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REGULAR ARTICLES

The impacts of novel lyophilized milk fermented with potential probiotic *Lactobacillus* sp. on the physiological indicators, meat characteristics, blood parameters, and gene expression in weaned rabbits

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Abstract
Rabbits have a delicate digestive system that makes them prone to gastrointestinal diseases. The goal of this research was to study the quality of life of weaned rabbits fed on novel lyophilized cow's skim milk fermented with potential probiotic *Lactobacillus* sp. After 12 weeks of dietary treatment, significant changes were observed in the physical characteristics of the treated rabbits (body weight, relative weight of carcass cuts and internal organs such as, hind legs, saddle, fore legs and liver weights) compared to the control group. Treated rabbits showed higher levels of superoxide dismutase (SOD), while malondialdehyde (MDA) levels were significantly reduced. All treatments led to significant reductions in blood biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides, High-density lipoprotein (HDL), and low-density lipoprotein (LDL), compared to the control group. In conclusion, current dietary supplemented *Lactobacillus* probiotics improved meat quality; however, their dietary combination will produce brighter red meat with superior keeping quality when compared to the control.

Keywords Probiotics · Novel lyophilized fermented milk · *Lactobacillus* · Rabbits · Animal health

Introduction

Small herbivores and rabbits are bred to produce meat and fur. However, because their digestive system is very weak, rabbits frequently develop gastrointestinal diseases (Zhu et al. 2015). As a result, more and more antibiotics are being added to their feed because it has been discovered that they can help animals grow and keep diseases at bay. Although this brings the disease under control quickly, antibiotics ultimately have negative influences on the production and the environment (Zou et al. 2016). As a result, antibiotic alternatives have received more attention from researchers. It has been discovered that probiotics, one of the antibiotic alternatives, have numerous health benefits for both people and animals. Probiotics were defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Joint 2002). A probiotic is a type of food supplement that contains live microorganisms that can have an advantageous effect on the host organism by helping to regulate the bacterial balance in the gut. (Fuller 1989). *Lactobacillus*, which are well-known friendly bacteria, can be found everywhere and are associated with habitats that contain high amounts of sugar or protein, like plants or spoiled food. Furthermore, some species of *Lactobacillus* can be found naturally in the digestive and urinary systems of both humans and animals (Aukrust et al. 1995). *Lactobacillus* is generally recognized as safe (GRAS) organism due to its non-threatening nature and long-standing presence in food manufacturing. Moreover, *Lactobacillus* typically has advantageous effects for the host (Tuohy et al. 2003) therefore, these microorganisms are commonly added to a variety of products intended to be consumed by either people or animals as probiotic supplements (Seegers 2002). *Lactobacillus* species are of great significance to the

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industrial sector, as their utilization in the production of fermented food products (including dairy, vegetable, and meat products), food bio preservation, and probiotic applications (Stefanovic et al. 2017). Because LAB are very selective when it comes to the numerous amino acids they require. Through their proteolytic system and their cell membrane proteinases, lactobacilli can degrade the proteins in their media. This action causes the release of peptides and free amino acids in the fermentation environment (Savijoki et al. 2006). The hydrolysis of milk proteins by Lactobacilli leads to the release of bioactive peptides (Schanbacher et al. 1998; Elkhtab et al. 2017). These bioactive peptides can interact with several receptors in the body, including those that control immunity, oxidative stress, and blood pressure. Numerous health advantages, including better digestion, and a lower risk of diseases, might result from these bioactive peptides (Haque et al. 2008; Mohanty et al. 2016). This study examined how physiological performance, meat quality, blood parameters, and gene expression are affected in weaned rabbits that were given novel lyophilized cow's skim milk fermented with potential probiotic *Lactobacillus* sp.

Materials and methods

Cultures

The Dairy Science Department's culture collection was utilized to get *Lactobacillus plantarum* Bu-Eg5 and *Lactobacillus rhamnosus* Bu-Eg6. According to (Elkhtab and Ismail 2021) these strains were isolated from traditional Egyptian dairy products, and they showed the typical lactic acid characteristics which are positive to the Gram stain, rods, not spore forming. and can coagulate milk. These strains were identified amplified ribosomal Deoxyribonucleic acid restriction analysis (ARDRA) and Deoxyribonucleic acid (DNA) sequencing techniques. Also, they showed in vitro probiotic behavior. *Lactobacillus plantarum* Bu-Eg5 showed antibacterial activity (using the agar diffusion test) against *Staphylococcus aureus*, *Salmonella typhimurium* and *Listeria monocytogenes* and the recorded inhibition zone (mm) were 8, 16 and 7 respectively, while *Lactobacillus rhamnosus* Bu-Eg6 recorded 10 mm inhibition zone against both *Staphylococcus aureus*, *Salmonella typhimurium*. These two strains were examined for the acid medium tolerance (pH 2 and 3 for 90 min using the pour-plating technique) and they showed little reduction in the log $_{10}$ CFU ml^{-1}. Also, they showed very good tolerance to 0.5% sodium tauroglycholate bile salt during three hours of incubation at 37◦C on MRS agar using pour-plating technique.

The *Lactobacillus* strains were activated three times on De Man, Rogosa, and Sharpe (MRS broth) medium (HIMEDIA, Pvt. Ltd., India), the strains were transferred to sterilized cow's skim milk at a rate of 1% and incubated at 37 °C until coagulation.

Raw cow's milk

Fresh cow's milk was obtained from the Faculty of Agriculture's herd, Benha University and then separated for the manufacturing of novel lyophilized cow's skim milk.

