Probiotics and Antimicrobial Proteins (2021) 13:1433–1442
https://doi.org/10.1007/s12602-021-09809-0

Enterocins as Novel Feed Additives in Rabbit Diet: Enterocin Ent M and Durancin Ent ED26E/7, Their Combination, and Effects on Microbiota, Caecal Fermentation, and Enzymatic Activity

M. Pogány Simonová[1] · A. Lauková[1] · Ľ. Chrastinová[2] · A. Kandričáková[1] · J. Ščerbová[1] · V. Strompfová[1] · R. Miltko[3] · G. Belzecki[3]

Accepted: 1 June 2021 / Published online: 10 June 2021

Abstract
The present study investigates the effects of enterocin Ent M and durancin Ent ED26E/7 applied separately and in combination on the intestinal microbiota, caecal enzymatic activity, and fermentaion of rabbits. Eighty rabbits (M91 meatline, aged 5 weeks, both sexes) were divided into groups E (Ent M; 50 µL/animal/day), D (Ent ED26E/7; 50 µL/animal/day), E + D (Ent M + Ent ED26E/7), and control (C). The additives were administered in drinking water for 21 days. Antimicrobial activity of Ent M and Ent ED26E/7 on coliforms (E, E + D: $P < 0.001$) and pseudomonads (D: $P < 0.05$) in feces was noted, compared to C. Ent M and Ent ED26E/7 application stimulated caecal enzymatic activity in rabbits. Pectinolytic (E vs. D, E + D: $P < 0.01$), inulolytic (E vs. E + D: $P < 0.01$; E vs. C: $P < 0.05$), and amylolytic (E vs. D, E + D. $P < 0.001$; E vs. C: $P < 0.01$) activities were influenced by Ent M, while cellulolytic (D vs. E + D: $P < 0.01$) and inulolytic (D vs. E + D, C: $P < 0.01$) activities by Ent ED26E/7 treatment. The cellulolytic and pectinolytic acitivities changed with time. Treatment × time interaction was detected for cellulose and xylan degradation. During Ent M and Ent ED26E/7 treatment, increased ammonia, lactic, butyric and iso-valeric acid, and lower acetic, propionic, iso-butyric, valeric, and caproic acid concentrations were noted. It can be concluded that Ent M and Ent ED26E/7 application can improve rabbit health due to reduced spoilage microbiota and enhanced caecal enzymatic activity.

Keywords Enterocin · Durancin · Microbiota · Caecal enzymes · Organic acids

Introduction

Production and consumption of animal products have significantly increased during the last few decades. High demand for livestock products, mainly meat, eggs, and milk, continues, which entails ensuring healthy and safe food for consumers [1]. The ban on antibiotics as growth promoters in response to widespread and alarming antibiotic resistance, mainly in food animals, has opened up research into new approaches to stabilize, control, and improve the health, growth performance, feed efficiency, potential pathogenic microbiota and dysbiosis, immunity, and product quality of livestock. Although many alternatives including prebiotics, probiotics or beneficial bacteria and their antimicrobial products, organic acids, and herbal extracts have been studied, some of them such as bacteriocins have still not been sufficiently studied, especially in some animal species. Bacteriocins are defined as ribosomally synthesized antimicrobial peptides, produced by Gram-negative and Gram-positive bacteria as well, mostly by lactic acid bacteria [2]. Despite the important positive traits of bacteriocins, namely, significant antimicrobial activity, natural origin, and non-toxicity for the host organism, their application in livestock, aquaculture, and veterinary medicine is reviewed only a few papers [3–7]. The application of bacteriocins as feed additives and alternatives to antibiotic growth promoters for rabbits

✉ M. Pogány Simonová
simonova@saske.sk

[1] Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovakia

[2] Department of Animal Nutrition, National Agricultural and Food Centre, Hlohovecká 2, 95141 Nitra-Lužianky, Slovakia

[3] The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Instytucka 3, 05 110 Jabłonna, Poland

is presented in several studies. During these experiments, already known and registered bacteriocins, mainly nisin and gallidermin but also some new, not commercial enterocins—bacteriocins produced by beneficial *Enterococcus faecium* isolates of rabbit- and non-rabbit origin were administered to rabbits, and their growth performance, microbiological, immunological, serum biochemical, and intestinal morphological parameters as well as meat quality were monitored [8–19]. While growth, immunity, blood biochemistry, and intestinal microbiota are the most often studied parameters in rabbits, several traits, e.g., jejunal morphometry and caecal fermentation, are not commonly tested, especially during dietary enterocin inclusion.

In rabbits, the caecum is the main fermentor organ, with dominance of phylum *Firmicutes* [20–22]. Caecal bacteria are helpful in nutrient digestion, due to the ability to produce bacterial enzyme which hydrolyze plant cell-wall components which are not decomposed by the host's intestinal digestive enzymes. These indigestible elements, especially lignins, cellulose, hemicellulose, and pectins, are hydrolyzed by bacterial enzymes into smaller compounds and fermented into end products, namely, ammonia, volatile fatty acids (VFA), intermediary metabolites (succinic, formic, lactic acid), and gases (CO_2, CH_4, H_2 [23]). The end product proportions are influenced by the caecal bacterial population and fermentative activities, and they change in relation to the animal's age (from birth through weaning to slaughter) and to feed composition (from milk to solid feed). Before weaning, bifidobacteria, lactobacilli, and colibacilli dominate in the caecum, because of the rabbits' milk intake. Around weaning, milk is gradually replaced with solid feed, rich in polysaccharides, leading to changes in microbiota in favour of anaerobic bacteria, enterobacteriaceae and archaea [21], with complex enzymatic activity for cellulose, hemicellulose, fibres, pectins, and starch degradation [24–26]. For this reason, rabbits are very fragile around the weaning period due to their complex caecal digestion and microbial fermentation, and even small dietary and/or environmental changes can lead to digestive disturbances and increased morbidity/mortality, also resulting in economic losses. Natural feed additives, mostly with antimicrobial character, can prevent dysbiosis by controlling the microbial population and improving digestive health and immunity, also strengthening rabbits' health and productivity.

