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Article

Use of active and inactive yeasts in lamb diets: intake, digestibility, and metabolism

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Abstract: This study aims to evaluate the use of active, inactive plus active, and inactive yeasts on the consumption of dry matter, water, and the apparent digestibility of dry matter, urinary parameters, and serum metabolites of lambs. Twenty crossbred lambs (Dorper x Santa Inês) were used, with an initial average body weight of 31.89 kg and seven months of age, distributed in a completely randomized design. Treatments were Control group (without yeasts); Active Flora® (ICC), live yeast plus inactivated yeast (Saccharomyces cerevisiae, 2.0 x 1010 UFC g-1) in the dose of 0.003 kg of animal dry matter -1 day-1,; Milk Sacc X® (Alltech®), active yeast - Saccharomyces cerevisiae strain 1026, 5.0 x 108 UFC g-1 - at the dose of 0.0015 kg of animal dry matter-1 day-1; and Rumen Yeast® (York Ag Products INC.), inactive yeast - Saccharomyces cerevisiae,1.5 x 104 UFC g-1 - in the dose of 0.0045 kg of animal dry matter-1 day-1. Variance analysis and the SNK (Student-Newman-Keuls) test were performed considering 5% significance. For glycemic concentrations over time, regression analysis at 5% signif icance were performed. The fecal score, as a non-parametric variable, was assessed by the Kruskal and Wallis test at a 5% significance level. There was a statistical difference (P< 0.05) in fecal dry matter (FDM), where the Active Flora® treatment was inferior to the others. The use of different yeasts did not change the intake and

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digestibility of dry matter, water, urinary parameters, and serum concentrations of energy, protein, and liver metabolites (P> 0.05). The average dry matter intake was 1.16 ± 0.16 kg day-1, whereas the dry matter digestibility was 85.40 ± 2.73%. It was concluded that active and/or inactive yeasts can be used as additives in the diet of lambs, maintaining the intake and digestibility of dry matter without causing metabolic disturbances.
Keywords: Saccharomyces cerevisiae, additives, small ruminants, yeast.

Introduction

Yeasts are single-celled fungi capable of changing the profile of rumen fermentation. The fungi culture stands out for acting as probiotics to stimulate rumen microbiome growth and to assimilate the oxygen in the rumen. The affinity between yeast cultures and oxygen improves the ruminal conditions for anaerobic microorganisms (PIRES, 2011).

Yeasts, mainly *Saccharomyces cerevisiae*, are a source of high-quality proteins, B vitamins, and minerals, especially selenium and zinc. These have two forms, an active form, that manages the concentrations of lactic acid in the rumen, providing positive factors to bacteria and absorbing oxygen in the rumen environment. And, the inactive form, present in the maintenance of the pH, improving the conditions of the rumen (PIRES, 2011).

Therefore, yeasts can stimulate the use of nutrients and change the characteristics of rumen fermentation, for instance, by improving the environment for cellulolytic bacteria. According to Patra (2012), the fermentative effects showed by this probiotic were increased in volatile fatty acids, decreased in lactate concentration, and maintained the rumen pH, avoiding acidosis, especially in diets with high concentrated proportions. The use of exogenous enzymes in diets for ruminants can result in better animal performance since there will be greater digestion and greater use of nutrients. The amylolytic enzymes in addition to increasing the degradation of starch, act as a supplement due to their highly fermentable substrate, contributing to increasing the activity of enzymes derived from the rumen microbiome (VALLEJO-HERNÁNDEZ et al., 2018).

The synergistic action of these additives may have the potential to contribute directly to a better ruminal environment with enzymes acting to promote greater digestion, nutrients usage, and yeasts, increasing the conditions of the rumen so that more action occurs on the effectiveness of the ruminal microbiome. In addition, according to Igarasi et al. (2008), diets containing high levels of grains may allow greater performance for the animals. However, these feeds are usually protected by the pericarp, which is a structure resistant to degradation. Moreover, the use of enzymes and yeasts can be an efficient strategy in the digestion and utilization of grains to improve animal performance and diet digestibility.

The use of enzymes can improve ruminal health and diet digestibility, especially in high-grain diets, consequently providing an increase in performance (AMIN and MAO, 2020). Geng et al. (2016) showed that the use of enzymes in high-grain diets leads to greater weight gain in calves. Also, with the use of enzymes, there was an increase in the concentration of protein and the quality of lamb meat (RUFINO et al., 2013). In addition, Erlaef et al. (2020) showed that the use of yeasts has improved the production and quality of sheep's milk.

