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Effects of polymer coated slow-release urea on ruminal fermentation and nutrient total tract digestion of beef steers

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ABSTRACT - The objective of this study was to evaluate the effects of polymer coated slow-release urea (SRU) in high-forage diets of beef steers on nutrient intake and digestibility, ruminal fermentation, microbial protein synthesis, and energy balance. Eight 24-mo-old rumen-fistulated castrated Nellore steers (average body weight = 418.0±40.0 kg) were used in a replicated 4 × 4 Latin square design. Animals were randomly distributed to receive one of the following diets: no urea inclusion; 1.0% inclusion of feed grade urea in the diet (dry matter [DM] basis); 1.0% inclusion of slow-release urea 1 in the diet (DM basis); and 1.0% inclusion of slow-release urea 2 in the diet (DM basis). Slow-release urea 2 had a similar composition to that of slow-release urea 1 and differed in that it contained 2.95% sulfur. A high-forage diet was provided (75% of total DM) and corn silage was used as the forage source. Diets with urea had increased crude protein (CP) intake, and CP and total digestible nutrients total tract digestion. Urea sources increased ruminal concentrations of ammonia nitrogen and acetate, and decreased butyrate concentrations. The polymer coated urea did not alter ruminal fermentation when compared with feed grade urea. Diets did not affect the energy balance of steers. Feed grade urea presented greater microbial protein synthesis than polymer coated slow-release urea. The partial replacement of soybean meal by 1% slow-release urea in a diet with 75% forage does not improve ruminal fermentation and microbial protein synthesis, and shows similar results as feeding feed grade urea to beef steers.

Key Words: ammonia, digestibility, microbial protein, Nellore, non-protein nitrogen, soybean

Introduction

Urea is the most common source of non-protein nitrogen (NPN) and is widely used in ruminant feeding because of its lower cost compared with true protein sources (e.g., soybean and cottonseed meal), representing an important source of rumen degradable protein (RDP). Urea supplementation is a common practice to meet the nitrogen requirement of animals fed high-forage diets. Ruminal fermentation of forage is slower than fermentation of non-fibrous carbohydrates (e.g., starch and sugars), and fermentation can be even slower when low-quality roughages are provided (Bergman, 1990). In the ruminal environment, dietary urea is rapidly hydrolyzed and metabolized into ammonia and CO_2 by urease, which increases ruminal ammonia concentrations during the first hour after feeding. However, when the rate of protein degradation exceeds the rate of carbohydrate utilization, large amounts of nitrogen can be lost as urea in the urine (Nocek and Russell, 1988). Furthermore, including feed grade urea in ruminant diets has potential negative effects due to the increased level of blood ammonia, and even death by ammonia toxicity if the diet is not appropriately mixed.

The goal of proper ruminant nutrition is to maximize microbial growth, improving the supply of amino acids to the small intestine and decreasing nutrient losses. Therefore, studies have been conducted aiming to obtain a greater synchrony between forage fermentation, urea hydrolysis, and ammonia utilization by ruminal microorganisms in order to improve the efficiency of NPN incorporation into microbial protein. Taylor-Edwards et al. (2009) demonstrated that polymer coated slow-release urea (SRU) can modulate (slower release and synchronized to form ammonia) the appearance of ammonia in the rumen

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environment when compared with feed grade diet. The SRU increased dry matter (DM) and nutrient intake of crossbreed steers fed a high-forage diet (Ribeiro et al., 2011) when compared with feed grade urea. Furthermore, Tedeschi et al. (2002) observed better feed conversion when growing steers were fed controlled release urea compared with urea in high-forage diets.

Considering the aforementioned facts, our hypothesis was that Nellore steers fed SRU would improve ruminal fermentation and microbial protein synthesis due to better utilization of ammonia. Therefore, this study was carried out to evaluate the effects of replacing soybean meal with polymer coated SRU in beef cattle diets on nutrient intake and total tract digestion, ruminal fermentation, microbial protein synthesis, and energy balance in Nellore steers.

Material and Methods

This study was approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Sciences of Universidade de São Paulo, in accordance with the ethical principles of animal experimentation (protocol no. 1910/2010)

Eight 24-mo-old rumen-fistulated castrated Nellore steers (average 418.0 kg±40.0 kg) were randomly assigned to a replicated 4 × 4 Latin square design. The experimental periods consisted of 11 d of adaptation and 7 d of data collection. Steers were randomly assigned to the following diets: Control, no urea inclusion; Urea, 1.0% inclusion of feed grade urea (Reforce N®, Petrobras Distribuidora S.A., Rio de Janeiro, RJ, Brazil) in the diet (DM basis); Slow-release urea 1 (SRU1: polymer coated urea synthetic polymer®, Petrobras, Distribuidora S.A., Rio de Janeiro, RJ, Brazil), 1.0% inclusion of SRU1 in the diet (DM basis); and Slow-release urea 2 (SRU2: polymer coated urea synthetic polymer®, Petrobras, Distribuidora S.A., Rio de Janeiro, RJ, Brazil), 1.0% inclusion of SRU2 in the diet (DM basis). Slow-release urea 2 had a similar composition to that of SRU1 and differed in that it contained 2.95% sulfur. Diets were formulated based on requirements described for an average daily gain (ADG) of 0.80 kg/d according to NRC (1996) (Table 1). The roughage:concentrate ratio of diets was set at 75:25, and corn silage was the roughage source. Diets were provided *ad libitum* once daily, at 7.00 h, as a total mixed ration. Steers were housed in a sand-bedded free-stall barn, with individual pens and forced ventilation during the entire experimental period. The feed and orts supplied to each steer were weighted daily and orts were restricted to 5-10% of intake on an as-fed basis, so as not to limit dry matte intake.

