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RESEARCH ARTICLE

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Effects of dietary supplementation of pre-weaned lambs with live yeast on rumen fermentation, gut development, performance, and blood health indicators

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ABSTRACT

This study evaluated the effects of supplementing pre-weaned lambs with live yeast (*Saccharomyces cerevisiae* CNCM I-1077; 3 x 10^9 CFU/kg DM) on rumen fermentation, blood metabolites, gut development, and performance. Forty-six male lambs were randomly assigned to two treatments in a completely randomised design: a control group (CTL) fed a starter concentrate, and a yeast group (YST) receiving the same concentrate supplemented with live yeast. Both groups had *ad libitum* access to diets and barley straw from two weeks of age until weaning at eight weeks. Rumen fluid and blood samples were collected at 7 and 10 weeks to assess fermentation parameters and metabolites, and rumen tissue samples were collected at slaughter to evaluate papillae development. Rumen fermentative activity increased from 7 to 10 weeks, with higher concentrations of volatile fatty acids (VFA), lactate, and ammonia-N ($p < 0.001$). YST lambs showed higher rumen concentrations of protozoa ($p = 0.007$) and anaerobic fungi ($p = 0.009$), and a 56% increase in total VFA concentration pre-weaning and slightly enhanced concentrate intake. Blood metabolites remained within a physiological range, and YST lambs showed higher β-hydroxybutyrate ($p < 0.001$) and total protein ($p = 0.054$) than CTL lambs. Yeast also reduced parakeratosis scores ($p < 0.001$) and increased papillae width ($p = 0.020$). However, growth performance was not significantly affected. These results suggest that live yeast supplementation during the pre-weaning period supports microbial colonisation and rumen papillae development, facilitating a smoother transition from liquid to solid feeding and improved rumen function post-weaning.

HIGHLIGHTS

- Yeast probiotics provided pre-weaning enhanced feed consumption, rumen microbial colonisation and fermentation.
- The treatment helped to prevent rumen hyper-parakeratosis and improved the morphology of rumen papillae.
- The positive effects did not translate into improvements in productive performance, probably due to the short production period.

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Introduction

Modern ruminant production systems are constantly exploring nutritional interventions to enhance productivity, health and overall sustainability. Meat sheep production is an important sector in many Mediterranean regions. Spain has a sheep population of 17.8 million, representing 25% of the EU's total sheep population (Eurostat 2023). However, the industry faces significant challenges, particularly in maintaining lamb production under climate change conditions that reduce land grazing capacity (Campo et al. 2016). As a result, lamb production systems often rely on indoor fattening and using diets rich in highly fermentable feeds during early life to shorten the production cycle. While effective for rapid growth, these practices may compromise optimal rumen development and animal health (Lara et al. 2018). Highly

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fermentable diets can lower rumen pH and increase the risk of digestive disorders such as subacute ruminal acidosis, parakeratosis and diarrhoea (González et al. 2016). Moreover, the type of diet consumed during the pre-weaning phase significantly influences the anatomical, physiological and microbiological development of the rumen, which in turn affects the success of the weaning process (Yáñez-Ruiz et al. 2015). Various nutritional strategies have been developed to mitigate these potential problems in young ruminants (Diao et al. 2019). Among them, the use of yeast-based probiotics has shown promising results in dairy calves (Lesmeister et al. 2004; Alugongo et al. 2017). However, the effects in lambs during the pre-weaning period, especially those raised under intensive feeding systems require further research.

In recent years, probiotics have gained special attention as a potential means to reduce the use of antibiotics due to their ability to enhance gut health and improve animal performance (Leistikow et al. 2022). Yeast *Saccharomyces cerevisiae* has been widely studied and is commonly included in the diets of high-yielding ruminants (Elghandour et al. 2024). Unlike yeast extracts, which primarily act as prebiotics and intestinal microbial modulators, live yeast is a facultative anaerobe that plays an important beneficial role in the rumen by scavenging oxygen and supporting the survival of another strictly anaerobic microorganisms. Upon cell lysis, it also supplies essential nutrients such as manno-oligosaccharide, proteins, B-complex vitamin, and minerals to other rumen microorganisms (Fonty and Chaucheyras-Durand 2006). The *S. cerevisiae* CNCM I-1077 strain is widely used as probiotic in ruminant nutrition due to its well-documented benefits on rumen fermentation, pH stabilisation, and the promotion of beneficial microbial populations. It offers unique advantages in supporting rumen development and function (Issakowicz et al. 2013; Chaucheyras-Durand et al. 2019). Furthermore, this strain has been shown to positively influence rumen anatomy by enhancing rumen fermentation, increasing butyrate production, and promoting the growth of rumen papillae, which is crucial for the efficient absorption and utilisation of nutrients later in life (Alugongo et al. 2017).

As noted above, most research on yeast probiotics in young ruminants has focused on calves rather than lambs (Issakowicz et al. 2013; Wang et al. 2023) and many studies have been performed under controlled experimental conditions in which the young ruminants are hardly exposed to some of the challenges (diseases, temperature changes, competition, etc.) that may occur in a commercial farm environment.

We hypothesised that supplementing lamb diets with live yeast (*S. cerevisiae*) during the pre-weaning period under farm commercial management conditions would enhance rumen development while reducing the detrimental effects of using highly fermentable diets. Therefore, the objective of this study was to evaluate the impact of yeast probiotics supplementation on rumen fermentation patterns, anatomical and microbiological development, blood health indicators and productivity in lambs.

Material and methods

Experimental design, diet, management, animals and growth performance

The experiment was conducted in a commercial sheep farm in the village of Puebla de Don Fadrique (37°54′35.5″N 2°25′17.7″W), Granada, Spain and lasted 12 weeks from birth to slaughter. The experimental design and sampling timeline are presented in Figure 1. A total of 46 newborn lambs were selected from the lambing group in the farm based on the following criteria: only singleton male lambs born within a 4–day window and with a similar birth weight (3.80 ± 0.1 kg) were included in this study. This selection minimised variability and ensured a standardised cohort for detailed biological sampling and production parameter evaluations. Selected lambs were randomly assigned at birth to one of two experimental treatments: lambs in the control treatment (CTL), which received a conventional starter concentrate without yeast supplementation or lambs in the yeast treatment (YST), that received the same starter concentrate supplemented with *S. cerevisiae* CNCM I-1077 (3×10^9 CFU at 165 mg/kg DM) from week 2 of age until weaning (week 8). This probiotic is a commercial product developed by Lallemand Animal Nutrition Co. specifically designed as a feed additive for ruminant species (cattle, sheep, goats and camelids) and has received approval following a thorough assessment and audit in accordance with the EU regulation (EC) No 1831/2003 (EFSA. 2019; Bampidis et al. 2019). The inclusion level was determined following the manufacturer recommendations, and the starter feed was made and pelleted two weeks prior the start of the experiment. All lambs were kept in two groups which were physically separated and raised with natural milk feeding with their ewes (Table 1). Lambs had *ad libitum* access to barley straw and fresh water during the entire duration of the experiment. During the milk-feeding period, lambs remained