Understanding the benefits and challenges in the use of enzymes in ruminant diets, we hypothesize that the inclusion of yeasts in diets with high grains improves ruminal fermentation, intake, and digestibility of dry matter, and change the metabolic profile of lambs. Thus, the aim is to analyze the effect of the inclusion of active, inactive, and active plus inactive yeasts in the diet of lambs under intake and digestibility of dry matter and metabolic profile.

Material and methods

The experiment was carried out at Fazenda Capim Branco in Uberlândia, West of Minas Gerais, which presented temperature and relative

humidity of 23.6°C and 80.7%, respectively, during the experimentation period, according to data from CLIMA (Climatology and Environmental Meteorology Laboratory) and according to the experimental protocol number 092/17 of the Ethics Committee on Use of Animals (CEUA) of the Federal University of Uberlândia.

It took place between February and March of 2019, for the period of twenty days, whereas fifteen days were used to adapt the animals to the diet and five days for data collection. Twenty crossbred lambs (Dorper x Santa Inês) were used, with an average initial body weight of 31.89 kg and seven months of age, assigned in a completely randomized design.

Before starting the experiment, the animals were weighed, identified, wormed using Zolvix® at a dose of 2.5 mg of Monepantel per kg of live weight, and vaccinated (rabies, leptospirosis, clostridia, and botulism). Later, they were allocated in metabolic cages with a feeder and a drinker according to the standards of the National Institutes of Science and Technology (INCT).

Treatments were Control group (without yeasts); Active Flora® (ICC), live yeast plus inactivated yeast (Saccharomyces cerevisiae, 2.0 x 1010 UFC g-1) in the dose of 0.003 kg of animal dry matter -1 day-1,; Milk Sacc X® (Alltech®), active yeast - Saccharomyces cerevisiae strain 1026, 5.0 x 108 UFC g-1 - at the dose of 0.0015 kg of animal dry matter-1 day-1; and Rumen Yeast® (York Ag Products INC.), inactive yeast - Saccharomyces cerevisiae,1.5 x 104 UFC g-1 - in the dose of 0.0045 kg of animal dry matter-1 day-1. For each treatment, the yeasts were previously weighed using a precision balance and then mixed on the concentrate in the feed supply.

Meals were at 8:00 am and 4:00 pm, and 50% of the total were offered in each period. All treatments were composed of corn silage (30.0%) and concentrate (70.0%). The diet was formulated according to the NRC (National Research Council, 2007) for a gain of 300g day-1 and considered leftovers between 5-10% of the total supply. The concentrate ingredients and chemical

composition of corn silage, concentrate, and diet, as well as the amylolytic enzyme are shown in Table 1.

Table 1 – Concentrate ingredients and chemical composition of corn silage, concentrate, diet, and amylolytic enzyme

Ingredients (kg)		Concentrate	
Corn meal		72	
Soybean meal		18	
Urea		2	
Mineral Mixture		5	
Amaize™		3	
Adsorber		0.002	
Nutrients (g kg-1)	Corn Silage	Concentrate	Diet
Dry matter	322	900.5	726.95
Crude protein	63	290.7	222.39
Total digestible nutrients	626.3	759.6	719.61
Neutral detergent fiber	546	81	220.5
Acid detergent fiber	326	-	-
Composition		Amaize™	
Amylase		Min. 600 FAU* g-1	

*A unit of the alpha-amylase enzyme, activity equivalent to the amount of enzyme that dextrinized 1 gram of soluble starch per minute, at pH 4.8 and 30ºC.

The enzyme product was added to the concentrate ingredients, according to the information in Table 1, using a 200 kg vertical mixer, and they were mixed for fifteen minutes.

After the adaptation period followed the data collection period. During all five days of collection, all the feed offered, leftovers and feces were weighed in an electronic balance with a precision of five grams and sampled.

Compound samples were made from simple ones from each animal, during the five days of collection.

The urine was sampled using buckets with screens so that the feces were retained and collected in plastic trays. The urine volume was measured using graduated plastic test tubes, with a capacity of two liters and an accuracy of 20 mL. The volume was quantified during 24 hours in each of the five days of collection.

Urine density was assessed using a Megabrix® portable refractometer (Fremont) with the aid of disposable pipettes, where 1 mL of urine was transferred from the collecting bucket to the optometer prism. This procedure was performed under fluorescent light, always in the same position. For each sample, the refractometer was cleaned and dried with paper towels so that there was no contamination.

