South African Journal of Animal Science 2024, VOL 54

Effects of the dietary supplementation of a native probiotic on the blood metabolites, gut parameters, and meat quality of growing lambs

C. Naseem[1,6], I. Rabbani[1#], M.A. Rashid[2], M.S. Yousaf[1], M. Imran[3], A. Anjum[4], I.H. Badar[5] & H. Rehman[1]

[1]Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[5]Department of Meat Science and Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[6]Department of Emerging Health Professional Technologies, Faculty of Allied Health Sciences, Superior University, Lahore, Pakistan

(Submitted 16 June 2024; Accepted 10 October 2024; Published December 2024)

Copyright resides with the authors in terms of the Creative Commons Attribution 4.0 South African Licence.

See: http://creativecommons.org/licenses/by/4.0/za

Condition of use: The user may copy, distribute, transmit and adapt the work, but must recognise the authors and the South African Journal of Animal Science.

Abstract

This study aimed to compare the efficacy of a native probiotic (indigenously produced *Saccharomyces cerevisiae*) with that of commercial *S. cerevisiae* in terms of the effects on blood metabolites, gut histology, the expression of genes related to the ruminal epithelium, ruminal electrophysiology, and meat quality in lambs. Thirty male Lohi lambs were allocated to three dietary treatments for three weeks (N = 10/treatment): the control (C; basal diet without supplementation); commercial yeast (CY; basal diet supplemented with 1 g/animal/day commercial *S. cerevisiae*); and indigenous yeast (IY; basal diet supplemented with 1 g/animal/day indigenous *S. cerevisiae*). The results showed that dietary supplementation of CY or IY significantly decreased plasma glucose and cholesterol, while jejunal villus height and ruminal papillae length and density increased significantly compared to the C lambs. Ruminal insulin-like growth factor-2 and monocarboxylate transporter-1 expression were higher in both IY and CY, which corresponded to a significantly increased short-circuit current (IY > CY). Regarding the meat parameters, $pH_{18\ h}$, water-holding capacity, and cooking loss were lower, while drip loss and redness were significantly higher in the supplemented groups. This study suggests that supplementation with IY produces beneficial effects in growing lambs. Supplementation with IY is a promising approach, and the use of native feed additives in animal farming should be further explored.

Keywords: gut histology, meat parameters, *Saccharomyces cerevisiae*, sheep, short-circuit current

[#]Corresponding author: imtiaz.rabbani@uvas.edu.pk

Introduction

With an ever-increasing human population, optimising livestock production and maintaining animal health are imperative. Despite the generally high efficiency of nitrogen utilisation, the variation in feeding practices and feed efficiency in ruminants suggests the possibility for improvements

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science http://dx.doi.org/10.4314/sajas.v54i6.01

(Calsamiglia *et al.*, 2010). Antibiotic growth promoters (AGPs) have been administered sub-therapeutically in livestock for better sustainability and improved production. However, growing public health concerns about antimicrobial resistance have led to a ban on the use of AGPs in livestock and poultry farming (Casewell *et al.*, 2003), prompting further research into alternatives to AGPs.

The development of the rumen is a complex progression of anatomical, physiological, and microbial alterations (Orgeur *et al.*, 1998). It is known that a three-week-old lamb can digest a wide variety of carbohydrates and is equipped with ruminal microbes found in adult sheep (Brown, 2009). However, these young lambs cannot digest the quantity of forage necessary for their sustainable growth, necessitating a shift in rumen fermentation towards metabolically efficient pathways. Furthermore, weaning is often associated with decreased feed intake, diarrhoea, parasitism, loss of body weight (BW), and other physiological responses (Uyeno *et al.*, 2010), and remains a challenge in livestock practice. Several studies have suggested the possible manipulation of these processes (Tripathi *et al.*, 2008; Hučko *et al.*, 2009), and the inclusion of probiotics in the feed of ruminants has shown varied yet promising outcomes for their production parameters (Desnoyers *et al.*, 2009).

Saccharomyces cerevisiae, a probiotic, is frequently added to livestock feed and has beneficial effects on animal health and productivity (Perdomo *et al.*, 2020). It can be employed in various forms and concentrations (Cuenca *et al.*, 2022). Furthermore, the components of *S. cerevisiae* are known to modulate the rumen ecosystem (Chaucheyras-Durand & Fonty, 2002), rumen fermentation (Bitencourt *et al.*, 2011), and ruminal papillae length (Xiao *et al.*, 2016). Yeast supplementation also stimulates the proportion of cellulolytic bacteria and enhances fibre digestibility in the rumen (Chaucheyras-Durand *et al.*, 2008), eventually leading to improved body weight gain (Shen *et al.*, 2009; Ma *et al.*, 2021), milk production (Bitencourt *et al.*, 2011), and immunological attributes (Kim *et al.*, 2020; Lee *et al.*, 2021).

Most developing countries import yeast to be supplemented in livestock feed for improved productivity. The economic and trade challenges posed by the recent COVID-19 pandemic have severely hampered global commerce regarding the cost and availability of feed additives for farm animals. It has also raised concerns among local livestock farmers and industry personnel regarding the reliance on imported items. In addition, all livestock animals harbour a complex ecosystem of microbes in their guts that is highly responsive to changes in diet, geographical location, and age (Guan *et al.*, 2008). It was therefore hypothesised that dietary supplementation with indigenous yeast would impart parallel results to those for commercial yeast. The objective of this study was thus to compare the effects of supplementation with the local strain of *S. cerevisiae* on gut histomorphometry, gene expression, electrophysiology, and meat quality with that of supplementation of commercial yeast in post-weaned lambs.

Materials and Methods

A four-week research trial (one-week adaptation and three-week supplementation) was conducted at the Small Ruminants Training Center of the Pattoki University of Veterinary and Animal Sciences (UVAS), in Lahore, Pakistan. All animals were treated for endo- and ectoparasites using a subcutaneous injection of ivermectin (0.1 mL per 5 kg BW) and were vaccinated against enterotoxaemia. The Animal Care and Use Committee of UVAS, Lahore approved the trial (DR/1136).