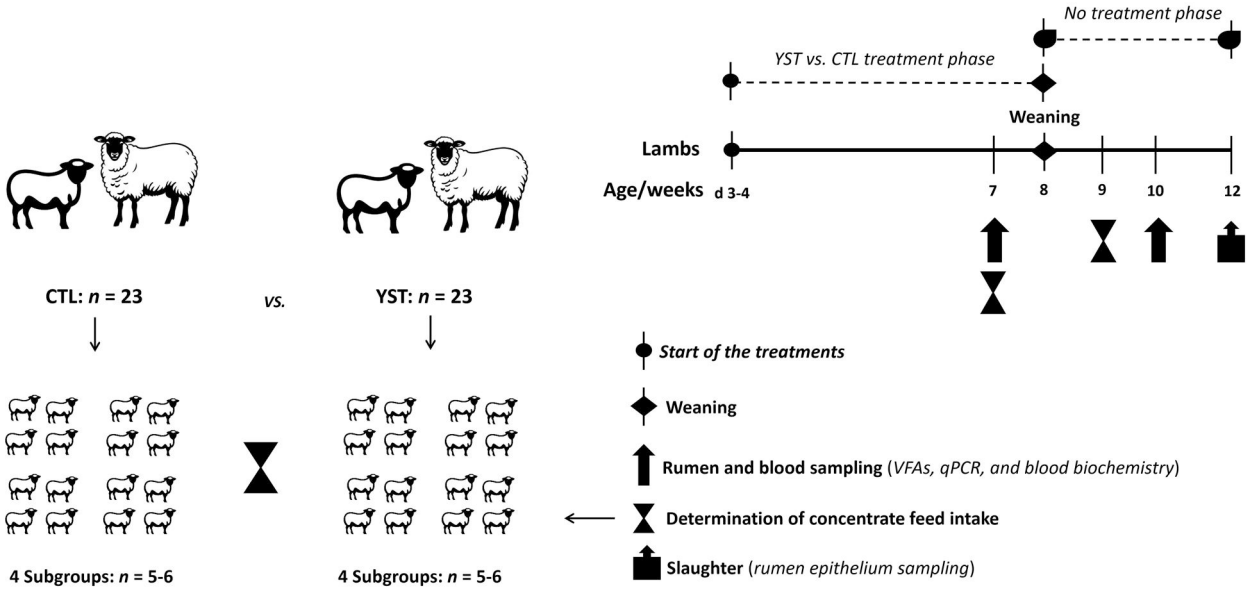

Figure 1. Experimental design and sampling timeline. CTL = lambs un-supplemented with live yeast; YST = lambs supplemented with live yeast (*S. cerevisiae* CNCM I-1077 at 3×10^9 CFU, 165 mg/kg DM).

Table 1. Diet ingredients and chemical composition of the starter and fattening concentrate feeds along with barley straw.

Item	Starter feed	Fattening feed	
Feed ingredients, %			
Corn	28.00	28.00	
Wheat grain	12.00	13.00	
Soybean meal (48%)	32.00	28.00	
Soybean hulls	19.00	18.00	
Beet pulp	2.00	4.00	
Molasses	2.00	2.00	
Wheat bran	3.00	5.00	
Palm oil	1.00	1.00	
Minerals and vitamins	1.00	1.00	
Chemical composition, g/kg DM	Starter feed[a]	Fattening feed[b]	Barley straw
DM	898	891	908
OM	912	942	942
CP	220	205	56.20
EE	35.50	35.00	23.30
Ash	88.30	57.80	85.00
NDF	285	249	536
ADF	96.50	87.90	305
Hemicellulose	188	161	231
Cellulose	63.80	61.40	273
Lignin	32.70	26.50	32.20
NSC	371	453	299
GE, MJ/kg DM	17.50	16.30	18.20

Abbreviations: DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fibre; ADF = acid detergent fibre; NSC = non-structural carbohydrates calculated as 1000 − (Ash + CP + EE + NDF); GE = gross energy (MJ/kg DM).

[a]Starter feed, which was supplemented with yeast (YST) or not (CTL), was given from the 2nd week of age until weaning (8 weeks). It contained (in mg/kg DM) Sepiolite clay 6, Mn 30, Zn 40, Se 0.2, Cu 6, Co 0.5, I 1, Niacin 3, Calcium pantothenate 2, Folic acid 0.05, Biotin 0.007, Vitamins E 20, B1 0.25, B6 0.45, C 95, A 8000 IU, D3 2500 IU.

[b]Fattening feed was not supplemented with yeast and provided from the 8th week of age until slaughter (12 weeks). It contained (in mg/kg DM) Sepiolite clay 250, Bentonite 102, Mn 50, Zn 50, Se 0.25, Cu 1, Co 0.5, I 1, Vitamins E 40, B1 1, A 7500 IU, and D3 1500 IU.

indoors with their dams, except for 6 h per day in which only the dams were allowed grazing outdoor. At the age of 8 weeks, lambs were weaned by abrupt separation from the dams, followed by relocation into separate pens where they were provided with a fattening concentrate without yeast supplementation in order to determine the persistency of the treatments. As the experiment was conducted in a commercial farm, it was not possible to measure individual concentrate feed intakes; instead, they were recorded in subgroups within each experimental group during the pre-weaning (week 7) and post weaning periods (week 9). In week 7, each experimental group ($n = 23$) was divided into four sub-groups of 5–6 lambs. Over seven consecutive days, these sub-groups were housed in a way that allowed only the lambs to access the concentrate feeders, preventing access by the dams. The same process was repeated only with the lambs during week 9 in order to assess the concentrate feed intake during the post-weaning period. All lambs were weighed at birth (wk 0), weaning (wk 8), post-weaning (wk 10) and slaughter (wk 12). Weighing was performed before the morning feeding using a digital scale. At the age of 12 weeks, the lambs were transported to a commercial abattoir located 17 km from the farm (37°48′49.6404″N, 2°31′22.7316″W). The transportation started at 8:00h and was conducted according to the EU regulations (Regulation (EC) No 1/2005) and animals were slaughtered after fasting for at least 4 h.

Samples collection

At 7 and 10 weeks of age, all lambs were sampled to assess the effects during the pre- and post-weaning stages, respectively. Feed was removed 3h prior to sampling in order to minimise the inter-animal variation due to differences in the moment of feeding. Rumen contents (approximately 50 mL) were obtained from each animal using a stomach tube, following the method previously described (Ramos-Morales et al. 2014). Rumen samples were filtered through two layers of cheesecloth, the pH was immediately measured, aliquots were kept for the analyses of volatile fatty acids (VFA) (0.8 mL of sample diluted with 0.8 mL of acid solution), ammonia-N and lactate (0.8 mL each diluted with 0.2 mL of trichloroacetic acid solution). An additional sub-sample was collected to quantify the abundance of the main rumen microbial groups by quantitative PCR (qPCR). All samples were frozen at −20 °C until further analyses. Blood samples (5 mL) from the jugular vein were collected, placed in tubes without anticoagulant and serum was obtained by centrifugation at 2,000 × g for 15 min at room temperature and then kept at −20 °C until analyses of serum metabolites. At the slaughterhouse, the hot carcase weight was recorded, and the dressing percentage was calculated. The different sections of the gastrointestinal tract (GIT) were separated in order to determine the weight of the total GIT, full and empty rumen, omasum, abomasum, and small intestine. Samples from rumen epithelium were cut in slices (1 cm wide) in triplicate and a photo was taken using optical stereomicroscope (M165FC Leica Microsystems). For morphological analysis, a sub-sample of rumen epithelium (2 × 2 cm) was collected from the central area of the ventral anterior sac. For each animal, three papillae were measured per sub-sample (nine measurements in total).

Feed chemical composition

The nutritional composition of the feed was determined following the methods described by the Association of Official Analytical Chemists (AOAC 2005) for the analysis of DM (method 934.01), ash (method 942.05), EE (method 920.39) and N (method 984.13) that was multiplied by 6.25 to convert to CP. The sequential analysis of NDF, ADF and ADL content was conducted using the Ankom^{220} model fibre analyser (ANKOM Technology) in accordance with Van Soest (1994) protocol. NDF analysis utilised the α-amylase enzyme, while the residual ash was used to express both NDF and ADF. The ADL content was determined by the solubilisation of cellulose in the ADF residue with 72% sulphuric acid. Gross energy (GE) content was determined using an oxygen bomb calorimeter (Model 6100 compensated jacket, Parr Instruments Co., Moline, IL) and all samples had been oven-dried prior to analysis.

