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Ruminal parameters and nitrogen balance in sheep fed diets containing residue from the extraction of tamarind pulp

Parâmetros ruminais e balanço de nitrogênio em ovinos alimentados com rações contendo resíduo da extração da polpa de tamarindo

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Abstract

This study measured the ruminal parameters, feed intake, fecal and urinary N production, and N balance (NB) in sheep fed increasing levels of residue from the extraction of tamarind pulp (RETP) on a 0.0%, 5.0%, 10.0%, and 15.0% dry matter (DM) basis. Four mixed-breed male sheep weighing 40.38 ± 2.10 kg of body weight were distributed in a 4 × 4 Latin square design. The animals were allocated to metabolism cages and fed twice a day. Each experimental period lasted 20 days. The pH and concentration of ammonia nitrogen (NH_3-N) in ruminal fluid were measured. Nitrogen intake (NI), fecal N (FN), urinary N (UN), absorbed N (AN), and NB were expressed in $g \cdot day^{-1}$, percentage of consumed nitrogen (CN), and $g \cdot kg^{-1}$ of metabolic weight ($g \cdot (kg^{0.75})^{-1}$. Statistical analysis of these variables was performed by evaluating the variance and regression at 5% probability. The levels of RETP did not alter ($p>0.05$) the pH level or NH_3-N concentration in the rumen fluid, but a quadratic behavior for the same values after feeding ($p<0.05$) was observed. The inclusion of RETP in the diet did not change ($p>0.05$) NI, UN, the AN in $g \cdot day^{-1}$ and $g \cdot (kg^{0.75})^{-1}$, NB in $g \cdot day^{-1}$ and $g \cdot (kg^{0.75})^{-1}$, or the percentage of CN. However, the AN as a percentage of CN showed a linear effect ($p<0.05$) with the inclusion of RETP in experimental diets. For FN in $g \cdot day^{-1}$ and $g \cdot (kg^{0.75})^{-1}$, no difference was observed ($p>0.05$) with the inclusion of RETP, but for FN expressed as a percentage of CN, a linear increase ($p<0.05$) was observed with the inclusion of RETP. Thus, we concluded that diets with up to 15.0% RETP do not alter the pH and NH_3-N in ruminal fluid, NI, UN, or NB. In addition, the inclusion of 15.0% RETP has a laxative affect.

Key words: Ammonia nitrogen, absorbed nitrogen, ruminal pH, urine, nitrogen balance

Resumo

Avaliou-se os parâmetros ruminais, o consumo, a produção fecal e urinária de nitrogênio e o balanço de nitrogênio (BN) em ovinos alimentados com níveis crescentes de resíduo da extração da polpa de tamarindo - REPT (0,0%, 5,0%, 10,0% e 15,0% na MS). Foram utilizados quatro ovinos sem padrão racial definido (SPRD), não castrados, com peso corporal (PC) médio de 40,38 kg ± 2,10 kg alocados

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em gaiolas de metabolismo, alimentados duas vezes ao dia. Foi utilizado um delineamento experimental em quadrado latino 4X4. Cada período experimental teve duração de 20 dias. Os parâmetros ruminais mensurados foram o pH e a concentração do nitrogênio amoniacal ($N\text{-}NH_3$) do líquido ruminal. Os dados de consumo de nitrogênio (N), N fecal, N urinário, N absorvido e BN expressos em g dia^{-1}; % do nitrogênio consumido (NC) e gramas por quilograma de peso metabólico (g $kg^{0,75\ -1}$) foram submetidos á análise de variância (ANOVA) e testados utilizando equação de regressão a 5% de probabilidade. Foi observado que os níveis de REPT não alteraram ($p>0,05$) o comportamento do pH e a concentração do $N\text{-}NH_3$ do líquido ruminal, porém foi observado que o tempo após alimentação alterou de forma quadrática ($p<0,05$) o valor de pH e a concentração do $N\text{-}NH_3$ do líquido ruminal dos ovinos. Foi observado que a inclusão do REPT na alimentação de ovinos não alterou ($p>0,05$) o consumo de N, N urinário e N absorvido em g dia^{-1}, g $kg^{\ 0,75\ -1}$ e o balanço de nitrogênio (BN) g dia^{-1}, g $kg^{\ 0,75\ -1}$ e % do nitrogênio consumido (NC). Porém o N absorvido em % do NC apresentou um efeito linear decrescente ($p<0,05$) com a inclusão do REPT nas rações experimentais. Para o nitrogênio fecal em g dia^{-1}, g $kg^{\ 0,75\ -1}$ não foi observado diferença ($p>0,05$) com a inclusão do REPT, porém para o N fecal expresso em % NC dos ovinos foi alterado ($p<0,05$) de maneira linear crescente com a inclusão do REPT nas rações. Assim, conclui-se que a inclusão de até 15,0% de resíduo da extração da polpa de tamarindo na alimentação de ovinos não altera o valor de pH e nitrogênio amoniacal do líquido ruminal, o consumo de nitrogênio, nitrogênio urinário e balanço de nitrogênio. A inclusão de 15,0% de resíduo da extração da polpa de tamarindo atua como agente laxativo.