Thirty male Lohi lambs (56 ± two days of age; 12 ± 2 kg BW) were randomly allocated to three dietary treatments (N = 10 per treatment): 1) control (C), 2) commercial yeast (CY), and 3) indigenous yeast (IY). The C lambs were given no additional supplements, the CY lambs were supplemented with commercial live yeast (Alltech Inc., Nicholasville, Kentucky, USA) at 1 g/animal/day (2×10^9 colony forming units (CFU) of *S. cerevisiae*), and the IY lambs were supplemented with local live yeast at 1 g/animal/day (2×10^9 CFU of *S. cerevisiae*). The yeast supplements (commercial or indigenous) were added to water and hand-fed with the respective dietary treatments twice daily at 07:00 and 17:00 for 21 consecutive days. All lambs were housed in individual pens of 1.5 × 1.5 m, bedded with sand. They were fed a starter diet (Table 1), and the orts were weighed daily. Free choice individual access to fresh water was ensured throughout the experimental period. At the end of the experiment, the lambs were slaughtered at the Meat Sciences Department of UVAS, Lahore, to collect the ventral rumen, jejunum, and meat samples.

Several strains of yeast, isolated from pulses through polymerase chain reaction (PCR), were subjected to various biochemical tests, including wet mount examinations, germ tube tests, and sugar utilisation assays, to facilitate their identification. In addition, a PCR was conducted using species-

specific primers to differentiate between *S. cerevisiae* and *S. bayanus* and/or *S. pastorianus* (Josepa *et al.*, 2000). The PCR was initiated by adding yeast colonies directly into a PCR reaction mix of 25 µL. The PCR results were visualised on 1.4% (w/v) agarose gel (Thermo Scientific). These methods allowed for the precise identification of the yeast strains, enabling the researchers to analyse and compare each strain's characteristics accurately.

Table 1 Ingredients and chemical composition of the starter diet on a dry matter basis

Ingredients	%
Corn grain ground	38.00
Wheat bran	22.50
Molasses	6.00
Canola meal	13.00
Corn gluten 30%	17.00
Mineral premix[1]	0.50
Salt	1.00
Lime	2.00
Nutrient levels	**%**
Dry matter	88.03
Crude protein	16.77
Metabolisable energy (M cal/kg)	2.51
Ash	4.86
Crude fibre	7.20
Ether extract	2.34
Calcium	1.24
Phosphorous	0.56

[1]Provided per kg: 140 g calcium, 70 g phosphorus, 1320 mg magnesium, 2200 mg iron, 140 mg cobalt, 3690 mg manganese, 4700 mg zinc, 61 mg iodine, 45 mg selenium, 12 g sulphur, 148 g sodium, 700 mg fluorine

The selected yeast was prepared by adding Sabouraud dextrose agar (SDA) to 10 mL of normal saline and pulsing the mixture. The resulting suspension formed two distinct layers, with the upper layer containing microorganisms and the lower layer solidified. The upper layer was incubated at 37 °C for 24 h, and a single colony was selected for further processing. To prepare the SDA, 6.2 g of the medium was added to 1 L of distilled water in a flask. The mixture was then heated to 121 °C in an autoclave for 15–20 minutes after adding 0.5% SDA to achieve a pH of 5.6. This method ensured optimal growth conditions for the selected yeast colony. Sabouraud dextrose broth was employed to support the growth of the yeast cells. The culture was centrifuged at 6000 rpm for ten minutes, and the supernatant was carefully removed. The resulting cell pellets were collected and washed with phosphate-buffered saline (PBS). The washed cells were then suspended in PBS, and the concentration of the cell suspension was adjusted to an optical density value of 2×10^9 CFU per millilitre (CFU/mL). The resulting suspension was then transferred to a sterile container and saved for supplementation. This method ensured the availability of a viable and concentrated yeast cell suspension for later use.

At the end of the experimental period, blood samples were collected from the jugular vein into vacutainers containing ethylenediaminetetraacetic acid (EDTA) and centrifuged (2000 × g for 15 minutes) to obtain plasma, which was stored at -20 °C until further analysis. The blood urea nitrogen (BUN; Biosystems, Spain), total protein, blood glucose, cholesterol (Human, Germany), β-hydroxybutyrate (BHBA, Randox, United Kingdom), and uric acid contents were analysed using commercially available kits with an EPOCH micro-plate spectrophotometer (Biotek Instruments Inc., Winooski, USA).

On completion of the experimental trial, six lambs from each treatment (N = 18) were slaughtered, and the reticulorumen and jejunum were removed from the abdominal cavity within ten minutes of death. For histology, sections of the tissues were fixed in 10% formalin, dehydrated using graded alcohol, cleared in xylol, stained with haematoxylin-eosin staining dye, and visualised at 4× magnification using an optical microscope (LX400, LABOMED, the Netherlands). As previously described, papilla length, width, and density were measured to evaluate rumen developmental parameters (Malik *et al.*, 2020). The villus height, villus width, and crypt depth of the jejunum were measured using the LABOMED Pixel Pro (Version 1, Leica Imaging Systems Ltd., Cambridge, United Kingdom) image processing and analysis system.

For electrophysiology, pieces of the ventral rumen (~200 cm^2) were collected, cleaned, stripped from the muscle layer, and transferred to the laboratory in a prewarmed (38 °C) and pre-gassed (95% O_2 and 5% CO_2) buffer solution (in mmol/L: 115 NaCl, 25 $NaHCO_3$, 0.4 NaH_2PO_4, 2.4 Na_2HPO_4, 5 KCl, 5 glucose, 1.2 $CaCl_2$, and 1.2 $MgCl_2$). The osmolarity and pH of the buffer were 280 ± 3 mosm/kg (Osmomat-030, Gonotec, Berlin, Germany) and 7.4, respectively. The tissue samples were cut into small pieces (4 × 4 cm), mounted on Ussing chambers with an exposed surface area of 3.14 cm^2, and bathed on each side with 16 mL of buffer solution. The time from slaughtering the lambs to mounting the tissues was approximately 20 minutes. After mounting, the tissues were allowed to further equilibrate for ten minutes under an open circuit, and then short-circuited to determine the short-circuit current (Isc) by fixing the voltage at 0 mV, as described by Rabbani *et al.* (2011). Electrical parameters were continuously obtained via modified KCl-filled agar bridges, monitored with an automatic computer-controlled voltage-clamp device (Mussler, Aachen, Germany), and measured by alterations in the potential difference (Pd) caused by the application of a bidirectional pulse of 100 µAmp every 0.2 seconds. The fluid resistance and junction potential were corrected before mounting the tissues.

