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REPLACEMENT OF COMMON UREA WITH PROTECTED UREA IN SHEEP SUPPLEMENT

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Abstract

The objective was to evaluate the effect of replacing common urea with protected urea in the supplement on the nutrient intake and digestibility and metabolic profile of sheep. Five adult sheep, with an average age of 4 years and an initial average body weight of 50 ± 4.03 kg, were used. The diet consisted of sorghum silage, supplemented with a multiple mixture of corn bran, soybean meal, white salt, mineral salt, common urea (CU) or protected urea (PU). The experimental design was a 5x5 Latin square. Treatments consisted of: control or 100% CU, 75% CU and 25% PU, 50% CU and 50% PU, 25% CU and 75% PU and 100% PU. Collections and analysis of feed, feces, urine and blood were carried out to assess intake, digestibility and blood metabolites. Analysis of variance and SNK test were applied considering 5% significance. Nonparametric data were analyzed by Kruskal-Wallis at a significance level of 5%. The replacement of common urea with protected urea did not (P> 0.05) alter the intake of dry matter, crude protein and water, and the dry matter digestibility. The production of urine and feces, and the density of urine also did not (P> 0.05) show statistical difference with the replacement of common urea with protected urea. As for protein and energy metabolites, only glycemia showed variation (P <0.05), with the proportion of 75% CU and 25% PU, indicating the highest concentration of glucose in relation to the replacement of 0, 50 and 100% PU in the supplement. There are no benefits from partial or total replacement of common urea with protected urea in sheep supplementation.

Keywords multiple mixture, non-protein nitrogen, nutrition, sheep, silage.

SUBSTITUIÇÃO DA UREIA COMUM POR UREIA PROTEGIDA EM SUPLEMENTOS PARA OVINOS

Resumo

Objetivou-se avaliar o efeito da substituição da ureia comum por ureia protegida no suplemento sob o consumo de nutrientes e digestibilidade da matéria seca e perfil metabólico de ovelhas. Foram utilizadas 5 ovelhas adultas, com idade média de 4 anos e peso corporal médio inicial de 50 ± 4,03 kg. A dieta foi composta por silagem de sorgo, sendo suplementada por mistura múltipla composta de milho moído, farelo de soja, sal branco, sal mineral, ureia comum (UC) ou ureia protegida (UP). O delineamento experimental foi em quadrado latino 5x5. Os tratamentos consistiram em: controle ou 100% UC, 75% UC e 25% UP, 50% UC e 50% UP, 25% UC e 75% UP e 100% UP. Foram realizadas coletas e análise de alimentos, fezes, urina e sangue para avaliação do consumo, digestibilidade e metabolitos sanguíneos. Foi realizado análise de variância e teste SNK considerando 5 % de significância. Os dados não paramétricos foram analisados por Kruskal-Wallis ao nível de significância de 5%. A substituição da ureia comum por ureia protegida não alterou o consumo de matéria seca, de proteína bruta e água e a digestibilidade da matéria seca (P>0,05). A produção de urina e fezes, e a densidade da urina também não apresentaram diferença estatística com a substituição da ureia comum pela protegida (P>0,05). Quanto aos metabólitos proteicos e energéticos somente a glicemia demonstrou variação (P<0,05), sendo que a proporção de 75%UC e 25%UP apresentou a maior concentração de glicose em relação a substituição de 0, 50 e 100% de UP no suplemento. Conclui-se que não há benefícios na substituição parcial ou total da ureia comum pela ureia protegida na suplementação de ovinos.

Palavras–chave mistura múltipla, nitrogênio não proteico, nutrição, ovinos, silagem.

INTRODUCTION

The most expensive nutrient in ruminant diets, protein is important in maintaining the rumen environment, microbial protein production, influencing nutrient intake, digestibility and metabolism (SALAMI et al., 2020). To meet the nutritional requirements of protein, sources of true protein and non-protein nitrogen are used. The main source of low-cost non-protein nitrogen is urea, which can be used in properties with a low technological level, accessible on the market and which does not compete with human food.

The supply of urea for ruminants has been investigated for some decades. Recent studies, such as Gunun et al. (2016) using urea with sugarcane bagasse, or in concentrates and multiple mixtures as in Gabriel et al. (2019) and Waruiru et al. (2017), demonstrate advantages in the use of urea, for example, the increase in nutrient digestibility. Thus, it can be used with silages such as sorghum, which has good energy value and average protein content (SANTIN et al., 2020).

