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Intake, digestibility and blood metabolites of lambs fed increasing levels of exogenous fibrolytic enzymes

Consumo, digestibilidade e metabólitos sanguíneos de borregas alimentadas com níveis crescentes de enzima fibrolítica exógena na dieta

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Highlights

Sheep fed exogenous fibrolytic enzymes showed changes in blood creatinine levels.
Fibrolytic enzymes don't affect dry matter intake or blood glucose in sheep.
No significant differences were observed in food intake.

Abstract

The present study aimed to evaluate how the increasing addition of exogenous fibrolytic enzymes in the diet of ewe lambs influenced feed intake, digestive capacity and blood metabolites. The trial was conducted in a 5×5 Latin square design with 5 treatments and 5 replications, using crossbred Santa Inês × Dorper ewe lambs with an average initial weight of 46.48 ± 5.60 kg and approximately 7 months of age. Over a period of 60 days, the animals were housed in individual metabolic cages. The treatments consisted of a control diet and four increasing levels of inclusion of fibrolytic enzymes (FIBROZYME®) (0.5,

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1.0, 1.5, and 2.0% of dry matter), administered daily at the time of diet offering. There were no significant differences in nutrient intake (g animal^{-1} day^{-1}), live weight (%PV^{-1}), and metabolic weight (PV$^{0.75}$) among treatments. There were also no significant differences in the apparent dry matter digestibility, fecal weight or fecal score. However, there was a significant difference in the blood metabolite creatinine but not in the animals' blood glucose levels. Therefore, the addition of exogenous fibrolytic enzymes does not influence the feed intake or blood glucose of ewe lambs but alters plasma creatinine.
Key words: Sheep. Fibrolytic enzyme. Consumption.

Resumo

O presente estudo teve como objetivo avaliar como a adição crescente de enzima fibrolítica exógena na dieta de borregas influencia o consumo alimentar, a capacidade digestiva e os metabólitos sanguíneos. O ensaio foi realizado em um quadrado latino (5x5) com cinco tratamentos e cinco repetições, utilizando borregas mestiças Santa Inês x Dorper com peso inicial médio de 46,48 ± 5,60 kg e aproximadamente sete meses de idade. Durante o período de sessenta dias, os animais foram alojados em gaiolas metabólicas individuais. Os tratamentos consistiram em uma dieta controle e quatro níveis crescentes de inclusão de enzima fibrolítica (FIBROZYME®) (0,5%, 1,0%, 1,5% e 2,0% da matéria seca), administrados diariamente no momento de oferta da dieta. Os resultados mostraram que não houve diferença significativa nos consumos de nutrientes (g animal^{-1} dia^{-1}), peso vivo (%PV^{-1}) e peso metabólico (PV^{0,75}) entre os tratamentos. Também não houve diferença significativa na digestibilidade aparente da matéria seca, no peso das fezes e no escore fecal. No entanto, houve diferença significativa no metabólito sanguíneo creatinina, mas não na glicemia dos animais. Conclui-se que a adição de enzima fibrolítica exógena não influencia o consumo alimentar e a glicemia das borregas, mas altera a creatinina plasmática.
Palavras-chave: Ovinos. Enzima fibrolítica. Consumo.

Introduction

The ability of ruminants to transform unusable plant biomass into foods, such as meat and milk, for human consumption is of significant social and agricultural importance. However, the effectiveness of this process depends mainly on the digestibility of plant cell walls (Adéola & Cowieson., 2011).

The use of additives during the ensiling process has been shown to be a promising technique for improving silage fermentation (Lynch et al., 2013). However, despite technological advances in this area, fiber digestibility still limits ruminants' access to energy available in forage, which can lead to excessive nutrient excretion into the environment (Beauchemin et al., 2003; D. A. de P. Silva et al., 2019).

The application of fibrolytic enzymes as additives has been considered a promising approach to improving fiber degradation in the rumen, which can lead to improved animal performance. The use of these enzymes aims to increase the availability of soluble carbohydrates from cell wall hydrolysis (Lynch et al., 2013), which can be used by ruminants as a source of energy. This can, in turn, improve animal feed utilization efficiency.

Fibrolytic enzymes are included in diets with a high concentrate content because these enzymes have more efficient activity in environments with an acidic pH, as is the case in the rumen of animals that consume concentrate-rich diets. This occurs because concentrate fermentation tends to reduce ruminal pH.

The use of enzymes from *Trichoderma longibrachiatum* extract FIBROZYME, a source of fibrolytic enzymes, assists in fiber fermentation processes for better nutrient use in the cattle diet. These fibrolytic enzymes can break down the fiber present in food, leading to an improvement in the digestibility of starch, a common component in concentrates. Studies have shown an increase in starch digestibility when fibrolytic enzymes are added to feed, suggesting that this strategy can contribute to the better use of nutrients present in the diet, especially in animals with concentrate-rich diets (Adesogan et al., 2014).

Fibrolytic enzymes have been widely studied by the scientific community (Adesogan et al., 2014). However, more studies are required on the real functionality of enzymes in ruminant nutrition, given that the ruminal environment is a complex and variable universe. Understanding the rumen ecosystem and the nature of its interactions with plant cell walls is a way to improve ruminant nutrition using exogenous enzymes (Adéola & Cowieson., 2011).

