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RESEARCH ARTICLE

Response of Probiotics and Yeast Added in Different Doses to Rations of Anatolian Merino Lambs on Fattening Performance, Meat Quality, Duodenum and Rumen Histology [1]

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Abstract

This study investigated the effects of dietary probiotics *(Lactobacillus reuteri* E81 [LRE], *Lactobacillus rhamnosus* GG [LRG]), yeast *(Saccharomyces cerevisiae* S81 [SCS]), and their combined supplementation on fattening performance (BW, DWG, FI, and FCR), meat quality, and rumen and duodenum histology in lambs. The study material comprised ninety 2.5-month-old Anatolian Merino lambs, and the trial was conducted for 70 days. Nine trial groups, each composed of 10 animals, were established. This study demonstrated that, when compared to the control group, the best fattening performance was achieved in the lambs that received 600 ppm of dietary LRE. Neither visceral organ weights nor rumen and duodenum histology was affected in the groups that received the tested feed supplements. Of the meat colour parameters investigated, the L* value was observed to have increased in the groups that were given feed supplements, excluding Groups LRE-600 and SCS-300. It was determined that the probiotic supplements had no effect on the a* and b* colour parameters, but affected the meat pH value. In conclusion, the assessment of the effects of different doses of dietary probiotics, yeast, and probiotic-yeast combinations on performance parameters, visceral organ weights, and meat quality in Anatolian Merino lambs showed that the best results were achieved in the group that received 600 ppm of LRE alone.

Keywords: *Probiotic, Yeast supplementation, Anatolian Merino lamb performance, Feed additive, Meat quality, Histology*

Anadolu Merinos Kuzularının Rasyonlarına Farklı Dozlarda İlave Edilen Probiyotiklerin ve Mayanın Besi Performansı, Et Kalitesi, Rumen ve Duodenum Üzerine Yanıtı

Öz

Yapılan çalışmada, kuzu rasyonlarına probiyotik *(Lactobacillus reuteri* E81 [LRE], *Lactobacillus rhamnosus* GG [LRG]), maya *(Saccharomyces cerevisiae* S81 [SCS]) ve karışımlarının ilavesinin besi performansı (CA, GCAA, Yem Tüketimi ve YYO) et kalitesi, rumen ve duodenum histolojisi üzerine etkisi araştırılmıştır. Araştırmada 2.5 aylık 90 adet Anadolu Merinosu koyun kullanılmış, çalışma 70 gün sürmüştür. Deneme her grupta 10'ar hayvan olacak şekilde 9 farklı gruptan (Kontrol, LE-300, LE-600, LR-300, LR-600, SC-300, SC-600, MİX-300 ve MİX-600) oluştu. Araştırma sonunda besi performansı üzerine kontrol grubuna kıyasla en iyi sonuçlar *L. reuteri* E81 600 ppm katkılı grupta elde edilmiştir. İç organ ağırlığı ve duodenum ile rumen histolojisi üzerine katkılı grupların etkisi olmazken, et renk parametreleri üzerine kontrol grubuna kıyasla L* parametresinde LRE 600 ve SCS 300 dışındaki gruplarda artış gözlenmiştir. Probiyotik uygulamasının, a* ve b* renk parametreleri üzerine etkisi olmazken, et pH değeri üzerinde oldukça etkili olduğu tespit edilmiştir. Sonuç olarak kuzu rasyonlarına ilave edilen probiyotik, maya ve karışımlarının besi performansı (CA, GCAA ve YYO), iç organ ağırlıkları ve et kalitesi üzerine en iyi sonuçlar *L. reuteri* E81 600 ppm gruplarda elde edilmiştir.

Anahtar sözcükler: *Probiyotik, Maya takviyesi, Anadolu Merinos kuzu performansı, Yem katkı maddesi, Et kalitesi, Histoloji*

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Introduction

The majority of the proteins required by the human body must be acquired by the consumption of animal proteins. The rapid growth of the global population hinders the access of some people to the proteins they need. Although there is continual research on how to increase the quantity and quality of food products in line with consumer preferences, a supply and demand equilibrium has not yet been established. Furthermore, the food safety approach, from the farm to the fork, requires the maintenance of the health of food-producing animals [1,2].

The gastrointestinal tract of newborn ruminants does not contain any microorganisms and is sterile. The rumen microbiota starts to form immediately after birth. As the animal grows, a microbial ecosystem containing very high concentrations of bacteria develops in the rumen [3]. The ruminal microbiota is very sensitive in the neonatal period and can be easily harmed by several factors. Changes in the rumen microbial environment can cause performance and health problems in most ruminants [4]. In this context, the incorporation of feed supplements in the ration of newborn lambs is aimed at enabling weight gain and supporting rumen development at the time of weaning [5]. For many years, antibiotics were used for these purposes, as growth promoters at sub-therapeutic doses. However, through legislative regulations, the European Union (EU) has banned the use of chemotherapeutic agents for these purposes, necessitating the development of new feed additives and alternative feeding and animal health strategies [6]. Listed among the newly developed alternative feed additives, probiotics are products that contain viable microorganisms, which effectively increase intestinal health by regulating the balance of intestinal microflora when received in sufficient amounts [7]. Research has shown that depending on the type and dose, probiotics increase performance [8,9], maintain microbial balance in the gastrointestinal tract [10], strengthen immune function [11], reduce stress [12], and increase food digestibility [13], intestinal microflora modulation [14], pathogen inhibition [15], immunomodulation and intestinal mucosal immunity [16]. Meat quality is affected by several parameters such as pre- and post-slaughter conditions, glycogen deposition [17], sex, breed, weight, and diet [18]. It is considered that probiotics may affect meat quality via their effects on animal health. However, the effect of probiotic supplementation on meat quality remains unclarified [19,20].