Feedstuffs and orts samples from each steer were collected daily from day 8 until day 11 and combined into composite samples. Fecal samples of each steer were collected twice daily from days 8 to 11 at 8.00 h and 16.00 h directly from the rectum, and composited into a single sample of each steer per period. All samples after collection were immediately frozen at −20 °C, for further analyses.

Feedstuffs, orts, and feces were dried at 55 ºC in a forced-air oven for 72 h and then ground to pass through a 2-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA, USA). Composite samples of feed supplied, orts, and fecal samples of each animal were analyzed for DM (method 95.15; AOAC, 2000); crude protein (CP), obtained by multiplying total nitrogen, determined using the micro Kjeldahl technique (method 984.13; AOAC, 2000), by a fixed conversion factor (6.25); and ether extract (EE), determined gravimetrically after extraction using petroleum ether in a Soxhlet apparatus (method 920.39; AOAC, 2000). The neutral (NDF) and acid (ADF) detergent fiber contents were determined using the methods described by Van Soest and Mason (1991). The NDF analyses were performed using α-amylase and without sodium sulfite in a fiber analyzer (model TE-149, Tecnal Equipamentos para Laboratorio Inc., Piracicaba, SP, Brazil).

Table 1 - Ingredient and chemical composition of experimental diets

Item	Diet			
	C	U	SRU1	SRU2
Ingredient (g kg^{-1} as fed)				
Corn silage[1]	748.6	748.2	748.2	748.2
Soybean meal	120.0	75.0	75.0	75.0
Ground corn	115.6	151.0	151.0	151.0
Dicalcium phosphate	5.4	5.4	5.4	5.4
Salt	2.5	2.5	2.5	2.5
Limestone	5.4	5.4	5.4	5.4
Mineral premix[2]	2.5	2.5	2.5	2.5
Urea	-	10.0	-	-
SRU1	-	-	10.0	-
SRU2	-	-	-	10.0
Composition (g kg^{-1} as fed)				
Dry matter (g kg^{-1} of DM)	488.2	489.3	489.3	489.3
Ash	67.3	75.1	75.1	75.1
Crude protein	143.6	152.1	152.1	152.1
Ether extract	26.1	25.9	25.9	25.9
Neutral detergent fiber	421.3	419.5	419.5	419.5
Acid detergent fiber	292.9	289.7	289.7	289.7
Non-fiber carbohydrates	350.5	364.3	364.3	364.3
Net energy (Mcal/kg of DM)[3]	14.9	14.6	14.6	14.6
Total digestible nutrients[3]	673.0	667.1	667.1	667.1

C - control; U - urea; SRU1 - polymer coated slow-release urea 1; SRU2 - polymer coated slow-release urea 2.

[1] Corn silage contained: dry matter - 347.0 g kg^{-1} as fed; neutral detergent fiber - 524.7 g kg^{-1} as fed; crude protein - 97.0 g kg^{-1} as fed; indigestible neutral detergent fiber - 173.0 g kg^{-1} as fed; ash - 57.0 g kg^{-1} as fed.

[2] Contained per kilogram of product: Ca - 180 g; P - 90 g; Na - 120 g; Mg - 20 g; S - 15 g; Cu - 100 mg; Zn - 2,500 mg; Mn - 1,000 mg; I - 80 mg; Co - 100 mg; Se - 20 mg.

[3] Estimated according to NRC (2001).

The estimate of total fecal excretion for each animal was determined based on concentration of indigestible ADF (iADF) as the internal marker, according to Casali et al. (2008). Dried and ground samples were placed in bags of non-woven fabric (100 g $m^{2\ -1}$) with dimensions of 4 × 5 cm and incubated for 288 h in the rumen of two cannulated Nellore steers previously adapted to a similar diet to that of the present experiment. After removal from rumen, bags were washed in running tap water, dried at 55 ºC in a forced-air oven for 72 h, and then analyzed for ADF concentration as previously described. Digestibility was calculated using the level of iADF in feed (corrected for orts) and feces.

