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In vitro and *in vivo* nutrient digestibility in sheep of rations with and without residue from the extraction of tamarind pulp

Digestibilidade *in vitro* e *in vivo* de nutrientes em ovinos para rações contendo ausência ou presença de resíduo da extração da polpa de tamarindo

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Abstract

This study evaluated the digestibility of nutrients by, and parameters associated with, *in vitro* fermentation using different inocula (sheep ruminal fluid and feces) as well as the *in vivo* digestibility in sheep that were fed rations with 50% concentrate containing either no (0%) residue from the extraction of tamarind pulp (RETP) or 15% RETP. To determine the *in vitro* digestibility (*IV*D) of nutrients, two sheep, weighing 40.38 ± 2.10 kg, were used as inoculum donors. To determine the *in vivo* digestibility of nutrients, we used four sheep and a 3×2 factorial experimental design, with three methods of digestion of nutrients and two experimental rations (0% and 15% RETP). The variables were subjected to analysis of variance and the variables that showed differences at 5% probability were further analyzed using the Tukey test at 5% significance. The *IV*D using different inocula did not significantly differ ($p>0.05$) from the *in vivo* digestibility in sheep for dry matter (DM), organic matter (OM), and neutral detergent fiber (NDF). The different methods for determining nutrient digestibility did not affect ($p>0.05$) the digestibility of DM, OM, crude protein (CP), NDF, and acid detergent fiber (ADF) in rations with 0% and 15% RETP. However, the *IV*D of CP for rations containing 0% and 15% RETP incubated with both inocula was lower ($p<0.05$) than the CP digestibility *in vivo*. The *in vivo* digestibility of ADF for rations containing 0% and 15% RETP was higher ($P<0.05$) than the *IV*D using sheep ruminal fluid and feces as inocula. The pH values and concentration of ammonia nitrogen (NH_3-N) after *in vitro* incubation for 24 h and the *in vivo* assay were not different ($p>0.05$) for the rations containing 0% and 15% RETP, but the pH and NH_3-N of both fermented and rumen contents differed ($p<0.05$) depending on the inocula used and the *in vivo* assay. In summary, the digestibility of DM, OM, and NDF can be determined by the *in vitro* fermentation method using the ruminal fluid or feces of sheep as inocula in rations containing 0% or 15% RETP. However, *in vitro* fermentation is not a suitable method for the determination of pH and NH_3-N concentration.

Key words: Dry matter, ammonia nitrogen, crude protein, pH, rumen

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Resumo

Avaliou-se a digestibilidade de nutrientes e os paramentos da fermentação *in vitro* com diferentes inóculos (líquido ruminal e fezes de ovinos) e *in vivo* em ovinos de rações com 50% de concentrado contendo 0% (ausência) ou 15% de resíduo da extração da polpa de tamarindo (REPT). Para a determinação da digestão *in vitro* dos nutrientes foram utilizados dois ovinos com peso corporal médio de 40,38 kg ± 2,10 kg, como doadores de inóculo e para a determinação da digestibilidade *in vivo* dos nutrientes foram utilizados quatro ovinos por tratamentos distribuídos em duas rações experimentais com 0% e 15% de REPT. O delineamento experimental foi fatorial 3X2, três métodos de digestão dos nutrientes e duas rações experimentais com 0 e 15% de REPT. As variáveis estudadas foram submetidas à análise de variância e para as variáveis que apresentaram diferença a 5% de probabilidade procedeu-se teste de Tukey a 5% de significância. Os valores de digestibilidade para os diferentes métodos *in vitro* com diferentes inóculos e *in vivo* em ovinos para a MS; MO e FDN não apresentaram (P>0,05) diferença entre si. As diferentes metodologias de determinação da digestão dos nutrientes não alteraram (p>0,05) os valores de digestibilidade da MS; MO; PB; FDN e FDA das rações com 0% e 15% do REPT. Entretanto, foi observado que a digestibilidade *in vitro* da PB das rações com 0% e 15% de REPT incubadas com ambos os inóculos apresentaram (p<0,05) valores menores em relação ao ensaio *in vivo* com ovinos. Foi observado que a digestibilidade *in vivo* em ovinos das rações com 0% e 15% de REPT para a FDA apresentaram (p<0,05) valores superiores em relação ao método de digestão *in vitro* para ambos os inóculos. Os valores de pH e concentração do nitrogênio amoniacal ($N\text{-}NH_3$) do conteúdo fermentado após a incubação *in vitro* e do ensaio *in vivo* com ovinos não apresentaram (p>0,05) diferença entre as rações experimentais, porém os dados de pH e $N\text{-}NH_3$ do conteúdo fermentado ou ruminal apresentaram (p<0,05) alteração para os diferentes inóculos e o ensaio *in vivo*. Conclui-se que a digestibilidade da MS, MO e FDN pode ser determinada pelo método da fermentação *in vitro* utilizando como inóculo o líquido ruminal ou as fezes de ovinos para rações com ausência (0%) ou com 15% de REPT. O método de fermentação *in vitro* não é indicado para a determinação do valor de pH e concentração do nitrogênio amoniacal.

