day adaptation (Menezes et al., 2011) and 9-day sampling.

The feed was provided in two daily meals at 6:00 a.m. and 6:00 p.m. and the leftovers were weighed daily to obtain a maximum of 5 to 10% orts. The leftovers were sampled, placed in labeled bags, and stored in the freezer for later analysis. The ingredients of the diets were sampled weekly. At the end of each experimental period, the feed samples, and leftovers were thawed and subjected to pre-drying at 60°C for 72h and were then ground in a Wiley-type knife mill with a 1 mm mesh. Composite samples were saved for each animal on a dry weight basis from each period.

For three days (9th to 11[th]day) in each experimental period, after providing the morning diet, total urine collection (24 h) was carried out, and the pH was measured every six hours to ensure that it was maintained at below 3.0. Funnel collectors were attached steers to collect urine samples, which was conductedin a container containing 500 mL of 10% sulfuric acid (McSweeney & Denman, 2007). At the end of each collection period, the weight and total volume of urine were determined, and the total N content was determined using the Kjeldahl method cited by Detmann et al. (2012) (method INCT-CA N-004/1). These samples were stored at −15°C for the later analysis of urea, creatinine, all an to in, and uric acid.

Four hours after the diet was consumed on the 11th day of each experimental period, blood was collected by jugular vein puncture in a test tube containing a separation gel with a coagulant activator (SST II Advance, BD Vacutainer, Brazil). These samples were stored at −15°C until later urea analysis.

Samples of feeds, orts, and feces were analyzed for dry matter (DM; method 934.01), organic matter (OM; method 930.05), crude protein (CP; method 968.06), and ether extract (EE; method 920.39) according to Association of Official Analytical Chemists (AOAC, 2000). For the analysis of NDF was used a heat-stable alpha amylase, without using sodium sulfite, and corrected for residual ash (Mertens, 2002). The NDF was also adjusted for the nitrogenous compounds contents by using the method described by Licitra, Hernandez and Van Soest (1996).

The quantification of non-fibrous carbohydrates (NFC) contents was performed according to Detmann and Valadares (2010) as follows: $NFC = 1000 - [(CP - CPu + U) + apNDF + EE + ash]$; where CPu = CP content from urea, U = urea content, and $apNDF$ = NDF corrected for residual ash and protein. The other terms were previously defined, and all of them are expressed as g kg^{-1} DM. The total digestible nutrients (TDN) were determined according to Weiss (1999): $TDN = CP_d + NFC_d + NDF_d + EE_d \times 2.25$ (subscript means digestible).

The N balance estimate was obtained by subtracting the fecal and urinary excretion values from ingested N. To determine the efficiency of dietary N compound utilization; the following indicators were used: N-urea in plasma, urinary excretion of N-urea, and N balance. The urea-N from plasma and urine was estimated using the factor 0.466, according to Cruz et al. (2006).

Urinary endogenous losses were estimated by regressing between the urinary excretion of nitrogen (Y) and nitrogen intake (X), represented by the intercept and the regression coefficient. Total endogenous losses, including fecal and urinary, were estimated by regressing the nitrogen balance (Y) and nitrogen intake (X), expressed in g per kg$^{0.75}$, represented by the regression intercept.

Analysis of purine derivatives (PD), allantoin and uric acid were performed using a colorimetric method by Fujihara, Ǿrskov, Reeds and Kyle (1987), which was described by Chen and Gomes (1992). PD excretion was calculated by multiplying the urine volume, which was estimated at 24h, by the PD concentration of the spot urine samples. Absorbed purines (Y, mmol d^{-1}) were calculated from the PD excretion (X, mmol d^{-1}), using the equation $Y = 0.85X + 0.385 BW^{0.75}$. Where, 0.85 is the recovery of absorbed purines as PD and 0.385 $BW^{0.75}$ is the endogenous contribution to purine excretion (Verbic, Chen, MacLeod & Ǿrkov, 1990).

The production of microbial nitrogen compounds was calculated using the equation $Nmic$ (g Nd^{-1}) = (70 x absorbed PD)/(0.83 x 0.116 x 1000). Where N is the content of purines (mg Nmmol^{-1}), 0.83 represents the intestinal digestibility of purines, and 0.116 is the average N-purines:N-total ratio in the bacteria that were isolated from the rumen (Chen & Gomes, 1992).

Ruminal fluid was collected from steers on three successive days (11th to 13th day) at 6:00 a.m. and 8:00 a.m. on consecutive days, and 10:00 a.m. and 12:00 p.m. on the same day. A total of 250 mL was collected from the anterior dorsal, anterior ventral, medium ventral, posterior-dorsal, and posterior-ventral locations within the rumen using a 50-mL syringe screwed to a stainless tube ending with a probe covered with a fine metal mesh. Ruminal fluid was acidified to a pH of 2 using a 40 mL aliquot fixed with 1 mL of H_2SO_4 (1: 1) and frozen (-20°C) for further analysis of the N-NH$_3$ concentration. A second aliquot (47 mL) was fixed with 3 mL of hydrochloric acid (6 N) and frozen for later evaluation of the concentration of VFA's. Analysis of VFA was performed using a gas chromatograph equipped with a flame ionization detector and auto-injector and fitted with a GP column (30 m × 0.250 mm, 0.25 μm; Chromosorb WAW).

