

Effects of indigenous *Saccharomyces cerevisiae* on intake, growth, gut histology, and serum biomarkers in pre-weaned Lohi lambs

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Abstract

The purpose of this study was to determine the comparative efficacy of locally-produced yeast to commercial yeast on growth performance, serum biomarkers, and gut histology. Thirty pre-weaned male Lohi lambs were divided into; 1) C (Control; starter diet with no supplement), 2) CY (starter diet with ewe milk supplemented with 1 g/animal/day commercial yeast), and 3) LY (starter diet supplemented with 1 g/animal/day local yeast). Animals were housed individually and fed a starter diet and orts were weighed daily. Dry matter intake (DMI) was determined daily while the body weights were determined on a weekly basis. At the end of the trial, blood samples were collected and analysed for blood urea nitrogen (BUN), glucose, β -Hydroxybutyrate (BHBA), and non-esterified fatty acids (NEFA), while ruminal fluid and tissues were collected for rumen fermentation parameters and histomorphometry. The results revealed that the DMI and average daily weight gain (ADWG) were higher in CY and LY compared to C. The feed efficiency was also better in CY and LY lambs compared to C lambs. The BHBA, BUN, total proteins (TP), cholesterol, creatinine, blood glucose, and tissue histomorphometry were similar in LY and CY supplementation. In conclusion, supplementation with LY produced comparable results to the CY.

Keywords Commercial yeast, jejunum, local yeast, rumen, sheep

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Introduction

Optimal growth and development of ruminants are imperative to meet the ever-increasing food demand of the growing population of the world. Sheep play a key role to cater for the nutritional demands of 220 million people in Pakistan and 31.6 million sheep serve as an important cohort for nutritional research. A number of previous studies have emphasised feed supplementation over the use of antimicrobials as feed additives in livestock to prevent the development of antimicrobial resistance in consumers and to improve digestibility and growth performance in ruminants (Ma *et al.*, 2021). For instance, saponin-rich plants such as *Sesbania sesban* have shown strong anti-protozoal activity while leaving the rest of the rumen biomass unaltered (Wang *et al.*, 2000). Similarly, supplementation of ruminant feed with *Eucalyptus* leaves (Manh *et al.*, 2012) and essential oils has improved the physiological status of animals.

Saccharomyces cerevisiae is frequently supplemented in ruminant feed with beneficial effects on gastrointestinal development (Alugongo *et al.*, 2017; Ma *et al.*, 2020), improved ruminal papillae length (Xiao *et al.*, 2016), and a stable rumen ecosystem (Chaucheyras-Durand & Fonty, 2002). This in turn improves dry matter intake (Ghazanfar *et al.*, 2015), body weight gain (Shen *et al.*, 2009; Ma *et al.*, 2021), and milk production (Bitencourt *et al.*, 2011). Generally, the constituents of *S. cerevisiae* serve as a probiotic to stabilize ruminal pH by suppressing lactate-producing bacteria and proliferating

lactate-utilizing bacteria (Guedes *et al.*, 2008; Amin & Mao, 2021), which in turn augments physiological (Ghazanfar *et al.*, 2015) and immunological attributes (Kim *et al.*, 2020; Lee *et al.*, 2021). It also affects blood glucose concentrations (Mirzad *et al.*, 2019), blood urea nitrogen, β -hydroxybutyrate (Kumprechtova *et al.*, 2019), and the lipid profile (Cakiroglu *et al.*, 2010) in ruminants.

In Pakistan, most feed additives including *S. cerevisiae* are imported from technologically advanced countries. However, due to the recent challenges of the COVID-19 pandemic and consequent constraints in shipment, the reliance of the livestock industry on import items has become a point of concern for farmers and stakeholders. The positive impact of yeast supplementation in ruminants has been well documented but the effectiveness of locally-produced live yeast and its impact on pre-weaned lambs remains to be explored. It was hypothesised that the indigenous yeast would produce comparable outcomes when supplemented in comparison to the commercial yeast and therefore, the objective of this study was to compare the effect of the local strain of *S. cerevisiae* on anthropometric parameters, serum biomarkers, ruminal fermentation parameters, and tissue histomorphometry with that of commercial yeast in lambs.

