Effect of slow release urea in sheep feed on intake, nutrient digestibility, and ruminal parameters

Ureia de liberação lenta na alimentação de ovinos sobre o consumo e coeficiente de digestibilidade dos nutrientes e parâmetros ruminais

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Abstract

This study aimed to evaluate the inclusion of 0.0%, 0.4%, 0.8%, and 1.2% slow release urea (SRU) in sheep feed on the intake and total digestibility coefficient (DC) of nutrients and ruminal parameters of sheep living in tropical regions. Four sheep with a body weight (BW) average of 30.8 ± 1.7 kg were assigned to a Latin square design. The animals were placed in metabolic cages and received two meals a day. Intake data and DC of nutrients of the sheep were subjected to analysis of variance and differences were tested using a regression equation to 5% probability. The inclusion of 0.0%, 0.4%, 0.8%, and 1.2% SRU in sheep feed did not change (p > 0.05) the intake of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral or acid detergent fiber (NDF, ADF), total carbohydrates (TC), and non-fibrous carbohydrate (NFC) expressed in g animal^{-1} day^{-1}, g (kg$^{0.75}$)$^{-1}$, and % BW. The mean values of 3.04%, 2.82%, 0.41%, 0.07%, 1.36%, 0.87%, 2.31%, and 1.00% of BW were obtained for the intake of DM, OM, CP, EE, ANF, ADF, TC, and NFC, respectively. The inclusion of 0.0%, 0.4%, 0.8%, and 1.2% of SRU in sheep rations had a quadratic manner (p<0.05) the DC of DM and CP, with maximum values of 69.67% and 63.59%, respectively, for 0.51% and 0.66% levels of SRU inclusion in the experimental diets. The pH of the rumen fluid did not vary (p > 0.05) for different levels of inclusion of SRU in the feed. However, the pH of the rumen fluid did differ (p<0.05) in relation to time (T) after feeding for all experimental diets. The inclusion levels of SRU in sheep feed did not affect (p>0.05) the concentration of ammonia nitrogen (NH$_3$-N) of the rumen fluid, but there was a quadratic effect (p < 0.05) with relation to T for all experimental diets. Thus, we conclude that the inclusion of 0.66% SRU provides the best digestibility value of CP and is the appropriate value for the digestion of DM. The inclusion of up to 1.2% of SRU in sheep rations did not change the intake of nutrients and ruminal parameters.
Key words: Crude protein. Dry matter. Feces. Non-protein nitrogen.

Resumo

Objetivou-se avaliar a inclusão de 0,0%; 0,4%; 0,8% e 1,2% de ureia de liberação lenta (ULL) na alimentação de ovinos em região tropical sobre o consumo e o coeficiente de digestibilidade total (CD)

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dos nutrientes e parâmetros ruminais. Foram utilizados quatro ovinos com peso corporal (PC) médio de 30,8 ± 1,7 kg, distribuídos em um delineamento em quadrado latino. Os ovinos foram alocados em gaiolas de metabolismo e receberam duas refeições por dia. Os dados de consumo e CD dos nutrientes dos ovinos foram submetidos à análise de variância e as diferenças observadas foram testadas com utilização de equação de regressão a 5% de probabilidade. A inclusão 0,0%; 0,4%; 0,8% e 1,2% de ULL na alimentação de ovinos não alterou (p>0,05) o consumo de matéria seca (MS), matéria orgânica (MO), proteína bruta (PB), extrato etéreo (EE), fibra em detergente neutro (FDN) e ácido (FDA), carboidratos totais (CHT) e carboidratos não fibrosos (CNF) expressos em g animal^{-1} dia^{-1}, g (kg^{0,75})$^{-1}$ e %PC. Os valores médios de 3,04%; 2,82%; 0,41%; 0,07%; 1,36%; 0,87%; 2,31% e 1,00% do PC foram obtidos para o consumo de MS; MO; PB; EE; FDN; FDA, CHT e CNF, respectivamente. A inclusão de 0,0%; 0,4%; 0,8% e 1,2% de ULL nas rações de ovinos influenciou de maneira quadrática (p<0,05) o CD da MS e PB, com valores de máximo de 69,67% e 63,59%, respectivamente para os níveis de 0,51 e 0,66% de inclusão de ULL nas rações experimentais. Os valores de pH do líquido ruminal não diferiram (p>0,05) para os níveis de inclusão da ULL na alimentação de ovinos. Contudo, o pH do líquido ruminal diferiu de maneira quadrática (p<0,05) em relação ao tempo (T) após a alimentação, para todas as rações experimentais. Os níveis de inclusão da ULL na alimentação dos ovinos não influenciaram (p>0,05) a concentração de nitrogênio amoniacal (N-NH$_3$) do líquido ruminal, contudo houve efeito quadrático (p<0,05) com relação ao tempo para todas as rações experimentais. Desta maneira, conclui-se a inclusão de 0,66% da ureia de liberação lenta propicia o melhor dados de coeficiente de digestibilidade da proteína bruta e teor adequado para a digestão da matéria seca. A inclusão de 1,2% de ureia de liberação lenta não altera o consumo dos nutrientes e os parâmetros ruminais.