This study focuses on two novel enterocins, enterocin Ent M produced by beneficial *E. faecium* AL41 strain, deponed in the Czech Collection of Microorganisms in Brno, Czech Republic, under catalogue number CCM8558 [9], originally named Ent AL41 and purified to homogeneity as Ent M [27], and durancin Ent ED26E/7 produced by *E. durans* ED26E/7 strain [28]. The objective of the study was to test the effects of these enterocins applied individually and in combination on the intestinal microbiota, caecal fermentation and enzymatic activity in rabbits. To best of our knowledge, studies presenting bacteriocin effects on these parameters in rabbits still needs to be expanded for better understanding of the relationships between the caecal environment and body health in rabbits.

Material and Methods

Animals and Housing

The experiment was performed in co-operation with our colleagues in Nitra (National Agricultural and Food Centre—NAFC). All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals approved by the Slovakian State Veterinary and Food Administration and the Ethical Committees of both institutions (permission code: SK CH 17,016 and SK U 18,016).

Eighty rabbits of meat line M91 (maternal albinotic line-crossbreed New Zealand White, Bouscat rabbit, Argente Champagnet rabbit, and paternal acromalictic line-crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in this experiment. The animals were weaned at 5 weeks of age (both sexes; equal male to female ratio per treatment) and were divided into experimental groups: E–enterocin Ent M, D–durancin Ent ED26E/7, and E + D–Ent M in combination Ent ED26E/7 and control group (C), with 20 animals in each. The average live weights of rabbits at the start of the experiment were 1211.0 ± 99.0 g in E and 1259.0 ± 89.0 g in D; in E + D, it was 1214.0 ± 99.0 g; and in C, it was 1199.0 ± 123.0 g. The animals were kept in standard cages, two rabbits per cage (type D-KV-72; 0.61 m × 0.34 cm × 0.33 m; Kovobel company, Domažlice, Czech Republic). A cycle of 16 h light and 8 h dark was used throughout the experiment. The temperature and humidity in the building were recorded continuously by a digital thermograph positioned at the same level as the cages. The heating and ventilation systems allowed the building air temperature maintained within 16 ± 4 °C and the relative humidity about 70 ± 5% throughout the experiment. The data were recorded continuously with a digital thermograph positioned at the same level as the cages.

Experiment Design

The animals were fed an untreated diet (pellets of 3.5 mm in average size), commonly used in the nutrition of growing rabbits (Table 1). The rabbits in group E were administered Ent M (prepared according to Mareková et al. [27], dose 50 µL/animal/day, with activity 25,600 AU/mL, in concentration 0.4 g/L, from day zero to day 21) in their drinking water. Rabbits in group D received durancin Ent ED26E/7

Table 1 Ingredients and chemical composition of granulated diet

Feed ingredients (%)		Chemical composition, minerals and vitamins	
Dehydrated lucerne meal	36.0	Dry matter[a]	882.1
Extracted sunflower meal	5.5	Crude protein[a]	164.2
Oats	13.0	Crude fiber[a]	155.5
Wheat bran	9.0	Fat[a]	33.3
Dry malting sprouts	15.0	Ash[a]	73.0
Extracted rapeseed meal	5.5	Nitrogen free extract[a]	466.8
Barley	8.0	Organic compounds[a]	809.1
DDGS	5.0	Acid detergent fibre (ADF)[a]	191.1
Sodium chloride	0.3	Neutral detergent fibre (NDF)[a]	339.5
Premix minerals[*]	1.7	Lignine[a]	42.3
Limestone	1.0	Hemicellulose[a]	148.5
		Cellulose[a]	148.8
		Starch[a]	127.2
		Calcium[a]	6.0
		Phosphorus[a]	5.9
		Magnesium[a]	2.3
		Sodium[a]	1.7
		Potassium[a]	7.5
		Iron[b]	403.9
		Zinc[b]	166.4
		Manganese[b]	156.7
		Copper[b]	22.9
		Metabolic energy (MJ/kg)	11.0

DDGS dried distiller grains with solubles

[*]Premix contains per kg: calcium 6.73 g; phosphorous 4.13 g; magnesium 1.90 g; sodium 1.36 g; potassium 11.21 g; iron 0.36 g; zinc 0.13 g; copper 0.03 g; and selenium 0.2 mg. Vitamin mixture provided per kg of diet: Vitamin A 1,500,000 IU; Vitamin D3 125,000 IU; Vitamin E 5000 mg; Vitamin B1 100 mg; Vitamin B2 500 mg; Vitamin B6 200 mg; Vitamin B12 0.01 mg; Vitamin K3 0.5 mg; biotin 10 mg; folic acid 25 mg; nicotinic acid 4000 mg; and choline chloride 100,000 mg

[a] - g/kg feed

[b] - mg/kg feed

(prepared according to Mareková et al. [27]; dose 50 µL/animal/day, with activity 12,800 AU/mL, in concentration 0.8 g/L) in their drinking water for 21 days. The activity of applied enterocins Ent M and Ent ED26E/7 was tested with the agar spot test according to De Vuyst et al. [29] against the principal indicator strain *E. avium* EA5 (isolated from piglet feces in our laboratory). The animals in group E + D were administered (days 0–21), a combination of Ent M (50 µL/animal/day, 25,600 AU/mL, in concentration 0.4 g/L) and Ent ED26E/7 (50 µL/animal/day, 12,800 AU/mL, in concentration 0.8 g/L). The doses of additives and their manner of application were decided outgoing from our previous in vitro studies testing the inhibitory activity of Ent M against target bacteria [9] and an experiment with rabbit-derived bacteriocin-producing strain *E. faecium* EF2019 (CCM7420; [12]). Based on our previous experiments, these additives can be dissolved in distilled water and/or phosphate buffer [27] and were applied firstly to 100 mL of drinking water through nipple drinkers in all cages, and after consuming this volume, the rabbits had access to water ad libitum. Control rabbits (group C) had the same conditions, but without additives being applied to their drinking water, and they were fed a commercial diet. The experiment lasted for 42 days.