The objective of this study was to investigate the effect of different doses (300 ppm and 600 ppm) of dietary probiotics *(Lactobacillus reuteri* E81 [LRE], *Lactobacillus rhamnosus* GG [LRG]), yeast *(Saccharomyces cerevisiae* S81 [SCS]), and combined probiotic + yeast supplementation on fattening performance (body weight [BW], daily weight gain [DWG], feed conversion ratio [FCR], and feed intake [FI]), visceral organ weights, rumen and duodenum histology (villus height [VH], villus width [VW], intestinal crypt depth [CD], tunica muscularis width [TMW], papilla ruminis height [PRH], papilla ruminis width [PRW], lamina propria width [LPW]), meat colour parameters, and pH value in lambs.

Material and Methods

Ethical Approval

This study was conducted according to the approval (dated 18.07.2019 and numbered 2019/12) of the Local Ethics Board for Animal Experiments of the Directorate of the Central Veterinary Control and Research Institute.

Lambs, Diet, and Experimental Design

The animal material of this study comprised 90 male Anatolian Merino weanling lambs, which were 2.5 months old (24.98±6.02) and raised under intensive breeding conditions at a private farm located in the central district of the Bayburt province. The pedigree and breeding records of the farm were regularly inspected. This study was conducted for a 56-day period that began after a fourteen-day acclimatization period of the animals at the Food, Agriculture, and Livestock Research and Application Centre of Bayburt University. After being weaned, lambs of the almost similar with each other body weight were assigned to nine groups, each including 10 animals Control (C = basal diet), LRE-300 (basal diet + 300 ppm *L. reuteri* E81), LRE-600 (basal diet + 600 ppm *L. reuteri* E81), LRG-300 (basal diet + 300 ppm *L. rhamnosus* GG), LRG-600 (basal diet + 600 ppm *L. rhamnosus* GG), SCS-300 (basal diet + 300 ppm *S. cerevisiae* S81), SCS-600 (basal diet + 600 ppm *S. cerevisiae* S81), MIX-300 (basal diet + 300 ppm L. reuteri E81 + 300 ppm *L. rhamnosus* GG + 300 ppm *S. cerevisiae* S81), and MIX-600 (basal diet + 600 ppm *L. reuteri* E81 + 600 ppm *L. rhamnosus* GG + 600 ppm *S. cerevisiae* S81). Excluding those included in the control group, the animals were given a daily amount of feed that contained feed supplements. Each day, at the same time (08:00 pm), the feed remaining in the feeders was weighed, and then replaced with new feed. The lambs were provided with a basal lamb ration in concentrated pellet form, the nutrients, and energy value of which are presented in *Table 1*. The basal ration, purchased from a private feed mill in the Balıkesir province, contained added probiotic *(L. reuteri* E81 [LRE], *L. rhamnosus* GG [LRG]), yeast *(S. cerevisiae* S81 [SCS], ($4x10^{10}$ CFU/g), or combined probiotic + yeast supplements, which were produced at the Food Engineering Department of Bayburt University. The feed used in this study was analyzed following the analysis methods of the AOAC [21].

Performance Parameters

To monitor the effects of the probiotic and yeast supplements added to the lamb ration, and to determine the weight gain of the lambs, each animal was weighed individually,

Table 1. *Nutrient content of the basal diet ration (%)*	
Raw Material	**Lamb Ration Content**
Barley	30
Corn	24
Soybean Meal	10
Wheat Bran	4
Cottonseed Meal	13
Molasses	8
Sunflower Meal	8
Premix	0.05
Salt	0.95
Dicalcium Phosphate	3
Dry Matter (%)	88
Crude Protein (%)	14
Crude Cellulose (%)	13
Crude Oil (%)	4.2
Ash (%)	9
ME (MJ/kg)	12,14
The vitamin and mineral premix provided the following (per kg): 4.000.000 IU vit. A, 800.000 IU vit. D_3, 5.000 IU vit. E, 400 mg vit. B_2, 2 mg vit. B_{12}, 5.000 mg vit. B_3, 1.000 mg D-pantothenic acid, 20.000 mg choline, 50 mg Co, 5400 mg Fe, 185 mg I, 6.900 mg Mn, 800 mg Cu, 6400 mg Zn, 14 mg Se	

every 7 days, before being given feed in the morning. The average daily feed intake of the groups was designated by weighing the feed remaining in the feeders each morning, calculating the amount of feed consumed per week, and dividing the weekly amount of feed consumed by 7. The feed conversion ratio (FCR) was determined based on the proportion of daily FI to daily weight gain (DWG).