The inclusion of fibrolytic enzymes in lambs' diet is anticipated to result in notable benefits, including improvements in feed consumption, digestive efficiency and blood metabolite composition. It is anticipated that the main function of the fibrolytic enzymes will be to break down dietary fiber, increasing the amount of nutrients available and facilitating food digestion.

The objective of this study was to evaluate how the addition of exogenous fibrolytic enzymes to lambs' diet influences food consumption, digestive capacity and blood metabolite levels.

Material and Methods

This study was conducted in the sheep and goat sector located at Fazenda Capim Branco, which belongs to the Federal University of Uberlândia (UFU), in Uberlândia, Minas Gerais. This research was carried out between February and May 2017, and all procedures were approved by the Ethics Committee for the Use of Animals (CEUA) of the UFU, registered under protocol nº 017/16.

Five Santa Inês × Dorper crossbred lambs with an average initial weight of 46.48 ± 5.60 kg, an average body condition score of 3.8 and around 7 months of age were used in the experiment. During the adaptation phase, the animals were dewormed, identified, weighed and randomly distributed in metabolic cages containing a feeder, drinker, trough with mineral salt and a device for collecting feces. The experiment lasted 75 days, divided into 5 periods of 15 days: 9 days of adaptation of the animals to the experimental diets and metabolic cages, 5 days of blood collection and fecal excretion, and 1 day to evaluate the animals' glycemic curve, totaling 6 days of data collection.

At the beginning and end of each experimental period, the animals were weighed using scales appropriate for their species. These weights were used to

calculate food consumption in grams per kilogram of live weight (g BW^{-1}), metabolic weight ($PV^{0.75}$) and the amount of leftovers.

The diets were formulated to meet the nutritional needs of medium-sized lambs, with an average daily gain of 200 g per animal (National Research Council [NRC], 2007). Feed was distributed twice a day, at 8:00 am and 4:00 pm, in the form of a total mixed ration (RTM), and the amount consumed was adjusted based on leftovers from the previous day, with an allowed margin of 5–10% surplus in relation to the total provided.

The animals had access to water and mineral salt ad libitum. The diets were composed of corn silage and concentrate in a proportion of 20% roughage and 80% concentrate. The concentrate was composed of ground corn, soybean meal, mineral salt and urea (Table 1).

Table 1
Proportion of ingredients in the composition of the experimental concentrate and bromatological composition of the silage and concentrate

Ingredients			g Kg^{-1}			
Ground Corn			605,0			
Soybean meal			360,0			
Urea			10,0			
Mineral Salt			25,0			
Foods	DM	CP	NDF	FDA	TDN	EE
Focused	897,0	245,0	248,0	73,0	818,8	314,0
Silage	340,0	65,0	566,0	336,0	631,7	24,0

Dry Matter = DM; Crude Protein = CP; Fiber in Neutral Detergent = NDF; Fiber in Acid Detergent=FDA; Total Digestible Nutrients = TDN; Ethereal Extract = EE; Minimum = min.; Maximum = max.

The enzyme used in this study was the commercial product FIBROZYME®, which contains xylanase with 100 $u*g^{-1}$ of the product (Table 2). Enzyme levels were calculated based on the manufacturer's recommendations, considering the daily dry matter intake per animal (kg $animal^{-1}$ day^{-1}). For the treatments, two higher levels and one lower level were used in relation to the commercial recommendation (0.0, 0.5, 1.0, 1.5 and 2.0% DM), in addition to the control group, in that the recommended level was 1% for each kg DM^{-1} consumed. The minimum dosage of xylanase enzyme activity units for each treatment was 101.2, 202.4, 303.6 and 404.8, corresponding to treatments of 0.5, 1.0, 1.5 and 2.0%, respectively. The enzyme was added to the feed when the animals were fed.

Table 2
Description of the FIBROZYME® product by the manufacturer

Composition	Minimum
Xylanase Enzyme	100u* g^{-1}

* One unit of xylanase enzymatic activity is equivalent to the amount of enzyme that releases 1 micromol of xylose per minute from xylan at pH 5.3 and 50°C. Appearance: FIBROZYME is a brown powder. Density: 620 kg m3 -1. Obtained from: Inactivated yeast, Yucca extract, Brewer's dry yeast, Dry fermentation product, *Trichoderma longibrachiatum.*

The feces were analyzed based on their scores, which were determined visually by a single evaluator. This process was crucial for several purposes, including monitoring gastrointestinal health, evaluating diet, controlling parasites, ensuring animal welfare and optimizing production. The scale used for analysis consisted of six categories: dry and dull stools (1), normal stools (2), slightly soft stools (3), soft stools losing their shape and sticking together, known as "grape bunch", (4), soft and non-normally shaped feces, similar to pig feces (5), and diarrheal feces (6) (Gomes et al., 2012). This analysis was carried out over five days of collection, providing a comprehensive and continuous view of the fecal condition of the animals.