Materials and Methods

A four-week research trial was conducted at the Small Ruminant Training Center, Pattoki, University of Veterinary and Animals Sciences, Lahore, Pakistan. All the animals were treated against endo- and ectoparasites using a sub-cutaneous injection of ivermectin (0.1 ml/5 kg) and were vaccinated against enterotoxaemia. This experiment was approved by the Animal Care and Use Committee (ACUC) UVAS, Lahore (DR/1136).

A total of 30 male Lohi lambs (body weight, BW, 5.0 ± 1.5 kg; age, 8 ± 2 days) were randomly allocated to three dietary treatments ($n = 10$ /treatment), 1) control (**C**), without any supplementation; 2) commercial yeast (**CY**), commercial live yeast (Alltech Inc., Nicholasville, Kentucky, USA) @ 1 g/animal/day (2×10^9 CFU); and 3) local yeast (**LY**), local live yeast @ 1 g/animal/day (2×10^9 CFU). For the supplemented group, the yeast, either commercial or local, was added to ewe milk and hand-fed twice a day at 07,00 and 17,00 @ 10% of BW for 28 consecutive days. From the 20th day of the trial, lambs were fed individually on a starter diet (Table 1) and orts were weighed daily. All the lambs were housed in individual pens of 1.5 \times 1.5 m, bedded with sand, and had free access to fresh water during the entire experimental period.

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

Ingredients	%
Ground maize	46.50
Soybean meal	21.0
Canola meal	14.5
Wheat bran	10.0
Molasses	5.0
Mineral mixture ¹	1
Lime	2.0
Nutrient levels ²	%
DM	89.65
CP	19.0
ME (MCal/kg)	2.79
Fat	2.81
NDF	14.22
ADF	6.25
Ca	1.09
P	0.58

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

²DM = dry matter; CP = Crude protein; ME = Metabolizable energy; NDF = neutral detergent fibre; ADF = acid detergent fibre

Multiple yeast strains were subjected to various biochemical tests, including wet mount examination, a germ tube test, a sugar utilization assay, and polymerase chain reaction (PCR) to facilitate their identification. Sabouraud dextrose broth was employed to support the growth of selected

yeast cells (indigenous yeast). The culture was centrifuged at 6000 rpm for 10 min and the supernatant was carefully removed. The resulting cell pellets were collected and subsequently 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 (OD) of 2×10^9 colony-forming units per millilitre (CFU/mL). The resulting suspension was then transferred to a sterile container and saved for further supplementation.

The DMI was determined daily while the animals were weighed weekly. Body condition score was performed on a weekly basis using a scale of 0 to 5 (Phythian *et al.*, 2012), where 0 was considered emaciated and 5 as obese. The faecal score was developed by visual scoring as described by Hu *et al.* (2012) and recorded daily on a scale from 1 to 4 where 1 was considered a solid pellet and 4 as watery.

At the end of the trial, 10 ml of blood sample was collected aseptically from the jugular vein, poured into non-EDTA vacutainers, and centrifuged for 15 min at $2200 \times g$ to harvest serum. The serum was stored at -20°C for analysis of glucose, blood urea nitrogen (BUN; Biosystems, Spain), cholesterol (Human, Germany), β -hydroxybutyrate (BHBA, Randox, UK), and total protein (portable refractometer, ATAGO U.S., Inc., Bellevue, WA). All blood chemistry parameters were analysed using commercially available kits with an EPOCH microplate spectrophotometer (Biotek Instruments Inc., Winooski, USA).

At the end of the experimental period, a gastric tube was used to collect ruminal fluid as reported by Lodge-Ivey *et al.* (2009) 4h after feeding. The pH of the ruminal fluid was measured immediately after the collection of fluid using a digital pH meter (NeoMet model pH-200L, Korea).