Approximately 500 mg ruminal samples from all three treatment groups were rinsed with distilled water, immediately frozen in liquid nitrogen, and stored at -80 °C. Total RNA from the ruminal samples was isolated using TRIzole reagent, following the manufacturer's protocol. A Thermo Scientific RevertAid™ H Minus First Strand complementary DNA synthesis kit was used to perform reverse transcription using approximately 1 µg of RNA from each sample. Then, 1 µL of complementary DNA was added to 2.5 µL of 10X ABM (ABM Inc. Canada) PCR buffer, 2 µL of 2.5 mM dNTPs, 0.5 µL of 10 µM each of forward primer and reverse primer (Table 2), and 0.25 µL Taq DNA polymerase to perform semiquantitative PCR. The PCR was performed using an Applied Biosystems SimpliAmp™ Thermal Cycler with a five-minute initial denaturation step at 95 °C, followed by 35 cycles of 95 °C for 30 seconds, annealing temperature for 30 seconds, 72 °C for 30 seconds, and a final extension period of 72 °C for five minutes. The annealing temperatures for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), insulin-like growth factor-2 (IGF-2), and monocarboxylate transporter-1 (MCT-1) were 60 °C, 59 °C, and 55 °C, respectively; GAPDH was used as a housekeeping gene to normalise the data. For size comparison, all bands were separated on 1% agarose gel with 5 µL on the ladder (ABM's 100 bp Opti-DNA marker). The PCR amplicons were visualised under ultraviolet (UV) light using a camera, and semiquantitative data analysis was performed.

Table 2 Primer sequences and resulting product size

Primers		Sequence	Product size
GAPDH	Forward	5′-CAAGGTCATCCATGACAACTTTG-3′	495 bp
	Reverse	5′-GTCCACCACCCTGTTGCTGTAG-3′	
IGF-2	Forward	5′-TGGACACCCTCCAGTTTGTCTGT-3′	100 bp
	Reverse	5′-TCGGAAGCAACACTCTTCCACGAT-3′	
MCT-1	Forward	5′-GGCATCTTATCAGGCAGTGG-3′	300 bp
	Reverse	5′-CCAGCCACACAGCAGTTTAATAG-3′	

GAPDH: glyceraldehyde 3-phosphate dehydrogenase, housekeeping gene; IGF-2: insulin-like growth factor-II; MCT-1: monocarboxylate transporter-1

Following chilling, the carcasses were deboned at 13 ± 1 °C, and the *longissimus dorsi* muscle was removed. Steaks of 2 cm thickness were labelled and used for various meat analyses. The pH of the *l. dorsi* muscle was measured 18 hours post-slaughter at three different sites using a portable pH meter (WTW, pH 3210 Germany) with a penetrating probe (AOAC, 2005 method 981.12). The pH meter was calibrated with buffers at pH 4.0, 7.0, and 10.0 (WTW Technical Buffer) at the start of each measurement, and the values were averaged for statistical analysis (Jaspal *et al.*, 2021). Compression was used to measure the water-holding capacity using a compressor (YYW-2, Nanjing Soil Instrument, Nanjing, China). A 5 g sample of raw meat was folded into filter paper (9 Whatman no. 40) and compressed at 373 N for five minutes. The weights of the meat samples before and after compression were recorded using a computerised compact weighing balance. The following formula was used to calculate the percentage weight loss after compression (Li *et al.*, 2012):

$$Expressible\ water\ (\%) = \frac{Initial\ weight\ -\ Final\ weight}{Initial\ weight} \times 100$$

Meat samples were stored at 0–4 °C for 12 days in vacuum-sealed plastic bags and weighed before and after the storage period using a digital compact weighing balance (SF-400, 7000 g). The weight difference was considered the drip loss (Warner, 2014):

$$Drip\ loss\ (\%) = \frac{Initial\ weight\ -\ Final\ weight}{Initial\ weight} \times 100$$

After measuring the colour parameters, the steaks were weighed using a digital compact weighing balance (SF-400, 7000 g) and vacuum-sealed into bags (150 × 200, PA/PE90) using a vacuum packing machine (Multivac® Baseline p100). The steaks were then placed in a water bath (Memmert WNB45, Germany) and cooked at 80 °C until all the steaks had attained a core temperature of 72 °C [25]. The core temperature was measured by inserting a digital thermometer probe (TP 101, -50 °C to 300 °C) into the steak. The meat samples were weighed again after cooking. The Warner Bratzler shear force (WBSF) was determined using a texture analyser (TA; XT plus® Stable Microsystems texture analyser, Godalming, United Kingdom). The cooked meat samples were cooled at 0–4 °C for 40 minutes before testing. They were cut into 6 cm long, 1 cm tall, and 1 cm wide strips, and the WBSF values were recorded in Newtons per square centimetre (N/cm^2).

As described by Jaspal *et al*. (2021), instrumental colour (L*, a*, and b*) was recorded using a Minolta Chroma Meter (Konica Minolta® CR-410, Japan) calibrated using a standard white tile (L* 95.18, a* 0.07, and b* 2.59). The steaks were placed in food-grade polystyrene trays, overwrapped with a plastic wrap of 250 mm thickness, and put into a horizontal display chiller (ALVO made by Technosight) at 0–4 °C for one hour. After blooming, the colour parameters lightness (L*), redness (a*), and yellowness (b*) were measured. Three measurements were collected from random sites across the steaks, avoiding flecks of marbling fat or connective tissue.

The collected data were subjected to the Schapiro–Wilk test to test normality and analysed by one-way analysis of variance (ANOVA) using Sigmaplot (Systat Software Inc., United Kingdom, Version 11.0). Tukey's post- hoc test was employed for pairwise comparisons between the treatment groups, and P <0.05 was considered significant. Where applicable, 'N' refers to the number of animals, while 'n' refers to the number of epithelial samples.

