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Active yeast supplementation on nutritional parameters of ewe lambs fed a high-concentrate diet

Suplementação com levedura ativa sobre os parâmetros nutricionais de borregas alimentadas com dieta altamente concentrada

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Highlights

Confined ewe lambs fed diets supplemented with active yeast exhibit higher intake.
Active yeast does not affect the digestibility of high-concentrate diets in ewe lambs.
Active yeast to confined ewe lambs diets enhances nitrogen absorption.
Diets containing active yeast improve energy metabolism in ewe lambs.

Abstract

This study aimed to evaluate the effects of active yeast supplementation on intake, apparent digestibility, nitrogen balance, and energy use in ewe lambs fed high-concentrate diets. Five Dorper × Santa Inês ewe lambs, with an average initial body weight of 54.1 ± 1.4 kg and 8 months of age, were housed in individual metabolism cages. Treatments included a control diet (without active yeast) and four levels of active yeast supplementation [Active Flora® - ICC, Louisville, Kentucky, USA, 2.0 × 10^{10} colony-forming units (CFU)], at inclusion rates of 0.15, 0.30, 0.45, and 0.60% of dry matter (DM) intake (kg $animal^{-1}$ day^{-1}). Diets consisted

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of 20% corn silage and 80% concentrate, based on DM. The experiment followed a 5 × 5 Latin square design with five animals and five evaluation periods. Each period lasted 15 days, including 10 days for adaptation and 5 days for data collection, totaling 75 days. Data were analyzed using analysis of variance and regression at a significance level of 5%. Results showed a quadratic effect of yeast supplementation levels on DM (P = 0.027) and organic matter (OM) intake (P = 0.029) in g animal^{-1} day^{-1}, as well as on total carbohydrate intake (P = 0.026). A linear increase was observed in DM (P = 0.041) and OM (P = 0.043) intake in percentage of body weight (BW) and in g kg^{-1} BW$^{0.75}$ (P = 0.031 and P = 0.032, respectively), as well as for crude protein (P = 0.037) and non-fibrous carbohydrate intake (P = 0.041). Apparent nutrient digestibility was unaffected by treatments. Nitrogen (N) intake (P = 0.036) and urinary N excretion (P = 0.003) exhibited quadratic responses to yeast levels, while fecal N excretion (P = 0.043) and absorbed N (P = 0.045) increased linearly. Gross energy (GE) intake (P = 0.009), metabolizable energy (P = 0.019), and the metabolizability of ingested GE (P = 0.024) showed quadratic responses to yeast supplementation. However, treatments did not affect fecal GE, digestible energy, or urinary GE. Supplementing active yeast at levels between 0.31% and 0.36% of dietary DM improved intake patterns, nitrogen absorption, metabolizable energy availability, and GE metabolizability without altering the apparent digestibility of nutrients in ewe lambs fed diets containing 80% concentrate.
Key words: Probiotic. *Saccharomyces cerevisiae.* Sheep. Starch.

Resumo

Objetivou-se avaliar os efeitos da adição de levedura ativa sobre o consumo, digestibilidade aparente, o balaço de nitrogênio e de energia de borregas alimentadas com dietas contendo alto teor de concentrado. Foram utilizadas 5 borregas mestiças Dorper x Santa Inês com peso médio inicial de 54,1 ± 1,4 kg e 8 meses de idade, alojadas em gaiolas individuais de metabolismo. Os tratamentos consistiram em um tratamento controle (sem levedura ativa) e quatro teores de inclusão de levedura ativa [Active Flora® - ICC, Louisville, Kentucky, Estados Unidos, 2,0 x 10^{10} unidades formadoras de colônia (UFC)], sendo 0,15; 0,30; 0,45 e 0,60% da matéria seca (MS) ofertada em kg animal^{-1} dia^{-1} de ração, contendo 20% de silagem de milho e 80% concentrado com base na MS. O delineamento experimental foi em quadrado latino 5 x 5, com 5 animais e 5 períodos. Cada período teve duração de 15 dias, sendo 10 para adaptação e 5 para coleta de dados, totalizando 75 dias de experimento. Os dados foram submetidos a análise de variância e de regressão com nível de significância de 5%. Houve efeito quadrático dos teores de levedura sobre os consumos de MS (P = 0,027), matéria orgânica (MO) (P = 0,029) em g animal^{-1} dia^{-1} e carboidratos totais (P = 0,026) e efeito linear crescente sobre os consumos de MS (P = 0,041) e MO (P = 0,043) em % de peso corporal (PC) e em g kg^{-1} PC^{0,75} (P = 0,031 e 0,032, respectivamente) e de proteína bruta (P = 0,037) e carboidratos não fibrosos (P = 0,041). A digestibilidade aparente dos nutrientes não foi influenciada pelos tratamentos. Houve efeito quadrático dos níveis de levedura ativa sobre o nitrogênio (N) ingerido (P = 0,036) e N urinário (P = 0,003), enquanto o N fecal (P = 0,043) e o N absorvido (P = 0,045) aumentaram linearmente. Houve efeito quadrático dos tratamentos sobre a energia bruta (EB) ingerida (P = 0,009), energia metabolizável (P = 0,019) e metabolizabilidade da EB ingerida (P = 0,024). Não houve efeito dos tratamentos sobre a EB fecal, energia digestível e EB urinária. A adição de levedura ativa entre 0,31 e 0,36% na matéria seca da ração aumenta os padrões de consumo, a absorção de nitrogênio, a disponibilidade de energia metabolizável e a metabolizabilidade de EB ingerida, sem alterar a digestibilidade aparente