Palavras-chave: Nitrogênio amoniacal, nitrogênio absorvido, pH do líquido ruminal, urina

Introduction

With the growing industrialization of Brazil, the production of residue and co-products from agro-industry is increasing. Some of these residues are unusable and considered pollutants. Most, however, can be used in ruminant feed, which reduces production costs and transforms residue materials of low nutritional content into market products with high value, such as meat and milk. The use of agro-industry residues as alternative foods facilitates livestock production and reduces the problems caused by the disposal of residues in the environment (GERON et al., 2011).

The fruit of the tamarind tree (*Tamarindus indica* L.) is distinguished by its excellent nutritional value, pleasant aroma, and sweet-sour taste. In addition, tamarind is widely used in the manufacture of soft drinks, ice creams, pastes, jams, and liqueurs, and is commonly used as an ingredient in condiments and sauces, particularly in the familiar agriculture of the Central−West and Northeast regions of Brazil (PEREIRA et al., 2004).

It is hypothesized that use of the residue from the extraction of tamarind pulp (RETP) in ruminant feed may change the dynamics of rumen fermentation because the protein in the pulp can be converted to ammonia (NH_3) by various proteolytic microorganisms present in the rumen. Although some species of bacteria can incorporate amino acids and peptides directly into microbial protein, approximately 40% to 70% of bacterial N passes through the ruminal NH_3 pool (HRISTOV; BRODERICK, 1994).

Several factors, including the proportion of roughage to concentrate in the diet, the frequency of feeding, and protein and carbohydrate sources in the diet, can alter rumen fermentation, the concentration of short-chain fatty acids and NH_3-N in the rumen fluid, the fluctuation of ruminal pH, and fiber digestion (ZEOULA et al., 2006; MAEDA et al., 2007).

Roughage is an important component in the diet of feedlot ruminants because fiber stimulates mastication and rumination (VAN SOEST, 1994). A study on sheep conducted by Geron et al. (2015) showed that rations with inappropriate levels of concentrate that do not adequately stimulate rumination can reduce saliva production, resulting

in both a decreased ruminal pH and digestibility of fiber.

According to Zeoula et al. (2014), many factors affect the degradation of crude protein (CP) in the rumen, including the chemical composition of and physical CP, microbial proteolytic activity, the access of bacteria to the protein, food retention time in the rumen, ruminal pH, food processing, room temperature, and the use of additives to modify ruminal fermentation.

Nitrogen balance (NB) is an important value that denotes N utilization by ruminants and its loss to the environment (HENRIQUE et al., 2003). Nitrogen balance is calculated by subtracting the amount of N excreted via feces and urine from the amount of N ingested. Further, NB is a marker of protein metabolism, which is used to evaluate the diet and determine if an animal's nitrogenous compounds are in equilibrium (GUIMARÃES JÚNIOR et al., 2007; GERON et al., 2013a).

Nitrogen balance may reflect how animals metabolize end-products and their N excretion, and is positively correlated with the concentration of urea found in urine, which is determined by the N concentration in plasma and intake of N by the animals (VAN SOEST, 1994).

