










Original Research

# Dairy-Derived and Bacteriocin-Producing Strain *Lactiplantibacillus plantarum* LP17L/1: An Assessment of Its Safety and Effect Using Broiler Rabbits

Andrea Lauková<sup>1,\*</sup>, Ľubica Chrastinová<sup>2,†</sup>, Iveta Plachá<sup>1</sup>, Valentína Focková<sup>1</sup>,  
Natália Zábolyová<sup>1</sup>, Eva Bino<sup>1</sup>, Ľubomíra Grešáková<sup>1</sup>, Rudolf Žitňan<sup>2</sup>,  
Zuzana Formelová<sup>2</sup>, Jana Ščerbová<sup>1</sup>, Grzegorz Belzecki<sup>3</sup>, Renata Miltko<sup>3</sup>,  
Monika Pogány Simonová<sup>1</sup>

<sup>1</sup>Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, 04001 Košice, Slovakia

<sup>2</sup>Department of Animal Nutrition, National Agricultural and Food Centre, 95141 Nitra-Lužianky, Slovakia

<sup>3</sup>Department of Animal Nutrition, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland

\*Correspondence: [laukova@saske.sk](mailto:laukova@saske.sk) (Andrea Lauková)

†Deceased.

Academic Editor: Baohong Zhang

Submitted: 26 February 2024 Revised: 26 March 2024 Accepted: 16 April 2024 Published: 5 July 2024

## Abstract

**Background:** Using bacterial (probiotic) strains can influence beneficial health statuses, e.g., through dairy products; however, they must be assessed as safe before consumption; broiler rabbits represent a suitable model for this purpose. This study evaluated the safety and effect (functionality) of the dairy-derived, plantaricin-producing strain *Lactiplantibacillus plantarum* LP17L/1 using broiler rabbits. The following parameters were assessed to evaluate safety and functionality: microbiota, phagocytic activity (non-specific immunity parameter), blood serum biochemistry, oxidative stress enzyme, growth performance, cecal hydrolytic activity, and jejunal morphometry. **Methods:** Previously validated methods were used for the analyses. The appropriate administrations approved the experiments. **Results:** Although only up to 1.0 colony forming unit (CFU)/g (log<sub>10</sub>) LP17L/1 reached the gastrointestinal tract of the rabbits, the total lactic acid bacteria and amylolytic streptococci were significantly increased ( $p < 0.001$ ). The other microbiota were not influenced, meaning there was no negative influence on phagocytic activity, growth parameters, and biochemistry in the analyzed blood serum. Conversely, lower GPx values were measured in the experimental group than in the control group, meaning LP17L/1 did not induce oxidative stress. LP17L/1 caused an increase in villi length to crypt depth ratio, while hydrolytic activity was also significantly increased ( $p < 0.01$ ,  $p < 0.001$ ). **Conclusions:** *L. plantarum* LP17L/1 was confirmed as safe. Regarding the functionality, no negative influence on the microbiota was noted, and the tested parameters were not negatively influenced. Demonstrating that the LP17L/1 strain is safe provides further chances for its industrial application. Additional studies are being conducted.

**Keywords:** probiotic strain; safety; model; food-derived animal

## 1. Introduction

Ewes milk and its products, such as milk lump cheeses, have a high nutritional value in the human diet [1]. Since sheep breeding is a tradition in Slovakia, ewe milk products are popular among consumers and frequently consumed [2,3]. Moreover, ewes milk lump cheese has received a traditional specialty guaranteed label (TSG) [4]. Similarly, stored ewes milk lump cheese also belongs to the popular products. These products possess naturally beneficial autochthonous microbiota, especially lactic acid bacteria (LAB) from the phylum Firmicutes [5]. Some bacterial strains can be isolated from this source and studied for their useful properties, leading to their potential application in dairy as a functional food [3]. However, primary strains and/or strain safety and functionality should be checked following the EFSA rules [6] to ensure their/its application in the future. *Lactiplantibacillus plantarum* LP17L/1 strain

was isolated from stored ewes cheese [7]. It represents a non-hemolytic ( $\alpha$ -hemolysis) strain that is susceptible to antibiotics. The LP17L/1 strain is deoxyribonuclease-negative and a non-biofilm-forming strain that sufficiently tolerates lower pH and oxgall–bile [3]. The production of the useful enzyme,  $\beta$ -galactosidase (an enzyme used in non-lactose milk processing for lactose intolerant individuals), was reported in the LP17L/1 strain alongside bacteriocin (postbiotic) anti-staphylococcal and anti-listerial activity [3,7]. Sequencing methods confirmed the taxonomy of this strain, and the strain has been deposited in GenBank under accession number (AN) ON114094 [7]. Moreover, it has also been deposited in the Czech Culture Collection (CCM, Brno, Czech Republic) as CCM 9208 [7]. The species *Lactiplantibacillus plantarum* belongs to the genus *Lactiplantibacillus*, family Lactobacillaceae, order Lactobacillales, class Bacilli, and phylum Firmicutes [8]. As has al-



ready been noted, the LP17L/1 strain should be safe, meaning it does not threaten the health status of the host. Experimental application using the pathogen-free male BALB/c mouse model confirmed the safety of the LP17L/1 strain. Furthermore, after one month of application in mice, a reduced count of coliforms was reported in the jejunum [3]. Broiler rabbits are suitable animal models that have already been used for this purpose in previous studies [9,10]. They also represent food-derived animals serving as confirmation models, e.g., in the safety aspect. In addition, e.g., *L. plantarum* - BiocenoI™ LP96 and flax-seed oil have been applied to decrease *E. coli* O8:K88ab:H9 infection in the gut of germ-free piglets [11]. Another strain, *L. plantarum* CLFP238, decreased lactococcosis in rainbow trout from 87% to 54% after 30 days of application [12]. Additionally, inflammation and normalized intestine were reduced after the experimental application of *L. plantarum* 299v (using a mouse model), thereby treating irritable bowel syndrome more effectively [13]. El-Shafei *et al.* [14] tested the *L. plantarum* strain for 8 weeks in four-week-old male New Zealand White rabbits and showed the appearance of goblet cells in the duodenum and caecum epithelia, thus suggesting an improvement in mucus production compared with control rabbits. The safe strain *L. plantarum* AD73 from goat milk showed sufficient counts in “chevre” cheese to warrant it being labeled probiotic cheese [15]. There are plans for this strain to be involved in a patent regarding dairy drinks, meaning its safety has to be assessed. Therefore, the main objective of this study was to use rabbits as an animal model to confirm the safety and functionality of the dairy-derived LP17L/1 strain by analyzing its colonization in the gastrointestinal tract to control microbiota, phagocytic activity, glutathione-peroxidase activity, enzymatic activity, growth performance, and jejunal morphometry, which are parameters that support the main objective. The original (noncommercial) plantaricin-producing strain was tested in this study, and it can be utilized in functional dairy food products, as formerly mentioned. Moreover, various additional potential applications of the LP17L/1 strain are indicated.