Visceral Organ Weights and Meat Quality Parameters

At the end of the experiment, a total of 27 lambs, including 3 randomly selected animals from each group, were sacrificed at the laboratory of the Food, Agriculture and Livestock Research and Application Centre of Bayburt University. The visceral organs of the sacrificed animals were weighed on a precision balance accurate to 0.001 g.

Meat quality parameters were investigated in 27 carcasses, including 3 from each group. Analyses were performed at the Food Engineering Department of Bayburt University on brisket and fat samples taken from carcasses aged 24 h.

The color parameters of the brisket and fat samples were taken from the lamb carcasses were determined using a colorimeter (CR-400, Minolta Co, Osaka, Japan). Colour saturation was determined according to the CIELAB space, based on three-dimensional colorimetry data, published by the International Commission on Illumination (CIE). Accordingly, colour saturation was assessed as follows: a*; a* = + 60 red, a*= - 60 green; b*; b* = + 60 yellow, b* = - 60 blue and L*; L* = 0 black, L* = 100 white (darkness/lightness). The pH values of the meat samples were determined by homogenizing 10 g of meat in 100 mL of distilled water with a laboratory homogenizer and using a pH-meter (Jenco Electronics 6173, Taiwan) calibrated with buffered solutions (pH 4.0 and pH 7.0).

Histomorphology

For histological analysis, at the end of the study period, three animals, randomly chosen from each group, were slaughtered. Tissue samples were taken from the duodenum and rumen and were fixed in 10% buffered formalin solution (saline). The tissues were dehydrated through a graded series of alcohol, cleared with xylene, and embedded in paraffin. Sections were cut at the 4-µm thickness and were stained with hematoxylin-eosin. The villus height (VH), villus width (VW), intestinal crypt depth (CD) and tunica muscularis width (TMW) values of the duodenum, and the papilla ruminis height (PRH), papilla ruminis width (PRW), lamina propria width (LPW), and tunica muscularis width (TMW) values of the rumen were measured in randomly selected five different areas of the duodenum and rumen specimens, using an oculometer at 10x and 20x magnification under a light microscope fitted with a stage micrometer. Each group was photographed using an Olympus BX-43 research microscope with an image analysis system (DP72-BSW). The nomenclature used in this study conforms to the Nomina Histologica.

Statistical Analysis

Firstly, a normality test was performed, and it was determined that the data were distributed normally. Variables are presented as means with standard errors. For data on rumen and duodenum histology, the non-parametric Kruskal-Wallis test was used since the number of samples did not provide any normality distribution. The Mann-Whitney U test with Bonferroni's correction was used as a post hoc test One-way ANOVA was utilized to determine the differences between the nine diet groups for FI, FCR, BW, and average DWG performances and meat colour. Duncan's multiple comparison test was performed for group means with a significance level of 0.05 using the IBM SPSS Statistics v25 software.

Results

The effects of the different doses of dietary probiotics, yeast, and combined probiotic + yeast supplementation on the performance of the lambs are shown in *Table 2* and *Table 3*. Data analysis demonstrated that, when compared to the control group, the best results for BW, DWG, and FCR were achieved in the groups that received 300 ppm and 600 ppm of dietary *L. reuteri* E81, and the results obtained in these two groups were found to be statistically significant ($P<0.05$). Nevertheless, no statistically significant difference was detected between the groups for feed intake and daily feed intake (FI) ($P>0.05$) *(Table 2, Table 3)*. Statistical analysis of visceral organ weight data showed

Table 2. *The effects of dietary probiotic, yeast, and combined probiotic and yeast supplementation on the fattening performance of lambs (Mean±SEM)*

Parameters	Control	LRE 300 ppm	LRE 600 ppm	LRG 300 ppm	LRG 600 ppm	SCS 300 ppm	SCS 600 ppm	Mix 300 ppm	Mix 600 ppm	P
BW (kg)	31.08±1.72^{dc}	38.44±1.69^{ab}	40.63±2.79^{a}	28.65±1.93^{ed}	36.25±1.95^{abc}	27.98±1.02^{ed}	18.97±1.15^{f}	34.50±2.21^{cb}	25.06±1.40^{e}	**
DWG (g)	0.27±0.03^{bc}	0.35±0.04^{ab}	0.41±0.04^{a}	0.28±0.03^{bc}	0.28±0.02^{bc}	0.16±0.02^{d}	0.17±0.02^{d}	0.33±0.02^{ab}	0.22±0.02^{dc}	**
FI (kg)	11.03±0.45	10.67±0.70	9.51±0.70	10.95±0.34	11.21±0.58	10.56±0.59	11.17±0.47	10.84±0.62	10.89±0.57	NS
FCR (kg/kg)	6.33±0.69^{b}	4.79±0.54^{bc}	3.26±0.26^{c}	6.00±0.64^{bc}	5.74±0.34^{bc}	10.19±0.30^{a}	10.09±1.75^{a}	4.81±0.34^{bc}	7.61±0.99^{ab}	**