The objective of our study was to evaluate the effect of RETP in the diet of sheep on ruminal parameters, feed intake, fecal and urinary N production, and NB.

Material and Methods

The study was conducted at the Universidade do Estado de Mato Grosso (UNEMAT) in the *University Campus* de Pontes e Lacerda, at the Animal Metabolism Sector and Food and Animal Nutrition Analysis Laboratory, located 15°15′05″S latitude and 59°13′26″W longitude and an altitude of 295 m.

Four mixed-breed male sheep, each with a body weight of 40.38 ± 2.10 kg, were used in the experiment. The sheep were allocated to metabolism cages containing individual feeders and waterers. The experiment rations were provided twice a day. The sheep were dewormed with an ivermectin-based product 15 days before the trial began.

Ten grams of a mineral mixture was added directly to the concentrate of each animal's meal twice a day (5 g salt·animal^{-1}·meal^{-1}). The chemical composition of the mineral supplement was as follows: 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 4800 mg Zn·kg^{-1}.

We used a 4 × 4 Latin square experimental design, with four animals, four periods, and four experimental rations with increasing levels of RETP (0.0%, 5.0%, 10.0%, and 15.0%). According to Gurjão (2006), tamarind and its by-products may have phenolic substances that can act as a laxative in animals, and so the rations did not exceed 15% RETP.

The RETP was obtained from an existing industry located in Pontes e Lacerda - MT. The residue consisted of the fruit peel, seed, and pulp, which was adhered to the seed after extraction of the fruit. The RETP was dried in the sun for 72 h.

The experimental diets (Table 1) contained corn silage and concentrate that consisted of ground corn, cassava flour, soybean meal, urea, and RETP (*Tamarindus indica* L.) in increasing amounts.

The experimental diets were formulated to contain 0.0%, 5.0%, 10.0%, and 15.0% RETP. The proportion of roughage used in the experimental diets was 50% corn silage and 50% concentrate. The rations were balanced with an average of 15% CP (isoproteic) and 69% total digestible nutrients (isocaloric) according to the National Research Council (NRC, 2007) for a moderate gain of approximately 0.150 kg·day^{-1}, as shown in Table 2.

Table 1. Chemical composition of experimental foods.

Variables	Experimental food					
	COS[1]	CGG[2]	CAF[3]	SOM[4]	RETP[5]	Ureia[6]
Dry matter %	25.12	91.68	90.74	90.35	83.49	97.68
Organic matter %	92.16	97.22	94.97	93.37	96.28	-[6]
Crude protein %	8.25	9.83	2.95	47.55	8.52	282.6
Ether extract %	1.61	4.34	0.30	1.51	1.09	-
Neutral detergent fiber %	68.58	13.63	10.30	17.80	50.62	-
Acid detergent fiber %	41.03	11.31	4.13	16.66	38.02	-
Crude fiber %	32.30	9.05	3.30	13.33	30.42	-
No-nitrogen extract %	52.52	74.00	88.42	31.23	57.47	-
Total carbohydrates %	82.30	83.05	91.72	44.31	86.67	-
No-fibrous carbohydrate %	13.72	69.42	81.42	26.51	36.04	-
Mineral matter %	4.79	2.78	5.03	6.38	2.50	-
Total digestible nutrients %	62.30	86.03	74.00	80.73	54.40	-

[1] corn silage, [2]corn ground grain, [3] cassava flour, [4] soybean meal, [5] residue from the extraction of tamarind pulp, [6] trace nutrient in the experimental food. Total carbohydrates (TC) = OM - [EE + CP] and not fibrous carbohydrate (NFC) = 100 - (CP +NDF + EE + MM) second Sniffen et al. (1992).

Table 2. Percentage and chemical composition of the experimental rations containing different levels of inclusion of residue from the extraction of tamarind pulp (RETP) provided to sheep.