The feces were collected in sieves attached to buckets to separate the feces and urine. This process was carried out daily, and the total production weight was recorded, with a 20% portion reserved. After each phase of the experiment, samples were prepared individually from each animal, which was stored in plastic bags and kept at −10°C. At the end of the study, these composite samples were thawed at room temperature for 12 h, placed in aluminum trays and taken to a forced ventilation oven at 55°C until they reached a constant weight to measure pre-dried matter. The samples were then ground in a knife mill with a 1 mm sieve and transferred to plastic containers for further analysis.

Samples of silage, concentrate and feed scraps were collected daily and weighed. At the end of the five days of collection, these samples were mixed and homogenized to obtain a composite sample for each animal in each experimental period. Subsequently, the samples were dried in a forced ventilation oven at 55°C, ground in a knife mill with a 1 mm sieve and stored for future laboratory analysis.

Laboratory analyses were carried out in the Animal Nutrition Laboratory of the UFT School of Veterinary Medicine and Animal Science. The methodology proposed by Cunniff (1995) was followed by determining dry matter, ash, ether extract and crude protein of the analyzed material. For the quantification of neutral detergent fiber (NDF), acid detergent fiber (FDA), cellulose (CEL), hemicellulose (HEMI) and lignin, the methodology proposed by Van Soest et al. (1991). The equation proposed by Van Soest et al. (1991) was used to estimate total carbohydrates (CHT), as follows:

$$CHT = 100 (\%CP + \%EE + \%MM)$$

The equation proposed by Berchielli et al. (2011) to estimate non-fibrous carbohydrates (NFC) in silage was used as follows:

$$CNF = \%CHT - \%NDFcp$$

where NDFap is the portion of NDF corrected for ash and protein.

Due to the presence of urea in the diets, the NFC of the concentrates were estimated as proposed by Van Soest et al. (1991), as follows:

$$\%NFC = 100\ [(\%CP - \%CP \text{ derived from urea} + \%\text{ urea}) + \%\text{ EE} + \%NDF + \%ash]$$

During the digestibility test, the equation proposed by Weiss (1999) was used to calculate the total digestible nutrients (TDN), as follows:

$$TDN = [DCP + DNFC + DNDFap + (DEE \times 2.25)]$$

where DCP, DNFC, DNDFap and DEE represent the intake of digestible crude protein, digestible NFC, NDF corrected for ash and digestible protein, and digestible ether extract, respectively.

After pre-drying the samples of leftovers and feces in an oven at 105°C for 24 h, the final dry matter and nutrient content were determined, allowing the apparent digestibility of these nutrients to be calculated.

Nutrient consumption was calculated using the formula proposed by Maynard et al. (1984), as follows:

$$NI = (Cons \times \%Cons) - (Lft \times \%Lft)$$

where NI is nutrient intake (kg); Cons is the amount of food consumed (kg); %Cons is the nutrient content in the food provided (%); Lft is the quantity of leftovers removed (kg); and %Lft is the nutrient content in the leftovers (%).

The digestibility coefficients of dry matter, organic matter, crude protein, ether extract, NDF, FDA, CEL, HEMI and gross energy were calculated using the following formula (J. F. C. Silva & Leão, 1979):

$$ADC = \frac{(Ingested - Excreted) \times 100}{Ingested}$$

where ADC is the apparent digestibility coefficient (%); Ingested is the average amount of nutrients in the food ingested (offered-leftovers) (kg day^{-1}); and Excreted is the average amount of nutrients in feces (kg day^{-1}).

Blood collections always took place before the first meal of the day, with three collections per experimental period of digestibility, to calculate the average of the evaluated blood parameters. Collections were made by jugular venipuncture with the aid of a vacuntainer® and a test tube with a capacity of 10 mL without anticoagulant. Then, the blood samples were centrifuged at 4000 rpm for 10 min, and the plasma obtained was stored in identified vials and stored at −8°C. The biochemical indicators determined in the blood were creatinine, urea, uric acid, total proteins, albumin, cholesterol, triglycerides, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP). Plasma biochemical analyses were carried out using commercial kits from Labtest®, using a Bioplus® 2000 spectrophotometer (Barueri, São Paulo, Brazil).

To measure glycemia for each experimental phase, blood collections were performed by jugular venipuncture in siliconized tubes with anticoagulant (sodium fluoride with 10% EDTA). The blood was centrifuged, and the plasma was stored in vials for glucose analysis. The pre-established collection times were 0 (before food supply), 3, 6, 9 and 12 h postprandial. The analysis of blood samples obtained during the experimental period was carried out in the Food Analysis Laboratory belonging to the Animal Husbandry course at UFU, Campus Umuarama.

The trial was submitted to a Latin square design (5 × 5) for the discontinued flow parameters and a Latin square design (5 × 5) with repeated measures over time for the continuous flow parameters (blood analyses), with the treatments being the inclusion levels of enzymes in the diets and repeated measurements over time (collection times at 0, 3, 6, 9 and 12 h) with 5 repetitions.