*Means within the same column showing different superscripts are significantly different (P<0.05), * Significant at the 0.05 level, ** Significant at the 0.01 level,* ***NS:*** *Not significant (P>0.05),* ***SEM:*** *Standard error of the mean (Lactobacillus reuteri E81 [LRE]), Lactobacillus rhamnosus GG [LRG]), yeast (Saccharomyces cerevisiae S81 [SCS]),* ***BW:*** *Body Weight,* ***DWG:*** *Daily Weight Gain,* ***FI:*** *Feed Intake,* ***FCR:*** *Feed conversion ratio*

Table 3. *The effects of dietary probiotic, yeast, and combined probiotic and yeast supplementation on weekly weight gain (kg) in lambs (Mean±SEM)*

Weeks	Control	LRE 300 ppm	LRE 600 ppm	LRG 300 ppm	LRG 600 ppm	SCS 300 ppm	SCS 600 ppm	Mix 300 ppm	Mix 600 ppm	SEM
Week 1	24.93±1.72^{b}	31.14±3.11^{a}	31.39±5.14^{a}	21.28±3.57^{cd}	29.21±3.13^{a}	25.90±1.49^{b}	19.43±2.97^{d}	24.30±3.85^{cb}	14.91±1.30^{e}	**
Week 2	26.92±2.12^{c}	34.41±2.74^{a}	33.64±4.56^{a}	22.90±3.58^{ed}	30.70±2.82^{b}	28.01±2.21^{bc}	21.41±3.36^{e}	25.20±3.76^{cd}	15.90±1.40^{f}	**
Week 3	27.90±2.57^{c}	35.51±2.79^{a}	36.55±3.57^{a}	25.41±3.44^{c}	32.40±2.56^{b}	30.80±1.80^{b}	22.8±3.07^{d}	26.21±3.85^{c}	17.20±1.38^{e}	**
Week 4	29.80±3.09^{c}	38.00±2.50^{a}	40.01±3.24^{a}	27.30±3.77^{c}	34.60±2.24^{b}	33.00±1.59^{b}	24.50±2.87^{d}	27.50±3.68^{c}	17.80±1.38^{d}	**
Week 5	31.30±3.74^{d}	40.40±2.25^{b}	43.63±2.48^{a}	28.70±3.71^{e}	37.20±1.49^{c}	35.60±1.31^{c}	25.60±2.88^{f}	28.20±3.64^{e}	19.00±1.48^{h}	**
Week 6	32.70±4.67^{d}	43.51±3.36^{b}	48.12±2.66^{a}	31.51±4.27^{e}	39.40±2.13^{c}	38.20±2.23^{c}	26.50±2.91^{f}	29.00±3.78^{e}	20.20±3.78^{h}	**
Week 7	35.20±5.58^{d}	46.50±4.34^{b}	51.09±3.59^{a}	34±5.38^{d}	41.40±2.64^{c}	40.30±2.97^{c}	28.50±3.81^{e}	30.30±4.18^{e}	22.00±2.31^{f}	**
Week 8	37.60±6.23^{c}	49.01±4.78^{a}	53.37±4.57^{a}	36.00±6.82^{cd}	43.50±4.39^{b}	42.30±5.10^{b}	30.20±4.52^{e}	31.81±4.92^{de}	23.40±2.84^{f}	**

*Means within the same column showing different superscripts are significantly different (P<0.05), * Significant at 0.05 level, ** Significant at 0.01 level,* ***NS:*** *Not significant (P>0.05),* ***SEM:*** *standard error of the mean (Lactobacillus reuteri E81 [LRE]), Lactobacillus rhamnosus GG [LRG]), yeast (Saccharomyces cerevisiae S81 [SCS])*

Table 4. *The effects of dietary probiotic, yeast, and combined probiotic and yeast supplementation on lamb meat quality (Mean±SEM)*