The cages allowed the feces separation; faecal samples were collected using nets mounted under the cages (two to three nets/group). At the start of the experiment (at day zero, without additives inclusion), we decided to collect 10 mixed samples from all experimental and control groups—initial microbial background). Because there were two animals housed in each cage (eight cages), at days 21 (3 weeks of additives application) and 42 (end of the experiment, 3 weeks of additives cessation), we collected one sample from under each cage, which were eight samples per net, i.e., per group. At days 21 and 42, rabbits were randomly selected for slaughter ($n = 8$). Rabbits were stunned with electronarcosis (50 Hz, 0.3 A/rabbit/4 s), immediately hung by the hind legs on the processing line and quickly bled by cutting the jugular veins and the carotid arteries. Caecum and appendix were sampled to test microbial profile and enzymatic activity.

Microbial Isolation and Analysis

To test microbiota, the samples of feces and appendix content (1 g) were treated using the standard microbiological diluton method (International Organization for Standardization, ISO). The appropriate dilutions in Ringer solution (pH 7.0; Oxoid Ltd., Basingstoke, Hampshire, England) were plated onto following media: M-Enterococcus agar (NF-V04503, Difco Laboratories, Detroit, MI, USA) for enterococci, DeMann-Rogosa-Sharpe agar (ISO 15,214, Merck KGAA, Darmstadt, Germany) for lactic acid bacteria (LAB), mannitol salt agar for coagulase-negative staphylococci (CoNS, ISO 6888, Difco), Baird-Parker agar enriched with egg yolk tellurite supplement (ISO 21,527–1, Difco) for coagulase-positive staphylococci and *S. aureus* (CoPS), Clostridium difficile agar with the supplement SR0096E 7% (v/v; Oxoid) defibrinated horse blood (SR0050, ISO 15,883, Oxoid) for *Clostridium* species (anaerobic cultivation), and MacConkey agar (ISO 7402, Oxoid) for coliforms. Pseudomonas were isolated on Pseudomonas agar (Biomark Laboratories, Dhayari, Pune, Maharashtra, India). Cultivation was performed at 30 °C and/or 37 °C for 24–48 h depending on the bacterial genera. The bacterial counts were expressed in log 10 of colony forming units per gram (log 10 CFU/g ± SD). Randomly picked up representants of

selected bacterial groups were confirmed by MALDI-TOF identfication system (Bruker Daltonics, Billerica, MA, USA).

Organic Acid Analyses and Measurement of Enzymatic Activity

Lactic acid (g/100 g) and volatile fatty acid (VFA) values (acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, and caproic acids) were determined (mmol/L) using Perkin Elmer Autosystem XL Gas Chromatograph (Perkin Elmer Inc., Waltham, MA, USA) from samples of caecal content (15 g) on days 21 and 42. A glass column (average diameter 3 mm, length 180 cm) was filled with N_2 (30 mL), H_2 (20 mL) and air (240 mL) and a sample (1 µL) for diffusion. As the standard column was used, isocapron acid (SP 1200 H_3PO_4) on Chromosorbe WAW (Restek Corporation, Bellefonte, PA, USA) was separated at 130 °C and at 125 °C on Chromatone N-AW-DMCS (Fabrimat, Paris, France). The value of pH was measured with a Jenway 3310 pH meter (Jenway, Essex, England).

The degradation of plant carbohydrates (cellulose, starch, inulin, pectin, xylan) was determined using the method previously described by Miltko et al. [30].

Statistical Analysis

Statistical analysis of tested parameters was performed using two-way analysis of variance (ANOVA), followed by a Bonferroni post-hoc test for pair-wise comparisons, where appropriate. The statistical model included the time and treatment effects and their interactions. The results are quoted as means ± SD. The results were compared between groups within the same days of samples collections to check the changes during the experiment within individual experimental groups. Differences between the mean values of the different dietary treatments were considered statistically significant at $P < 0.05$. All statistical analyses were performed using GraphPad Prism statistical software (GraphPad Prism version 6.0, GraphPad Software, San Diego, CA, USA).

Results

The animals remained in good health condition throughout the trial. All tested bacterial counts in feces were influenced by time, except *Clostridium*-like sp. (Table 2). Significant reduction in coliforms was noted after both Ent M and Ent ED26E/7 addition (day 21; E, D, E + D: $P < 0.001$) and in pseudomonas during durancin Ent ED26E/7 application (D: $P < 0.05$), compared to control data. The tendency to reduce several bacterial strains was also recorded 3 weeks after treatment cessation (day 42): decrease in CoPS (E vs. D, C: $P < 0.01$, E vs. E + D: $P < 0.001$), LAB (D vs. E + D: $P < 0.001$, D vs. C: $P < 0.01$) and coliforms (E + D vs. E, D: $P < 0.05$) was found.

In caecal samples, slightly (numerical, not significant) reduced counts of coliforms, pseudomonas, and clostridia were recorded during enterocin addition, both separately and in combination (Table 3).