Palavras-chave: Matéria seca, nitrogênio amoniacal; rúmen; proteína bruta, pH

Introduction

The efficiency of livestock production systems depends, among other factors, on the adequate supply of nutrients to the animals. The nutritional quality of food has been defined as the product of its voluntary intake and the digestibility and efficiency of use of ingested nutrients (SILVEIRA, 2006).

The most consistent parameter used to evaluate the potential of a certain diet to meet the nutritional requirements of a particular animal in a specific management system system is the animal performance. To understand this, researchers have developed mechanistic and accurate systems of nutritional assessment and prediction of animal production, which are based on detailed analyses of food and animal physiology (SILVA et al., 2003).

Tilley and Terry (1963) developed an *in vitro* digestion technique for ruminant diets that simulates animal digestion and which has been used to predict *in vivo* digestibility. However, this method depends on cannulated animals or on ruminal inoculum collection with a vacuum suction pump and it also evaluates multiple foods simultaneously; these factors can lessen the operating costs of this technique.

The use of *in vitro* techniques for the prediction of *in vivo* digestibility is supported by high correlation coefficients (SILVA; QUEIROZ, 2002), which make it possible to compare digestibility in different species or between forage cuts taken during different periods of growth (SANTOS et al., 2000; SILVA; QUEIROZ, 2002).

In addition, studies evaluating the use of feces from either the same or different species as an alternative inoculum to ruminal liquid have met with relative success (ALCALDE et al., 2001; SILVA et al., 2003; POSADA et al., 2012).

A study performed by Alcalde et al. (2001) evaluated the *in vitro* digestibility (*IV*D) of foods, using ruminal fluid or cattle feces as inocula and showed that cattle feces can be used in a 100/300 dilution to evaluate the *in vitro* digestion of wheat bran, corn ground grain, soybean meal, and cost-cross hay.

The energy and protein levels in a diet are the key factors affecting the growth and efficiency of rumen bacteria (VAN SOEST, 1994). However, other factors also contribute to ruminal fermentation, including pH and passage rate; these depend on feed intake level and the quality and proportion of roughage in the overall diet (ZEOULA et al., 2014), as well as the type and processing of carbohydrates and protein in the food.

According to Geron et al. (2011), alternative foods, including organic residues, can improve the efficiency of the production systems of small and medium ruminants. The use of non-traditional food sources from agribusiness, including RETP in ruminant feed, can change the dynamics of ruminal fermentation. More specifically, protein in the diet can be converted to ammonia through the action of various proteolytic microorganisms in the rumen (HRISTOV; BRODERICK, 1994). Some species of bacteria can incorporate amino acids and peptides directly into microbial proteins, with about 40-70% of nitrogen (N) bacteria passing by the rumen ammonia pool.