Parameters	Variables	Control	LRE 300 ppm	LRE 600 ppm	LRG 300 ppm	LRG 600 ppm	SCS 300 ppm	SCS 600 ppm	Mix 300 ppm	Mix 600 ppm	P
Meat	L*	48.31±4.94^{ab}	51.53±5.05^{a}	49.0±3.21^{ab}	49.64±4.43^{a}	49.40±3.18^{a}	49.00±3.07^{ab}	51.12±1.62^{a}	50.00±2.57^{a}	45.99±3.25^{b}	*
	a*	22.20±1.92	20.30±3.41	20.46±2.21	22.28±2.27	22.70±2.36	22.02±2.25	22.97±1.77	23.07±3.21	23.13±3.64	NS
	b*	11.32±3.46	9.32±4.50	7.96±2.39	10.25±2.39	10.62±2.67	8.66±3.39	8.80±3.19	8.55±3.01	8.22±1.82	NS
Fat	L*	84.12±1.29^{ab}	79.94±1.62^{bc}	78.48±3.19^{c}	83.50±3.62^{ab}	84.46±4.14^{ab}	85.49±1.02^{a}	85.78±4.09^{a}	86.13±4.21^{a}	82.16±3.55^{abc}	**
	a*	4.25±1.95^{bcd}	7.14±0.91^{a}	5.24±1.63^{abc}	4.99±1.69^{bcd}	3.78±1.45^{dc}	5.27±0.88^{abc}	3.01±1.23^{d}	3.65±1.56^{dc}	5.99±0.82^{ab}	**
	b*	7.84±1.41	8.97±2.36	7.44±3.01	7.73±1.62	7.29±2.73	8.45±1.21	6.57±1.25	7.06±1.58	8.73±1.97	NS
pH		6.12±0.06^{b}	5.91±0.10^{bc}	5.87±0.09^{c}	5.98±0.08^{bc}	6.02±0.10^{bc}	6.01±0.21^{bc}	6.00±0.04^{bc}	5.96±0.08^{bc}	6.47±0.17^{a}	**

*Means within the same column showing different superscripts are significantly different (P<0.05), * Significant at 0.05 level, ** Significant at 0.01 level,* ***NS:*** *Not significant (P>0.05).* ***SEM:*** *Standard error of the mean (Lactobacillus reuteri E81 [LRE]), Lactobacillus rhamnosus GG [LRG]), yeast (Saccharomyces cerevisiae S81 [SCS])*

that combined dietary probiotic + yeast supplementation did not affect the skin, pluck (liver/heart/lungs), spleen and adrenal gland weights, and ruminal pH value *(Table 5)*.

The results obtained for meat colour parameters revealed that, when compared to the control group, the L* value was higher in the treatment groups, excluding Groups LRE-600 and SCS-300, but no effect was observed on the a* and b* colour parameters. The investigation of fat tissue colour parameters revealed an increase in the L* and a* values in Groups SCS-300, SCS-600, and MIX-300, a decrease in the same parameters in Groups LRE-300, LRE-600, and MIX-600, and no effect on the b* value in any of the treatment groups *(Table 4)*.

In the present study, the histological values of the duodenum histology were measured under a microscope. When compared to the control group, the treatment groups (A, B, C, Mix) showed no significant difference for villus height, villus width, and crypt depth *(Table 6)*. However, the width of the tunica muscularis had decreased in the control group *(Fig. 1-1a)*, and increased in Groups A, B, C, and Mix. The highest level of increase was detected in Group B 600 *(Fig. 1-1b)* and Mix 300 *(Fig.1-1c)*. There was no significant difference between the A, B, C, and Mix groups for the rumen measurements. However, the width of the tunica muscularis had decreased in the control group *(Fig. 1-1a)*, and increased in Groups A, B, C *(Fig. 1-3a)*, and Mix. The highest level of increase was detected in Group B 600

Table 5. *The effects of dietary probiotic, yeast, and combined probiotic and yeast supplementation on visceral organ weights (kg) and ruminal pH value in lambs (Mean±SEM)*

Parameters	Control	LRE 300 ppm	LRE 600 ppm	LRG 300 ppm	LRG 600 ppm	SCS 300 ppm	SCS 600 ppm	Mix 300 ppm	Mix 600 ppm	P
Head	2.51±0.48	2.67±0.29	2.83±0.12	2.71±0.30	2.67±0.25	2.67±0.29	2.50±0.61	2.48±0.22	2.02±0.11	NS
Feet	1.60±0.54	1.35±0.16	1.35±0.19	1.57±0.40	1.76±0.21	2.00±0.01	1.41±0.59	1.61±0.33	1.15±0.33	NS
Skin	5.00±1.00	6.00±1.00	6.33±0.57	6.33±0.57	6.00±1.00	5.66±1.52	5.00±2.00	5.16±1.25	4.33±0.57	NS
Pluck	1.76±0.48	1.96±0.39	2.23±0.11	2.10±0.22	1.75±0.49	2.24±0.46	1.78±0.39	1.97±0.30	1.92±0.86	NS
Liver	0.87±0.36	0.98±0.15	1.17±0.09	1.05±0.25	1.05±0.46	1.13±0.31	0.80±0.10	1.03±0.17	0.96±0.55	NS
Lungs	0.52±0.40	0.73±0.19	0.85±0.11	0.81±0.13	0.75±0.13	0.75±0.12	0.63±0.07	0.73±0.07	0.74±0.28	NS
Heart	0.19±0.01	0.22±0.06	0.21±0.05	0.22±0.02	0.19±0.04	0.24±0.03	0.15±0.01	0.41±0.42	0.21±0.03	NS
Kidneys	0.12±0.04	0.13±0.02	0.14±0.02	0.13±0.02	0.12±0.03	0.15±0.04	0.11±0.02	0.13±0.03	0.13±0.05	NS
Adrenal Glands	0.11±0.08	0.10±0.01	0.15±0.05	0.13±0.08	0.19±0.07	0.14±0.09	0.08±0.02	0.09±0.02	0.18±0.17	NS
Ruminal pH	5.24±0.14	5.46±0.04	5.35±0.31	5.61±0.86	5.49±0.25	5.57±0.13	5.40±0.35	5.66±0.52	5.58±0.63	NS
Spleen	0.09±0.01	0.09±0.01	0.12±0.02	0.09±0.01	0.09±0.01	0.10±0.01	0.10±0.02	0.11±0.03	0.11±0.03	NS