Rumen fermentation parameters

Concentrations of individual VFA in the rumen samples were determined using a gas chromatography system coupled with a flame ionisation detector (Auto-System, Perkin Elmer, Waltham, MA) as previously described (Playne 1985). The ammonia-N concentration was measured using the colorimetric method of Weatherburn (1967), with absorbance readings taken on a microplate reader (VICTOR X5; 2030 Multimode, PerkinElmer, Waltham, MA, USA). Lactic acid concentration was determined using the colorimetric method of Barker and Summerson (1941), with absorbance measured on a UV–Vis spectrophotometer (Model UV-1280, Shimadzu Corporation, Kyoto, Japan).

Blood metabolites and rumen epithelium

The metabolites quantified in serum samples included β-hydroxybutyrate (BHB) (Cat. No. 21525), glucose (Cat No. 11803), lactate (Cat. No. 23736), total protein (Cat. No. 11553), albumin (Cat. No. 11573), blood urea nitrogen (BUN) (Cat. No. 11516), which were measured using an auto-analyser (BA400, Bio-Systems, Barcelona, Spain). pH, HCO_3, PCO_2, Anion GAP, TCO_2, Na, K and Cl levels were performed using a Roche Omni C Analyser (reference, 100–192) at the Analytical Services of University of León, Spain. Papillae length and width were measured under a dissecting microscope as described by Lane et al. (2000) and were analysed using Leica Application Suite X Life Science software (Belanche et al. 2023). The parakeratosis level of the ruminal epithelium was evaluated using a visual scale ranging from 1 (representing a clean epithelium without parakeratosis) to 5 (indicating a dark epithelium with extreme parakeratosis).

Microbial quantification

The abundance of microbial populations was quantified using qPCR with specific primer sets and PCR conditions that targeted specific genes of total bacteria, protozoa, archaea, and anaerobic fungi as previously described (Belanche et al. 2016). DNA was extracted using a commercial kit (DNEASY POWERSOIL PRO KIT – Cat. No. 47016, Qiagen Ltd., Barcelona, Spain), the primer sets used were as follows: the 16S rRNA for total bacteria

Table 2. Rumen fermentation and concentration of the main microbial groups of lambs fed experimental diets un-supplemented (CTL) or supplemented with live yeast (YST).

	Pre weaning (week 7)		Post weaning (week 10)			*P*-value		
Items	CTL	YST	CTL	YST	SEM	Treatment	Time	T x T
Rumen fermentation								
pH	6.65	6.32	6.45	6.29	0.086	0.011	0.185	0.325
Lactate, μg/mL	13.20	14.60	21.80	24.80	0.887	0.375	<0.001	0.816
Ammonia-N, mg/dL	1.17^{c}	1.32^{c}	4.90^{a}	3.77^{b}	0.238	0.046	<0.001	0.004
Total VFA, mM	43.50^{c}	67.80^{b}	96.30^{a}	101^{a}	3.370	<0.001	<0.001	0.006
Acetate, %	50.60^{b}	51.70^{a,b}	49.60^{b}	53.90^{a}	0.833	0.006	0.404	0.028
Propionate, %	35.50	33.30	36.10	29.20	1.440	0.005	0.195	0.074
Isobutyrate, %	1.62^{a}	1.30^{b}	1.29^{b}	1.20^{b}	0.060	0.001	0.001	0.054
Butyrate, %	8.37	9.92	8.91	12.14	0.716	0.003	0.047	0.220
Isovalerate, %	1.38	1.45	1.57	2.01	0.171	0.134	0.032	0.272
Valerate, %	2.47^{a}	2.25^{a}	2.43^{a}	1.45^{b}	0.152	<0.001	0.007	0.013
Acetate / Propionate ratio	1.50^{b}	1.64^{b}	1.43^{b}	2.10^{a}	0.124	0.005	0.082	0.016
Microbial population, log^{10} copy/mg DM								
Bacteria	11.00^{b}	10.90^{b}	11.00^{ab}	11.10^{a}	0.047	0.048	<0.001	<0.001
Archaea	8.41^{a}	8.39^{a}	8.14^{b}	7.60^{c}	0.089	0.003	<0.001	0.005
Anaerobic fungi	3.34	4.12	2.30	3.69	0.357	0.009	0.072	0.456
Protozoa	8.98	9.64	9.70	10.00	0.196	0.007	0.003	0.289

Abbreviations: CTL = un-supplemented with live yeast; YST = supplemented with live yeast (*S. cerevisiae* CNCM I-1077 at 3×10^9 CFU, 165 mg/kg DM); Yeast = the main effect of the diet (supplemented or not with live yeast); Time = the main effect of the time pre- (wk 7) and post weaning (wk 10); T x T = the interaction between treatment and time; Total VFA = total volatile fatty acids; SEM = standard error of the mean.
[a–c]Values within a row with different superscripts differ significantly at $p < 0.05$ ($n = 23$).

(forward primer GTGSTGCAYGGYTGTCGTCA and the reverse primer ACGTCRTCCMCACCTTCCTC), mcrA gene for archaea (forward primer TTCGGTGGATCDCARAGRGC and the reverse primer GBARGTCGWAWCCGTAGAATCC), the 18S rRNA for anaerobic fungi (forward primer GAGGAAGTAAAAGTCGTAACAAGGTTTC and the reverse primer CAAATTCACAAAGGGTAGGATGAT), and the 18S rRNA for protozoa (forward primer GCTTTCGWTGGTAGTGTATT and the reverse primer CTTGCCCTCYAATCGTWCT). Cycling conditions were set to 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 55 s, with a final extension at 72 °C for 1 min. The absolute amount of each microbial group was expressed as DNA copies/mL of fresh matter, which was determined using serial dilutions of known amounts of standards. The qPCR standards utilised comprised the plasmid ZR Plasmid Miniprep Classic (Cat. No. D4015, Zymo research) with inserted 16S, mcrA, or 18S gene fragments corresponding to each microbial group as previously described. Standards were employed to determine the absolute abundance of microbial groups, which was expressed as the logarithm (Log^{10}) of the number of gene copies per microgram of DNA. The qPCR SYBR Green mix used was Bio-Rad iQ SYBR Green Supermix (Cat. No. 1708882, Bio-Rad Laboratories, Inc.).

Statistical analyses

Residuals of all observation parameters were checked for normality using the Shapiro-Wilk statistic, as well as for variance homogeneity using the test of Levene, and visually assessed using quantile-quantile plots by InfoStat version 2020 (Balzarini et al. 2008). The data were confirmed to be normally distributed. For body weight gain and gut anatomical development data, a one-way ANOVA was used as follows:

$$Y_{ij} = \mu + D_i + e_{ij}$$

where μ is the overall mean, D_i is the fixed effect of the diet (i = CTL vs YST), and e_{ij} is the residual error. This model was also used to analyse the effects on the concentrate feed intake but considering the pen as the experimental unit. For rumen fermentation and blood parameter, a two-way ANOVA was applied as follows:

$$Y_{ijk} = \mu + D_i + T_j + (D \times T)_{ij} + A_k + e_{ijk}$$

where μ is the overall mean, D_i is the fixed effect of the diet (i = CTL vs YST), T_j is the fixed effect of the sampling time (j = 7 vs 10 wks of age), $(D \times T)_{ij}$ is the interaction term, A_k is the random effect of the animal (k = 1 to 46), and e_{ij} is the residual error. When significant effects were detected, means were compared by Fisher's Least Significant Difference LSD test (Fisher 1935). *P*-values less than 0.05 were considered statistically significant. Additionally, $0.05 \leq p < 0.01$ was indicative of a tendency.

