

Ruminal fermentation and digestion of cattle diets with total and partial replacement of soybean meal by a slow-release urea product

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Abstract: One *in vitro* assay and one *in vivo* trial with ruminally cannulated Holstein steers were conducted to evaluate the effects of a dietary substitution of soybean meal by a urea and slow-release urea source of fermentation and degradation of diets for cattle. The experimental diets consisted of the total mixed rations defined as the control with soybean meal (SBM), U (urea), SRU (slow-release urea), and SRU+U+AA (0.42% + 0.42% + 1% amino acids methionine and lysine). The dietary substitution of SBM by U or SRU reduced ($P < 0.05$) the total gas production (V), microbial mass and degradation at 72 h incubation under the *in vitro* conditions, as well as the degradation rate (c) and the total volatile fatty acids (VFA) in the rumen of the steers; however, when the dietary substitution of SBM was by U+SRU+AA, those values did not decrease. In the steers, the dietary substitution of SBM by U and SRU reduced the ruminal degradation rate and the total VFA, and increased the ammonia N, but when SBM was substituted by U+SRU+AA in the diets, these changes were not observed. No advantage of SRU over U was found. The dietary substitution of SBM by U, SRU, U+SRU+AA did not modify the molar proportion of the VFA in the rumen nor were there changes in the nutrient digestion or excretion. Both the *in vitro* assay and the *in vivo* trial indicated that replacing SBM with U or SRU increases the ruminal ammonia N concentrations and reduces the degradation rate in the rumen, although those undesirable findings were not found when the SBM was replaced by U+SRU+AA. Therefore, it is feasible to replace the SBM with a combination of urea, slow-release urea, lysine and methionine in the diet for the ruminants.

Keywords: degradation; duodenal flow; nitrogen; nutrient excretion

Current concerns about the impact of cattle farming systems on the environment have stimulated interest in increasing the nitrogen (N) efficiency use in cattle farming for milk and meat production and reducing N excretion in manure (Foskolos and Moorby 2018). The optimal efficiency and desired animal productivity with a minimum amount of dietary crude protein (CP) can be achieved if

adequate amounts of a rumen-degradable protein (RDP) are provided. Supporting maximal growth of ruminal microorganisms and providing the necessary profile and amounts of amino acids (AA) require complementary protein and non-protein N (NPN) feed supplements (Hackmann and Firkins 2015). It is well documented that microorganisms that ferment structural carbohydrates only

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require ammonia as their N source, while species that degrade non-structural carbohydrate sources will benefit from the preformed AA (Russell et al. 1992). Therefore, the synchrony of ruminal protein and carbohydrate digestion is critical for reducing the feed costs, increasing the feed efficiency and easing concerns over the nutrient disposal. Feed-grade urea (U) has proved to be an effective replacement for plant proteins in the rations for the growing and fattening beef cattle and for dairy cattle in several countries. Because urea is reduced to ammonia in the rumen at a rate faster than the rumen microflora can utilise it, an undesired synchrony of the ruminal protein and carbohydrate digestion may occur.

Slow-release urea (SRU) products to reduce the rate of ammonia release from U are currently available. Substantial research has been developed to evaluate the effects of these SRU products (Chegeni et al. 2013; Benedeti et al. 2014; Giallongo et al. 2015; Gardinal et al. 2017; Corte et al. 2018) in cattle. Previous studies found that although SRU released more N than soybean meal (SBM), which induced a higher ammonia N concentration in the rumen, SRU can replace SBM in diets without affecting the growth performance of beef steers or milk production of dairy cattle (Pinos-Rodriguez et al. 2010a; Pinos-Rodriguez et al. 2010b).

In most, but not all, of these experiments, SRU was added to the diets that were similar to the control diets in the type and quantity of ingredients, or SRU was added to the diets to partially or totally replace U. Research has shown that a dietary supply of readily fermentable carbohydrates improves the capture of ammonia in the rumen, thereby increasing the microbial protein synthesis (Seo et al. 2013). Because U and SRU release ammonia at different rates (Pinos-Rodriguez et al. 2010a; Pinos-Rodriguez et al. 2010b), these NPN sources can be combined, even more so, if additional non-structural carbohydrates and AA are included in the diets. It was hypothesised that it is feasible to replace SBM by conventional U in combination with an SRU product and amino acids in the diet of dairy cattle without affecting the rumen fermentation, digestion, nutrient flow or excretion. Thus, the objective of this study was to evaluate the effects of the total and partial replacements of SBM by U, SRU and a combination of U+SRU+AA on the fermentation and degradation of the diets for cattle and the nutrient excretion under the *in vitro* and *in vivo* conditions.