Foods	Inclusion levels of residue from the extraction of tamarind pulp in the experimental rations			
	0%	5%	10%	15%
Corn silage	50.00	50.00	50.00	50.00
Corn ground grain	15.40	16.00	23.00	23.00
Cassava flour	19.00	14.00	4.00	0.00
Soybean meal	15.00	14.40	12.40	11.40
Residue from the extraction of tamarind pulp	0.00	5.00	10.00	15.00
Ureia	0.60	0.60	0.60	0.60
Chemical composition				
Dry matter	58.06	57.70	57.41	57.06
Organic matter	93.10	93.19	93.45	93.53
Crude protein	15.03	15.08	1495	14.80
Ether extract	1.76	1.81	2.11	2.14
Neutral detergent fiber	41.02	43.01	45.11	47.05
Acid detergent fiber	25.54	27.20	29.15	30.72
Crude fiber	20.43	21.76	23.32	24.58
No-nitrogen extract	59.14	57.85	56.44	55.46
Total carbohydrates	78.01	79.99	78.08	78.30
No-fibrous carbohydrate	37.00	34.98	32.97	31.25
Mineral matter	4.74	4.59	4.28	4.14
Total digestible nutrients	70.57	69.92	69.35	68.30

TDN estimated from the values of the chemical composition of food.

The ration was weighed each day and the animals had free access to the food, and so refusals represented only 10% of the total feedings. The animals were fed at 6:00 a.m. and 6:00 p.m.

Samples of corn silage were collected from different sites in the silo to determine the DM content. During the experimental period, feed leftover samples were collected for each animal, period, and treatment.

For the measurement of total feces, a collection bag was attached to each sheep during the experimental period. Each animal's feces were weighed every morning and homogenized and composite samples representing 10% of the total weight were taken (GERON et al., 2013a). The samples were then placed in plastic bags that were identified with the 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. For the collection, 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 (ZEOULA et al., 2003).

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 the 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).

For the collection of total urine, plastic buckets covered with screens to prevent contamination with hair, feed, and feces, were placed below the metabolism cages. Twenty milliliters of hydrochloric acid (HCl 1:1) was added to each bucket to prevent bacterial degradation of purine derivatives and uric acid precipitation. Urine was collected at the same time each morning. Urine samples (10% of total production) were stored in the refrigerator (5°C) for further analysis (ZEOULA et al., 2006).

After the experimental period concluded, feed and feces samples were dried in a ventilated oven (55°C for 72 h), ground to 1 mm diameter, and then mixed in equal quantities based on dry weight to form composite samples. The N content of feed, urine, and feces was calculated using the semi-micro Kjeldahl method, using 6.25 as the conversion factor for CP, as described by Silva and Queiroz (2002). Mineral matter (MM) was determined by incineration in a muffle furnace at 600°C and then obtaining the value of organic matter (OM) by difference, and the ether extract (EE) content was determined by extraction washing with petroleum ether, as described by Silva and Queiroz (2002). The concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van Soest et al. (1991).

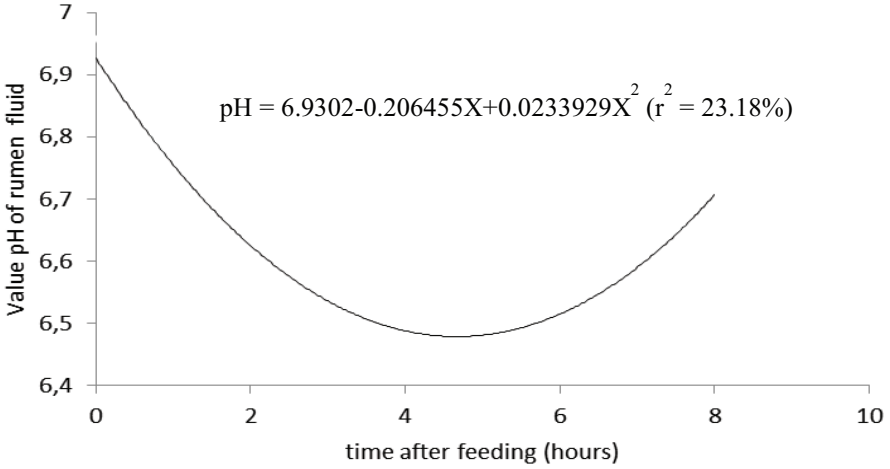
The NB, or retained nitrogen, was obtained using the following formula: NB = [(N supplied g - N scraps g) - (N feces g + N urine g)] as described by Zeoula et al. (2006). The absorbed nitrogen (AN) was calculated using the following equation: AN = [(N provided g - N scraps g) - (N feces g)], and N intake (NI) by the equation: NI = [(N provided g - N scraps g)], as written by Moreno et al. (2010).