Results

Rumen fermentation and microbiology

The interaction between sampling time (pre- *vs*. post-weaning) and yeast supplementation significantly influenced rumen microbial fermentation (Table 2). The supplementation with yeast increased the rumen total

Table 3. Serum metabolites and electrolytes of acid-base balance of lambs fed experimental diets un-supplemented (CTL) or supplemented with live yeast (YST).

	Pre weaning (week 7)		Post weaning (week 10)			*P*-value		
Items	CTL	YST	CTL	YST	SEM	Treatment	Time	T x T
Blood metabolites								
BHB mg/dL	2.22	3.04	2.27	3.77	0.303	<0.001	0.199	0.268
Glucose, mg/dL	94.60	94.00	101	99.40	2.810	0.676	0.027	0.845
Lactate, mg/dL	32.40	36.00	19.40	15.80	3.020	0.993	<0.001	0.219
Total protein, g/L	57.60	58.60	56.20	58.80	0.935	0.054	0.502	0.363
Albumin, g/L	34.70	34.50	33.60	32.20	0.649	0.206	0.008	0.314
Globulin, g/L	22.90	24.10	22.50	26.60	0.952	0.007	0.267	0.123
A/G ratio	1.56	1.48	1.55	1.28	0.065	0.008	0.101	0.145
BUN, mg/dL	35.70	32.80	35.60	36.80	2.130	0.689	0.356	0.331
Electrolytes								
pH	7.36	7.34	7.64	7.62	0.046	0.523	<0.001	0.943
PCO_2, mmHg	38.40[b]	64.30[a]	21.00[c]	26.00[c]	3.280	<0.001	<0.001	0.003
TCO_2, mmol/L	30.40	30.70	28.70	29.70	0.620	0.315	0.025	0.507
Anion GAP, mmol/L	11.30	12.90	10.10	10.80	0.645	0.059	0.014	0.533
HCO_3, mmol/L	28.40	28.60	27.60	28.60	0.601	0.379	0.435	0.513
Na, mmol/L	146	148	145	145	0.799	0.306	0.011	0.066
K, mmol/L	5.39[b]	5.90[a]	5.45[b]	5.20[b]	0.103	0.223	0.003	<0.001
Cl, mmol/L	111	112	116	110	2.150	0.235	0.558	0.127

Abbreviations: CTL = un-supplemented with live yeast; YST = supplemented with live yeast (*S. cerevisiae* CNCM I-1077 at 3×10^9 CFU, 165 mg/kg DM); Yeast = the main effect of the diet (supplemented or not with live yeast); Time = the main effect of the time pre (wk 7) and post weaning (wk 10); T x T = the interaction between treatment and time; BHB = β-hydroxybutyrate concentration; A/G ratio = albumin to globulin ratio; PCO_2 = partial pressure of carbon dioxide; TCO_2 = total carbon dioxide; HCO_3 = bicarbonate; SEM = standard error of the mean.
[a–c]Values within a row with different superscripts differ significantly at $p < 0.05$ ($n = 23$).

VFA concentration, making this effect particularly evident during the pre-weaning period (interaction, $p = 0.006$). During the post-weaning period yeast supplementation resulted in higher proportions of acetate and acetate-to-propionate ratio (interactions, $p = 0.028$, 0.016, respectively), while reducing ammonia-N concentrations (interaction, $p = 0.004$). Yeast supplementation induced a shift in fermentation patterns, leading to lower rumen pH ($p = 0.011$) and overall shift from propionate towards butyrate production ($p = 0.003$). From week 7 to week 10 of age, substantial increases in the rumen total VFA ($p < 0.001$), lactate ($p < 0.001$), and ammonia-N concentrations ($p < 0.001$), along with increased molar proportions of butyrate ($p = 0.047$) and isovalerate ($p = 0.032$) in detriment to isobutyrate ($p = 0.001$) and valerate ($p = 0.007$) over time. Quantitative PCR data showed a significant interaction between sampling time and yeast supplementation in rumen microbial populations. Yeast supplementation did not modify the abundance of bacteria and archaea during the pre-weaning period, but it increased those of bacteria (interaction $p < 0.001$) and lowered archaea levels (interaction $p = 0.005$) during the post-weaning period. Yeast supplementation increased the concentrations of anaerobic fungi ($p = 0.009$) and protozoa ($p = 0.007$) in both sampling times.

Blood parameters

Substantial differences were observed between samples obtained pre (week 7) and post-weaning (week 10) in some of the blood metabolites analysed (Table 3): a progressive decrease in lactate ($p < 0.001$), albumin ($p = 0.008$), and increase in glucose ($p = 0.027$) and pH ($p < 0.001$). Supplementation with yeast promoted an increase in blood BHB ($p < 0.001$), total proteins ($p = 0.054$), and globulin ($p = 0.007$) and decreased the albumin/globulin ratio ($p = 0.008$).

For blood electrolytes, significant interactions between the YST supplementation and sampling time were observed for certain blood ions. As a result, YST supplementation increased the blood PCO_2 (interaction $p = 0.003$) and K levels ($p = 0.001$) before weaning, but differences disappeared after weaning. Moreover, a progressive decrease in anion GAP ($p = 0.014$) was observed over time, whereas no differences were noted between groups in relation to the blood pH or HCO_3 levels.

Feed intake, productive performance and anatomical development of the digestive system

Although forage was offered *ad libitum*, concentrate feed intake was recorded during one week for each period. Results showed that lambs fed the YST diet showed significantly higher concentrate intake than those in the CTL group during the pre-weaning ($p = 0.024$) and post-weaning period ($p = 0.004$, Table 4). All the animals were in good health thorough the course of the experiment, and their daily growth rate remained consistent in both pre- and post-weaning periods (average 235 and 298 g/d, respectively).

Table 4. Concentrate dry matter intake, and productive performance of lambs fed experimental diets un-supplemented (CTL) or supplemented with live yeast (YST).

	Treatment			
Items	CTL	YST	SEM	*P*-value
Concentrate intake, kg/day				
Week 7	0.386	0.402	0.004	0.024
Week 9	0.879	0.913	0.005	0.004
Body weight, kg				
Week 1	3.880	3.940	0.047	0.276
Week 8 (weaning)	16.800	17.400	0.248	0.194
Week 10	20.900	21.700	0.369	0.081
Total BW gain	17.100	17.800	0.373	0.120
Average daily gain, kg/day				
Week 1 to 8	0.220	0.240	0.023	0.369
Week 8 to 10	0.295	0.300	0.019	0.672

Abbreviations: CTL = un-supplemented with live yeast; YST = supplemented with live yeast (*S. cerevisiae* CNCM I-1077 at 3×10^9 CFU, 165 mg/kg DM); DMI = dry matter intake. Significance was declared at $p < 0.05$. Differences between dietary treatments (CTL vs YST) were tested using Fisher's Least Significant Difference (LSD) test.

Similar BWs were noted for both treatments at week 1 and 8 of age (Table 4), while lambs supplemented with YST tended to have greater BW at week 10 of age ($p = 0.081$), resulting on a numerically higher total BW gain over the entire duration of the experiment. However, no differences between treatments were noted in terms of average BW gain during the pre- and post-weaning periods.

