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Nitrogen metabolism and microbial synthesis in sheep fed diets containing slow release urea to replace the conventional urea

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ABSTRACT. This study aimed to evaluate the effects of adding slow release urea to replace conventional urea in diets for feedlot sheep on nitrogen metabolism and microbial protein synthesis. The substitution levels used as treatments were 0, 20, 40, 60 and 80%. We used 25 Santa Ines x SRD sheep distributed in the treatments in a completely randomized design. The animals were given 50% Tifton-85 hay and 50% concentrate, comprising diets with approximately 12% crude protein. The ingestion, digestion and excretion of nitrogen were not affected by the addition of slow release urea to the diet, in which the digested nitrogen accounted for 72.98% of the ingested. The concentration of plasma urea-N showed a quadratic variation, with the maximum at the level of 72.18% substitution. The microbial protein production and conversion efficiency of the protein into total digestible nutrients were not affected by the addition of slow-release urea in the diets. The replacement of conventional urea with slow release urea in the diet changes the concentrations of urea-N in plasma, however, does not affect the nitrogen balance, nor microbial synthesis and efficiency.

Keywords: nitrogen balance, microbial efficiency, non-protein nitrogen.

Metabolismo do nitrogênio e síntese microbiana em ovinos alimentados com dietas contendo ureia de liberação lenta em substituição à ureia convencional

RESUMO. Objetivou-se avaliar os efeitos da inclusão de ureia de liberação lenta em substituição à ureia convencional em dietas para ovinos confinados sobre o metabolismo de nitrogênio e síntese microbiana. Os níveis de substituição utilizados como tratamentos foram 0; 20; 40; 60 e 80%. Foram utilizados 25 ovinos Santa Inês x SRD, distribuídos nos tratamentos na forma de delineamento inteiramente casualizado. Os animais foram alimentados com 50% de feno de capim *tifton*-85 e 50% de concentrado, compondo dietas de aproximadamente 12% de proteína bruta. A ingestão, excreção e digestão de nitrogênio não foram influenciadas pela inclusão de ureia de liberação lenta na dieta, em que o nitrogênio digerido representou 72,98% do ingerido. A concentração de N-ureico no plasma variou de forma quadrática, com ponto máximo no nível de 72,18% de substituição. A produção de proteína microbiana e a eficiência de conversão da proteína em nutrientes digestíveis totais não foi afetada pela inclusão de ureia de liberação lenta nas dietas. A substituição da ureia convencional pela de ureia de liberação lenta na dieta provoca variação nas concentrações de N-ureico no plasma, entretanto, não afeta o balanço de nitrogênio nem a síntese e a eficiência de síntese microbiana.

Palavras-chave: balanço de nitrogênio, eficiência microbiana, nitrogênio não proteico.

Introduction

Protein is considered a key nutrient in ruminant nutrition, not only by providing amino acids to the animal, but also as a source of nitrogen (N) for microbial protein synthesis (OLIVEIRA JUNIOR et al., 2004a). The final protein supply to the small intestine is formed by dietary protein (rumen undegraded protein) and microbial protein (CALDAS NETO et al., 2008). Microbial protein synthesized in the rumen can supply more than 50% of the amino acids absorbed by ruminants, being considered a protein of high biological value (AFRC, 1993). Therefore, optimization of microbial synthesis is one of the main objectives sought by researchers in ruminant nutrition.

Microbial growth is dependent on the rate of protein degradation and availability of ammonia ($N\text{-}NH_3$) in the rumen (BROOKS et al., 2012). These authors reported that the lack of rumen degradable protein (RDP) in the diet caused a decrease in microbial N production, microbial efficiency and degradation of peptides. Much of the

RDP in the diet can be supplied by urea, a product that stands out for its low cost, availability and ease of use. Urea is hydrolyzed by microbial enzymes to produce N-NH_3, which is converted into microbial protein, thus providing additional protein to the host animal (CALSAMIGLIA et al., 2008).

Besides the presence of N-NH_3 in the rumen, it is also necessary to have available energy. The inadequate supply of RDP in relation to fermentable carbohydrates causes negative effects on fiber digestion and, consequently, loss of energy (KLEVESAHL et al., 2003; VAN KESSEL; RUSSELL, 1996) and reduction of microbial efficiency (HOOVER; STOKES, 1991). This is one reason that limits the use of urea in ruminant diets because it is soluble and makes N-NH_3 available in the rumen very quickly and, most often, there is no balance with the availability of energy from the degradation of carbohydrates.

