

## VOLUNTARY INTAKE, APPARENT DIGESTIBILITY AND NITROGEN BALANCE IN EWES FED DIETS WITH DIFFERENT NEUTRAL DETERGENT FIBER LEVELS

GILBERTO DE LIMA MACEDO JUNIOR<sup>1</sup>, LUCIANO FERNANDES SOUSA<sup>2</sup>, FERNANDA NASCIMENTO DE GODOI<sup>3</sup>,  
JUAN RAMON OLALQUIAGA PEREZ<sup>4</sup>, PATRICIA MARIA DE FRANÇA<sup>5</sup>, THAIS ROMANO VASCONCELOS  
ALMEIDA<sup>6</sup>, OITI JOSÉ DE PAULA<sup>7</sup>, ROBERTA DE MOURA ASSIS<sup>8</sup>

<sup>1</sup>Professor, PhD, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil – gilbertomacedojr@gmail.com

<sup>2</sup>Adjunct Professor, collaborator at Fundação Universidade Federal do Tocantins, Araguaína, TO, Brazil

<sup>3</sup>PhD candidate at Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

<sup>4</sup>Tenure Professor at Universidade Federal de Lavras, Lavras, MG, Brazil

<sup>5</sup>Scholarship holder, FAPESP, Post-Doctorate Program of Faculty of Agrarian and Veterinary Sciences of Universidade Estadual Paulista, Jaboticabal, SP, Brazil.

<sup>6</sup>Professor at Instituto Federal Fluminense, Bom Jesus do Itabapoana, RJ, Brazil.

<sup>7</sup>PhD, Technical Coordinator of Núcleo de Inseminação Artificial e Transferência de Embriões, NIARTE, Três Corações, MG, Brazil.

<sup>8</sup>Professor at Universidade Federal de Goiás, Campus Jataí, Jataí, GO, Brazil

### ABSTRACT

This research was carried out to investigate the effect of different levels of forage neutral detergent fiber (fNDF) in the diet offered to ewe on the intake and apparent digestibility of the diet and on nitrogen balance. Sixteen adult, non-pregnant and non-lactating Santa Ines ewes, weighting  $45.01 \pm 5.15$  kg, were used. The experimental design used was a 4x4 latin square, with four treatments (8.67%; 17.34%; 26.01% and 34.69% of fNDF), four animals and four periods. The intake of dry matter (DM), organic matter (OM), gross energy (GE), digestible energy (DE), metabolizable energy (ME), crude protein (CP), digestible protein (DP), neutral detergent fiber

(NDF) and acid detergent fiber (ADF) were influenced positively by the levels of fNDF of the treatments. The apparent digestibility of DM, OM, GE and ADF were influenced by treatments. Nitrogen balances were positive at all levels of forage NDF. This study made it clear that there is a physiological limit to the use of concentrate in diets offered to ewe, being necessary a minimum amount of forage NDF in the diet to reach the maximum efficiency of nutrients use; however, this limit seems to be inferior at the lowest level of fNDF in the current study.

KEYWORDS: animal nutrition; metabolism; ruminants.

### CONSUMO, DIGESTIBILIDADE APARENTE E BALANÇO DE NITROGÊNIO EM OVELHAS ALIMENTADAS COM DIFERENTES NÍVEIS DE FIBRA EM DETERGENTE NEUTRO

### RESUMO

Avaliou-se a influência de diferentes níveis de fibra em detergente neutro de origem forrageira (FDNf) na dieta de ovelhas sobre o consumo, digestibilidade aparente e balanço de nitrogênio. Foram utilizadas 16 ovelhas adultas, não gestantes e não lactantes, da raça Santa Inês, com peso vivo de  $45,01 \pm 5,15$  kg, distribuídas em delineamento quadrado latino (4x4), com quatro tratamentos (8,67%; 17,34%;

26,01% e 34,69% de FDNf), 4 animais e 4 períodos, perfazendo 4 quadrados latinos. As variáveis referentes ao consumo de matéria seca (MS), matéria orgânica (MO), energia bruta (EB), energia digestível (ED), energia metabolizável (EM), proteína bruta (PB), proteína digestível (PD), fibra em detergente neutro (FND) e fibra em detergente ácido (FDA) bem como os coeficientes de digestibilidade

aparente da MS, MO, EB e FDA foram influenciados positivamente pelos níveis crescentes de FDNf dos tratamentos. O balanço de nitrogênio foi positivo em todos os níveis de FDNf. O trabalho evidenciou que existe um limite fisiológico para a utilização de concentrado na dieta de

ovelhas, sendo necessária uma quantidade mínima de FDNf para maior eficiência no aproveitamento dos nutrientes; no entanto, esse limite parece ser inferior ao nível mais baixo de FDNf do presente estudo.