Palavras-chave: Fezes. Matéria seca. Nitrogênio não proteico. Proteína bruta.

Introduction

Protein requirements in ruminants, as in other animals, are met by amino acids absorbed by the small intestine, though the majority of absorbable protein (50-80%) arises from microbial protein (MicP) synthesized in the rumen (BACH et al., 2005).

The concept of using non-protein nitrogen (NPN) has progressed greatly in recent years. In the past, it was used as much as possible, the NPN in replacing of true protein because it did not affect the production and health of the animals. In addition, the substitution of NPN for vegetable or animal protein in the diet decreases the cost of nitrogen, and as a consequence, the price of feed (MENDES et al., 2010; OLIVEIRA et al., 2001; SILVEIRA et al., 2012).

The concept that the ammonia nitrogen (NH$_3$-N) release rate should match the carbohydrate digestion rate has become increasingly important (CASTANEDA et al., 2009) and has led the industry to pursue the development of slow-release NPN compounds with less risk of poisoning (PIRES et

al., 2004). In recent years, several sources of NPN were evaluated as alternatives to replace urea, and among the best known are the biuret, creatinine, the diureido, and isobutane, as well as other products in the experimental test phase (PIRES et al., 2004; XIN et al., 2010).

Finding effective methods of protecting urea has been a challenge, and therefore the industry has developed compounds with polymers (Optigen® and Optigen®1.200) and polyurethane (XIN et al., 2010), encapsulated urea (TAYLOR-EDWARDS et al., 2009), and urea physically encapsulated by vegetable waxes (Optigen® RII), among others.

The use of NPN of gradual and/or slow release in the rumen can be a strategy to reduce the use of true protein sources and livestock urea in ruminant diets, with advantages including reducing the risks of urea intoxication, increasing space for the inclusion of other ingredients in the diet, replacing true protein sources that are of high cost and limited availability, and improving the synchronization of nutrients in the rumen, without compromising the production performance of ruminants (SOUZA et al., 2010).

Optigen® is a pelleted urea that is covered with a biodegradable polymer capable of controlled release. This highly concentrated source of nitrogen (42% N) can alter rumen function by providing nitrogen to rumen bacteria at a speed that optimizes its conversion into microbial protein (MARTHA, 2013).

Puga et al. (2001) evaluated sheep fed diets containing both high forage and protected urea and found a significant increase in the intake and digestibility of nutrients with the inclusion of the protected urea, which is likely because optimal microorganism activity in fiber fermentation occurred in the rumen.

However, Paris et al. (2013) studied the use of slow-release urea (SRU) in mineral salt supplementation of cattle grazing Tifton 85, and showed that the replacement of common urea by SRU resulted in no increase in average daily gain and gain by area. For this reason, the authors recommended the use of mineralized salt with common urea because it costs less.

A study conducted by Akay et al. (2004) evaluated the *in situ* degradation of SRU (Optigen®) compared to common urea, soybean meal, and soybean grain, and showed that SRU had a disappearance curve similar to that of soybean meal, but slower than common urea, with an intermediate rate of disappearance in the first 16 hours of rumen fermentation, followed by a slow disappearance rate between 16 to 30 hours, similar to that observed for soybean meal.

In an evaluation of sheep fed diets containing SRU (which replaced conventional urea) on nitrogen metabolism and microbial synthesis, the substitution of common urea by SRU did not alter intake, fecal and urinary excretion of nitrogen, and the synthesis and microbial efficiency (ALVES et al., 2014a). Thus, the authors recommended that common urea should be used for sheep in confinement.

Silveira et al. (2012) evaluated the partial substitution of soybean meal by SRU in commercial dairy herds and found that the use of Optigen® (SRU) in the diets of lactating cows provided more ruminal nitrogen because of the continuous supply of N from the SRU, which resulted in similar milk production and an increase in protein synthesis in the microbial rumen, without inducing increased milk urea nitrogen. The authors also concluded that every 150 g of SRU in dairy cow diets replaced 1.0 kg of soybean meal in the ration, and in this way, when soybean meal is expensive, the inclusion of SRU in diets of dairy cows is economically more profitable.

