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Exogenous enzymes in sheep diet: nutritional and physiological parameters

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ABSTRACT. The objective of this study was to evaluate the effect of adding exogenous enzymes to the diet on nutrient intake and digestibility, nitrogen balance, ingestive behavior and rumen movement of ewe lambs. Five ewe lambs, Dorper x Santa Inês crossbred, with an average age of 7 ± 1 months, average weight of 36.40 ± 2.36 kg were assigned in a 5x5 Latin square design. The treatments consisted of adding exogenous enzymes to the concentrate: Allzyme, Fibrozyme®, Amaize®, Mix and Control. Analyses of variance were applied and means were compared by the SNK test, and non-parametric Kruskal Wallis test at 5% significance. The dry matter intake in relation to body weight, crude protein intake and nitrogen intake were higher with the use of amylolytic enzyme compared to the other treatments ($p < 0.05$). Nitrogen balance was higher with the use of amylolytic enzymes and the Allzyme® enzyme complex ($p < 0.05$). A longer time spent in total chewing was observed with the inclusion of fibrolytic and amylolytic enzymes without changes in rumen movement. The use of exogenous enzymes promotes better use of nutrients, with high digestibility of dry matter, neutral detergent fiber and crude protein.

Keywords: starch; fiber; *Ovis aries*; protein.

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Introduction

Exogenous enzymes are concentrated enzyme extracts obtained mainly by fungal (e.g. *Aspergillus oryzae*) or bacterial (e.g. *Bacillus subtilis*) fermentation. Exogenous enzymes have the function of catalyzing specific reactions due to their high degree of affinity for a particular substrate and site of action (Gurung, Ray, & Rai, 2013), transforming macromolecules into simpler precursors. Considered as food additives, their use in the diet for ruminants aims to improve the efficiency of microbial protein synthesis and nutrient use (Thammiah, Samanta, Senani, & Sridhar, 2017).

According to Sujani and Seresinhe (2015), research on the use of exogenous enzymes in ruminant feed has taken place since the 1960s, however, due to the high cost of production, there has been little development in this area. However, with technological advances in the last decade, it has become possible to reduce the cost of production of exogenous enzymes, increasing the amount of these products on the market, and calling researchers' attention to further studies on the form of use (e.g., in concentrate, total diet) and inclusion levels.

For sheep, studies such as those by Bhasker, Nagalakshmi, and Rao (2013) with exogenous fibrolytic enzymes demonstrated that the use of these enzymes did not change nutrient intake, ruminal pH and total rumen nitrogen concentration. On the other hand, Bueno, Martínez, García, García, and Pérez (2013), also examining the effect of exogenous fibrolytic enzymes, observed a reduction in dry matter intake, but without changing the digestibility and feed conversion of nutrients. Mota et al. (2011) evaluated amylolytic enzymes and reported no significant effect of their use on nutrient intake and average daily gain of lambs.

According to Beauchemin and Holtshausen (2011), the addition of exogenous enzymes aims to improve the digestibility and ruminal degradability of fiber, starch and protein, animal performance and reduce feed costs. However, the answers regarding its benefits in the rumen environment and its impact on intake, digestibility and performance are contradictory. Furthermore, there are gaps in the knowledge of which group of these exogenous enzymes (e.g., fibrolytic, amylolytic or proteolytic) performs better, and whether it is possible to use a mixture to explore the synergistic effect between them.

In this context, the hypothesis was that the use of exogenous enzymes affects the intake and digestibility of nutrients, the ruminal parameters and the ingestive behavior of ewe lambs. Therefore, this study aimed to evaluate the use of different exogenous enzymes in the feed of lambs on nutrient intake and digestibility, ruminal parameters and ingestive behavior.

Material and methods

The experiment was conducted at the Goat and Sheep Sector, Experimental Farm Capim Branco, Federal University of Uberlândia, from September 23 to December 7, 2016, and carried out under the approval of the Ethics Committee on Animal Use of the Federal University of Uberlândia according to protocol number CEUA/UFU 093/16.

Five crossbred Dorper x Santa Inês ewe lambs, with an average age of 7 ± 1 months and average weight of 36.40 ± 2.36 kg, were distributed in a 5 × 5 Latin square design. Ewe lambs were housed in individual metabolic cages of 2 m^2. provided with feeders and drinking fountains, following the recommendations of the National Institute of Science and Technology (INCT). The total experimental period was 75 days, divided into five phases, each consisting of ten first days for adaptation and five days for data collection.