Data variance and regression were analyzed using the Statistics and Genetic Analysis System program - SAEG (UFV, 2007). Treatment mean differences were determined by the Tukey test. Tests that had a $p \leq 0.05$ were considered statistically significant. Statistical analyses of ruminal parameters (pH and NH_3-N) were performed in a split-plot design, with treatments in the plots and collection times as subplots.

Results and Discussion

The different levels of RETP (0.0%, 5.0%, 10.0%, and 15.0%) in the diets did not affect ($p>0.05$) the pH of rumen liquid, which had an average pH of 6.67. However, the pH of ruminal fluid differed significantly ($p<0.05$) relative to the time elapsed since feeding, with the pH demonstrating a quadratic behavior dependent on the level of inclusion of RETP (Figure 1), where $pH = 6.9302 - 0.206455X + 0.0233929X^2$ and $r^2 = 23.18\%$.

Figure 1. Value of the pH of the rumen fluid of sheep fed different levels of residue from the extraction of tamarind pulp in relation to time (hours) after the first meal of the day.



The minimum average value of the ruminal pH was 6.47 at 4h24min after feeding, with the pH ranging from 6.48 at 4h00min after feeding to 6.96 at 0h00min before the morning feeding. This decrease in ruminal fluid pH after feeding was correlated with an increased production of short-chain fatty acids from the fermentation of dietary carbohydrates in the rumen (ZEOULA et al., 2006). The return of the pH to near neutrality after 4h24min was probably due to the buffering mechanisms of the rumen, including salivary activity, fiber rumination, and acid absorption by the rumen epithelium. A rising pH of 6.75 was observed at 2h00min after the morning feeding in diets with average values of 44.05% NDF and 34.05% no-fiber carbohydrate (NFC), which is consistent with Van Soest's (1994) findings that diets must contain sufficient amounts of fiber to stimulate salivation and the buffering effects of rumination (Figure 1).

The inclusion of 0.0%, 5.0%, 10.0%, and 15.0% RETP in the diets did not change ($p>0.05$) the concentration of NH_3-N of rumen fluid, which had an average value of 19.01 mg·100 mL^{-1} (Figure 2), a value above the ideal level of 15.00 mg·100 mL^{-1} needed for maximum ruminal fermentation activity, and greater than the concentration of 5.00 mg·100 mL^{-1}, which can limit microbial growth (PEREIRA et al., 2009).

However, the NH_3-N concentration in rumen fluid after the morning feeding exhibited a quadratic behavior ($p<0.05$) for all inclusion levels, where $NH_3\text{-}N = 19.0644 + 1.04094X - 0.17578X^2$ and $r^2 = 20.87\%$ (Figure 2). The maximum mean value of NH_3-N in the rumen fluid was 20.61 mg·100 mL^{-1} obtained at 3h00min after the morning feeding.

The inclusion of RETP did not change ($p>0.05$) the intake of N and fecal excretion of N, calculated in g·day^{-1} and g·$(kg^{0.75})^{-1}$ (Table 3). However, NI

($g \cdot day^{-1}$) increased by 7.36% and 6.32% for the diets containing 10.0% and 15.0% RETP, respectively, compared to the rations with no RETP. This may be due to the RETP increasing FN excretion by 16.05% (in the diet with 10.0% RETP) and 25.69% (in the diet with 15% RETP), compared to the diet without RETP. Thus, the sheep took in more N either because it was not absorbed by the digestive tract, or because of the laxative effect of tamarind (GURJÃO, 2006). According to Geron et al. (2013b), the inclusion of 0.0%, 5.0%, 10.0%, and 15.0% RETP did not change ($p>0.05$) CP intake in $g \cdot day^{-1}$; however, a variation of 5.71% was observed in the intake of CP in rations containing RETP compared to the diets without RETP.