Rumen fluid samples (200 mL) were collected on day 11 of each period, at 0, 2, 4, 6, 8, 10, and 12 h after the morning feeding. Immediately after collection, rumen fluid pH values were determined using a pH meter (model MB-10, Marte Científica, Santa Rita do Sapucaí, MG, Brazil). Aliquots of samples were mixed with 20% metaphosphoric acid (0.25 Mol/L HPO_3) and then centrifuged at 7000 × *g*. The supernatant was stored at −20 °C in identified plastic tubes for subsequent analysis of volatile fatty acids (VFA). The remaining aliquots were mixed with sulfuric acid (0.5 mol/L H_2SO_4) and stored at −20 °C for subsequent determination of ammonia nitrogen concentration (NH_3-N) by the colorimetric phenol-hypochlorite method (Broderick and Kang, 1980).

Volatile fatty acids were measured using a gas chromatograph (model GC-2014, Shimadzu, Tokyo, Japan) equipped with a capillary column (Stabilwax®, Restek Corporation, Bellefonte, PA, USA). Gases used were helium (8.01 mL/min flow) as the carrier gas, hydrogen (pressure of 60 kPa) as the fuel gas, and synthetic air (pressure of 40 kPa) as the oxidizer gas. The steamer temperature was set at 220 °C and ionization detector flames at 250 °C. The separation column was set at 145 °C for 3 min, which was then raised by 10 °C/min up to 200 °C.

Energy values were calculated as follows: digestible energy (DE) intake = gross energy (GE) intake × GE digestibility (Havartine and Allen, 2006); net energy intake was calculated from DE using ME according to NRC (2001). Net energy for gain was calculated according to NRC (2001); and net energy available for maintenance was calculated as NE intake − NE gain.

Urine samples of 50 mL were collected from all animals on day 9 of each period, 4 h after the morning feeding by manual stimulation of the prepuce. The urine was filtered and 10 mL aliquots were immediately diluted into 40 mL of 0.036 N sulfuric acid to prevent bacterial lysis of purine derivatives and precipitation of uric acid.

Creatinine concentrations were determined with commercial kits (Laborlab®, São Paulo, SP, Brazil), through a kinetic calorimetric enzymatic reaction using an automatic biochemistry analyzer (model SBA- 200, Celm, São Caetano do Sul, SP, Brazil). Total daily urinary volume was estimated by dividing the daily creatinine urinary excretion by the creatinine concentration value in spot urine samples, as described by Chizzotti et al. (2007). The daily urinary excretion of creatinine was estimated from the proposition of 27.76 mg kg^{-1} BW for Nellore steers (Rennó, 2003). Thus, the total daily excretion of creatinine and creatinine concentration (mg/dL) in the spot urine sample and the total daily urine volume (L/d) were estimated. Urinary allantoin was determined using the modified colorimetric method of Fujihara et al. (1987), described by Chen and Gomes (1992).

Total excretion of purine derivatives (mmol/d) was calculated as the sum of allantoin and uric acid excreted in urine. The absorbed purine derivatives (PD_{abs}, mmol/d) were calculated as follows: $PD_{abs} = (PD - 0.385*BW^{0.75})/0.84$, in which $0.385*BW^{0.75}$ represents the endogenous excretion of PD (Chen and Gomes, 1992); and 0.84, the recovery of PD_{abs}. The ruminal synthesis of nitrogen compounds ($N_{mic,}$ g N/d) was calculated based on absorbed purine derivatives, using the equation described by Chen and Gomes (1992): $N_{mic} = (70*PD_{abs})/(0.83*0.134*1,000)$, considering 70 as the N purine derivative content (mg N/mol); 0.134 as the purine derivatives N/microbial N ratio (Valadares et al., 1999); and 0.83 as the intestinal digestibility of microbial purines (Chen and Gomes, 1992).

Data were analyzed to check the normality of residuals and homogeneity of variance by using the UNIVARIATE procedure of SAS (Statistical Analysis System, version 9.1.3). Afterwards, data were analyzed by using the MIXED procedure of SAS, according to the model below:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_l + c\gamma_{kl} + e_{ijk},$$

in which y_{ijkl} = observation on steer *k* given treatment *i* at period *j* in square *l*; α_i = fixed effect of treatment *i* (*i* = 1 to 4); β_j = fixed effect of period *j* (*j* = 1 to 4); γ_l = fixed effect of square *l* (*l* = 1 or 2); $c\gamma_{kl}$ = random effect of steer within square (*k* = 1 to 8); and e_{ijk} = random error associated with each observation. Ruminal fermentation data (pH, NH_3, and VFA) were analyzed as repeated measures in the MIXED procedure of SAS (Statistical Analysis System, version 9.1.3) (0, 2, 4, 6, 8, 10, or 12 h post-feeding) and each variable was evaluated according to the model below:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_l + c\gamma_{kl} + e(a)_{ijkl} + \delta_m + \alpha\delta_{im} + \beta\delta_{jm} + \gamma\delta_{lm} + c\gamma\delta_{klm} + e(b)_{ijklm}$$

in which y_{ijkl} = observation on steer *k* given treatment *i* at period *j* in square *l;* α_i = fixed effect of treatment *i* (*i* = 1 to 4);