In this line of research, we developed the slow-release urea (GALO et al., 2003), type of coated urea with controlled release of N-NH_3, which theoretically can improve the rumen functionality and modify metabolic profile. The slow-release urea is in the form of pellets, so that the ammonia-N is slowly released within eight h after intake (XIN et al., 2010), unlike the conventional urea, which is hydrolyzed in 20-60 minutes. According to Broderick et al. (2009), supplementation of slow-release urea in diets for ruminants fed high levels of rapidly fermentable carbohydrates can improve the ability of microbial protein synthesis.

This study aimed to evaluate the effects of replacing conventional urea with slow release urea in diets for feedlot Santa Ines x SRD sheep on metabolic parameters and microbial synthesis.

Material and methods

The experiment was conducted at the Sheep and Goat Farming sector, Department of Rural Technology and Animal - DTRA, State University of Southwest Bahia, Itapetinga Campus. We used 25 male Santa Ines x SRD sheep, non-castrated, ear tagged, with initial body weight of 21.1 ± 1.2 kg and approximate age of four months.

Animals were dewormed and confined in pens of 1.20 x 0.80 m (0.96 m^2) with slatted floor, with access to individual feeder and drinker, distributed into a completely randomized design. The experimental period was 93 days; the first 21 days for adaptation of animals to facilities, management and diets, and the other 72 days were used for data collection.

It was evaluated the replacement of conventional urea (CU) with slow-release urea (SRU) [Optigen®II], so that the equivalent protein from these sources were equivalent: [T1: 1.5% CU in total DM of the diet - SRU 0%, T2: 20% replacement of CU with SRU, T3: 40% replacement of CU with SRU, T4: 60% replacement of CU with SRU, T5: 80% replacement of CU with SRU]. The diets were composed of Tifton 85 hay (*Cynodon* spp.) and concentrate made up of ground corn grain, sugar cane molasses, conventional urea, slow-release urea and mineral mix (Table 1), in order to meet the nutritional requirements recommended by the NRC (2007) for average daily gain of 200 g (Table 2).

Table 1. Chemical composition of ingredients of experimental diets.

Variable	FT-85	Substitution level of conventional urea (%)				
		0	20	40	60	80
Dry matter - DM(%)	89.31	88.56	88.62	88.36	88.56	88.47
% in DM						
Organic matter	93.39	93.40	93.43	93.43	93.31	93.43
Crude protein	6.46	18.24	18.08	17.80	17.62	17.51
Ether extract	1.58	2.04	2.08	2.23	2.34	2.22
Total carbohydrate[1]	85.35	73.12	73.27	73.40	73.35	73.70
Non-fiber carbohydrate [2]	9.76	62.96	62.43	61.07	61.69	61.75
Neutral detergent fiber	78.53	18.87	18.82	19.82	18.50	17.97
NDFcp	75.59	15.86	16.18	17.31	16.29	16.22
Acid detergent fiber	54.66	6.41	5.59	5.37	5.33	5.05
Lignin	10.48	1.41	1.51	1.63	1.52	1.59
Mineral matter	6.61	6.60	6.57	6.57	6.69	6.57

[1]According to Sniffen et al. (1992); [2] According to Hall (2000).

Table 2. Proximate and chemical composition of diets containing slow-release urea in place of conventional urea for finishing Santa Ines x SRD crossbred sheep.