dos nutrientes em borregas alimentadas com dieta contendo 80% de concentrado.
Palavras-chave: Amido. Ovinos. Probiótico. *Saccharomyces cerevisiae.*

Introduction

The use of additives in diets for confined ruminants is critical due to their ability to positively influence ruminal fermentation patterns, enhance nutrient use efficiency, and reduce the risk of metabolic disorders associated with the high energy density of rations (Hernández et al., 2014). Ionophores are among the most used additives in confinement systems, serving as antibiotics targeting gram-positive bacteria in the rumen. Their mode of action promotes improved energy efficiency, and nitrogen compound use, and reduces the risk of acidosis (Azzaz et al., 2015).

However, the use of ionophores has become increasingly restricted and is already prohibited in some countries, with global bans likely in the near future. These restrictions are justified by concerns over potential risks to human health, food safety and quality, and environmental sustainability (Elghandour et al., 2019). This scenario underscores the urgency of identifying alternative products fulfilling similar functions.

Over the years, several alternatives to ionophores have been explored, including exogenous enzymes, essential oils, and live microorganisms. Among these, active yeasts have gained prominence as natural zootechnical additives due to their probiotic function in the rumen (Elghandour et al., 2024). *Saccharomyces cerevisiae* plays a key role in removing oxygen (Elghandour et al., 2019), competing for substrates with lactic acid-producing bacteria, and providing microbial growth factors (Amin & Mao, 2021). These actions enhance ruminal fermentation, potentially improving nutrient digestibility.

Numerous studies have demonstrated the efficacy of yeast supplementation in ruminants, particularly in cattle (Puniya et al., 2015; Ran et al., 2018; É. Rodrigues et al., 2013; Sartori et al., 2017; Zhang et al., 2022). However, research focusing on sheep remains limited and inconsistent, making it challenging to predict its effects on this species (Sales, 2011; Mohammed et al., 2018).

This study hypothesizes that active yeast supplementation positively alters fermentation patterns, increasing nutrient intake and digestibility in confined sheep. The objective was to evaluate the effects of active yeast addition on nutrient intake, apparent digestibility, and nitrogen and energy balances in ewe lambs fed high-concentrate diet.

Material and Methods

Location, animals, and treatments

The experiment was conducted in the Sheep and Goat Sector at the Capim Branco Farm, part of the Universidade Federal de Uberlândia (UFU) in Uberlândia, Minas Gerais, Brazil. The region has an average annual temperature of 22.3 °C and rainfall of 1342 mm. All procedures were approved by the

Ethics Committee on the Use of Animals (CEUA) at UFU under protocol number 145/16.

Five Dorper × Santa Inês crossbred ewe lambs, with an average initial weight of 54.1 ± 1.4 kg and 8 months of age, were used. Animals were identified, weighed, and treated for endoparasites with Zolvix® (2.5 mg monepantel per kg body weight - BW). They were then randomly assigned to individual metabolic cages equipped with feeders, drinkers, and devices for feces and urine collection.

Treatments included a control diet (no active yeast) and four active yeast levels (0.15, 0.30, 0.45, and 0.60% dry matter - DM). These levels were selected to include approximately two levels below and two above the manufacturer's recommendation. The active yeast product, Active Flora® (ICC, Louisville, Kentucky, USA), contained 2.0×10^{10} colony-forming units (CFU) g^{-1} of *Saccharomyces cerevisiae* strains. Yeast levels were calculated based on daily DM intake (kg $animal^{-1}$ day^{-1}) and mixed with the concentrate at feeding time.