According to a study conducted by Gurjão (2006), tamarind pulp has a CP content of more than 8.50%; it is widely used in the pharmaceutical industry as a laxative, probably because of its phenolic compounds. Several factors can affect the extent of CP degradation in the rumen, including the chemical and physical composition of the CP, microbial proteolytic activity, access of bacteria to protein, food retention time in the rumen, ruminal pH, food processing, ambient temperature, and modifying substances of ruminal fermentation (VAN SOEST, 1994; GERON et al., 2015).

In summary, the objectives of this study were to evaluate the *IV*D of two different inocula (sheep ruminal fluid and feces) and the *in vivo* digestibility in sheep of rations containing 0% or 15% RETP. In addition, fermentation parameters (pH and NH_3-N) were evaluated *in vitro* and *in vivo*.

Material and Methods

The experiment was conducted in the Animal Metabolism Sector and the Food and Animal Nutrition Analysis Laboratory, belonging to the Universidade do Estado de Mato Grosso - UNEMAT, located in Pontes e Lacerda city. The protocol of the ethics committee was the 001/2015 CEUA/UNEMAT.

A 3×2 factorial design was used, with three ways to determine digestibility (*in vitro* digestion with inocula of sheep ruminal fluid or feces and digestion *in vivo* in sheep), and two experimental rations containing 0% and 15% RETP.

According to Gurjão (2006), tamarind and its by-products may contain phenolic substances that can have a laxative effect in animals, and so the experimental rations were limited to 0% and 15% inclusion of 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 that was adhered to the seed after extraction of the fruit. The RETP was dried in sunlight for 72 h.

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

The *in vivo* digestibility assay used four mixed-breed male sheep, each weighing 40.38 ± 2.10 kg, for each experimental ration. The sheep were allocated to metabolic cages containing individual feeders and waterers. The experimental rations were provided twice a day. The sheep were dewormed with an ivermectin-based product 15 days before the trial began.

Table 1. Chemical composition of the experimental diets.

Variables	Experimental foods					
	CSI[1]	CGG[2]	CFL[3]	SME[4]	RETP[5]	Urea
Dry matter (DM) %	25.12	91.68	90.74	90.35	83.49	97.68
Organic matter (OM) %	92.16	97.22	94.97	93.37	96.28	-[6]
Crude protein (CP) %	8.25	9.83	2.95	47.55	8.52	282.6
Ether extract (EE) %	1.61	4.34	0.30	1.51	1.09	-
Neutral detergent fiber (NDF) %	68.58	13.63	10.30	17.80	50.62	-
Acid detergent fiber (ANF) %	41.03	11.31	4.13	16.66	38.02	-
Total carbohydrates (TC) %	82.30	83.05	91.72	44.31	86.67	-
Non-fiber carbohydrates (NFC) %	13.72	69.42	81.42	26.51	36.04	-
Total digestible nutrients (TDN)%	62.30	86.03	74.00	80.73	54.40	-

[1] CSI: corn silage, [2] CGG: corn ground grain, [3] CFL: cassava flour, [4] SME: soybean meal, [5]RETP: residue from extraction of tamarind pulp, [6] trace nutrient in the experimental food. Total carbohydrates (TC) = OM - [EE + CP] Sniffen et al. (1992) and non-fiber carbohydrate (NFC) = 100 - (CP + NDF + EE + MM) Weiss (1999).

The animals were housed in metabolic cages for a total of 21 days, with 15 days of adaptation and six days of data collection. At the end of the trial, the data obtained were tabulated with the values of *IV*D of rations using inocula sheep ruminal fluid or feces.

The rations with 0% and 15% RETP were balanced to present an average of 15% CP (isoproteic) and 69% total digestible nutrients (TDN; isocaloric), as recommended by the NRC (2007), for a moderate gain of 0.150 kg animal^{-1} day^{-1}, as shown in Table 2.

Table 2. Percentage and chemical composition of the experimental diets containing 0% and 15% residue from extraction of tamarind pulp (RETP).