Fecal scores were determined according to Gomes et al. (2012), with feces observations on a scale of 1 to 6, being: 1 - dry and dull feces, 2 - normal feces, 3 - slightly softened feces, 4 - soft feces losing their shape, 5 - soft feces and without their normal shape, 6 - diarrheal feces.

The water intake was measured daily. The offered water was placed in 20-liter buckets, replaced whenever necessary, and one bucket for evaporation control. Thus, the leftovers in all buckets were measured by a graduated test tube to estimate water intake by the difference between water supplied, evaporated, and leftovers.

The daily sampled feed and feces gave samples that were homogenized at the end of the collection period and stored in a freezer at a temperature of -15ºC. The feed and feces were pre-dried in a ventilated oven at 55ºC for 72 hours and crushed, in a Willey knife mill, in particles of one millimeter. The analysis of the dry matter at 105ºC was according to Silva and Queiroz (2002). The intake and apparent digestibility of dry matter were calculated according to Maynard et al. (1984).

For glycemic, blood samples were collected on the last day of data collection, at 8:00 am (before the first meal), then at 11:00 am, 2:00 pm, 5:00 pm, and, at last, 8:00 pm. Exceptionally on that day, the second meal was only offered after the 8:00 pm harvest. These samples were collected through a jugular venipuncture, using Vacutainer® tubes containing fluoride and EDTA.

Blood samples were collected before the first feed by a jugular vein puncture with the help of a vacutainer into tubes without anticoagulant. Following that, the blood samples were immediately centrifuged at 2.700×g for 20 min. The plasma samples were pipetted and frozen at -18°C for later analysis of total protein, albumin, globulin, uric acid, urea, creatinine, cholesterol, triglycerides, glucose, fructosamine, aspartate aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase, using a commercial kit from Lab Test® in an automated biochemical analyzer (Bioplus® 2000).

The mathematical model was $Y_{ij} = \mu + T_i + e_{ij}$; where Y is the observation; μ is the general mean; T_i is the fixed effect of treatment i and e_{ij} is the random error ij.

The data were analyzed using the statistical software SAEG (System for Statistical and Genetic Analysis), version 9.0. The data were tested for normality (SHAPIRO and WILK, 1965) and homoscedasticity (LEVENE, 1960) of the residue variance. Variance analysis and SNK (Student-Newman - Keuls) test were performed with a 5% significance level for type I error. For fecal score, the Kruskal-Wallis test (1952) was used at the 5% significance level. The analysis of glycemia by harvest time was assessed using regression analysis testing the linear and quadratic effects at 5% significance.

Results and discussion

There was no statistical difference (P> 0.05) for the variables of dry matter intake (DMI) in kg per day, as a percentage of body weight and as a

function of metabolic weight, and digestibility of dry matter (DDM). This was because yeasts have the function of capturing oxygen and keeping ruminal pH stable by using lactic acid to provide a favorable environment for the growth of ruminal microorganisms, especially cellulolytic bacteria (FONTY and CHAUCHEYRAS-DURAND, 2006). However, for diets with higher proportions of concentrate, the improvement in the rumen environment is not highlighted, not changing the diet, the intake, and the digestibility of dry matter.

Table 2 – Dry matter intake and digestibility in lambs with or without active, inactive, or active plus inactive yeasts in the diet

Item	Control	Active®	Milk Sacc X®	Rumen Yeast Y®	P-Value	Mean	CV
DMI (kg day^{-1})	1.19	1.17	1.19	1.11	0.9973	1.16	17.34
DMI (%BW)	3.32	3.94	4.26	3.48	0.4297	3.88	18.79
DMI (BW^{0,75})	89.58	91.35	97.81	82.31	0.2886	90.26	13.50
DDM (%)	83.34	86.17	85.79	85.32	0.6397	85.40	2.73

DMI: dry matter intake; BW: body weight; DDM: digestibility of dry matter; CV: coefficient of variation (%).

The average DMI of 1.16 kg day-1 was within the recommended for an animal category, which is 1.0-1.3 kg day-1 according to NRC (2007) (Table 2). The DMI concerning body weight (DMI % of BW) recommended by the NRC (2007) is 3.51%, therefore, the average is 10% above the recommended. The increased intake concerning body weight was because the inclusion of the amylolytic enzyme in the diet produced no effect on yeasts. The use of the enzyme improves diet digestion, ensuring better nutrient absorption, and increases feed efficiency (OLIVEIRA, 2012).