At the completion of the trial, lambs ($n = 5$) from each treatment were slaughtered at the UVAS slaughtering facility to collect ventral rumen and jejunum tissue samples. Tissue samples were fixed in 10% formalin, dehydrated by graded alcohol in ascending order, and cleared in xylol. After tissue sectioning, samples were stained with a haematoxylin-eosin staining dye, and images were taken at 4X using an optical microscope (LX400, LABOMED, The Netherlands) fitted with a digital camera (DC, 1355-F050, CMEX Euromex, The Netherlands). To assess rumen developmental parameters; papillae length, papillae width, and surface areas were measured as described earlier by Malik & Rashid (2020). Jejunal histomorphometry was assessed in terms of villus height, villus width, and crypt depth by using a microscope and an image processing and analysis system, LABOMED Pixel Pro (Version 1, Leica Imaging Systems Ltd., Cambridge, United Kingdom).

The collected data were tested for a normal distribution using the Kolmogorov–Smirnov test and analysed using one-way analysis of variance (SPSS; version 20.0 IBM Corp., Armonk, New York, USA). Tukey's post-hoc test was employed to determine differences between treatments and the results were considered significant at $P < 0.05$.

Results and Discussion

The results of the current study showed that the average daily weight gain (ADWG), dry matter intake (DMI), and feed efficiency were improved ($P < 0.05$) in sheep supplemented with either LY or CY compared to C (Table 2). As expected, the supplementation of yeast substantially increased the DMI, which is in agreement with the previous findings (Abd El-Ghani, 2004; Stella *et al.*, 2007), who reported that the DMI was higher in goats supplemented with yeast. An increase in DMI with yeast supplementation is generally attributed to its favourable capacity to create anaerobic conditions in the rumen, which aids ruminal microorganisms and fibre digestibility (Chaucheyras-Durand *et al.*, 2008). Similar results were observed for the ADWG, in which CY and LY increased the ADWG by 8.5% and 11%, respectively, over the C group. These findings are in accordance with the previously published claims that yeast supplementation enhances DMI and increases fibre digestibility in the rumen as a result of cellulolytic bacteria (Newbold *et al.*, 1996).

No effect ($P > 0.05$) of yeast supplementation was observed for body condition score (BCS), which was also reported by Dann *et al.* (2000) in dairy cows. Despite the fact that all the animals were acclimatized and randomly selected, the faecal score in C (although within the normal range) was higher than CY and LY ($P < 0.05$) and showed improvement with supplementation, probably as a result of better feed conversion (Ghazanfar *et al.*, 2015) or an improved faecal microbiome (Huebner *et al.*, 2019). Although previous studies have shown age-related improvements in faecal score rather than yeast supplementation (He *et al.*, 2017), *S. cerevisiae* treatment does lower the diarrhoeal score in piglets (Trckova *et al.*, 2014), primarily by improving the immune response.

Table 2 Growth performance and a faecal score of lambs supplemented with local yeast (LY) or commercial yeast (CY)

Parameters	Treatment			SEM	P-Value
	C	CY	LY		
DMI (g/day)	211.36 ^{ab}	231.14 ^a	234.65 ^a	3.933	<0.001
ADWG (g)	171.8 ^b	187.9 ^a	190 ^a	2.378	0.001
Feed Efficiency	1.25 ^b	0.95 ^a	0.92 ^a	0.035	<0.001
BCS	2.50	2.55	2.66	0.057	NS
Faecal Score	2.32 ^a	1.80 ^b	1.72 ^b	0.086	0.005

The data are presented as mean \pm standard error of mean (SEM); values in the same row with different superscripts are significantly different at $P < 0.05$; CY= Commercial yeast, LY= Local yeast

For the rumen fermentation parameters, rumen pH did not change ($P > 0.05$) with supplementation. A slightly increased rumen pH in CY and LY could be due to an increase in the number of cellulolytic microbes or due to decreased VFA concentration, which negatively correlates with the rumen pH (Throne *et al.*, 2009). BHBA was substantially higher in the group supplemented with CY compared to the control group, while BUN was higher in LY than the control (Table 3). Interestingly, BHBA markedly increased in CY compared to the C but was similar to LY, despite a trend towards an increase. This could be due to strain differences that might have stimulated the ruminal milieu in different ways. However, both the yeast-supplemented groups were similar. An increasing trend of BHBA in LY compared to C ($P = 0.08$) motivates further investigations and experiments with LY. There was also an increase in BUN in CY and LY compared to C, suggesting an increase in the microbial protein turnover and ammonia level in the rumen (Chuelong *et al.*, 2011).