Table 2 Bacterial counts (log 10 CFU/g ± SD) in the feces of rabbits during enterocin EntM (E), durancin EntED26E/7 (D), and their combinative (E + D) application

Parameter	Day	E	D	E+D	C	Significance of effects		
						Treatment	Time	Treatment × time
Enterococcus sp.	21	3.54 ± 0.35	2.67 ± 1.10	3.49 ± 0.23	3.61 ± 0.73	0.1428	0.0008	0.0693
	42	3.07 ± 1.36^{a}	2.49 ± 0.29^{ab}	2.00 ± 1.62^{b}	2.00 ± 0.39^{b}			
LAB	21	3.62 ± 0.32	3.26 ± 0.14	3.19 ± 0.32	3.23 ± 0.89	0.0293	0.0280	0.0043
	42	3.50 ± 0.26^{ab}	3.05 ± 0.14^{a}	3.99 ± 0.59^{b}	3.77 ± 1.13^{b}			
CoNS	21	3.07 ± 0.87	2.47 ± 0.19	2.42 ± 0.98	3.05 ± 0.89	0.1212	<0.0001	0.2847
	42	3.75 ± 0.21^{a}	3.90 ± 0.40^{b}	3.31 ± 0.87^{c}	3.61 ± 0.22^{b}			
CoPS	21	3.45 ± 0.51	3.16 ± 0.29	3.42 ± 0.39	3.25 ± 0.42	0.0006	<0.0001	0.0002
	42	2.18 ± 0.37^{a}	2.73 ± 0.23^{b}	3.31 ± 0.17^{c}	2.82 ± 0.21^{b}			
Coliform bacteria	21	1.56 ± 0.14^{a}	1.67 ± 0.77^{b}	2.13 ± 0.61^{b}	4.79 ± 0.69^{c}	<0.0001	<0.0001	<0.0001
	42	1.64 ± 0.70^{a}	1.56 ± 0.45^{b}	0.94 ± 0.09^{c}	1.20 ± 0.57^{b}			
Pseudomonas-like sp.	21	4.94 ± 0.59^{ab}	4.41 ± 0.36^{ab}	4.55 ± 0.82^{a}	5.14 ± 0.41^{b}	0.0028	<0.0001	<0.0001
	42	3.65 ± 0.17^{a}	5.19 ± 1.19^{b}	3.32 ± 0.45^{a}	3.34 ± 0.10^{a}			
Clostridium-like sp.	21	4.20 ± 0.84	4.50 ± 0.39	5.12 ± 0.92	4.54 ± 0.69	0.1080	0.6213	0.3059
	42	4.64 ± 0.65	4.96 ± 0.11	4.32 ± 1.16	4.84 ± 0.65			

Mean values within lines with different superscript letters are significantly different ($p < 0.05$) using by Bonferroni's post hoc test

LAB lactic acid bacteria, *CoNS* coagulase-negative staphylococci, *CoPS* coagulase-positive staphylococci, *sp.* species

Table 3 Bacterial counts (log 10 CFU/g ± SD) in the caecum of rabbits during enterocin EntM (E), durancin EntED26E/7 (D), and their combinative (E + D) application

Parameter	Day	E	D	E + D	C	Significance of effects		
						Treatment	Time	Treatment × time
Enterococcus sp.	21	2.16 ± 1.00	1.95 ± 0.14	1.91 ± 1.13	1.59 ± 0.97	0.9100	0.2433	0.3491
	42	1.42 ± 0.73	1.20 ± 0.29	1.57 ± 0.65	2.10 ± 0.77			
LAB	21	2.05 ± 0.99	1.45 ± 0.39	1.70 ± 1.01	2.17 ± 1.06	0.4952	0.0823	0.8994
	42	2.28 ± 0.34	2.11 ± 0.16	2.35 ± 0.39	2.48 ± 0.50			
CoNS	21	2.63 ± 0.17	2.59 ± 0.80	2.93 ± 0.73	2.44 ± 0.23	0.6269	0.1548	0.9317
	42	2.43 ± 0.53	2.40 ± 0.35	2.43 ± 0.34	2.20 ± 0.21			
CoPS	21	0.90 ± 0.00	1.59 ± 1.04	1.44 ± 0.19	0.99 ± 0.00	0.0699	0.0212	0.1755
	42	1.61 ± 0.34^{a}	1.66 ± 0.74^{ab}	1.93 ± 0.38^{b}	1.87 ± 0.66b			
Coliform bacteria	21	0.98 ± 0.10	0.90 ± 0.00	1.26 ± 0.71	1.40 ± 0.93	0.0282	0.0161	0.0670
	42	0.90 ± 0.00^{a}	2.09 ± 0.33^{b}	1.32 ± 0.53^{ab}	2.07 ± 1.34^{ab}			
Pseudomonas-like sp.	21	3.12 ± 0.57	2.63 ± 1.23	2.92 ± 0.36	3.33 ± 0.45	0.9510	0.2109	0.5023
	42	3.42 ± 0.92	3.52 ± 0.36	3.69 ± 1.33	2.98 ± 0.97			
Clostridium-like sp.	21	1.63 ± 0.59	1.92 ± 0.19	2.86 ± 0.74	2.75 ± 1.35	0.0932	0.3536	0.3623
	42	2.39 ± 0.99	2.40 ± 0.26	2.37 ± 0.26	2.96 ± 1.28			

Mean values within lines with different superscript letters are significantly different ($p < 0.05$) using by Bonferroni's post hoc test

LAB lactic acid bacteria, *CoNS* coagulase-negative staphylococci, *CoPS* coagulase-positive staphylococci, *sp.* species

No antimicrobial effects of tested Ent M and Ent ED26E/7 were noted in the appendix. Bacterial counts of coagulase-negative staphylococci (CoNS) were affected by the treatment, with significant increase in groups E and D compared to C ($P < 0.05$; Table 4). Time influenced LAB alone, while the treatment × time interaction was significant only for coliforms. Surprisingly, the highest counts of coliforms were detected in E + D group (day 21; $P < 0.05$).