Although it has the benefits mentioned, urea has low palatability and risk of intoxication due to its rapid hydrolysis in the rumen (ABO-DONIA et al., 2021). As an alternative to mitigate the risks of poisoning due to gradual release in the rumen medium, it is possible to use protected urea in the diet. Protected urea has up to 1.2% dry matter of the sheep diet and does not change the intake and digestibility of nutrients (GERON et al., 2016). However, the combination and partial replacement of common urea with protected urea has been poorly explored. The combination of these can maximize the production of microbial protein due to the synergism of energy and protein, used as a substrate by ammonia-degrading amylolytic and cellulolytic bacteria (LI et al., 2021).

Therefore, our hypothesis is that the partial or total replacement of common urea with protected urea in the supplement causes no changes in nutrient intake and digestibility, and metabolites of sheep. Therefore, this study aimed to evaluate the effect of replacing common urea with protected urea on nutrient intake and digestibility, fecal and urinary parameters and metabolites of sheep.

MATERIAL AND METHODS

The experiment was conducted in the Goats and Sheep Sector of the Capim Branco Experimental Farm, at the Federal University of Uberlândia, Minas Gerais State,

from January 6th to February 17th, 2016, and carried out with the approval of the Ethics and Animal Use Committee of the Federal University of Uberlândia, according to protocol number CEUA/UFU 017/16.

Five ewes, adult, non-pregnant and non-lactating, Dorper x Santa Inês, with an average age of four years and average weight of 50 ± 4.03 kg, were distributed in a 5 × 5 Latin square design. Animals were orally dewormed with 5mL ZOLVIX® and housed in individual metabolic cages of 2m², provided with feeding and drinking troughs, following the recommendations of the National Institute of Science and Technology (INCT). The total experimental period was 45 days, divided into five phases, each consisting of four first days for adaptation and five days for collection.

The treatments consisted of replacing common urea (CU) with protected urea in the multiple mixture; the control with 100% CU, 75% CU and 25% PU, 50% CU and 50% PU, 25% CU and 75% PU and 100% PU.

All diets were based on sorghum silage forage. Supplementation was given *ad libitum* (400 g/day per ewe, total capacity of the salt lick) with a multiple mixture consisting of ground corn, soybean meal, white salt, mineral salt, common urea or protected urea. The percentage of each food in the multiple mixture as a function of the treatments and the chemical composition of the diet are listed in Table 1.

Table 1: Composition of multiple mixtures with different proportions of common and protected urea.

Ingredients (g/kg)	Treatments				
	CU 100%	CU 75% PU 25%	CU 50% PU 50%	CU 25% PU 75%	PU 100%
Corn bran	150	150	150	150	150
Soybean meal	150	150	150	150	150
White salt	300	300	300	300	300
Mineral salt	300	300	300	300	300
Common urea	100	75	50	25	NI
Protected urea	NI	25	50	75	100
	Nutrients (%)				
Feed	DM	CP	NDF	ADF	TDN
Sorghum silage	31.25	5.99	50.18	31.39	63.89
Nutrients (%)	CU 100%	CU 75% PU 25%	CU 50% PU 50%	CU 25% PU 75%	PU 100%
Dry matter	89.36	89.47	89.2	88.95	90.02
Crude protein	40.02	40.36	39.98	40.41	40.32

CU: common urea; PU: protected urea; NI: not included; DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; TDN: total digestible nutrients.

Forage was supplied in two meals: at 08h00 and 16h00, and supplementation only at 8h00. At each new phase, animals were weighed to adjust the feed supply, being readjusted to keep approximately 10% leftovers in the trough.

Water was supplied every day in the morning, in plastic buckets, in the amount of six liters per animal, and the volume of water was measured through a two-liter plastic cylinder accurate to 20 mL. Water leftovers were measured daily through a plastic cylinder accurate to 20 mL. A six-liter reference bucket placed daily on a flat surface, away from the animals, was used to measure the amount of water lost by evaporation. The reference bucket was measured using a plastic cylinder accurate to 20 mL. The calculation of water consumption was made by the difference between what was offered, leftovers and evaporated.