The data were subjected to Shapiro and Wilk (1965) and Bartlett (1937) tests to test the assumptions of normality and homoscedasticity, respectively. After accepting the assumptions, the results were subjected to regression analysis to associate the levels of enzymes in the diet and the collection times with the results. A significance level of 0.05 probability of type I error was observed for decision-making regarding the model that best explained the results.

Results and Discussion

Table 3 presents the results of nutrient intake, revealing that the average dry matter intake (DMI) obtained was 874.0 g/day, while, according to the NRC (2007), this animal category should consume around 1.4 kg/day. This represents a difference corresponding to 60.2%. One possible explanation for this reduction in nutrient intake is the physiological age and body condition score (BCS) of the animals, which had an average BCS of 4.5 on a scale of 5. When the energy density of the feed is higher than the animal's needs, consumption is limited by energy demand, with no rumen repletion, which increases osmotic pressure in the rumen, exposes the food matrix to ruminal microorganisms, enhances fermentation, increases digestibility and reduces DMI (Berchielli et al., 2011).

Table 3
Nutrient consumption by lambs fed with different levels of exogenous fibrolytic enzyme in the diet

	Treatments					Value from P			
Variables	0%	0.5%	1.0%	1.5%	2.0%	L	Q	DL	CV(%)
Dry matter, *g day*$^{-1}$	942.01	902.32	889.57	775.25	859.15	0.160	0.539	0.503	15.84
Dry matter, *%PV*	1,908	1,795	1,764	1,562	1,706	0.102	0.439	0.568	14.56
Dry matter, *PV*$^{0.75}$	50.5	47.72	46.95	41.41	45.39	0.114	0.460	0.546	14.77
Crude Protein, *g day*$^{-1}$	216.8	202.1	201.6	169.6	195.5	0.180	0.442	0.466	18.89
Ethereal Extract, *g day*$^{-1}$	22.27	14.8	14.08	14.08	17.93	0.392	0.087	0.914	44.98
Neutral Detergent Fiber, *g day*$^{-1}$	143.07	128.53	140.24	113.79	127.59	0.178	0.250	0.985	17.37
Acid Detergent Fiber, *g day*$^{-1}$	56.3	48.4	52.1	44.8	50.4	0.228	0.336	0.335	18.95
Hemicellulose, *g day*$^{-1}$	86.1	80.3	87.2	69.5	77.3	0.172	0.916	0.236	17.31
Cellulose, *g day*$^{-1}$	52.3	42.89	48.38	40.24	45.93	0.261	0.304	0.304	20.09
Total carbohydrates, *g day*$^{-1}$	544.8	526.5	513.9	442.6	490.1	0.157	0.639	0.541	17.97
Non-Fibrous Carbohydrate, *g day*$^{-1}$	463.3	429.7	423.1	370.7	415.8	0.214	0.438	0.666	19.77

BW: live weight; Probability of the treatment effect; Effect: L: Linear and Q: Quadratic. Deviation from linearity (DL); Coefficient of variation (CV).

According to the NRC (2007), lambs with an average body weight of 46.48 kg consume 3.5% of their live weight in dry matter. However, an average consumption of 1.74% in relation to live weight and 49.39 g kg^{-1} was observed at PV$^{0.75}$ in relation to metabolic weight. These values were lower for lambs because the diet provided contained 80% concentrate and 20% roughage, making it very energetic and, therefore, reducing consumption. Furthermore, the animals in the study were already in the process of deposition of fat, which can also influence consumption. Adipose tissue plays an important role in controlling satiety through the production of the hormone leptin, which acts on anorexigenic neurons in the brain, inhibiting appetite (Nelson & Cox, 2014).

Even with a DMI below the recommended level, the average consumption of crude protein (CP, 197.12 g day^{-1}) is above that recommended by the NRC (2007) for this animal category, which is 175 g animal^{-1} day^{-1}. In other words, the animals ingested an amount of protein approximately 12.64% higher than that recommended. Considering that the supply of fibrolytic enzymes intensifies fiber degradation in the ruminal environment and that the use of a diet with adequate crude

protein content provides amino acids in the ruminal environment, these mechanisms together provide an improvement in the supply of microbial protein to the small intestine (Rodrigues et al., 2022).

By observing energy intake by animals through the ether extract intake (EE), it is possible to ensure that they are receiving the appropriate amount of energy in their diet, which is essential for their growth, development and production. Additionally, EE monitoring can be useful in identifying potential health problems, such as metabolic or digestive diseases, that may affect food intake. Throughout the experiment, the NDF, FDA, HEMI and CEL intake remained constant, which may suggest that the digestive process of the animals studied presented a certain stability or that there was an adaptation of the organism to the different diets offered during thIS research.

In the present study, the animals consumed an average amount of 130.64 g animal^{-1} day^{-1} of NDF. According to Cunniff (1995), the sheep diet must contain at least 25% NDF to ensure digestive health and prevent metabolic disorders. Adequate intake of NDF contributes to maintaining the health of the animals' gastrointestinal tract, promoting adequate chewing and reducing the risk of ruminal acidosis. No problems of nutritional origin were observed, especially due to the lack of NDF.