MATERIAL AND METHODS

Experiment protocols, under the supervision and approval (NOM-062-ZOO-1999) of an Academic Committee, were conducted in compliance with the Animal Protection Law enacted by Mexico.

Experimental diets. Four diets for lactating dairy cows (685 kg BW (body weight), daily milk production 50 kg) were formulated so that the added urea and SRU replaced the SBM using the CNCPS V6.1.12 (The Cornell Net Carbohydrate and Protein System, Cornell University, Ithaca, NY, USA). The diets contained 52.5% forage (DM (dry matter) basis) (Table 1) and were defined as follows (on the DM basis): control (6.9% SBM, 22.2% maize grain); U (4.3% SBM, 0.42% U, 24.3% maize grain); SRU (4.3% SBM, 0.42% SRU, 24.3% maize grain); and SRU+U+AA (0% SBM, 0.42% U, 0.42% SRU, 27.2% maize grain, 0.1% methionine, 0.9% lysine). The feed-grade urea was used and referred to as U. The SRU product is based on a matrix of urea pills and lipids (Optigen® Alltech Inc., Kentucky, USA). The methionine and lysine added to the diets were DL-Met (Mepron® M85, Degussa Corporation Germany) and L-lysine (AminoShure®-L, Balchem Corporation, New Hampton NY), respectively. The criteria for the supplemental rumen-protected methionine and lysine together when the SBM was totally replaced by NPN was based on the findings of Bas et al. (1990), who indicated that when the dietary protein is replaced by NPN, the amount of the true dietary protein escaping the ruminal degradation is reduced. Moreover, the findings of Trinacty et al. (2009) evidenced that methionine and lysine are the most limiting and co-limiting AA in cattle.

***In vitro* assay.** A manual system was used to measure the *in vitro* incubation gas production at 39 °C, following Theodorou et al. (1994). The rumen fluid was collected 3 h after the morning feeding through the cannula from the cranial dorsal rumen of two Holstein cows (650 kg) fitted with a rumen cannula and adapted for 20 d to feed diets of 50 : 50 forage to concentrate. The incubation was conducted in glass flasks (125 ml) with 90 ml of a medium (a trypticase peptone/micro and macro mineral/buffer/resazurin solution described by Longland et al. (1995)), 10 ml of a ruminal inoculum and 500 mg of DM of the experimental diets. The samples for each treatment and time were incubated in triplicate. The gas pressure was obtained by manometric readings (0 to 1 kg/cm²),

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Table 1. The ingredients and chemical composition of the diets

Ingredients (% DM basis)	Experimental diets			
	SBM	U	SRU	U+SRU+AA
Corn silage	29.5	29.5	29.5	29.5
Alfalfa hay	21.0	21.0	21.0	21.0
Oat straw	1.8	1.8	1.8	1.8
Corn grain, flaked	22.2	24.3	24.2	27.1
Cottonseed meal	10.8	10.8	10.8	10.8
Soybean meal 44% CP solvent extract	6.9	4.3	4.3	–
Corn gluten meal	2.3	2.3	2.3	2.3
Cane molasses	2.1	2.1	2.1	2.1
Fish meal	2.0	2.0	2.0	2.0
Megalac ¹	0.9	1.0	1.0	1.0
Calcium carbonate	0.25	0.25	0.25	0.24
Mineral premix ²	0.18	0.18	0.18	0.18
Salt	0.03	0.03	0.03	0.03
Urea 281% CP	–	0.42	–	0.42
Slow-release urea ³ 274% CP	–	–	0.42	0.42
DL- methionine ⁴	–	–	–	0.1
L-lysine HCL ⁵	–	–	–	0.9
Chemical composition (% DM basis)	–	–	–	–
Dry matter	61.9	61.4	61.4	61.5
Crude protein	18.1	18.0	18.2	18.0
Soluble protein % CP	29.0	34.0	33.0	27.0
Metabolizable Energy (MJ/kg DM)	11.6	11.5	11.4	11.7
Neutral detergent fibre	32.1	32.0	32.1	31.8
Acid detergent fibre	0.7	0.7	0.7	0.5
Ash	6.3	6.2	6.3	6.3