Ingredient (%)	Substitution level of conventional urea (%)				
	0	20	40	60	80
Proximate composition					
Tifton 85 hay	50.00	50.00	50.00	50.00	50.00
Ground corn grain	43.00	42.97	42.94	42.90	42.86
Molasses	4.00	4.00	4.00	4.00	4.00
Conventional urea	1.50	1.20	0.90	0.60	0.30
Slow release urea	0.00	0.33	0.66	1.00	1.34
Mineral mix[1]	1.50	1.50	1.50	1.50	1.50
Chemical composition					
Dry matter - DM (%)	88.94	88.97	88.84	88.94	88.89
% in DM					
Matter	93.39	93.41	93.41	93.35	93.41
Crude protein	12.35	12.27	12.13	12.04	11.99
NDIN[2] (% total-N)	13.90	14.78	13.96	13.63	14.00
$NPN_{estimated}$[3] (% total-N)	50.81	50.76	50.71	50.76	50.81
Ether extract	1.81	1.83	1.91	1.96	1.90
Total carbohydrate	79.23	79.31	79.37	79.35	79.52
Neutral detergent fiber	48.70	48.68	49.18	48.52	48.25
NDFcp[4]	45.73	45.89	46.45	45.94	45.91
Acid detergent fiber	30.54	30.13	30.02	30.00	29.86
Non-fiber carbohydrate	36.36	36.10	35.41	35.72	35.75
Lignin	5.94	5.99	6.05	6.00	6.03
Mineral matter	6.61	6.59	6.59	6.65	6.59

[1]Composition: Calcium (0.48%); Phosphorus (0.35%), Sodium (0.59%); Sulfur (0.072%); Copper (590 ppm); Cobalt (40 ppm); Chrome (20 ppm); Iron (1,800 ppm); Iodine (80 ppm); Manganese (1,300 ppm); Selenium (15 ppm); Zinc (3,800 ppm); Molybdenum (300 ppm). [2]NDIN: neutral detergent insoluble nitrogen; [3]$NPN_{estimated}$: non-protein nitrogen based on ingredients of the diet and NPN content tabulated (VALADADRES FILHO et al., 2010); [4]NDFcp: neutral detergent fiber corrected for ash and protein.

The diets had forage: concentrate ratio of 50:50, being offered at 7:00 and 16:00 hours, to provide remains of 10% in relation to the consumption of the previous day, in addition to supplying water ad libitum. The amount of feed offered was registered daily, and the remains was removed individually and weighed, to evaluate the average daily consumption.

The collection of feces, urine and blood was held from the 85th to the 93rd experimental day. To carry out the collection of feces, the animals were adapted for five days to collection bags made up of sheepskin leather attached by harnesses, continuing with the total collection of feces from each animal for three consecutive days. During the collection and weighing of feces, always at 7:30 and 15:30 hours, were taken samples equivalent to 10% total weight, which were stored in a freezer for later analysis.

On the 93rd experimental day, spot urine samples were collected during spontaneous voiding of animals, approximately four hours after supplying the morning feeding. The samples were filtered through gauze and a 10 mL aliquot was separated and diluted in 40 mL of sulfuric acid (0.036 N) (VALADARES et al., 1999) which was aimed at quantifying the concentrations of urea, nitrogen, creatinine, allantoin, uric acid, xanthine and hypoxanthine in the urine. Samples with pH above three had the pH adjusted to avoid bacterial destruction of purine bases in the urine and precipitation of uric acid.

Simultaneously, four hours after morning feeding, individual blood samples were collected by jugular puncture, and kept in tubes (Vacutainer TM) ethylenediaminetetraacetic acid (EDTA), which were then centrifuged at 3500 rpm for 15 minutes to obtain plasma, which was placed in Eppendorf tubes and freezer stored until analysis.

We conducted sampling of food supplied and leftovers, placing the samples in plastic bags and kept in a freezer for later analysis. Later, they were thawed, and pre-dried in a forced air oven at 55°C for 72 hours. They were then homogenized to form composite samples per animal and ground in a Wiley mill to particles of 1 mm.

The content of dry matter (DM), crude protein (CP), ether extract (EE), mineral matter (MM) and neutral detergent insoluble nitrogen (NDIN) were determined according to Silva and Queiroz (2002), and neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin according to the methodology described by Van Soest et al. (1991). The organic matter (OM) was obtained by the formula: OM (% DM) = 100 - MM (% DM).

The content of total carbohydrates (TC) was calculated according to the equation proposed by Sniffen et al. (1992): TC (% DM) = 100 - (CP + EE + MM), while the non-fiber carbohydrates (NFC) were calculated according to Hall (2000).

The amount of total digestible nutrients (TDN) was obtained from the summative equation: TDN = DCP + 2.25 x DEE + DNDFcp + DNFC (NRC, 2001), in which: DCP = digestible crude protein; DEE = digestible ether extract; DNDFcp = digestible neutral detergent fiber (corrected for ash and protein); DNFC = digestible non-fiber carbohydrate.