Furthermore, carbohydrates are essential nutrients in the diet of ruminants, providing energy for cellular metabolism and meeting the animals' energy needs. The average total carbohydrate (CHT) intake in this study was 503.58 g animal^{-1} day^{-1}, while the average intake of NFC was 420.52 g animal^{-1} day^{-1}. NFC provides a quick and efficient source of energy for animals. The consumption of CCNF represented 83.50% of the CHT; thus, the consumption of carbohydrates of fibrous origin was 16.49%. The quantity and quality of carbohydrates in ruminants' diets are fundamental to the animals' health and performance (Neiva et al., 2021).

The results of nutrient digestibility can be found in Table 4. There were no significant differences ($P > 0.05$) between treatments. In a meta-analysis carried out by Adesogan et al. (2014), the activity of 18 types of commercial fibrolytic enzymes was analyzed, demonstrating an optimal activity of 78–83% at 50–77°C, and 61% of these enzymes present optimal activity at a pH between 4 and 5. The ruminal temperature is between 39 and 42°C, and the pH can vary from 5.5 to 7, depending on the diet, sampling time and feeding frequency of the host (Sniffen et al., 1992).

Table 4
Apparent digestibility of nutrients (%) by lambs fed with different levels of exogenous fibrolytic enzyme in the diet

Variables	Treatments					P-value		CV	DL
	0.0%	0.5%	1.0%	1.5%	2.0%	Q	L		
Dry matter	81.73	75.02	82.09	80.70	81.50	0.587	0.565	0.234	8.26
Crude Protein	89.64	89.91	92.21	91.75	91.77	0.136	0.484	0.679	2.96
Ethereal extract	89.91	90.00	90.93	90.99	91.19	0.355	0.883	0.946	2.89
Fiber in Neutral Detergent	70.30	66.84	72.32	72.29	73.60	0.355	0.790	0.684	12.46
Acid detergent fiber	65.67	69.87	66.78	68.26	69.60	0.469	0.908	0.541	8.68
Hemicellulose	73.30	75.82	75.91	74.90	76.19	0.569	0.722	0.846	7.76
Cellulose	63.70	68.32	64.83	66.45	67.81	0.489	0.895	0.515	9.49
Total Carbohydrates	86.48	86.67	87.81	87.71	88.38	0.193	0.948	0.901	2.84
Non-Fibrous Carbohydrate	93.31	93.35	93.45	93.67	94.08	0.432	0.754	0.999	1.73
Total Digestible Nutrients	88.44	88.63	90.09	90.11	90.42	0.121	0.757	0.839	2.57

Probability of treatment effect – P; Effect: Q: Quadratic and L: Linear. Deviation from linearity (DL); Coefficient of variation – CV.

The enzyme used is xylanase, which is considered a fibrolytic enzyme. Xylanase can break down xylan, one of the main components of plant cell walls, into smaller sugars, such as xylose. The function of xylanase, therefore, is to hydrolyze xylan, a complex and insoluble polysaccharide, into simple sugars that can be easily metabolized (Subramaniyan & Prema, 2002). With the addition of the xylanase enzyme to high-concentrate diets, such as the one used in this study (80 C:20 V), it aims to increase the digestion of starch present in the diet. Starch is crucial in these diets, and its efficient digestion can lead to greater energy availability for animals. Xylanase acts on the fibrous components of the diet, improving the breakdown of starch and facilitating its absorption and metabolism in the animal body. Therefore, the inclusion of xylanase can improve the efficiency of digestion and the use of nutrients, contributing to the performance of animals fed high-concentrate diets.

The addition of the xylanase enzyme to the diet did not have a significant effect on nutrient digestibility in the animal, suggesting that xylanase is not a critical enzyme for the digestion and absorption of nutrients from the diet in question. However, there are conflicting results in the literature regarding the use of exogenous fibrolytic enzymes on animal performance. Some studies have reported increased digestibility of certain dietary components with the addition of fibrolytic enzymes, while others observed no positive effects (Al-Mamun & Uddin, 2017). For example, some studies have shown no

improvements in the ability to digest and assimilate nutrients from silage and alfalfa hay using enzymes, while others have demonstrated an increase in NDF digestibility with increasing enzyme level (Rodrigues et al., 2022). Furthermore, according to Brito (2010), improving the digestibility of a dietary component can provide substrates for bacteria that degrade other components, potentially increasing the degradability of other nutrients in the rumen environment.

The average percentage of digested dry matter was 80.21%, indicating greater efficiency in food conversion. Furthermore, the average digestibility of NDF was 71.07%, while the average digestibility of NFC was even higher, corresponding to 93.57%. This significant improvement in digestion can be attributed to the abundance of fermentable carbohydrates in the diet, as well as the source of fiber used, which was corn silage. Corn silage, with its average starch content of 25–30%, played a key role in increasing digestibility (Rojo et al., 2007).

No significant differences were observed in crude protein digestibility (DPB) with the addition of the xylanase enzyme. However, in concentrate-rich diets, plant fiber can limit DPB by reducing amino acid availability. The presence of the enzyme xylanase in the diet suggests that it can help break down these fibers, releasing nutrients, including amino acids, previously inaccessible to the animal. This implies that xylanase can improve the nutritional efficiency of the diet, allowing the animal to better utilize nutrients for growth, development and production.

