

Combination of active and inactive yeast and its use as an additive for confined ewe lambs: nutritional parameters

Combinação de levedura ativa com inativa e seu uso como aditivo para borregas confinadas: parâmetros nutricionais

Tays Raniellen Miranda Feitosa^{1*}; Luciano Fernandes Sousa¹; Deborah Alves Ferreira¹; Karla Alves Oliveira²; Erica Beatriz Schultz³; Marcela Rodrigues de Oliveira⁴; Marco Túlio Santos Siqueira⁵; Lucas Eduardo Gonçalves Vilaça⁶; Jhone Tallison Lira de Sousa¹; Gilberto de Lima Macedo Junior⁷

Highlights

The additive does not affect the feed intake of confined lambs.

Probiotics and prebiotics does not influence the digestibility of the lamb's diet.

Active and inactive yeasts does not change the nitrogen and energy retention in lambs.

Abstract

This study aimed to evaluate the effects of adding increasing levels of a combination of active and inactive yeast (Milk Sacc X[®] - Alltech[®], Maringá, Paraná, Brazil, 5.0 x 10⁸ CFU) on the consumption and apparent digestibility of dry matter (DM) and its components, as well as the nitrogen and energy balances of lambs fed high-concentrate diets. Five Dorper x Santa Inês crossbred lambs, averaging an initial weight of 40.40 ± 0.15 kg, were housed in individual metabolic cages. The treatments included five

¹ Drs. in Animal Science, Center of Agricultural Sciences, Universidade Federal do Norte do Tocantins, UFNT, Araguaína, TO, Brazil. E-mail: tays.feitosa@ufnt.edu.br; luciano.sousa@mail.uft.edu.br; deborah.alvesferreira@mail.uft.edu.br; jhonelira@hotmail.com

² Dr^a in Animal Science, Instituto Federal do Triângulo Mineiro, IFTM, Uberaba, MG, Brazil. E-mail: karla.alves.oliveira@hotmail.com

³ PhD in Animal Science, Department of Animal Science, Universidade Federal de Viçosa, UFC, Viçosa, MG, Brazil. E-mail: erica.schultz@ufv.br

⁴ Student of the Master's course in Animal Production, Universidade Federal de Uberlândia, UFU, Minas Gerais, Brazil. E-mail: marcelaro1002@outlook.com

⁵ M.e in Animal Science, Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP/FCAV, Jaboticabal, SP, Brasil. E-mail: marcotulio.s.siqueira@gmail.com

⁶ Student of the Master's course in Animal Production, Universidade Federal de Uberlândia, UFT, Uberlândia, Minas Gerais, Brazil. E-mail: luedugovi@hotmail.com

⁷ Dr. in Animal Science, UFT, Uberlândia, MG, Brazil. E-mail: gilbertomacedojr@gmail.com

* Author for correspondence

levels of yeast combination 0, 0.15, 0.3, 0.45, and 0.6% of the DM offered in kg animal⁻¹ day⁻¹ blended into a diet with a 20:80 ratio of corn silage to concentrate based on DM. Employing a 5x5 Latin square design, the study involved five animals over five periods, thus creating 25 experimental units. Regression analysis conducted at a significance level of 5% indicated no effects of the treatments on consumption variables; however, there was a significant lack of model fit (LMF), with the average DM consumption being 1343.0 g animal⁻¹ day⁻¹. The treatments also showed no effect on the apparent digestibility variables, which averaged 86.9% for DM. Similarly, the nitrogen balance variables were unaffected by the yeast levels, as indicated by the LMF effect showing that the data did not fit the regression model; the average retained nitrogen was 45.3 g animal⁻¹ day⁻¹. Energy measurements, including gross energy (GE) ingested, digestible energy (DE), metabolizable energy (ME), and metabolizability, did not fit the regression model, with averages of 5549, 2685, 2504 kcal animal⁻¹ day⁻¹, and 42.6%, respectively. Neither fecal nor urinary GE excretion was influenced by the treatments. In conclusion, the combination of active and inactive yeast does not alter the intake, nutrient digestibility, nitrogen, or energy balance of lambs fed a high-concentrate diet.

Key words: Prebiotic. Probiotic. *Saccharomyces cerevisiae*. Sheep. Starch.