The treatments consisted of the inclusion of exogenous enzymes in the concentrate: Control (no enzyme), Allzyme® (enzyme complex), Fibrozyme® (fibrolytic enzyme), Amaize® (amylolytic enzyme) and Mix (enzyme complex: 150g Allzyme® + 180g Fibrozyme® + 150g Amaize®) The level of inclusion of the exogenous enzymes Allzyme®, Fibrozyme® and Amaize® in the feed was respectively 150, 180 and 150 g per kilogram of concentrate, according to the manufacturer. The guarantee levels of the composition of exogenous enzymes are listed in Table 1.

Table 1. Guarantee levels and composition of exogenous enzymes.

Composition (u g^{-1})	Allzyme®[1*]	Fibrozyme®[3*]	Amaize®[2*]	Mix
Pectinase	min. 400	-	-	min. 400
Protease	min. 700	-	-	min. 700
Phytase	min. 300	-	-	min. 300
Beta-glucanase	min. 200	-	-	min. 200
Xylanase	min. 100	min. 100	-	min. 100
Cellulase	min. 40	-	-	min. 40
Amylase	min. 30	-	min. 600	min. 600

*Data provided by Alltech®. [1]A unit of enzyme activity equivalent to the amount of enzyme that catalyzes one gram of soluble substrate per minute at pH 4.8 and 30°C; [2]A unit of alpha-amylase enzyme activity equivalent to the amount of enzyme that catalyzes one gram of soluble starch per minute, at pH 4.8 and 30ºC; [3]A unit of xylanase enzyme activity equivalent to the amount of enzyme that releases one micromole of xylose per minute from xylan at pH 5.3 and 50°C.

All diets had the same composition, that is, basal diet of roughage (corn silage) and concentrate (corn meal, soybean meal, urea, mineral salt and enzymes) in the proportion 30% roughage and 70% concentrate, calculated according to the *Nutrient requirements of small ruminants* (Nacional Research Council [NRC], 2007) for an average daily gain of 200 g. day^{-1}, varying only in the inclusion of enzymes in each treatment. The percentage of each food in the concentrate according to the treatments and the chemical composition of the food and diet are listed in Table 2.

Table 2. Concentrate ingredients and chemical composition of food and diet.

Ingredients (%)	Treatments				
	Control	Allzyme®	Fibrozyme®	Amaize®	Mix*
Corn meal	80	80	80	80	80
Soybean meal	15	15	15	15	15
Mineral salt	3	3	3	3	3
Urea	2	2	2	2	2
Enzyme (g)	-	150	180	150	150
Composition (g kg^{-1})			Silage	Concentrate	Diet
Dry matter			384	895.3	741.91
Crude Protein			73.6	199.8	161.94
Neutral detergent fiber			544.3	85.5	223.14
Total digestible nutrients			-	803	

*Mix – Enzyme complex (75g Allzyme® + 75g Amaize® + 90g Fibrozyme®)

Diets were supplied in two meals a day: at 08h00 and 16h00. In each experimental period, animals were weighed to adjust the feed supply, being readjusted to keep approximately S10% leftovers in the trough. Ovinofos® mineral salt specific for sheep was supplied ad libitum.

Samples of the offered feed and leftovers were weighed daily and stored in plastic bags at −20°C for further analysis. The total fecal output was 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 assessed according to Gomes et al. (2012), in which scale one (1) the feces are dry and opaque; on scale two (2) feces are normal; on scale three (3) the feces are slightly softened; on scale four (4) the feces are softened, losing their shape and glued together (a bunch of grapes); on scale five (5), the feces are soft and without normal shape (swine feces); and on scale six (6) the feces are diarrheic.

Six liters of water were offered daily, measured in a plastic cylinder, with 20 mL graduation and maximum capacity of two liters, with the leftovers measured after 24 hours in the same cylinder. The amount of water lost by evaporation during 24 hours was measured daily in the shed with a reference bucket containing six liters of water measured in a plastic cylinder, with 20 mL graduation and maximum capacity of two liters, in a place free of access to the animals, on a flat surface of the same height as the buckets in the cages.