## 2. Materials and Methods

### 2.1 Animal Model and Experimental Design

A total of 24 rabbits (meat lines M91 and P91) were weaned on day 35, including both sexes (equal male-to-female ratio per treatment), were divided into the experimental group (EG) and the control group (CG), 12 animals in each. The average body weight of rabbits at the beginning of the experiment was 1472 g. The experiment was conducted alongside colleagues in Nitra-Lužianky from the National Agricultural and Food Centre (NAFC). The guidelines in the Guide for the Care and Use of Laboratory Animals approved by the Slovak Veterinary and Food Administration and Ethical Commissions of both institutions (permission code: SK CH 17016 and SK U 18016) were ac-

cepted for care and experimental animal procedures. The animals were fed a commercial diet for growing rabbits (total energy value 10.99 MJ/Kg) using the following values: Dry matter content 895.38 g/kg, crude fiber 152.43 g/kg, fat 35.77 g/kg, N-free extract 153.64 g/kg, organic matter 834.97 g/kg, ash 60.41 g/kg, starch 139.78 g/kg, calcium 11.74 g/kg, phosphorus 5.89 g/kg, magnesium 2.56 g/kg, sodium 1.80 g/kg, kalium 10.17 g/kg, iron 260.39 mg/kg, and zinc 122.90 mg/kg. Rabbits were maintained in standard cages (type D-KV-72; 0.61 m × 0.34 m × 0.33 m; Kovobel company Domažlice, Czech Republic), with two animals per cage. A cycle of 16 h light and 8 h dark was applied throughout the experiment. The temperature and humidity in the menagerie were recorded continuously by a digital thermograph positioned at the same level as the cages. The heating and ventilation systems allowed the menagerie air temperature to be maintained throughout the experiment at  $16 \pm 4$  °C, with the relative humidity at about  $70 \pm 5\%$ .

The strain was applied to the drinking water of the experimental rabbits at a dose of 500 µL per day per rabbit for 30 days. Feces for bacterial background control were sampled on day 0/1. Blood was also sampled for the appropriate analyses. Rabbits ( $n = 4$ ) were culled on day 30, and four rabbits from each group (one rabbit/one replicate) were selected based on daily weight measurements to ensure similar animal weights. Animals were sacrificed electro-stunning (50 Hz, 0.3 A/rabbit/4 s) in an experimental slaughterhouse by dissecting the carotid and jugular veins, as previously described by Pogány Simonová *et al.* [9]. The caecum and appendix were removed and treated for microbiota enumeration, as described in Section 2.2. Cecal samples were also taken to analyze hydrolytic activities. *Musculus longissimus thoracis et lumborum* (MLTL) was separated by removing the skin, fat, and connective tissue before being chilled and stored at 4 °C for 24 h until analysis.

### 2.2 Preparation of Strain LP17L/1 for Application

As previously introduced [3,7], the strain *Lactiplantibacillus plantarum* LP17L/1 was isolated from stored ewes cheese. LP17L/1 was marked by rifampicin to differentiate it from the total lactic acid bacteria count. To prepare its rifampicin variant for application, the same protocol was used as previously reported for the strain *Enterococcus faecium* EK13 by Stropfová *et al.* [16]. Briefly, LP17L/1 was grown overnight in MRS broth (De Man Rogosa-Sharpe broth, pH,  $6.0 \pm 0.2$ , Merck, Darmstadt, Germany) to reach  $A_{600} = 1.00$ . Then, it was centrifuged at  $10,000 \times g$  for 30 minutes. The supernatant was removed, and the obtained cells were re-suspended in Ringer solution (pH 7.0, Merck) to the requested cell concentration of  $10^9$  colony forming units (CFUs)/mL. The strain count was checked after cell dilutions in Ringer solution, and their spreading on MRS agar (Merck, Darmstadt, Germany) was enriched

**Table 1. The tested biochemical parameters in rabbit blood serum on day 30 (application of LP17L/1 strain).**

<i>n</i> = 8	Reference values	CG	EG	<i>p</i> -value
Total protein (g/L)	53–85	55.16 ± 1.76	56.25 ± 3.49	0.444
Albumin (g/L)	26–46	39.10 ± 1.41	39.49 ± 2.01	0.662
Creatinine (μmol/L)	44–141	42.59 ± 4.44*	50.58 ± 8.57*	0.035*
Glucose (mmol/L)	5.5–8.6	8.36 ± 0.56	8.09 ± 0.89	0.471
Cholesterol (mmol/L)	0.28–2.1	1.61 ± 0.36**	1.12 ± 0.16**	0.003**
Triglycerides (mmol/L)	Up to 1.44	0.98 ± 0.30	0.74 ± 0.29	0.124
ALT (μkat/L)	0.33–1.19	0.39 ± 0.09	0.43 ± 0.12	0.466
AST (μkat/L)	0.23–0.93	0.26 ± 0.08	0.24 ± 0.05	0.356
ALP (μkat/L)	0.3–2.13	2.95 ± 0.74	2.65 ± 0.39	0.329
Na (sodium, mmol/L)	-	142.1 ± 3.52	140.3 ± 3.19	0.284
K (kalium, mmol/L)	-	4.35 ± 0.72	4.69 ± 0.74	0.381
Ca (calcium, mmol/L)	2.2–4.2	3.56 ± 0.23	3.66 ± 0.14	0.315
P (phosphorus, mmol/L)	1.2–2.4	2.26 ± 0.42	2.37 ± 0.12	0.355
Mg (magnesium, mmol/L)	0.8–1.20	0.96 ± 0.09	0.99 ± 0.044	0.325
Chloride (Cl, mmol/L)	92–120	98.81 ± 3.65	95.68 ± 3.24	0.082