Daily, in each experimental period, samples of the supplied feed and leftovers were weighed and stored in plastic bags at –20°C for further analysis.

Total feces retained in metabolic cages were collected daily for five days of each collection period. Total feces were weighed and sampled daily in each experimental period. Before storing the feces in plastic bags at –20°C for further analysis, the fecal score was assigned, according to Gomes et al. (2012), in which scale one (1) feces are dry and dull; on scale two (2) feces are normal; on scale three (3) feces are slightly softened; on scale four (4) feces are softened, with no shape and glued together (bunch of grapes); on scale five (5), feces are soft and without a normal shape (swine feces); and on scale six (6) feces are diarrheic.

Urine was sampled using buckets with screens to retain feces, which were collected in plastic trays. Urine volume was measured using plastic graduated cylinders, with a capacity of two liters and accurate to 20 mL. The volume excreted by each animal during 24 hours was quantified, where 20% daily total of all collecting buckets was sampled in each one of the five days of collection. After this period, the total amount sampled from each experimental unit was homogenized. Subsequently, these samples were filtered through disposable paper filters, stored in plastic bottles identified for each treatment, and freezer-stored at -15ºC for future analysis.

Urine density was measured using a portable Megabrix® (Fremont) hand refractometer with the aid of disposable pipettes, where 1 mL urine was transferred from the collecting bucket to the optometer prism. This procedure was performed under fluorescent light, always in the same position. Between the measurement of each

sample, the refractometer was sanitized and dried with a paper towel so that there was no interference with the readings.

Samples of feed, leftovers and feces were pre-dried in a ventilated oven at 55ºC for 72 hours and crushed in a Wiley knife mill, to one-millimeter particles. Analysis of dry matter, mineral matter and crude protein were made according to AOAC (1990). The calculation of intake and apparent digestibility of dry matter was performed according to equations proposed by Maynard et al. (1984).

Blood samples were always taken before the first meal of the day, on the first three days of each experimental period. Blood samples were taken to measure glucose, by jugular venipuncture, in 4 mL tubes, containing sodium fluoride and EDTA anticoagulant. For biochemical evaluations of energy metabolites (cholesterol, triglycerides and glucose) and proteins (total proteins, albumin, creatinine and urea), blood was collected by venipuncture with the aid of a Vacutainer® and a 10 mL test tube without anticoagulant. Subsequently, blood samples were centrifuged for 10 minutes at 4,000 rpm, the plasma obtained was pipetted using an automatic pipette, and stored in properly identified Eppendorf tubes, stored in a freezer until analyses were carried out. Blood analyses were performed using commercial Labtest® kits, in a Bioplus® 2000 spectrophotometer.

The statistical model was: $Y_{ijkl} = \mu + \tau_i + P_j + A_k + \varepsilon_{ijkl}$

In which: Y_{ijkl} = observation ijkl; μ = overall mean; τ_i = fixed effect of treatment i; P_j = random effect of period j; A_k = random effect of animal k ; ε_{ijkl} = random error. Analysis of variance was applied considering 5% significance ($p<0.05$). When relevant for comparison between means, the SNK test at 5% significance was used ($p<0.05$). The fecal score variable, as it is a non-parametric variable, was evaluated by the Kruskal-Wallis test (1952) at a significance level of 5%. All analyses were run in SAS software.

RESULTS AND DISCUSSION

There was no difference in daily dry matter intake as a function of : body weight; metabolite weight; water intake; protein and crude protein intake with partial or total replacement of common urea with protected urea in the supplement ($P>0.05$) (Table 2).

The use of protected urea to replace common urea proposes the slow release of nitrogen compounds, increased synergism with a fiber carbohydrate degradation

profile and greater microbial protein production, reducing feed conversion, dry matter and crude protein intake (SALAMI et al., 2021). However, the multiple supplement was composed of a source of true protein, the soybean meal (Table 1) (VALADARES FILHO, 2018), contributing to the maintenance of protein intake and microbial protein production, not modifying the intake of dry matter, protein and crude protein when the common urea is replaced with protected urea.

Table 2: Nutrient intake and nutrient digestibility of sheep subjected to replacement of common urea with protected urea in the supplement.