The concentrations of creatinine and uric acid in urine, and urea in urine and in blood plasma were estimated using commercial kits (Bioclin). The conversion of the urea in urea-N was performed by multiplying the values by 0.4667. The purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) were estimated by colorimetric methods according to the technique of Fujihara et al. (1987), described by Cx'hen and Gomes (1992), and the total N content estimated by the Kjeldahl method (SILVA; QUEIROZ, 2002).

Excretion of total purine (TP) was estimated by the sum of the quantities of allantoin, uric acid, xanthine and hypoxanthine excreted in the urine. The absorbed microbial purine (X, mmol day^{-1}) was calculated from the purine derivatives excretion in the urine (Y, mmol day^{-1}) using the equation:

$$Y = 0.84X + (0.150BW^{0.75} e^{-0.25X})$$

Being 0.84 the recovery of purines absorbed as urinary purine derivatives and 0.150 $BW^{0.75}$ is the endogenous contribution for purine excretion (VERBIC et al., 1990).

The intestinal flow of microbial nitrogen (g N day^{-1}) was calculated according to the microbial purine absorbed (X, mmol day^{-1}) according to the equation:

$$Y\left(g\ N\ day^{-1}\right) = \frac{X\left(mmol\ day^{-1}\right)x70}{0.83x0.116x1{,}000}$$

where 70 is the nitrogen content in purines (70 mg of N $mmol^{-1}$ purine), 0.83, is the intestinal digestibility of microbial purines, and 0.116 is the ratio purine N: total N in the microbial mass (CHEN; GOMES, 1992).

The N balance (N-retained, g day^{-1}) was calculated by the formula: $NB = N_{ing} - N_{urinary} - N_{fecal}$, where: NB = nitrogen balance (g); N_{ing} = nitrogen ingested (g); $N_{urinary}$ = nitrogen excreted in the urine (g); N_{fecal} = nitrogen excreted in the feces (g).

The experimental design was completely randomized with five treatments and five replications, adopting the mathematical model: Yij = m + Ti + Eij, where: Yij = observed value for the characteristic analyzed, m = overall mean, Ti = effect of adding slow release urea to replace conventional urea, and Eij = random error common to all observations.

The results were analyzed using the SAEG (2007) version 9.1, with performance of descriptive statistics for mean, standard deviation and coefficient of variation program, in addition to regression analysis by means of the F test α 0.05 level to review the effect of replacing conventional urea by slow-release urea nitrogen metabolism and microbial protein synthesis. The criteria used to select the model were based on the significance of the regression coefficients and the coefficient of determination.

Results and discussion

The mean intakes of nitrogen (N) were not affected ($p > 0.05$) by the inclusion of slow-release urea, with mean value of 21.39 g day^{-1} or 1.57 g kg^{-1} $BW^{0.75}$ (Table 3). Typically, which may cause variation in the intake of N is its concentration in the diets (CAVALCANTE et al., 2006) or differentiated consumption of DM (OLIVEIRA JUNIOR et al., 2004b), so the fact that the diets were isonitrogenous and the animals showed similar consumption (978.45 g DM day^{-1}) justifies the observation. The results are consistent with those reported by Zeoula et al. (2006) and Alves et al. (2012), when worked with isonitrogenous diets fed to sheep and found no difference in the amounts of N ingested.

The amount of nitrogen excreted in the feces was not different ($p > 0.05$), with mean value of 5.79 day^{-1} g or 0.42 g kg^{-1} $BW^{0.75}$ (Table 3). This may be due to the lack of effect ($p > 0.05$) of including slow-release urea on digested-N, with mean value of 15.6 g day^{-1} g or 1.14 kg^{-1} $BW^{0.75}$.

When there is no difference in digestibility of N or CP of the diet, the fecal N excretion can be related to the amount ingested, as verified in studies where N excretion increased (CARVALHO et al., 2010) or remained similar (ALVES et al. 2012; ZEOULA et al. 2006) according to its ingestion. According to Manatt and Garcia (1992), there is a balance between nitrogen intake and excretion, which can be obtained in different levels of N consumption.