β_j = fixed effect of period j (j = 1 to 4); γ_l = fixed effect of square l (l = 1 or 2); $c\gamma_{kl}$ = random effect of steer within square (k = 1 to 8); e_{ijk} = random error associated with each observation of main plot (a); δ_m = fixed effect of time m (m = 0, 2, 4, 6, 8, 10, or 12); $\alpha\delta_{im}$ = fixed effect of interaction between treatment i and time m; $\beta\delta_{jm}$ = fixed effect of interaction between period j and time m; $\gamma\delta_{lm}$ = fixed effect of interaction between Latin square l and time m; $c\gamma\delta_{klm}$ = random effect of interaction among steer k within each Latin square and time m; and $e(b)_{ijklm}$ = random error associated with each observation of subplot (b). To determine differences among diets, the following orthogonal contrasts were performed: (1) control vs. diets containing urea (U, SRU1 and SRU2); (2) urea vs. SRU1 and SRU2; and (3) SRU1 vs. SRU2.

Results of repeated measures analyses were subjected to three covariance structures: compound symmetric, first-order autoregressive, and unstructured. The covariance structure was chosen based on the smallest Akaike's information criterion values. Means were adjusted by LSMEANS and significance level was set at $P \leq 0.05$.

Results

Diets containing urea increased CP intake and CP and TDN total tract digestion when compared with control diet (Table 2; C1). Feed grade urea and polymer coated urea (SRU1 and SRU2) presented similar results for nutrient intake and total tract digestion. Moreover, SRU1 and SRU2 did not differ in intake and digestion of nutrients.

Urea sources increased ruminal concentration of ammonia and acetate; animals fed diets containing urea sources had a lower butyrate ruminal concentration (Table 3). No differences were observed among urea sources

Table 2 - Effects of different urea sources on nutrient intake and total tract digestion of Nellore steers

Item	Diet				SEM	P-value		
	C	U	SRU1	SRU2		C1	C2	C3
Intake (kg d^{-1})								
Dry matter	7.43	7.40	7.87	7.67	0.18	0.377	0.157	0.484
Organic matter	6.99	6.90	7.32	7.13	0.17	0.564	0.186	0.489
Crude protein	0.87	0.94	1.01	0.97	0.02	<0.001	0.072	0.253
Ether extract	0.16	0.16	0.17	0.17	0.01	0.662	0.225	0.291
Non-fiber carbohydrates	3.13	3.00	3.20	3.08	0.07	0.697	0.176	0.340
Neutral detergent fiber	2.81	2.79	2.93	2.89	0.07	0.532	0.256	0.788
Total digestible nutrients	4.93	4.84	5.14	5.01	0.12	0.678	0.203	0.508
Total tract digestion (g kg^{-1} as fed)								
Dry matter	603.3	628.4	620.8	639.9	1.01	0.191	0.927	0.434
Organic matter	622.7	648.0	637.9	656.4	0.94	0.179	0.966	0.405
Crude protein	654.7	688.3	705.6	710.9	0.87	0.004	0.212	0.769
Ether extract	836.9	847.5	842.6	851.5	0.51	0.326	0.964	0.484
Neutral detergent fiber	507.2	550.4	552.1	534.6	1.88	0.141	0.793	0.574
Non-fiber carbohydrates	705.0	715.0	682.5	742.1	1.62	0.794	0.935	0.130
Total digestible nutrients[1]	603.9	645.4	636.5	652.2	0.97	0.025	0.957	0.462

C - control; U - urea; SRU1 - polymer coated slow-release urea 1; SRU2 - polymer coated slow-release urea 2; SEM - standard error of the mean.
C1 - control vs. diets containing urea (C vs. U + SRU1 + SRU2); C2 - urea vs. SRU1 and SRU2 (U vs. SRU1 + SRU2); and C3 - SRU1 vs. SRU2.
[1] Estimated according to NRC (2001).

Table 3 - Effects of different urea sources on ruminal fermentation of Nellore steers

Item	Diet				SEM	P-value[1]					
	C	U	SRU1	SRU2		Diet	Time	Diet × Time	C1	C2	C3
pH	6.42	6.45	6.41	6.39	0.05	0.668	<0.001	0.051	0.980	0.247	0.644
NH_3-N (mg dL^{-1})	16.37	23.21	21.03	20.99	1.53	0.001	<0.001	0.155	<0.001	0.147	0.980
Total VFA (mmol L^{-1})	97.60	100.46	100.03	99.54	1.85	0.727	0.868	0.998	0.276	0.773	0.856
VFA (mmol/100 mmol)											
Acetate (C2)	72.02	72.95	72.23	72.83	0.34	0.043	0.119	0.910	0.039	0.206	0.123
Propionate (C3)	17.32	17.08	17.73	16.77	0.27	0.028	0.086	0.898	0.632	0.562	0.003
Butyrate	10.65	9.97	10.03	10.41	0.17	0.008	0.850	0.995	0.006	0.202	0.102
C2:C3[2]	4.23	4.33	4.17	4.39	0.08	0.070	0.012	0.912	0.384	0.541	0.015

C - control; U - urea; SRU1 - polymer coated slow-release urea 1; SRU2 - polymer coated slow-release urea 2; SEM - standard error of the mean.
C1 - control vs. diets containing urea (C vs. U + SRU1 + SRU2); C2 - urea vs. SRU1 and SRU2 (U vs. SRU1 + SRU2); and C3 - SRU1 vs. SRU2.
VFA - volatile fatty acids.
[1] P-value for diet, time, and their interaction (Diet × Time).
[2] Acetate:propionate ratio.