The diet was formulated to meet the nutritional requirements of medium-sized 8-month-old ewe lambs with an average daily gain of 300 g $animal^{-1}$ day^{-1} (National Research Council [NRC], 2007). It consisted of 20% roughage and 80% concentrate on a DM basis (Table 1). The total mixed ration (TMR) was offered in two equal portions daily at 08:00 a.m. and 04:00 p.m. Feed intake was adjusted based on leftovers from the previous day, allowing for 5–10% leftovers. As the yeast was mixed with the concentrate, and leftovers primarily consisted of fibrous material, total yeast intake was ensured.

Table 1
Ingredients of the concentrate and the chemical composition of the feed and total mixed ration (TMR)

Ingredient (g kg^{-1} DM)	Concentrate	Corn silage	TMR
Ground corn	600.0	-	480.0
Soybean meal	360.0	-	288.0
Mineral mixture	25.0	-	20.0
Urea	10.0	-	8.0
Bromatological composition (g kg^{-1} DM)			
Dry matter (g kg^{-1} NM)	906.45	283.16	781.79
Organic matter	929.56	960.56	935.76
Mineral matter	70.44	39.44	64.24
Crude protein	233.26	92.46	205.10
Ether extract	15.22	32.85	18.75
Neutral detergent fiber	139.73	503.22	212.43
Acid detergent fiber	35.12	254.69	79.03
Total carbohydrates	685.62	835.25	715.55
Non-fibrous carbohydrates	612.44	367.20	563.39
Total digestible nutrients	846.40	211.60	719.44
NDIP	32.49	27.35	31.46
ADIP	16.38	1.17	13.34
Active Flora®	CP min. 44% DM**		

*Minimum and maximum values per kilogram of the product guaranteed by the manufacturer. Calcium: 120 g (min), 150 g (max); Phosphorus: 80 g (min); Sulfur: 15 g (min); Magnesium: 15 g (min); Sodium: 110 g (min); Cobalt: 172 mg (min); Copper: 1875 mg (min); Iron: 1500 mg (min); Iodine: 156 mg (min); Manganese: 3440 mg (min); Selenium: 36 mg (min); Zinc: 6250 mg (min); Fluorine: 800 mg (max). **Minimum percentage of crude protein in DM guaranteed by the manufacturer. NM = Natural matter; NDIP = Neutral detergent insoluble protein; ADIP = Acid detergent insoluble protein; TMR = Total mixed ration.

Experimental design, measurements, and analytical methods

The experiment lasted 75 days, divided into five 15-day periods in a 5 × 5 Latin square design, totaling 25 experimental units. Each period included 10 days for animal adaptation to the experimental diets and metabolism cages, followed by 5 days of data collection. Daily samples of silage, concentrate, TMR, and feed leftovers were collected, weighed, and stored. At the end of each period, samples were mixed and homogenized to create composite samples for each animal, which were then stored at -20 °C.

Urine collection involved the addition of 100 mL of 2N hydrochloric acid (HCl) to collection buckets the day before sampling to prevent nitrogen losses from volatilization. For each animal, a composite urine sample representing 20% of the total daily volume was prepared for each experimental period, stored in plastic bottles, and kept at -10 °C.

Fecal samples were collected daily, with total fecal output recorded. A 20% aliquot of the daily fecal output was taken to create composite samples for each animal per period, which were stored in individual plastic bags at -10 °C.

The determination of DM (method 967.03), mineral matter (MM) (method 942.05), crude protein (CP) (method 981.10), and ether extract (EE) (method 920.39) followed the protocols outlined by the Association of Official Analytical Chemists [AOAC] (2016). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG) were measured using the sequential method described by Van Soest et al. (1991). Neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), and NDF corrected for ashes and protein (NDFcp) were analyzed according to Licitra et al. (1996).

Total carbohydrate (TC) content was estimated using the equation proposed by Sniffen et al. (1992), as follows:

$$TC = 100 - (\%\ CP + \%\ EE + \%\ MM)$$

where: *TC* = total carbohydrates; *CP* = crude protein; *EE* = ether extract; and *MM* = mineral matter.

Non-fibrous carbohydrates (NFC) content in silage and feces was estimated using the equation recommended by Detmann et al. (2012), as follows:

$$NFC = 100 - (\%\ CP + \%\ EE + \%\ MM + \text{NDFcp})$$

where: *NDFcp* = neutral detergent fiber corrected for ashes and protein.