Manufacturing of the novel fermented cow's skim milk

The novel fermented cow's skim milk was prepared as described by (Hameed et al. 2021) with modification. Briefly, the process of separating cream from cow's milk was carried out at the Moshtohor Faculty of Agriculture's dairy processing facility at Benha University. The bulk of skim milk was split into three sections, sealed into sterile glass containers (1 L each), then heated in an autoclave at 121 °C for 5 min. After cooling to room temperature, the sections were labeled as follows: the first section was inoculated with 1% (w/v) of *Lb. plantarum* Bu-Eg5 (T1), the second was inoculated with 1% (w/v) of *Lb. rhamnosus* Bu-Eg6 (T2), and the third was inoculated with a 1:1 mixture of *Lb. plantarum* Bu-Eg5 and *Lb. rhamnosus* Bu-Eg6 (T3). The inoculation was done in a sterile environment, and the inoculated cow's skim milk was then incubated at 37 °C until it coagulated (~24 h). The pH of T1, T2 and T3 reached 4.11, 4.42 and 4.32 respectively after 24 h of incubation (Hameed et al. 2021).

Lyophilization of the novel fermented cow's skim milk

The process of lyophilization of the novel fermented cow's skim milk, which is detailed in (Hameed et al. 2021a), was done with a VaCo 5-D freeze dryer (manufactured by Zirbus Technology in Germany; serial number COM98754) at a temperature of -40 °C, under vacuum with a minimum pressure of 0.011 kPa (see Figs. 1 and 2).

The chemical composition and the microbiological quality of the novel fermented lyophilized cow's skim milk are presented in Hameed et al. (2021).

Ethics approval and consent to participate

The experiments on rabbits were conducted at the Rabbit Research Unit of the Faculty of Agriculture, Benha University after getting the approval by the Scientific

Fig. 1 Laboratory lyophilizer equipment (model: VaCo 5-D, S/N: COM98754, Zirbus Technology, Germany)

Ethics Committee of the Animal Production Department in November of 2020.

Rabbits experimental design

The experiment involved 60 APRI male rabbits at four weeks of age with an approximate weight of 450 ± 5 g. The rabbits were divided into four groups of fifteen rabbits each. Each group was housed in five cages of 45 × 55 × 30 cm in size (three rabbits per cage). The groups of rabbits were Control fed with only the standard isocaloric and isonitrogenous diet given; T1, given the standard diet plus 1000 mg/kg of the novel lyophilized cow's skim milk fermented with *Lb. plantarum* Bu-Eg5 on the top; T2, given the standard diet plus 1000 mg/kg of the novel lyophilized cow's skim milk fermented with *Lb. rhamnosus* Bu-Eg6 on the top; and T3, given the standard diet plus 1000 mg/kg of the novel lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* Bu-Eg5 and *Lb. rhamnosus* Bu-Eg6 (1:1) on the top. All rabbits were given the same nutrient requirements as outlined by the Nutritional Research Council Nutrient Requirements of Rabbits (Smith et al. 1966), as present in Table 1.

Evaluation of rabbits growth indicators and carcass characteristics

The body weight (BW) was recorded for each individual rabbit at the start and end of the experiment using a digital balance. The average daily gain (ADG) was calculated by dividing the gained body weight (the final body weight of the animal – the start body weight of the animal) by the number of the experimental days. At the end of the experiment, five animals of each group were chosen randomly for additional testing and slaughtered to determine the carcass characteristics and the internal organs weight. As a percentage of the total body weight, the carcass's weights including that of the saddle, hind legs, thoracic neck, lung, liver, spleen and kidney were recorded.

Fig. 2 lyophilized novel fermented cow's skim milk

Table 1 Components and calculated analyses (g/kg) of the experimental standard nutrient requirements (Atallah et al. 2021)

Ingredients	Content
Alfalfa hay	350
Yellow corn	200
Soybean meal	96
Wheat bran	300
Corn Stover	30
Di-calcium phosphate	12.5
L-Lysine HCl	2.1
DL-Methionine	2
Sodium chloride	5
Vitamin/mineral premix[a]	1.5
Total	1000.0
Calculated analysis (g/ kg on dry matter basis)	
Digestible energy (MJ/kg)	11.6
Crude protein	179
Crude fiber	125
Crude fat	32.0
Ca	10.9
Available P	5.9
Methionine	4.2
Lysine	9.0

*For every 1 kg of premix, it is comprised of 20,000 IU of vitamin A, 15,000 IU of vitamin D3, 8.33 g of vitamin E, 0.33 g of vitamin K, 0.33 g of vitamin B1, 1.0 g of vitamin B2, 0.33 g of vitamin B6, 8.33 g of vitamin B5, 1.7 mg of vitamin B12, 3.33 g of pantothenic acid, 33 mg of biotin, 0.83 g of folic acid, and 200 g of choline chloride