Figure 2. The concentration of ammoniacal nitrogen (NH_3-N) of sheep's rumen fluid fed to the different levels of residue from the extraction of tamarind pulp in relation of time (hours) after the first meal of the day.

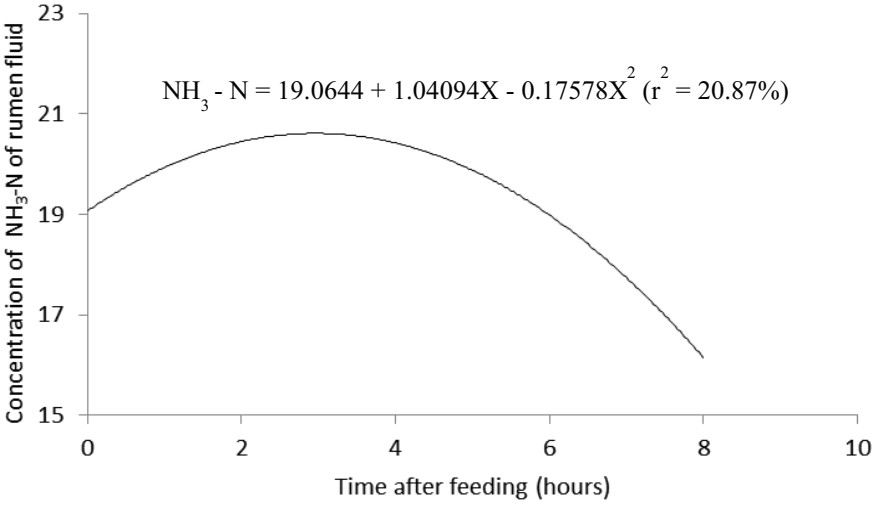


Table 3. Values intake, fecal and urinary excretion of nitrogen, nitrogen balance, absorbed nitrogen ($g\ day^{-1}$) and nitrogen balance compared with nitrogen absorbed to sheep fed with different levels residue from the extraction of tamarind pulp.

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Variables	Inclusion levels residue from the extraction of tamarind pulp in the experimental rations (%)				Regression	% CV
	0%	5%	10%	15%		
NI g day^{-1}	52.17	55.64	56.36	55.69	Y=54.96	6.93
NI g $kg^{0,75\ -1}$	3.33	3.43	3.47	3.55	Y=3.44	8.00
FN g day^{-1}	12.61	14.53	15.02	16.97	Y=14.78	14.78
FN g $kg^{0,75\ -1}$	0.82	0.89	0.92	1.08	Y=0.93	15.66
FN % CN	24.07	25.99	26.61	30.50	[1]	10.10
UN g day^{-1}	11.51	11.04	12,46	1093	Y=11.49	21.37
UN g $kg^{0,75\ -1}$	0.74	0.68	0.77	0.70	Y=0.72	23.65
UN % CN	1.41	1.23	1.37	1.26	Y=1.32	22.62
NB g day^{-1}	28.04	30.07	28.88	27.79	Y=28.70	9.43
NB g $kg^{0,75\ -1}$	1.77	1.86	1.78	1.77	Y=1.79	8.90
NB % CN	54.08	54.13	51.24	49.86	Y=52.33	10.65
NA g day^{-1}	39.55	41.12	41.34	38.72	Y=40.18	5.85

... Continuation

AN g $kg^{0,75\ -1}$	2.51	2.54	2.55	2.47	Y=2.52	6.35
AN % CN	75.94	74.01	73.39	69.50	²	3.69
NB NI^{-1}	0.54	0.54	0.51	0.50	Y=0.52	10.65
NB AN^{-1}	0.71	0.73	0.70	0.72	Y=0.71	8.17

¹Y= 23.8034 + 0.398556X (r^2 = 38.90%); ²Y= 76.1966 - 0.398556X (r^2 = 38.90%)

NI: Nitrogen intake; g $(kg^{0,75})^{-1}$: grams per kilogram of metabolic weight; % CN: percentage of consumed nitrogen; FN: fecal nitrogen; UN: urinary nitrogen; NB: nitrogen balance; AN: absorbed nitrogen. % CV: coefficient of variation.