Siqueira et al. (2020) studying the use of active and inactive yeasts in the diet of lambs, did not found the difference between the use or not of yeasts under the DMI, and the DMI % of BW, with the averages being 1.20 kg day-

1 and 3.29 %, respectively. These values are similar to what was obtained in this study, which reinforces the amylolytic enzyme effect since the yeast effect was not significant.

Another factor related to intake above the recommendation is the feed composition in the diets. As well as concentrate increased, the concentration of non-fibrous carbohydrates increased, and the fiber diet decreased. By reducing fiber concentration in the diet, there is no intake limitation due to the filling effect, leading to an increased passage rate due to a higher proportion of feeds with smaller particle size and high degradation rates, leading to the maximization of consumption (MACEDO JÚNIOR et al., 2012).

Regarding DDM, the average was 85.40% and the DMI was within the recommended, it can be inferred that there was a good use of feed. As showed by Malekkhahi et al. (2014), evaluating the effect of yeasts on lambs consuming diets with high amounts of concentrate and corn silage, the average DDM was 81.00%. Mendes et al. (2010), studying the nutrients digestibility from diets containing a high proportion of concentrate and different sources of neutral detergent fiber for lambs, found an average DDM of 87.20%. Siqueira et al. (2020) found 81.77% of DDM for lambs that consumed diets containing active and inactive yeasts.

The digestibility values in this study were the association response between the amylolytic enzyme and the high amounts of corn bran and corn silage offered in the diet because both have starch in their compositions. The in vitro digestibility of dry matter (DDM) of corn bran and corn silage obtained by Lopes et al. (2010) were 89.40% and 73.10%, respectively. The DM digestibility of corn starch acquired by Queiroz et al. (2008), using lambs, was 93.00%. Thus, the inclusion of the enzyme additive in the diet may have increased the digestibility of corn starch present in the feeds consumed and consequently increased the digestibility, since the amylases help in the degradation of glucose polysaccharides and are used in ruminant nutrition to

increase the degradation of indigestible portions of some foods, with synergism between the rumen endogenous enzymes (NEIVA, 2018).

The inclusion of active and/or inactive yeasts did not change the water intake (WI) and water intake concerning dry matter consumption (WI / DMI) (P> 0.05) (Table 3). According to the NRC (2007), water intake is calculated by the equation: WI = 3.86 x DMI - 0.99. Therefore, as the DMI obtained was 1.16 ± 0.17 kg day-1, the recommended water intake would be between 2.83 and 4.14 L. However, the average was 3.18 L day-1. Water intake values can be associated with high DMI and DDM values, and it is possible to state that WI did not limit these variables, since water deprivation for sheep can cause numerous metabolic disorders and reduce dry matter intake (RIBEIRO and BENEDETTI, 2011).

Table 3 – Water intake, urine, and fecal parameters of lambs

Item	Control	Active®	Milk Sacc X®	Rumen Yeast®	P-Value	Mean	CV
WI (L day^{-1})	3.04	3.45	3.25	3.09	0.9775	3.18	14.47
WI/DMI (L Kg^{-1} day^{-1})	2.86	3.06	2.66	2.95	0.8857	2.88	10.98
UV (L day^{-1})	1.47	1.74	1.36	1.20	0.7257	1.44	14.75
UD (g mL^{-1})	1.0216	1.0220	1.0236	1.0206	0.8679	1.022	0.57
FMNM (Kg day^{-1})	0.500	0.532	0.514	0.567	0.8293	0.528	22.77
FMDM (Kg day^{-1})	0.172	0.159	0.176	0.162	0.7042	0.167	15.72
FDM (%)	32.99A	28.32B	32.19A	33.54A	0.0001	31.76	3.48
FS*	2.60	2.68	2.28	2.92	0.2130	2.62	-

WI: water intake; DMI: dry matter intake; UV: urine volume; UD: urine density; FMNM: fecal mass in the natural matter; FMDM: fecal mass in the dry matter; FDM: fecal dry matter; FS: Fecal Score; CV: coefficient of variation (%); * Nonparametric variable.

For the urinary parameters of volume (UV) and density (UD), there was no significant difference for the use or not of yeasts (P> 0.05) (Table 3).