The measurements and analyses of samples collected at slaughter (Table 5), showed that YST lambs had numerically higher BW than CTL lambs ($p = 0.112$), which resulted in a tendency to have higher carcase weight ($p = 0.081$) without affecting the dressing percentage. Supplementation with yeast did not affect the weight of total gastrointestinal tract (GIT); however, it modified the anatomical development of some sections, reflected in a lower weight of the empty rumen ($p < 0.001$) and an increased weight of the empty omasum ($p < 0.001$). The rumen epithelium was darker in the CTL lambs (Figure 2) than in those supplemented with YST ($p < 0.001$). Moreover, a greater presence of rumen mucosal ulcerations was detected in CTL than in YST lambs (Figure 2(b)). Also, the YST lambs exhibited wider rumen papillae compared to the CTL group ($p = 0.020$), while no differences were noted in terms of papillae length.

Table 5. Final body weight (week 12), slaughter performance, gut anatomical development of lambs fed experimental diets un-supplemented (CTL) or supplemented with live yeast (YST).

	Treatment			
Items	CTL	YST	SEM	*P*-value
Slaughter performance				
Animal live weight, kg	23.30	24.00	0.243	0.112
Hot carcase weight, kg	11.80	12.20	0.139	0.081
Dressing percentage, %	50.50	50.80	0.335	0.646
Gut anatomical development, % of live body weight				
Total GIT	18.60	19.40	0.330	0.116
Full rumen	9.63	9.24	0.319	0.398
Empty rumen	3.02	2.31	0.065	<0.001
Full abomasum	1.23	1.23	0.088	0.992
Empty abomasum	0.70	0.73	0.036	0.468
Full omasum	0.31	0.38	0.011	0.009
Empty omasum	0.25	0.32	0.011	<0.001
Small intestine	8.33	9.02	0.220	0.036
Rumen papillae				
Length, mm	3.14	3.13	0.127	0.920
Width, mm	1.13	1.32	0.051	0.020
Rumen epithelium color[a]	3.15	1.96	0.195	<0.001

Abbreviations: CTL = un-supplemented with live yeast; YST = supplemented with live yeast (*S. cerevisiae* CNCM I-1077 at 3×10^9 CFU, 165 mg/kg DM); Total GIT = total gastrointestinal tract; SEM = standard error of the mean; Significance was declared at $p < 0.05$. Differences between dietary treatments (CTL vs YST) were tested using Fisher's Least Significant Difference (LSD) test.

[a]Visual scale ranging from 1 (representing a clear epithelium without parakeratosis) to 5 (indicating a dark epithelium with extreme parakeratosis).

Discussion

Rumen microbial fermentation

Rumen development in preparation for weaning is one of the most critical physiological challenges for young ruminants. The ruminant forestomach possesses a unique structure and function that sets it apart from the stomachs of monogastric animals in both morphology and metabolic processes (Ramkrishna and Tiwari 1979). At birth, lambs have physically and metabolically underdeveloped rumen (Bhatt et al. 2009). The rumen development can be chronologically divided into 3 phases: pre-ruminant phase (0–3 wk) in which animals are fed on milk, which bypasses the rumen through the oesophageal groove; transition phase (3–8 wk); and ruminant phase (from 8 wk onward), when animals consume only solid diets (Lane et al. 2000). A smooth transition from pre-ruminant to ruminant animal is needed to ensure a correct anatomical, microbiological, and physiological development to face the weaning nutritional challenge and ultimately to warrant optimal performances later in life (Heinrichs and Lesmeister 2005).

Introducing a starter concentrate diet rich in highly fermentable substrates promotes the growth of bacteria that produce VFA, which are crucial for ruminal epithelial development (Liu et al. 2019). In addition, providing forage stimulates rumen muscularization and rumination, increases rumen volume and motility, and helps maintain the integrity and health of the rumen wall (Montoro et al. 2013). However, in intensive lamb production systems, the benefits of starter feed for rumen development may be compromised when it is offered *ad libitum* as finely ground, highly palatable grain-based feed with limited digestible

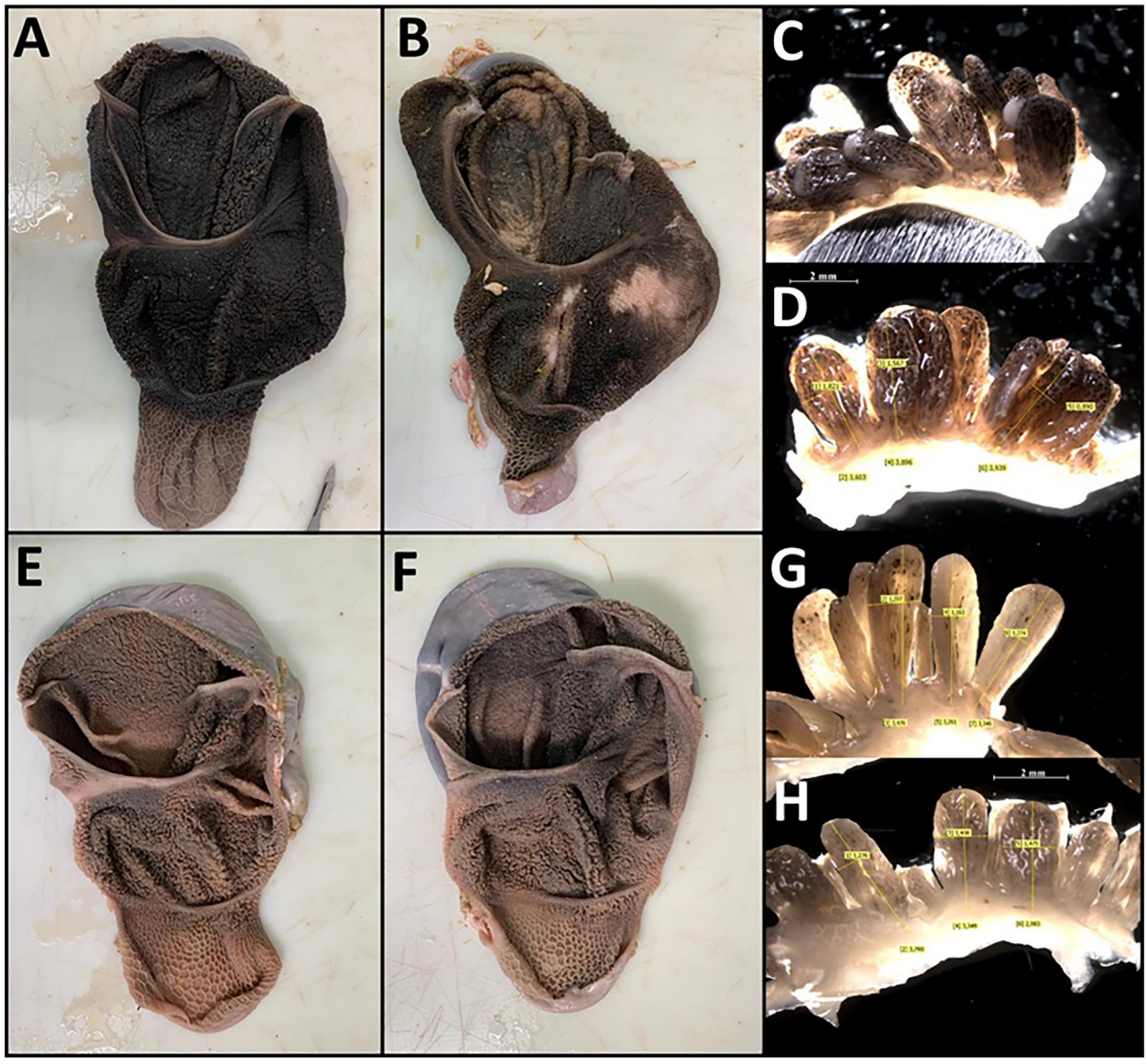

Figure 2. Illustrations of rumen mucosa and rumen papillae of 12–week-old lambs fed the control diet (a, b, c and d) or supplemented (e, f, g and h) with live yeast (*Saccharomyces cerevisiae* CNCM I-1077 at 3×10^9 CFU, 165 mg/kg) from week 2 to week 8 of age (weaning).

forage, which can lead to digestive disorders such as ruminitis and parakeratosis (Zhang et al. 2024).