In general, increased enzymatic activity was noted in the caecal content of rabbits administered Ent M and Ent ED26E/7 alone (groups E and D), except for the amylolytic activity in group D (day 21; Table 5). The most significant changes were noted after EntM addition in pectinolytic (E vs. D, E + D: $P < 0.01$), amylolytic (E vs. D, E + D. $P < 0.001$; E vs. C: $P < 0.01$), and inulolytic (E vs. E + D: $P < 0.01$; E vs. C: $P < 0.05$) activity. Ent ED26E/7 supplementation mostly

Table 4 Bacterial counts (log 10 CFU/g ± SD) in the appendix of rabbits during enterocin EntM (E), durancin EntED26E/7 (D), and their combinative (E + D) application

Parameter	Day	E	D	E + D	C	Significance of effects		
						Treatment	Time	Treatment × time
Enterococcus sp.	21	3.84 ± 1.72	4.47 ± 0.56	3.90 ± 0.91	3.33 ± 1.70	0.6601	0.0814	0.9892
	42	2.85 ± 1.63	3.54 ± 1.17	2.99 ± 2.10	2.68 ± 1.10			
LAB	21	4.03 ± 0.96	4.49 ± 0.54	4.00 ± 1.14	3.45 ± 1.50	0.2337	0.0250	0.2724
	42	2.01 ± 0.25	3.54 ± 0.84	3.18 ± 1.45	3.16 ± 0.91			
CoNS	21	3.97 ± 1.31^{a}	3.78 ± 1.20^{a}	3.00 ± 0.90^{ab}	2.15 ± 0.39^{b}	0.0038	0.0531	0.2478
	42	4.00 ± 1.16^{a}	2.17 ± 0.50^{b}	1.88 ± 0.74^{b}	2.12 ± 0.59^{b}			
CoPS	21	4.11 ± 0.75	4.19 ± 0.98	3.33 ± 1.83	3.20 ± 1.74	0.7980	0.2326	0.8997
	42	3.15 ± 1.72	3.15 ± 1.06	3.06 ± 1.83	3.00 ± 1.08			
Coliform bacteria	21	1.50 ± 1.07^{ab}	1.43 ± 1.05^{ab}	2.97 ± 1.84^{a}	0.90 ± 0.00^{b}	0.1052	0.6375	0.0465
	42	1.13 ± 0.47^{a}	1.08 ± 0.35	2.13 ± 2.01^{ab}	3.25 ± 1.86^{b}			
Pseudomonas-like sp.	21	4.25 ± 0.99	4.57 ± 0.68	3.64 ± 1.53	4.41 ± 1.25	0.7067	0.8082	0.8548
	42	4.14 ± 0.87	3.95 ± 1.06	3.96 ± 1.19	4.44 ± 0.39			
Clostridium-like sp.	21	2.67 ± 1.00	3.31 ± 0.90	4.21 ± 1.60	3.19 ± 1.45	0.1661	0.0064	0.9194
	42	1.66 ± 0.60	2.38 ± 0.42	2.62 ± 1.11	2.19 ± 0.32			

Mean values within lines with different superscript letters are significantly different ($p < 0.05$) using by Bonferroni's post hoc test

LAB lactic acid bacteria, *CoNS* coagulase-negative staphylococci, *CoPS* coagulase-positive staphylococci, *sp.* species

Table 5 The effect of enterocin EntM (E), durancin EntED26E/7 (D), and their combinative (E + D) application on the digestion rate of carbohydrates in caecum of rabbits

Parameter	Day	E	D	E + D	C	Significance of effects		
						Treatment	Time	Treatment × time
CMC (μmol glucose/g DM of ceacum digesta/min)	21	11.36 ± 2.82^{ab}	13.22 ± 3.88^{a}	8.64 ± 1.69^{b}	8.40 ± 1.48^{b}	0.1432	< 0.0001	0.0217
	42	6.82 ± 0.47	5.92 ± 0.80	6.89 ± 0.74	6.83 ± 0.90			
Xylan (μmol xylose/g DM of ceacum digesta/min)	21	14.62 ± 0.98^{ab}	16.73 ± 2.64^{a}	11.58 ± 1.86^{b}	11.75 ± 3.13^{b}	0.0200	0.4730	0.0160
	42	16.05 ± 1.10	13.03 ± 0.82	14.20 ± 2.67	13.43 ± 1.20			
Pectin (μmol xylose/g DM of ceacum digesta/min)	21	14.11 ± 1.19^{a}	12.84 ± 2.69^{ab}	11.17 ± 1.33^{b}	10.94 ± 1.87^{b}	0.0155	0.0002	0.0759
	42	11.45 ± 2.02^{a}	7.72 ± 1.28^{b}	9.86 ± 1.00^{ab}	9.90 ± 1.34^{ab}			
Starch (μmol xylose/g DM of ceacum digesta/min)	21	16.69 ± 3.55^{a}	9.27 ± 1.84^{b}	9.22 ± 2.34^{b}	10.63 ± 1.93^{b}	< 0.0001	0.5066	0.6115
	42	16.41 ± 1.21^{a}	10.13 ± 2.93^{b}	7.06 ± 2.06^{b}	10.07 ± 1.48^{b}			
Inulin (μmol xylose/g DM of ceacum digesta/min)	21	6.02 ± 2.58^{a}	4.12 ± 1.49^{ab}	3.41 ± 0.70^{b}	3.73 ± 0.55^{b}	0.2803	0.8682	0.0588
	42	4.14 ± 0.14	4.22 ± 0.40	4.83 ± 0.70	4.37 ± 0.49			