Resumo

Objetivou-se avaliar os efeitos da adição de níveis crescentes da combinação de levedura ativa e inativa sobre o consumo e digestibilidade aparente da matéria seca (MS) e seus componentes, e balanços de nitrogênio e de energia de borregas alimentadas com dietas de alto concentrado. Foram utilizadas 5 borregas mestiças Dorper x Santa Inês com peso inicial médio de 40,40 ± 0,15 kg, que foram alojadas em gaiolas individuais de metabolismo. Os tratamentos consistiram em 5 níveis de levedura ativa mais inativa [Milk Sacc X[®] - Alltech[®], Maringá, Paraná, Brasil, 5,0 x 10⁸ Unidades formadoras de colônia (UFC)], sendo 0; 0,15; 0,3; 0,45 e 0,6% da MS ofertada em kg animal⁻¹ dia⁻¹ de ração, que continha relação 20:80 de silagem de milho: concentrado com base na MS. O delineamento experimental utilizado foi um quadrado latino 5 x 5, com 5 animais e 5 períodos, totalizando 25 unidades experimentais. Os dados foram submetidos a análise de regressão com nível de significância de 5%. Não foram detectados efeitos dos tratamentos sobre as variáveis de consumo, ademais, houve significância para falta de ajuste do modelo (FAM), sendo a média para consumo de MS de 1343.0 g animal⁻¹ dia⁻¹. Não houve efeito dos tratamentos sobre as variáveis de digestibilidade aparente, com média de 86,89% para digestibilidade da MS. As variáveis de balanço de N não foram afetadas pelos níveis do aditivo, pois, o efeito para FAM indica que os dados não se ajustaram ao modelo de regressão. O N retido médio foi de 45,3 g animal⁻¹ dia⁻¹. Os dados de energia bruta (EB) ingerida, energia digestível (ED), energia metabolizável (EM) e metabolizabilidade não se ajustaram ao modelo de regressão, com médias de 5549, 2685, 2504 kcal animal⁻¹ dia⁻¹ e 42,6%, respectivamente. As EB fecal e urinária não foram influenciadas pelos tratamentos. A combinação de levedura ativa com inativa não altera o consumo, digestibilidade de nutrientes, balanço de N e de energia de borregas alimentadas com dieta de alto concentrado.

Palavras-chave: Amido. Ovinos. Probiótico. Prebiótico. *Saccharomyces cerevisiae*.

Introduction

Ensuring safe food for consumers, alongside promoting animal welfare and environmental preservation, are primary goals in animal production, where probiotics and prebiotics are increasingly used to boost production rates (Anadón et al., 2019). Probiotics, defined as mono or mixed cultures of live microorganisms, enhance animal health by improving gut microbiota properties (Fuller, 1992). Prebiotics, selectively utilized by host-beneficial microbes, promote their growth and activity, thereby improving host health (Gibson et al., 2017).

Yeasts, both active and inactive, are frequently evaluated in ruminant diets due to their ability to interact with and support the ruminal and intestinal microflora. Active yeasts aid ruminal metabolism by scavenging free oxygen and competing with lactic acid-producing bacteria, such as *Streptococcus bovis* and *Lactobacillus* sp., for starch utilization, often outperforming these bacteria (Elghandour et al., 2019; Amin & Mao, 2021). Inactive yeast, comprising dead cells, provides growth factors crucial for ruminal microbes, including essential amino acids, B vitamins, lipids, and minerals, while its cell wall consists primarily of α -D-mannan, chitin, and β -D-glucan (Araújo et al., 2009; Li et al., 2006).

Fomenky et al. (2017) noted that yeast cells could pass through the rumen intact, potentially benefiting the intestinal microbiota by inhibiting toxins and pathogenic microorganisms. Mannans from yeasts can adsorb pathogenic gram-negative bacteria through a process called lectin-mannan interaction, facilitating their removal from the digestive tract and preventing attachment to the intestinal mucosa, thus potentially

enhancing food digestibility and nutrient supply (Araújo et al., 2009).

While numerous studies confirm the benefits of yeast products in dairy cows and beef cattle, research on sheep remains limited, highlighting a need for further investigation into the effects of yeast additives on this animal group. Consequently, this study hypothesizes that yeast additives can alter ruminal fermentation processes, thereby increasing consumption and improving nutrient digestibility and feed efficiency in confined sheep. The aim was to assess the effects of various levels of active and inactive yeast on consumption, apparent nutrient digestibility, nitrogen and energy balances, ingestive behavior, and blood metabolites in ewe lambs fed high-concentrate diets.

Materials and Methods

Location, animals, and treatments

The experiment was conducted at the sheep and goat sector of Capim Branco Farm, owned by Universidade Federal de Uberlândia (UFU), in Uberlândia, Minas Gerais. The region has an average annual temperature of 22.3 °C and rainfall of 1342 mm. All procedures adhered to ethical standards approved by the Ethics Committee on the Use of Animals (CEUA) of UFU, under protocol number 145/16. The study involved five crossbred Dorper x Santa Inês ewe lambs, each weighing an average of 40.40 ± 0.15 kg and aged 8 months, which were identified, weighed, and treated for endoparasites before being randomly assigned to individual metabolic cages equipped with feeders, drinkers, and collection systems for feces and urine.