**PALAVRAS-CHAVE:** metabolismo; nutrição animal; ruminantes.

## INTRODUCTION

Animal production is strictly related to nutrition, which depends primarily on four factors: nutritional requirements, composition and digestibility of food and amount of nutrients that the animal consumes (ALLISON, 1985). Dry matter intake is referred to as the most important and decisive factor in animal performance, because it delimits nutrient intake needed to meet the requirements for maintenance and production.

Fiber is used to characterize the food (VAN SOEST, 1994) and establish the limits for ingredient inclusion in diets; however, there is no consensus regarding the definition of fiber or the concentration of dietary fiber able to optimize the energy intake by sheep.

The presence of fiber in the diet for ruminants, in greater or lesser proportion, affects important characteristics of food, relating the energy value and digestibility with rumen fermentation and probably it also controls intake (VAN SOEST, 1994).

Fiber digestibility of forages is not constant for all animals, and the sources of variation are found in plant structure, chemical composition, roughage:concentrate ratio as well as environmental conditions such as high air temperatures. Despite the great interest in knowing the ideal level of fiber in the diet for woolless sheep, few studies have been conducted with this purpose.

MACEDO JUNIOR et al. (2009), studying pregnant sheep, observed that the lower and higher limits of neutral detergent fiber in the forage (NDFf)

are 20 and 35%, respectively. This study aimed to evaluate whether increasing levels of NDF influence on consumption and digestibility of nutrients, as well as nitrogen balance of Santa Ines sheep in maintenance, since dietary NDF limits are unknown for this animal category.

## MATERIAL AND METHODS

The experiment was carried out at the Sheep Production Sector and the chemical analyzes performed at the Laboratory of Animal Research, Department of Animal Science, Universidade Federal de Lavras.

We used 16 Santa Ines sheep with average weight and standard deviation of  $45.01 \pm 5.15$  kg in a 4x4 Latin square with four animals, four treatments and four periods per square. The animals were housed in individual metabolic cages supplied with feeder, drinker and salt trough.

Diets were formulated according to ARC (1980), in order to meet the daily nutritional requirements and allow 10% leftovers, and were constituted of four NDFf levels: diet A - 8.67% of NDFf; diet B - 17.34 % of NDFf; diet C - 26.01% of NDFf; and diet D - 34.69% of NDFf. The animals' diet consisted of chopped (42-day cutting interval) *coast-cross* hay (*Cynodon dactylon* L. Pers.), ground corn grain (*Zea mays* L.), soybean meal (*Glicine max*) (46 % CP) and commercial mineral salt (Table 1), being offered in two daily meals (at 8 a.m. and 3 p.m.), the first accounting for 60% of the total feed offered daily.

Table 1. Nutritional composition of the diet ingredients, expressed in dry matter basis

Feedstuff	Contribution of the ingredients					
	DM (%)	CP (%)	NDF (%)	ADF (%)	Ca (%)	P (%)
<i>Coast cross</i> hay	91.30	8.53	78.63	33.93	0.733	0.434
Corn grain	86.77	10.56	21.58	4.03	0.063	0.311
Soybean meal	88.40	45.62	20.70	10.17	0.452	0.781
Limestone	99.99	-	-	-	35.84	-
Common salt	99.82	-	-	-	-	-
Mineral/Vitamin Supplement <sup>1</sup>	94.36	-	-	-	23.00	9.00

<sup>1</sup> Nutrients / kg of supplement: calcium = 230 g; phosphorus = 90 g; sulfur = 15 g; magnesium = 20 g; sodium = 48 g; cobalt = 100 mg; copper = 700 mg; iron = 2,000 mg; iodine = 80 mg; manganese = 1,250 mg; selenium = 200 mg; zinc = 2,700 mg; fluorine = 900 mg; vitamin A = 200,000 IU; vitamin D3 = 60,000 IU; vitamin E = 60 IU. DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; Ca = calcium; P = phosphorus.