Rabbits meat quality parameters analysis

Current instrumental quality parameters such as pH after 24 h, drip loss after 24 and 48 h., cooking loss, Warner-Bratzler Shear Force (WBSF), and color were measured on *Longissimus lumborum* muscles collected from compared rabbit groups (Imbabi et al. 2021a, b). Drip loss (24 and 48 h) as follows: meat samples are excised from the carcass and promptly weighed. The sample weight of approximately 80 g. Each sample is placed in netting and suspended inside an inflated bag—ensuring it remains free of contact with the bag's surface. The samples are stored under chilled conditions (typically 1–5 °C). and cooking losses were calculated using the protocols of Honikel (1998); Honikel and Hamm (1994), with minor modifications described in our previously published articles (Imbabi et al. 2021a, b). The L*, a*, and b* values of the instrumental color were determined using a Chroma Meter CR400 (Konica Minolta Sensing, Japan). The mean of five measurements was computed. These parameters were further used to calculate the meat sample's saturation (chroma) and hue (hue angle). Higher chroma levels denote more saturation of the sample's primary hue, according to the chroma equation C = (a*2 + b*2)1/2. Higher numbers indicate less red meat even if the hue angle (or colour intensity) may be determined using the equation HA = [arctangent (b*/a*)] (AMSA 2012). Warner-Bratzler Shear Force (WBSF) was done on the rabbit hindquarter guided by (Stock and Board 1995), shelf-life parameters including pH and Malondialdehyde (MDA) were measured (Osman et al. 2021). Rabbit hindquarter muscle was trimmed from the bone and minced. The minced meat was then placed in a sterile falcon tube. To determine pH values, three tubes were indicated for each checkpoint (4 checkpoints; days 1, 3, 6, and 9) and refrigerated at 5 °C (Osman et al. 2021). pH was determined by a pH meter (J3510, Jenway, UK), at least three measurements were taken in three different sample regions.

Antioxidant variables assay

Phosphate buffer saline (PBS) with heparin (0.16 mg/mL) solution at pH 7.4 was used to wash the liver tissues of rabbits for removing any clots of blood. The washed liver tissues were diluted and homogenized by PBS at a rate of (1 g tissues:5 ml PBS) then centrifuged at 4000 rpm at 4 °C for 15 min. The collected supernatant was stored at -20 °C for further biochemical analysis of superoxide dismutase (SOD) (Nishikimi et al. 1972), catalase (CAT) (Aebi 1984), malondialdehyde (MDA) (Ohkawa et al. 1979) and total antioxidant capacity (TAC) (Koracevic et al. 2001).

Rabbits blood indicators

Blood was collected from rabbits and divided into two parts. The first part was collected with 10% ethylene diamine tetraacetate (EDTA) to prevent clotting for measuring haemoglobin (Hgb), hematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCH), and white blood cell count (WBC) and red blood cell count (RBC) by using the methods of (Jain 1986). The second part was collected and left to clot at 4 °C then centrifuged for 10 min at 3000 rpm to obtain the blood serum. The serum was stored at -20 °C until further determinations. (Morgenstern et al. 1966) methods were used to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT). High-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides and total cholesterol were determined spectrophotometrically by using specific kits produced by Pasteur Laboratories (Egyptian American Co. for Laboratory Services, Egypt).

Separation of RNA and synthesis of cDNA

The liver tissues were collected from five rabbits per treatment, then immediately frozen using liquid nitrogen and stored at -80 °C. The expression of seven target genes for the growth hormone receptor (GHR), fibroblast growth factor-1 (FGF-1), and transforming growth factor beta-1

Table 2 Primers are used for quantitative real-time PCR analysis of genes expressions

Gene	Primers sequences (5'→3')	Product size (bp)
UXT	F: GCGGGACTTGCGAAAGGT R: AGCTTCCTGGAGTCGTTCAATG	100
IGF-1	F: AACAAGCCCACAGGATACGG R: TCCAGCCTCCTCAGATCACA	98
GHR	F: ACGTGTCGAGCCAAGCTTTA R: GTCTTCTGCTGTCCCAGACC	91
FGF1	F: GTGTTTGTTCCTGGAACGGC R: CGTTTTTCTTCAGCCCCACG	98
TGFB1	F: TGTCCACCTGCAAGACCATC R: CCGCAGTTTGGACAGGATCT	86

IGF-1: insulin-like growth factor 1; *GHR*: growth hormone receptor; *FGF1*: fibroblast growth factor 1; *TGFB1*: transforming growth factor beta-1

Table 3 The impacts of lyophilized cow's skim milk that has been fermented with *Lactobacillus* sp. on the growth of recently weaned rabbits

Growth Parameters	*Lactobacillus* supplementation[1]				SEM	*P*-value
	Control	T1	T2	T3		
BW 4 (g)	455	455	456	455	6.77	0.98
BW 8 (g)	850[c]	850[c]	1000[b]	1200[a]	50.00	< 0.0001
BW 12 (g)	1350[b]	1366[b]	1566[ab]	1680[a]	129.29	0.037
ADG 4–8 (g/d)	14.10[c]	14.10[c]	19.40[b]	26.60[a]	1.75	< 0.0001
ADG 8–12 (g/d)	17.86	18.45	20.24	17.40	3.51	0.74
ADG 4–12 (g/d)	15.98[b]	16.28[b]	19.82[ab]	21.82[a]	2.26	0.034

[1]There were four groups: a control group that only consumed the basal diet, T1, a group that ate the basal diet plus 1000 mg/kg of lyophilized cow's skim milk fermented with *Lb. plantarum*, T2, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with *Lb. rhamnosus*, and T3, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus*. All data are presented as the mean ± SEM, with a, b, and c indicating significant differences between means ($P \leq 0.05$)

(TGFB-1), as well as insulin-like growth factor-1 (IGF-1), was then assessed using qRT-PCR in the liver and spleen tissues by utilizing the qRT-PCR reagents from TransGen Biotech, Beijing, China, and the ABI 7500 Realtime Detection System from Applied Biosystems, Foster City, California, USA. Each PCR reaction contained 8 µL of ddH2O, 10 µL of 2x TransStart Top/Tip Green Qpce, 0.4 µL of each primer (10 pM), 0.4 µL of a passive reference dye diluted 50 times, and 0.8 µL of cDNA (100 ng). The denaturation and amplification cycle consisted of an initial denaturing at 95 °C for 30 s followed by 40 amplification cycles of 15 s at 95 °C, 30 s at 57 °C, and 85 s at 72 °C, and then thermal denaturing, to generate melting curves. Details about the gene-targeting primers are shown in Table 2.