According to Reece (2006), a sheep's UV varies between 0.1 and 0.4 L for each 10 kg of live weight. In this study, the animals had an average of 31.89 kg, which corresponds to 0.32 - 1.28 L. Although it is shown that UV is 13% above the limit proposed by Reece (2006), the lambs were not negatively affected.

According to Hendrix (2005), sheep urine density varies between 1.020 and 1.040. Thus, the UD average of 1.022 was within the recommended. Additionally, regarding the values of DMI, WI, and UV, it can be inferred that the animals did not present water restriction, therefore, there was good digestion and metabolism of nutrients, since water participates in the chemical reactions of hydrolysis of protein, fat, and carbohydrates.

There were no differences between the fecal mass in the natural matter (FMNM) and the weight of fecal mass in the dry matter (FMDM) (Table 3) (P> 0.05). The mean FMNM was 0.528, a result lower than the assessment by Vieira (2008), pointing that an adult sheep produces between 0.8 and 1.5 kg of FMNM kg day-1. Ruminant feces can be quantitatively affected by the temperature of the environment, the quality of the feed, and animal characteristics. The weight of the fecal volume excreted can be influenced by the passage and digestion rate in the rumen (SANTOS and NOGUEIRA, 2012). Therefore, this result may be associated with the high digestibility of the diet and the fact that the animals used in the experiment are in the growth phase.

For fecal dry matter (FDM), the average was 31.76%. Van Cleef et al. (2010) recommend values for the sheep species between 37 and 44%, therefore, the numbers verified in this test are 5.24% lower. Lower values of FDM suggest that having a higher water content in the feces might indicate a higher feed passage rate. According to Martins et al. (2006), the passage rate of the liquid phase can be influenced by the water intake level. The increased passage rate is demonstrated by the increased consumption above the recommendation (Table 2). As the amylolytic enzyme was one of the ingredients of the concentrate and the yeasts were mixed at the time of

feeding, these additives may have promoted the highest passage rate, reflecting lower values of FDM. Siqueira et al. (2020) obtained an average of FDM for lambs fed with yeast in the ratio of 32.15%, which may reinforce that the use of yeasts can lead to a reduction in the percentage of FDM.

There was a difference in the FDM when the Control, Milk Sacc X®, and Rumen Yeast® treatments were higher than the Active® treatment ($P< 0.05$) (Table 3). When the control treatment, without the addition of yeasts, was also shown to be superior, the amylolytic enzyme may have influenced it. Vigne et al. (2019), evaluating starch digestibility in confined steers, which were consuming feed with different doses of enzyme complexes and diets with high energy density, found that feeds containing high proportions of NFC (Non-Fiber Carbohydrates) can cause an increase in the gastrointestinal tract passage rate and cause an effect of osmolarity, generating water retention in the intestinal lumen and consequently reducing DMI according to inclusion levels of enzymes in the diet. Therefore, the addition of the amylolytic ingredient in the diet concentrate may have caused this process and contributed to the FDM values that were obtained. The Active® treatment, which had active and inactive yeasts, was inferior to the others, probably because the interaction between active and inactive yeasts adapts to the rumen environment and increases the fiber digestibility (PIRES, 2011).

Despite changes in FDM, with or without the use of yeasts, the fecal score did not show statistical differences ($P> 0.05$) (Table 3). The value considered normal for this variable by Gomes et al. (2012) is 2, so all values found in this experiment are within the normal range. The fecal score evaluation based on shape and consistency is essential in the assessment of possible disorders in the gastrointestinal tract and their impacts on animal health and performance. The fiber in the roughage offered by all treatments may have influenced the fecal score since the fiber of the diet makes the stools firmer (FERREIRA et al., 2013).

Regarding metabolism, there was no difference for the energy metabolites of lambs fed with or without the use of yeasts (P> 0.05) (Table 4). Cholesterol values are within the reference values established for sheep by Silva et al. (2020). Cholesterol has an exogenous origin through food and endogenous synthesized in the small intestine, fat cells, and liver. That is an indicator of the bloodstream lipid levels. Triglycerides are synthesized in the liver and act as the main form of storage of fatty acids in adipose tissue, constituting sources of energy reserve (KANEKO et al., 2008). As the food offered had great energy potential due to the high cluster of corn bran in the concentrate, this ingredient may have promoted such stabilization in the values of cholesterol and triglycerides. It demonstrates that both the use and non-use of yeasts provide adequate energy storage for the lambs.