Treatments included five levels (0, 0.15, 0.3, 0.45, and 0.6%) of a combination of active and inactive yeast, specifically Milk Sacc X[®] (Alltech[®], Maringá, Paraná, Brasil). This commercial premix, containing 5.0×10^8 colony forming units (CFU) g⁻¹ of *Saccharomyces cerevisiae* strains, also includes zinc, copper, selenium, chromium, and prebiotic and probiotic elements. Yeast levels were calculated based on the dry matter (DM) offered in kg animal⁻¹ day⁻¹, with doses corresponding to 0, 2.8, 5.8, 9.2, and 11.6 g animal⁻¹ day⁻¹ of the product, mixed with the concentrate at feeding time.

The diet, formulated to meet the nutritional needs of medium-sized ewes targeting an average weight gain of 300 g animal⁻¹ day⁻¹ according to the National Research Council [NRC] (2007), maintained a 20:80 roughage to concentrate ratio based on DM (Table 1). The total mixed ration (TMR) was split into two daily feedings at 8:00 a.m. and 4:00 p.m. Feed consumption was adjusted based on the previous day's leftovers, allowing for a 5 to 10% residual.

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

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 NM1)	906.5	283.2	781.8
Organic matter	929.6	960.6	935.8
Mineral matter	70.4	39.4	64.2
Crude protein	233.3	92.5	205.1
Ether extract	15.2	32.9	18.8
Neutral detergent fiber	139.7	503.2	212.4
Acid detergent fiber	35.1	254.7	79.0
Total carbohydrates	685.6	835.3	715.5
Non-fibrous carbohydrates	612.4	367.2	563.4
PIND	32.5	27.4	31.5
PIAD	16.4	1.2	13.3

DM = Dry matter; MN = Natural matter; PIND = Protein insoluble in neutral detergent; PIAD = Protein insoluble in acid detergent; TMR = Total mixed ration.

Measurements and analytical methods

The experiment spanned 75 days, organized into five 15-day periods. The initial ten days allowed for animal acclimatization to the experimental diets and metabolic cages, with data collection occurring over the last five days of each period. Animal weights were recorded at the start and end of each period, before the morning feeding, to calculate consumption in g kg⁻¹ of body weight (BW) and metabolic weight (BW^{0.75}).

During the collection phases, daily samples of silage, concentrate, total mixed ration (TMR), and leftover feed were taken, weighed, and then combined and homogenized at the end of each period to create a composite sample for each animal. These samples were stored at -20°C. To prevent nitrogen loss by volatilization, 100 mL of 2N hydrochloric acid (HCl) was added to urine collection buckets the day before each collection. A 20% sample of the urine collected per animal during each period was stored at -10°C in plastic bottles. Fecal samples were collected daily, with the total weight recorded and a 20% aliquot taken for composite sample preparation. These fecal samples were stored at -10°C in individual plastic bags.

Analytical determinations of dry matter (DM), mineral matter (MM), crude protein (CP), and ether extract (EE) were performed using methods 967.03, 942.05, 981.10, and 920.39, respectively, as specified by the Association of Official Analytical Chemists [AOAC] (2016). Fiber analyses, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG), were conducted using the sequential method outlined by Van Soest et al. (1991). Neutral

and acid detergent insoluble nitrogen (NIDN and NIDA, respectively), and NDF corrected for ash and protein (NDF_{cp}) followed the methods of Licitra et al. (1996).

Total carbohydrates (TC) were estimated using the equation of Sniffen et al. (1992), $TC = 100 - (\%CP + \%EE + \%MM)$. Non-fibrous carbohydrates (NFC) in silage and feces were calculated based on Detmann et al. (2012), $NFC = 100 - (\%CP + \%EE + \%MM + \%NDF_{cp})$. For concentrates and leftover feeds containing urea, NFC was determined using the formula of Hall (2000) formula, $\%NFC = 100 - [(\%CP - \%CP \text{ derived from urea} + \%urea) + \%EE + \%NDF_{cp} + \%MM]$. Intakes were obtained using the formula of Maynard et al. (1984), as follows:

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

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 of J. F. C. Silva and Leão (1979), as follows:

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

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 (D. J. Silva & Queiroz, 2002). The nitrogen balance (NB), or retained nitrogen, was calculated

based on the formula proposed by Zeoula et al. (2006). This calculation considers the nitrogen consumed (NC), nitrogen excreted in feces (NF), and nitrogen excreted in urine (NU):

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

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

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

Methane production was estimated using the following equation from Blaxter and Clapperton (1965):

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

wherein: MP = methane production in kcal 100 kcal⁻¹ energy consumed; and AD = apparent digestibility of food gross energy.