Due to the presence of urea in the concentrate, the values of NFC of this dietary compound, offered feed, and leftovers were estimated as proposed by Hall (2000), in which:

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

Total digestible nutrients (TDN) were calculated using the equation suggested by Weiss (1999), as follows:

$$TDN = [DCP + DNFC + NDFcp + (DEE\ x\ 2.25)]$$

Where: *DCP* = digestible crude protein; *DNFC* = digestible non-fibrous carbohydrates; *NDFcp* = neutral detergent fiber corrected for ashes and digestible protein; and DEE = digestible ether extract.

Intakes were obtained using the following formula:

$$INut = (Intake\ x\ \%\ Intake) - (kg\ Leftovers\ x\ \%\ Leftovers)$$

wherein: *INut* = nutrient intake (kg); *Intake* = amount of food offered (kg); *% Intake* = nutrient content within the amount of food offered (%); *Leftovers* = amount of leftovers removed (kg); *%Leftovers* = nutrient content within the amount of leftovers removed (%). Apparent digestibility coefficients were determined using the formula proposed by J. F. C. Silva and Leão (1979), as follows:

$$ADC = \frac{(Intake - Excreted)}{Intake\ x\ 100}$$

wherein: *ADC* = apparent digestibility coefficient (%); *Intake* = average amount of nutrients within the ingested food (offered – leftovers) (kg day^{-1}); *Excreted* = average amount of nutrients within feces removed (kg day^{-1}).

Nitrogen (N) content in urine was determined using the Kjeldahl method (Silva & Queiroz, 2002). Nitrogen balance (NB), or retained nitrogen, was calculated using the formula proposed by Zeoula et al. (2006), which accounts for nitrogen consumed (NC),

nitrogen excreted in feces (NF), and nitrogen excreted in urine (NU):

$$NB = (N\ offered\ g - N\ in\ leftovers\ g) - (N\ in\ feces\ g + N\ in\ urine)$$

Nitrogen intake (NI) was calculated by subtracting the N content in feed leftovers from the N content in the feed offered. Nitrogen absorbed was determined by subtracting fecal nitrogen (NF) from NI.

Gross energy (GE) was measured using an adiabatic calorimeter (Parr®, model 6200, Moline, Illinois, USA) with a calorimetric bomb, following the direct energy determination method. GE was analyzed for the feed offered, feed leftovers, and feces. Digestible energy (DE) was calculated using the formula by Blaxter and Clapperton (1965), where DE equals the GE ingested minus the GE excreted in feces. Metabolizable energy (ME) was determined as DE minus the sum of GE lost in urine and the energy associated with methane production (MP). The metabolizability coefficients were calculated as the ratio of ME ingested to GE ingested.

Methane production was estimated using the equation proposed by Blaxter and Clapperton (1965), as follows:

$$MP = 0.67 + 0.062\ x\ AD$$

Where: *MP* = methane production expressed as kcal per 100 kcal of energy consumed; and AD = apparent digestibility of gross energy in feed.

Gross energy in urine was calculated using the equation proposed by Street et al. (1964), as follows:

$$GE\ urine\ (kcal\ g^{-1}) = 0.027 + 0.119\ x\ (\%\ N\ in\ urine)$$

Statistical procedures

The following statistical model was used:

$$Y_{ijk} = \mu + D_i + A_j + P_k + e_{ijk}$$

Where: Y_{ijl} = value corresponding to the observation of repetition *i* within treatment, in row *j*, and column *k*; μ = overall average; D_i = fixed effect of treatments (*i* = 0.0, 0.15, 0.30, 0.45, and 0.6%); A_j = effect of rows (animals) (*j* = 1, 2, 3, 4, and 5); P_k = effect of columns (periods) (*k* = 1, 2, 3, 4, and 5); e_{ijk} = random error associated with the observation.

Data were evaluated for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965) and for homoscedasticity of variances using Levene's test (Levene, 1960). Once these assumptions were confirmed, the data were subjected to regression analysis to evaluate the significance of linear and quadratic effects, using the SAS statistical software package (Statistical Analysis System Institute [SAS Institute], 2015), at a 5% significance level.