The variables studied were analyzed by statistical procedures using the PROC MIXED procedure of SAS (Statistical Analysis System, version 9.1.), adopting 0.10 as the critical level of probability for a type I error, according to the following model:

$$Y_{ijk} = \mu + T_i + A_j + P_k + \varepsilon_{ijk}$$

where Y_{ijk} = dependent variable measured in animal j that was subjected to the i treatment in period k; μ = general mean, Ti = fixed effect of treatment i, Aj = random effect of animal j, Pk = random effect of period k, and ε_{ijk} = random unobserved error assuming a normal distribution.

Dunnett test was used to compare each treatment group (urea levels) with the mean of the control group (characterized by a traditional 'multiple supplement'). Comparisons between urea levels in the supplements were conducted by the decomposition of sum of squares in orthogonal contrasts to linear, quadratic effects, and cubic when appropriate (p < 0.10), with subsequent adjustment

of regression equations. The contrasts were: I – All treatments versus control; II – Spineless cactus without urea versus spineless cactus with urea; III – Linear effect in urea levels; IV – Quadratic effect in urea levels. Ruminal pH, rumen ammonia nitrogen (RAN), and volatile fatty acids (VFAs) were analyzed as the effects of repeated measures over time, according to the following model:

$$Y_{ijk} = \mu + T_i + (t \star p)_{ik} + \sigma_{ij} + P_k + \varepsilon_{ijk},$$

where $Y_{ijk} =$ observation$_{ijk}$; μ overall mean; $T_i =$ effect of treatment i; $p_k =$ effect of period k; $(t \star p)_{ik} =$ effect of interaction between treatment i and period k; $\sigma_{ij} =$ random error with mean 0 and variance σ^2_σ, the variance between animals (subjects) within treatment and it is equal to the covariance between repeated measurements within animals; $\varepsilon_{ijk} =$ random error with the mean 0 and variance σ^2, the variance between measurements within animals (Kaps & Lamberson, 2004).

Results and discussion

DM intake showed a quadratic behavior (p < 0.10) with a maximum value of 6.97 kgd^{-1} estimated with spineless cactus enriched with 1.8% urea (Table 2). The increased urea levels promoted a linear increase of N intake (p < 0.001) (Table 2). There was a quadratic effect (p < 0.10) on N balance, with maximum retention of 50.9 g Nd^{-1} with the inclusion of 2.1% urea (Table 3). In diets with spineless cactus without urea and those with 1% urea, the lowest (p < 0.001) PUN content was found (Table 2).

The behavior observed for nitrogen intake in animals fed spineless cactus without urea and those fed spineless cactus enriched with 1% urea was due to both the dry matter intake of the animals and the protein content of the diet. According to Costa et al. (2011), the association between nitrogen intake and the balance of nitrogen compounds should not be interpreted directly, because the animal's body can absorb not all of the existing nitrogen in the supplement. In addition to the protein supply in the

intestine, other mechanisms are involved in the balance of nitrogen compounds, in particular, diets with protein sources degradable in the rumen promoting its increase. Excess ruminal ammonia can generate losses in both of energy and nitrogen, increasing the animal's energy cost, since the conversion of ammonia into urea costs 12 kcalg^{-1} of nitrogen to the animal (Van Soest, 1994).

According to Hoffman et al. (2001), there is a linear relationship between the amount of nitrogen intake and its excretion, even in feces and urine. The results obtained in this study are in agreement with this statement, since the treatment without urea promoted lower nitrogen excretion via urine, while the other treatments promoted the linear excretion of urinary nitrogen, according to the inclusion of urea in the diet.

The lower PUN concentrations in animals fed spineless cactus without urea and those receiving spineless cactus enriched with 1% urea can be explained because urea transferred from blood to the ruminal epithelium is rapidly degraded by ureolytic bacteria adhered to the ruminal epithelium. Thus, there is a potential difference between the rumen and the bloodstream, ensuring a favorable gradient to the transfer, often passively (Van Soest, 1994). According to Chizzotti et al. (2006), plasma urea nitrogen has a high correlation with dietary protein contents, thus justifying the linear increase in inclusion urea levels.