In summary, SRU contributes available N to the rumen more slowly than urea, but faster than the N of protein foods of vegetable origin. This study aimed to evaluate the inclusion of SRU (Optigen®) in sheep feed on consumption, nutrient digestibility, and ruminal parameters (pH and NH_3-N concentration) of sheep in tropical regions.

Material and Methods

The study was conducted at the University *Campus* of Pontes e Lacerda in Setor de Metabolismo Animal (SeMA) and in Laboratório de Análise de Alimentos e Nutrição Animal (LAANA) belonging to Universidade do Estado de Mato Grosso (UNEMAT). The climate is classified as Tropical Continental, alternately wet and dry Aw second Koppen (GERON et al., 2015a). Room temperature values, maximum and minimum temperature, dry and wet bulb, and relative humidity for the execution period of the study are shown in Table 1.

Four mixed breed castrated sheep with a body weight (BW) average of 30.8 ± 1.7 kg were used in the experiment. The sheep were housed in metabolic cages that had individual feeders and waterers. Fifteen days before the beginning of the trial period, the sheep were wormed with Ivermectina® based products.

A Latin square (4 × 4) design was used with four animals, four periods, and four experimental diets with varying inclusion levels of SRU in order to evaluate the effects of SRU on the intake and coefficient of total digestibility of nutrients and ruminal fermentation in sheep.

Table 1. Ambient temperature and relative humidity for the trial of sheep fed diets containing inclusion levels of slow release urea (SRU) in the state of Mato Grosso.

Period	Ambient weather variables					
	RT	RT min	RT max	DBT	WBTU	RU (%)
Morning	23.15	-	-	25.30	22.05	75.00
Afternoon	34.75	-	-	40.35	31.85	52.00
Average	28.95	22.60	36.83	32.83	26.95	63.50

RT: room temperature; RT min: minimum temperature; RT max: maximum temperature; DBT: dry bulb temperature; WBT: wet bulb temperature and RH: relative humidity in%.

The SRU was acquired in the local market in the state of Mato Grosso, and the trade name of the product evaluated was Optigen®. Inclusion levels of 0.0%, 0.4%, 0.8%, and 1.2% of SRU in the DM were used, and the maximum level of inclusion of SRU in the experimental ration represented a value of 57% of nitrogen from the replacement of soybean meal by NPN. The inclusion levels of SRU in the sheep feed were based on a literature review that showed that the inclusion of 0.4% of SRU in dairy cows did not alter nutrient intake and milk production (SOUZA et al., 2010). In addition, Alves et al. (2014b) showed that the substitution of conventional urea by SRU in 80% of sheep feed led to a reduction in carcass yield and subcutaneous fat.

The feed concentrates used in the preparation of the experimental diets were ground grain corn, soybean meal, and SRU (Optigen®), and the roughage provided to the animals was corn silage. The chemical composition of the foods is shown in Table 2.

Table 2. Chemical composition of the experimental foods.

Variables	Experimental foods			
	CS[1]	CGG[2]	SM[3]	SRU[4]
Dry matter %	28.40	91.68	90.35	100.00
Organic matter %	92.16	97.22	93.37	99.00
Crude protein %	8.25	9.83	47.55	282.00
Ether extract %	1.61	4.34	1.51	-[5]
Neutral detergent fiber %	68.58	13.63	17.80	-
Acid detergent fiber %	41.03	11.31	16.66	-
Total carbohydrates %	82.30	83.05	44.31	-
No-fibrous carbohydrate %	13.72	69.42	26.51	-
Mineral matter %	4.79	2.78	6.38	-
Total digestible nutrients %	62.30	86.03	80.73	-

1CS: corn silage, 2CGG: corn grain ground, ³SM: soybean meal, ⁴SRU: slowly release of urea (Optigen®); ⁵ nutrient trace within the experimental food.

The roughage:concentrate used was 65:35. The diets were balanced to submit on average 14.0% of crude protein (isoproteic) and 69.5% of total digestible nutrients (isoenergetic), as recommended by the NRC (2007) for a net gain of 100 g animal[-1] day^{-1} to castrated sheep (Table 3).