Results and Discussion

The inclusion of active yeast had a quadratic effect on the intakes of dry matter (DM), organic matter (OM), and total carbohydrates (TC) ($P < 0.05$), with maximum intake levels estimated at 1400, 1287, and 971 g day^{-1}, respectively. These corresponded to active yeast inclusion levels of 0.35%, 0.34%, and 0.34% (Table 2). These findings can be attributed to the ability of active yeast to enhance fermentation in starch-rich diets by removing ruminal oxygen and stabilizing acidosis. As noted by Beauchemin

et al. (2003), subclinical acidosis is common in animals fed high-starch diets, where the accumulation of short-chain fatty acids leads to a drop in ruminal pH when their production exceeds absorption. Clinical acidosis, characterized by a sharp pH decline, results from lactic acid accumulation produced by starch-fermenting bacteria (Kozloski, 2017).

Table 2
Intakes of dry matter (DMI), organic matter (OMI), crude protein (CPI), neutral detergent fiber (NDFI), acid detergent fiber (ADFI), total carbohydrates (TCI), and non-fiber carbohydrates (NFCI) for ewe lambs fed diets containing increasing active yeast levels

Parameter (g animal^{-1} day^{-1})	Treatment					*p*-value			CV (%)
	0%	0.15%	0.30%	0.45%	0.60%	L	Q	LFM	
[1]DMI	992.3	1340.2	1367.3	1338.5	1225.0	0.016	0.027	0.737	18.62
[2]OMI	920.5	1236.0	1257.7	1230.2	1121.4	0.018	0.029	0.746	18.76
[3]CPI	192.7	260.9	270.0	269.0	247.5	0.037	0.067	0.854	22.62
[4]NDFI	237.6	273.8	274.3	263.6	224.6	0.105	0.071	0.969	19.11
[5]ADFI	99.0	95.9	113.4	100.0	83.6	0.237	0.139	0.481	22.05
[6]TCI	699.9	934.3	953.2	923.2	842.7	0.017	0.026	0.743	18.39
[7]NFCI	555.6	722.7	804.7	741.7	717.5	0.041	0.075	0.800	22.86

L = Linear effect; Q = Quadratic effect; LFM = Model lack-of-fit effect; CV = Coefficient of variation. Equations: 1 - $Y = 1020.12 + 217.32X - 31.07X^2$ ($R^2 = 0.929$); 2 – $Y = 945.71 + 197.39X - 28.50\ X^2$ ($R^2 = 0.928$); 3 - $Y = 197.43 + 43.89X$ ($R^2 = 95.15$); 4 – $Y = 254.77$; 5 – $Y = 98.39$; 6 – $Y = 718.75 + 147.60X - 21.55X^2$ ($R^2 = 0.928$); 7 - $Y = 564.54 + 123.31X$ ($R^2 = 0.929$).

Active yeast also competes with starch-fermenting bacteria, such as Streptococcus bovis and Lactobacillus, for substrates, effectively limiting lactic acid production (Chaucheyras-Durand et al., 2008; Amin & Mao, 2021). This competition, along with ruminal pH stabilization, likely improved rumen fermentation and stimulated feed intake.

Similar outcomes have been observed in other ruminants. Hassan et al. (2016) reported increased feed intake in pre-weaned calves supplemented with 2.5 g of yeast. Kholif et al. (2017) found higher intake in dairy goats fed 4 g of yeast, and Oliveira et al. (2010) observed increased DM and OM intakes in dairy cows receiving 10 g of active yeast. Conversely, Neumann et al. (2008), working with sheep in a creep feeding system, found no differences in intake with live yeast supplementation. Similarly, Rodrigues et al. (2021) reported no effect on intake variables in sheep fed a high-concentrate diet supplemented with 3 g of active yeast.

The maximum intake for DM, OM, and TC was achieved at an average yeast inclusion level of 0.35%, beyond which intake decreased. According to Mousa et al.

(2012), animal responses to *Saccharomyces cerevisiae* supplementation depend on the type and dose of the additive used. The quadratic effect observed in this study may be due to the presence of culture medium in yeast products, which supports the production of secondary metabolites (Siddiqui et al., 2012), such as phenolic compounds. These metabolites can modulate microbial populations in the rumen, potentially affecting fermentation and reducing intake by forming antinutritional enzyme complexes (Lima et al., 2010). Additionally, higher yeast doses may reduce feed palatability, further limiting intake.

Research on yeast supplementation in sheep and goat diets indicates that typical dosages range between 0.5 and 15 g per animal per day (Pienaar et al., 2015; Kholif et al., 2017; Rodrigues et al., 2021; Gloria-Trujillo et al., 2022; Elghandour et al., 2024). In this study, yeast product dosages varied from 0 to 14 g per animal daily, confirming that no overdosing occurred, consistent with findings in the literature. However, the observed decrease in DM intake beyond the 0.35% inclusion level (approximately 4.4 g per animal per day of yeast product) raises new questions regarding optimal yeast supplementation in confined sheep diets. This highlights the importance of evaluating the substrate used in the yeast culture medium, as it may influence ruminal microbiota interactions with feed particles by forming antinutritional factors, potentially impacting intake behavior.