Results and Discussion

The means and standard errors of the serum metabolites are shown in Table 3. Supplementation of either IY or CY did not affect the BHBA, BUN, uric acid, or total protein concentrations (P >0.05). However, the glucose and cholesterol concentrations were significantly lower in the IY group than in the C group, although the IY and CY groups did not differ significantly from each other.

Supplementation of both indigenous and commercial yeasts increased the rumen papilla length and density, as well as the jejunal villus height and villus height-to-crypt ratio (P <0.05), while the rumen papilla width and jejunal crypt depth, although largest in the IY group, remained non-significantly

different between the groups. The jejunal villus width was significantly lower in both treatment groups (CY < IY, non-significant) than in the control (Table 4).

The Isc was significantly higher in the CY and IY groups (CY > IY) than in the C group. The transepithelial Pd was significantly lower in the IY group than in the C group but not the CY group. A higher tissue conductance (Gt) was observed for supplemented groups (CY > IY), and this was significantly different from the value found for the C group (Table 5).

Table 3 Effect of *Saccharomyces cerevisiae* supplementation on rumen fermentation parameters and blood metabolites in growing Lohi lambs

Parameters	Treatment groups			SEM	*P*-value
	Control	CY	IY		
BHBA (mmol/l)	0.45	0.44	0.42	0.01	0.13
BUN (mg/dl)	20.65	19.43	18.61	0.87	0.65
Total protein (g/dl)	6.82	7.40	7.38	0.11	0.08
Glucose (mg/dl)	78.71^{a}	75.60^{ab}	73.56^{b}	0.76	0.01
Cholesterol (mg/dl)	83.03^{a}	74.82^{b}	76.94^{b}	1.24	0.02
Uric acid (mg/dl)	3.03	2.80	2.76	0.05	0.10

Data presented as the mean ± standard error of the mean (SEM). Values in the same row bearing different superscripts are significantly different ($P < 0.05$). CY: commercial yeast; IY: indigenous yeast; BHBA: β-hydroxybutyrate; BUN: blood urea nitrogen

Table 4 Ruminal and jejunal histomorphometry of growing Lohi lambs

Parameters		Groups			SEM	*P*-value
		Control	CY	IY		
Jejunum	**Villus height (µm)**	344.90^{b}	404.95^{ab}	415.42^{a}	11.62	0.02
	Villus width (µm)	78.66^{a}	59.03^{b}	66.83^{b}	8.12	<0.01
	Crypt depth (µm)	117.87	119.54	133.64	3.60	NS
	Villus to crypt ratio	2.99^{b}	3.40^{ab}	3.77^{a}	0.12	0.03
Rumen	**Ruminal papilla length (µm)**	1011.44^{c}	1413.19^{b}	1779.48^{a}	80.78	<0.01
	Ruminal papilla width (µm)	392.39	412.93	429.87	14.05	NS
	Papilla density (no/cm^2)	71.50^{b}	83.80^{a}	85.73^{a}	10.57	<0.01

Data are presented as the mean ± standard error of the mean (SEM). Values in the same row bearing different superscripts are significantly different ($P < 0.05$). CY: commercial yeast; IY: indigenous yeast

Table 5 Electrophysiological parameters from the isolated ruminal epithelium of growing Lohi lambs

Parameters	Groups			SEM	*P*-values
	Control	CY	IY		
Isc (µAmp/cm^2)	43.62^{c}	53.19^{b}	61.16^{a}	1.21	<0.01
Pd (mV)	2.17	2.20	2.19	0.17	NS
G_t (mS/cm^2)	20.03^{b}	28.30^{a}	27.42^{a}	1.09	<0.01

Data are presented as the mean ± standard error of the mean (SEM). Values in the same row bearing different superscripts are significantly different ($P < 0.05$). Isc: short-circuit current; Pd: potential difference; G_t: tissue conductance; CY: commercial yeast; IY: indigenous yeast; N/n = 18/36

The results of the gene expression analysis of the ruminal samples from the slaughtered animals are shown in Figure 1 A. The expression levels of both the IGF-2 and MCT-1 genes of the two supplemented groups were almost double those of the C group. However, these expression levels did not differ significantly between the CY and IY groups. Corresponding to the gene expression, an addition of 40 mM Na-acetate on the mucosal side increased the Isc from 27.90 ± 1.46 μAmp/cm^2 to 62.33 ± 2.23 μAmp/cm^2 in IY and from 28.11 ± 1.01 μAmp/cm^2 to 55.22 ± 3.30 μAmp/cm^2 in CY (Figure 1 B).

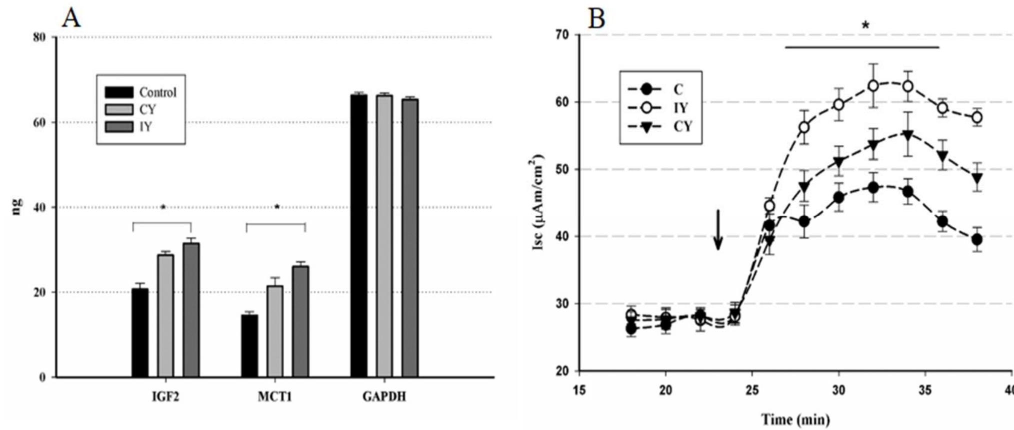

Figure 1 Effects of *Saccharomyces cerevisiae* supplementation on (**A**) the expression of epithelial development genes in the rumen, and (**B**) the effect of the addition of 40 mM Na-acetate (indicated by the arrow) on the mucosal side on the short-circuit current (Isc). C: control; CY: commercial yeast; IY: indigenous yeast; IGF2: insulin-like growth factor; MCT1: monocarboxylate transporter 1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase (N/n = 18/36)

Both indigenous and commercial yeast supplementation significantly affected the measured meat quality parameters. Meat pH, cooking loss, drip loss, and WBSF did not differ significantly between the CY and IY groups. However, the water-holding capacity was significantly higher in the IY group than in the C and CY groups. Furthermore, meat from the IY group was significantly lighter in colour than meat from the C and CY groups, while the redness was higher (P <0.05) in both the supplemented groups than in the C group. Supplementation with either CY or IY did not affect the yellowness of the meat (Table 6).