The experiment was conducted in four consecutive and immediate periods, with 15 days for animal's adaptation to the diets and five days of sampling. Hay and concentrate were sampled daily, the latter comprising the concentrated premix in order to obtain the chemical composition of experimental diets (Table 2).

All fecal excretion was collected, weighed and sampled in 20%, every day, before the morning

feeding, and packed in plastic bags properly identified and maintained at -20°C. The urine excreted per animal, in buckets with 100 ml of sulfuric acid at 2N to avoid nitrogen loss, was measured and stored at -20°C, at a rate of 10% of the total volume. The leftovers were collected, identified, weighed and sampled individually for further analysis.

Table 2. Nutritional composition and contribution of the ingredients on the nutritional characteristics of diets

Item	Diet with NDFf levels			
	8.67%	17.34%	26.01%	34.69%
	Chemical Composition			
Dry Matter (%)	83.44	83.10	82.49	83.22
Neutral Detergent Fiber (%)	8.67	17.34	26.01	34.69
Acid Detergent Fiber %	10.92	15.99	32.35	38.90
Crude Protein (%)	19.59	20.47	20.61	21.02
Mineral Matter (%)	4.41	4.49	4.60	4.70
Organic Matter (%)	95.59	95.51	95.40	95.29
Metabolizable Energy (kcal/kg)	2,954	2,948	2,945	2,813
	Proportion of the ingredients in the diets (%)			
<i>Coast cross</i> hay	10.00	20.00	30.00	40.00
Corn grain	66.50	56.50	46.50	37.50
Soybean meal	22.50	22.50	22.50	22.50
Mineral/Vitamin Supplement	1.0	1.0	1.0	1.0

The following values were determined in stool samples, feed leftovers and feed: dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), gross energy (GE) and mineral matter (MM). Analyses of crude protein (CP) and gross energy (GE) were carried out in urine samples. Chemical analyzes were performed using the methodology described by SILVA & QUEIROZ (2002). The metabolizable energy (ME) values were obtained by the difference between digestible energy and energy losses, resulting from the formation of methane and urine, following formulas proposed by SNIFFEN et al. (1992)

$$\begin{aligned} \text{ME} &= \text{GEI} - (\text{FGE} + \text{UGE} + \text{ELD}) \\ \text{ELD} &= \text{GLD} \times \text{GEI} / 100 \\ \text{GLD} &= 4.28 + 0.059 \text{ CDGE} \end{aligned}$$

The coefficients of apparent digestibility (CD)

of nutrients were obtained by the formula, according to the methodology used by COELHO DA SILVA (1979) and MAYNARD et al. (1984):

$$\text{CD} = \frac{(\text{Kgcons} \times \% \text{cons}) - (\text{kglo} \times \% \text{lo}) - (\text{kgfc} \times \% \text{fc})}{(\text{Kgcons} \times \% \text{cons}) - (\text{kglo} - \% \text{lo})} \times 100$$

The values of all the variables were analyzed by regression and means comparison, according to Tukey test, using the Statistical Analysis System (SAS).

## RESULTS AND DISCUSSION

Dry matter and other nutrients intake by sheep fed diets with different levels of NDF can be verified in Table 3.

Table 3. Mean values of nutrient intake of experimental diets

Intake <sup>1</sup>	Diet with NDFf levels				
	8.67%	8.67%	8.67%	8.67%	
Dry matter <sup>2</sup>	1198.07b	1380.02a	1389.04a	1448.04a	10.88
Organic matter <sup>2</sup>	1146.25b	1318.46a	1382.65a	1324.70a	10.91
Neutral detergent fiber <sup>2</sup>	351.03c	488.00b	541.17ab	604.47a	13.63
Acid detergent fiber <sup>2</sup>	134.77c	212.45b	529.74a	553.07a	7.17
Crude protein <sup>2</sup>	232.33b	288.14a	316.5a	313.05a	10.58
Digestible protein <sup>2</sup>	173.237c	216.60b	242.25a	242.12ab	12.00
Gross energy <sup>3</sup>	5116.5b	6084.5a	6447.2a	6062.0a	11.40
Digestible energy <sup>3</sup>	3962.4b	4522.3ab	4764.3a	4363.0ab	13.88
Metabolizable energy <sup>3</sup>	3554.1b	4084.9ab	4279.7a	3916.1ab	13.89

1 - Means followed by the same letter in line do not differ by Tukey test ( $P > 0.05$ ); 2 - g / sheep / day; 3 - kcal / sheep / day.