Table 3 Rumen fermentation parameters of lambs supplemented with local yeast (LY) and commercial yeast (CY)

Parameters	Treatments			SEM	P-Value
	C	CY	LY		
Rumen pH	5.89	6.15	6.06	0.068	NS
BHBA (mmol/l)	0.27 ^b	0.47 ^a	0.39 ^{ab}	0.035	0.038
BUN (mg/dl)	14.36 ^b	16.42 ^{ab}	17.41 ^a	0.508	0.047

The data are presented as mean \pm standard error of mean (SEM); values in the same row with different superscripts are significantly different at $P < 0.05$; CY= Commercial yeast, LY= Local yeast

For serum biochemical parameters, the total protein, cholesterol, and creatinine were not affected by yeast supplementation (Table 4). However, glucose decreased in CY, while uric acid decreased in LY, as compared to the C treatment. Serum total protein and cholesterol were similar among the treatments; these findings are in agreement with Wojcik (2010) and Elaref *et al.* (2020), respectively. The blood glucose showed a substantial decrease in CY compared to C with supplementation, which is contradictory to that previously reported by Sowińska *et al.* (2016). However, this trend could be due to an increase in fibre digestibility and less propionate production. During the process of digestion, ruminal microbes breakdown the fibres and the prime VFA produced from fibre fermentation is acetic acid, instead of propionic acid. Propionic acid is known as glucogenic VFA and high production leads to higher glucose in the blood and vice versa (Yost *et al.*, 1977). Serum creatinine was similar among all the groups, which has been reported before (Elaref *et al.*, 2020).

Table 4 Serum biochemical parameters of lambs supplemented with local yeast (LY) and commercial yeast (CY)

Parameters	Treatments			SEM	P-Value
	C	CY	LY		
Total protein (g/dl)	6.07	6.41	6.42	0.081	NS
Glucose (mg/dl)	74.54 ^a	69.97 ^b	70.59 ^{ab}	0.745	0.027
Cholesterol (mg/dl)	70.88	68.51	68.75	1.051	NS
Creatinine (mg/dl)	1.14	0.98	1.02	0.057	NS
Uric acid (mg/dl)	6.68 ^a	5.97 ^{ab}	5.87 ^b	0.141	0.041

The data are presented as mean \pm standard error of mean (SEM); values in the same row with different superscripts are significantly different at $P < 0.05$; CY= Commercial yeast, LY= Local yeast

Rumen papillae length and density were improved in both treatment groups compared to the control group ($P < 0.05$); however, rumen papillae width was similar (Table 5). In the jejunum, villus height and the villus height to crypt ratio was substantially improved in supplemented groups compared to the control group; villus width and crypt depth were similar across treatments. Representative ruminal and jejunal histology samples from each treatment group are shown in Figure 1. These findings suggest that the yeast supplementation increased the digestibility of the fibre and, consequently, the availability of VFA in the gut milieu, thereby resulting in an adaptive response at the level of the epithelium (Steele *et al.*, 2016). One of the proposed mechanisms for enhancing the growth of epithelium by VFA is through indirect effects of multiple hormones, including epidermal growth factor and IGF-1 (Cui *et al.*, 2019).

Table 5 Rumen and jejunum histomorphometry of lambs supplemented with local yeast (LY) and commercial yeast (CY)

	Parameter	C	CY	LY		
Rumen	Ruminal Papillae length (μm)	845.94 ^b	1236.96 ^a	1369.06 ^a	76.96	0.003
	Ruminal Papillae width (μm)	282.25	318.12	323.00	20.00	NS
	Ruminal Papillae density (no/cm ²)	69.25 ^b	80.25 ^a	83.25 ^a	2.14	0.004
Jejunum	Villus Height (μm)	300.60 ^b	400.59 ^a	408.08 ^a	18.97	0.015
	Villus Width (μm)	76.15	73.05	73.39	1.28	NS
	Crypt depth (μm)	134.37	115.70	112.70	6.015	NS
	Villus height to crypt ratio	2.24 ^b	3.41 ^a	3.6 ^a	0.207	0.001

The data are presented as mean \pm standard error of mean (SEM); values in the same row with different superscripts are significantly different at $P < 0.05$; CY= Commercial yeast, LY= Local yeast