In diets with a high concentrate content, the presence of vegetable fibers can compromise the digestion of the ether extract, as these fibers can hinder the access of lipolytic enzymes to dietary lipids. In this sense, the addition of xylanase can help break down these fibers, allowing the release of lipids and consequently increasing their availability for absorption by the animal. However, the digestibility of the ether extract (DEE) did not show a significant difference in the present study. The high-concentrate diet used in this study resulted in an average of 71.07% digestibility of NDF, which can be explained by the high fermentative potential of the diet. This condition provided adequate energy for rumen microorganisms, resulting in greater efficiency in the digestions evaluated.

The digestibility of ADF as well as the digestibility of CEL and HEMI, did not show significant differences. The ADF is a measure that includes CEL, HEMI and lignin, and the addition of xylanase to high-concentrate diets can improve ADF digestibility. This is because the degradation of HEMI by xylanase can increase the availability of nutrients to rumen bacteria, thereby improving the fermentation and digestion of carbohydrates.

CEL is a fibrous carbohydrate fraction that is part of dietary fiber and consists mainly of glucose chains linked by beta-1,4-glycosidic bonds. The inclusion of the xylanase enzyme can help break these bonds and improve CEL fermentation and digestion. Furthermore, xylanase can break the glycosidic bonds present in the xylan present in HEMI. However, the effectiveness of the enzyme used in this study had no significant effect on the digestibility of ADF, CEL and HEMI.

The quality of animal feces is determined using the fecal score (Table 5), which considers the consistency and appearance of the feces. The addition of xylanase to the diet can improve nutrient digestion, reducing the amount of undigested material excreted in the feces and consequently improving feces quality. However, no significant differences were observed between fecal weight and fecal score since there were no differences in dry matter intake. The average fecal score was 1.9, which is close to the ideal value recommended for healthy feces, which is 2 (Gomes et al., 2012).

Table 5
Stool weight and fecal score

Variables	Treatments					P-value		CV	DL
	0.0%	0.5%	1.0%	1.5%	2.0%	Q	L		
Stool weight(g)	0.461	0.517	0.374	0.338	0.377	0.13	0.779	0.39	36.02
Fecal Score	2.4	1.8	1.8	1.8	1.9	0.14	0.131	0.726	24.39

Linear Effect (L); Quadratic Effect (Q); Deviation from linearity (DL); Coefficient of variation (CV).

Table 6 presents the results of the blood biochemical profiles of the animals evaluated. It is important to highlight that diet plays a crucial role in regulating total cholesterol levels in blood plasma, which reflects the amount of circulating lipids. The diet with a high proportion of concentrate used favored the production of propionic acid in the rumen but did not affect serum cholesterol levels, which remained within the ranges considered normal (Varanis et al., 2020).

Table 6
Blood biochemistry of lambs fed increasing levels of fibrolytic enzyme in the diet

Variables	Treatments					VR	P-value		DL	CV(%)
	0.0%	0.5%	1.0%	1.5%	2.0%		L	Q		
Creatinine, *mg dL^{-1}*	1.04	1.01	1.01	0.98	0.94	0.61-1.66	0.036	0.687	0.912	6.66
Urea, *mg dL^{-1}*	56.60	53.26	56.26	52.46	54.06	8.4-61.5	0.227	0.584	0.155	5.97
Uric acid, *mg dL^{-1}*	0.77	0.68	0.72	0.82	0.68	0-1.4	0.824	0.851	0.231	20.17
Total Protein, *g L^{-1}*	6.28	6.07	6.30	6.57	6.44	5.4-11.0	0.136	0.730	0.281	5.60
Albumin, *g L^{-1}*	3.84	3.83	3.67	3.81	3.78	1.9-3.6	0.720	0.590	0.710	7.51
Triglycerides, *mg dL^{-1}*	56.80	55.93	55.66	57.2	55.8	7-43	0.935	0.945	0895	11.08
Cholesterol, *mg dL^{-1}*	35.46	38.46	38.20	38.93	37.66	36.3-94.0	0.558	0.446	0.933	15.15
Alkaline Phosphatase, *UI L^{-1}*	203.53	204.46	182.66	182.06	184.66	58-727.7	0.358	0.747	0.859	23.24
AST, *UI L^{-1}*	260.80	192.06	178.60	188.19	230.46	47-353.5	0.444	0.026	0.945	27.48
GGT, *UI L^{-1}*	51.06	43.80	50.73	48.06	49.80	31-154	0.771	0.247	0.036	8.46

1Y = 1.042800-0.044400X (R2=94.00%); Aspartateaminotransferase (AST); Gammaglutamyltransferase (GGT); Alkaline Phosphatase (FA); Linear Effect (L); Quadratic Effect (Q); Deviation from linearity (DL); Coefficient of variation (CV); Reference Value (VR): Varanis et al. (2020).

Based on Rodrigues et al. (2022), it can be inferred that blood triglyceride levels are indicative of the availability of energy provided by the diet and its quality. Triglyceride levels were not affected by the diet provided, suggesting that energy processing in the diet was adequate and did not result in changes in lipid levels in the animals' bloodstream during the experimental period.