The gross energy of urine was calculated using the equation proposed by Street et al. (1964):

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

Experimental design and statistical procedures

The experiment employed a 5 x 5 Latin square design, comprising 25 experimental units. The statistical analysis was conducted according to the following model:

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

Wherein: Y_{ijk} = observed value for the repetition in treatment i , row j , and column k ; μ = general mean; D_i = effect of treatments (diets) ($i = 0.0, 0.15, 0.3, 0.45, \text{ and } 0.6\%$); A_j = effect of rows (animals) ($j = 1, 2, 3, 4, \text{ and } 5$); P_k = effect of columns (periods) ($k = 1, 2, 3, 4, \text{ and } 5$); e_{ijk} = random error associated with the observation.

The data underwent the normality test (Shapiro & Wilk, 1965) and homoscedasticity test of variances (Levene, 1960). Upon confirming these assumptions, regression analysis was conducted to assess the significance of linear and quadratic effects using the SAS statistical package, with a significance level set at 5%.

Results and Discussion

No significant effects of the treatments were observed on intake parameters ($P \geq 0.05$). The data did not fit the regression model, as shown by a significant lack of model fit (LMF) ($P < 0.05$) (Table 2). This result may be attributed to the more pronounced impact of nutrient supply, which promotes microbial growth and eliminates oxygen from the ruminal environment, when high-fiber diets are used (Ogunade et al., 2019). Conversely, these effects are less pronounced in high-concentrate diets (Fonty & Chaucheyras-Durand, 2006), resulting in no changes to feed intake.

Table 2

Intake 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) in ewe lambs fed a high-concentrate diet with increasing levels of active and inactive yeast as an additive

Parameter	Additive (% DM)					CV (%)	P-value		
	0	0.15	0.3	0.45	0.6		L	Q	LFM
DMI (g animal ⁻¹ day ⁻¹)	1338.4	1836.2	1431.9	1023.8	1084.5	11.87	0.161	0.006	0.000
DMI (% BW)	3.2	4.3	3.3	2.3	2.5	11.66	0.212	0.006	0.000
DMI (g kg ⁻¹ BW ^{0.75})	90.5	108.9	85.1	59.9	63.3	1155	0.192	0.006	0.000
OMI (g animal ⁻¹ day ⁻¹)	1233.3	1699.6	1323.8	941.7	995.9	12.29	0.159	0.007	0.000
OMI (% PC)	2.9	3.9	3.1	2.1	2.3	12.21	0.233	0.008	0.000
OMI (g kg ⁻¹ PC ^{0.75})	74.3	100.8	78.6	55.1	58.3	12.06	0.206	0.007	0.000
CPI (g animal ⁻¹ day ⁻¹)	300.0	393.3	302.5	234.9	246.2	10.25	0.383	0.017	0.000
NDFI (g animal ⁻¹ day ⁻¹)	276.7	302.2	280.4	154.6	210.8	17.77	0.480	0.570	0.004
ADFI (g animal ⁻¹ day ⁻¹)	122.4	158.0	126.0	93.7	108.9	13.18	0.759	0.169	0.001
TCI (g animal ⁻¹ day ⁻¹)	854.7	1202.8	939.4	637.4	677.1	13.79	0.134	0.006	0.000
NFCI (g animal ⁻¹ day ⁻¹)	670.6	970.5	760.2	525.4	548.7	15.47	0.098	0.007	0.001

DM = Dry matter; CV = coefficient of variation; L = Linear effect; Q = Quadratic effect; LFM = Lack of fit to the model.

Similarly, no significant effects of the treatments were observed on apparent digestibility parameters ($P \geq 0.05$) (Table 3). The average dry matter digestibility (DMS) was 86.89%, aligning with findings from Siqueira et al. (2020) and Rodrigues et al.

(2021), who reported average apparent DM digestibility values of 84.51% and 85.79%, respectively, in studies supplementing sheep fed high-concentrate diets with a combination of yeast and inactive yeast.