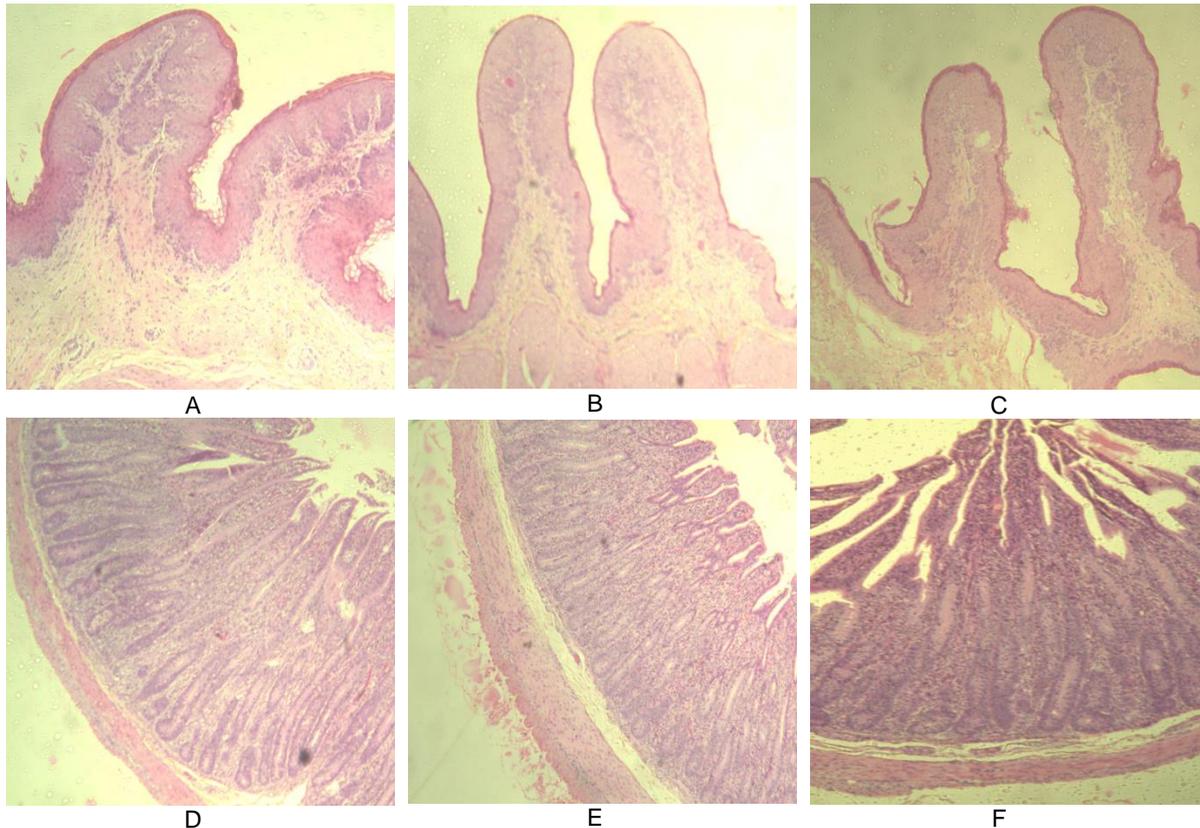


Figure 1 Rumen and jejunum histology; A) control - rumen papillae, B) commercial live yeast - rumen papillae, C) local live yeast – rumen papillae, D) control - jejunum, E) commercial live yeast - jejunum, F) local live yeast - jejunum

Conclusion

Supplementation of *S. cerevisiae* produced from local sources improved anthropometric parameters and serum biomarkers and promoted ruminal and jejunal development in lambs, the results of which were comparable to the results of the commercial yeast. However, further studies are required to determine the cost-effectiveness of local *S. cerevisiae* to benefit the stakeholders.

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Author's Contributions

AA prepared indigenous yeast; CN conducted the research trial and collected the data for the experiment; MA, MI conducted the statistical analyses, interpreted the results, and wrote the initial draft of this manuscript; IR, HR, SY, AR developed the original hypotheses, designed the experiments, and finalized the manuscript; all authors read and approved the manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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