CG, the control group of rabbits; EG, the experimental group of rabbits; creatinine, \**p* < 0.05; cholesterol, \*\**p* < 0.01; the other values are not significant.

with rifampicin (100 μL/L). The dose of the strain was selected based on our previous experiences of beneficial strain application [10]. The concentration of the LP 17L/1 strain cells applied in EG was 10<sup>9</sup> CFUs/g per rabbit, as formerly mentioned.

### 2.3 Microbiota Evaluation Using Standard Microbial Analysis

Fecal samples (1 g, *n* = 6) from EG and CG were diluted in Ringer solution (pH 7.0, Darmstadt, Merck, Germany) using the standard microbiological dilution method (1:9 ratio [17]). The appropriate dilutions (10<sup>-1</sup>–10<sup>-6</sup>) were spread on the selective media according to the International Organization for Standardization (ISO). The LP17L/1 strain colonies on MRS agar enriched with rifampicin (100 μg/L, De Man–Rogosa–Sharpe agar, Merck, Darmstadt, Germany) were counted. The total LAB was enumerated using MRS agar (SO 15, 214, Merck, Darmstadt, Germany). Enterococci on M-Enterococcus medium (Difco Laboratories, Detroit, MI, USA) (ISO NF-V04503) were counted. M17 agar (Difco, Detroit, MI, USA) was enriched with starch (30 g/per L) to enumerate amylolytic streptococci. Mannitol Salt agar (MSA, ISO 688, Difco, MI, USA) was applied to count coagulase-negative staphylococci (CoNS), and Baird-Parker agar supplemented with tellurite and yolk egg was used to enumerate coagulase-positive staphylococci (CoPS, Detroit, MI, USA). Coliforms were counted on MacConkey agar (Difco, Detroit, MI, USA). Cecal and appendix samples were treated as was formerly described for feces and plated using the same cultivation media. The bacterial counts were expressed in colony-forming units per gram (CFUs/g) ± SD (standard deviation, log10).

### 2.4 Phagocytic Activity in Blood and Glutathione–Peroxidase Activity

Blood (*n* = 8) from the marginal ear vein (*vena auricularis*) was sampled from rabbits into Eppendorf tubes with micro-spheric hydrophilic (MSH) particles and heparin to test phagocytic activity (PA), as previously described by Pogány Simonová *et al.* [9]. Sampling was performed on day 30 (end of the strain application). The 50 μL of MSH particle suspension (ARTIM, Prague, Czech Republic) was mixed with 100 μL of blood in Eppendorf tubes and incubated at 37 °C for 1 h. Blood smears were prepared and stained using May–Gruenwald and Giemsa–Romanowski. To validate PA, the direct microscopic counting procedure was performed to calculate the number of white cells containing at least three engulfed particles per 100 white cells (monocytes/granulocytes). PA was expressed as a percentage (%). Moreover, an index of phagocytic activity was included in the analysis (IFA).

Glutathione–peroxidase (GPx (*n* = 8)) was determined by the colorimetric method (Spectrophotometer UV-2550, Shimadzu, Tokyo, Japan) using the commercial kit Randox RS504 (Randox Laboratory, Hong Kong, China) after blood sampling with heparin.

### 2.5 Faecal Hydrolytic Activity Measurements, Biochemical Evaluation of Blood Serum, and Growth Performance

Hydrolytic activities of amylolytic, cellulolytic, xylanolytic, pectinolytic, and inulolytic (expressed in μmol/g/DM/min, meaning in micromol per gram of dry matter per minute) were processed as previously described by Lauková *et al.* [18]. These enzymes were extracted using the procedure described by Huhtanen and Khalili [19] and measured according to the method by Miltko *et al.* [20].

**Table 2. Fecal bacteria and LP17L/1 strain counts on day 0/1 and day 30 (CFUs/g, log<sub>10</sub>).**

Day 0/1, n = 10; day 30, n = 6	Day 0/1	Day 30 (CG)	Day 30 (EG)
LP 17L/1	nt	nt	0.9 ± 0.01
LAB	2.34 ± 0.62***	3.61 ± 0.20***	4.00 ± 0.32
Enterococci	2.18 ± 0.58***	3.58 ± 0.31***	3.89 ± 0.59
CoNS	3.12 ± 0.27**	3.84 ± 0.37***	3.93 ± 1.02
CoPS	2.44 ± 0.45**	4.17 ± 0.04**	3.86 ± 1.96
Amylolytic streptococci	5.82 ± 0.37***	7.08 ± 0.04***	7.10 ± 0.00
Coliforms	1.27 ± 0.72***	4.02 ± 0.47***	4.61 ± 1.04

CFUs, colony forming units; LAB, lactic acid bacteria: day 0/1 vs. EG30, \*\*\* $p < 0.001$ ; day 0/1 vs. CG30, \*\*\* $p < 0.001$ ; EG30 vs. CG30 = NS; Enterococci: day 0/1 vs. EG30, \*\*\* $p < 0.001$ ; day 0/1 vs. CG, \*\*\* $p < 0.001$ , EG30 vs. CG30 = NS; coagulase-negative staphylococci; CoNS = NS; coagulase-positive staphylococci: CoPS, day 0/1 vs. EG30, \*\* $p < 0.01$ ; day 0/1 vs. CG30, \*\*\* $p < 0.001$ ; CG30 vs. EG30 = NS; Amylolytic streptococci: day 0/1 vs. EG30, \*\*\* $p < 0.001$ , day 0/1 vs. CG30, \*\*\* $p < 0.001$ , EG30 vs. CG30 = NS; Coliforms: day 0/1 vs. EG30, \*\*\* $p < 0.001$ ; day 0/1 vs. CG30, \*\*\* $p < 0.001$ , EG30 vs. CG30 = NS; CG, the control group of rabbits; EG, the experimental group of rabbits; nt, not tested; NS, not significant; SD, standard deviation; vs., versus/comparing.