Table 3

Apparent digestibility of dry matter (DDM), organic matter (DOM), crude protein (DCP), neutral detergent fiber (DNDF), total carbohydrates (DTC), non-fiber carbohydrates (NFCD), and total digestible nutrients (TDN) in ewe lambs fed a high concentrate diet with increasing levels of active yeast as an additive

Parameter (%)	Additive (% DM)					CV (%)	P-value		
	0	0.15	0.3	0.45	0.6		L	Q	LFM
DDM	89.3	90.7	87.1	84.3	83.1	5.95	0.802	0.632	0.662
DOM	90.6	91.9	88.7	86.6	85.2	5.45	0.887	0.597	0.700
DCP	90.8	91.3	86.7	85.2	84.2	5.38	0.423	0.992	0.595
DNDF	80.7	79.7	74.7	62.0	67.3	14.03	0.275	0.782	0.275
DTC	89.0	91.5	88.7	85.7	84.1	6.48	0.778	0.396	0.696
TDN	94.8	94.8	94.1	93.1	91.4	3.15	0.861	0.442	0.999

CV = coefficient of variation; L = Linear effect; Q = Quadratic effect; LFM = Lack of fit to the model.

Few studies have explored the association of active and inactive yeast, and limited information is available on the combined effects of probiotic and prebiotic supplementation on digestive function in ruminants fed high-energy diets. According to Zapata et al. (2021), the distinct modes of action of probiotics (live yeast cells) and prebiotics (fiber, cell wall material, mannan-polysaccharides, and derivatives of yeast cell wall hydrolysis) may result in additive effects on digestion and fermentation when used together. In their study, supplementation of lambs fed a high-energy diet with 3 g (equivalent to 0.26% of ingested DM) of active yeast, inactive yeast, or a probiotic-prebiotic combination improved digestion and fermentation patterns of the diet.

The apparent digestibility averages were high across all variables studied, indicating efficient diet utilization. The diet contained high levels of highly fermentable carbohydrates, a highly soluble non-protein nitrogen source in the rumen, and high biological value non-degradable protein, derived from crushed corn, urea, and soybean meal, respectively (Table 1). Products containing active and inactive yeast are commonly used to enhance the ruminal environment and improve animal performance. They achieve this through various mechanisms, including the supply of nutrients from the intracellular contents and cell walls, which serve as substrates for microbial proliferation and activity (Chaucheyras-Durand et al., 2008; Sales, 2011; Alzahal et al., 2014; Malekkhahi et al., 2016).

Yeasts can pass through the ruminant's foregut without being degraded, moderately influencing the intestinal microbiota and

colonic gene expression (Fomenky et al., 2017; Bach et al., 2018, 2019), thereby exerting potential effects at the intestinal level. *Saccharomyces cerevisiae* can inhibit toxins and pathogenic microorganisms, improving intestinal microflora, nutrient supply, and food digestibility. These effects may have contributed to the notable apparent digestibility of crude protein (CP) and neutral detergent fiber (NDF). By enhancing microbial proliferation, the utilization of fiber by ruminal microorganisms increased, as did the flow of microbial protein and nutrient absorption in the small intestine.

These findings align with those of Song et al. (2021), who reported no effects of supplementing confined sheep with 5 g of yeast culture containing both live cells and yeast cell walls on the apparent digestibilities of dry matter (DM), organic matter (OM), and CP. Similarly, Rodrigues et al. (2021) observed no significant effects of supplementing sheep with 15 g of active and inactive yeast on the apparent digestibility of DM, corroborating our results.

Nitrogen (N) balance variables were not influenced by the levels of active and inactive yeast ($P \geq 0.05$) (Table 4). The lack of model fit (LMF) indicates that the data did not conform to the regression model, consistent with the significant LMF observed in the intake variables (Table 2). The effects of probiotics and/or prebiotics on N digestion have shown variability in previous studies. When effects are detected, they are often attributed to enhanced ruminal microbial synthesis, which increases microbial N flux to the duodenum and improves N absorption efficiency (Williams & Newbold, 1996; Hristov et al., 2010). Supporting this, Garcia Diaz et al. (2018) reported reduced ruminal ammonia

concentrations in sheep fed a grain-rich diet supplemented with a combination of active and inactive yeast at a dose of 2 g/kg DM,

resulting from increased microbial protein flux to the small intestine.

Table 4

Nitrogen balance in ewe lambs fed a high-concentrate diet with increasing levels of active and inactive yeast as an additive

Parameter (g animal ⁻¹ day ⁻¹)	Additive (% DM)					CV (%)	P-value		
	0	0.15	0.3	0.45	0.6		L	Q	LFM
N ingested	63.5	82.8	64.3	51.5	54.4	8.60	0.334	0.012	0.000
N fecal	4.2	5.5	6.3	5.3	6.5	33.95	0.300	0.534	0.483
N urinary	11.8	22.5	12.4	7.1	8.2	21.74	0.193	0.008	0.000
N absorbed	59.3	77.3	58.0	46.1	47.9	10.46	0.581	0.030	0.000
N retained	47.5	54.8	45.5	39.0	39.7	10.78	0.953	0.224	0.014

CV = coefficient of variation; L = Linear effect; Q = Quadratic effect; LFM = Lack of fit to the model.