Crude protein (CP) and non-fibrous carbohydrate (NFC) intakes increased linearly with active yeast inclusion ($P < 0.05$), with a tendency toward a quadratic effect for these variables ($P = 0.05–0.10$). The rise in CP intake could be attributed to the high protein content in yeast (Table 1), which ranges from 28.70% to 38.28% according to Araújo et al. (2009). Yeast's effects on the ruminal microbiota, such as stabilizing ruminal pH and providing substrates for microbial growth (Amin & Mao, 2021), likely enhanced the degradation of protein, soluble sugars, and starch. This, in turn, increased microbial protein synthesis, absorption through the ruminal wall, and flow to the intestine (Fereli et al., 2010), resulting in greater CP and NFC intake.

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) intakes (NDFI and ADFI, respectively) were not significantly affected by active yeast inclusion ($P \geq 0.05$). Feed intake is influenced by numerous factors, including the animal's physiological state, diet composition, feed quality and quantity, palatability, fiber digestion, and digesta flow rate (Hassan et al., 2016). In this study, the low roughage content in the diet, leading to reduced fiber levels (Table 1), along with the high nutritional quality of the feed, may explain the lack of a significant impact of the fibrous fraction on intake.

The apparent digestibility of nutrients was not significantly influenced by the treatments ($P \geq 0.05$) (Table 3), with mean values for all variables remaining high, regardless of yeast inclusion levels. The mean apparent digestibility of DM was 86.96%. These results suggest that digestibility was primarily influenced by the type of diet provided, which was rich in easily and rapidly degradable substrates.

Table 3
Digestibility of dry matter (DDM), organic matter (DOM), crude protein (DCP), neutral detergent fiber (DNDF), total carbohydrates (DTC), and non-fiber carbohydrates (DNFC) for ewe lambs fed diets containing increasing active yeast levels

Parameter (%)	Treatment					*p*-value			CV (%)
	0%	0.15%	0.30%	0.45%	0.60%	L	Q	LFM	
[1]DDM	86.96	88.71	85.81	85.72	87.61	0.653	0.693	0.544	5.27
[2]DOM	88.57	89.78	86.97	86.93	88.90	0.528	0.576	0.585	5.06
[3]DCP	83.38	87.90	84.87	85.23	86.19	0.636	0.698	0.499	6.69
[4]DNDF	74.84	75.91	69.53	68.78	70.43	0.440	0.656	0.662	12.35
[5]DTC	85.43	86.51	83.21	82.35	85.43	0.436	0.506	0.573	6.63
[6]DNFC	94.51	94.23	93.89	92.58	94.52	0.400	0.409	0.619	3.22

L = Linear effect; Q = Quadratic effect; LFM = Model lack-of-fit effect; CV = Coefficient of variation. Equations: 1 – Y = 86.96; 2 – Y = 88.23; 3 – Y = 85.51; 4 – Y = 71.90; 5 – Y = 84.59; 6 – Y = 93.95.

According to McCarthy et al. (1989), ruminal microorganisms dynamically regulate degradation rates to maintain a balance in substrate availability for the microbiota. In this study, the combination of NFC sources, such as ground corn, with highly soluble urea likely enhanced ruminal degradation and microbial protein synthesis, increasing the flow of microbial protein to the small intestine. Additionally, the rumen undegradable protein (RUP) in the diet was of high biological value, further contributing to the high overall digestibility.

Elghandour et al. (2019) noted that active yeast primarily improves fermentation by assimilating ruminal oxygen, promoting cellulolytic bacterial growth, and enhancing fiber digestion. However, studies by Mousa et al. (2012) and Sartori et al. (2017) demonstrated that the positive effects of *Saccharomyces cerevisiae* supplementation on digestibility are more pronounced in diets containing 30–50% forage. Higher fiber content, associated with less digestible particles, contrasts with the low-fiber, high-concentrate diet used in this study, potentially explaining the lack of impact on digestibility.