Fecal metabolic nitrogen (FMN) compounds corresponded to 3.56 g Nkg^{-1} DM intake and were estimated from the equation $\hat{Y} = -3.5581 + 0.9381X$ ($r^2 = 0.97$), with Y = digestible nitrogen and X = nitrogen intake. The estimation of endogenous urea nitrogen (EUN) of 0.265 g Nkg^{-1} of metabolic weight was obtained by urinary total nitrogen excretion (Y) and nitrogen intake (X) ($\hat{Y} = -0.2652 + 0.4743X$, $r^2 = 0.43$). Endogenous losses (fecal and urinary) of nitrogen (0.277 g Nkg^{-1} of metabolic weight) were obtained by regression between N balance (Y) and N intake (X) ($\hat{Y} = -0.2768 + 0.6108X$, $r^2 = 0.72$).

Table 2. Dry matter intake (DMI) and N balance (NB) in crossbred steers fed multiple supplements containing spineless cactus enriched with urea.

Item	Control	Urea levels, %				SEM	Contrasts[a] (P value)			
		0	1	2	3		I	II	III	IV
DMI, kg d^{-1}	7.18	6.01★	6.89	6.85	6.59	0.46	0.077	0.033	0.188	0.061
NI, g d^{-1}	135	57.2★	102★	129	155★	5.47	< 0.001	< 0.001	< 0.001	0.840
FN, g d^{-1}	32.4	27.1★	30.9	30.2	29.9	2.44	0.051	0.035	0.162	0.114
UN, g d^{-1}	29.1	9.68★	25.0	51.4★	77.2★	5.83	0.074	< 0.001	< 0.001	0.348
NB, g d^{-1}	73.8	20.5★	46.0★	47.4★	47.4★	7.69	< 0.001	0.004	0.014	0.069
NB, % of N intake	54.5	35.9★	45.2	36.3★	30.4★	5.16	0.004	0.781	0.211	0.106
PUN, mg dL^{-1}	20.0	8.30★	14.1★	17.6	22.4	1.52	0.005	< 0.001	< 0.001	0.668

SEM, standard error of the mean; NI, nitrogen intake; FN, fecal nitrogen; UN, urinary nitrogen; PUN, plasma urea nitrogen. [a]I – All treatments *vs.* Control; II - Spineless cactus without urea *vs.* Spineless cactus with urea; III - Linear effect in urea levels; IV - Quadratic effect in urea levels. ★Significant by Dunnett test (p < 0.10).

The fecal metabolic nitrogen (FMN) andthe endogenous urinary losses estimated in this study (3.56 g Nkg^{-1} DM intake and 0.265 g N per $kg^{0.75}$) was different than those reported by Valadares, Gonçalves, Rodrigues, Valadares and Silva (1997) in Zebu steers (5.98g N kg^{-1} DM intake and 0.220 g N per $kg^{0.75}$).Total endogenous losses of nitrogen (0.277 g N per $kg^{0.75}$) were similar to those found by Valadares et al. (1997) of 0.246 g of N per $kg^{0.75}$. According to Benedeti et al. (2014), urinary excretion of N increases linearly with the inclusion of urea in the diets. Therefore, an increase in urinary N loss with increasing levels of urea in the diets is due to rapid ruminal hydrolysis resulting in ruminal ammonia nitrogen escape from the rumen. The effects of N balance, as evidenced by the total losses, reflected positively with the inclusion of urea in the diets.

There was a quadratic effect (p < 0.001) on ruminal pH over time, with a minimum pH of 6.45 at 4.08 hours after feeding (Table 3). Also, RAN content showed a quadratic behavior (p < 0.10) over time when comparing the control treatment and urea levels (1, 2 and 3% DM). The maximum RAN content of 25.0; 11.8; 23.2 and 24.3 mg $dL^{-1}$was estimated at 2.94; 2.76; 3.20 and 3.40 hours after feeding, respectively (Table 4).

The lack of response to rumen pH in this study demonstrated an improvement in the balance of the rumen environment with the presence of spineless cactus in the 'multiple supplements'. The average rumen pH of 6.57 (Table 4) remained at levels considered adequate (6.2–7.0) by Hoover (1986), which is justified by the presence of effective fiber from hay, the mucilage from spineless cactus, and the urea inclusion in the diets.

The fiber and mucilage stimulate salivation, preventing the pH decrease, and the urea promotes alkalization in the rumen due to the ammonia-N produced. This ammonia produced was theresult of an increased non-protein nitrogen (NPN) intake, deriving from urea, which probably led to a higher content of RAN (ranging from 11.8 to 24.3 mg dL^{-1}) in the cattle fed 'multiple supplements' containing 1 to 3% urea. This RAN content corroborates with the amount (10 to 20 mg dL^{-1}) suggested by Leng (1990) for maximum microbial growth.