On average, 8.8% of ingested N was excreted in feces, while urinary losses accounted for 19.6%. The higher proportion of urinary N excretion compared to fecal N can be attributed to the CP content of the diet (20.51%) and the inclusion of urea to meet the animals' growth requirements. This likely led to increased urea degradation and amino acid deamination, reflecting limited synergy between the degradation of carbohydrates and nitrogen compounds under these conditions (Zeoula et al., 2006). Excess ruminal ammonia, combined with its absorption through the ruminal wall, increases hepatic urea production, leading to greater urinary N losses. Additionally, this process contributes to a higher caloric

increment (Van Soest, 1994), as it requires energy expenditure, which Martin and Blaxter (1965) calculated at 88.4 kcal/mol for sheep.

The data for ingested gross energy (GE), digestible energy (DE), metabolizable energy (ME), and metabolizability did not fit the regression model, as indicated by a significant lack of model fit (LMF) ($P < 0.05$) (Table 5). These variables were associated with intake parameters, as presented in Table 2, which also influenced the fecal apparent metabolizability (FAM). Fecal GE and urinary GE were not significantly affected by the treatments ($P \geq 0.05$), likely reflecting the patterns observed in the apparent digestibility coefficients (Table 3).

Table 5

Energy balance in ewe lambs fed a high-concentrate diet with increasing levels of active and inactive yeast as an additive

Parameter (kcal animal ⁻¹ day ⁻¹)	Additive (% DM)					CV (%)	P-value		
	0	0.15	0.3	0.45	0.6		L	Q	LFM
GE intake	5489	7421	5831	4339	4666	9.30	0.131	0.004	0.000
GE feces	2790	3013	2956	2740	2824	10.45	0.498	0.400	0.401
DE	2699	4408	2875	1599	1843	14.36	0.130	0.001	0.000
GE urine	184	172	175	170	199	36.10	0.578	0.505	0.951
EM	2514	4236	2700	1429	1643	14.27	0.088	0.001	0.000
ME/GE (%)	45.0	56.4	45.2	32.5	34.1	8.50	0.442	0.005	0.000

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.

Energy intake, metabolization, and losses are directly related to the amount of organic matter consumed. Since there were no changes in the intake or digestibility of organic matter the primary source of the energy required by the animals gross energy consumption, the energy available for metabolic processes, and metabolizability remained consistent and unchanged.

Conclusion

The combination of active and inactive yeast does not affect nutrient intake, digestibility, or the nitrogen and energy balances in ewes fed a high-concentrate diet.

Acknowledgments

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

References

- Alzahal, O., Dionissopoulos, L., Laarman, A. H., Walker, N., & McBride, B. W. (2014). Active dry *Saccharomyces cerevisiae* can alleviate the effect of subacute ruminal acidosis in lactating dairy cows. *Journal of Dairy Science*, 97(12), 7751-7763. doi: 10.3168/jds.2014-8212
- Amin, A. B., & Mao, S. (2021). Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: a review. *Animal Nutrition*, 7(1), 31-41. doi: 10.1016/j.aninu.2020.10.005
- Anadón, A., Ares, I., Martínez-Larrañaga, M. R., & Martínez, M. A. (2019). Prebiotics and probiotics in feed and animal health. In Gupta, R., Srivastava, A., & Lall, R. (Eds.), *Nutraceuticals in Veterinary Medicine* (pp 261-285). Springer, Cham.
- Araújo, L. D. F., Dias, M. V. C., Brito, E. D., & Oliveira, S., Jr. (2009). Enriquecimento proteico de alimentos por levedura em