Table 4 – Lamb's profile of energy metabolites fed diets containing active and inactive yeasts in the feed

Item	Control	Active®	Milk Sacc X®	Rumen Yeast®	P-Value	Mean	CV	RV*
Cholesterol (mg dL^{-1})	30.4	22.86	21.46	16.4	0.3191	22.78	22.04	14-126
Triglycerides (mg dL^{-1})	22.8	22.06	19.36	21.93	0.3974	22.41	24.04	5-71
Frutosamine (µmol L^{-1})	177.89	170.75	176.18	198.98	0.3533	180.95	14.19	119-451
Glucose (mg dL^{-1})	58.92	51.32	61.88	58.8	0.3749	57.73	24.46	30-94

CV: coefficient of variation; RV*: reference value according to Silva et al. (2020).

The fructosamine values were also within the reference values of Silva et al. (2020). Fructosamine can be used for glycemic control, since it can monitor the glucose for two weeks, showing that the use of yeasts did not promote changes in glycemic control. The glucose values did not differ

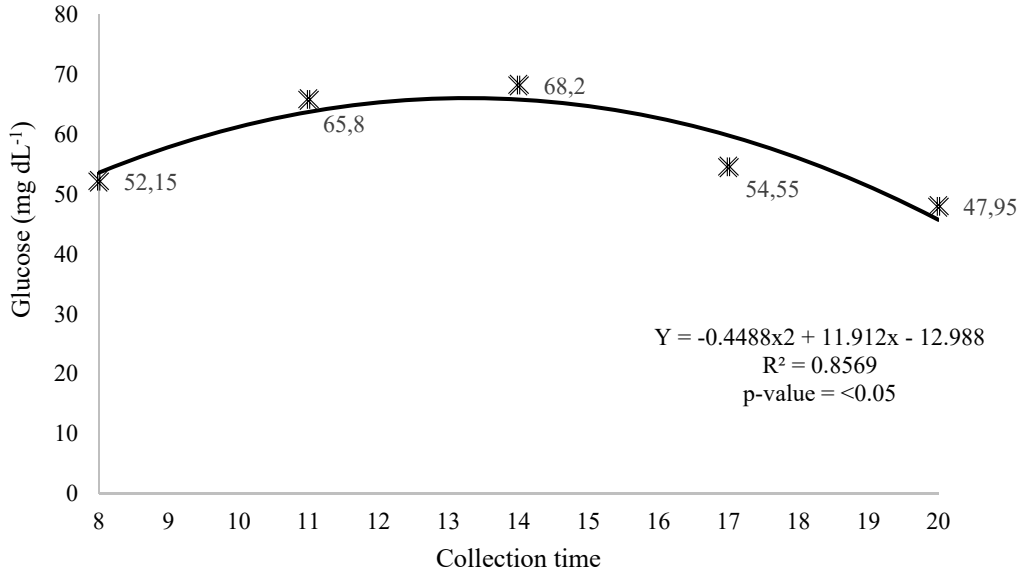
significantly with the use or not of active and or inactive yeasts in the feeding of lambs (P> 0.04) (Table 4).

All glucose values were in reference to Silva et al. (2020) (Table 4). The maintenance of plasma glucose in ruminants is directly related to glycemic stability since dietary carbohydrates are widely used in the rumen (SILVA et al., 2020). Thus, its precursors are non-carbohydrate compounds, such as volatile fatty acids (VFAs) propionate, which are absorbed in the rumen and converted into glucose (gluconeogenesis process) to be used as an energy source by the animal. This process is carried out mainly in the liver, where occurs the synthesis of glucose, which is released into the bloodstream, thus maintaining blood glucose.

The formation of propionate occurs through microbial fermentation of carbohydrates. Therefore, when there is ingestion of highly fermented foods, as is the case of starch present in the corn bran based diet, there are increases in microbial activities, which provide changes in the final amounts of VFAs and ammonia from rumen fermentation, reflecting the use of other nutrients in the diet (GOULARTE et al., 2011). Thus, the inclusion of the amylolytic enzyme may have helped the stabilization of glycemic levels, since it contributed to the digestion and utilization of the starch that was ingested by the animals, providing greater microbial fermentation and, consequently, satisfactory amounts of VFAs. The noticed stabilization can be considered positive, since the glycosidic indices were measured in reference to Silva et al. (2020), inferring that the animals showed good glycemic stability and energy supply.