Table 6 Meat parameters of growing Lohi lambs (N = 18)

Parameters	**Groups**			**SEM**	***P*-value**
	Control	**CY**	**IY**		
Meat pH	5.63^a	5.43^b	5.40^{ab}	0.03	<0.01
Water-holding capacity (%)	19.72^a	17.79^b	16.28^b	0.28	<0.01
Drip loss (%)	2.30^b	2.59^a	2.64^a	0.04	<0.01
Cooking loss (%)	33.68^a	32.01^a	30.88^b	0.38	<0.01
Shear force (N/cm^2)	34.86^a	28.99^b	28.04^b	0.83	<0.01
L*	39.96^b	42.29^{ab}	43.39^a	0.51	0.01
a*	20.40^b	21.53^a	21.66^a	0.21	0.02
b*	9.08	8.79	8.71	0.20	NS

Data are presented as the mean ± standard error of the mean (SEM). Values in the same row bearing different superscripts are significantly different (P <0.05). CY: commercial yeast; IY: indigenous yeast; L*: lightness; a*: redness; b*: yellowness

The positive effects of local yeast supplementation on intake and growth have already been reported (Naseem *et al.*, 2023). This study aimed to assess whether dietary supplementation with indigenously sourced yeast produced beneficial results for gut histology, rumen development parameters, and meat quality in growing lambs. Early rumen development is vital for nutrient absorption and utilisation by ruminants, and significantly impacts animal production performance (Graham & Simmons, 2005). Yeast supplementation in growing lambs improves growth through its positive effects on rumen development. In this study, non-significant effects were observed for BHBA, BUN, total protein, and uric acid, consistent with the findings of Kumprechtova *et al.* (2019), suggesting that the animals did not show any negative energy balance. The decrease in blood glucose concentration found in our study is contrary to the results of previous studies, where glucose increased in response to yeast supplementation due to increased feed digestibility (Knollinger *et al.*, 2022). This outcome could be age-related, and a decrease in cholesterol suggests increased fibre digestibility and less propionate production.

The rumen epithelium plays a crucial role in providing a conducive environment for ruminal microbes by absorbing volatile short-chain fatty acids (VFAs) without cytosolic acidification (Penner *et al.*, 2009), transporting nitrogen, and recycling urea (Abdoun *et al.*, 2006). The rumen papilla length and width are essential for determining rumen development (Lesmeister *et al.*, 2004). While many earlier studies have reported the beneficial effects of yeast supplementation on the rumen epithelia (Garcia Diaz *et al.*, 2018; Tekce *et al.*, 2021), similar positive effects related to ruminal papilla length and density were observed in the present study for both supplemented groups compared to the C group. Although there were no significant changes in the ruminal papilla width, the papilla length was higher in the IY group than in the other groups. These outcomes suggest that yeast supplementation, either commercial or indigenous, increases fibre digestibility and the availability of VFAs in the gut milieu, which in turn stimulates an adaptive response at the epithelial level and eventually leads to a greater surface area for the absorption of nutrients (Steele *et al.*, 2016). However, contrary to previous studies, no effects of the treatments on the ruminal papilla width were seen. This disparity could be due to either the addition of selenium (Shahid *et al.*, 2020) or zinc (Zengin *et al.*, 2022) with yeast supplementation or higher doses of yeast supplementation (Wang *et al.*, 2022) in previous studies. In terms of villus height, width, and height-to-crypt ratio, the jejunal histology was significantly different in the yeast-supplemented animals compared to the C group, suggesting an enhanced absorptive surface area for nutrients. These findings agree with those of Ahmed *et al.* (2016), who reported an increase in villus length and width following selenium yeast supplementation in goats. However, our findings are not in agreement with Khorasani *et al.* (2021), who did not find any significant difference in the villus length or crypt depth of jejunal tissues in lambs supplemented with *Megasphaera* and *Saccharomyces*.

Tissue conductance and transepithelial Pd are essential for ascertaining biological tissues' electrical properties and barrier functions. Collectively, these parameters influence the Isc, which signifies the transcellular epithelial transport in ruminal tissues or isolated cells (Stumpff *et al.*, 2011). Our study showed that supplementation with either commercial or indigenous yeast improved the epithelial absorptive response, as reflected by increased Gt and Isc values. Fu *et al.* (2021) reported that yeast supplementation upregulated zonula occludens-1 and down-regulated toll-like receptor 4 in the jejunal mucosa of weaned pigs, essential parameters for gut barrier function. Improvement in gut barrier function has also been confirmed by several researchers who investigated the effects of yeast supplementation on various species of animals and a variety of gut segments (Lodemann *et al.*, 2006; Awad *et al.*, 2008). One possible reason for the improved Gt and Isc values found in our study could be that the yeast supplementation increased the dry matter intake and fibre digestibility by cellulolytic bacteria in the rumen (Newbold *et al.*, 1996), and the resultant changes in fermentation parameters such as the pH, VFA concentration, and osmotic pressure altered the rumen epithelium and its response (Martens *et al.*, 2012). Likewise, supplementation with probiotics is reported to increase the mRNA expression of hepatic insulin-like growth factor-1 (IGF-1) and ruminal MCT-1 in lambs (Izuddin *et al.*, 2019), which was also observed in the present study for the ruminal expression of IGF-2 and MCT-1. These findings agree with Nishihara *et al.* (2019), who attributed ruminal epithelial growth to IGF-2 in weaned calves. Although the up-regulation of MCT-1 in the rumen is generally associated with differences in dietary treatment and the availability of butyric acid (Kuzinski & Röntgen, 2011), the increased expression of MCT-1 in the present study was solely due to yeast supplementation, as the diet was uniform across the experimental groups.