Urine was collected daily during the experimental periods using plastic buckets containing 100 mL sulfuric acid (H_2SO_4) to avoid possible fermentations and losses of nitrogen (N) by volatilization. After 24 hours, using disposable Pasteur pipettes, drops of urine were transferred from the collecting bucket to the prism of a Megabrix® portable handheld refractometer, with which the urine density was measured. This procedure was performed under fluorescent light, in the same position. After measuring each sample, before inserting another, the refractometer prism was sanitized and dried with a paper towel, in order to avoid interference between the evaluations. After measuring the density, urine volume was measured in plastic cylinders with 20 mL graduation and maximum capacity of two liters, and the collected samples were filtered through disposable paper filters, and later stored in plastic containers at -20ºC for later analysis.

Samples of offer, leftovers and feces were pre-dried according to the INCT – CA G-001/1 method and ground in a Wiley mill using 1mm sieves. After, samples were analyzed for determination of dry matter (DM) content, according to INCT - CA G-003/1, crude protein according to INCT - CA N-001/1, neutral detergent fiber (NDF) by INCT – CA F-001/1 and acid detergent fiber (ADF) by INCT– CA F-003/1 method. Hemicellulose content was determined by the difference between NDF and ADF. For urine samples, the nitrogen content was determined by the Kjeldahl method, according to Detmann et al. (2012) described in INCT - CA N-001/1.

The intake of dry matter, crude protein, neutral detergent fiber, acid detergent fiber and hemicellulose were calculated by the difference between the offer and the leftovers. Water intake was determined from the difference between the offered and the leftovers, taking into account the amount of water evaporated. The apparent digestibility of dry matter, crude protein and neutral detergent fiber was estimated by the difference between the amount consumed and the feces divided by the intake (Salman, Ferreira, & Soares, 2010).

Nitrogen balance (NB), nitrogen retained (NR), nitrogen intake (NI) and the ratio of ingested N and N retained (NING/NRET) were calculated using formulas described by Vieira et al. (2017) where, NB = [(N supplied g – N from leftovers g) – (N in feces g + N in urine g)]; NI = (N supplied g – N from leftovers g); and NRET/NING = (NB/NI).

The ingestive behavior was evaluated on the last day of each experimental period, and the time spent on ingestive activities (food and water), rumination and idle, every five minutes, for 24 hours was measured, according to the methodology proposed by Barros, Monteiro, Dittrich, Fernandes, and Pinto (2011). The chewing time was calculated by the sum of the feeding and rumination times. Feeding, rumination and chewing efficiencies were calculated by dividing dry matter intake by the total feeding, rumination and chewing times.

Rumen movement was determined by auscultation with the aid of a stethoscope for 5 consecutive minutes as described by Lira et al. (2013). Auscultation was always performed by the same observer on the third day of the collection period, one hour after the first meal.

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

Where: Y_{ijkl} = observation ijkl; μ = overall mean; T_i = fixed effect of treatment i; P_j = fixed effect of period j; A_k = random effect of animal k; ε_{ijkl} = random error. Data were subjected to the Shapiro Wilk normality test and the Bartlett homoscedasticity test. Mean values were tested by SNK test considering 5% significance ($p < 0.05$) for type I error. For the fecal score data, the non-parametric Kruskal Wallis test was applied considering 5% significance ($p < 0.05$).

Results and discussion

The use of exogenous enzymes changed ($p < 0.05$) the intake of dry matter, dry matter in relation to body weight, dry matter in relation to metabolic and protein weight, without modifying ($p > 0.05$) the intake of neutral detergent fiber, acid detergent fiber and hemicellulose (Table 3).

The highest intake of dry matter was found for treatments with inclusion of Amaize® (amylolytic) and Fibrozyme® (fibrolytic) compared to diets without enzymes or with an enzyme complex. This is because, according to Freiria et al. (2018), by providing fibrolytic and amylolytic enzymes separately, these in the ruminal environment have the ability to provide a higher energy content to microorganisms, promoting an increase in the number, for example, of fibrolytic and non-fibrolytic bacteria, and thus increasing the colonization and digestion capacity of fiber (e.g. cellulose) and non-fiber (e.g. starch) carbohydrates. For this reason, there is an increase in the rumen emptying speed and the rate of passage of the digesta, allowing an increase in dry matter intake and intake of dry matter in relation to body and metabolic weight.