Similar findings have been reported in other studies. Pienaar et al. (2015), investigating apparent digestibility in a standard finishing diet for lambs, found no effect of active yeast supplementation (0.22 g kg^{-1} DM) on digestibility variables. Gloria-Trujillo et al. (2022) also reported no changes in digestibility coefficients with the inclusion of 0, 3, 5, and 10 g per animal per day of *Saccharomyces cerevisiae* in starch-rich diets for lambs. Thus, the low NDF content of the diet likely limited the potential benefits of yeast supplementation on nutrient digestibility in this study.

Active yeast levels had a quadratic effect on nitrogen (N) intake and urinary N excretion ($P < 0.05$) (Table 4), with maximum values of 59.36 g and 14.68 g per animal per day at inclusion levels of 0.36% and 0.34% yeast, respectively. Fecal N and absorbed N increased linearly with yeast inclusion ($P <$

0.05). Retained N, although positive, was not significantly affected by treatments (P ≥ 0.05). The observed increase in N intake aligned with the pattern of CP intake (Table 2), leading to higher N absorption, and increased urinary and fecal N excretions.

Table 4
Nitrogen balance in ewe lambs fed diets containing increasing active yeast levels

Parameter (g animal^{-1} day^{-1})	Treatment					*p*-value			CV (%)
	0%	0.15%	0.30%	0.45%	0.60%	L	Q	LFM	
[1]N ingested	43.77	55.69	57.77	58.63	52.59	0.002	0.036	0.900	17.53
[2]N fecal	4.25	5.02	6.65	6.47	5.18	0.043	0.072	0.660	32.40
[3]N urinary	8.03	13.13	15.06	12.98	11.57	0.001	0.003	0.540	22.28
[4]N absorbed	39.51	50.66	52.12	52.17	47.41	0.045	0.074	0.831	19.80
[5]N retained	3.49	37.53	36.07	39.19	35.84	0.223	0.306	0.741	22.04

L = Linear effect; Q = Quadratic effect; LFM = Model lack-of-fit effect; CV = Coefficient of variation. Equations: 1 - $Y = 44.27 + 8.44X - 1.18X^2$ ($R^2 = 0.973$); 2 – $Y = 4.01 + 1.34X$ ($R^2 = 0.866$); 3 – $Y = 8.34 + 3.70X - 0.54X^2$ ($R^2 = 0.930$); 4 – $Y = 40.26 + 7.10X - 0.99X^2$ ($R^2 = 0.935$); 5 – $Y = 36.03$.

On average, fecal N losses accounted for 10.26% of ingested N, while urinary losses represented 22.63%. The greater urinary N losses can be attributed to the high CP content (20.51%) and the presence of urea in the diet, which enhanced urea degradation and amino acid deamination (Zeoula et al., 2006). Excess ammonia, absorbed through the ruminal wall, increased hepatic urea synthesis, resulting in greater urinary N losses (Van Soest, 1994). This process is energetically demanding, with energy losses for urea synthesis in sheep calculated at 88.4 kcal mol^{-1} (Martin & Blaxter, 1965).

The effects of active yeast on microbial N metabolism remain unclear, as noted by Fonty and Chaucheyras-Durand (2006) and Chaucheyras-Durand et al. (2008). Ammonia concentration, a commonly used parameter to evaluate yeast effects on N metabolism, is highly variable. Wallace and Newbold (1995) suggested that deamination in the rumen is energetically inefficient, as energy spent hydrolyzing peptides and amino acids into ammonia could instead support microbial protein synthesis.

Research in adult animals has indicated that active yeast may reduce ruminal ammonia by limiting proteolytic bacterial activity. This mechanism, observed in vitro, involves competition between yeast and bacteria for energy and an inhibitory effect of yeast-derived peptides on bacterial peptidases. Contrarily, other studies suggest that yeast could enhance microbial growth by increasing proteolytic activity and reducing N losses if dietary soluble N and carbohydrate levels are balanced (Fonty & Chaucheyras-Durand, 2006; Chaucheyras-Durand et al., 2008).

In this study, increasing active yeast levels enhanced N intake and absorption but also increased N losses, without affecting the overall N balance. Additionally, since apparent CP digestibility coefficients were unaffected, we concluded that active yeast, despite increasing intake, did not influence CP digestibility or N retention. Notably, the average apparent digestibility of CP was high (Table 3), likely due to the high solubility of urea in the dietary concentrate, indicating no detrimental effect on fecal and urinary N losses.