The sheep had access to water via individual troughs. Ten grams of mineral mixture per animal was fed daily and added directly to the concentrate at the time of feeding. Twice daily, 5 g salt meal[-1] animal[-1] was provided. The chemical composition of the mineral salt used in this study was 120 g Ca kg^{-1}; 85 g P kg^{-1}; 16 g S kg^{-1}; 148 g Na kg^{-1}; 50 mg Co kg^{-1}; 500 mg Cu kg^{-1}; 16 mg Se kg^{-1}; and 4,800 mg Zn kg^{-1}.

Table 3. Percentage and chemical composition of the experimental rations containing different levels of inclusion of the slowly release of urea (SRU) provided to sheep.

Foods	Inclusion levels of slowly release of urea in the experimental rations			
	0.0%	0.4%	0.8%	1.2%
Corn silage	65.00	65.00	65.00	65.00
Corn grain ground	22.00	24.10	26.10	28.30
Soybean meal	13.00	10.50	8.10	5.50
Slowly release of urea (Optigen)	0.00	0.40	0.80	1.20
Total	100.00	100.00	100.00	100.00
Chemical composition expressed in %				
Dry matter	50.38	50.44	50.51	50.57
Organic matter	93.43	93.53	93.63	93.74
Crude protein	13.71	13.85	14.04	14.14
Ether extract	2.20	2.25	2.30	2.36
Neutral detergent fiber	49.89	49.73	49.58	49.41
Acid detergent fiber	31.32	31.14	30.97	30.79
Total carbohydrates	77.53	78.16	78.76	79.44
No-fibrous carbohydrate	27.64	28.43	29.18	30.02
Mineral matter	4.55	4.45	4.36	4.25
Total digestible nutrients	69.92	69.70	69.49	69.28

The rations were provided at will (*ad libitum*), but the remains were controlled so that they were approximately 10.0%, which standardized the delivery of food and avoided wasting, but still allowed the animal to reach its daily ingestive capacity. The experimental diets were divided into two meals a day and provided at 06h and 18h. The monitoring of food remains was carried out daily before the first meal of the day.

The nutrient digestibility trial lasted 84 days, and was divided into four periods lasting 21 days (i.e., 15 days for animal adaptation, 5 days for the collection of food remains and feces samples, and a day of ruminal fluid collection). During the collection period, total feces and food remains generated per animal day-1, beyond sampling of food were measureds. In the period of adaptation and collection, management was performed as described by Geron et al. (2013).

A napa bag was adapted and used to collect all feces during the experimental period from each animal. The feces of the animals were weighed every morning and homogenized, and composite samples corresponding to 10% of the total sample weight

were taken (GERON et al., 2015a). The samples of feces were placed in plastic bags, identified per animal and experimental period, and stored in a freezer at -10 ° C for further analysis.

On the last day of each collection period, samples of ruminal fluid were collected. The samples were taken before the first feeding (6 h), which was established as time zero (0), and then at 2, 4, 6, and 8 h post feeding, with five samples per animal per period. A vacuum pump (40 mm Hg pressure) attached to a silicone hose (2.0 m long × 12.0 mm diameter) lubricated with mineral oil (Nujol) was introduced into the mouth of the animal (GERON et al., 2015b) and used to collect the samples. For NH_3-N determination, the sample was filtered to obtain 100 mL of ruminal fluid. Immediately after collection, the pH of the sample was measured from 50 mL of ruminal fluid using a digital pH meter. One milliliter of sulfuric acid (H_2SO_4, 1:1) was then added to stop fermentation. The NH_3-N level was determined by distillation with potassium hydroxide 2 N, using the method described by Fenner (1965), and later modified by Vieira (1980).

Samples of food, remnants, and feces were dried at 55 ° C for 72 hours and processed in a Wiley mill using a 1 mm sieve and then homogenized in equal amounts, based on dry weight, to form composite samples of feces remnants and food, per period experimental^{-1} animal^{-1} experimental rations^{-1}.

Samples of food, remnants, and feces were subjected to analysis of dry matter (DM - method 934.01), organic matter (OM - method 924.05), crude protein (CP - method 920.87), and ether extract (EE - method 920.85) according to AOAC (1990).

The determination of the neutral detergent fiber (NDF) of the food, remnants, and feces was performed according to Van Soest et al. (1991), without the use of sulfite and without correcting the NDF with respect to the mineral matter content of the fiber. The acid detergent fiber (ADF) was carried out according to method 973.18 (AOAC, 1990).

The determination of total carbohydrates (TC) of the food, remnants, and feces was obtained by the equation: TC = OM - [EE + CP] (SNIFFEN et al., 1992). The non-fiber carbohydrate content (NFC) of the food, remnants, and feces was determined by the equation: NFC = 100 - (CP + NDF + EE + MM), wherein the MM: mineral material second Sniffen et al. (1992).