The urinary N was not influenced ($p > 0.05$) by the addition of slow-release urea, with mean value of 4.84 g day^{-1} or 0.36 g kg^{-1} $BW^{0.75}$ (Table 3). In accordance with Van Soest (1994), the amount of N excreted in the urine is related to the CP content of the diet, which could enhance the excretion of urea in the urine when there is an increase in the intake of N, because this behavior is associated with a higher production of urea in the liver. On the other hand, a low intake of N leads to reduced excretion of urea in the urine to maintain serum urea pool, which is under physiological homeostatic control. The diets used in this study were isonitrogenous and with no effects on N intake, this explains the similarity in the amount of N lost via urine.

Table 3. Mean values of nitrogen balance and plasma urea-N of Santa Ines x SRD crossbred sheep fed diets containing slow-release urea in place of conventional urea.

Variable	Substitution level of conventional urea (%)					P	Regression	CV %
	0	20	40	60	80			
			N ingested					
g day^{-1}	19.82	21.74	20.33	22.59	22.48	0.11	$\hat{Y}$ = 21.39	8.89
g kg^{-1} $BW^{0.75}$	1.45	1.56	1.53	1.67	1.62	0.21	$\hat{Y}$ = 1.57	9.56
			N fecal					
g day^{-1}	5.19	5.55	6.01	6.03	6.17	0.42	$\hat{Y}$ = 5.79	15.69
g kg^{-1} $BW^{0.75}$	0.38	0.40	0.45	0.45	0.44	0.36	$\hat{Y}$ = 0.42	15.64
			N digested					
g day^{-1}	14.62	16.19	14.31	16.57	16.30	0.08	$\hat{Y}$ = 15.60	8.25
g kg^{-1} $BW^{0.75}$	1.07	1.16	1.08	1.22	1.17	0.13	$\hat{Y}$ = 1.14	9.29
% of the ingested	73.87	74.46	70.73	73.31	72.56	0.25	$\hat{Y}$ = 72.98	3.66
			N urinary					
g day^{-1}	5.58	4.50	4.04	6.22	3.84	0.19	$\hat{Y}$ = 4.84	36.43
g kg^{-1} $BW^{0.75}$	0.41	0.32	0.31	0.46	0.28	0.21	$\hat{Y}$ = 0.36	37.71
			N retained					
g day^{-1}	9.05	11.69	10.27	10.35	12.47	0.07	$\hat{Y}$ = 10.76	17.16
g kg^{-1} $BW^{0.75}$	0.67	0.84	0.77	0.77	0.89	0.13	$\hat{Y}$ = 0.79	17.21
% of the ingested	45.77	53.77	50.19	46.01	55.53	0.18	$\hat{Y}$ = 50.25	14.89
% of the digested	61.99	72.09	71.48	62.83	76.53	0.18	$\hat{Y}$ = 68.98	15.48
			Plasma urea-N					
mg dL^{-1}	11.73	14.90	15.51	18.24	17.34	0.00	[1]	7.80

[1] $y = 11.772 + 0.1588SRU - 0.0011SRU^2$, $R^2 = 93.45$; CV = coefficient of variation.

N retention is a result of the difference between N intake and excretion of N in feces and urine. The lack of change in the amount of N ingested and excreted determined the similarity in the values of N retention, with recorded means of 10.76 g day^{-1}, 0.79 g kg^{-1} $BW^{0.75}$, 50.25% of the N ingested and 68.98% of the N digested. According to Kolb (1984), the determination of N balance or N retention is useful to assess whether the animal is in nitrogen balance and whether under certain dietary conditions occurs gain or loss of N. Whereas young animals were used in this study, it was expected a positive N balance once the animals are in the growth phase.

The percentage of N retained both in relation to ingested as digested N were higher than the mean values (44.31 and 32.82% of the N ingested; 55.41 and 52.40% of the N digested) reported Zeoula et al. (2006) and Alves et al. (2012), respectively. Greater retention may indicate better utilization of dietary N and possibly higher muscle deposition and weight gain. It is noteworthy that younger animals are prone to better use the N ingested, resulting in a greater retention.