AA = amino acids; CP = crude protein; SBM = soybean meal; SRU = slow-release urea; U = urea

¹Church & Dwight Co., Princeton, NJ, USA

²Vitasal: Ca 17 %, P 12 %, Mg 5%, Na 7%, Cl 10.5%, K 0.04 %, S 504 ppm, Mn 400 ppm, Fe 2939 ppm, Zn 6000 ppm, Cu 1000 ppm, I 500 ppm, Se 40 ppm, Co 60 ppm, Vit. A 35 000 UI, Vit. D 150 000 UI, Vit. E 150 ppm

³Optigen[®], Alltech Inc., Nicholasville, KY, USA

⁴DL Met (Mepron[®] M85, Degussa Corporation, Germany)

⁵L-lysine (AminoShure[®]-L, Balchem Corporation, New Hampton, NY, USA)

while the volume was measured by the headspace volume with a graduated syringe (10 ml). The determinations were undertaken 0, 2, 4, 6, 8, 10, 12, 16, 22, 24, 28, 34, 42, 48, 50, 58, 68, 72, 90, and 92 h after the addition of the ruminal inoculum. To quantify the gas production derived from the culture medium and the ruminal inoculum, four flasks were used as blanks. The gas volume was calculated following Theodorou et al. (1994). The DM cumulative gas production profiles were assessed with the logistic model described by Malafaia et al. (1999):

$$V(t) = V_F / (1 + \exp [2 + 4R (L - t)]) \quad (1)$$

where:

V is the total gas production by the digested fraction at time t , its respective gas production rate R and the duration of the initial gas volume L . V_F is the asymptotic gas volume corresponding to maximum digestion of the incubated material (ml)

The microbial biomass yield was calculated after 48 h of incubation using the equations quoted by Blummel et al. (1997) as follows:

$$\text{The microbial biomass yield} = \text{the substrate truly degradable} - \text{the amount of substrate truly degraded} \quad (2)$$

$$\text{The partition factor (mg/ml)} = \frac{\text{the } in\ vitro \text{ truly degraded substrate}}{\text{volume of the gas produced}} \quad (3)$$

Using the procedure of Menke and Steingass (1988), the metabolisable energy (ME) was estimated using 24 h gas production and the following equation:

$$\text{ME (mega joules/kg DM)} = 2.20 + 0.1357 \text{ gas production} + 0.0057 \text{ crude protein} + 0.0002859 \text{ fat}^2 \quad (4)$$

The ruminal fluid samples and DM residuals were collected from three additional glass flasks per treatment to evaluate the *in vitro* ruminal fermentation and degradation after 72 h of incubation.

In vivo trial. Four Holstein steers (328.5 ± 12.5 kg) fitted with ruminal and duodenal cannulas were

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used. The steers were housed individually in pens, fed twice a day (8:00 and 16:00 h) with free access to fresh clean water and feed. The feed intake and refusal (3% of the total intake) were manually recorded. Four experimental periods, 21 d each, consisted of a 7-d diet adjustment period followed by a 14-d collection period (7 d for the faeces collection and duodenal samples and 7 d for the *in sacco* disappearance evaluation). The treatments consisted of the experimental diets used in the *in vitro* assay. During d 18–21 of each sampling period, the *in sacco* DM disappearance of the experimental diets was calculated per steer (Vanzant et al. 1998) using 16 Dacron bags (10 cm × 20 cm; 53 µm pore size; ANKOM Technology Corp., Fairport, NY, USA). Bags with 5 g (DM basis) of the corresponding diet were placed in the rumen and removed at 0, 3, 6, 12, 24, 48 and 72 h later. The Gompertz model, as described by Susmel et al. (1999), was used to estimate the diet DM kinetics:

$$\text{dis}(t) = (a + b) \exp[-(-C) \exp(-Dt)] \quad (5)$$

where:

dis is the disappearance of the material (g/kg) from the bag at time t ; a is the rumen-soluble fraction (g/kg) at $t = 0$ (h); b is the insoluble, but potentially disappearing, fraction (g/kg); C is the fractional disappearance rate of a and b ; and D is a parameter to measure the disappearance rate.