The determination of the total digestibility coefficient (DC) of nutrients was obtained by the equation: [((nutrient contained in feed supplied g - remnants of nutrient g) - nutrient contained in feces g) / intake of nutrient g] × 100 as described by Geron et al. (2015a).

The variables were subjected to analysis of variance through the SAEG software (UFV, 2007), with a p value of 0.05 considered significant. When significant effects of inclusion levels of SRU in the experimental feeds were observed, regression analysis was then performed, with the linear and quadratic effect considered at 5% significance. For the pH and concentration of ammonia in the rumen fluid, statistical analysis that considered the subdivision of the parcel was undertaken, which included the time of evaluation after feeding the animals. This method of frequentist analysis, was considered with the regression for each level of inclusion of SRU beyond the average of experiments rations (SOUZA et al., 2016).

Results and Discussion

Inclusion levels of 0.0%, 0.4%, 0.8%, and 1.2% of SRU in sheep feeding did not change (p>0.05) the intake of DM, OM, CP, EE, NDF, ADF, TC, and CNF (Table 4). The average values for the intake of DM, OM, CP, EE, NDF, ADF, TC, and NFC were 927.80, 868.67, 127.97, 21.86, 418.59, 267.52, 713.62, and 307.40 g animal^{-1} day^{-1}, respectively. The replacement of soybean meal (true protein) by SRU, which has a disappearance rate similar to that of soybean meal (AKAY et al., 2004), may have contributed to the maintenance of the rumen

environment (MARTHA, 2013) and the average results observed for the intake of the different nutrients of the experimental rations in the present study.

The average values of intake of the different nutrients in the experimental rations were consistent with those reported in the literature for diets containing 13 to 15% crude protein and diets containing 35 to 50% concentrate, with a DM intake variation of 580.56 to 1371.44; OM, 553.14 to 1346.10; and CP, 77.37 to 352.24, expressed in g animal⁻¹ day⁻¹ (GERON et al., 2013, 2015a; PRADO et al., 2004).

Table 4. Daily average intake of dry matter (IDM), organic matter (IOM), crude protein (ICP), ether extract (IEE), neutral detergent fiber (INDF) and acid (IADF), total carbohydrates (ITC), and non-fiber carbohydrates (INFC) by sheep fed with rations containing slowly release of urea (SRU).

Variables	Inclusion levels of the slowly release of urea in experimental rations				Regres.	CV (%)
	0.0%	0.4%	0.8%	1.2%		
IDM g animal⁻¹ day⁻¹	946.37	956.80	971.77	836.26	Y = 927.80	13.37
IDM % BW	3.09	3.20	3.12	2.76	Y = 3.04	17.56
IDM g (kg^{0,75})⁻¹	72.68	72.25	73.71	64.74	Y = 70.85	14.47
IOM g animal⁻¹ day⁻¹	886.78	895.82	904.59	787.51	Y = 868.67	13.14
IOM % BW	2.90	2.86	2.91	2.60	Y = 2.82	14.79
IOM g (kg^{0,75})⁻¹	68.11	67.65	68.68	60.97	Y = 66.35	14.33
ICB g animal⁻¹ day⁻¹	129.10	132.22	134.51	116.03	Y = 127.97	13.00
ICP % BW	0.42	0.42	0.43	0.38	Y = 0.41	14.46
ICP g (kg^{0,75})⁻¹	9.91	9.99	10.21	8.98	Y = 9.77	14.02
IEE g animal⁻¹ day⁻¹	22.16	21.65	21.95	21.68	Y = 21.86	14.33
IEE % BW	0.072	0.069	0.071	0.071	Y = 0.071	16.74
IEE g (kg^{0,75})⁻¹	1.70	1.63	1.66	1.68	Y = 1.66	16.11
INDF g animal⁻¹ day⁻¹	422.21	459.54	413.81	378.78	Y = 418.59	19.77
INDF % BW	1.38	1.47	1.34	1.23	Y = 1.36	20.96
INDF g (kg^{0,75})⁻¹	32.45	34.97	31.53	29.33	Y = 32.00	20.63
IADF g animal⁻¹ day⁻¹	277.43	264.00	267.83	260.83	Y = 267.52	12.66
ISDF % BW	0.91	0.84	0.91	0.86	Y = 0.87	14.20
IADF g (kg^{0,75})⁻¹	21.31	19.90	20.31	20.21	Y = 20.43	13.75
ITC g animal⁻¹ day⁻¹	735.52	690.50	755.32	673.13	Y = 713.62	11.68
ITC % BW	2.40	2.20	2.43	2.22	Y = 2.31	12.88
ITC g (kg^{0,75})⁻¹	56.50	52.12	57.40	52.12	Y = 54.53	12.50
INFC g animal⁻¹ day⁻¹	313.31	289.91	325.58	300.80	Y = 307.40	6.46
INFC % BW	1.02	0.93	1.05	0.99	Y = 1.00	9.01
INFC g (kg^{0,75})⁻¹	24.05	21.92	24.74	23.29	Y = 23.50	8.32