The reduced dry matter intake for the control treatment and the Allzyme® and Mix enzyme complexes was due to the absence of enzymes (Control), and by the reduction in enzymatic activity and interaction between them, in addition to the effect of diluting their concentration when using enzyme complexes. According to Meale, Beauchemin, Hristov, Chaves, and McAllister (2014), the use of enzyme complexes has the function of including the enzymatic action on various foods and compounds. In turn, Meale et al. (2014) address that the increase in enzyme variability can lead to competition for binding sites with rumen microorganisms and, consequently, a reduction in the action potential or complementarity of enzymes.

Table 3. Intake and digestibility of dry matter in ewe lambs fed or not enzymes in the concentrate.

Item	Treatments					p-value	OV	CV
	Control	Allzyme®	Fibrozyme®	Amaize®	Mix*			
DMI (kg day^{-1})	0.878 B	0.899 B	1.065 A	1.095 A	0.830 B	0.0076	0.953	11.51
DMI (%BW)	2.18 AB	2.25 AB	2.57 AB	2.67 A	2.07 B	0.0231	2.35	11.86
DMI BW ^{0,75}	54.85 AB	56.60 AB	65.14AB	67.53 A	52.14 B	0.0167	59.25	11.73
NDFI (kg day^{-1})	0.292	0.274	0.316	0.301	0.248	0.2053	0.286	15.41
NDFI (%BW)	0.71	0.68	0.75	0.73	0.62	0.3833	0.700	15.49
HEMII (kg day^{-1})	0.173	0.184	0.205	0.199	0.162	0.1677	0.185	15.35
ADFI (kg day^{-1})	0.118	0.089	0.111	0.101	0.086	0.2794	0.101	25.20
CPI (kg day^{-1})	0.141 B	0.186 AB	0.168AB	0.199 A	0.165 B	0.0075	0.172	11.83
DMD (%)	81.96	82.57	82.66	84.61	82.52	0.3381	82.87	2.44
NDFD (%)	63.10	63.46	60.83	62.58	59.35	0.6274	61.82	7.81
CPD (%)	80.83 BC	84.83 A	79.92 C	85.14 A	83.94AB	0.0349	82.93	3.35

DMI: dry matter intake; BW: body weight; NDFI: neutral detergent fiber intake; HEMII: hemicellulose intake; ADFI: acid detergent fiber intake; CPI: crude protein intake; DMD: dry matter digestibility; NDFD: neutral detergent fiber digestibility; CPD: crude protein digestibility; OV: overall mean; CV: coefficient of variation. Different uppercase letters, in the same row, are statistically different by SNK test at 5% significance.

The overall mean of dry matter intake (DMI) and dry matter intake in relation to body weight (DMI/BW) are respectively 10 and 3.54% below the value recommended by the NRC (2007) for ewe lambs. The reduction in DMI and DMI/BW may be directly related to the nature of the diet offered (Table 2), since it contained 30% roughage and 70% concentrate. Concentrate diets have higher particle density, increasing the osmotic pressure in the rumen with consequent greater exposure of the feed matrix to rumen microorganisms, accelerating fermentation, increasing digestibility and reducing DMI (Kozloski, 2011).

The increase in protein intake occurred following the response pattern of dry matter intake, that is, it was higher for Amaize® compared to the other treatments (Table 3) ($p < 0.05$). This reinforces the possibility of reducing enzyme activity and their interaction, in addition to the effect of diluting their concentration when using enzyme complexes.

For all treatments, animals presented an average crude protein intake (CPI) of 26.5% higher than the recommended by the NRC (2007), which is 0.136 kg day^{-1} for this animal category. Protein requirements of ruminant animals are met by the intestinal absorption of amino acids from the synthesis of microbial protein in the rumen and dietary protein undegraded in the rumen (Rotta et al., 2016). This microbial protein production is directly related to carbohydrate fermentation in the rumen and is highly energy dependent. The most readily available source of energy in the rumen environment is non-structural carbohydrates (Medeiros & Marino, 2015), which in the diet for the studied animals was starch from both corn silage and concentrate (Table 2).

Regarding the intake of plant cell wall components, neutral detergent fiber, neutral detergent fiber in relation to body weight, acid detergent fiber and hemicellulose had the same response pattern regardless of

the use or not of exogenous enzymes (p >0.05) (Table 3). This demonstrates that the use or not of enzymes or enzyme complex does not alter the intake of cell wall components.