Lamb meat is a nutritious, easily digestible food product and is considered a delicacy in many parts of the world. In the current study, supplementation with either CY or IY significantly altered various parameters related to meat quality. Meat pH, an essential parameter for carcass quality and linked to glycolysis after slaughter, was significantly lower in the supplemented groups than in the control group. This attribute is desirable for better meat flavour and colour (Abril *et al.*, 2001). These findings agree with those of Mariezcurrena-Berasain *et al.* (2022), who observed a non-significant decrease in meat pH after 24 hours in the carcasses of lambs supplemented with selenium-enriched yeast. One plausible reason for the lower pH in the supplemented groups could be that they had higher muscle glycogen reserves, because of better dry matter intake and increased nutrient absorbability from the gut. The reduced meat pH also significantly decreased the water-holding capacity in the supplemented groups, consistent with previous studies, showing a negative correlation between pH and drip loss (Huff-Lonergan *et al.*, 2002; Sowińska *et al.*, 2016). In contrast, water-holding capacity and drip loss were increased in lambs subjected to transportation stress in previous studies (Bond *et al.*, 2004; Kadim *et al.*, 2006).

Meat colour, a significant concern from the consumer's perspective, showed a significant improvement in redness with yeast supplementation. A possible reason for this outcome could be the antioxidative properties of *S. cerevisiae* that have previously been found to prevent the oxidation of polyunsaturated fatty acids and preserve muscle protein integrity (Jia *et al.*, 2022). In our study, a lower WBSF (P <0.05) was observed for both the CY and IY groups compared to the C group, which reflects the tenderness of the meat and is a desirable trait for lamb meat. Although this observation was not aligned with the reduced water-holding capacity that we observed, other factors may have also be involved in this outcome, and these require further research.

Conclusions

In conclusion, dietary supplementation with CY or IY improved rumen fermentation and development parameters by upregulating MCT-1 and IGF-2, corresponding to an increased Isc and improved meat parameters. However, further research is needed to ascertain the cost-effectiveness and bioavailability of IY for optimised inclusion in the diet of farm animals. Our findings suggest that IY can be a suitable alternative to CY in local settings.

Acknowledgments

This work was financially supported by funding project number 8977, granted by the Higher Education Commission (HEC) of Pakistan under the national research programmes for universities. We gratefully acknowledge the support rendered by the UVAS administration for providing animals for tissue samples. We thank the German Academic Exchange Service (DAAD) for providing the equipment for the electrophysiological parameters.

Authors' contributions

CN, IR, and MSY conceptualised and designed this study. AAA assisted in the production of indigenous *S. cerevisiae* and validated the supplements. CN, MI, and IHB executed the experiments and analysed the samples, while IR supervised the project. MI assisted in the execution and interpretation of the molecular expression, while IHB assisted in meat analysis. IR, MSY, and HR interpreted and critically revised the manuscript for intellectual input. IR reviewed and edited the final draft. All authors have read and agreed to the published version of the manuscript.

Conflict of interest declaration

The authors declare that they have no conflict of interest.

References

Abdoun, K., Stumpff F. & Martens H., 2006. Ammonia and urea transport across the rumen epithelium: a review. Anim. Health Res. Rev. 7 (1–2), 43–59. DOI: 10.1017/S1466252307001156

Abril, M., Campo M., Önenç A., Sañudo C., Albertí P. & Negueruela A., 2001. Beef colour evolution as a function of ultimate pH. Meat Sci. 58 (1), 69–78.

Ahmed, Z., Malhi M., Soomro S., Gandahi J., Arijo A., Bhutto B. & Qureshi T., 2016. Dietary selenium yeast supplementation improved some villi morphological characteristics in duodenum and jejunum of young goats. J. Anim. Plant Sci. 26 (2), 382–387.

Awad, W., Ghareeb K. & Böhm J., 2008. Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing *Enterococcus faecium* and oligosaccharides. Int. J. Mol. Sci. 9 (11), 2205–2216.

Bitencourt, L.L., Silva J.R.M., Oliveira B.M.L. d., Dias Júnior G.S., Lopes F., Siécola Júnior S., Zacaroni O.d.F. & Pereira M.N., 2011. Diet digestibility and performance of dairy cows supplemented with live yeast. Sci. Agric. 68 301–307.

Bond, J., Can L. & Warner R., 2004. The effect of exercise stress, adrenaline injection and electrical stimulation on changes in quality attributes and proteins in *Semimembranosus* muscle of lamb. Meat Sci. 68 (3), 469–477.

Brown, T.H., 2009. The early weaning of lambs. J. Agric. Sci. 63 (2), 191–204. DOI: 10.1017/S0021859600063152

Calsamiglia, S., Ferret A., Reynolds C.K., Kristensen N.B. & van Vuuren A.M., 2010. Strategies for optimizing nitrogen use by ruminants. Animal. 4 (7), 1184–1196. DOI: 10.1017/S1751731110000911

Casewell, M., Friis C., Marco E., McMullin P. & Phillips I., 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J. Antimicrob. Chemother. 52 (2), 159–161.

Chaucheyras-Durand, F. & Fonty G., 2002. Influence of a probiotic yeast (*Saccharomyces cerevisiae* CNCM I-1077) on microbial colonization and fermentations in the rumen of newborn lambs. Microb. Ecol. Health Dis. 14 (1), 30–36.

Chaucheyras-Durand, F., Walker N. & Bach A., 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. AFST 145 (1–4), 5–26.

Cuenca, M., Chauca J., García C. & Sigüencia H., 2022. *Saccharomyces cerevisiae* as a replacement alternative to growth-promoting antibiotics in animal feed. Arch. Zootec. 71 (273), 61–69.