Statistical analyses of the obtained data

To discriminate between the dietary treatments, one-way ANOVA and Tukey's multiple comparison tests (Tukey, 1953) were performed using SPSS (version 20; IBM, Chicago, IL, USA). The *p*-value of ≤ 0.05 was set for determining the significance between values.

Results

Rabbits' growth indicators and carcass characteristics

The effects of a diet based on lyophilized cow's skim milk fermented with either *Lb. plantarum* Bu-Eg5, *Lb. rhamnosus* Bu-Eg6 or their combination on the growth indicators of rabbits are reported in Table 3.

At the start of the experiment, there were no substantial differences between the groups of 4-week-old rabbits in terms of body weight. However, the results revealed that the body weight of the T2 and T3 groups after 8 and 12 weeks was significantly greater than that of the control and T1. The average daily gains (ADG) from weeks 4–8 and 4–12 were seen in T2 and T3. Notably, the combination of *Lb. plantarum* and *Lb. rhamnosus* was more effective than the control and other treatments ($p \leq 0.05$).

Table 4 presents the impacts of probiotics on the relative body weight of rabbits regarding their carcass characteristics. Supplementation of 1000 mg of lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus* had significant impacts on the relative weights of carcass cuts and internal organs ($p \leq 0.05$). The group supplemented with *Lb. plantarum* and *Lb. rhamnosus* showed the highest final body weight at 12 weeks and highest carcass relative weight ($p \leq 0.05$) compared to the control. Furthermore, the combination treatment displayed

Table 4 The impacts of lyophilized cow's skim milk fermented with *Lactobacillus* sp. on the proportions of different parts of the body and internal organs of newly weaned rabbits

Growth Parameters	*Lactobacillus* supplementation[1]				SEM	*P*-value
	Control	T1	T2	T3		
Carcass cuts						
Live body weight(g)	1350[b]	1366[b]	1566[ab]	1680[a]	129.29	0.037
Carcass (%)	41.88	42.89	42.96	42.15	2.39	0.925
Hind legs rate (%)	15.48[b]	15.88[b]	15.96[b]	18.12[a]	0.97	0.039
Saddle rate (%)	9.07[b]	9.69[b]	9.88[b]	12.13[a]	0.90	0.015
Forelegs rate (%)	5.26[b]	6.18[ab]	5.86[ab]	7.83[a]	0.63	0.04
Thoracical neck rate (%)	8.81	8.77	8.30	8.45	0.37	0.35
Body organs (%)						
Liver (%)	3.11[b]	3.44[ab]	3.41[ab]	4.27[a]	0.49	0.08
Kidney (%)	0.75	0.69	0.69	0.67	0.071	0.55
Spleen (%)	0.28	0.28	0.28	0.27	0.02	0.77
Lung (%)	0.72	0.69	0.68	0.61	0.10	0.61
Heart (%)	0.07	0.06	0.06	0.06	0.007	0.59

[1]There were four groups: a control group that only consumed the basal diet, T1, a group that ate the basal diet plus 1000 mg/kg of lyophilized cow's skim milk fermented with *Lb. plantarum*, T2, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with *Lb. rhamnosus*, and T3, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus*. All data are presented as the mean ± SEM, with a, b, and c indicating significant differences between means ($P \leq 0.05$)

Table 5 The impacts of novel lyophilized cow's skim milk fermented with *Lactobacillus* sp. on the antioxidant enzymes of newly weaned rabbits

Antioxidant Variables	*Lactobacillus* (LB) supplementation[1]				SEM	*P*-value
	Control	T1	T2	T3		
SOD (U/mg)	153[d]	204[c]	208[b]	241[a]	3.67	< 0.0001
CAT (U/mg)	3.46[c]	3.87[b]	3.91[b]	4,28[a]	0.02	< 0.0001
MDA (nM/g)	243[a]	217[b]	214[bc]	208[c]	2.52	< 0.0001

[1]There were four groups: a control group that only consumed the basal diet, T1, a group that ate the basal diet plus 1000 mg/kg of lyophilized cow's skim milk fermented with *Lb. plantarum*, T2, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with *Lb. rhamnosus*, and T3, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus*. All data are presented as the mean ± SEM, with a, b, and c indicating significant differences between means ($P \leq 0.05$)

a higher rate in hind legs, saddle, and fore legs ($p \leq 0.05$) than the controls. The liver weight in all treatments was also higher than in the controls ($p \leq 0.05$).

Antioxidant enzymes activity

The indicators of antioxidants of rabbits fed on a basal diet supplemented with novel lyophilized cow's skim milk fermented with *Lb. plantarum* Bu-Eg5, *Lb. rhamnosus* Bu-Eg6, and their combination are presented in Table 5. Treatment-treated rabbits had higher concentrations of SOD and CAT than the control group. while MDA concentrations were different from those in SOD and CAT.

Rabbits blood indicators

The blood biochemical indicators of rabbits fed on a basal diet supplemented with novel lyophilized cow's skim milk fermented with *Lb. plantarum* Bu-Eg5, *Lb. rhamnosus* Bu-Eg6, and their combination are presented in Table 6. The levels of blood biochemical indicators (ALT, AST, total cholesterol, triglycerides, HDL, and LDL) were significantly reduced by *Lb. plantarum* Bu-Eg5, *Lb. rhamnosus* Bu-Eg6, and their combination compared to control. The changes in creatinine were negligible and not significantly different from the control.