Blood samples were transferred into Eppendorf tubes, centrifuged (3000 ×g, 30 minutes), and delivered to Slovak-Laboratory (SK-Lab) company Lučenec (Slovakia) for analysis using previously validated methods. To assess the nitrogen profile, the total protein (TP in g/L), albumin (g/L), and creatinine (μmol/L) levels were tested. Regarding the enzymatic–hepatic profile, the following parameters were tested: alanine aminotransferase (ALT in μkat/L), aspartate transferase (AST in μkat/L), and alkaline phosphatase (ALP in μkat/L). To test the energy profile, glucose (mmol/L), cholesterol (mmol/L), and triglycerides (mmol/L) were evaluated. The following minerals were involved in mineral profile testing: sodium (Na), kalium (K), chlorides (CL), calcium (Ca), phosphorus (P), and magnesium (Mg), measured in mmol/L. The reference values for the rabbits are summarized in Table 1.

Body weight (BW) was measured every week during the experiment.

### 2.6 Fatty Acid Content in *Musculus Longissimus Thoracis* and *Lumborum* (MLTL), *Jejunal Morphometry Testing*, and *Statistical Analysis*

Animals were sacrificed, as it is indicated in Section 2.1. *Musculus longissimus thoracis et lumborum* (MLTL) was removed from each rabbit and processed by removing the skin, fat, and connective tissue, chilled, and stored at 4 °C for 24 h until analysis. The FA composition in the MLTL samples was determined using the previously described method by Ouhayoun [21], using gas chromatography of fatty acid methyl ester (FAME) on GC 6890 N (Agilent technologies, Swiss, AG Basel). Results are expressed as a percentage of total FA content. Morphometry testing was performed as previously described by Žitňan *et al.* [22].

The treatment effect regarding the tested parameters was statistically analyzed using a one-way analysis of variance (ANOVA) with Tukey's *posthoc* test (unpaired). Data are expressed as the mean and standard deviation SD of the mean. Different superscript letters indicate a significant difference ( $p < 0.05$ ). Statistical analyses were performed using GraphPad Prism version 6.0 (Inc., San Diego, CA, USA).

## 3. Results

### 3.1 Microbiota Assessment

On day 30, the LP17L/1 strain showed low colonization in feces, reaching up to 1.0 CFU/g (log<sub>10</sub>) (Table 2). However, the total LAB count in the EG increased significantly ( $p < 0.001$ ) compared to the LAB count on day 0/1. The total LAB count was also significantly increased in the EG ( $p < 0.001$ ) compared to CG (Table 1). On day 30, an increase in LAB was also noted in the CG ( $p < 0.001$ ) compared to day 0/1 (Table 2). The enterococci counts increased significantly ( $p < 0.001$ ) in both groups compared to day 0/1. The CoNS counts were not influenced. Enterococci and CoNS were well-balanced in both groups on day 30. Similarly, the total counts of amylyolytic streptococci were well-balanced in both groups, and high counts were detected in both groups (Table 1). On day 30, amylyolytic streptococci were significantly increased in the feces ( $p < 0.001$ ) of both groups compared to day 0/1. Regarding the CoPS, a mathematical decrease (0.31 log cycle) was noted on day 30 between the CG and the EG. However, a significant increase in CoPS was noted ( $p < 0.01$ ) in the EG and the CG compared to day 0/1. Coliforms were not influenced/reduced.



**Table 3. Cecal bacteria and LP17L/1 strain counts on day 30 (in CFUs/g log10).**

<i>n</i> = 4	CG	EG
LP 17L/1	nt	0.9 ± 0.0
LAB	2.49 ± 0.37	2.72 ± 0.51
Enterococci	1.7 ± 0.27	1.00 ± 0.2
CoNS	3.12 ± 0.80	4.07 ± 0.35
CoPS	3.56 ± 0.59	3.89 ± 0.176
Amylolytic streptococci	5.82 ± 0.57**	4.46 ± 0.69**
Coliforms	2.47 ± 0.19	2.45 ± 0.35

LAB, lactic acid bacteria; Enterococci; coagulase-negative staphylococci (CoNS); coagulase-positive staphylococci (CoPS); Amylolytic streptococci, EG30 vs. CG30, \*\**p* < 0.01; the counts of other tested bacteria on day 30 in the EG and CG were not significant (NS). nt, not tested; CG, the control group of rabbits; EG, the experimental group of rabbits; SD, standard deviation; vs., versus/comparing.

**Table 4. The bacteria and LP17L/1 strain counts on day 30 are in the appendix (CFUs/g log10).**

<i>n</i> = 4	CG	EG
LP 17L/1	nt	0.9 ± 0.0
LAB	2.23 ± 0.14	2.03 ± 0.70
Enterococci	0.95 ± 0.06	0.98 ± 0.05
CoNS	3.71 ± 0.63	3.45 ± 0.66
CoPS	3.23 ± 0.76	3.29 ± 0.60
Amylolytic streptococci	5.01 ± 2.23	4.83 ± 0.19
Coliforms	1.59 ± 0.97	3.21 ± 1.22

LAB, lactic acid bacteria; Enterococci; coagulase-negative staphylococci (CoNS); coagulase-positive staphylococci (CoPS); the counts of tested bacteria on day 30 comparing the EG and CG were not significant. nt, not tested; CG, the control group of rabbits; EG, the experimental group of rabbits; SD, standard deviation.

The LP17L/1 strain count in the caecum was the same as in the feces (Table 3). Bacterial counts were mostly lower than in the feces. LAB, enterococci, and CoPS were well-balanced in both groups on day 30, and CoNS and CoPS counts were even similar to those in the feces. Amylolytic streptococci were decreased in the caecum of the EG (*p* < 0.01) compared to the CG. The coliform count in the caecum was not influenced, similarly to that in the feces.