The average percent intake of neutral detergent fiber in relation to body weight (NDFI/BW) was 0.700, close to that recommended by Ribeiro et al. (2020) of 0.780, which reported that lambs consuming 0.780% neutral detergent fiber as a percentage of body weight performed satisfactorily, without metabolic disorders, such as metabolic acidosis compared to fiber intake recommended by Mertens (2002) of 1.20 to 1.36% in relation to body weight.

Despite the increase in dry matter intake, there was no difference between the use or not of exogenous enzymes in the digestibility of dry matter and neutral detergent fiber (p >0.05). Zilio et al. (2019) analyzed the use of exogenous enzymes (fibrolytic and amylolytic) for dairy cows with a diet of 48% roughage and 52% concentrate, and also reported no significant effect on nutrient digestibility. Therefore, the effect of using exogenous enzymes is not evidenced in diets with a high proportion of fermentable carbohydrates.

The average percentages of 82.87 and 61.82%, respectively, for dry matter and neutral detergent fiber digestibility highlight the high proportion of concentrate in the diet (70%), that is, components that are readily digested by rumen microorganisms. Ítavo et al. (2002) stated that in diets with dry matter digestibility values lower than 66%, food intake is determined by physical factors, that is, they are related to the physical distension of the rumen-reticulum. On the other hand, in diets with values above 66% digestibility, it is the physiological factors that control food intake, that is, the balance between nutrient supply and demand. Therefore, for all diets, intake was controlled by the physiological effect.

Protein digestibility showed a significant difference between treatments (p <0.05) (Table 3), being higher for Allzyme® and Amaize® treatments compared to Control, Mix and Fibrozyme®. The increase in protein digestibility in the treatment with Allzyme® complex was due to the presence and concentration of proteolytic enzymes in this complex, although they were also present in the Mix treatment, which due to the diluting effect of the mixtures did not cause a positive effect on protein digestibility, since proteases are characterized by catalyzing the breaking of peptide bonds in proteins. With the use of the amylolytic enzyme (Amaize®), the increase in protein digestibility occurred, according to Meale et al. (2014), due to the increase in energy intake for rumen microorganisms, and thus greater digestion, synthesis and quantity of proteins of microbial origin.

As a function of protein intake and digestibility, there was a significant effect of nitrogen intake and nitrogen balance (p <0.05), without changing the nitrogen concentration in feces and urine (p >0.05) (Table 4). Nitrogen intake (NI) was higher for the treatment using the Amaize® enzyme than without the use of enzymes or with enzyme complexes (Table 4) (p <0.05).

Table 4. Nitrogen intake, excretion and balance of ewe lambs fed or not enzymes in the concentrate.

Item	Treatments					p-value	OV	CV
	Control	Allzyme®	Fibrozyme®	Amaize®	Mix*			
NI (g day^{-1})	22.67 C	29.79 B	28.09 B	33.22 A	26.19 BC	0.0009	27.99	10.04
N in feces (g day$^{-1)}$	4.31	4.55	5.11	4.59	4.25	0.5872	4.56	19.85
N in urine (g day^{-})[1]	7.37	8.99	11.10	10.01	10.65	0.1193	9.62	22.87
NR/NI	0.40	0.57	0.46	0.54	0.48	0.2559	0.49	24.41
NB (g day^{-1})	9.42 B	17.27 A	12.76AB	17.95 A	12.91AB	0.0171	14.06	26.12

NI: nitrogen intake; N: nitrogen; NB: nitrogen balance; OV: overall mean; CV: coefficient of variation. Different uppercase letters, in the same row, are statistically different by SNK test at 5% significance.

Values of nitrogen excretion for the same animal category presented by Moreno et al. (2010); Bringel et al. (2011); Morgado, Bertocco Ezequiel, Galzerano, and Santos (2014) ranged from 4.0 and 8.5 g day^{-1}, with the average found in this study approximately 25% above the recommended level, indicating that the animals mobilized energy for N excretion in urine. Regarding fecal nitrogen, the average value found in the literature varies between 2.8 and 12.3 g day^{-1} (Moreno et al., 2010; Bringel et al., 2011; Morgado et al., 2014) therefore, they are similar and are within the expected range.

In ruminants, nitrogen compounds degraded in the rumen are converted to ammonia by the action of microbial enzymes. Intake of rapidly fermentable foods, such as starch, increases microbial activity, causing substantial variation in the final fermentation products (volatile fatty acids and ammonia). Thus, when a diet rich in rapidly fermentable ingredients was combined with the enzymatic treatment (Amaize®), its degradation was optimized and the microbial activity increased, due to the greater energy input.