Desnoyers, M., Giger-Reverdin S., Bertin G., Duvaux-Ponter C. & Sauvant D., 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92 (4), 1620–1632. DOI: 10.3168/jds.2008-1414

Fu, R., Liang C., Chen D., Yan H., Tian G., Zheng P., He J., Yu J., Mao X. & Huang Z., 2021. Effects of dietary *Bacillus coagulans* and yeast hydrolysate supplementation on growth performance, immune response and intestinal barrier function in weaned piglets. J. Anim. Physiol. Anim. Nutr. (Berl.). 105 (5), 898–907.

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.

Graham, C. & Simmons N.L., 2005. Functional organization of the bovine rumen epithelium. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288 (1), R173–R181.

Guan, L.L., Nkrumah J.D., Basarab J.A. & Moore S.S., 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiol. Lett. 288 (1), 85–91.

Hučko, B., Bampidis V., Kodeš A., Christodoulou V., Mudřik Z., Poláková K. & Plachý V., 2009. Rumen fermentation characteristics in pre-weaning calves receiving yeast culture supplements. Czech J. Anim. Sci. 54 (10), 435–442.

Huff-Lonergan, E., Baas T.J., Malek M., Dekkers J.C., Prusa K. & Rothschild M., 2002. Correlations among selected pork quality traits. J. Anim. Sci. 80 (3), 617–627.

Izuddin, W.I., Loh T.C., Samsudin A.A., Foo H.L., Humam A.M. & Shazali N., 2019. Effects of postbiotic supplementation on growth performance, ruminal fermentation and microbial profile, blood metabolite and GHR, IGF-1 and MCT-1 gene expression in post-weaning lambs. BMC Vet. Res. 15 (1), 315. DOI: 10.1186/s12917-019-2064-9

Jaspal, M.H., Badar I.H., Amjad O.B., Yar M.K., Ijaz M., Manzoor A., Nasir J., Asghar B., Ali S. & Nauman K., 2021. Effect of wet aging on color stability, tenderness, and sensory attributes of *longissimus lumborum* and *gluteus medius* muscles from water buffalo bulls. Animals. 11 (8), 2248.

Jia, X., Li J., Li S., Zhao Q., Zhang K., Tang C., Yang Y., Ma Q., Wang J. & Zhao Z., 2022. Effects of dietary supplementation with different levels of selenium yeast on growth performance, carcass characteristics, antioxidant capacity, and meat quality of Tan sheep. Livest. Sci. 255, 104783.

Josepa, S., Guillamon J.M. & Cano J., 2000. PCR differentiation of *Saccharomyces cerevisiae* from *Saccharomyces bayanus*/*Saccharomyces pastorianus* using specific primers. FEMS Microbiol. Lett. 193 (2), 255–259.

Kadim, I., Mahgoub O., Al-Kindi A., Al-Marzooqi W. & Al-Saqri N., 2006. Effects of transportation at high ambient temperatures on physiological responses, carcass and meat quality characteristics of three breeds of Omani goats. Meat Sci. 73 (4), 626–634.

Khorasani, O., Chaji M. & Baghban F., 2021. Effects of buffer and *Megasphaera elsdenii* as pH regulators, on histomorphometry and histopathology of small and large intestine of Arabian fattening lambs fed with high concentrate diets. J. Anim. Sci. Res. 31 (3), 73–87.

Kim, E.T., Lee H.G., Kim D.H., Son J.K., Kim B.W., Joo S.S., Park D.S., Park Y.J., Lee S.Y. & Kim M.H., 2020. Hydrolyzed yeast supplementation in calf starter promotes innate immune responses in Holstein calves under weaning stress condition. Animals 10 (9), 1468. DOI: 10.3390/ani10091468

Knollinger, S.E., Poczynek M., Miller B., Mueller I., de Almeida R., Murphy M.R. & Cardoso F.C., 2022. Effects of autolyzed yeast supplementation in a high-starch diet on rumen health, apparent digestibility, and production variables of lactating Holstein cows. Animals 12 (18). DOI: 10.3390/ani12182445

Kumprechtova, D., Illek J., Julien C., Homolka P., Jancik F. & Auclair E., 2019. Effect of live yeast (*Saccharomyces cerevisiae*) supplementation on rumen fermentation and metabolic profile of dairy cows in early lactation. J. Anim. Physiol. Anim. Nutr. (Berl.). 103 (2), 447–455. DOI: 10.1111/jpn.13048

Kuzinski, J. & Röntgen M., 2011. The mRNA and protein expression of ruminal MCT1 is increased by feeding a mixed hay/concentrate diet compared with hay ad libitum diet. Arch. Anim. Breed. 54 (3), 280–286.

Lee, J.J., Kyoung H., Cho J.H., Choe J., Kim Y., Liu Y., Kang J., Lee H., Kim H.B. & Song M., 2021. Dietary yeast cell wall improves growth performance and prevents of diarrhea of weaned pigs by enhancing gut health and anti-inflammatory immune responses. Animals 11 (8), 2269. DOI: 10.3390/ani11082269

Lesmeister, K., Tozer P. & Heinrichs A., 2004. Development and analysis of a rumen tissue sampling procedure. J. Dairy Sci. 87 (5), 1336–1344.

Li, C., Liu D., Zhou G., Xu X., Qi J., Shi P. & Xia T., 2012. Meat quality and cooking attributes of thawed pork with different low field NMR T21. Meat Sci. 92 (2), 79–83.

Lodemann, U., Hübener K., Jansen N. & Martens H., 2006. Effects of *Enterococcus faecium* NCIMB 10415 as probiotic supplement on intestinal transport and barrier function of piglets. Arch. Anim. Nutr. 60 (1), 35–48.

Ma, J., Wang C., Wang Z., Cao G., Hu R., Wang X., Zou H., Kang K., Peng Q., Xue B., Wang L., Zhu Y. & Zhu X., 2021. Active dry yeast supplementation improves the growth performance, rumen fermentation, and immune response of weaned beef calves. Anim. Nutr. 7 (4), 1352–1359. DOI: 10.1016/j.aninu.2021.06.006

Malik, M.I., Rashid M.A., Yousaf M.S., Naveed S., Javed K. & Rehman H., 2020. Effect of physical form and level of wheat straw inclusion on growth performance and blood metabolites of fattening goat. Animals. 10 (10), 1861.