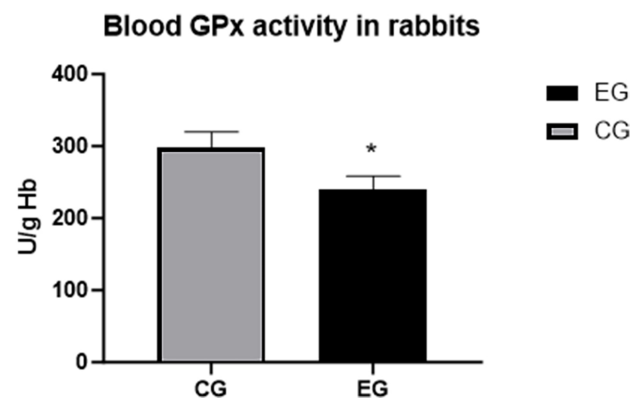
Similarly, as in the feces and caecum, the LP17L/1 strain was found in the appendix at counts of up to 1.0 CFU/g log10 (Table 4). Notably, amylolytic streptococci were found at almost the same level in both groups and the caecum (Table 4). In general, bacterial counts in the appendix were not influenced. The counts of CoNS and CoPS were similar to those in caecum and feces.

### 3.2 Phagocytic Activity, GPx, Biochemical, Growth Performance, and Cecal Hydrolytic Activity

On day 30, the phagocytic activity (PA) values were nearly identical in both groups (CG: 70.00 ± 1.41%; EG:

70.67 ± 1.75%) (Table 5). This means that applying the LP17L/1 strain did not stimulate PA but also did not negatively influence it. The same situation was noted for IPA (Table 5).

The GPx value was significantly lower in the EG (231.0 ± 35.09 U/gHb) compared to the GPx value in the CG (298.0 ± 58.18 U/gHb, *p* < 0.05) (Table 5, Fig. 1). This result confirms that applying the LP17L/1 strain over 30 days did not affect the health of the rabbits and did not promote oxidative stress.



**Fig. 1. Glutathione-peroxidase (GPx) activity, \**p* < 0.05 (CG:EG).**

Regarding the biochemistry of the blood serum samples (see Table 1), the total protein value was measured at the lower level of the reference range (RR) (55.16 ± 1.76 g/L). In the EG, a slightly higher total protein value was noted (56.25 ± 3.49 g/L). The albumin, glucose, AST, and ALP values were not influenced. Albumin values were nearer the upper limit of the reference range (Table 1). The glucose value was at the top of the reference range. AST values were in the lower limit of the reference value (RV), and ALP values were well-balanced in both groups but higher than the upper limit of the reference value. The ALT values were also balanced; slightly higher in the EG (2.65 ± 0.39 μkat/L) than in the CG (2.95 ± 0.74 μkat/L) and in the lower level of RR. Triglycerides were measured in the physiological range up to the reference limit, whereas a lower value was measured in the EG compared to the CG (Table 6). Creatinine values were nearer the lower limit of the reference range, although slightly higher in the EG than in the CG (*p* < 0.05). Cholesterol values were in the physiological range, nearer the upper limit. However, a lower value was measured in the EG (1.12 ± 0.16 mmol/L) than in the CG (1.61 ± 0.36 mmol/L, *p* < 0.001). Regarding the mineral profile, chlorides were within the physiological range with a slightly lower value in the EG (95.68 ± 3.24 mmol/L) compared to CG (98.81 ± 3.65 mmol/L). K, Ca, P, and Mg values were not influenced; they were balanced in both groups, with slightly higher values in the EG than

**Table 5. Phagocytic activity (%) and glutathione–peroxidase results (U/gHb) ± SD.**

<i>n</i> = 8	Control group (CG)	Experimental group (EG)
Phagocytic activity (PA) on day 30	70.00 ± 1.41	70.67 ± 1.75
Index of phagocytic activity (IPA)	3.53 ± 0.15	3.63 ± 0.16
GPx	298.0 ± 58.18*	231.0 ± 35.09*

The PA and IPA values were not significant (NS). GPx, CG vs. EG, \**p* < 0.05. CG, the control group of rabbits; EG, the experimental group of rabbits; SD, standard deviation; NS, not significant; vs., versus/comparing.

**Table 6. Hydrolytic activity on day 30.**

<i>n</i> = 4	CG	EG
Amylolytic activity	20.30 ± 6.91***	46.72 ± 9.99***
Xylanolytic activity	22.84 ± 6.79***	37.17 ± 8.81***
Cellulolytic activity	7.21 ± 1.60***	17.11 ± 2.02***
Pectinolytic activity	7.11 ± 1.28***	24.11 ± 3.91***
Inulolytic activity	3.26 ± 0.86	3.86 ± 1.34

Hydrolytic activity in µmoL of released product/g DM of caecum/min ± SD; EG, the experimental group of rabbits; CG, the control group of rabbits; amylolytic activity, cellulolytic activity, pectinolytic activity, xylanolytic activity, \*\*\**p* < 0.001; inulolytic activity.

in the CG. A similar result was found for Na; however, a marginally lower value was measured in the EG than in the CG (Table 1).

Regarding the hydrolytic activity in the caecum on day 30 (Table 6), the EG exhibited increased activity compared to the CG. Amylolytic activity reached the highest value (*p* < 0.001) in the EG compared to the CG. Higher amylolytic activity values are associated with higher amylolytic streptococci values. The xylanolytic activity was also higher in the EG (*p* < 0.001) compared to the CG. Likewise, pectinolytic activity was higher in the EG (*p* < 0.001) than in the CG (Table 7). A higher cellulolytic activity was found in the EG (*p* < 0.001) than in the CG. The inulolytic activity was not influenced (Table 6).