Continue ...

Foods	Rations without (0%) or with (15%) RETP	
	0%	15%
Corn silage	50.00	50.00
Corn ground grain	15.40	23.00
Cassava flour	19.00	0.00
Soybean meal	15.00	11.40
Residue from the extraction of tamarind pulp	0.00	15.00
Urea	0.60	0.60
Total	100.00	100.00
Chemical composition expressed in %		
Dry matter	58.06	57.06

... Continuation

Organic matter	93.10	93.53
Crude protein	15.03	14.80
Ether extract	1.76	2.14
Neutral detergent fiber	41.02	47.05
Acid detergent fiber	25.54	30.72
Total carbohydrates	78.01	78.30
Non-fiber carbohydrates	37.00	31.25
Total digestible nutrients[1]	70.57	68.30

[1] Total digestible nutrients (TDN) estimated from the values of the chemical composition of food.

The animals were fed *ad libitum* so that approximately 10% of the rations were leftover every day. The meals were divided into two portions and offered at 6:00 a.m. and 6:00 p.m. daily. 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. During the period of adaptation and collection, management was performed as described by Silva and Leão (1979).

For the development method of the *in vitro* digestion assay, four replicates, or fermentation batteries, were performed. In each battery, three tubes were placed in an experimental ration containing 0% or 15% RETP which was then incubated with two types of inoculum (sheep ruminal fluid and feces). The samples then underwent 24 h of *in vitro* fermentation, as adapted from the technique of Smith et al. (2010). The *IV*D of nutrients assay was conducted after the test in *vivo* digestion.

For the *IV*D of nutrients, two mixed-breed sheep each weighing 40.38 were used as ruminal fluid and feces inoculum donors. Ruminal fluid was collected using a gavage and vacuum pump, as described by Geron et al. (2013).

The basal diet provided to the donor sheep contained 50% roughage (corn silage) and 50% concentrate with 0% RETP (Table 2). Diets were fed *ad libitum* so that approximately 10% of the ration was leftover each day. The basal ration was provided as two meals provided at 7:00 a.m. and 5:00 p.m.

On the day that ruminal fluid and feces samples were collected, the sheep were fed at 7:00 a.m., and 2 h later, the ruminal fluid was collected. Approximately 0.6 L of ruminal fluid per sheep was obtained to form a composed mixture. After collection, the ruminal fluid was filtered through four layers of gauze and placed in a thermal bottle containing CO_2. Then, 0.5 L of the filtered ruminal fluid from each animal was used to make the inoculum, which was used in the *in vitro* incubation of the experimental rations. Feces were collected directly from the rectum and then a 200/200 (tampon/feces) dilution was used, as described by Alcalde et al. (2001). Feces collection was performed 30 min before the collection of ruminal fluid, as feces accumulate in the rectum of animals.

The *IV*D of the nutrients in rations with 0% and 15% RETP was determined by the following formula: *IV*D nutrient = sample weight (g nutrient in DM) - [residue weight (g nutrient in DM) - white weight (g nutrient in DM)] / sample weight (g nutrient in DM) × 100 as documented by Silva and Queiroz (2002).

The *in vivo* digestibility of nutrients was determined by the equation: *In vivo* digestibility = [((g nutrient in provided feed - g of remaining

nutrient) - g nutrient in feces) / g nutrient intake] × 100 as described by Silva and Leão (1979).

After filtering the contents of the tubes, the pH of the fermented content was measured using a digital pH meter after 24 h of *in vitro* incubation.

Sulfuric acid 1:1 (0.2 mL) was added to a 20 mL aliquot of fermented content to acidify the medium and stop fermentation. After 24 h of *in vitro* incubation, the concentration of NH_3-N in the fermented content was determined by distillation with potassium hydroxide (KOH, 2 mol L^{-1}), according to the technique described by Preston (1995).