*Means within the same column showing different superscripts are significantly different (P<0.05), * Significant at 0.05 level, ** Significant at 0.01 level,* ***NS:*** *Not significant (P>0.05),* ***SEM:*** *Standard error of the mean (Lactobacillus reuteri E81 [LRE]), Lactobacillus rhamnosus GG [LRG]), yeast (Saccharomyces cerevisiae S81 [SCS])*

Table 6. *Intestinal morphology of the trial groups (Mean±SEM)*

Groups	VH	VW	CD	TMW
Control	1120.00±151.33	104.00±2.17	128.00±4.00	220.00±24.33^{a}
LRE300 ppm	1012.00±97.08	88.00±10.58	108.00±20.79	336.00±13.86^{bc}
LRE600 ppm	980.00±40.00	88.00±10.58	112.00±8.00	372.00±36.00^{bc}
LRG300 ppm	892.00±56.43	96.00±12.00	84.00±6.93	328.00±38.16^{bc}
LRG600 ppm	920.00±40.0	93.33±10.91	80.00±4.00	428.00±14.42^{c}
SCS300 ppm	980.00±40.00	72.00±0.00	108.00±12.00	356.00±28.00^{bc}
SCS600 ppm	864.00±13.86	88.00±10.58	92.00±10.58	280.00±34.18^{ab}
MIX300 ppm	972.00±12.00	96.00±0.00	84.00±6.93	380.00±58.92^{bc}
MIX600 ppm	900.00±120.00	112.00±8.00	104.00±4.00	400.00±8.00^{c}
P	NS	NS	NS	*

NS: *P>0.05, * P<0.05,* ^{a,b,c} *Means within the same column showing different superscripts are significantly different (P<0.05),* ***VH:*** *villus height,* ***VW:*** *villus width,* ***CD:*** *intestinal crypt depth,* ***TMW:*** *tunica muscularis width (Lactobacillus reuteri E81 [LRE], Lactobacillus rhamnosus GG [LRG]), yeast (Saccharomyces cerevisiae S81 [SCS])*

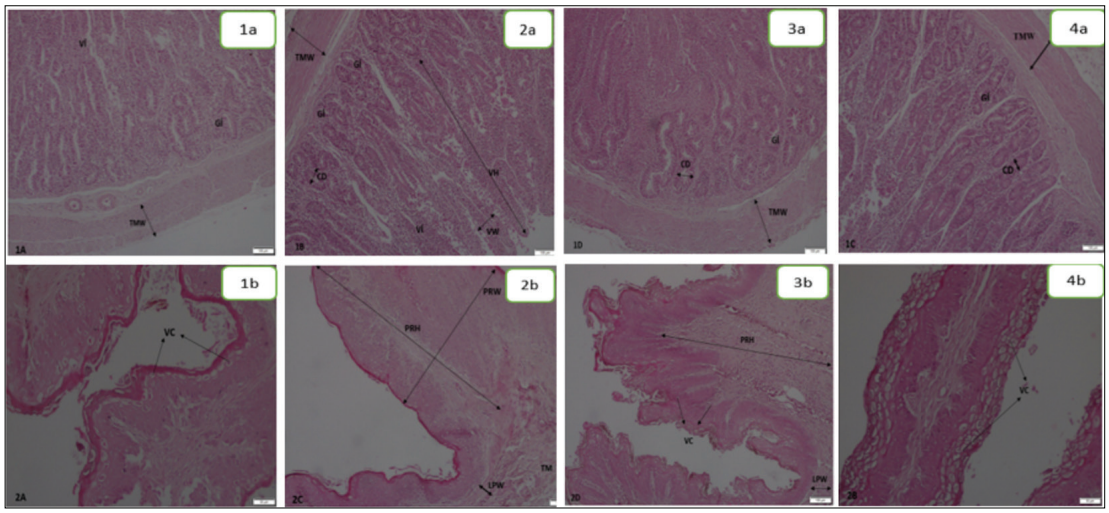

Fig 1. Photomicrographs of the duodenum (1a, 2a, 3a, 4a). TMW (Tunica muscularis width), GI (Glandula intestinalis), CD (Crypt depth). Haematoxylin-eosin, bar = 100 µm. Photomicrographs of the rumen (1b, 2b, 3b, 4b). *Black arrows:* VC (Vesicular cells). Haematoxylin-eosin, bar = 50 µm