On day 30, the live body weight of rabbits reached an average of 2791.8 g ± 44.67 in the EG and an average of 2469.16 ± 49.69 g in the CG. Thus, a higher body weight was noted in the EG (322.6 g higher), meaning that the LP17L/1 strain probably stimulated increased taste and hunger in the EG rabbits.

### 3.3 Fatty Acids Content in *Musculus Longissimus Thoracis and Lumborum* (MLTL) and Jejunal Morphometry

The total saturated fatty acids (SFAs) value reached 34.890 ± 2.149% from the total FAs in the EG on day 30. In the CG, the SFA value was 33.580 ± 0.849%. In the total monounsaturated fatty acids (MUFAs), the value for the EG measured 49.448 ± 0.975%, while 47.443 ± 1.731% was calculated for the CG. The total essential fatty acids (EFAs) value in the EG reached 8.808 ± 1.264%, while 9.200 ± 0.946% was measured in the CG. For the total polyunsatu-

**Table 7. Jejunal morphometry on day 30.**

<i>n</i> = 4	CG	EG
Villus circumference µm	1580.0 ± 39.74	1584.0 ± 39.79
Villus cut surface µm <sup>2</sup>	81546.0 ± 285.56	81705.0 ± 258.8
Villus height µm	684.0 ± 26.15	686.0 ± 26.19
Crypt depth µm	179.0 ± 13.37	175.0 ± 13.22
Villus height vs. crypt depth	3.83	3.92

CG, the control group of rabbits; EG, the experimental group of rabbits; SD, standard deviation. The values are not significantly influenced; vs., versus/comparing.

rated fatty acids (PUFAs), 10.858 ± 0.747% was measured in the EG, while 12.368 ± 1.106% was found in the CG.

Thus, the strain LP17L/1 beneficially influenced all tested morphometry parameters. Only in crypt depth was a slight difference found in the CG. However, the final villus height to crypt depth ratio was slightly higher in the EG than in the CG (3.92 vs. 3.83, Table 7), although it was not significantly different.

## 4. Discussion

Confirming the safety and functionality of bacterial strains, which could be industrially applied for health benefits, is paramount. Lactobacilli do not represent the predominant bacteria in the digestive tract of rabbits after weaning; however, they are part of the gastrointestinal tract (GIT) microbiota. This study confirms that although the LP17L/1 strain was not high, the GIT and lactic acid bacteria (LAB) levels increased in the tested rabbits. The highest amylolytic activity in the caecum of the EG rabbits is associated with detecting high amylolytic activity. Following our aim to evaluate the safety of the strain, it is beneficial to know that microbiota were not negatively influenced. Regarding the antimicrobial activity evaluation after beneficial strain application in rabbits, the influence on the microbiota using probiotics and bacteriocin-producing enterococci as bacteria belonging to the lactic acid bacteria group has been previously reported [9,10]. After applying *E. faecium* AL41, antimicrobial activity was noted against pseudomonads in feces and caecum, and a significant decrease in coliforms was noted (*p* < 0.05) [10]. Repeatedly, coliforms, clostridia, and staphylococci were decreased following *E. faecium* EF2019 = CCM7420 application [9].

Based on our previous results, phagocytic activity (PA) tended to increase after beneficial strain application, e.g., after the application of beneficial strain *Enterococcus faecium* EF9A (isolated from the Hungarian Pannon White rabbit). A significantly higher PA value was noted in the EG ( $p < 0.001$ ) than in the CG [23]. Following the aim of the present study, controlling LP17L/1 strain safety, its application did not have negative/side effects on PA, thus indicating the possibility of its further incorporation in dairy products with no side effects and/or negative impacts on immunity. Moreover, LP17L/1 strain application did not promote oxidative stress. On day 30, the GPx value in the EG was significantly lower ( $p < 0.05$ ) than in the CG, which also confirms the safety of the LP17L/1 strain.

The beneficial effect on jejunal morphometry also supports the safety and beneficial properties of the applied strain [24]. Lee *et al.* [24] reported that two peptides, the expression of which requires cyclinJ, mediated the recovery phase during which enterocytes (after their damage) regain their original shape and volume. This study noted a tendency to improve the villus height to crypt depth ratio in the EG compared to the CG, indicating a beneficial effect after LP17L/1 strain application on enterocyte reparation (jejunal morphometry). When the morphometry was improved, it showed better intestinal functionality and nutrient absorption, leading to better health status and meat quality in rabbits/animals [24]. In general, intestinal health (intestinal morphology, microbial balance) belongs to the parameters that confirm host health status. In our previous studies, following the beneficial application of probiotic and bacteriocin (postbiotic) active strains with beneficial properties, usually, jejunal morphometry was beneficially influenced as the villus height to crypt depth ratio increased [9,10]. When *E. faecium* AL41 = CCM8558-producing Enterocin M was applied in rabbits, a significant increase in PA ( $p < 0.01$ ) was noted on day 21 (3 weeks application) and day 42 (3 weeks after cessation, indicating a prolonged effect), compared to the CG. Similarly, PA was significantly increased after applying the autochthonous strain *E. faecium* 2019 (CCM 7420). As formerly mentioned, El-Shafei *et al.* [14] reported that probiotic strains could increase villus length/height and decrease crypt depth in the small intestine. These effects are beneficial for the digestion and absorption of nutrients, thus directly affecting mucosa morphology, digestive enzyme activity, and, consequently, growth performance. In previous studies by Pogány Simonová *et al.* [9] and Lauková *et al.* [10], the average weight gain was 11.2%. Similarly, body weight was increased after applying the LP17L/1 strain. Probiotic strains also increased hydrolytic activity, as shown in our previous study [10]. The stimulating effect of the strain LP17L/1 on metabolism in broiler rabbits was also noted in this study. These results confirmed and contributed to maintaining health status. In the case of the enhanced mucus layer covering the epithelial lining of the gut, it can

serve as an antibacterial shield that prevents the binding of enteric pathogens, while goblet cells also have a role in the defense of the intestinal mucosa. Cukrowska *et al.* [25] even reported on using lactobacilli, whereby an *in vivo* experiment in mice showed (on the cellular level) that they can shift the cytokine balance in favor of an anti-allergic immune response.