Both neutral detergent fiber and acid detergent fiber intake increased with the addition of NDFf to the diet. The variation in NDF intake ranged from 0.68 to 1.34%, while in ADF intake ranged from 0.29 to 1.22% in relation to live weight of the sheep consuming diets containing 8.67% and 34.69 % of NDF. The greatest variation in ADF intake was probably due to the selection of the ration ingredients by the animals, which was different among treatments, since the amount of forage increased in the treatments. In treatments with lower roughage proportion, there was almost total consumption, and sometimes complete intake of hay, while the other treatments were consumed partially and the remains contained a greater proportion of stems, therefore, more FDA. This was due to the high ability of sheep to select feed ingredients (VAN SOEST, 1994).

MACEDO JUNIOR et al. (2009) found that the NDFI and ADFI showed linear increase with increasing levels of NDF in the diet. The NDF intake was 34.4g/BW<sup>0.75</sup> and 22.4g/BW<sup>0.75</sup> in diets containing 35% and 9% NDFf, respectively. SILVA SOBRINHO et al. (2002), by working with different levels of concentrate in the diet of wool and woolless sheep, observed that the animals fed diets with 60% concentrate presented a similar NDF intake, with values of 35.5g/BW<sup>0.75</sup>. In the present study, the NDF intake was 33.35g BW<sup>0.75</sup>, in the diet with 34.69% of NDF, corresponding to 40% roughage. VAN SOEST (1994) stated that dairy cows should consume at least 27% roughage in the diet for proper functioning of the rumen. Also, according to this author, there is a high correlation between DM intake and NDF content in the diet, showing that when the level of NDF increases, DM intake decreases. However, in the present study, the ratio

was in reverse, that is, DM intake increased according to increase of total forage levels in the experimental diets. This result can be explained by a better rumen condition with the increase of roughage in the diet. According to VAN SOEST (1994), fiber is an important factor for rumen microbiota balance, favoring not only the consumption but also the digestibility of the diet. Nevertheless, if fiber levels in the diet compromise the supply of fermentable non-fibrous carbohydrate, there could be reduced DM intake (FORBES, 2007).

Regression analysis of the DMI as a function of increasing levels of NDFf in the diets, presented in Table 4, showed a positive effect on the DMI, OMI, NDFI, ADFI, GEI and CPI. The NDF intake in g/kg BW<sup>0.75</sup> was linear ( $Y: 15.6277 + 0.5242X$ ;  $R^2 = 96.30$ ), which does not allow the conclusion that there was a physical limitation of the rumen in consumption regulation, indicating that it was possibly regulated by the energy level of diet (physiological solution). The DMI can be limited by the energy level of diet as well as the amount of fiber (roughage) in the diet, especially when lower quality roughage is used. BENEVIDES et al. (2007) observed that Tanzania grass should not be grazed by sheep for a period exceeding the time required for the emission of 2.5 new leaves per tiller. According to MACEDO JUNIOR et al. (2006), there is a minimum physiological limit for the inclusion of forage in the diet of ruminants. It can be verified in Table 3 that diets containing less than 20% NDF present reduction in dry matter intake. The same behavior was observed in several studies (ALVES et al., 2000; FURUSHO GARCIA et al., 2003; BURGER et al., 2006; CARVALHO et al., 2006; DADO & ALLEN 2006; MACEDO JUNIOR et al., 2006; e MACEDO JUNIOR et al., 2007).

Table 4. Regression equations adjusted for nutrient intake in relation to the levels of NDF (X) and their coefficients of variation (CV) in the different diets

Intake	Regression equations <sup>1</sup>	R <sup>2</sup> (%)	CV(%)
Dry matter <sup>2</sup>	Y: 892.7762 + 42.0727X - 0.7996X <sup>2</sup>	99.98	10.88
Organic matter <sup>2</sup>	Y: 855.5143 + 40.0857X - 0.7651X <sup>2</sup>	99.97	10.91
Neutral detergent fiber <sup>2</sup>	Y: 200.6975 + 20.0058X - 0.2451X <sup>2</sup>	98.74	13.63
Acid detergent fiber <sup>2</sup>	Y: 4.4696 + 0.0516X	93.84	7.17
Crude protein <sup>2</sup>	Y: 145.6084 + 11.6977X - 0.1979X <sup>2</sup>	94.06	10.58
Digestible protein <sup>2</sup>	Y: 145.6084 + 11.6977X - 0.1979X <sup>2</sup>	99.99	12.00
Gross energy <sup>3</sup>	Y: 3437.0118 + 231.8890X - 0.4973X <sup>2</sup>	99.90	11.40
Digestible energy <sup>3</sup>	Y: 2841.0546 + 155.1643X - 3.1946X <sup>2</sup>	98.45	13.88
Metabolizable energy <sup>3</sup>	Y: 2520.9576 + 143.6548X - 2.9725X <sup>2</sup>	99.19	13.89