Mean values within lines with different superscript letters are significantly different ($p < 0.05$) using by Bonferroni's post hoc test

influenced cellulolytic (D vs. E + D: $P < 0.01$) and inulolytic (D vs. E + D, C: $P < 0.01$) activity. After treatment cessation (day 42), higher activity of tested enzymes was noted for xylan, pectin (E vs. D: $P < 0.01$), and starch (E vs. D, C: $P < 0.01$; E vs. E + D: $P < 0.001$) degradation using Ent M. During Ent ED26E/7 administration, the lowest pectinolytic (D) and amylolytic (E + D) activity was detected in all experimental and control groups. Fermentation processes in the caecum were influenced only by time, in the case of ammonia (NH_3) and lactic acid (LA) production (Table 6). While increased NH_3 level was noted during Ent ED26E/7 application (D, E + D), higher LA values were recorded when using the two additives separately (E, D). Lower levels of most tested volatile fatty acids (VFA), namely, acetic, propionic, iso-butyric, valeric, and caproic acid, were found during Ent M and Ent ED26E/7 supplementation, while the

Table 6 The effect of enterocin EntM (E), durancin EntED26E/7 (D), and their combinative (E + D) application on organic acids concentrations and on the digestion rate of carbohydrates in caecum of rabbits

Parameter	Day	E	D	E + D	C	Significance of effects		
						Treatment	Time	Treatment × time
pH	21	5.83 ± 0.14	5.76 ± 0.11	5.69 ± 0.19	5.85 ± 0.21	1.0000	0.8117	0.9999
	42	6.08 ± 0.06	6.15 ± 0.19	6.31 ± 0.20	6.21 ± 0.09			
NH_3 (mmol/L)	21	12.907 ± 1.741	13.645 ± 1.220	13.807 ± 3.095	12.249 ± 2.052	0.8322	0.0005	0.9629
	42	22.032 ± 2.131	20.552 ± 5.237	22.525 ± 4.032	19.072 ± 2.262			
Lactic acid (g/100 g)	21	28.675 ± 10.787	24.875 ± 10.498	17.057 ± 1.266	17.500 ± 2.440	0.5461	0.0006	0.1689
	42	10.450 ± 0.660	10.675 ± 2.274	13.375 ± 3.452	12.300 ± 2.440			
Acetic acid (mmol/100 mL)	21	12.399 ± 0.666	11.149 ± 1.345	11.579 ± 1.685	12.529 ± 3.258	0.9492	0.0074	0.9994
	42	6.823 ± 1.104	6.087 ± 1.377	6.592 ± 1.070	7.471 ± 1.086			
Propionic acid (mmol/100 mL)	21	0.721 ± 0.179	0.512 ± 0.119	0.540 ± 0.136	0.746 ± 0.404	0.9999	0.9119	0.9994
	42	0.451 ± 0.051	0.447 ± 0.070	0.419 ± 0.104	0.452 ± 0.095			
Iso-butyric acid (mmol/100 mL)	21	0.002 ± 0.001	0.004 ± 0.002	0.006 ± 0.005	0.012 ± 0.015	1.0000	0.9903	1.0000
	42	0.026 ± 0.007	0.022 ± 0.010	0.029 ± 0.006	0.029 ± 0.015			
Butyric acid (mmol/100 mL)	21	3.398 ± 0.554	3.276 ± 0.673	3.413 ± 0.657	2.956 ± 0.682	0.9989	0.3986	0.9999
	42	1.934 ± 0.468	1.873 ± 0.434	1.777 ± 0.608	1.662 ± 0.659			
Iso-valeric acid (mmol/100 mL)	21	0.284 ± 0.160	0.119 ± 0.064	0.056 ± 0.020	0.073 ± 0.025	1.0000	0.9906	1.0000
	42	0.117 ± 0.017	0.111 ± 0.019	0.109 ± 0.019	0.115 ± 0.048			
Valeric acid (mmol/100 mL)	21	0.091 ± 0.027	0.096 ± 0.014	0.097 ± 0.027	0.104 ± 0.030	1.0000	0.9929	1.0000
	42	0.105 ± 0.005	0.108 ± 0.021	0.124 ± 0.016	0.111 ± 0.016			
Caproic acid (mmol/100 mL)	21	0.080 ± 0.028	0.122 ± 0.024	0.072 ± 0.043	0.125 ± 0.077	1.0000	0.9740	1.0000
	42	0.054 ± 0.020	0.043 ± 0.015	0.036 ± 0.017	0.045 ± 0.041			

concentrations of butyric and iso-valeric acids increased through the treatment period. The highest butyric and lowest iso-butyric levels were found after Ent M addition (E). Surprisingly, the lowest iso-valeric concentration was noted during combined addition of Ent M and Ent ED26E/7 (E + D), in contrast to their separate application (groups E and D).

In general, increased enzymatic activity was noted in the caecal content of rabbits administered Ent M and Ent ED26E/7 alone (groups E and D), except for the amylolytic activity in group D (day 21; Table 5). The most significant changes were noted after EntM addition in pectinolytic (E vs. D, E + D: $P < 0.01$), amylolytic (E vs. D, E + D. $P < 0.001$; E vs. C: $P < 0.01$) and inulolytic (E vs. E + D: $P < 0.01$; E vs. C: $P < 0.05$) activity. Ent ED26E/7 supplementation mostly influenced cellulolytic (D vs. E + D: $P < 0.01$) and inulolytic (D vs. E + D, C: $P < 0.01$) activity. After treatment cessation (day 42), higher activity of tested enzymes was noted for xylan, pectin (E vs. D: $P < 0.01$) and starch (E vs. D, C: $P < 0.01$; E vs. E + D: $P < 0.001$) degradation using Ent M. During Ent ED26E/7 administration, the lowest pectinolytic (D) and amylolytic (E + D) activity was detected in all experimental and control groups.