(feed grade urea vs. polymer coated slow-release urea) in ruminal fermentation. However, SRU1 provided a higher propionate concentration when compared with SRU2. No interaction effect was observed.

Experimental diets did not affect energy balance or energy efficiency utilization (Table 4). However, animals fed feed grade urea had greater microbial protein synthesis when compared with coated urea.

Discussion

Inclusion of urea in the animal diet, regardless of the source (SRU or U), resulted in higher CP intake and digestibility compared with control treatment (Table 2; C1). In this study, the higher CP intake for diets with urea was due to a higher concentration of CP in the diets of the animals when compared with control (15.21 vs. 14.36 g kg^{-1} of DM, respectively), since no difference (P<0.05) was observed for total DM intake. The higher CP digestibility is explained by the higher proportion of protein found after inclusion of urea. According to other authors (Taylor-Edwards et al., 2009; Highstreet et al., 2010), the protein fraction of the diet is more soluble and digestible. In a previous work, Lazzarini et al. (2009) stated that CP digestibility directly reflects the amounts of highly degradable nitrogen compounds in the diet. However, we did not observe statistically significant differences among the urea sources (SRU and U) for nutrient intake and digestibility (P>0.05). Previous studies (Puga et al., 2001; Galina et al., 2003; Galo et al., 2003; Xin et al, 2010) showed that SRU supplementation may improve the intakes of DM and nutrients when compared with U due to a higher activity of fibrolytic bacteria, resulting from an improved energy and N utilization by these microorganisms (Pinos-Rodríguez et al., 2010; Xin et al., 2010), with a consequent increase in the fiber fermentation (Taylor-Edwards et al., 2009; Xin et al., 2010; Holder et al., 2013). Lean et al. (2005) analyzed data from continuous culture fermenter studies and reported enhanced microbial CP synthesis and increased total tract digestion of CP and DM when a slow-release urea was used. In this work, we observed only a tendency (P = 0.072) of higher CP intake for animals fed the SRU diets. Lopez-Soto et al. (2014) demonstrated that the proportion of starch and fiber has a great influence on ruminal microbial growth and therefore on nutrient intake and digestibility for diets containing SRU and feed grade urea (FGU).

Table 4 - Effects of different urea sources on efficiency of energy utilization, energy balance, and microbial protein synthesis of Nellore steers

Item	Diet				SEM	P-value		
	C	U	SRU1	SRU2		C1	C2	C3
Energy intake (MJ d^{-1})								
GE	119.75	117.7	125.23	121.92	0.71	0.626	0.160	0.489
DE	72.13	73.89	77.4	78.12	0.51	0.173	0.244	0.852
NE_L	44.39	43.01	44.94	44.22	0.29	0.850	0.410	0.742
Production								
EBWC (kg d^{-1})	0.89	0.92	1.00	1.18	0.09	0.412	0.379	0.401
NE_G (MJ d^{-1})	19.75	18.33	20.00	19.58	0.26	0.798	0.432	0.842
Balance								
NE_L A. Maint[1] (MJ d^{-1})	24.60	24.69	24.94	24.60	0.05	0.493	0.570	0.143
Efficiency								
NEProd/DE[2]	0.26	0.24	0.25	0.24	0.01	0.578	0.792	0.879
Microbial protein synthesis (mmol d^{-1})								
Creatinine	3.63	3.08	3.98	4.06	0.19	0.835	0.182	0.055
Allantoin	70.40	72.45	67.93	70.87	4.86	0.749	0.096	0.470
Uric acid	4.00	4.53	3.93	3.43	0.24	0.958	0.187	0.386
Total excreted PD	75.92	78.55	72.33	75.82	5.01	0.758	0.093	0.509
P_{abs}	57.92	64.72	76.03	45.30	5.97	0.763	0.092	0.498
N_{mic} (g d^{-1})	36.46	40.73	34.98	28.52	2.03	0.684	0.048	0.289
UV (L d^{-1})	8.28	9.45	7.76	7.11	0.45	0.844	0.134	0.142
ALA:PD (%)	93.61	93.67	94.46	93.63	0.32	0.667	0.576	0.381
Microbial CP (g d^{-1})	227.84	254.59	218.68	178.23	12.72	0.685	0.048	0.290

C - control; U - urea; SRU1 - polymer coated slow-release urea 1; SRU2 - polymer coated slow-release urea 2; SEM - standard error of the mean.
C1 - control vs. diets containing urea (C vs. U + SRU1 + SRU2); C2 - urea vs. SRU1 and SRU2 (U vs. SRU1 + SRU2); and C3 - SRU1 vs. SRU2.
GE - gross energy; DE - digestible energy; NE_L - net energy for lactation; EBWC - empty body weight change; NE_G - net energy for gain; PD - purine derivatives; P_{abs}, - absorbed microbial purines; N_{mic} - microbial nitrogen; UV - urinary volume; ALA - allantoin.
[1] NE_L available for maintenance = $NE_L - NE_G$
[2] NE_G BW gain/digestible energy intake.