Elevated levels of the enzyme aspartate aminotransferase (AST) are often associated with severe and widespread liver damage, especially when accompanied by other signs, such as jaundice. However, in the context of this study, no signs of liver damage were observed. The AST enzyme results obtained indicate that the levels are within normal standards in accordance with the reference ranges established for the specific category of animals evaluated. (Varanis et al., 2020). Alkaline phosphatase (ALP) is an enzyme present in all organs, but mainly in the liver. Its concentration reflects liver health, and the animals studied presented values within the recommendations. The concentration of the GGT enzyme was also within the recommended values for the species and category evaluated, indicating the liver health of the animals (Varanis et al., 2020).

Serum albumin levels were stable and within the physiological ranges of the study. Blood proteins are synthesized mainly in the liver and reflect nutritional status and liver functionality (Neiva et al., 2021). Table 6 shows that the total protein level was within the reference value, suggesting that the animals were ingesting an adequate amount of metabolizable protein to maintain their serum levels. The serum uric acid levels observed in the animals in this experiment are within the reference range (0–1.4), indicating balance in the rumen environment and adequate activity of the microbiota to produce endogenous protein, grow and multiply. This can be attributed to the availability of food protein, fiber and energy, as well as highly fermentable carbohydrates in the ruminal environment and large intestine (Varanis et al., 2020).

The urea levels obtained in this experiment are within the recommended values, suggesting that the diet offered to the animals met their nutritional needs in relation to crude protein content. Only creatinine showed a significant difference ($P < 0.05$), with a linear decreasing behavior of 0.22 mg/dL for each 0.5% inclusion of the fibrolytic enzyme. The observed values (1.02 mg/dL) are below the range considered normal for the species (1.2 to 1.9 mg/dL), as indicated by Rodrigues et al. (2022). The plasma creatinine level approached the minimum recommended value, suggesting adequate kidney function. This is because changes in plasma creatinine concentration are entirely caused by variations in creatinine excretion, which reflects renal function (Meyer & Harvey, 2004).

Table 7 of the study shows the results of the glycemic profile of lambs subjected to different levels of fibrolytic enzymes. The data demonstrate that there was no significant difference ($P > 0.05$) in blood glucose concentration. This can be attributed to the similarity of the diets used in the different treatments and the regulatory mechanisms of glycemia in ruminants, as explained by Kozloski (2011).

Table 7
Glycemia of lambs (mg/dL) fed with increasing levels of fibrolytic enzyme in the diet

	Time (H)					P - Value			
	0	3	6	9	12	L	Q	DL	CV(%)
0.0%	78.20	85.60	77.60	76.00	72.20	0.395	0.594	0.787	
0.5%	66.00	77.00	73.80	80.60	68.20	0.752	0.223	0.714	19.76
1.0%	78.60	81.60	88.20	81.40	77.60	0.931	0.369	0.882	
1.5%	65.40	79.40	78.20	69.40	69.40	0.937	0.238	0.633	
2.0%	82.80	90.20	65.00	84.20	82.80	0.813	0.373	0.102	
L	0.662	0.556	0.295	0.791	0.260				
Q	0.159	0.176	0.072	0.744	0.461				
DL	0.130	0.744	0.271	0.205	0.375				

Linear Effect (L); Quadratic Effect (Q); Deviation from linearity (DL); Coefficient of variation (CV).

Ruminants have several hepatic gluconeogenic metabolic routes that provide biochemical versatility to maintain glycemic levels in circulation during the postprandial and fasting period (Araújo et al., 2020). Glycemic control is regulated by insulin and glucagon, influenced by cortisol, which keeps the average within the normal range (D. A. de P. Silva et al., 2019). Plasma glucose values were close to normal (50–80 mg/dL), as suggested by Varanis et al. (2020).

Conclusion

The present study showed no effect of the addition of fibrolytic enzymes on food consumption, feed efficiency or glycemia in sheep. However, a decrease in plasma creatinine concentration was observed without causing metabolic damage to the animals.

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References

Adéola, O., & Cowieson, A. J., (2011). Opportunities and challenges in using exogenous to improve nonruminant animal production. *National Center for Biotechnology Information, 89*(10),3189-3218. doi: 10.2527/jas.2010-3715

Adesogan, A. T., Ma, Z. X., Romero, J. J., & Arriola, K. G. (2014). Ruminant nutrition symposium: improving cell wall digestion and animal performance with fibrolytic enzymes. *Journal of Animal Science, 92*(4), 1317-1330. doi: 10.2527/jas.2013-7273

Al-Mamun, M., & Uddin, M. M. (2017). Effect of exogenous fibrolytic enzymes on growth performance and digestibility in Black Bengal goat. *Journal of Animal Science Advances,* (1), 2426-2433. doi: 10.32649/ajas.2021.176226

Araújo, C. M., Oliveira, K. A., Macedo, G. de. L. Jr., Silva, S. P. da & Silva, D. A. de P. (2020). Parâmetros nutricionais e bioquímicos de ovinos consumindo volumoso extrusado com diferentes teores de Uruchloa brizantha em comparação a silagem de milho tradicional. *Caderno de Ciências Agrárias, 12*(2020), 1-11. doi: 10.35699/2447-6218.2020.25810

Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. P*roceedings of the Royal Society, 160*(901), 268-282.