The value of FN expressed as a percentage of the CN showed increasing linear behavior ($p<0.05$) with the addition of RETP in the diets, which corroborates the pharmaceutical industry's statement that tamarind has laxative activity (GURJÃO, 2006).

The evaluation of the metabolism of nitrogenous compounds in sheep fed diets containing 0.0%, 33.0%, 67.0%, and 100.0% detoxified mammon meal was conducted by Silva et al. (2010), who did not observe ($p>0.05$) changes in NI, which averaged 26.70 $g \cdot day^{-1}$ over the different treatment diets.

According to Van Soest (1994), losses of FN in ruminants can range between 6% and 8% of the CP intake. The average CP intake in diets containing different levels of RETP was 343.52 $g \cdot day^{-1}$ according to Geron et al. (2013b), and the average value of FN was 14.78 $g \cdot day^{-1}$ (Table 3), which gives an estimated loss of 4.30%. The limited mobilization of N from muscle reduces urinary and fecal N excretion (VAN SOEST, 1994), which avoids waste and environmental contamination.

Varying levels of RETP did not affect ($p>0.05$) UN, which had means of 11.49 $g \cdot day^{-1}$, 0.72 $g \cdot (kg^{0.75})^{-1}$, and CN of 1.32%. The EE values of the diets, which varied from 1.76% to 2.14%, and increased proportionally to the level of RETP, did not affect the fermentation of DM and OM in the rumen, which influenced the UN values in this study. However, a study conducted by Ozino et al. (2013), which evaluated different sources of fiber in a forage-based cactus diet in sheep, demonstrated that Tifton 85 hay containing cottonseed showed higher UN excretion ($p<0.05$) compared to the diet without cottonseed. The authors suggest that diets with cottonseed containing 4.20% of EE may have reduced N availability due to the reduction of organic matter digestibility from the addition of fiber (OZINO et al., 2013).

Ruminal ammonia can be absorbed through the rumen wall in its non-ionized form (NH_3), but not its ionized form (NH_4^+) (SANTOS; PEDROSO, 2011). Therefore, reductions in ruminal pH favor NH_4^+, which reduces its absorption, while an increase in rumen fluid pH favors NH_3, which increases ammonia absorption. As there was no effect ($p>0.05$) of inclusion of RETP in the diet on the pH of the rumen fluid, these data corroborate the average value of 11.49 $g \cdot day^{-1}$ obtained for the excretion of UN.

The different levels of RETP did not affect ($p > 0.05$) the NB, or retained N, of sheep with average values of 28.70 $g \cdot day^{-1}$, 1.79 $g \cdot (kg^{0.75})^{-1}$, and 52.33% CN (Table 3). According to Ozino et al. (2013), sheep consuming diets containing 13% CP and diverse fiber sources had a mean value of 13.35 $g \cdot day^{-1}$ NB, lower than that observed in this study. Specifically, we observed a value of 56.66% for the NB expressed as percentage CN, a value similar to the observed mean (52.33% CN) in diets containing different levels of RETP (Table 3).

For AN in $g \cdot day^{-1}$ and $g \cdot (kg^{0.75})^{-1}$ (Table 3), no effect ($p>0.05$) was observed in sheep fed different levels of RETP. However, for AN in percentage CN, a decreasing linear effect was observed ($p<0.05$) with the addition of RETP to the diets, an effect

probably explained by the increase ($p<0.05$) in FN.

For the ratios of $NB \cdot NI^{-1}$ and $NB \cdot NA^{-1}$, no effect was observed ($p<0.05$) with the inclusion of RETP (Table 3). Nitrogen retention, i.e., NB in relation to the AN, reflects the utilization of N in tissue protein synthesis, the formation of new tissues or enzyme systems, or the replacement of old tissue or epithelia (EZEQUIEL et al., 2000). The efficiency of this activity depends on the composition of the N compound that reaches the tissue after intestinal absorption. The results obtained in this study indicate that the proteins or other N forms in RETP were used equivalently, regardless of the level of inclusion of RETP.

The inclusion of up to 15.0% RETP in sheep diets does not alter the pH, NH_3-N from rumen fluid, NI, UN, or NB. The inclusion of 15.0% RETP does, however, have a laxative affect.

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