Glucose concentrations throughout the day had a quadratic effect (P> 0.05) (Graph 1).

Graph 1 – Glucose concentrations throughout the day (24-Hour Clock) of lambs fed diets containing active and inactive yeasts



As gluconeogenesis uses an indirect pathway, its production is slow, which justifies the increased blood glucose at 11:00 and the peak at 14:00 hours. According to Caldeira (2005), the peak of glucose in ruminants occurs between three to six hours after feeding, thus, as the first meal was offered at 08:00, the elevation of the levels noticed at 11:00 and 14: 00 are justified. At 17:00 and 20:00, a reduction in glycemic levels was observed, which may be due to animal fasting, since the plasma glucose levels tend to decrease due to the oxidative use of the tissues dependent on this sugar, such as the central nervous system (KOZLOSKI, 2017).

For the concentration of hepatic metabolites, there was no significant difference regarding the use or not of active and/or inactive yeasts in the feeding of lambs (P> 0.05) (Table 5). All liver metabolite values were within the species recommendation according to Silva et al. (2020). Such stabilization may have occurred because the animals were healthy and had no changes in energy metabolites.

Table 5 - Hepatic enzymes for lambs fed diets containing amylolytic enzymes and active and inactive yeasts

Item	Control	Active®	Milk Sacc X®	Rumen Yeast®	P-Value	Mean	CV	RV*
Alkaline phosphatase (U/L^{-1})	195.46	189.33	155.13	154.86	0.8235	173.70	25.86	49-826.90
GGT (U/L^{-1})	95.53	80.86	71.00	73.60	0.4284	80.25	14.39	25-146
AST (U/L^{-1})	120.46	134.17	103.28	127.67	0.8397	121.39	23.12	41-298

GGT: gamma-glutamyl transferase; AST: aspartate aminotransferase; CV: coefficient of variation; RV*: reference value according to Silva et al. (2020).

Alkaline phosphatase participates in the digestion of fatty acids in the liver, has a hepatic origin, and performs hydrolysis of phosphate substrates. GGT, on the other hand, evaluates the level of enzymes in the blood and measures liver function. It has a hepatic and renal origin, and the renal origin GGT is excreted in the urine. AST is indicative of possible lesions that could compromise the liver. For ruminants, it is the main indicator for liver damage and metabolic disorders (KANEKO et al., 2008). As there was no change in the values obtained by these variables, it is possible to infer that the use of active and inactive yeasts does not compromise the functioning of the liver and does not predispose lambs to liver damage or metabolic disorders.

There was no significant difference for protein metabolites with or without the use of active and/or inactive yeasts in the lamb diet ($P > 0.05$) (Table 6). Blood proteins are synthesized mainly by the liver; therefore, the synthesis rate is directly related to animal nutritional status. Albumin is the most abundant protein in plasma being synthesized in the liver and is considered an important protein reserve. Its level is one of the indicators of diet protein content (KANEKO et al., 2008). As the mean values obtained for albumin and total proteins are within the range for sheep recommended by Silva et al. (2020), it is possible to infer that the use of yeasts had a positive

effect on the protein metabolism of animals, indicating that the animals had normal protein intake and good nutritional status during the study.

Table 6 – Protein metabolites of lambs fed diets containing amylolytic enzymes and active and inactive yeasts

Item	Control	Active®	Milk Sacc X®	Rumen Yeast®	P-Value	Mean	CV	RV*
Total protein (g dL^{-1})	3.36	3.28	3.44	3.66	0.6370	3.43	14.01	3.1-10.7
Urea (mg dL^{-1})	100.66	121.13	103.53	105.46	0.2963	107.69	16.44	10-92
Uric acid (mg dL^{-1})	0.05	0.00	0.04	0.11	0.4570	0.05	4.88	0-1.7
Albumin (g dL^{-1})	4.26	4.19	3.88	3.59	0.2429	3.98	13.96	1.1-5.2
Creatinine (mg dL^{-1})	0.95	0.98	0.91	0.88	0.6253	0.93	0.88	0.4-1.7

CV: coefficient of variation; RV*: reference value according to Silva et al. (2020).

The creatinine values are also within the recommended for sheep according to Silva et al. (2020). Creatinine is an important indicator of renal function since its levels suffer little or no influence from factors such as diet, age, or sex (SILVA, 2017). As uric acid and urea have renal excretion and the levels of creatine found were indicative of a good renal function, it can be inferred that these two metabolites were easily excreted in the urine. In addition to the values of creatinine, CH2O, UV, and UD also indicate that the animal renal function was not impaired or restricted by the use of yeasts.