Maximum (p < 0.10) acetate concentration of 70.9 mmol $mL^{-1}$was estimated with spineless cactus enriched with 1.5% urea (Table 5). Propionate and butyrate over time showed maximum concentration (p < 0.001) of 17.5 and 7.8 mmol mL^{-1} at 4.5 and 4.9 hours after feeding, respectively (Table 5). Maximum values (p < 0.10) for microbial protein (463 g CPd^{-1}) and microbial efficiency (103 g $CPkg^{-1}$ TDN) were estimated with 1.9 and 1.6% urea levels, respectively (Table 5).

Table 3. Ruminal pH of cattle fed multiple supplements.

Item	Control	Urea levels, %				Effect (P value)		
		0	1	2	3	Treat.	Time	Treat. x Time
pH	6.50	6.60	6.59	6.55	6.60	0.449	< 0.001	0.854
					Time effect			
Item		Collection times, h				Contrasts (P value)		
		0	2	4	6	Linear	Quadratic	Cubic
pH		6.76	6.57	6.42	6.52	< 0.001	< 0.001	0.132

Treat – treatment.

Table 4. Rumen ammonia nitrogen (RAN, mg dL^{-1}) as a function of collection times.

Item	Control	Urea levels, %				Contrasts[a](P value)			
		0	1	2	3	I	II	III	IV
		After feeding, hours							
0	10.4	1.02★	3.74★	6.94	8.66	0.010	0.003	0.751	0.781
2	29.5	2.30★	15.8★	19.6	24.2	0.001	<0.001	0.176	0.469
4	17.6	5.24★	5.94★	23.4	21.4	0.005	0.003	0.657	0.017
6	13.3	2.32★	4.28★	10.1	16.5	0.062	0.006	0.522	0.830

[a]I – All treatments *vs.* Control; II - Spineless cactus without urea *vs.* Spineless cactus with urea; III - Linear effect in urea levels; IV - Quadratic effect in urea levels. ★Significant by Dunnett test (p <0.10).

Table 5. Volatile fatty acids (mmol mL^{-1}) in crossbred steers fed multiple supplements containing spineless cactus enriched with urea.

Item	Control	Urea levels, %				SEM	Effect (P value)		
		0	1	2	3		Treat.	Time	Treat. x Time
Acetate (A)	63.1	67.6	67.9	72.9★	65.9	3.91	0.022	0.062	0.678
Propionate (P)	15.6	15.8	16.5	16.7	15.1	1.18	0.193	0.001	0.745
Butyrate(B)	7.10	7.23	6.53	7.30	6.67	0.53	0.502	< 0.001	0.437
A:P ratio	4.05	4.43	4.23	4.58	4.50	0.20	0.323	0.003	0.976
MP, g d^{-1}	493	360★	439	463	429	26.2	0.028	0.012	0.062
MP, g kg^{-1} TDN	106	91.0	97.5	106	91.2	5.93	0.124	0.264	0.710

SEM, standard error of the mean; Treat, treatment; MP,Microbial protein; MP,Microbial protein efficiency; TDN, Total digestible nutrients.

Consequently, the highest concentration (70.9 $mmolmL^{-1}$) of acetic acid was estimated with spineless cactus enriched with 1.5% urea. Despite the higher content of soluble carbohydrates and NPN content of the diets, there was no effect on the propionate and butyrate concentration. This result isprobably explained by the soluble carbohydrates and pectin present in the spineless cactus are preferably fermented by the microorganisms, producing both acetate and ethanol (Valadares Filho & Pina, 2011).

The animals supplemented with spineless cactus without urea showed a lower microbial protein synthesis. This result was probably related to the lower DM intakeand a restriction of N supply, generating an improper fermentation profile. Possibly, the nutritional composition of the diet led to a higher energy:protein ratio, which in turn may have limited the protein degradation rate, causing a reduction in microbial protein synthesis and efficiency. On the other hand, the reduction in the microbial protein and microbial efficiency using spineless cactus plus 2% urea was related to the excess of RAN and the lack of available energy in the rumen. The most energetic ingredient of diets, spineless cactus, had its content reduced with the addition of urea.

Nocek and Russell (1988) reported that the efficiency of microbial growth depends on the energy partition in maintenance and growth and is inversely related to the residence time of the microorganisms in the ruminal environment. In this sense, the faster the passage of microorganisms through the gastrointestinal tract, the less energy used for maintenance, therefore promoting a greater efficiency of microbial synthesis. In thetropical conditions, the analyses of a Brazilian data set suggested that 120 g kg^{-1} of TDN would be more reliable (Valadares et al., 2010). In the current study, the average value for microbial protein efficiency (103 g CP kg^{-1} TDN) obtained by 'multiplesupplements' containing spineless cactus plus 1.6% urea was slightly close to this data.

Conclusion

Thus, 'multiple supplements' containing spineless cactus enriched with 1.6 up to 1.8% urea in replacement of a traditional supplement is recommended for cattle due to improving the dry matter intake and rumen fermentation, favoring the microbial protein synthesis.

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