1 - Significant at 5% probability; 2 - g / sheep / day; 3 - kcal / sheep / day.

VAN SOEST (1994), as well as SILVEIRA (2002), mention that there is a reduction in DM intake when large amounts of soluble carbohydrates or starch are used in the diets. The NRC (1985) suggests that forage intake is insufficient to keep the rumen fermentation on the physiological parameters when diets have more than 70% concentrate. In this assay, increased levels of 60 to 90% concentrate in the diet had a positive effect on physiological parameters (digestibility and N balance) and on a parameter highly correlated with animal production (DM intake).

The sheep in this study had an average dry matter intake of 1007.94 g /animal/day or 2.2% BW, showing that dry matter intake was close to that recommended by NRC (1985), i.e., that dry matter intake should be 1 kg or 2.0% of body weight. Even under negative effect of soluble carbohydrates during the trial period, random variations were observed in the DMI, which suggested a case of sub-clinical ruminal acidosis, although no proven clinical cases were found.

Energy and crude protein consumption were reduced (Table 3) with the decrease of NDFf

in the experimental diets, possibly influenced by the lower DM intake in these treatments. As the levels of NDFf increased, there was a quadratic effect on DEI and MEI (Table 4). However, the treatment with the smallest amount of NDFf (8.67%) had the lowest intake of digestible and metabolizable energy. The NRC (1985) recommends that for adult sheep in maintenance, with 50 kg of live weight, the DEI is 2400 kcal and MEI is 2000 kcal per day. The smallest values of DEI and MEI in this study were 3962.4 kcal/animal/day and 3554.1 kcal/animal/day, respectively, showing that even the lowest intakes obtained here were higher than the recommendations by NRC (1985). Once more it becomes clear that there was a negative effect of high amounts of soluble carbohydrates in rumen balance.

The average coefficients of apparent digestibility of dry matter (DMD), organic matter (OMD), gross energy (GED), crude protein (CPD), neutral detergent fiber (NDFD) and acid detergent fiber (DADF) can be observed in Table 5.

Table 5. Mean coefficients of apparent digestibility of nutrients in diets containing different concentrations of neutral detergent fiber

Coefficients of digestibility (%)	Diet with NDFf levels				
	8.67%	8.67%	8.67%	8.67%	8.67%
Dry matter	78.13a	74.24ab	73.62b	71.95b	5.65
Organic matter	79.34a	75.87ab	75.14b	73.48b	5.23
Gross energy	77.12a	74.06ab	73.87ab	72.06b	5.77
Crude protein	74.73a	74.95a	76.58a	77.17a	6.38
Neutral detergente fiber	57.42a	55.78a	55.69a	59.13a	14.80

Means followed by the same letter in the same line do not differ by Tukey test ( $P > 0.05$ ).

When DM intake increased, there was a reduction in the diet digestibility, probably due to the increase of NDFf, increasing the amount of structural carbohydrate and thus deteriorating its quality, because these carbohydrates have lower digestibility than non-structural ones. VALADARES FILHO (1985) stated that soluble carbohydrates and structural carbohydrate present approximately 90% and 50% digestibility, respectively. Therefore, the increase of fiber in the diet may have caused the reduction of DMD. BERCHIELLI et al. (2006) verified that the quality of forage, the form of presentation and the

amount of soluble carbohydrates may interfere with the DMD.

The CP digestibility was not affected by the levels of NDFf of the diets, probably due to problems that affected DM intake and may have affected the energy/protein relationship

The regression equations of DMD, OMD, GED, CPD and NDFD, in relation to NDFf levels, are shown in Table 6. The regression equations for DMD and OMD were linear, increasing as the NDFf level decreased in the experimental diets.