The effect of novel lyophilized cow's skim milk fermented with *Lb. plantarum*, *Lb. rhamnosus*, and their combination on haematological parameters of weaned rabbits are presented in Table 7. The results showed that supplements of 1000 mg of lyophilized cow's skim milk fermented with *Lb. plantarum*, *Lb. rhamnosus*, and their combination caused no significant change in any of the assessed haematological parameters of the rabbits compared with control. However, the levels of RBCs and haemoglobin in the combination of (*Lb. rhamnosus* and *Lb. plantarum*) rabbits were higher than in the control and other treatment groups ($p \leq 0.05$). In addition, HCT and PLT were numerically high in rabbits supplied with *Lb. rhamnosus* and the combination compared to those supplied with *Lb. plantarum* and the control ($p \leq 0.05$).

Table 6 The impacts of novel freeze-dried cow's skim milk fermented with *Lactobacillus* sp. on the biochemical parameters of weaned rabbits

Blood Biochemical	*Lactobacillus* supplementation[1]				SEM	*P*-value
	Control	T1	T2	T3		
AST (U/L)	58.39^{a}	46.16^{ab}	37.20^{b}	33.53^{b}	4.42	0.016
ALT (U/L)	70.07	56.22	65.15	56.66	6.64	0.427
Total Cholesterol (mg/dl)	69.75^{a}	60.98^{ab}	56.72^{b}	52.66^{b}	2.70	0.011
Triglyceride (mg/dl)	89.35^{a}	76.64^{a}	57.07^{b}	50.00^{b}	4.52	0.001
HDL (mg/dl)	30.32^{c}	22.97^{b}	33.50^{b}	37.33^{a}	0.51	< 0.0001
LDL (mg/dl)	45.76^{a}	37.03^{ab}	35.16^{b}	26.39^{b}	0.43	< 0.0001
Creatinine (mg/dl)	1.16	1.23	1.24	1.16	0.10	0.91

[1]There were four groups: a control group that only consumed the basal diet, T1, a group that ate the basal diet plus 1000 mg/kg of lyophilized cow's skim milk fermented with *Lb. plantarum*, T2, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with *Lb. rhamnosus*, and T3, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus*. All data are presented as the mean ± SEM, with a, b, and c indicating significant differences between means ($P \leq 0.05$)

Table 7 The impacts of novel lyophilized cow's skim milk fermented with *Lactobacillus* sp. on hematological parameters of weaned rrabbits

Hematological parameters	*Lactobacillus* supplementation[1]				SEM	*P*-value
	Control	T1	T2	T3		
Hgb (g/dl)	10.30^{c}	11.50^{b}	10.88^{bc}	12.33^{a}	0.23	
RBcs (10^6/cmm)	4.77^{c}	5.42^{b}	4.94^{c}	6.53^{a}	0.11	< 0.0001
HCT (vol%)	32.36^{ab}	35.76^{a}	32.90^{ab}	29.73^{b}	1.09	0.001
MCV (fl.)	68.76	65.96	66.70	63.43	1.87	0.028
MCH (pg)	21.83	21.26	22.03	21.26	0.61	0.317
MCHC (%)	31.83	32.20	33.00	33.63	0.83	0.46
PLT (10^3/cmm)	465.66^{ab}	537.00^{a}	423.00^{b}	471.00^{ab}	21.99	0.037
WBCs (10^3/cmm)	5.78	5.90	5.86	5.85	0.86	0.32
Lym %	43.06	53.93	70.53	76.26	11.92	0.25
Mid %	7.7	5.9	4.26	3.26	1.84	0.40
Gran%	49.23	49.16	25.20	20.46	10.16	0.24

[1]There were four groups: a control group that only consumed the basal diet, T1, a group that ate the basal diet plus 1000 mg/kg of lyophilized cow's skim milk fermented with *Lb. plantarum*, T2, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with *Lb. rhamnosus*, and T3, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus*. All data are presented as the mean ± SEM, with a, b, and c indicating significant differences between means ($P \leq 0.05$)

Rabbits' meat characteristics

The dietary influence of novel lyophilized cow's skim milk fermented with *Lactobacillus* sp. including *Lb. plantarum*, *Lb. rhamnosus*, and their combination on rabbit meat quality and shelf life is shown in Table 8; Figs. 3A & B.

In comparison to the control group, the only parameters that showed significant changes after dietary probiotic supplementation were pH (24 h), WBSF, and keeping quality ($P \leq 0.05$). *Longissimus lumborum* meat from rabbits fed a diet containing *Lb. rhamnosus* had the lowest pH (24 h), while rabbits fed a combination of *Lb. plantarum* and *Lb. rhamnosus* had the lowest drip loss (24 h) and the highest WBSF values ($P \leq 0.05$). Furthermore, at most incubation points, most rabbit groups served with *Lactobacillus* probiotic had lower malondialdehyde (MDA) levels in their meat than the control ($P > 0.05$). Meat from rabbits fed *Lb. rhamnosus*, followed by meat from rabbits fed on the combination of *Lb. plantarum* and *Lb. rhamnosus*, always has significantly lower pH values than control and *Lb. plantarum* supplemented rabbits during the keeping quality test ($p \leq 0.05$). While meat from rabbits fed dietary *Lb. plantarum* had significantly lower MDA values than control meat on the first, third, and sixth days of incubation ($p \leq 0.05$). Other estimated meat quality traits, on the other hand, did not differ between rabbit groups.

The expression of genes that influence growth rate

The combination of *Lb. rhamnosus* and *Lb. plantarum* treatment showed a considerable rise in mRNA expression in the liver as demonstrated in Fig. 4 when compared with the control. The greatest liver mRNA expressions for IGF-1, FGF1, TGFB1, and GHR were detected in the LBRP group.