Dietary supplementation with live yeast has been described to improve rumen fermentation by acting as oxygen scavenger and ultimately modulating the rumen microbial populations (Amin and Mao 2021). As a result, it stimulates the growth of rumen anaerobes like cellulolytic and lactate-utilizing bacteria, and fibrolytic protozoa, all of which are involved in the digestion of fibre and carbohydrates (Wiedmeier et al. 1987; Yang et al. 2004; Xue et al. 2022), and thus an increase in DMI and VFA production, particularly butyrate (Zhang et al. 2024). However, the effects of yeast supplementation in animals are modulated by several factors including the yeast strain, dosage, physiological state of the animal, and the type of diet and feeding system, which may lead to variable effects in ruminants (Ghazanfar et al. 2017; Amin and Mao 2021).

In this study, significant differences were observed in the concentrations of total VFA (+77.6%), lactate (+67.6%), and ammonia-N (3.48 times higher) between the pre- and post-weaning periods likely due to increased consumption of the solid diet and the subsequent effects on the rumen physiological development (Yáñez-Ruiz et al. 2015). Interestingly, YST supplementation resulted in 3.3% increase in concentrate intake and a 55% increase in VFA production during the pre-weaning phase, with no significant differences in lactate concentrations between groups. These effects were accompanied by a significant

decrease in rumen pH at both pre-weaning (−0.33) and post-weaning (−0.16). Nevertheless, rumen pH values remained within the physiological range, indicating no harmful impact on microbial diversity or activity (Commun et al. 2009; Amin and Mao 2021). These results align with Chaucheyras-Durand et al. (2019), who observed increased total VFA production in yeast-supplemented lambs at 6 weeks of age, with rumen pH remaining between 6.0 and 7.0. These observations suggest that yeast supplementation enhances the feed consumption to a small extent and rumen fermentation process to a greater extent during the pre-weaning period.

Yeast supplementation also modified the rumen fermentation profile, increasing the molar proportion of acetate while decreasing that of propionate both before and after weaning. These findings were consistent with those of Magrin et al. (2018), who observed similar impact in finishing bulls fed a high-concentrate diet with live yeast supplementation at a dosage of 1 x 10^{10} CFU/day, and Zhang et al. (2023), who reported comparable effects in young dairy goats fed a TMR diet with live yeast supplementation at a dosage of 5×10^9 CFU/g. However, previous studies have shown inconsistent effects of yeast supplementation on VFA molar proportions. Such variability may be attributed to differences in animal age (and consequently rumen development stage), diet composition, yeast strain, supplementation dose, and sampling time.

Yeast supplementation resulted in higher propionate and lower acetate-to-propionate ratio in steers fed 50:50 forage-to-concentrate diet (Williams et al. 1991), lactating dairy cows fed 43:57 (Erasmus et al. 1992), non-lactating cows fed 60:40 and 70:30 forage-to-concentrate ratios (Guedes et al. 2008; Pinloche et al. 2013), as well as, growing calves consuming low-quality hay (Parra et al. 2021). Chaucheyras-Durand et al. (2019) observed higher proportion of propionate and lower proportion of acetate after weaning in lambs fed a starter diet with 6 x 10^6 CFU/g live yeast from day 8 of age and reared with artificial milk replacer. Similar findings were reported in dairy calves fed with active dry yeast at 2×10^9 CFU/day from 3 (pre-weaning) to 10 (post-weaning) wks of age (Takemura et al. 2020). On the other hand, a meta-analysis conducted by Wang et al. (2023) noted that probiotic yeast supplementation did not significantly affect total VFA or propionate proportion. The effects observed in our study on acetate and propionate molar proportions can be explained by an increased cellulolytic activity in the rumen in animals raised in a system where they consumed mostly starter concentrate feed and not much forage. The positive effect of the supplementation with yeast on cellulolytic bacteria has been shown in previous studies (Pinloche et al. 2013). As described earlier, yeast has been reported to have the ability to consume oxygen in the rumen, which promotes anaerobic conditions within the rumen, facilitating the establishment of anaerobic microbes, particularly fibrolytic bacteria, protozoa, and anaerobic fungi as observed in our study. Thus, the establishment of a diverse and adequate microbiota in the rumen is expected to result if forage consumption is enhanced around weaning (Amin and Mao 2021, Su et al. 2022). Interestingly, our study demonstrated that the modulatory impact of yeast supplementation on rumen fermentation patterns during the pre-weaning phase persisted into the post-weaning period, even without further yeast supplementation, which represents both a practical and financial advantage. The literature on the medium- to long-term effects of interventions applied pre-weaning is very scarce and generally focuses on specific topics (i.e. methane emissions), and types of interventions (i.e. direct rumen fluid inoculation) (Abecia et al. 2013; De Barbieri et al. 2015; Meale et al. 2021). Recently, Huuki et al. (2022) demonstrated, using twin dairy calves, that oral administration of rumen fluid until weaning induced transient changes in early rumen microbiome maturation and later production performance, although the mechanisms that mediating these effects were not clarified. The persistence later in life of the impact of yeast supplementation provided until weaning deserves further research investigation in longer-term studies.

The reduction in ruminal ammonia-N concentrations by 23% in lambs fed YST, particularly during the post-weaning phase, suggests that early yeast supplementation led to lasting improvements in N utilisation efficiency. This effect might be linked to a better synchronisation between N and energy availability for the rumen microbes, which could optimise microbial protein synthesis (Newbold et al. 1996; Cotta and Russell 1997). Live yeast has been described as enhancing microbial activity by stabilising the rumen environment and promoting the proliferation of beneficial bacteria that efficiently capture ammonia-N and incorporate it into microbial protein (Phesatcha et al. 2021). These results aligned with previous studies indicating that live yeast supplementation significantly reduces ruminal ammonia-N concentrations in lambs fed a starter diet either before or after weaning (Chaucheyras-Durand and Fonty 2002), as well as in lactating cows fed corn silage–based diets supplemented with 5g/day *S.*

cerevisiae SC47 (Pinloche et al. 2013). Lower rumen protein degradation and ammonia-N concentration, along with increased total VFA production indicate an improved balance between nitrogen release from protein degradation and energy availability from carbohydrate fermentation. Given that VFAs serve as the primary energy source for ruminants, this balance likely enhances N utilisation efficiency, supporting more efficient microbial protein synthesis while minimising nitrogen losses (Chaucheyras-Durand and Fonty 2001; Min et al. 2019; Putri et al. 2021). The persistence of these effects post-weaning, despite the cessation of yeast supplementation, suggests that early-life microbial modulation play a key role in shaping a more efficient rumen environment.

In relation to rumen microbiota, it should be noted that our microbial analyses were limited to quantifying broad microbial groups using qPCR, without taxonomic resolution at the genus or species level. Future studies applying sequencing could provide a more detailed understanding of which specific bacterial taxa drive the observed changes in rumen fermentation and microbial ecology. The increase in concentrations of total bacteria and protozoa in the rumen from week 7 to week 10 indicates continuous and progressive development of rumen microbial populations during the post-weaning phase. This trend was observed in studies conducted by Chaucheyras-Durand and Fonty (2001; 2021) who reported significant increases in total anaerobic bacteria (from 21 to 120 days of age) and protozoa (at 56 days of age) in lambs supplemented with live yeast. The higher rumen concentration of fibrolytic microbes such as protozoa and anaerobic fungi in YST-supplemented lambs appear to enhance the fibre degradation in the rumen and could explain their higher butyrate and acetate molar proportions in comparison to CTL lambs. These effects, along with the higher blood concentration of BHB may indicate that yeast supplementation also had positive effects on rumen energy metabolism.