The concentration of ruminal NH_3-N increased for animals fed diets containing urea, but no interaction between time and diet was found. The NH_3-N production rate is related to the solubility and NPN content of the degraded CP (NRC, 2001). Because the experimental treatments had a higher concentration of urea, which is totally degraded in rumen, and no differences were found in DMI, the higher level of ammonia in the rumen was expected. Similarly, Shain et al. (1998) and Milton et al. (1997) observed an increasing NH_3-N ruminal concentration according to dietary urea inclusion.

Slow-release urea is hydrolyzed more slowly to ammonia than conventional urea, and could potentially be used more efficiently by rumen microorganisms and consequently decrease concentrations of ruminal NH_3-N (Galo et al., 2003; Taylor-Edwards et al., 2009; Xin et al., 2010; Bourg et al., 2012). However, in the present study, NH_3-N concentrations did not differ (P>0.05) between diets containing U and SRU. According to Owens and Zinn (1988), energy is a limiting factor in the microbial protein synthesis. López-Soto et al. (2014) demonstrated similar results when using different ratios of starch to ADF in diets with SRU and feed grade urea. In this study, the energy may have been a limiting factor to the growth of ruminal microorganisms for animals fed diets containing urea. Xin et al. (2010) noted a 15.6% greater microbial efficiency in SRU diet than diets with FGU and lower concentration of NH_3-N. In a subsequent study with a roughage:concentrate ratio of 50:50, we observed that feeding SRU diets resulted in lower NH_3-N concentrations when compared with U diets (data not published).

Animals fed diets containing urea had higher concentrations of acetate and lower butyrate concentration in the rumen. These results might be explained by a possible selective effect of urea sources on ruminal microorganisms. Some ruminal microorganisms, especially the fibrolytic bacteria, have a greater affinity for NPN. Therefore, supplementation with NPN sources may select these bacteria, and change the pattern of fermentation. Moreover, the NDF total tract digestion was approximately 38.5 g kg^{-1} higher for animals fed urea than for animals fed control diet, leading to high acetate concentrations. Xin et al. (2010) found similar results and suggested that higher acetate and lower butyrate concentrations in diets containing urea (FGU and SRU) resulted in lower conversion of acetate to butyrate in the rumen (Sharp et al., 1982; Sutton et al., 2003). Khattab et al. (2013) observed higher acetate concentration when feeding urea, and an increase in microbial protein synthesis.

Similar to other studies (Taylor-Edwards et al., 2009; Xin et al., 2010; Ding et al., 2014), when comparing SRU with FGU diets, we did not observe differences in concentration and molar proportions of VFA. According to Taylor-Edwards et al. (2009), replacing urea with SRU rarely affects any ruminal metabolite concentrations other than ammonia, at least in situations in which reduced ammonia concentrations presumably do not limit microbial growth. Our findings suggest that the urea source does not affect total production or ruminal concentrations of VFA. However, when comparing the SRU diets, feeding SRU1 resulted in a higher proportion of propionate and lower C2:C3, which may be due to the presence of sulfur (2.95%) in its composition. More detailed studies are necessary to elucidate this relationship. According to NRC (1996), sulfur supplementation is necessary when NPN is included in the diet due to the microbial synthesis of sulfur amino acids.

When analyzing the synthesis of microbial protein, we observed that animals fed SRU diets showed lower values of microbial CP and N_{mic} (g d^{-1}; Table 4; C2) than steers fed diets with FGU. These findings are opposed to what was expected. According to Russell et al. (2009), cellulolytic ruminal bacteria are unable to grow on other N sources in the absence of NH_3 and the stimulation of cellulolytic species by precursors of various N sources suggests a quantitative dependence on NH_3-N-release rate for optimum growth (Cherdthongand Wanapat, 2010). Thus, the use of SRU should result in a better synchrony between the urea hydrolysis and ammonia utilization by ruminal bacteria (Holder el al., 2013), which would be demonstrated by higher N_{mic} and microbial CP values for diets with SRU. Mehrez et al. (1977) stated that the ammonia concentration in the rumen needs to be 23.5 mg dL^{-1} for maximal fermentation rate. In this trial, the highest concentration of NH_3-N (23.21 mg dL^{-1}) was associated with the U group, which is close to values mentioned by Mehrez et al. (1977). However, in a later study of our group (data not published) conducted to evaluate the inclusion of 2% urea in diets with a roughage:concentrate ratio of 50:50, animals fed the U diet had higher concentrations of ruminal NH_3-N (24.0 mg dL^{-1}) compared with the SRU diets (SRU1: 20.7 mg dL^{-1}; SRU2: 16.4 mg dL^{-1}). On the other hand, SRU diets showed numerically higher values of microbial CP and N_{mic}. Thus, microbial protein synthesis in the rumen may have been limited by the low availability of energy (López-Soto et al., 2014) and not because part of the NPN could leave the rumen without being converted to NH_3 by reducing its incorporation into microbial protein as other authors have suggested (Galo et al., 2003; Firkins et al., 2007).