For the *in vivo* study, ruminal fluid was collected on the last day of the experiment and the pH and concentration of NH_3-N were measured. Ruminal fluid was collected at time 0 (before feeding) and at 8 h (after the first feeding). Samples were obtained using a vacuum pump with 40 mm Hg pressure and a silicone tube (2.0 m long × 12.0 mm diameter), which was lubricated with mineral oil (Nujol) before being introduced into the mouths of the animals (ZEOULA et al., 2003). Approximately 100 mL of ruminal fluid was collected from each animal and filtered through a double cotton cloth. Samples were homogenized and the pH was measured immediately after each collection. After that, about 50 mL of ruminal fluid was transferred to a vial and 1 mL of sulfuric acid (H_2SO_4, PA - 98.0%) added to stop fermentation, and this sample was used to determine the concentration of NH_3-N according to the method described by Fenner (1965), and later modified by Vieira (1980).

Samples of roughage (corn silage) were dried at 55°C for 72 h, and then processed in a Wiley mill using a 1 mm sieve.

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 the value of organic matter (OM) was obtained 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 using the method described by Van Soest et al. (1991), which does not use sulfite and does not correct the NDF and ADF values relative to the mineral content of the fiber.

The TDN of the experimental rations was obtained according to Sniffen et al. (1992), in which TDN = DP + 2.25 × DEE + DTC (DP: digestible protein; DEE: digestible ether extract; DTC: digestible total carbohydrates).

The total carbohydrate (TC) content of feed and feces was determined using the following equation: TC = OM - (EE + CP) (SNIFFEN et al., 1992). The non-fiber carbohydrate (NFC) content of feed and feces was determined by the formula NFC = 100 - (CP + NDF + EE + MM) the according Weiss (1999), without protein correcting of NDF.

The *in vitro* and *in vivo* nutrients digestibility, pH and NH_3-N values (from samples taken both *in vitro* and *in vivo*), were subjected to analysis of variance using the SAEG software (UFV, 2007). Treatment mean differences were determined by the Tukey test, and tests that had a $p \leq 0.05$ were considered statistically significant.

Results and Discussion

No significant difference ($p>0.05$) was observed between the use of sheep ruminal fluid or feces inocula to determine *IV*D and the *in vivo* digestibility in sheep on the values of DM, OM, and NDF (Table 3). This result suggests that the use of sheep feces for determining the *IV*D of DM, OM, and NDF may be conducted with confidence because the *in vitro* assay with feces did not differ from the *in vitro* assay with ruminal fluid inoculum or the

in vivo digestibility in sheep for these nutrients. Furthermore, the collection of sheep feces to use as inoculum in the *in vitro* assay is fast and simple, which reduces stress to the animals.

Table 3. *In vitro* digestibility (*IV*D) of the nutrients obtained from the use of ruminal fluid or feces of sheep (inocula) and *in vivo* digestibility coefficient (DC) of nutrients in sheep fed rations with 0% and 15% residue from extraction of tamarind pulp (RETP).

Rations	*in vitro* DC		*in vivo* DC	Average of DC	% CV
	Ruminal fluid	Sheep feces			
			Dry matter		
0.0% RETP	59.56Aa	51.42Aa	62.60Aa	57.86	11.42
15.0% RETP	51.88Aa	49.43Aa	54.34Aa	51.88	11.42
Average of R	55.72	50.43	58.47	-	-
			Organic Matter		
0.0% RETP	63.49Aa	55.84Aa	63.64Aa	60.99	9.88
15.0% RETP	54.81Aa	54.26Aa	58.45Aa	55.84	9.88
Average of R	59.15	55.05	58.47	-	-
			Crude protein		
0.0% RETP	63.36Ab	59.74Ab	76.28Aa	66.46[1]	9.72
15.0% RETP	60.28Ab	58.00Ab	69.65Aa	62.64[1]	9.72
Average of R	61.82	58.87	72.97	-	-
			Neutral detergent fiber		
0.0% RETP	42.54Aa	32.08Aa	44.09Aa	39.57	24.30
15.0% RETP	39.73Aa	29.90Aa	40.08Aa	36.57	24.30
Average of R	41.14	30.99	42.09	-	-
			Acid detergent fiber		
0.0% RETP	30.76Ab	24.48Ab	40.95Aa	32.06[1]	14.00
15.0% RETP	27.45Ab	25.09Ab	33.22Aa	28.59[1]	14.00
Average of R	29.14	24.79	37.09	-	-