In this Gompertz model, the fractional disappearance rate varies as a function of time, and the average value (i.e., a constant comparable to the exponential rate of the disappearance) is derived as:

$$c = D/C \quad (6)$$

The remaining DM at each incubation time was used to fit a nonlinear regression model with the “NLIN” option of SAS (Statistical Analysis Systems) (1999).

Chromium sesquioxide (0.3% as DM) was included in the diets as a digesta marker. During the collection period, the protocol described by Zinn et al. (1980) was followed. The individual samples consisted of approximately 500 ml of duodenal chyme and 200 g (wet basis) of faecal material. We calculated the Microbial Organic Matter (MOM) and Microbial N (MN) leaving the abomasum with the purines as the microbial markers (Zinn and Owens 1986). We used chromium oxide as a digesta

marker to calculate the DM duodenal flow and faecal excretion. The organic matter fermented in the rumen was estimated as the difference between the amount of total OM reaching the duodenum and the microbial OM (MOM) reaching the duodenum subtracted from the organic matter (OM) intake. The feed N escaping to the small intestine was considered equal to the total N leaving the abomasum minus the ammonia N and microbial N (MN), and, thus, included the endogenous contributions. The apparent digestibility in the forestomach, small intestine, and hindgut was subsequently calculated based on the nutrient intake and flow. In addition, on the final day of each collection period, ruminal samples (100 ml), through the cannula from the cranial dorsal rumen, were obtained from each steer 4 h after feeding.

Chemical analysis. The samples of the experimental diets and the composited samples of the duodenal and faeces were analysed in duplicate for the DM (oven drying at 90 °C until no further weight loss), ash, Kjeldahl N, fat (AOAC 2006); NDF (neutral detergent fibre) (Mertens 2002; adjusted for the insoluble ash). In addition, the faeces and duodenal samples from each steer were analysed for chromium oxide by atomic absorption spectrophotometry (Spectra AA-10 plus, Varian Analytical Instruments, San Fernando, CA, USA) and purines (Zinn and Owens 1986). In the fresh ruminal fluids from steers at 3 h post diurnal feeding, the pH was measured using a glass electrode connected to a pH meter (Orion 250-A, Orion Research Inc., Beverly, MA, USA). The ruminal fluids sampled from the cranial dorsal section for the *in vitro* assay and metabolic trial were filtered through four layers of gauze, acidified (1 ml of 25% w/v *m*-phosphoric acid per 4 ml of ruminal fluid), centrifuged (17 000 × *g* for 10 min) and stored frozen at –20 °C for further analysis of the volatile fatty acids (VFA; Erwin et al. 1961) with a gas chromatograph (Claurus 500, Perkin Elmer) and ammonia-N concentrations (McCullough 1967) with a UV-VIS spectrophotometer (630 nm, CARY I-E, VARIAN).

Statistical analysis. Data from the *in vitro* assay were analysed with the MIXED procedure of SAS (1999), using a completely randomised model with three treatments, triplicates (glass flasks) as an error term, and incubation time as a repeated measure. The data from the metabolic trial were analysed as a 4 × 4 Latin square with four treatments (control, U, SRU and U+SRU+AA) with the ‘MIXED’

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procedure of SAS (1999) where the model included the steer (random), the period (random), and the treatments (fixed). Tukey's procedure was used to compare the means. The distribution of the residuals was tested for normality with the UNIVARIATE procedure of SAS (1999). The differences between the treatments were declared when the probability (P) was below 0.05.

RESULTS

In the *in vitro* assay, gas production rate and molar proportion of the acetate, propionate and butyrate were similar among the treatments. The SRU product induced less ammonia-N when compared to the U. The dietary substitution of SBM by U or SRU reduced ($P < 0.05$) the total gas production (V), microbial mass and degradation at 72 h incuba-

tion and increased ($P < 0.05$) the ammonia-N and VFA molar concentration under the *in vitro* conditions, although the dietary substitution of SBM by U+SRU+AA had similar values of the gas produced, microbial mass production, ammonia N and VFA concentrations (Table 2).