Item	Treatments					P-value	OM	CV (%)
	100% CU	75%CU : 25% PU	50%CU : 50%PU	25%CU : 75%PU	100% PU			
DMI (Kg/day)	0.890	0.860	0.860	0.900	0.900	0.8721	0.880	7.79
DMIBW (%)	1. 85	1.85	1.81	1.89	1.93	0.8563	1.87	7.64
DMIMW (g/Kg$^{0.75}$)	48.76	48.35	47.65	49.54	50.26	0.8436	48.91	7.40
IH2O /DMI (L/Kg)	1.350	0.890	0.690	0.920	0.950	0.3614	0.960	49.85
PI (g/day)	0.250	0.231	0.227	0.225	0.237	0.2587	0.234	16.05
DMD/PI (g/g)	3.75	3.82	4.02	4.20	3.97	0.4521	3.93	21.49
CPB (g/day)	64.68	63.88	67.11	70.27	63.92	0.3621	65.97	10.68
DMD (%)	45.31	46.39	44.11	48.32	49.57	0.1274	46.74	0.87

DMI: dry matter intake; DMIBW: dry matter intake according to body weight (%); DMIMW: dry matter intake according to metabolic weight; IH2O /DMI: water intake according to dry matter intake; PI: protein intake; CMS/PI: dry matter intake according to protein intake; DMD: dry matter digestibility; CPB: crude protein intake; OM: overall mean; CV: coefficient of variation.

The mean dry matter intake was within the recommended by the NRC (2007), between 1.83 to 1.93% body weight. Likewise, the crude protein intake was within the recommendations of the NRC (2007), around 63 to 69g per day for sheep maintenance. The maintenance of nutrient intake demonstrates that the diets met the nutritional requirements of the ewes.

Dry matter digestibility was maintained with the replacement of common urea with protected urea in the supplement (P>0.05) (Table 2). Due to the slow release of ammonia in the rumen environment, the addition of protected urea allows better use of nitrogen for growth by ruminal microorganisms, increasing nutrient digestibility (JIN et al., 2018). Although the sheep diet was based on sorghum silage, a source of fiber carbohydrates with a slow degradation rate (SANTINI et al., 2020), the use of soybean meal in the supplement, in addition to contributing to the maintenance of intake, also favored the maintenance of digestibility, therefore, was a source of

nitrogen for the maintenance of microbial growth even with the total use of common urea, without relation to total or partial replacement with protected urea.

Partial or total replacement of common urea with protected urea in the supplement did not change the water intake (P>0.05) (Table 3). According to Forbes (1968), in relation to dry matter intake, the predicted mean water intake would be between 2.15 to 2.66 liters per day, 34% higher than observed (Table 3). It is important to point out that the animals were ingesting sorghum silage, which is characterized by being a wet bulky and, therefore, presenting a high percentage of water in its composition, which causes the animal water intake to be reduced, since they are already ingesting water through food. Added to this factor, we emphasize that the equation proposed by Forbes (1968) recommended in the NRC (2007) does not describe the reality of tropical food and environmental conditions.

Urinary parameters of volume and density, as well as fecal parameters did not change (P>0.05) with the partial or total replacement of common urea with protected urea in sheep supplement (Table 3). Values of urine volume and density were within normal limits, according to Reece (2008) and Hendrix (2005), between 100 and 400 mL per 10 kg body weight, and between 1,020 and 1,040 g/mL, respectively. These values reinforce that water intake was at adequate levels, providing urine dilution, and the need to adapt the equations to the environment and tropical foods.

Table 3: Urinary and fecal parameters of sheep subjected to replacement of common urea with protected urea in the supplement.

	Treatments							
Item	100% CU	75%CU:25% PU	50%CU: 50%PU	25%CU: 75%PU	100% PU	P-value	OM	CV (%)
IH_2O (L)	1.04	1.45	1.54	1.78	1.19	0.7851	1.40	44.68
V.Urine (L)	1.66	1.99	2.17	2.54	1.88	0.3678	2.05	21.26
D. Urine	1.0194	1.0196	1.0180	1.0150	1.0170	0.1012	1.017	0.39
FNM (Kg)	1.45	1.47	1.49	1.30	1.59	0.1568	1.46	14.97
FS	2.48	2.72	2.24	2.04	2.64	0.3987	2.42	20.75
FDM (%)	48.06	45.20	47.44	45.45	45.39	0.1247	46.31	7.24
FWDM (g)	707.80	656.77	709.39	594.94	748.99	0.5410	683.5	20.98

IH_2O: water intake; V: volume; D: density; FNM: feces in natural matter; FS: fecal score; FDM: fecal dry matter; FWDM: feces weight in dry matter; OM: overall mean; CV: coefficient of variation.