A sequencing analysis of the rumen microbial diversity could help to better understand the impact of YST supplementation on the main metabolic pathways in the rumen. A related study conducted by Ogunade et al. (2019) applied shotgun metagenomic sequencing to evaluate the effects of live yeast supplementation on genetic and functional potential of the rumen microbiota in beef steers. Pinloche et al. (2013) used Serial Analysis of V1 Ribosomal Sequence Tag (SARST-V1) and found that yeast supplementation significantly improved rumen function by stimulating the growth of carbohydrate-fermenting bacteria Moreover, it enhanced the activity of carbohydrate-active enzymes in the rumen, with 10 metabolic pathways showing enrichment.

In ruminants, H_2 is primarily produced by microbial fermentation and is largely utilised by methanogenic archaea to convert CO_2 into CH_4. While archaea help remove excess H_2 and support plant cell wall degradation, CH_4 production also reduces overall energy utilisation efficiency (Wolin et al. 1997). Live yeast may influence rumen microbial dynamics by shifting H_2 utilisation away from methanogenesis towards reductive acetogenesis, whereby homoacetogenic bacteria compete with archaea for H_2, promoting acetate production and reducing methane synthesis (Chaucheyras-Durand et al. 2008). An *in vitro* study demonstrated that live yeast supplementation enhances the growth of acetogenic bacteria, increases H_2 utilisation, and stimulates acetate production (Chaucheyras-Durand et al. 2008). These effects may explain the lower rumen archaeal concentrations observed in YST lambs during the fattening period, even after yeast supplementation ceased.

Blood metabolites and health

Blood biochemical parameters are closely linked to animal health and serve as indicators of the body's metabolic status (Vranković et al. 2018). In ruminants, from birth to weaning, glucose is the primary energy source due to the limited utilisation of VFA by the underdeveloped rumen (Heinrichs and Lesmeister 2005). However, as the intake of solid feed increases and rumen development progresses, the contribution of VFA to the animal's energy requirements also increases, and most metabolic BHB is formed from butyrate uptake by the ruminal papillae (Weigand et al. 1975). Like other ketone bodies, BHB serves as a major energy source for the body and is also an indicator of rumen development (Belanche et al. 2020; Rico and Barrientos-Blanco 2024).

Yeast supplementation resulted in a 37% increase in blood BHB concentration pre-weaning and a 66% increase post-weaning, which is consistent with the elevated rumen total VFA concentrations and butyrate molar proportion. Moreover, the strong correlation observed between VFA production, butyrate proportion, and BHB concentrations indicates that a nutritional strategy based on yeast probiotics during the pre-weaning phase enhances both feed consumption and rumen microbial fermentation. It should be noted that there is a lack of studies examining the impact of live yeast on BHB levels in lambs around the weaning

period. Certain studies have documented a notable rise in BHB levels in finishing calves (7–8 months of age) supplemented with live yeast (Kosenda et al. 2023), as well as during the transition period in dairy cows (Benedetti et al. 2024).

The concentrations of various serum parameters, including total protein, albumin, BUN, lactate, and glucose, are essential indicators of protein and energy metabolism, while the albumin-to-globulin ratio can serve as an immunological indicator and reflect the overall health status of the animals. The concentration of BUN is an indirect measure of dietary protein composition and utilisation, the body's capacity to catabolize proteins, and the kidney's ability to excrete urea (Burtis and Bruns 2014). In our study, no significant differences were noted in albumin, glucose, lactate, or BUN levels between groups. However, higher total protein and globulin levels were detected in YST lambs, indicating that yeast supplementation did not negatively affect protein metabolism. These findings align with previous research in growing goats, bulls, and cows, where blood albumin, glucose, and BUN levels were unaffected by yeast supplementation (Yalcin et al. 2011; Geng et al. 2016; Ogbuewu and Mbajiorgu 2023).

Acid-base balance disorders were not observed in any of the experimental groups. Throughout the post-weaning phase, blood pH remained slightly alkaline. However, during the pre-weaning period, YST lambs exhibited higher PCO_2 and anion gap values compared with CTL lambs (+65% and +14%, respectively). Lactic acid concentration was also numerically higher during this period, but YST-fed lambs recovered during the subsequent phase. Metabolic acidosis is characterised by decreased serum pH, increased PCO_2, reduced HCO_3 concentrations, and base deficiency (Carlson 1997). Such symptoms were not observed in this study, except for the high concentration of PCO_2 in YST-fed lambs during the pre-weaning phase, which was later normalised, indicating a good overall health and physiological state of the animals. Furthermore, electrolyte balance is closely associated with acid-base balance (Sobiech et al. 2005). In the absence of acid-base disorders, there were no major changes in the blood concentrations of Na, K, and Cl in lambs during either the pre-weaning or post-weaning phases, and values remained within normal physiological ranges (Bartko et al. 1975), except for a significant increase in K levels during the pre-weaning phase in YST lambs. To our knowledge, there are no previous studies related to the use of the *S. cerevisiae* on acid-base balance or electrolytes in ruminants.

Lamb growth performance

In this study, despite the positive effects of yeast supplementation on rumen microbiological and functional development, no differences in BW were observed before weaning. Interestingly, YST lambs tended to consume more concentrate during the post-weaning period (+3.9%) than CTL lambs, which was reflected in their BW, as YST lambs tended to be heavier at 10 weeks of age (+3.6%). This suggests that the positive effects of YST supplementation during the pre-weaning phase persisted to some extent into the fattening period, although no differences in BW were observed between treatments at slaughter. Our findings align with previous reports showing limited or no effects of yeast supplementation on growth performance. Chaucheyras-Durand et al. (2019) and Mavrommatis et al. (2024) in early-life lambs, Smith et al. (2020) in beef cattle, and Villot et al. (2019) in dairy calves all reported that supplementation with *S. cerevisiae* strains CNCM I-1077 or I-1079 did not significantly affect BW or ADG. In the same context, a meta-analysis conducted by Sales (2011) found no effect of active dry yeast on sheep growth performance. Other studies have also indicated that yeast supplementation in ruminant diets did not noticeably influence growth performance (Kawas et al. 2007; Titi et al. 2008; Bayat et al. 2015). Moreover, most studies evaluating active yeast supplements reported no effects on carcase weight or dressing percentage (Kawas et al. 2007; Payandeh and Kafilzadeh 2007; Ogbuewu et al. 2019; Burt et al. 2023). On the other hand, environmental factors such as mineral contamination may also influence growth, health and tissue metabolism and could modify responses to dietary additives. Recent multi-omics work in goats indicated that excess molybdenum exposure markedly alters muscle energy and amino-acid metabolism and reduces muscle fibre size, while environmental sulphur and molybdenum stress have been shown to disrupt mineral homeostasis, impair liver function and induce systemic inflammation, all of which can depress BW gain and confound nutritional interventions (Zhou and Shen 2025a, 2025b). These studies imply that variability in background mineral exposure or pasture contamination could mask or modify growth responses to probiotics/yeast in field conditions; therefore, while our study demonstrates clear local effects on rumen fermentation, papillae and some blood markers, testing yeast supplementation under different environmental mineral-stress scenarios would be valuable to evaluate whether such supplementation can mitigate those adverse effects. In our study a modest increase

in the final BW (+3.0%) and carcase weight (+3.4%) was observed for lambs on the YST diet, suggesting a marginal improvement in productivity.