Fermentation processes in the caecum were influenced only by time, in the case of ammonia (NH_3) and lactic acid (LA) production (Table 6). While increased NH_3 level was noted during Ent ED26E/7 application (D, E + D), higher LA values were recorded when using the two additives separately (E, D). Lower levels of most tested volatile fatty acids (VFA), namely, acetic, propionic, iso-butyric, valeric, and caproic acid, were found during Ent M and Ent ED26E/7 supplementation, while the concentrations of butyric and iso-valeric acids increased through the treatment period. The highest butyric and lowest iso-butyric levels were found after Ent M addition (E). Surprisingly, the lowest iso-valeric concentration was noted during combined addition of Ent M and Ent ED26E/7 (E + D), in contrast to their separate application (groups E and D).

Discussion

The antimicrobial effect of bacteriocins is well known. The broad antimicrobial spectrum of enterocins against Gram-negative but mostly Gram-positive bacteria has been already presented in many in vitro and in vivo studies, including experiments with rabbits [9–12, 16, 19], similarly to our present results. Most studies report that Gram-positive bacteria tend to be more sensitive to enterocins than Gram-negative ones [31], because of the stronger membrane of the latter bacteria. These findings were also confirmed in previous experiments with enterocins Ent 7420, Ent 4231, Ent M, and nisin inclusion in rabbit diets, when the inhibition of staphylococci, enterococci and clostridia was reported [9, 10, 12, 19]. Kritas et al. [32] found lower frequency of *Clostridium perfringens*, but also of *E. coli* in probiotic-treated rabbits. In the present study, stronger antimicrobial effect of both enterocin Ent M and durancin Ent ED26E/7 against coliforms and pseudomonas was detected even during their addition (day 21), similar to other trials [10, 12, 19], whereas the inhibition of Gram-positive bacteria, i.e., staphylococci, clostridia, and lactic acid bacteria (LAB), was noted only after cessation of treatment (day 42). This observation confirms the antimicrobial potency of the tested enterocins; they represent a promising way of preventing possible gastrointestinal infections and dysbiosis caused by *E. coli* or clostridia, such as multifactorial epizootic rabbit enteropathy (ERE). To this day, the aetiology of ERE is still not exactly identified, and many factors remain unknown regarding the infection and its negative economic impact (high morbidity, mortality, diarrhoea incidence, weight loss). In general, numerical (not significant) inhibition/reduction of clostridia, pseudomonas, and coliforms was detected in this study, similar to our previous results [9, 10]. Caecal microbiota and changes in bacterial counts can also influence fermentation processes in the caecum and help in nutrition digestion, due to the ability to synthesize bacterial enzymes for plant cell-wall degradation and/or fibre hydrolyzation. Higher counts of the tested bacteria were detected in the appendix than in the caecum; these data again confirm our results recorded in our previous experiment with Ent M and its producer strain *Enterococcus faecium* AL41 application in rabbits [9]. In that trial, reduced bacterial counts were found in the appendix of treated animals compared to control, which also accords with results obtained during nisin treatment [10] and combined application of Ent M with sage extract [16]. We expected similar results in our present experiment with Ent M and Ent ED26E/7 as well, but surprisingly no inhibitory effect on tested bacteria was observed. While the antimicrobial activity of nisin, Ent M, and Ent ED26E/7 has already been presented and repeatedly confirmed under in vitro conditions, and nisin and Ent M have also been tested under in vivo conditions in the rabbit ecosystem, durancin Ent ED26E/7 itself was first applied to rabbits in this experiment, and further studies are needed to explain its effect within the gastrointestinal tract of rabbits. The appendix is closely related to rabbit immunity via the development of gut-associated lymphoid tissue (GALT) and can be improved/enhanced by microbiota, which play an essential role in rabbit appendix development and diversity in the primary antibody repertoire [33]. Information about the microbiota in the rabbit appendix is generally scarce. For this reason, monitoring microbiological changes during bioactive substance administration can help us better understand the complexity of digestion and immune response in rabbits.

The caecal microbial population changes in relation to age (before and after weaning) started from the second day of

treatment onwards, with quantified total bacteria (*Firmicutes* and *Bacteroides-Prevotella* groups) as well as archaea identified only from the seventh day onwards [22]. The authors further noted that *Firmicutes* reached their maximum between days 14 and 21 (about 80–90%; [20, 34], while *Bacteroides-Prevotella* sp. increased from day 14 to 21, remained stable until day 35 and then decreased until day 70 to a similar level as at day 14. In accord with these observations, stable counts of aerobic bacterial species from *Firmicutes* were noted during this experiment (between 35 and 77 days of age, 3 weeks of additive application and 3 weeks after treatment cessation). However, we did not monitor the strictly anaerobic *Bacteroides-Prevotella* sp. counts, as we expected them to increase during the trial. The importance of caecal bacteria lies mostly in their ability to produce hydrolyzing enzymes and their role in degradation of plant cell-wall components, which cannot be decomposed by the host digestive enzymes. Changes in caecal microbial composition are caused not only by ageing, but also by diet changing from milk to solid feed [25], whereas stable levels of bacterial community are reached during weaning [24]. Our results show no effect of age on caecal enzymatic activity (except for xylanolytic activity) at the end of the experiment, in contrast to data obtained previously during Ent M and sage extract application in rabbits [17]. Although the production of amylolytic and cellulolytic enzymes by *Clostridium* sp. has been described [35, 36], enzymes produced by strictly anaerobic *Bacteroides* and *Bifidobacterium* showed stronger xylanolytic, pectinolytic, cellulolytic, and amylolytic activity [35, 37]. Because we observed no significant changes in the counts of tested aerobic bacteria, including clostridia, we hypothesize higher fermentative activity by the anaerobic caecal microbiota. However, the diversity of the bacterial population in the rabbit caecum is very complex, and most of these bacteria are still attributed to a new uncultivated bacterial species not found in the databases [20, 21, 23].