Table 6. Regression equations for the coefficients of apparent digestibility and nitrogen balance in relation to the levels of NDFf in the diet (X)

Coefficients of digestibility (%)	Regression equations <sup>1</sup>	CV (%)	R <sup>2</sup> (%)
Dry matter	Y: 0.7925 – 0.0022X	5.65	89.00 <sup>1</sup>
Organic matter	Y : 0.8054 – 0.0021X	5.23	91.86 <sup>1</sup>
Gross energy	Y: 0.7830 – 0.0018X	5.77	90.94 <sup>1</sup>
Crude protein	Y: NS	6.38	
Neutral detergente fiber	Y: NS	14.80	
N consumed (g/sheep/ day)	Y: 23.2976 + 1.8716X – 0.0316X <sup>2</sup>	10.58	99.99 <sup>1</sup>
N fecal (g/sheep/ day)	Y: NS	24.34	
N urinary (g/sheep/ day)	Y: NS	22.46	
N retained (g/sheep/ day)	Y: 8.4125 + 1.1625X – 0.0168X <sup>2</sup>	19.53	99.90 <sup>1</sup>

1 - Significant at 5% probability; NS - non significant.

The GED was linear, decreasing as NDFf contents of the diets increased. Clearly, GED was determined by DM and OM digestibility, which had very similar responses. Thus, such observations were possibly due to the increase of NDFf in the diet, since this nutrient has the lowest digestibility among

all the others. The treatments did not affect the NDFD, corroborating BOLZAN et al. (2002), who also did not observe when the amounts of concentrate in the diets increased.

Table 7 shows the mean values of nitrogen (N) intake, fecal N, urinary N, N retention in

relation to nitrogen retention (% of intake) in sheep fed with different concentrations of neutral detergent fiber in forage source and Table 6 presents the regression equations of N retention, fecal N, urinary N and N intake, according to the level of NDF in the diet.

Table 7. Daily average values of nitrogen balance in sheep fed with different concentrations of neutral detergent fiber in diets

VARIABLE	Diet with NDFf levels				
	8.67%	17.34%	26.01%	34.69%	CV (%)
N consumed (g/animal)	37.17b	46.15a	50.64a	50.09a	10.58
Fecal N (g/animal)	9.46a	11.49a	11.88a	11.35a	24.34
Urinary N (g/animal)	10.43a	11.33a	11.31a	10.31a	22.46
N retained (g/animal)	17.29c	23.32b	27.45ab	28.43a	19.53

Means followed by the same letter in the same line do not differ by Tukey test (P> 0.05)

Nitrogen intake was lower in the treatments with the lowest NDF level. As the diets were formulated to be isonitrogenous, the reduction in DM intake in this treatment can be considered as the main responsible factor for the reduction in nitrogen intake.

There was no influence of NDFf levels in N (g/sheep/day) lost in feces and urine. Animals fed diets with lower levels of NDF, 8.67 and 17.34%, showed the lowest amount of N retained, which can be explained by the fact that these treatments had lower N intakes, due to the reduction of the DM, DP and ME intakes, and, consequently, lower N retention, since the protein: energy relation may have affected significantly nitrogen retention. Possibly, the excess of rapid fermentation carbohydrates affected the growth of bacteria, reducing N retention; thus, when the amounts of NDF increased, higher amounts of retained N were observed, suggesting interference of low levels of NDFf, which promoted a drop of DMI in treatments with the lowest levels of NDF and, therefore, also influenced N retention.

Another possible factor is the displacement of the digestion site to the large intestine (LI). FURLAN et al. (2006) reported that corn digestibility is lower in LI. In the present study, the concentrate contained ground corn and soybean in its composition. The N absorption in the LI is low since the microbiota formed in this organ is lost in the feces. Comparing the results of fecal N and urinary N of this study with the values found by PIRES et al. (2008), which were 7.63 g/day and 8.57g/day fecal N for sheep with one or two fetuses, respectively, and 4.99g/day and 5.31g/day for urinary N for sheep with one or two fetuses, respectively, it is evident that the amount of N in this work is numerically greater,

indicating high N losses in urine and feces. The average value of N retained in relation to N consumed was 51.10%, exceeding the value of 26.32% verified by ROCHA (2002), working with Santa Ines sheep.

## CONCLUSION

There is evidence of a physiological limit for the use of concentrate in the diet for sheep, requiring a minimum NDFf for more efficient nutrients use.

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