Monnerat et al. (2013) evaluated the effect of supplementation with 12.5 g kg^{-1} BW of yeast in beef cattle fed high-concentrate diets on N balance and reported no significant effects of the additive on the respective parameters. In a more recent study, supplementation with 15 g of yeast per animal per day was evaluated in cows fed high- and low-starch diets. Results showed that microbial N production was higher in cows on the high-starch diet supplemented with yeast, and ammoniacal N concentration was lower in the same group. The combination of NFC energy supply and yeast presence in the rumen promoted enhanced microbial proliferation, likely increasing the duodenal flow of methionine, and altering the availability of amino acids for absorption in the small intestine (Dias et al., 2018). According to Bach et al. (2019), live yeast can bypass ruminal degradation and reach the small intestine, benefiting the intestinal microbiota by inhibiting pathogenic bacteria. Additionally, the experimental diet contained soybean meal (Table 1), a high-quality source of RUP, which, together with yeast, may have contributed to improved absorption of essential amino acids in the intestine.

A quadratic effect of treatments was observed for gross energy (GE) intake, metabolizable energy (ME), and the metabolizability of ingested GE ($P < 0.05$), with maximum values of 5932 kcal, 2850 kcal per animal per day, and 47.35%, corresponding to yeast inclusion levels of 0.33%, 0.32%, and 0.31%, respectively (Table 5). No significant effects were found for fecal GE, digestible energy (DE), or urinary GE ($P \geq 0.05$), likely reflecting the lack of influence on apparent digestibility coefficients (Table 2), which were also unaffected by active yeast levels. The high energy density of the diet likely diminished the additive effect of yeast on these parameters.

Table 4
Energy balance for ewe lambs fed diets containing increasing active yeast levels

Parameter (kcal animal^{-1} day^{-1})	Treatment					*p*-value			CV (%)
	0%	0.15%	0.30%	0.45%	0.60%	L	Q	LFM	
[1]GE ingested	4580.2	5792.1	5759.3	5746.4	5124.5	0.007	0.009	0.639	13.00
[2]GE fecal	2684.3	3051.4	2754.5	3072.5	2962.8	0.281	0.476	0.105	9.46
[3]DE	1868.1	2402.2	2602.7	2334.9	1912.1	0.982	0.044	0.963	28.48
[4]GE urinary	150.7	201.2	151.3	171.1	206.3	0.936	0.761	0.429	47.74
[5]ME	1776.0	2620.0	2884.7	2553.9	2041.7	0.018	0.019	0.916	30.59
[6]ME/GE (%)	36.18	42.59	49.69	43.4	37.54	0.025	0.024	0.704	21.00

CV = coefficient of variation; L= Linear effect; Q= Quadratic effect; LFM = Lack-of-fit to the model; GE = Gross energy; DE = Digestible energy; ME = Metabolizable energy; ME/GE = Metabolizability coefficient. Equations: 1 – $Y = 4670.85 + 764.29\,X - 115.79\,X^2$ ($R^2 = 0.920$); 2 – $Y = 2905.10$; 3 – $Y = 2224.00$; 4 – $Y = 176.14$; 5 – $Y = 1809.85 + 661.17X - 105.03X^2$ ($R^2 = 0.977$); 6 – $Y = 35.76 + 7.46X - 1.20X^2$ ($R^2 = 0.903$).

The quadratic trends for ingested GE, ME, and metabolizability of ingested GE paralleled the pattern observed for OM intake (Table 2). As OM intake, the primary source of energy for the animal increased, corresponding increases in GE intake, available metabolizable energy, and metabolizability were also observed. The metabolizability coefficient reflects the relationship between metabolizable energy and gross energy in the diet.

In a study by Kholif et al. (2017), the effects of 4 g of yeast, 4 g of an exogenous enzyme, or 8 g of a 1:1 mixture of the two additives were evaluated in a diet with a roughage-to-concentrate ratio of 40:60 for lactating goats. The study found that yeast supplementation improved digestible energy and metabolizable energy compared to the control treatment. This effect was attributed to yeast's ability to eliminate oxygen on the surfaces of freshly ingested feed, thereby lowering the redox potential in the rumen. Additionally, yeast provides soluble compounds, such as organic acids, amino acids, peptides, and vitamins, which are essential for the activity and efficient growth of ruminal bacteria.

Conclusion

Inclusion of active yeast at levels between 0.31% and 0.36% of dietary dry matter increased nutrient intake, nitrogen absorption, metabolizable energy availability, and energy metabolizability, without affecting the apparent digestibility of nutrients in ewes fed an 80% concentrate diet. Yeast levels exceeding 0.36% may negatively impact these parameters.

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To Professor Dr. Luciano Fernandes Sousa *(in memoriam).*

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