- fermentação semissólida: alternativa na alimentação animal. *Revista Tecnologia & Ciência Agropecuária*, 3(3), 47-53.
- Association Official Analytical Chemists (2016). *Official methods of analysis* (20nd ed.). AOAC.
- Bach, A., Guasch, I., Elcoso, G., Chaucheyras-Durand, F., Castex, M., Fàbregas, F. E., Garcia-Fruitos, E., & Aris, A. (2018). Changes in gene expression in the rumen and colon epithelia during the dry period through lactation of dairy cows and effects of live yeast supplementation. *Journal of Dairy Science*, 101(3), 2631-2640. doi: 10.3168/jds.2017-13212
- Bach, A., López-García, A., González-Recio, O., Elcoso, G., Fàbregas, F., Chaucheyras-Durand, F., & Castex, M. (2019). Changes in the rumen and colon microbiota and effects of live yeast dietary supplementation during the transition from the dry period to lactation of dairy cows. *Journal of Dairy Science*, 102(7), 6180-6198. doi: 10.3168/jds.2018-16105
- Blaxter, K. L., & Clapperton, J. L. (1965). Prediction of the amount of methane produced by ruminants. *British Journal of Nutrition*, 19(1-2), 511-522. doi: 10.1079/BJN19650046
- Chaucheyras-Durand, F., Walker, N. D., & Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. *Animal Feed Science and Technology*, 145(1-4), 5-26. doi: 10.1016/j.anifeedsci.2007.04.019
- Detmann, E., Souza, M. A., & Valadares, S. C., Fº. (2012). *Métodos para análise de alimentos*. Suprema.
- Elghandour, M. M. Y., Tan, Z. L., Abu Hafsa, S. H., Adegbeye, M. J., Greiner, R., Ugbogu, E. A., Monroy, C., & Salem, A. Z. M. (2019). *Saccharomyces cerevisiae* as a probiotic feed additive to non and pseudo-ruminant feeding: a review. *Journal of Applied Microbiology*, 128(3), 658-674. doi: 10.1111/jam.14416
- Fomenky, B. E., Chiquette, J., Bissonnette, N., Talbot, G., Chouinard, P. Y., & Ibeagha-Awemu, E. M. (2017). Impact of *Saccharomyces cerevisiae* boulardii CNCMI-1079 and *Lactobacillus acidophilus* BT1386 on total lactobacilli population in the gastrointestinal tract and colon histomorphology of Holstein dairy calves. *Animal Feed Science and Technology*, 234, 151-161. doi: 10.1016/j.anifeedsci.2017.08.019
- Fonty, G., & Chaucheyras-Durand, F. (2006). Effects and modes of action of live yeasts in the rumen. *Biologia*, 61(6), 741-750. doi: 10.2478/s11756-006-0151-4
- Fuller, R. (1992). The effect of probiotics on the gut micro-ecology of farm animals. In Wood, B. J. B. (Ed.), *The Lactic Acid Bacteria Vol. 1* (pp.171-192). Springer, Boston, MA.
- Garcia-Diaz, T., Ferriani-Branco, A., Jacovaci, F. A., Cabreira-Jobim, C., Bolson, D. C., & Pratti-Daniel, J. L. (2018). Inclusion of live yeast and mannan-oligosaccharides in high grain-based diets for sheep: ruminal parameters, inflammatory response and rumen morphology. *PLoS One*, 13(2), e0193313. doi: 10.1371/journal.pone.0193313
- Gibson, G. R., Hutkins, R. W., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S.,

- Cani, P. D., Verbeke, K., & Reid, G. (2017). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews: Gastroenterology & Hepatology*, 14(8), 491-502. doi: 10.1038/nrgastro.2017.75
- Hall, M. B. (2000). *Calculation of non-structural carbohydrate content of feeds that contain non-protein nitrogen*. (Bulletin, 339). University of Florida.
- Hristov, A. N., Varga, G., Cassidy, T., Long, M., Heyler, K., Karnati, S. A., Corl, B., Hovde, C. J., & Yoon, I. (2010). Effect of *Saccharomyces cerevisiae* fermentation product on ruminal fermentation and nutrient utilization in dairy cows. *Journal of Dairy Science*, 93(2), 682-692. doi: 10.3168/jds.2009-2379
- Levene, H. (1960). Robust tests for equality of variances. In I. Olkin (Ed.), *Contributions to probability and statistics; essays in honor of harold hotelling* (pp. 278-292). Redwood City, CA.
- Li, J., Li, D. F., Xing, J. J., Cheng, Z. B., & Lai, C. H. (2006). Effects of β -glucan extracted from *Saccharomyces cerevisiae* on growth performance and immunological and somatotropic responses of pigs challenged with *Escherichia coli* lipopolysaccharide. *Journal of Animal Science*, 84(9), 2374-2381. doi: 10.2527/jas.2004-541
- Licitra, G., Hernandez, T. M., & Van Soest, P. J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, 57(4), 347-358. doi: 10.1016/0377-8401(95)00837-3
- Malekshahi, M., Tahmasbi, A. M., Naserian, A. A., Danesh-Mesgaran, M., Kleen, J. L., Alzahal, O., & Ghaffari, M. H. (2016). Effects of supplementation of active dried yeast and malate during sub-acute ruminal acidosis on rumen fermentation, microbial population, selected blood metabolites, and milk production in dairy cows. *Animal Feed Science and Technology*, 213, 29-43. doi: 10.1016/j.anifeedsci.2015.12.018
- Martin, A. K., & Blaxter, K. L. (1965). The energy cost of urea synthesis in sheep. In K. L. Blaxter (Ed.), *Energy metabolism* (pp. 83-91). London.
- Maynard, L. A., Loosli, J. K., Hintz, H. F., & Warner, R. G. (1984). *Nutrição animal* (3a ed.). Freitas Bastos S.A.
- National Research Council (2007). *Nutrient requirements of small ruminants*. National Academy Press.
- Ogunade, I. M., Lay, J., Andries, K., McManus, C. J., & Bebe, F. (2019). Effects of live yeast on differential genetic and functional attributes of rumen microbiota in beef cattle. *Journal of Animal Science and Biotechnology*, 10(1), 1-7. doi: 10.1186/s40104-019-0378-x
- Rodrigues, G. R. D., Schultz, E. B., Siqueira, M. T. S., Fonseca, A. L., Oliveira, M. R., Silva, D. A. P., & Macedo, G. L., Jr. (2021). Use of active and inactive yeasts in lamb diets: intake, digestibility, and metabolism. *Veterinária Notícias*, 27(2), 19-43.
- Sales, J. (2011). Effects of *Saccharomyces cerevisiae* supplementation on ruminal parameters, nutrient digestibility and growth in sheep: A meta-analysis. *Small Ruminant Research*, 100(1), 19-29. doi: 10.1016/j.smallrumres.2011.05.012

- Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality. *Biometrika*, 52(3/4), 591-609. doi: 10.1093/biomet/52.3-4.591
- Silva, D. J., & Queiroz, A. C. (2002). *Análise de alimentos (métodos químicos e biológicos)* (2a ed.). UFV. Imp. Univ.
- Silva, J. F. C., & Leão, M. I. (1979). *Fundamentos de nutrição de ruminantes*. Livroceres.
- Siqueira, M. T. S., Ruela, P. A. C., Oliveira, K. A., Paula Silva, D. A. de, Sousa, L. F., & Macedo, G. D. L., Jr. (2020). Avaliação dos parâmetros nutricionais e metabólicos de borregas alimentadas com leveduras na ração. *Caderno de Ciências Agrárias*, 12, 1-10. doi: 10.35699/2447-6218.2020.23902
- Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science*, 70(11), 3562-3577. doi: 10.2527/1992.70113562x
- Song, B., Wu, T., You, P., Wang, H., Burke, J. L., Kang, K., Yu, W., Wang, M., Li, B., He, Y., Huo, Q., Li, C., Tian, W., Li, R., Li, J., Wang, C., & Sun, X. (2021). Dietary supplementation of yeast culture into pelleted total mixed rations improves the growth performance of fattening lambs. *Frontiers in Veterinary Science*, 8, 657816. doi: 10.3389/fvets.2021.657816
- Street, J. C., & Butcher, J. E., & Harris L. E. (1964). Estimating urine energy from urine nitrogen. *Journal of Animal Science*, 23(4), 1039-1041. doi: 10.2527/jas1964.2341039x
- Van Soest, P. J. (1994). *Nutritional ecology of the ruminant* (2nd ed.). Cornell University Press.
- 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
- Williams, P. E. V., & Newbold, C. J. (1996). Rumen probiosis: the effects of novel microorganisms on rumen fermentation and ruminant productivity. In P. C. Garnsworthy, & D. J. A. Cole (Eds.), *Recent developments in ruminant nutrition 3*. (pp. 351-367). Sevenoaks.
- Zapata, O., Cervantes, A., Barreras, A., Monge-Navarro, F., González-Vizcarra, V. M., Estrada-Angulo, A., Urías-Estrada, J. D., Corona, L., Zinn, R. A., Martínez-Alvarez, I. G., & Plascencia, A. (2021). Effects of single or combined supplementation of probiotics and prebiotics on ruminal fermentation, ruminal bacteria, and total tract digestion in lambs. *Small Ruminant Research*, 204, 106538. doi: 10.1016/j.smallrumres.2021.106538
- Zeoula, L. M., Fereli, F., Prado, I. N., Geron, L. J. V., Caldas, S. F. Neto, Prado, O. P. P. P., & Maeda, E. M. (2006). Digestibilidade e balanço de nitrogênio de rações com diferentes teores de proteína degradável no rúmen e milho moído como fonte de amido em ovinos. *Revista Brasileira de Zootecnia*, 35(5), 2179-2186. doi: 10.1590/S1516-35982006000700039