Mean concentrations of plasma urea-N (Table 3) showed a quadratic trend ($p < 0.05$), with a peak of 23.23 mg dL^{-1} with the inclusion of 72.18% slow-release urea in the place of conventional urea. The highest concentration of this metabolite with the inclusion of slow-release urea may have occurred, possibly because this type of urea provide balance in the availability of ammonia-N in the rumen, thus keeping constant the concentration of urea-N in plasma. In diets with a higher share of conventional urea, the supply of ammonia-N may have occurred shortly after consumption and the peak of plasma urea-N has occurred before four hours after eating, when blood was collected from the animals.

The urea-N in plasma is used as a parameter to indicate the N not used, particularly when the dietary N is of rapid release, as is the case for urea (RENNÓ et al., 2008; ZIGUER et al., 2012). The concentration of plasma urea-N can also be related to the content of non-fiber carbohydrates (NFC) in the diet, since this type of carbohydrate quickly provides energy to be used by rumen microorganisms. According to Valadares et al. (1999), when the NFC content of the diet is less than 35%, the utilization efficiency of the ammonia-N is reduced by increasing absorption by the rumen wall and N concentration in the bloodstream. As in this work, the content of NFC was similar between diets and was greater than 35%, this variable did not interfere with the concentration of plasma urea-N.

The concentration of plasma urea-N in animals may vary depending on the species or category of animal, and further studies are required to establish an ideal range for every situation. When too high may indicate waste of N and energy expenditure, and when too low may indicate N deficiency in the diet. The values of urea-N in plasma (11.73 to 18.24 mg dL^{-1}) are in agreement to those reported by Alves et al. (2012) who evaluated diets with increasing levels of urea in sheep feeding. The ranges observed in this study may indicate efficient use of N from the diet, since they promoted no changes in urinary excretion of N nor N retention.

Higher concentrations of plasma urea-N in sheep were found by Ribeiro et al. (2003, 2004) and Peixoto et al. (2010). Ribeiro et al. (2003) evaluated the metabolic profile of grazing Corriedale ewe lambs at different times of the year and found mean plasma urea-N of 37.94 mg dL^{-1}. Similar results were verified by Ribeiro et al. (2004) who examined the metabolic profile of Border Leicester x Texel ewes during pregnancy and lactation, and reported values of urea-N in plasma from 5.20 mmol L^{-1} (31.14 mg dL^{-1}) to 7.61 mmol L^{-1} (45.57 mg dL^{-1}). Similarly, Peixoto et al. (2010) on Ile De France sheep reported values of plasma urea-N ranging from 35.68 to 38.11 mg dL^{-1}.

In beef cattle, Valadares et al. (1997) found that the concentration range of plasma urea-N from 13.52 to 15.15 mg dL^{-1} corresponded to the maximum microbial efficiency, reporting that it probably represents the limit from which would occur nitrogen loss in these animals. Oliveira et al. (2001) worked with lactating Holstein dairy cows and showed values of plasma urea-N of 19-20 mg dL^{-1} as limits from which would occur dietary N losses.

Urinary excretions of allantoin, uric acid, xanthine and hypoxanthine were not affected ($p > 0.05$) by the levels of slow-release urea in the diet (Table 4). This was reflected in the mean values of total purines, which also did not differ $p > 0.05$). Similar trend was observed for microbial purines absorbed, represented by the mean absorption of 5.04 mmol day^{-1}. The mean percentage value of purine derivatives in relation to the total purines were 49.07; 7.90 and 43.03% to allantoin, uric acid, and xanthine ± hypoxanthine, respectively.

According to Yu et al. (2002), the main factors that can affect the excretion of allantoin, uric acid, xanthine and hypoxanthine are the sources of dietary protein and energy, body weight of the animals, feed additives and animal species. This information contributes to explain the results, since the absence of effect on the excretion of purine derivatives may be related to the similarity between the food sources and the weight of the animals used in the experiment.

Table 4. Microbial protein synthesis in Santa Ines x SRD crossbred sheep fed diets containing slow-release urea in place of conventional urea.