Discussion

This research focused on assessing the impacts of a new form of lyophilized cow's skim milk that was fermented with *Lb. plantarum* Bu-Eg5 and *Lb. rhamnosus* Bu-Eg6 on the physiological condition, meat quality, blood characteristics, and

Table 8 The impacts of novel lyophilized cow's skim milk fermented with *Lactobacillus* sp. on meat quality of weaned rabbits

Meat quality parameters	*Lactobacillus* supplementation[1]				SEM	*P*-value[2]
	Control	T1	T2	T3		
pH (24 h)	5.71 [a]	5.74 [a]	5.64 [b]	5.71 [a]	0.014	0.001
Drip loss (24 h)	5.32 [a]	4.51 [ab]	3.43 [ab]	2.17 [b]	0.643	0.057
Drip loss (48 h)	6.17	5.49	5.00	3.60	0.970	0.406
Cooking loss	7.42	8.13	8.54	8.62	0.541	0.479
Lightness (L*)	53.98	53.72	56.84	53.63	0.851	0.160
Redness (a*)	10.93	10.51	8.26	11.23	0.781	0.160
Yellowness (b*)	5.51	5.93	6.29	6.27	0.630	0.834
Color Chroma (c)	12.32	12.13	10.41	12.87	0.723	0.296
Hue angle (h˚)	26.69	29.44	38.24	29.20	3.099	0.186
WBSF[3]	3.59 [b]	3.68 [b]	4.16 [ab]	4.69 [a]	0.275	0.047
Keeping quality test						
MDA Day1 (nM/g)	31.34	25.67	30.88	31.36	1.605	0.131
MDADDay3	48.27 [ab]	27.54 [b]	49.64 [a]	39.09 [ab]	4.277	0.041
MDADDay6	53.26 [a]	31.50 [b]	49.67 [a]	43.10 [ab]	3.802	0.026
MDADDay9	63.74	43.93	52.28	49.27	6.858	0.301

[1]There were four groups: a control group that only consumed the basal diet, T1, a group that ate the basal diet plus 1000 mg/kg of lyophilized cow's skim milk fermented with *Lb. plantarum*, T2, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with *Lb. rhamnosus*, and T3, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus*. All data are presented as the mean ± SEM, with a, b, and c indicating significant differences between means ($P \leq 0.05$)

gene expression of weaned rabbits. Recently, probiotic combinations with various components have become popular for raising young rabbits, and these include active elements that are known to have an impact on the body's systems. In addition, unlike antibiotics, probiotic treatments do not damage the beneficial bacteria found in the intestines, instead they replace the harmful microorganisms which are the cause of many stomach issues. This research sought to determine the positive consequences of supplementing diet with *Lb. plantarum*, *Lb. rhamnosus* and their combination. The growth of rabbits was boosted due to dietary supplementation with *Lb. rhamnosus* and *Lb. plantarum*, as well as their combination likely due to increased nutrient digestibility and absorption, as well as a favorable anabolic state, improved pathogen resistance, lower serum cholesterol, higher serum protein, and augmented rabbit growth (Dawood et al. 2016; Saputri et al. 2018; Fijan et al. 2019; Pogány Simonová et al. 2020). Previous studies (Matusevičius et al. 2006; Kritas et al. 2008; Simonová et al. 2008; Bhatt et al. 2017; Cunha et al. 2017) have documented the impact of probiotics on the growth of rabbits, including proprietary probiotic formulations and new beneficial microorganisms, which generally demonstrate an increase in body weight. (Ayyat et al. 2018; Abd El-Aziz et al. 2021) further discovered that supplementation of diets with yeast or probiotics had an effect on carcass characteristics in juvenile rabbits. The growth rate of New Zealand White rabbits increased when they were fed diets with *Saccharomyces cerevisiae*, according to (Shehata et al. 2012). However, the results of (Khanna et al. 2014) diverge from these, as they observed that the average weight of the fore and hind portions of the rabbit carcass was significantly higher in the yeast-treated groups compared to the control groups.

Typically, biochemical parameters are measured as part of diagnostic procedures, but they can also be used to monitor an animal's health. In the present study, blood biochemical parameters recorded positive values affected by *Lb. plantarum* Bu-Eg5 and *Lb. rhamnosus* Bu-Eg6, and all parameters fell within the physiological ranges. The treatment with this combination of probiotics might have a beneficial effect on bringing down ALT and AST levels. Overall, it appeared that the probiotics used in this study improved kidney and liver function. Haematological parameters seemed to get better with the probiotics used in this study. This difference may have to do with how each probiotic strain works and which microbiota groups it targets. One of the most important things is that Probiotics can alter the immune system of the host, primarily through the modification of white blood cell numbers. For instance, a study involving rabbits showed that the administration of *Lb. rhamnosus* GG led to increased white blood cell levels when compared to doses of *Lb. Plantarum* and *Lb. reuteri.* These higher values can indicate a lower risk of developing infections and other illnesses (Alziadi and Gatea 2018).