Mariezcurrena-Berasain, M.D., Mariezcurrena-Berasain M.A., Lugo J., Libien-Jiménez Y., Pinzon-Martinez D., Salem A. & García-Fabila M., 2022. Effects of dietary supplementation with organic selenium-enriched yeast on growth performance, carcass characteristics, and meat quality of finishing lambs. Trop. Anim. Health Prod. 54 (1), 49.

Martens, H., Rabbani I., Shen Z., Stumpff F. & Deiner C., 2012. Changes in rumen absorption processes during transition. AFST. 172 (1), 95–102. DOI: 10.1016/j.anifeedsci.2011.12.011

Naseem, C., Rabbani I., Rashid M., Arif M., Yousaf M., Imran M., Anjum A. & Rehman H., 2023. Effects of indigenous *Saccharomyces cerevisiae* on intake, growth, gut histology, and serum biomarkers in pre-weaned Lohi lambs. S. Afr. J. Anim. Sci. 53 (4), 607–614.

Newbold, C.J., Wallace R. & McIntosh F., 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. Br. J. Nutr. 76 (2), 249–261.

Nishihara, K., Suzuki Y., Kim D. & Roh S., 2019. Growth of rumen papillae in weaned calves is associated with lower expression of insulin-like growth factor-binding proteins 2, 3, and 6. Anim. Sci. J. 90 (9), 1287–1292. DOI: 10.1111/asj.13270

Orgeur, P., Mavric N., Yvore P., Bernard S., Nowak R., Schaal B. & Lévy F., 1998. Artificial weaning in sheep: consequences on behavioural, hormonal and immuno-pathological indicators of welfare. Appl. Anim. Behav. Sci. 58 (1–2), 87–103.

Penner, G.B., Aschenbach J.R., Gabel G., Rackwitz R. & Oba M., 2009. Epithelial capacity for apical uptake of short chain fatty acids is a key determinant for intraruminal pH and the susceptibility to subacute ruminal acidosis in sheep. J. Nutr. 139 (9), 1714–1720. DOI: 10.3945/jn.109.108506

Perdomo, M., Marsola R., Favoreto M., Adesogan A., Staples C. & Santos J., 2020. Effects of feeding live yeast at 2 dosages on performance and feeding behavior of dairy cows under heat stress. J. Dairy Sci. 103 (1), 325–339.

Rabbani, I., Siegling-Vlitakis C., Noci B. & Martens H., 2011. Evidence for NHE3-mediated Na transport in sheep and bovine forestomach. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301 (2), R313–319. DOI: 10.1152/ajpregu.00580.2010

Shahid, A.B., Malhi M., Soomro S.A., Shah M.G., Kalhoro N.H., Kaka A., Mal R., Soomro M.A., Samo S.P. & Sanjrani M.N., 2020. Influence of dietary selenium yeast supplementation on fermentation pattern, papillae morphology and antioxidant status in rumen of goat. Pak. J. Zool. 52 (2).

Shen, Y.B., Piao X.S., Kim S.W., Wang L., Liu P., Yoon I. & Zhen Y.G., 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J. Anim. Sci. 87 (8), 2614–2624. DOI: 10.2527/jas.2008-1512

Sowińska, J., Tański Z., Milewski S., Ząbek K., Wójcik A., Sobiech P. & Illek J., 2016. Effect of diet supplementation with the addition of Saccharomyces cerevisiae upon stress response in slaughter lambs. Acta Vet. 85 (2), 177–184.

Steele, M.A., Penner G.B., Chaucheyras-Durand F. & Guan L.L., 2016. Development and physiology of the rumen and the lower gut: Targets for improving gut health. J. Dairy Sci. 99 (6), 4955–4966. DOI: 10.3168/jds.2015-10351

Stumpff, F., Georgi M. I., Mundhenk L., Rabbani I., Fromm M., Martens H. & Gunzel D., 2011. Sheep rumen and omasum primary cultures and source epithelia: barrier function aligns with expression of tight junction proteins. J. Exp. Biol. 214 (Pt 17), 2871–2882. DOI: 10.1242/jeb.055582

Tekce, E., Bayraktar, B., Aksakal V., Dertli E., Kamiloğlu, A., Karaalp M., Timurkaan S. & Mehmet G., 2021. Response of probiotics and yeast added in different doses to rations of Anatolian Merino lambs on fattening performance, meat quality, duodenum and rumen histology. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 27 (1).

Tripathi, M.K., Karim S.A., Chaturvedi O.H. & Verma D.L., 2008. Effect of different liquid cultures of live yeast strains on performance, ruminal fermentation and microbial protein synthesis in lambs. J. Anim. Physiol. Anim. Nutr. (Berl.). 92 (6), 631–639. DOI: 10.1111/j.1439-0396.2007.00759.x

Uyeno, Y., Sekiguchi Y. & Kamagata Y., 2010. rRNA-based analysis to monitor succession of faecal bacterial communities in Holstein calves. Lett. Appl. Microbiol. 51 (5), 570–577. DOI: 10.1111/j.1472-765X.2010.02937.x

Wang, J., Zhao G., Zhuang Y., Chai J. & Zhang N., 2022. Yeast (*Saccharomyces cerevisiae*) culture promotes the performance of fattening sheep by enhancing nutrients digestibility and rumen development. Fermentation. 8 (12), 719.

Warner, R., 2014. Measurement of meat quality. Measurements of water-holding capacity and color: objective and subjective. 2, 164–171.

Xiao, J., Alugongo G., Chung R., Dong S., Li S., Yoon I., Wu Z. & Cao Z., 2016. Effects of *Saccharomyces cerevisiae* fermentation products on dairy calves: Ruminal fermentation, gastrointestinal morphology, and microbial community. J. Dairy Sci. 99 (7), 5401–5412.

Zengin, M., Kısadere İ., Usta M., Bacaksız O.K., Demir E. & Azman M.A., 2022. Effects of adding ground or steam-flaked corn and zinc-enriched yeast to grower pellet feed on fattening performance, development of rumen papilla, and some blood parameters in lambs. RBZ. 51.