Table 7. *Rumen morphology of the trial groups (Mean±SEM)*

Groups	PRH	PRW	LPW	TMW
Control	1540.00±530.28	560.00±66.57^{bc}	224.00±8.00	1000.00±105.83
LRE300 ppm	1360.00±80.00	516.00±12.00^{b}	240.00±0.00	976.00±112.00
LRE600 ppm	1360.00±80.00	464.00±8.00^{ab}	172.00±16.00	1072.00±136.00
LRG300 ppm	1440.00±334.07	452.00±22.27^{ab}	260.00±639.40	828.00±114.47
LRG600 ppm	1516.00±68.00	656.00±64.00^{c}	260.00±64.00	1008.00±72.00
SCS300 ppm	1400.00±160.00	520.00±8.00^{b}	108.00±24.00	1196.00±148.00
SCS600 ppm	1340.00±163.71	556.00±76.00^{bc}	188.00±22.27	1104.00±120.00
MIX300 ppm	1372.00±191.17	332.00±17.44^{a}	272.00±73.43	1024.00±209.04
MIX600 ppm	1420.00±100.00	376.00±8.00^{a}	304.00±52.00	920.00±260.00
P	NS	*	NS	NS

NS: *P>0.05,* ****** *P<0.05,* ^{a,b,c} *Means within the same column showing different superscripts are significantly different (P<0.05),* ***PRH:*** *papilla ruminis height,* ***PRW:*** *papilla ruminis width,* ***LPW:*** *lamina propria width,* ***TMW:*** *tunica muscularis width (Lactobacillus reuteri E81 [LRE]), Lactobacillus rhamnosus GG [LRG], yeast (Saccharomyces cerevisiae S81 [SCS])*

(Fig. 1-2a) and Mix 300 *(Fig.1- 4a)*. There was no significant difference between the A, B, C, and Mix groups for the rumen measurements. However, the papilla ruminis width (PRW) *(Table 7)* had significantly decreased in Groups Mix 300 *(Fig. 1-4b)* and Mix 600, and significantly increased in Group B 600 *(Fig. 1-2b)* with a moderate increase in the other groups *(Fig. 1-3b)*. Furthermore, it was ascertained that while only a few vesicular cells were observed in the rumen in the control group *(Fig. 1-1b)*, the number of these cells had significantly increased in all treatment groups, especially in the mix group *(Fig. 1-4b)*.

Discussion

The present study was aimed at determining the possible advantages that probiotic and yeast feed supplements may offer in increasing post-weaning life expectancy, fattening performance, and the marketability of animals, through the investigation of the effects of different doses (300 ppm and 600 ppm) of dietary probiotics *(L. reuteri* E81 and *L. rhamnosus* GG), yeast *(S. cerevisiae* S-81), and combined probiotic + yeast supplementation *(Table 2, Table 3)*. Some studies have reported that, when incorporated in lamb feed, probiotics increase feed intake, improve growth performance [22-24], support the improvement of rumen ecology, regulate digestion, and thereby, increase feed intake [25-29]. On the other hand, other studies suggest that the supplementation of ruminant rations with probiotics does not affect fattening performance [5,18,30,31]. In the present study, data analysis showed that, when compared to the control group, the best results for BW, DWG, and FCR were achieved in the groups that received 300 ppm and 600 ppm of dietary *L. reuteri* E81, and the results obtained in these two groups were found to be statistically significant (P<0.05). Nevertheless, no statistically significant difference was observed between the groups for feed intake and daily feed intake (FI) (P>0.05) *(Table 2, Table 3)*. These results are in agreement with the results of some research reports [22-24] but do not concur with the results of other reports [5,18,30,31]. Such differences in research data have been attributed to differences in the feed provided to the animals, the type and dose of the probiotics added to the ration, and the feeding strategies followed by farmers [5].

In ruminants, the rumen, as the site of fermentation and hydrophilic reactions, plays an important role in the utilization of nutrients through microbial digestion. Digested plant polymers (cellulose, lignin, and hemicellulose) provide basic energy compounds and play an important role in ruminant nutrition. For optimum fattening performance, the aim is to maintain a healthy and balanced microbial environment in the rumen [32,33]. Previous research has shown that visceral organ weights vary with weight gain and age [34]. While some research indicates that the visceral organ weights of lambs are not affected by dietary probiotic supplementation [35-39], other research suggests that probiotic supplements increase visceral organ weights [18]. In this study, combined dietary probiotic + yeast supplementation was observed not to have any impact on the skin, pluck (liver/heart/lungs), spleen and adrenal gland weights, and ruminal pH value *(Table 5)*. It has been reported that, when compared to the control group, lambs given dietary probiotics presented with improved height and width measurements of the rumen papilla, whereas no effect was observed on the height of the duodenal, jejunal and ileal villi [40]. In another study, probiotic supplementation positively affected the ruminal epithelium by reducing the thickness of the stratum corneum [41]. It has been stated that the addition of probiotics to feeds affects the histology and morphology of the ruminal papilla by increasing the amount of short-chain fatty acids in the rumen [41,42]. Duodenal values were measured under a microscope. The treatment groups (A, B, C, Mix) showed no significant difference from the control group for villus height, villus width, and crypt depth *(Table 6)*. However, the width of the tunica muscularis had decreased in the control group *(Fig. 1-1a)*