To our knowledge, no studies have attempted to accurately estimate the changes in rabbit meat quality caused by dietary supplementation with *Lb. rhamnosus*, *Lb. plantarum* or their combination of the two. However, previous research focused on determining the impact of the direct application of these probiotics, *Lb. plantarum* on the physicochemical and microbiological quality indicators of fermented meat products such as goat meat Dendeng (Radiati et al. 2020)

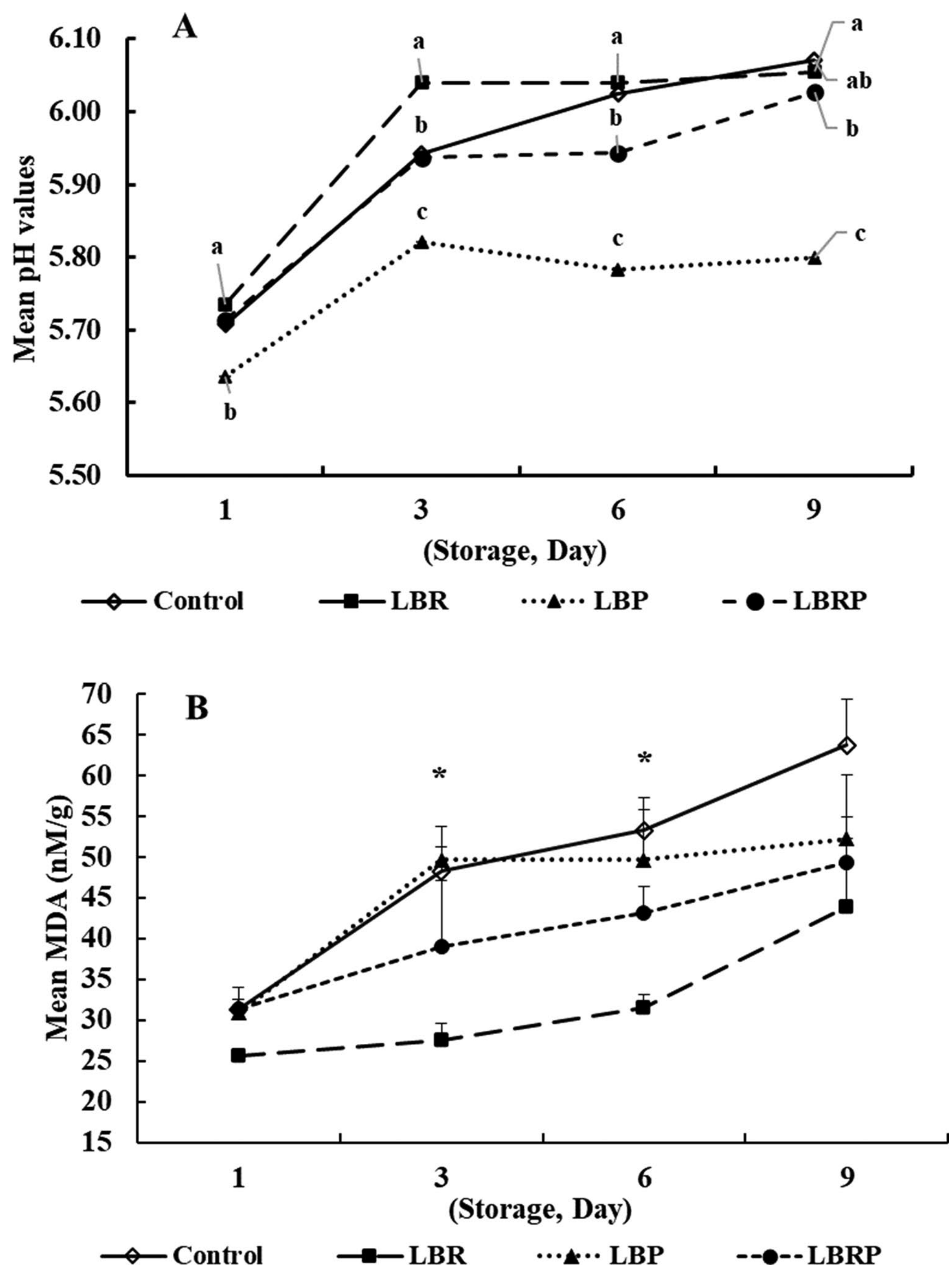

Fig. 3 The impact of probiotic supplementation, including *Lb. rhamnosus* (LBR), *Lb. plantarum* (LBP), and their combination (LBRP), on rabbit meat pH (**A**) and oxidative stability (**B**) (MDA, nM/g) during a nine-day shelf-life test. Different small letter (a, b, and c) at the same incubation point indicates significant difference ($P \leq 0.05$)

and Italian sausage (Campaniello et al. 2020). Nonetheless, meat quality changes caused by current probiotics, *Lactobacillus rhamnosus* (Chen et al. 2018), and *Lb. plantarum* (Radzi 2015), or similar probiotic microorganisms such as *Lb. acidophilus* (Dev et al. 2020), *Bacillus coagulans* and *subtilis* (Zhou et al. 2010; Park and Kim 2014), yeast (Zhang et al. 2005), and synbiotic (Zhang et al. 2012) have primarily been studied in broiler (Suo et al. 2012) and sheep (Tekce et al. 2021). The current study found that supplemented *Lactobacillus* probiotics did not affect most assessed meat quality traits, except for pH (24 h), WBSF, and the keeping quality test. Previously, dietary *Lb. rhamnosus* supplementation of broiler meat showed no effect on the pH and shearing force of breast meat (Chen et al. 2018), which is consistent with the current study findings. Similar results were obtained when *Lb. rhamnosus* was added to a lamb diet, resulting in no changes in instrumental meat color and a slight decrease in meat pH ($P > 0.05$) (Tekce et al. 2021). Contrary to current results, feeding *Lb. plantarum* to pigs did not reduce the pH of pork meat (Suo et al. 2012). However, similar to the current findings, no significant changes in instrumental color values were observed, and drip loss (24 and 48 h) values of pork meat showed a decreasing trend ($P > 0.05$) when compared to the control (Suo et al.