The intramuscular fat was characterized by the highest percentage of MUFAs (47.443% in the CG vs. 49.448% in the EG) and a lower % of PUFAs (CG: 12.368%; EG: 10.858%). Almost identical values of PUFAs were reported in the control rabbits by Pogány Simonová *et al.* [26]. Similarly, the control value of PUFAs was nearly the same at 12.480% in this study (12.368%). In this study, the total saturated fatty acids were the highest in the EG (LP17L/1 strain). A similar result was reported by Pogány Simonová *et al.* [27] in rabbits following the administration of the postbiotic—Enterocin M.

Serum biochemistry was not negatively influenced in rabbits, indicating the safety of the LP17L/1 strain. However, a higher glucose value could be associated with higher energy diet intake. Beneficial strains were previously reported to influence the biochemical parameters [28].

The strain *L. plantarum* LP17L/1 is a safe and functioning strain previously applied in yogurts for its potential as a functional food [7]. Its count in yogurt made from cow milk is not high, yet it remained stable during all experimental evaluations with no negative impact on the pH of the yogurt [7]. Functional food can beneficially influence body functions, boosting health by reducing the risk of diseases and/or by improving a specific physiological response [5]. Moreover, Dvorožňáková *et al.* [29] reported that the LP17L/1 strain showed high immune-modulatory potential on CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes in trichinellosis. This means that the modulation of gut lymphocyte immunity following *T. spiralis* infection with LP17L/1 strain showed a beneficial effect on the host's anti-parasitic defenses. The anti-parasitic defenses (damage and killing of newborn larvae with reactive oxygen species from macrophages) of the host were stimulated by increased macrophage metabolic activity induced by the probiotic strain LP17L/1 treatment [30]. Evidence also shows the benefits and perspectives of administering the safe and functional *L. plantarum* LP17L/1 strain.

## 5. Conclusions

Although the LP17L/1 strain was found in lower counts in the GIT of rabbits, the total LAB and amyolytic streptococci were increased with no negative influence on other microbiota, growth parameters, biochemistry, and/or jejunal morphometry; however, it promoted a beneficial impact on the cecal hydrolytic activity. Phagocytic activity was not negatively influenced. Moreover, the LP17L/1 strain did not promote oxidative stress, which provides further evidence of its safety. Assessing the safety of *Lac-*

*tiplantibacillus plantarum* LP17L/1 using broiler rabbits, provides a chance for its future industrial application, although additional studies are still required.

## Availability of Data and Materials

Data are a part of the manuscript submitted. And/or they can be asked from the corresponding author.

## Author Contributions

AL designed the research study. AL, ECH, IP, VF, NZ, MPS, ĽG, RŽ, GB, and RM performed the research. ECH and ZF provided help and advice on animals. EB and JŠ provided help with microbiological samples treatment. AL, MPS, RM, IP, and ĽG analyzed the data. AL wrote the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors contributed to editorial changes in the manuscript.

## Ethics Approval and Consent to Participate

The guidelines stated in the Guide for the Care and Use of Laboratory Animals approved by the Slovak Veterinary and Food Administration and Ethical Commissions of both institutions (permission code: SK CH 17016 and SK U 18016) were accepted for care and experimental procedures involving animals.

## Acknowledgment

We would like to acknowledge our colleagues from Nitra-Lužianky, Dr. Ľubomír Ondruška, Dr. Rastislav Jurčík, and Ing. Pecho for their help with animals bleeding and slaughtering. We also thank our colleague Mr. Peter Jerga for his kindness to help us in slaughterhouse. And we also are thankful for our laboratory assistant Mrs. Dana Melišová for laboratory skills. We also thank to our colleague in Dairy Research Institute in Žilina for giving us LP17L/1 strain as isolate for study.

## Funding

This research was funded by the Slovak Research and Development Agency, the projects APVV-02-204 and APVV-17-0028.

## Conflict of Interest

The authors declare no conflict of interest.

## References

[1] Kováčová M, Výrostková J, Dudříková E, Žigo F, Semjon B, Regecová I. Assessment of quality and safety of farm level produced cheeses from sheep and goat milk. *Applied Sciences*. 2021; 11: 3196.