[1] $p<0.05$ (significant); Capital letters differentiate ($p<0.05$) in columns; Lowercase letters differentiate ($p<0.05$) in the lines. % CV: % coefficient of variation. Average of R: average of rations.

There was no significant difference ($p>0.05$) between the 0% and 15% RETP rations on the *in vitro* and *in vivo* digestibility values of DM, OM, CP, NDF, and ADF (Table 3). This finding is consistent with a study by Silva et al. (2013) that showed that different levels of RETP did not change ($p>0.05$) the total digestibility of DM, OM, CP, EE, NDF, and ADF. Similarly, the present study of the *IV*D using ruminal fluid and feces inocula with rations containing 0% and 15% RETP, demonstrated that diets with 15% RETP did not change ruminal fermentation or the digestibility of nutrients.

Geron et al. (2014) found that the *IV*D of nutrients in rations with varying levels of concentrate showed no statistical difference ($p>0.05$) among the levels of concentrate with an average of 59.74%. This finding is similar to that of the present study, which showed that for the ration with 0% RETP, the *IV*D had an average of 59.56% when sheep ruminal fluid was used.

In contrast, the *IV*D of CP in the rations with 0% and 15% RETP incubated with both sheep ruminal fluid and feces inocula showed lower values

($p<0.05$) compared to the CP digestibility obtained *in vivo*. Factors including the concentrate:roughage ratio, addictive substances (PRADO et al., 2010), and anti-nutritional factors, can change the ruminal fermentation process and microorganism synthesis in ruminal fluid. According to Maeda et al. (2011), the use of urea in silage sugarcane subjected to *in vitro* fermentation can change ($p<0.05$) DM digestibility and, consequently, CP digestibility. In addition, the use of bacteria in silage sugarcane led to a reduction in NDF content in the fermentation substrate. Thus, in this study, we used the same rations for both the *in vitro* and *in vivo* digestibility tests, and the difference observed ($p<0.05$) for the *IV*D of CP among the digestion methods may be linked to the dynamic absorption of ruminal metabolites in the *in vivo* study. This absorption helps to maintain the rumen environment, since in the *in vitro* study, the short chain fatty acids and NH_3-N promoted a reduction in bacterial activity and, consequently, a reduction in the hydrolysis of some nutrients.

The digestibility of ADF *in vivo* in sheep for the rations with 0% and 15% RETP was higher ($p<0.05$) than that of the *in vitro* digestion method, for both ruminal fluid and feces inocula (Table 3). These observations indicate that the *in vitro* assay is a tool that can be used in animal nutrition to predict the nutritional value of rations, but variations and fluctuations in the data, especially with CP and ADF, may occur due to differences in absorption kinetics depending on the methodology employed. However, this study demonstrated that the use of sheep feces may be a viable alternative source of bacterial inoculum to determine the *IV*D of rations containing a 50:50 roughage:concentrate ratio.

ADF digestibility in rations containing 0% and 15% RETP may have been influenced by the ADF content in the rations, which, according to Van Soest (1994), is associated with lower food digestibility. Therefore, the ADF content of the rations (Table 2) corroborated the variation observed in ADF digestibility (Table 4). In diets containing similar proportions of forage and concentrate, ADF digestibility decreased when the DM and ADF intake levels increased owing to less retention time in the rumen (RAMOS et al., 2000; GERON et al., 2013).

Table 4. pH and ammonia nitrogen (NH_3-N) values for the *in vitro* and *in vivo* assays for diets containing 0% and 15% residue from extraction tamarind pulp (RETP).