Nitrogen balance (NB), or nitrogen retained, was higher in the treatments using Amaize® and Allzyme®, the same treatments where there was higher CPD (Table 3), indicating greater use of protein by the animals. Moreover, it is noteworthy that NB showed positive values in all treatments, despite the increase in N excretion in urine, proving that there was a better use of dietary protein, once nitrogen excretion (urine and feces) was lower than the nutrient intake.

There was a significant difference for water intake and water intake divided by dry matter intake ($p < 0.05$) according to the addition of enzymes in the diet for ewe lambs (Table 5). The highest water intake was for the treatment with the Allzyme® enzyme complex and the lowest intake for the Mix enzyme complex.

Table 5. Water intake and excretion of feces and urine of ewe lambs fed or not enzymes in the concentrate.

Item	Treatments					p-value	OV	CV
	Control	Allzyme®	Fibrozyme®	Amaize®	Mix*			
WI (L day^{-1})	2.90BC	3.50 A	2.92BC	3.40AB	2.73 C	0.0083	3.09	10.36
CH_2O/CMS (L kg^{-1} day^{-1})	3.28AB	3.13AB	3.87 A	2.84 B	3.34AB	0.0199	3.29	12.16
FNM (kg day^{-1})	0.307	0.407	0.371	0.429	0.317	0.2943	0.366	27.86
FMS (kg day^{-1})	0.155	0.169	0.159	0.178	0.148	0.5135	0.162	17.37
FS*	1.83	2.47	2.27	2.32	1.95	0.1828	2.16	-
Urine (L day^{-1})	1.43	1.34	1.51	1.07	1.06	0.1416	1.28	24.60
UD (g mL^{-1})	1.017	1.020	1.017	1.023	1.022	0.2787	1.020	0.47

*Non-parametric statistics; WI: water intake; WI/DMI: ratio of water intake to dry matter intake; FNM: feces on a natural natter basis; FDM: feces on a dry matter basis; FS: fecal score; UD: urine density; OV: overall mean; CV: coefficient of variation. Different uppercase letters, in the same row, are statistically different by SNK test at 5% significance.

According to the NRC (2007), the increase in voluntary water intake is proportional to the increase in the intake and digestibility of dietary protein. Therefore, the treatments with Allzyme® and Amaize®, due to the higher intake and digestibility of protein, showed an increase in water intake by ewe lambs compared to the control and the Mix enzyme complex added to the feed (Table 5).

Considering water intake as a function of dry matter intake, the average was 9.66% higher than the minimum recommended by the NRC (2007), 2 to 3 $L.kg^{-1}$ DM, this increase is due to dietary composition; diets with a higher proportion of concentrate (89.53% DM) increase the need for water intake per kg dry matter for the need for hydration in the diet, as there is a reduction in the intake of preformed water in foods. In accordance with the NRC (2007), the recommended water intake is 2.69 liters per day, that is, the animals ingested an average amount of water 14.9% higher than necessary. The difference between the estimated and observed values was because the equation proposed by Forbes (1968) does not consider animals in tropical regions with higher temperatures, leading to greater water loss by heat exchange and consequently increasing water intake. Therefore, we emphasize the need for equations that are suitable for sheep in tropical climate.

There was no difference for fecal weight on a natural matter basis (FNM) and fecal weight on a dry matter basis (FDM) with the use or not of exogenous enzymes in the feed for ewe lambs ($p > 0.05$) (Table 5). This result can be related to the fact that digestibility was kept high and stable between treatments (Table 3). There was no difference between the values of fecal score (FS) presented by the ewe lambs as a function of the different supplements with exogenous enzymes, the overall mean of the FS of ewe lambs under study was 2.16, considered normal considering the method of evaluation in values of fecal score (Gomes et al., 2012).

As for the urine volume (UV) and urine density (UD), there was no difference with the use or not of exogenous enzymes in the diet ($p > 0.05$) (Table 5). The overall mean of urine production was 1.28 L day^{-1}. For Reece (2006), in sheep, urine excretion should be between 100 and 400 mL for every 10 kg body weight. The studied animals had an average weight of 36.4 kg, that is, the urine excretion should vary be 364-1.456 mL, thus being able to affirm that the average urine excretion presented by the ewes was compatible with the recommended range. As well as the average urine density. according to Antonelli et al. (2012), for sheep with 1.015 to 1.045.