- [2] Herian M. Benefit of sheep milk products to human health. *Milk Letters (Mlékařské Listy)*. 2014; 143: 1–6. (In Slovak)
- [3] Lauková A, Tomáška M, Fraqueza MJ, Szabóová R, Bino E, Ščerbová J, *et al.* Bacteriocin-Producing Strain *Lactiplantibacillus plantarum* LP17L/1 Isolated from Traditional Stored Ewe's Milk Cheese and Its Beneficial Potential. *Foods*. 2022; 11: 959.
- [4] The Slovak Spectator. Available at: <https://spectator.sme.sk> (Accessed: 6 August 2011).
- [5] Quintieri L, Fanelli F, Monaci L, Fusco V. Milk and Its Derivatives as Sources of Components and Microorganisms with Health-Promoting Properties: Probiotics and Bioactive Peptides. *Foods*. 2024; 13: 601.
- [6] EFSA. Scientific Opinion on Lactiferm (*Enterococcus faecium*) as a feed additive for weaned piglets and calves. *EFSA Journal*. 2012; 10: 2574.
- [7] Lauková A, Tomáška M, Drončovský M, Bino E, Dvorožňáková E, Chrastinová L, *et al.* Ewes stored cheese, a source of autochthonous, beneficial, bacteriocin-producing bacteria and their application potential. In Proceedings of lectures of the 17th Symposium of the Society for Probiotics and Prebiotics. Prague, Czech Republic. 2023. (In Slovak)
- [8] Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, *et al.* A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*. 2020; 70: 2782–2858.
- [9] Pogány Simonová M, Chrastinová L, Lauková A. Autochthonous Strain *Enterococcus faecium* EF2019(CCM7420), Its Bacteriocin and Their Beneficial Effects in Broiler Rabbits-A Review. *Animals*. 2020; 10: 1188.
- [10] Lauková A, Chrastinová L, Pogány Simonová M, Stropfiová V, Plachá I, Čobanová K, *et al.* *Enterococcus faecium* AL 41: Its Enterocin M and Their Beneficial Use in Rabbits Husbandry. *Probiotics and Antimicrobial Proteins*. 2012; 4: 243–249.
- [11] Nemcová R, Borovská D, Koščová J, Gancarčíková S, Mudroňová D, Buleca V, *et al.* The effect of supplementation of flax-seed oil on interaction of *Lactobacillus plantarum*–BiocenoL™ LP96 and *Escherichia coli* O8:K88ab:H9 in the gut of germ-free piglets. *Research in Veterinary Science*. 2012; 93: 39–41.
- [12] Waugh AWG, Forsheng RR, MacFarlane SM, Doyle JSG, Churchill TA, Sydora BC, *et al.* Effect of *Lactobacillus plantarum* 299v treatment in animal model of irritable bowel syndrome. *Microbial Ecology in Health and Disease*. 2009; 21: 5.
- [13] Balcázar JL, Vendrell D, de Blas I, Zarzuela IR, Girones O, Muzquir JL. Protection of rainbow trout (*Oncorhynchus mykiss*) from lactococcosis by probiotic bacteria *Lactobacillus plantarum* CFLP238. *Comparative Immunology and Microbial Infection Diseases*. 2008; 3: 337–345.
- [14] El-Shafei AA, Younis TM, Al-Gamal MA, Hesham AM. Impact of probiotic (*Lactobacillus plantarum*) supplementation on Productive and physiological performance of growing rabbits under Egyptian conditions. *Egyptian Journal of Rabbit Science*. 2019; 29: 125–148.
- [15] Ngamsomchat A, Kaewkod T, Konkit M, Tragoolpua Y, Bovonsombut S, Chitov T. Characterization of *Lactobacillus plantarum* of Dairy-Product Origin for Probiotic Chèvre Cheese Production. *Foods*. 2022; 11: 934.
- [16] Stropfiová V, Marciňáková M, Simonová M, Gancarčíková S, Jonecová Z, Sciranková L, *et al.* *Enterococcus faecium* EK13—an enterocin a-producing strain with probiotic character and its effect in piglets. *Anaerobe*. 2006; 12: 242–248.
- [17] Acharaya T. Microbe Online. 2024. Available at: <https://microbionline.com/author/tankeshwar/> (Accessed: 25 February 2024).
- [18] Lauková A, Styková E, Kubašová I, Stropfiová V, Gancarčíková S, Plachá I, *et al.* Enterocin M-Producing *Enterococ-*



- cus faecium* CCM 8558 Demonstrating Probiotic Properties in Horses. *Probiotics and Antimicrobial Proteins*. 2020; 12: 1555–1561.
- [19] Huhtanen P, Khalili H. The effect of sucrose supplements on particle-associated carboxymethylcellulase (EC 3.2.1.4) and xylanase (EC 3.2.1.8) activities in cattle given grass-silage-based diet. *The British Journal of Nutrition*. 1992; 67: 245–255.
- [20] Miltko R, Belzecki G, Kowalik B, Skomial J. Presence of carbohydrates-digesting enzymes throughout the digestive tract of sheep. *Turkish Journal of Veterinary and Animal Science*. 2016; 40: 271–277.
- [21] Ouhayon J. Rabbit meat characteristics and qualitative variability. *Cuni Sciences*. 1992; 7: 1–15.
- [22] Žitňan R, Voigt J, Kuhla S, Wegner J, Chudy A, Scoenhausen U, *et al.* Morphology of small intestinal mucosa and intestinal weight change with metabolic type of cattle. *Veterinary Medicine*. 2008; 53: 525–532.
- [23] Pogány Simonová M, Lauková A, Chrastinová E, Plachá I, Szabóová R, Kandričáková A, *et al.* Beneficial effects of *Enterococcus faecium* EF9A administration in rabbit diet. *World Rabbit Science*. 2020; 28: 169–179.
- [24] Lee KZ, Lestradet M, Socha C, Schirmeier S, Schmitz A, Spenlé C, *et al.* Enterocyte Purge and Rapid Recovery Is a Resilience Reaction of the Gut Epithelium to Pore-Forming Toxin Attack. *Cell Host & Microbe*. 2016; 20: 716–730.
- [25] Cukrowska B, Motyl I, Kozáková H, Schwarzer M, Górecki RK, Klewicka E, *et al.* Probiotic *Lactobacillus* strains: in vitro and in vivo studies. *Folia Microbiologica*. 2009; 54: 533–537.
- [26] Pogány Simonová M, Chrastinová E, Chrenková M, Formelová Z, Kandričáková A, Bino E, *et al.* Benefits of Enterocin M and Sage Combination on the Physico-chemical Traits, Fatty Acid, Amino Acid, and Mineral Content of Rabbit Meat. *Probiotics and Antimicrobial Proteins*. 2020; 12: 1235–1245.
- [27] Pogány Simonová M, Lauková A, Chrastinová E, Kandričáková A, Ščerbová J, Stropfová V, *et al.* Effect of enterocin M and durancin ED26E/7 supplementation on blood parameters, immune response and jejunal morphometry in rabbits. *Journal of Animal Physiology and Animal Nutrition*. 2022; 106: 378–386.
- [28] Kalma RP, Chauhan HD, Shrivastava AK, Pawar MM. Growth and blood profile of broiler rabbits on probiotics supplementation. *Indian Journal of Small Ruminants*. 2018; 24: 66–69.
- [29] Dvorožňáková E, Vargová M, Hurníková Z, Lauková A, Revajová V. Modulation of lymphocyte subpopulations in the small intestine of mice treated with probiotic bacterial strains and infected with *Trichinella spiralis*. *Journal of Applied Microbiology*. 2022; 132: 4430–4439.
- [30] Vargová M, Hurníková Z, Revajová V, Lauková A, Dvorožňáková E. Probiotic Bacteria can Modulate Murine Macrophage's Superoxide Production in *Trichinella Spiralis* Infection. *Helminthologia*. 2020; 57: 226–234.