In the *in vivo* trial, the dietary substitution of SBM by U or SRU decreased ($P < 0.05$) DM the degradation rate and VFA molar concentration in the rumen of the steers and increased ($P < 0.05$) the ammonia-N concentration in the ruminal fluid of the steers (Table 2), although those changes due to the dietary substitution of SBM by U or SRU were not induced by U+SRU+AA. The total degradation of the DM and molar proportion of the acetate, propionate and butyrate in the rumen of the steers' feed diets with SBM, U, SRU or U+SRU+AA were similar. The nutrient intake (DM, OM, NDF and N) and the ruminal, intestinal and total tract digestion of the

Table 2. The effects of the urea and slow-release urea on the ruminal fermentation and degradation of the diets under the *in vitro* and *in vivo* conditions

	Experimental diets				SEM
	SBM	U	SRU	U+SRU+AA	
<i>In vitro</i>					
Total gas V (ml/100 mg DM)	37.1 ^a	31.8 ^b	31.4 ^b	39.4 ^a	5.61
Gas production rate R (h)	0.28	0.29	0.28	0.27	0.019
Microbial mass (mg/g DM)	1.94 ^a	1.80 ^b	1.81 ^b	1.97 ^a	0.054
Degradation 72 h (g/100 g DM)	69.2 ^a	64.6 ^b	65.1 ^b	71.6 ^a	3.37
Ammonia-N (mmol/100 ml)	22.9 ^c	30.2 ^a	26.7 ^b	23.5 ^c	1.02
Volatile fatty acids (mmol/l)	21.5 ^a	19.7 ^b	19.9 ^b	22.6 ^a	0.41
Acetate (mol/100 mol)	68.0	68.4	67.9	67.1	1.05
Propionate (mol/100 mol)	21.1	21.2	21.5	22.2	0.95
Butyrate (mol/100 mol)	10.9	10.6	10.6	10.7	0.90
Acetate: propionate ratio	3.2	3.2	3.2	3.0	0.07
<i>In vivo</i>					
Total degradation $a + b$ (g/100 g DM)	79.3	77.9	78.1	80.1	2.19
Degradation rate c (/h)	0.62 ^a	0.53 ^b	0.56 ^b	0.63 ^a	0.061
pH	6.95	6.82	6.81	6.90	0.035
Ammonia N (mmol/100 ml)	22.4 ^c	29.1 ^a	25.9 ^b	22.9 ^c	1.06
Volatile fatty acids (mmol/l)	79.9 ^a	76.3 ^b	74.8 ^b	80.6 ^a	2.18
Acetate (mol/100 mol)	72.1	71.8	72.4	71.9	0.61
Propionate (mol/100 mol)	16.9	16.3	17.1	17.0	0.51
Butyrate (mol/100 mol)	11.1	11.9	10.5	11.1	0.35
Acetate: propionate ratio	4.3	4.5	4.2	4.2	0.10

AA = amino acids; DM = dry matter; SBM = soybean meal; SEM = standard error of means; SRU = slow-release urea; U = urea; V = total gas production

^{a-c} means bearing different superscripts in a row differ ($P < 0.05$)

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nutrients were also similar among the treatments. Replacing SBM with U, SRU or U+SRU+AA did not modify the OM, N, or NDF faecal excretion in the steers (Table 3).

DISCUSSION

For all the cases of the *in vitro* assay, the ammonia-N concentrations were necessary for the maximum microbial protein synthesis per unit of the fermented substrate (Mehrez and Orskov 1977) and in the normal range (8.8 to 56.1 mmol/100 ml) as reported in the literature (Rogers et al. 1986; Chegeni et al. 2013). Compared with the U, the SRU product reduces the ruminal rate of the N release while ensuring that the entire N is completely available within the rumen (Sinclair et al. 2012). There was a consistent depression of the total gas and microbial mass produced and an increment of the ammonia-

N by the dietary addition of U or SRU as compared to SBM. This depression of the gas production when the nitrogenous compounds are fermented is mainly due to the production of ammonia, which neutralises the acids and reduces the gas production from the buffer (Spanghero et al. 2018). Those undesirable reductions of the microbial mass values by U or SRU when compared to SBM were alleviated when AA were added. The higher microbial efficiency by SBM or the combination of U+SRU+AA when compared to U or SRU alone may be explained by the use of a peptide or an amino acid nitrogen to form true proteins to enhance the microbial growth (Russell et al. 1992; Xin et al. 2010; Gardinal et al. 2017). The improvement in the microbial efficiency by SMB and U+SRU+AA when compared to U and SRU could explain the greater degradation and molar concentration of the total VFA in the ruminal fluids.