Beauchemin, K. A., Colombatto, D., Morgavi, D. P., & Yang, W. Z. (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science, 81*(2), 37-47. doi: 10.2527/2003.8114_suppl_2E37x

Berchielli, T. T., Pires, A. V., & Oliveira, S. G. (2011). *Nutrição de ruminantes.* Funep.

Brito, F. O. (2010). *Enzimatic complex levels in ruminant diets.* Dissertação de mestrado, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brasil.

Cunniff, P. (1995). *Association of official analytical chemists* (16nd ed.). Official Methods of Analysis.

Gomes, S. P., Borges, I., Borges, A. L. C. C., Macedo, G. de L., Jr., Campos, W. E., & Brito, T. S. (2012). Tamanho de partícula do volumoso e frequência de alimentação sobre o metabolismo energético e proteico em ovinos, considerando dietas com elevada participação de concentrado. *Revista Brasileira de Saúde e Produção Animal, 13*(3), 732-744. doi: 10.1590/S1519-99402012000300013

Kozloski, G. V. (2011). *Bioquímica dos ruminantes* (3a rev. e amp.). Editora da UFSM.

Lynch, J. P., Jin, L., Church, J. S., Baah, J., & Beachemin, K. A. (2013). Fibrolytic enzymes and a ferulic acid esterase-producing bacterial additive applied to alfalfa hay at baling: effects on fibre digestibility, chemical composition and conservation characteristics. *Grass and Forage Science, 70*(1), 85-93. doi: 10.1111/gfs.12093

Maynard, L. A., Loosli, J. K., Hintz, H. F., & Warner, R. G. (1984). *Nutrição animal* (3a ed.). F. Bastos.

Meyer, D. J., & Harvey, J. W. (2004). *Veterinary laboratory medicine: interpretation & diagnosis.* Saunders.

National Research Council (2007). *Nutrient requirements of small ruminants.* NRC.

Neiva, M. C., Schultz, E. B., Sousa, L. M., Oliveira, K. A., Sousa, L. F., & Macedo, G. de L., Jr. (2020). Exogenous enzymes in sheep diet: nutritional and physiological parameters. *Acta Scientiarum. Animal Sciences, 44*(1), 1-9. doi: 10.4025/actascianimsci.v44i1.56504

Nelson, D. L., & Cox, M. M. (2014). *Princípios de bioquímica de lehninger* (6a ed.). Artmed.

Rodrigues, G. R. D., Siqueira, M. T. S., Dutra, T. O., Schutz, E. B., Sousa, L. F., & Macedo Júnior, G. de L, (2022). Uso de enzima amilolítica associada a enzimas proteolíticas e fibrolíticas na dieta para ovinos. *Revista Agrária Acadêmica, 5*(3), 2-14. doi: 10.32406/v5n3/2022/59-72/agrariacad

Rojo, R., Mendoza, G. D., & Plata, F. X. (2007). Comparison of method of application on the effect of amylolytic enzymes on in vitro ruminal starch digestion. *Journal of Applied Animal Research, 32*(1), 81-84. doi: 10.1080/09712119.2007.9706852

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality. *Biometrika, 52*(3), 591-609. doi: 10.2307/2333709

Silva, D. A. de P., Santana, A. G., Araújo, C. M., Oliveira, K. A., Siqueira, M. T. S., & Macedo, G. de L., Jr. (2019). Avaliação dos efeitos nutricionais e metabólicos da substituição de silagem de milho por ração extrusada de Capim-marandu (*Urochloa brizantha*) em ovinos. *Caderno de Ciências Agrárias, 12*(2020), 1-9. doi: 10.35699/2447-6218.2020.19833

Silva, J. F. C., & Leão, M. I. (1979). *Fundamentos de nutrição de ruminantes.* Livroceres.

Sniffen, C. J., O'connor, D. J., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: carbohydrate and protein availability. *Journal of Animal Science, 70*(11), 3562-3577. doi: 10.2527/1992.70113562x

Subramaniyan, S., & Prema, P. (2002). Biotechnology of microbial xylanases: enzymology, molecular biology, and application. *Critical Reviews in Biotechnology, 22*(1), 33-64. doi: 10.1080/07388550290789450

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Animal Science, 74*(10), 3583-3597. doi: 10.3168/jds.s0022-0302(91)78551-2

Varanis, L. F. M., Schutz, E. B., Oliveira, K. A., Sousa, L. F., Cruz, W. F. G., & Macedo, G. de L., Jr. (2020). Intervalos de referência de bioquímicos séricos para cordeiros do nascimento a um ano nos trópicos. *Semina: Ciências Agrárias, 42*(3), 1725-1740. doi: 10.5433/1679-0359.2021v42n3Supl1p1725

Weiss, W. P. (1999). *Energy prediction equations for ruminant feeds.* Cornell University.