Conclusions

The partial replacement of soybean meal by 1% slow-release urea in a diet with 75% forage does not improve ruminal fermentation or microbial protein synthesis, and shows similar results as feeding feed grade urea to beef steer but without the potential hazards associated with feed grade urea.

References

AOAC - Association of Official Analytical Chemistry. 2000. Official methods of analysis. 17th ed. AOAC International, Arlington, VA.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiology Review 10:567-589.

Bourg, B. M.; Tedeschi, L. O.; Wickersham, T. A. and Tricarico, J. M. 2012. Effects of a slow-release urea product on performance, carcass characteristics, and nitrogen balance of steers fed steam-flaked corn. Journal Animal Science 90:3914-3923.

Broderick, G. A. and Kang, J. H. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. Journal of Dairy Science 63:64-75.

Casali, A. O.; Detmann, E.; Valadares Filho, S. C.; Pereira, J. C.; Henriques, L. T.; Freitas, S. G. and Paulino, M. F. 2008. Influence of incubation time and particles size on indigestible compounds contents in cattle feeds and feces obtained by in situ procedures Revista Brasileira de Zootecnia 37:335-342.

Chen, X. B. and Gomes, M. J. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an overview of technical details. International Feed Research Unit; Rowett Research Institute, Bucksburnd, Aberdeen. 21p. (Occasional Publication).

Cherdthong, A. and Wanapat, M. 2010. Development of urea products as rumen slow release feed for ruminant production: A review. Australian Journal of Basic and Applied Sciences 4:2232-2241.

Chizzotti, M. L.; Valadares Filho, S. C.; Valadares, R. F. D.; Chizzotti, F. H. M.; Marcondes, M. I. and Fonseca M. A. 2007. Consumo, digestibilidade e excreção de uréia e derivados de purinas em vacas de diferentes níveis de produção de leite. Revista Brasileira de Zootecnia, 36:138-146.

Ding, L. M.; Lascano, G. J. and Heinrichs, A. J. 2014. Effect of precision feeding high- and low-quality forage with different rumen protein degradability levels on nutrient utilization by dairy heifers. Journal Animal Science 93:3066-3075.

Firkins, J. L.; Yu, Z. and Morrison, M. 2007. Ruminal nitrogen metabolism: Perspectives for integration of microbiology and nutrition for dairy. Journal of Dairy Science 90(Suppl. E):E1-E16.

Fujihara, T.; Orskov, E. R.; Reeds, P. J. and Kyle, D. J. 1987. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. Journal of Dairy Science 109:7-12.

Galina, M. A.; Perez-Gil, F.; Ortiz, R. M. A.; Hummel, J. D. and Ørskov, R. E. 2003. Effect of slow release urea supplementation on fattening of steers fed sugar cane tops (*Saccharum officinarum*) and maize (*Zea mays*): ruminal fermentation, feed intake and digestibility. Livestock Producion Science 83:1-11.

Galo, E.; Emanuele, S. M.; Sniffen, C. J.; White, J. H. and Knapp, J. R. 2003. Effects of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. Journal of Dairy Science 86:2154-2162.

Harvatine, K. J. and Allen, M. S. 2006. Effects of fatty acid supplements on milk yield and energy balance of lactating dairy cows. Journal of Dairy Science 89:1081-1091.

Highstreet A.; Robinson, P. H.; Robison, J. and Garrett, J. G. 2010. Response of Holstein cows to replacing urea with with a slowly rumen released urea in a diet high in soluble crude protein. Livestock Science 129:179-185.

Holder, V. B.; El-Kadi, S. W.; Tricarico, J. M.; Vanzant, E. S.; McLeod, K. R. and Harmon, D. L. 2013. The effects of crude protein concentration and slow release urea on nitrogen metabolism in Holstein steers. Archives of Animal Nutrition 67:93-103.

Khattab, I. M.; Salem, A. Z. M.; Abdel-Wahed, A. M. and Kewan, K. Z. 2013. Effects of urea supplementation on nutrient digestibility, nitrogen utilisation and rumen fermentation in sheep fed diets containing dates. Livestock Science 155:223-229.

Lazzarini, I.; Detmann, E.; Sampaio, C. B.; Paulino, M. F.; Valadares Filho, S. C.; Souza, M. A. and Oliveira, F. A. 2009. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. Revista Brasileira de Zootecnia 38:2021-2030.

Lean, I. J.; Miller-Webster, T. K.; Hoover, W.; Chalupa, W.; Sniffen, C. J.; Evans, E.; Block, E. and Rabiee, A. R. 2005. Effects of BioCholor and fermenten on microbial protein synthesis is continuous culture fermenters. Journal of Dairy Science 88:2524-2536.