Urea is synthesized in the liver and developed using ammonia from amino acid catabolism and the recycling of rumen ammonia. The recycling process begins when the ammonia is absorbed by the rumen wall and

transported through the enterohepatic circulation via the portal vein to the liver, where it is metabolized into urea (KANEKO et al., 2008). The average value obtained for this variable was 17% above the maximum value established by Silva et al. (2020), possibly being related to the low values found for the uric acid variable, since uric acid acts directly on the microbial protein synthesis of ruminal microorganisms. With lower amounts of uric acid, there is less microbial synthesis and consequently less use of ruminal ammonia, which promotes increased ammonia liberation and directly influences the amount of urea present in the plasma and liver.

Increased urea levels may indicate pre- and post-renal problems, such as decreased blood flow in the kidney, filtration deficiency, or obstruction in the urinary tract (GONZÁLES and SILVA, 2006). However, according to Santarosa et al. (2016), such problems in sheep are usually accompanied by clinical cases, such as decreased urine volume, increased urinary protein excretion, and urinary acidification, with changes in urinary density. In this experiment, there were no changes in the UV, UD, and, as the creatinine values indicated, there was good renal function. The probability of having renal deficiencies in the animals during the experimental period was ruled out. Thus, the urea variable is above the reference (SILVA et al. 2020) and it did not provide disturbances for the animals.

The concentration of uric acid was within the recommended for sheep (SILVA et al. 2020). Besides regulating the synthesis of microbial protein, uric acid helps the regulation of the animals' defense mechanisms and in their concentration levels - which may vary according to the source of protein and energy in the diet -, it can also facilitate the consumption of dry matter, live weight, and in the use of food additives (SILVA, 2017).

Conclusion

The inclusion of active, inactive, and active plus inactive yeasts did not alter the dry matter intake and apparent digestibility. The Active® was inferior to the others FDMs, however, it did not cause any disturbance to the lambs. The use of active and inactive yeasts did not cause metabolic problems and it can be used in the diet of growing lambs.

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Uso de leveduras ativas e inativas na dieta de cordeiros: consumo, digestibilidade e metabolismo

Resumo: Objetivou-se avaliar o efeito de leveduras ativas, inativas mais ativas e inativas sobre o consumo e digestibilidade aparente da matéria seca, de água, dos parâmetros urinários e dos metabólitos séricos de borregas. Utilizou-se 20 borregas mestiças Dorper x Santa Inês, com peso corporal médio inicial de 31,89 kg e sete meses de idade, distribuídas em delineamento inteiramente casualizado. Os tratamentos consistiram em *Controle*, sem uso de enzimas, e no uso das leveduras *Active Flora*® (levedura viva junto a levedura inativada - *Saccharomyces Cerevisiae*, com 2,0 x 10^{10} UFC g^{-1}), *Milk Sacc X*® (levedura ativa - *Saccharomyces C.* 1026, 5,0 x 10^{8} UFC g^{-1}) e *Rúmen Yeast*® (levedura inativa - *Saccharomyces C.* com 1,5 x 10^{4} UFC g^{-1}). Foi realizado uma análise de variância e um teste SNK considerando 5% de significância. Para as concentrações glicêmicas ao longo do tempo foi realizada uma análise de regressão a 5% de significância. O escore fecal, por ser uma variável não paramétrica, foi avaliado pelo teste Kruskal e Wallis à significância de 5%. Observou-se a diferença estatística ($P< 0,05$) na matéria seca fecal (MSF), onde o tratamento Active Flora® se mostrou inferior aos demais. O uso de diferentes leveduras não modificou o consumo e a digestibilidade da matéria seca, de água, dos parâmetros urinários e das concentrações séricas dos metabólitos energéticos, proteicos e hepáticos ($P> 0,05$). A média de consumo da matéria seca foi de 1,16±0,16 kg dia^{-1}, já a digestibilidade da matéria seca foi de 85,40 ±2,73%. Concluiu-se que as leveduras ativas e/ou inativas podem ser utilizadas como aditivos na dieta de borregas, mantendo o consumo e a digestibilidade da matéria seca sem causar distúrbios metabólicos.
Palavras-chave: *Saccharomyces cerevisiae*, aditivos, levedura, pequenos ruminantes.

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