With respect to ruminal parameters and ingestive behavior, there was a significant difference for chewing efficiency ($p < 0.05$), however, without changing the efficiency of ingestion and chewing of ewe lambs fed or not exogenous enzymes ($p > 0.05$) (Table 6). Vigne et al. (2019) analyzed the inclusion of increasing levels of enzyme complex for diets with a high proportion of concentrate (85%) and also found no significant difference for time spent on feeding and rumination.

Table 6. Rumen movement and ingestive behavior of ewe lambs fed or not enzymes in the concentrate.

Item	Treatments					p-value	OV	CV
	Control	Allzyme®	Fibrozyme®	Amaize®	Mix*			
RM (mov 5 min.$^{-1}$)	7.20	6.60	7.00	6.80	6.75	0.3695	6.87	7.40
Intake (min. day^{-1})	184.00	193.00	154.00	167.00	176.00	0.2547	174.80	17.47
Rumination (min. day^{-1})	361.00	315.00	345.00	332.00	351.00	0.2968	340.80	16.43
Idle (min. day^{-1})	895.00	932.00	941.00	941.00	913.00	0.3179	924.00	7.52
Chewing (min. day^{-1})	545.00	508.00	499.00	499.00	527.00	0.3025	515.60	13.48
Ing. Ef. (g min.$^{-1}$)	5.01	4.80	7.33	6.25	5.72	0.1224	5.82	26.06
Rum. Ef. (g min.$^{-1}$)	2.51	2.87	3.20	3.28	2.51	0.1132	2.88	18.55
Che. Ef. (g min.$^{-1}$)	1.63 B	1.79 AB	2.19 A	2.10 A	1.67 B	0.0376	1.88	15.99

RM: rumen movement; Ing. Ef.: ingestion efficiency; Rum. Ef.: rumination efficiency; Che. Ef.: chewing efficiency; OV: overall mean; CV: coefficient of variation. Different uppercase letters, in the same row, are statistically different by SNK test at 5% significance.

Figueiredo et al. (2013) stated that the rumination activity in adult animals takes around 480 min. per day, and may vary between 240 and 540 min. per day. The result obtained may be related to the fact that the animals are confined in metabolic cages, which facilitated animal access to food, thus reducing ingestion time and increasing idle time. In addition, the lambs received diets made up of 70% concentrate, a food that is more processed and therefore more easily ingested and digested. According to Figueiredo et al. (2013), diets with this characteristic have a large amount of non-fiber carbohydrates and are quickly digested in the rumen, which generated low stimulus for rumination, thus explaining the low time spent by animals in this activity.

The time spent by the animals with chewing was 515.4 min. Figueiredo et al. (2013) stated this activity is essential for the production of saliva that acts on rumen buffering, which prevents metabolic disorders like acidosis. The animals did not show any sign of metabolic disturbance, which corroborates the values of rumen movement (RM) found, since normal RM is indicative of ruminal homeostasis.

Also, no differences were detected for the rumination variables as a function of dry matter intake (Rum Ef DM) and intake efficiency as a function of dry matter (Ing Ef DM). According to Van Soest (1994), rumination time is influenced by the nature of the diet and is proportional to the cell wall content of roughages. Therefore, as the animals received a diet with a large amount of concentrate, the rumination time was only 340.8 minutes, which demonstrates efficiency when considering that the average obtained for Rum Ef DM was 2.88 grams per minute. The reduction in chewing efficiency for the control group or the Mix treatment is a reflection of the reduction in the amount consumed and consequently processed. The close values between the Mix treatment and the control group reinforce the diluting effect of the enzyme mixture for the complex, reducing its effectiveness of action to improve the use of diets.

The use of exogenous proteolytic and amylolytic enzymes is recommended in diets with a high proportion of concentrate for sheep, increasing the use of nutrients. The use of the Mix enzyme complex dilutes the effect of exogenous enzymes. To use such enzyme complex, further studies testing doses higher than those studied here should be conducted in the sheep diet.

Conclusion

The use of exogenous enzymes or exogenous enzyme complexes Amaize and Allyzme increases nutrient intake and digestibility, as well as nitrogen balance and chewing efficiency, without causing deleterious effects on the rumen physiology and ingestive behavior of ewe lambs. The addition of an enzyme mixture does not improve the intake and use of nutrients, similar to the non-use of exogenous enzymes in the diet for ewe lambs.

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