g (kg^{0,75})⁻¹: grams per kilogram of metabolic weight; % BW: body weight; g animal⁻¹ day⁻¹: grams animal per day; Regres.: regression equation; and % CV: coefficient of variation.

Likewise, a study by Alves et al. (2014a) that assessed the nitrogen metabolism and microbial synthesis of sheep with a body weight of 21 kg fed diets containing 20 to 80% replacement of soybean meal by SRU, showed that the substitution of soybean meal by SRU did not change the intake of

PB, with an average of 133.69 g animal^{-1} day^{-1}, a value similar to that observed in this study (127.97 g animal^{-1} day^{-1}) (Table 4). This observation supports the use of SRU in ruminant feed as a substitute for true protein (soybean meal), which has a high commercial value, especially during the soybean of season.

Inclusion levels of 0.0%, 0.4%, 0.8%, and 1.2% SRU in sheep feed did not change (p> .05) the DC of the OM, EE, NDF, ADF, TC, and NFC (Table 5). The average figures for the DC of the OM, EE, NDF, ADF, TC, and NFC were 70.40%, 85.54%, 44.87%, 36.32%, 71.84%, and 96.23%, respectively.

However, the inclusion levels of the SRU in sheep feed changed (p < 0.05) the DC of the DM and CP in a quadratic manner (Table 5). The highest points of the DC of DM and CP obtained were 69.92% and 63.55%, respectively, for the inclusion levels of the SRU of 0.51 and 0.66%, respectively.

The highest value for the DC of CP of 63.55% estimated by the regression equation for the level of 0.66% SRU was below the average of 72.98% for the DC of CP found by Alves et al. (2014a) who evaluated the replacement of true protein (soybean meal) by SRU. Most likely, the greater body weight of the animals in this study provided a lower DC value of CP in relation to those values reported in the literature. However, for sheep with a body weight of 30.8 kg, a level of 0.66% SRU corresponded to a replacement of approximately 33.08% of true protein (soybean meal), which resulted in the best value for the digestibility coefficient of CP (Table 5).

Table 5. Coefficients of variation (CV) and total digestibility coefficients (DC) for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid fiber (ADF), total carbohydrates (TC) and non-fibrous carbohydrate (NFC) for sheep fed with rations containing slowly release of urea (SRU).

Variables	Inclusion levels of the slowly release of urea in experimental rations				Regres.	CV %
	0.0%	0.4%	0.8%	1.2%		
DCDM	66.82	66.87	71.88	63.28	[1]	5.02
DCOM	7047	69.01	73.93	68.18	Y = 70.40	4.67
DCCP	52.16	59.26	65.47	54.79	[2]	10.08
DCEE	82.37	89.01	86.57	84.21	Y = 85.54	10.16
DCNDF	40.72	45.12	52.27	41.34	Y = 44.87	19.51
DCADF	31.34	34.87	43.98	35.11	Y = 36.32	20.02
DCTC	70.75	71.07	74.97	70.57	Y = 71.84	5.73
DCNFC	92.99	91.19	95.02	93.73	Y = 96.23	1.26

[1]DCDM = 65.8869 + 14.8252X - 13.5159X^2 (R^2= 62.33%) p<0.05; [2]DCCP = 51.3629 + 36.8574X - 27.7826X^2 (R^2 = 68.22%) p<0.05; Regre.: regression equation.

According to Ribeiro et al. (2014), the ruminal degradation rate of an energy source is the main limiting factor in the use of NPN, and therefore the synchronization between the nitrogen source (protein) and energy in the rumen is essential to maximize rumen fermentation and reduce the loss of nitrogen by the rumen wall, thereby increasing the efficiency of microbial synthesis, a finding corroborated by the results of this study. Once the experimental rations showed traditional foods, which have a positive associative effect (GERON et al., 2013), and consequently a synchronization between energy and protein sources.