and increased in Groups A, B, C *(Fig. 1-3a),* and Mix. The highest level of increase was detected in Groups B 600 *(Fig. 1-2a)* and Mix 300 *(Fig. 1-4a)*. No significant difference was observed between Groups A, B, C, and Mix for the rumen measurements. However, the papilla ruminis width (PRW) *(Table 7)* had significantly decreased in Groups Mix 300 *(Fig. 1-4b)* and Mix 600 and significantly increased in Group B 600 *(Fig. 1-2b)* with a moderate increase in the other groups *(Fig. 1-3b)*. Furthermore, it was observed that while few vesicular cells were present in the rumen in the control group *(Fig. 1-1b),* the number of these cells had significantly increased in all of the treatment groups, especially in Group Mix *(Fig. 1-4b)*. The differences in research results are attributed to differences in the sheep breeds and types and doses of probiotics used in these studies.

Lamb meat is a high-quality, lean, easily digestible, and nutritious food product. Therefore, consumers who prefer lean meat tend to purchase lamb meat. As an integral component of meat, fat has a considerable effect on meat's sensory properties [43]. Meat colour is determined by the amount of myoglobin and hemoglobin and the level of lipid oxidation in muscle tissue [44]. Previous research on the effects of dietary probiotic supplements on meat quality have shown that probiotics increase the water catch capacity of meat, and thereby maintain the juiciness and increase the quality of meat [30,45,46]. Similarly, research carried out on lambs has also demonstrated an increase in the water catch capacity of lamb meat as a result of dietary probiotic supplementation [47]. Lower water holding capacity causes a lighter meat colour. The results of previous research on meat colour parameters vary, in that while it has been indicated that, in comparison to the control group, probiotic feed supplements decrease the L* value, increase the a* value, and do not affect the b* value [43], it has also been suggested that dietary probiotic supplementation does not affect meat colour parameters [26,48]. The results obtained in the present study demonstrated that, when compared to the control group, the L* value was higher in the treatment groups, excluding Groups LRE-600, Mix 600, and SCS-300, but no effect was observed on the a* and b* colour parameters. pH is an important parameter for meat quality and is affected by glycolysis level and lactic acid formation under pre- and post-slaughter conditions [17]. Volatile fatty acid formation in the rumen can also affect glycogen deposition and thereby, may ultimately affect the pH value [49]. In the present study, probiotic feed supplementation showed a significant effect on meat pH value ($P<0.01$). As shown in *Table 4,* the lowest pH value was determined to be 5.87±0.09 in Group LRE- 600. A pH value above 6.0 may cause some quality problems [50]. While the pH value was above 6.0 in the control group, the highest pH value was determined in the Group Mix-600. Probiotic supplementation is used to regulate the intestinal flora, reduce the stress of animals, balance the ruminal pH [51], improve rumen fermentation, and increase feed intake [52]. In this study, although the ruminal pH did not change with dietary probiotic supplementation, Group LRE-600 presented with the highest level of weight gain and the lowest meat pH value. Probiotic supplementation may affect glycogen accumulation. The investigation of fat tissue colour parameters in the present study revealed an increase in the L* and a* values in Groups SCS-300, SCS-600, and MIX-300, a decrease in the same parameters in Groups LRE-300, LRE-600, and MIX-600, and no effect on the b* value in any of the treatment groups *(Table 4)*. While these results agree with some literature [43], they contradict the results of other reports [26,48]. These differences in research results are attributed meat colour differences caused by growth and fat content, which eventually bring about differences in meat colour parameters.

In conclusion, this study on the effects of the addition of different doses (300 ppm and 600 ppm) of probiotics *(L. reuteri* E81 [LRE], *L. rhamnosus* GG [LRG]), yeast *(S. cerevisiae* S81 [SCS]), and probiotic + yeast combinations to lamb rations has demonstrated the best fattening performance results (BW, DWG, and FCR) to have been achieved in the treatment group that received 600 ppm of *L. reuteri*, with no effect of the tested supplements on duodenum and rumen histology and visceral organ weights. Also, the meat pH value had improved in the group given 600 ppm of *L. reuteri* E81. Today, the increasing global population has increased the demand for animal products. Thus, the livestock industry is striving to lower production costs, reduce feed consumption, improve fattening performance, and produce high-quality products that meet consumer preferences. In this context, it is apparent that further research should be carried out on the potential of dietary probiotic and yeast supplementation in ruminants.

Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Author Contributions

Conceptualization: TE, BB, AV, DE; Data curation: TE, BB; Formal analysis: KM, TE; Investigation: TE, BB, DE, KA, GM; Methodology: TE, BB, AV, DE, KA, KM, GM; Duodenum and Rumen Histology: TS; Project administration: TE, BB, AV, DE, KA, KM, GM; Writing-original draft: TE, BB, AV, DE, KA, KM, GM.

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