The mean fecal score was close to normal, according to Gomes et al. (2012). There was no difference in fecal output in natural matter and in dry matter, as dry matter intake and digestibility were similar regardless of the replacement of common urea with protected urea in the supplement.

For the fecal dry matter (FDM), the values were above those found for sheep by Silva et al. (2020) of 35.95% with the use of extruded feed. The increase in the percentage of dry matter in feces is related to lower water intake in diets with sorghum silage, moist food, when compared to extruded diets, dry food.

Regarding metabolites, the replacement of common urea with protected urea in the supplement did not change protein metabolism (P>0.05) (Table 4). Varlyakov et al. (2015) demonstrated that the supply of protected urea can increase circulating albumin, urea and total protein concentrations in one-year-old lambs. Protein metabolites are indicative of dietary protein intake, that is, with the use of common urea or partial or total replacement with protected urea, they promoted the same protein intake in sheep, remaining within the recommended range for the species, according to Silva et al. (2020).

Table 4: Energy and protein metabolites in sheep with replacement of common urea with protected urea in the supplement.

Item	Treatments					P-value	OM	CV (%)	RF*
	100% CU	75%CU:25% PU	50%CU: 50%PU	25%CU: 75%PU	100% PU				
Glucose (mg/dL)	55.60[b]	64.40[a]	53.20[b]	51.80[b]	50.00[b]	0.0241	55.00	9.25	30-94
Urea (mg/dL)	27.48	34.99	32.84	39.51	33.40	0.6589	33.64	20.23	10-92
TP (mg/dL)	5.37	8.68	7.57	8.04	8.78	0.5024	7.69	25.17	3.1-10.7
Albumin (g/dL)	1.55	1.78	2.10	1.60	1.88	0.2589	1.78	28.37	1.1-5.2
Creatinine (mg/dL)	1.01	1.01	1.08	1.07	1.06	0.4764	1.05	27.84	0.4-1.7
Uric acid (mg/dL)	0.31	0.82	0.28	0.28	0.43	0.3314	0.33	48.12	0-1.7
Trigly. (mg/dL)	21.43	22.00	18.23	21.59	19.73	0.1924	20.59	19.95	5-71
Cholesterol (mg/dL)	40.63	35.16	47.16	48.96	47.13	0.4278	43.01	28.30	14-126

Different letters in the same row indicate significant differences by SNK test at 5% level of significance. TP: total protein; Trigly: Triglycerides; OM: overall mean; CV: coefficient of variation. * Valores de referência por Silva et al. (2020).

For energy metabolites, partial or total replacement of common urea with protected urea did not change serum cholesterol or triglyceride levels (P>0.05) (Table 4). Cholesterol and triglycerides are indicators of circulating lipid levels and storage of

fatty acids in the adipose tissue that constitute energy reserves (KANEKO et al., 2008). Therefore, when meeting the nutritional requirements with dry matter intake within the recommended range, there was no need to mobilize reserves and change serum levels of cholesterol and triglycerides, these parameters being within the recommended range for the species, according to Silva et al. (2002).

There was a statistical difference ($P<0.05$) for glycemia, with the use of 75% of common urea and 25% protected urea being superior (64.40 mmol/L) to the other treatments in relation to the glycemic contribution of the animals. According to Wattiaux and Armentano (2015), most propionate is converted into glucose in the liver. But the liver can also use amino acids for glucose synthesis. Probably the other treatments were not efficient in the energy: protein ratio in the short term such as 75% CU and 25% PU, reducing the production of microbial protein, and thus, the source of amino acids for glucose synthesis.

CONCLUSION

The replacement of common urea with protected urea in different proportions of the supplement does not change the intake and digestibility of nutrients, and protein metabolites. However, the use of 75% common urea and 25% protected urea increases the blood glucose level. Therefore, there are no benefits of partial or total replacement of common urea with protected urea in sheep supplementation.

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