The pH values of the rumen fluid did not differ (p>0.05) for the inclusion levels (0.0%, 0.4%, 0.8%, and 1.2%) of SRU in sheep feed (Table 6). However, the pH of the rumen fluid varied (p<0.05) in relation to T for all experimental rations (Figure 1A and 1B). The average regression equation for the pH value of rumen liquid was pH = 7.0320 - 0.1520T + 0.0175T^2 for the different experimental rations. The minimum point estimate for the pH value was 6.70 at 4:18 a.m. after the morning feeding, and the maximum point calculated by the equation was 7.03 for the time 0h before the morning feeding. The time obtained for the maximum pH value of rumen liquid estimated by equation coincides with the data shown in Table 6, in which the mean maximum observed value was 7.08 for time 0h before the morning feeding.
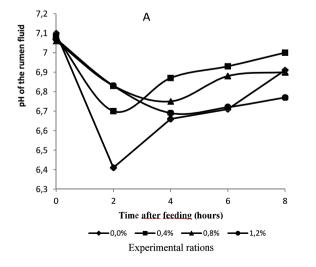
According to Ortolani (1981), a volume of 300 mL of ruminal liquid must be obtained from cattle using an esophageal catheter in order to avoid any interference with the pH value because volumes less than this may result in an alkalizing trend. In this study, there was great difficulty in both harvesting ruminal liquid through the gavage in the hours following food intake and collecting 100 mL of ruminal fluid because of the small size of sheep compared to cattle. For this reason, the pH value of the ruminal fluid was above 7.0 at time 0h because of how the ruminal fluid was collected, as volumes of less than 300 mL can be contaminated by saliva, a buffer.

Table 6. Values of pH of the rumen fluid of sheep fed rations containing inclusion levels of slow release urea (SRU) in function of the time of collection after the morning feeding.

Collection time - hours	Inclusion levels of the slowly release of urea in experimental rations				Average
	0.0	0.4%	0.8%	1.2%	
0	7.10	7.08	7.06	7.07	7.08
2	6.41	6.70	6.83	6.83	6.69
4	6.66	6.87	6.75	6.69	6.74
6	6.71	6.92	6.88	6.72	6.81
8	6.91	7.00	6.90	6.77	6.90
Average	6.76	6.91	6.88	6.82	6.84

Figure 1. Observed values (A) and regression equations (B) the pH of the rumen fluid of sheep fed rations containing inclusion levels of slow release urea (SRU) in function of time after feeding in the morning.

Experimental rations	Equations estimated for the pH of the rumen fluid of sheep fed the experimental diets
0.0% SRU	pH = 6.9997 − 0.2297X + 0.0282X^2 (r^2 = 65.53%)
0.4% SRU	pH = 7.0149 − 0.1094X + 0.0141X^2 (r^2 = 54.35%)
0.8% SRU	pH = 7.0394 − 0.1149X + 0.0127X^2 (r^2 = 83.07%)
1.2% SRU	pH = 7.0651 − 0.1426X + 0.0134X^2 (r^2 = 98.55%)
Average	pH = 7.0320 − 0.1520X + 0.0175X^2 (r^2 = 78.67%)

Likewise, Geron et al. (2013) obtained a maximum pH value of rumen fluid of 6.98 at time 0h before the morning feeding to sheep fed diets containing 20%, 40%, 60%, and 80% levels of concentrate. Furthermore, the authors observed that the different concentrate levels did not alter the pH of the rumen fluid of sheep.

In this study, the average minimum estimated value for the ruminal pH was 6.70 at 4:18 a.m. after feeding, and the range of variation of ruminal pH values was 6.41 (at 2h after meals) to 7.10 (0h before the morning feeding). The reduction in rumen fluid pH may be correlated with an increased production of volatile fatty acids from the fermentation of dietary carbohydrates in the rumen (GERON et al., 2008; ZEOULA et al., 2006). Most likely, the resumption of rumen fluid pH to near-neutral values after the break from 4 a.m. to 4:18 a.m. was due to mechanisms buffering the pH of the rumen, including salivary action from the rumination of fiber, the absorption of acids by the rumen epithelium (GERON et al., 2015b), and sensory stimuli such as the viewing and smelling of food (ZEOULA et al., 2003) that may result in ruminal ammonia concentration before the sheep are fed.
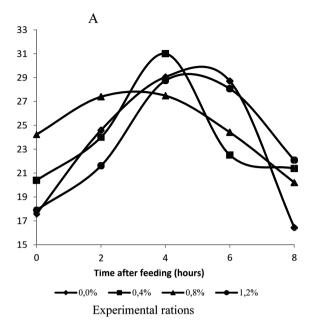
An average pH of 6.90 (Table 6) was observed for 8h after the morning feeding, a finding likely explained by the rations containing a sufficient amount of fiber to stimulate rumination and hence salivation, which has a buffering action. The experimental rations of this study showed an average of 49.65% NDF and 28.82% NFC, which may have contributed to the rumen fluid pH results (Figure 1A).