Variable	Substitution level of conventional urea conventional (%)					P	Regression	CV (%)
	0	20	40	60	80			
	Urinary excretions (mmol day^{-1})							
Allantoin	2.82	2.87	2.55	3.00	2.38	0.99	$\hat{Y}$ = 2.72	32.87
Uric acid	0.34	0.49	0.49	0.49	0.40	0.99	$\hat{Y}$ = 0.44	44.28
Xanthine and hypoxanthine	2.61	2.45	2.39	2.33	1.95	0.99	$\hat{Y}$ = 2.35	29.30
Total purine (TP)	5.77	5.81	5.42	5.82	4.73	0.99	$\hat{Y}$ = 5.51	27.04
	Microbial purine (mmol day^{-1})							
Absorbed	5.24	4.24	5.11	5.31	4.08	0.99	$\hat{Y}$ = 5.04	43.23
	Purine derivatives (% of TP)							
Allantoin	48.62	48.11	47.47	50.81	50.33	0.99	$\hat{Y}$ = 49.07	13.58
Uric acid	5.88	8.19	8.61	8.51	8.32	0.99	$\hat{Y}$ = 7.90	30.31
Xanthine ± hypoxanthine	45.51	43.71	43.93	40.68	41.36	0.99	$\hat{Y}$ = 43.03	17.43
	Microbial production (g day^{-1})							
Microbial N	3.81	3.97	3.72	3.86	2.97	0.99	$\hat{Y}$ = 3.66	43.23
Microbial CP	23.79	24.82	23.23	24.13	18.54	0.99	$\hat{Y}$ = 22.90	43.23
	Microbial efficiency							
g CP kg^{-1} TDN	37.01	36.93	40.33	36.23	27.52	0.99	$\hat{Y}$ = 35.60	45.75

CV = coefficient of variation; g CP kg^{-1} TDN = grams of crude protein per kilogram of total digestible nutrients consumed.

The microbial production (Table 4) followed the same pattern observed for the urinary excretion of total purine and microbial purines absorbed, i.e., was not influenced by the inclusion of slow-release urea ($p > 0.05$), which is in according to the observations of Puchala and Kulasek (1992), evaluating microbial synthesis in sheep. According to the authors, there is a high correlation between excretion of purine derivatives in urine and microbial nitrogen flow in the duodenum.

The microbial synthesis may vary depending on the substrate availability in the rumen for fermentation, in particular the N and organic matter. Because of the similarity in the types of ingredient and diet composition, probably there was no difference in the amounts and proportions of substrates available in the rumen for fermentation, which contributed to the similarity of microbial synthesis between treatments.

The mean values of microbial production corresponded to 3.66 and 22.90 g day^{-1} N and CP, respectively (Table 4). The low microbial protein production may be related to the low NDF content of diets, because as stated by Russel et al. (1992), microbial protein production decreases accordingly to the NDF content of diets. It is also possible that higher passage rates of the digesta coupled with the reduced rate and extent of degradation of ground corn has made available lower amount of substrate for fermentation in the rumen and, therefore, microbial synthesis was reduced. Moreover, the absence of a vegetable protein source in the diet reduced the amount of amino acids and peptides available to rumen microorganisms, which may have limited microbial protein synthesis.

The results of microbial efficiency (g CP kg^{-1} TDN) observed in this experiment (Table 4) were not influenced ($p > 0.05$) by the inclusion of slow-release urea, with a mean values of 35.60 g CP kg^{-1} TDN. The synthesis of microbial protein depends in large part on the availability of carbohydrates and N in the rumen (CLARK et al., 1992; NRC, 2001), so that microbial growth is maximized by synchronization between the availability of fermentable energy and degradable N in the rumen (NRC, 1996; RUSSELL et al., 1992).

In the diets of this study were used carbohydrate sources with different degradability, molasses as a source of soluble carbohydrate (energy readily available), corn as a source of starch (rapidly degradable energy), and Tifton 85 hay as a source of slowly degradable energy. Therefore, possibly, the energy released by these carbohydrate sources did not limit the use of N released by rumen microorganisms, regardless of the speed of the release, i.e., has favored the use of N from both types of urea used.

Conclusion

The replacement of conventional urea with slow-release urea of up to 80% has no influence on the intake of nitrogen. The nitrogen concentration in the plasma increases up to 72.18% inclusion of slow-release urea to replace the conventional urea in the diet, however, the fecal and urinary nitrogen excretion have no change. Likewise, this substitution does not alter the microbial synthesis and efficiency.

Based on the results herein, there are no advantages of using slow-release urea in relation to conventional urea. Thus, in feedlot production systems for growing lambs is recommended conventional urea in the place of slow-release urea.

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