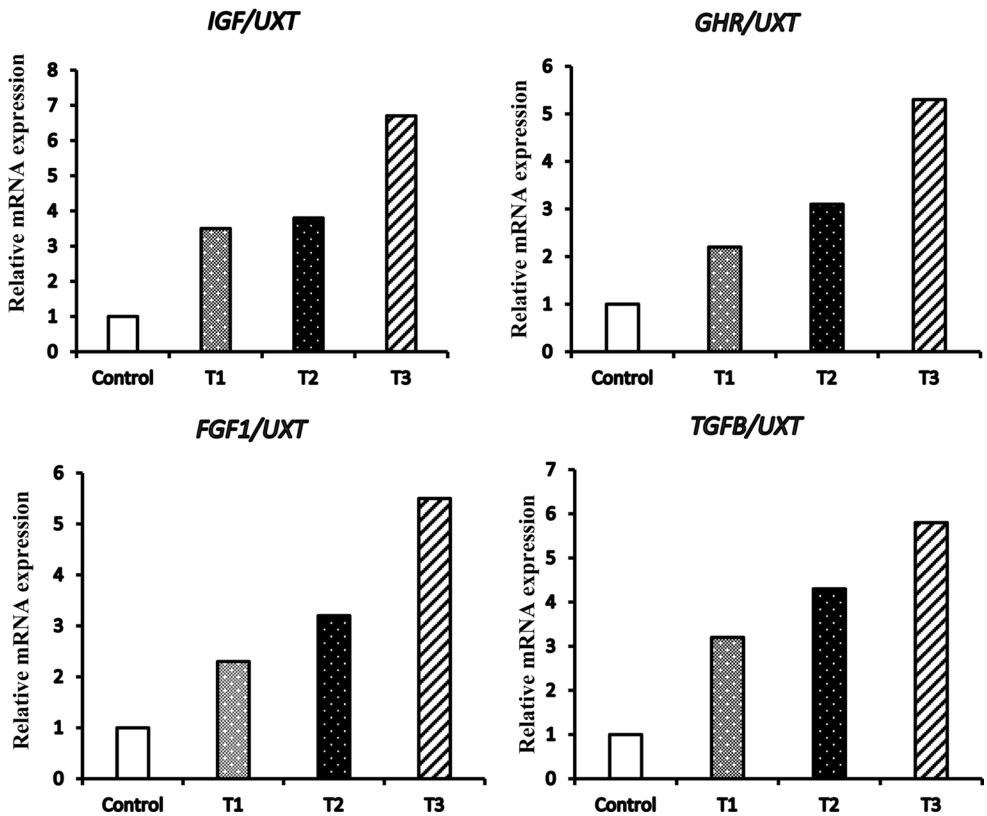

Fig. 4 The mRNA expression of IGF-1, GHR, FGF1, TGFB1, and TCIRG1 in liver tissue was validated through qRT-PCR among the control group that only consumed the basal diet, T1, a group that ate the basal diet plus 1000 mg/kg of lyophilized cow's skim milk fermented with *Lb. plantarum*, T2, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with *Lb. rhamnosus*, and T3, a group that ate the basal diet plus 1000 mg/kg lyophilized cow's skim milk fermented with a combination of *Lb. plantarum* and *Lb. rhamnosus*. *IGF-1*: insulin-like growth factor 1, *GHR*: growth hormone receptor, *FGF1*: fibroblast growth factor 1, *TGFB1*: transforming growth factor beta-1. cDNA samples, liver/group ($n=5$)

2012). Interestingly, meat from rabbits fed the *Lactobacillus* mixture had similar technological characteristics and a slightly brighter red color (L* and a*) than meat from other rabbit groups, but it had a higher WBSF value, indicating it would be tougher. Finally, the oxidative stability of meat from rabbits fed various *Lactobacillus* probiotics was superior along various estimated points of keeping quality tests, especially those supplemented with *Lb. rhamnosus*. In terms of the pH value estimated during the keeping quality test, meat from rabbits fed *Lb. plantarum* and the *Lactobacillus* combination always has significantly lower pH values than the control and *Lb. rhamnosus*-supplemented rabbits. *Lactobacillus* probiotics' beneficial effect on meat quality has always been attributed to the production of bacteriocins, but the mechanism of action is still unknown (Chen et al. 2018). Increased protein expressions, including cytoskeleton and molecular chaperones related to carbohydrate and energy metabolism, in broilers after dietary supplementation with probiotics *E. faecium*, was linked to enhanced meat quality (Zheng et al. 2014). Thus, current keeping quality results suggest that *Lb. rhamnosus* may produce more effective prebiotic substances with superior biological functions than *Lb. plantarum* or their combination. It also supports previous findings in chickens where dietary *Lb. rhamnosus* had a better effect on meat quality than *Enterococcus faecium* (Chen et al. 2018). However, the medium range of meat pH and oxidative stability values observed in rabbits fed the *Lactobacillus* mixture could indicate synergistic actions between *Lb. plantarum* and *Lb. rhamnosus* in this rabbit group, which advantageously gathered their related antioxidative and acidification properties. In conclusion, current dietary supplemented Lactobacillus probiotics improved meat quality; however, their dietary combination will produce brighter red meat with superior keeping quality when compared to the control.

Conclusion

In summary, the study's findings revealed that the group of weaning rabbits that received a diet supplemented with 1,000 mg/kg of a combination of *Lb. plantarum* and *Lb. rhamnosus* (1:1) had the greatest improvements in growth performance, meat quality, blood biochemical parameters, gene expression, and antioxidant enzymes.

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Data availability The data that support the results of this study are available upon request from the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

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