The levels of SRU inclusion in sheep feed did not affect ($p > 0.05$) the concentration of NH_3-N of the rumen fluid, and had no effect with respect to T (Table 7). The NH_3-N concentration of ruminal fluid in response to the different experimental diets had an average value of 19.01 mg of NH_3-N 100 mL^{-1} of rumen fluid (Figure 2A), which remained above the optimum number of 15 mg 100 mL^{-1} needed for maximum ruminal fermentation activity (GERON et al., 2013, 2015b; PEREIRA et al., 2009), and above the concentration of 5.0 mg 100 mL^{-1}, a limit established by the literature that does not inhibit microbial growth (PEREIRA et al., 2009). However, the NH_3-N concentration in rumen liquid for all experimental diets behaved in quadratic form ($p < 0.05$) in relation to T (h) after the first feed (Figure 2B).

Table 7. Ammonia nitrogen concentration values (NH_3-N) from rumen fluid of sheep fed rations containing inclusion levels of slow release urea (SRU) in function of the time of collection after the morning feeding.

Collection time - hours	Inclusion levels of the slowly release of urea in experimental rations				Average
	0.0	0.4%	0.8%	1.2%	
0	17.59	20.39	24.24	17.90	20.03
2	24.59	24.00	27.39	21.61	24.40
4	29.05	31.00	27.48	28.75	29.07
6	28.70	22.51	24.41	28.06	25.92
8	16.45	21.38	20.21	22.09	20.03
Average	17.59	20.39	24.24	17.90	20.03

Figure 2. Observed concentration (A) and regression equations (B) of ammonia nitrogen (NH3-N) from rumen fluid of sheep fed diets with the inclusion of levels of slow-release urea in function of time after feeding in the morning.

Experimental rations	Equations estimated for the pH of the rumen fluid of sheep fed the experimental rations
0.0% SRU	$NH_3\text{-}N = 16.723 + 6.2786X - 0.7734X^2$ ($r^2 = 93.66\%$)
0.4% SRU	$NH_3\text{-}N = 20.191 + 3.5916X - 0.4459X^2$ ($r^2 = 62.75\%$)
0.8% SRU	$NH_3\text{-}N = 24.403 + 1.9994X - 0.3189X^2$ ($r^2 = 98.77\%$)
1.2% SRU	$NH_3\text{-}N = 16.832 + 4.6258X - 0.4855X^2$ ($r^2 = 87.89\%$)
Average	$NH_3\text{-}N = 19.537 + 4.1246X - 0.5061X^2$ ($r^2 = 94.41\%$)

The average regression equation of the different inclusion levels of the SRU for NH_3-N concentration of ruminal fluid, as a function of T, was as follows: NH_3-N $= 19.537 + 4.1246T - 0.5061T^2$ (Figure 2B). The highest estimated concentration of NH_3-N was 27.94 mg 100 mL^{-1} of rumen fluid at 4:06 a.m. after the morning feeding, and the minimum concentration was 19.54 mg 100 mL^{-1} of rumen fluid at 0h (before the morning feeding).

The overall average observed value of NH_3-N rumen fluid was 20.03 mg 100 mL^{-1} of rumen fluid (Table 7), which remained within the optimum range of 19 to 23 mg 100 mL^{-1} needed for maximum ruminal fermentation activity (GERON et al., 2013; ZEOULA et al., 2003), and above the concentration of 5.0 mg 100 mL^{-1}, a value established in the literature (PEREIRA et al., 2009) that does not limit microbial growth.

According to Zeoula et al. (2003), increases in ruminal ammonia concentrations before sheep are fed can be attributed in part to saliva secretion, which is stimulated even before food intake, by sensory stimuli such as food visualization and smell. Therefore, the handling and movement associated with weighing the food prior to delivery can condition animals, stimulate salivation, and may have caused an increase in ammonia concentration moments before feeding.

Conclusion

The inclusion of up to 0.66% of SRU in sheep feed provides better CP digestibility. The inclusion of up to 1.2% of SRU in sheep rations did not change the intake of nutrients and ruminal parameters.

Acknowledgements

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