Rations	*in vitro* digestibility		*in vivo* digestibility in sheep	Average of digestibility	% CV
	Ruminal fluid	Sheep feces			
			pH of the fermented content		
0.0% RETP	6.85Ab	7.24Aa	6.75Ab	6.95[1]	2.76
15.0% RETP	7.05Ab	7.36Aa	6.72Ab	7.04[1]	2.76
Average of R	6.95	7.30	6.74	-	-
			NH_3-N concentration of the fermented content		
0.0% RETP	23.68Aa	24.62Aa	16.10Ab	21.47[1]	9.82
15.0% RETP	26.02Aa	24.03Aa	18.81Ab	22.95[1]	9.82
Average of R	24.85	24.33	17.46	-	-

[1] $p<0.05$ (significant); Capital letters differentiate ($p<0.05$) in columns; Lowercase letters differentiate ($p<0.05$) in the lines. % CV: % Coefficient of variation. Average of R: average of rations.

The pH value and concentration of NH_3-N after *in vitro* incubation of 24 h and the *in vivo* test with sheep showed no difference ($p>0.05$) between the rations containing 0% and 15% RETP (Table 4).

However, the pH value and NH_3-N concentration of the fermented rumen content was significantly different ($p<0.05$), depending on which inocula was used in the *in vitro* and *in vivo* assays (Table 4).

The pH values of the fermented content for the *in vivo* and *in vitro* studies with ruminal fluid were similar ($p>0.05$) with averages of 6.95 and 6.74, respectively. However, these two assays differed ($p<0.05$) from the *in vitro* study using feces as inoculum, which showed a mean value of 7.30 for the different rations (0% and 15% RETP). This contrast indicates that the use of feces as inoculum for *in vitro* fermentation can result in a lower concentration of active bacteria due to the reduced production of short chain fatty acids and, consequently, a lower pH value compared to the *in vitro* assay with ruminal fluid and *in vivo* study in sheep.

Gilaverte et al. (2011) evaluated the ruminal parameters and performance of Santa Inês sheep that were fed citrus pulp and wet brewery residue and found that the ration containing wet brewery residue resulted in a ruminal fluid pH of 6.58 10 h after the morning feeding. This pH value was lower than that observed in this study, likely due to the difference in the forage:concentrate ratio, since Gilaverte et al. (2011) used diets with 30% forage that may have favored a lower pH of ruminal fluid after feed intake.

The concentrations of NH_3-N for the *in vitro* fermentation of sheep ruminal fluid or feces inocula were 24.85 and 24.33 mg 100^{-1} mL fermented content, respectively, which were higher values ($p<0.05$) than that of the *in vivo* study in sheep, which showed an average value of 17.46 mg 100^{-1} mL. This difference between the *in vitro* and *in vivo* methods suggests that the kinetics of protein degradation and its derivatives, as well as microbial protein synthesis and absorption of ruminal N (VAN SOEST, 1994), are factors that may have contributed to the observed difference in the concentration of NH_3-N after 24 h of *in vitro* fermentation.

Geron et al. (2013) evaluated the feed intake, digestibility, and ruminal parameters of lambs fed increasing levels of concentrate in tropical environments, and showed that increasing levels of concentrate did not change ($p>0.05$) the concentration of NH_3-N in ruminal fluid, which had an average value of 17.61 mg 100^{-1} mL. This value was below the optimum range of 19.0 to 23.0 mg 100^{-1} mL needed for maximum ruminal fermentation activity (ZEOULA et al., 2003), and above the concentration of 5.0 mg 100^{-1} mL that does not limit microbial growth, as established in the literature.

In conclusion, DM, OM, and NDF digestibility can be determined by an *in vitro* fermentation method that uses sheep ruminal fluid or feces inocula, for rations containing 50% concentrate and either 0% or 15% RETP. However, the *in vitro* fermentation method is not suitable for determining the pH and concentration of NH_3-N in rations with 50% forage and 50% concentrate containing RETP.

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