López-Soto, M. A.; Rivera-Méndez, C. R.; Aguilar-Hernández, J. A.; Barreras, A.; Calderón-Cortés, J. F.; Plascencia, A.; Dávila-Ramos, H.; Estrada-Angulo, A. and Valdes-García, Y. S. 2014. Effects of combining feed grade urea and a slow-release urea product on characteristics of digestion, microbial protein synthesis and digestible energy in steers fed diets with different starch:ADF ratios. Asian-Australasian Journal Animal Science 27:187-193.

Mehrez, A. Z.; Orskov, E. R. and McDonald, I. 1977. Rates of rumen fermentation in relation to ammonia concentration. British Journal of Nutrition 38:437-443.

Milton, C. T.; Brandt, R. T. Jr. and Titgemeyer, E. C. 1997. Urea in dry-rolled corn diets: finish steer performance, nutrient digestion, and microbial protein production. Journal of Animal Science 75:1415-1424.

Nocek, J. E. and Russel, J. B. 1988. Protein and energy as an integrated system. Relationship of ruminal availability to microbial contribution and milk production. Journal of Dairy Science 71:2070-2107.

NRC - National Research Council. Nutrient requirements of beef cattle. 1996. 7th ed. National Academic Press, Washington, D.C.

NRC - National Research Council. Nutrient requirements of dairy cattle. 2001. 7th ed. National Academic Press, Washington, D.C.

Owens, F. N. and Zinn, R. 1988. Protein metabolism of ruminant animal. p.227-249. In: The ruminant animal: digestive physiology and nutrition. Church, D. C., ed. Simon & Schuster, Englewood Cliffs.

Pinos-Rodríguez, J. M.; Peña, L. Y.; González-Muñoz, S. S.; Bárcena, R. and Salem, A. 2010. Effects of a slow-release coated urea product on growth performance and ruminal fermentation in beef steers. Italian Journal Animal Science 9:16-19.

Puga, D. C.; Galina, H. M.; Perez-Gil, R. F.; Sangines, G. L.; Aguilera, B. A. and Haenlein, G. F. W. 2001. Effect of a controlled-release urea supplement on rumen fermentation in sheep fed a diet of sugar cane tops (*Saccharum officinarum*), corn stubble (*Zea mays*) and King grass (*Pennisetum purpureum*). Small Ruminant Research 39:269-276.

Rennó, L. N. 2003. Consumo, digestibilidade total e parcial, produção microbiana, parâmetros ruminais e excreções de uréia e creatinina em novilhos alimentados com dietas contendo quatro níveis de uréia ou dois de proteína. Thesis (D.Sc.). Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Ribeiro, S. S.; Vasconcelos, J. T.; Morais, M. G.; Itavo, C. B. C. F. and Franco, G. L. 2011. Effects of ruminal infusion of a slow-release polymer-coated urea or conventional urea on apparent nutrient digestibility, in situ degradability, and rumen parameters in cattle fed low-quality hay. Animal Feed Science and Technology 164:53-61.

Russell, J. B., Muck, R. E. and Weimer, P. J. 2009. Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. FEMS Microbiology Ecology 67:183-197.

Shain, D. H.; Stock, R. A.; Klopfenstein, T. J. and Herold, D. W. 1998. Effect of degradable intake protein level on finishing cattle performance and ruminal metabolism. Journal of Animal Science 76:242-248.

Sharp, W. M.; Johnson, R. R. and Owens, F. N. 1982. Ruminal VFA production with steers fed whole or ground corn grain. Journal Animal Science 55:1505-1514.

Sutton, J. D.; Dhanoa, M. S.; Morant, S. V.; France, J.; Napper, D. J. and Schuller, E. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. Journal Dairy Science 86:3620-3633.

Taylor-Edwards, C. C.; Elam, N. A.; Kitts, S. E.; McLeod, K. R.; Axe, D. E.; Vanzant, E. S.; Kristensen, N. B. and Harmon, D. L. 2009. Influence of slow-release urea on nitrogen balance and portal-drained visceral nutrient flux in beef steers. Journal of Animal Science 87:209-221.

Tedeschi, L. O.; Baker, M. J.; Ketchen, D. J. and Fox. D. G. 2002. Performance of growing and finishing cattle supplemented with a slow-release urea product and urea. Canadian Journal of Animal Science 82:567-573.

Valadares, R. F. D.; Broderick, G. A. and Valadares Filho, S. C. 1999. Effect of replacing alfafa with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. Journal of Dairy Science 82:2686-2696.

Van Soest, P. J. and Mason, V. C. 1991. The influence of Maillard reaction upon the nutritive value of fibrous feeds. Animal Feed Science and Technology 32:45-53.

Xin, H. S.; Schaefer, D. M.; Liu, Q. P.; Axe, D. E. and Meng, Q. X. 2010. Effects of polyurethane coated urea supplement on *in vitro* ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. Journal of Animal Science 23:491-500.