The results of the *in vivo* trial were consistent with the *in vitro* assay, especially in terms of the

Table 3. The effects of the slow-release urea on the feed intake, duodenal flow and digestion of the nutrients

	Experimental diets				SEM
	SMB	U	SRU	U+SRU+AA	
Feed intake (kg/d)					
DM	7.8	7.6	8.2	7.7	3.17
OM	7.3	7.0	7.4	7.2	2.94
NDF	5.9	5.6	6.0	5.7	2.40
N	0.231	0.239	0.245	0.220	0.09
Ruminal digestion (g/100 g DM)					
OM	54.4	55.0	55.8	58.1	4.10
NDF	49.0	50.1	50.2	52.0	3.43
N	66.3	66.1	68.1	69.9	4.61
Intestinal digestion (g/100 g DM)					
OM	25.1	23.6	23.9	23.8	2.19
NDF	37.8	34.5	36.0	34.5	4.43
N	17.7	16.7	16.0	14.2	1.78
Total tract digestion (g/100 g DM)					
OM	79.5	78.6	79.7	81.9	5.21
NDF	86.8	84.6	86.2	86.5	3.40
N	84.0	82.8	84.1	84.1	5.97
Faeces excretion (g/d)					
OM	1.5	1.5	1.5	1.3	0.12
NDF	0.791	0.853	0.830	0.779	0.079
N	0.037	0.041	0.039	0.035	0.014

AA = amino acids; DM = dry matter; OM = organic matter; N = nitrogen; NDF = neutral detergent fibre; SBM = soybean meal; SEM = standard error of means; SRU = slow-release urea; U = urea

^{a,b}means bearing different superscripts in a row differ ($P < 0.05$)

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reduction in the degradation rate and molar concentration of the total VFA, and the increment of ammonia-N in the ruminal fluid of the steers' feed diets with U and SRU when compared to those with SBM and U+SRU+AA. In agreement with our results, Benedeti et al. (2014) found that SRU provided higher concentrations of NH₃-N throughout the day than SBM in low concentrate diets, but it did not affect the N balance, the N utilisation efficiency or the microbial efficiency. We hypothesised that the substitution of SBM by U+SRU+AA would not affect the feed intake, digestion, nutrient flow or excretion. Consequently, the substitution of SBM by U+SRU+AA would cause a ruminal synchrony of the availability of the energy from the diet and N from U+SRU+AA. Since the values of the ruminal digestibility are related to the availability of N during the ruminal fermentation, it can be inferred that U+SRU+AA was similar to SBM for the total N release for the ruminal bacteria as no differences were observed for the ruminal digestibility of N among the different sources of N. In addition, the lack of effects observed for the intestinal digestibility of the nutrients showed that the changes in the proportion of the non-protein N of the diets did not cause changes in the nutrient digestion after the abomasum. Therefore, the microbial protein likely met 100% of the metabolisable protein required by the steers (Benedeti et al. 2014; Corte et al. 2018). Similar to our data, Giallongo et al. (2015) found no effect of the SRU supplementation and the SRU plus rumen-protected methionine on the NDF digestibility, the feed intake or the milk production and the quality in the dairy cows. Thus, in the current trial, fibre digestibility did not benefit from the increased NPN supplied by U or SRU. Neither diets supplemented with U+SRU+AA had beneficial effects on the feed intake, digestion or excretion of the nutrients as compared to SBM, U or SRU. In agreement with our results, in dairy cows (Calomeni et al. 2015) and beef steers (Gardinal et al. 2016), no advantages on the ruminal fermentation and nutrient digestion were observed with the supplementation of polymer-coated slow-release urea when compared with feed-grade urea.

In conclusion, replacing SBM by U or SRU did not benefit the microbial mass, digestion or nutrient excretion in the cattle. Some evidence indicated that it is feasible to replace SBM by U+SRU+AA without affecting the microbial mass, degradation rate or rumen fermentation. Therefore, the replacement of

SBM by SRU+U may be of practical value, provided that adequate amounts of lysine and methionine can be used for the optimal microbial efficiency and to obtain the desirable cattle productivity.

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