Anatomical development of the digestive system

Understanding the anatomical and physiological development of the rumen and lower gut is essential for improving ruminant growth and health, as it depends on factors such as the establishment of ruminal microbiota, initiation of feed consumption, and efficiency of absorption processes (Baldwin et al. 2004; Xiao et al. 2016). In our experiment, YST supplementation did not affect total GIT weight but had a significant impact on specific digestive sections. The empty rumen of YST lambs was 24% smaller, while the full rumen weight was similar between groups, suggesting a greater digesta content in the YST lambs. A plausible explanation for this difference in rumen digesta weight is an increased forage intake, as indicated by the higher acetate molar proportion, which promotes rumen muscularization and rumination and enhances rumen volume and motility (Montoro et al. 2013). However, this hypothesis cannot be fully confirmed in our study because individual feed intake could not be measured under farm conditions.

The rumen epithelium typically exhibits an olive-green to greenish-brown colour (Harfoot 1981). However, the darkening observed in CTL lambs (Figure 2(a, b)) may reflect pathological changes driven by complex interactions between diet and microbial activity. High-concentrate and low-fibre starter diets commonly used in intensive systems can disrupt microbial equilibrium, favouring lactate-producing bacteria (e.g. *Streptococcus bovis*), which increase lactate accumulation (Steele et al. 2011; Jin et al. 2021). Prolonged acidosis damages the epithelium, leading to pathological changes such as parakeratosis, characterised by thickened, clumped tissue and mucosal ulcerations (as noted in some CTL lambs, Figure 2(b)), which impairs rumen function, nutrient absorption, and barrier integrity (Steele et al. 2009). This darkening likely results from cellular necrosis and oxidative stress. High-grain diets increase corneum thickness, which is a hallmark of parakeratosis (Delano et al. 2002; Steele et al. 2011; Voulgarakis et al. 2024), and disrupt keratinocyte differentiation, leading to keratohyalin granule retention (Steele et al. 2012). Reduced microbial diversity further exacerbates mucosal injury and permeability, allowing toxins and inflammatory mediators to infiltrate and aggravate epithelial damage (Steele et al. 2011, 2012). These changes, intensified by inflammation and microbial metabolic by-products, manifest as a dark, brownish-black epithelium, an adaptive yet pathological response to ruminal stress. The transitional phase from milk to solid feed is a sensitive window for regulating rumen development and keratinisation. While concentrate starter introduction can promote epithelial development by regulating gene expression linked to cell proliferation (Sun et al. 2021). In our study, the evaluation of the colour of the epithelium using a scoring scale from 1 to 5, showed that those in CTL lambs were substantially darker (3.15 vs. 1.96) than YST treated lambs.

Ruminal butyrate plays a dual role: at physiological level, it favours epithelial proliferation, papillae development, and provides energy *via* its conversion to ketone bodies (BHB) and anti-apoptotic effects (Baldwin et al. 2004; Niwińska et al. 2017). It also regulates gene expression critical for epithelial integrity and keratinocyte differentiation (Rémond et al. 1995; Brady 2004). Notably, YST-fed lambs in this study exhibited elevated butyrate levels compared to CTL (12.2 vs. 8.64 mM) reaching the optimal range (10–20 mM) necessary for epithelial development and VFA absorption while preventing hyper-keratinisation. However, excessive butyrate (>30 mM) under low-pH conditions, common in high-grain diets, disrupts the normal transition from differentiated keratinocytes to terminally differentiated keratinocytes, leading to parakeratosis (Baldwin et al. 2004; Liu et al. 2019; Zhang et al. 2024). This abnormal keratinisation, or hyperkeratosis, results in the persistence of nucleated cells in the stratum corneum, thereby compromising barrier function and nutrient absorption (Beharka et al. 1998). Zhang et al. (2024) demonstrated that a high-concentrate starter diet in neonatal lambs induces parakeratosis by blocking proper keratinocyte differentiation due to excessive butyrate, while Steele et al. (2009) observed that in dairy cows, a high-grain diet reduces ruminal pH, diminishes motility, and causes papillae keratinisation, ultimately impairing VFA absorption (Niwińska et al. 2017; Aschenbach et al. 2019). Although rumen pH values remained within the physiological range across all animals, lambs on the YST diet experienced lower rumen pH than those on the CTL diet, likely due to a higher total VFA concentration. Recent studies in small ruminants have shown that microbiome-targeted interventions can simultaneously enhance fermentation, papilla morphology, and epithelial barrier function. In goats fed a high-concentrate diet, Zhou and Shen (2025c) reported that probiotic supplementation increased final body weight and ADG, elevated total VFA (particularly butyrate), and improved papilla width. Moreover, probiotics upregulated ruminal

tight-junction proteins (Claudin-1, Claudin-4, Occludin, and ZO-1) and promoted anti-inflammatory signalling (higher IL-10, lower IL-1β and TNF-α), consistent with enhanced epithelial barrier integrity. These findings provide mechanistic evidence consistent with our observation that early yeast supplementation enhanced papilla morphology and reduced parakeratosis, suggesting that microbiome modulation during the pre-weaning period may simultaneously improve fermentative capacity and ruminal epithelial health.

Preventing parakeratosis helps maintain optimal ruminal health and enhancing nutrient absorption capacity (Aschenbach et al. 2019), which may translate into improved productivity, particularly in animals undergoing longer fattening periods or intended for replacement purposes (Amin and Mao 2021). Although parakeratosis was prevented in YST lambs, there were no differences in productive performance. This outcome can be attributed to the short interval between post-weaning and slaughter in the intensive fattening system used. Nevertheless, the beneficial effects of yeast supplementation may be especially relevant for replacement animals, which require optimal rumen development to overcome with diverse challenges throughout their longer productive lifespan.

Conclusions

Based on these results, it can be concluded that supplementation with live yeast *S. cerevisiae* CNCM I-1077 before weaning positively influenced lambs raised with their dams in an intensive production system. Yeast supplementation promoted rumen colonisation by protozoa and anaerobic fungi, increased feed intake, and enhanced fermentation, as reflected by higher ruminal concentrations of total VFA, acetate, and butyrate, together with elevated plasma BHB. It also prevented parakeratosis of the rumen epithelium and increased rumen papillae width. However, such positive effects did not translate into improved productive performance, most likely due to the short fattening period applied in this production system. Future studies should assess whether these benefits persist over longer production periods, investigate the underlying microbial and molecular mechanisms, and evaluate the effectiveness of yeast supplementation under different environmental and nutritional conditions.

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Ethical approval

The lambs used in this study received attentive care and were managed by proficient individuals in accordance with the regulations outlined in the Spanish Royal Decree 53/2013 concerning the protection of animals utilised for scientific purposes, and with the endorsement of the Andalusian regional Government (approval number A/18/03/2019/042), which served as the designated authority overseeing these matters.

Credit authorship contribution statement

Mahmoud Hassan: Conceptualisation, validation, Methodology, investigation, visualisation, formal analysis, data curation, writing – original draft; **Alejandro Belanche**: Conceptualisation, validation, formal analysis, data curation, resources, writing – review and editing, and supervision; **Eva Romera-Rocio**: Methodology; **Bernardo Rodríguez**: Methodology; **Ines Rivelli**: review and editing; **Marine Gauthier**: Methodology, investigation; **David R. Yáñez-Ruiz**: Conceptualisation, validation, formal analysis, resources, writing – review and editing, supervision, project administration, and funding acquisition. All authors read and approved the final version.

Disclosure statement

Marine Gauthier is employed by Lallemand Animal Nutrition. The authors have not stated any other conflicts of interest.

Generative AI statement

The author(s) declare that no generative AI was used in the creation of this manuscript.

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Data availability statement

None of the data were deposited in an official repository. However, the data supporting the study's findings can be obtained upon request.

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