In general, higher enzymatic activity is detected in young rather than adult rabbits, mostly in rabbits receiving Ent M and Ent ED26E/7. These data contrast with our previous results obtained from Ent M and sage extract application in rabbits [17], but they correspond to higher starch, pectin, xylan and cellulose fermentation with increasing age, as presented by Lavrenčič et al. [38]. Marounek et al. [39] found lower amylolytic, but higher cellulolytic, xylanolytic, and inulolytic activity in younger rabbits, in accord with our results. In general, enhanced enzymatic activity noted in experimental rabbits receiving both Ent M and Ent ED26E/7 confirm that they induce a more stable caecal ecosystem, with the capability of better microbial fermentation and nutrient utilization, also improving the growth rate and feed conversion (data not shown; [8]).

During fermentation processes, volatile fatty acids (VFA) are produced and continuously supply from 30 to 50% of adult rabbits´ energy requirements. These VFA are detected in a specific ratio in rabbits, with a predominance of acetate, followed by butyrate and propionate (respectively 77 mmol/100 mL; 17 mmol/100 mL and 6 mmol/100 mL on average; [22]). Fibre content in feed can affect this ratio, with higher acetate and lower butyrate proportions noted with increasing fibre level. Fermentative parameters are also age-related: Combes et al. [21] found increased total VFA concentration with increasing age (between 28 and 70 days of age), and in parallel, reduced propionate to butyrate ratio (decrease in propionate and increase in butyrate level). In contrast to these findings, our data did not show any age-related changes in the main VFA proportions. On the other hand, we noted some treatment effect on the propionate/butyrate ratio, when increased butyrate and decreased propionate concentrations were detected during enterocin addition, with lower levels of acetate. There are various studies describing the effects of bioactive compounds on VFA and organic acid production in the rabbit caecum; while some authors observed beneficial effects mostly of probiotics and herbal extracts on caecal fermentation processes [40], others describe no or negative impact of additives on their tested parameters [10, 41]. Until now, only a few studies have presented the effects of bacteriocins and/or enterocins on VFA and lactic acid production in the rabbit caecum; while enterocin Ent 4231 did not influence caecal VFA content [19], nisin and Ent M addition decreased the tested VFA and lactic acid levels [9, 10, 17]. Our results also confirm the previous findings, except for lactic, butyric, and isovaleric acid concentrations, which were found to be higher compared to control data. The highest butyric and lowest iso-valeric, caproic, and lactic acid levels measured in rabbits receiving Ent M and Ent ED26E/7 in combination may indicate the strengthening effect of both additives. It was also interesting to find that combined administration of Ent ED26E/7 and Ent M affected iso-valeric acid levels in the opposite way compared to their separate application. Further experiments are needed to determine possible synergistic and/or antagonistic effects of both additives on the tested parameters. Stimulation of caecal enzymatic activity increases caecal pH via higher VFA concentrations, thus indicating reduction in intestinal pathogens and beneficial influence on nutrient digestibility. Increased butyric, isovaleric, and lactic acid levels found in our experiment did not cause any reduction in caecal pH and bacterial population, as also previously described by Phuoc and Jamikorn [40] during probiotic application and by Lauková et al. [10] after nisin administration to rabbits.

Antibacterial effects of enterocin Ent M and durancin Ent ED26/7 were found in the gastrointestinal tract of rabbits, demonstrated by reduced counts of coliforms and pseudomonas in faeces and lower levels of coliforms and

clostridia in the caecum. In the appendix, coagulase-negative staphylococci significantly increased. Chymus samples from young rabbits showed higher caecal enzymatic activity compared to adult animals. Fermentation processes in the caecum were stimulated by Ent M and Ent ED26ED/7 administration. Synergistic/strengthening effects of enterocins were observed in rabbits receiving the combination of Ent M and Ent ED26E/7. Based on the experimental rabbits' good health, the reduced potential pathogenic microbiota in their intestines, and their stimulated caecal enzymatic activity, we conclude that enterocin Ent M and durancin Ent ED26E/7 could be used as promising novel feed additives in rabbit nutrition.

Acknowledgements We are grateful to Mrs. M. Bodnárová and Mr. P. Jerga for their skilful technical assistance and also to Dr. V. Párkányi, Dr. R. Jurčík, and Dr. Ľ. Ondruška from the National Agricultural and Food Centre in Nitra for caecal sampling and Mr. J. Pecho for slaughtering.

Author Contibution AL: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Validation, Writing—review and editing. ĽCh: Data curation, Methodology. AK, JŠ, VS, RM, GB: Methodology. MPS: Project administration, Writing—original draft preparation, Writing—review and editing.

Funding This work was supported by the Slovak Grant Agency VEGA, grunt numbers 2/0006/17 and 2/0005/21.

Data Availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest The authors declare no competing interests.

References

1. OECD FAO (2018) OECD – FAO Agricultural Outlook 2018–2027. OECD Publishing, Paris. https://doi.org/10.1787/agr_outlook-2018-e
2. Franz CM, van Belkum MJ, Holzapfel WH, Abriouel H, Gálvez A (2007) Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. FEMS Microbiol Rev 31:293–310. https://doi.org/10.1111/j.1574-6976.2007.00064.x
3. Ahmad V, Khan MS, Jamal QMS, Alzohairy MA, Al Karaawi MA, Siddigui MU (2017) Antimicrobial potential of bacteriocins: in therapy, agriculture and food preservation. Int J Antimicrob Agents 49:1–11. https://doi.org/10.1016/j.ijantimicag.2016.08.016
4. Bemena LD, Mohamed LA, Fernandes AM, Lee BH (2014) Applications of bacteriocins in food, livestock health and medicine. Int J Curr Microbiol App Sci 3:924–949. https://doi.org/10.1039/D0RA06161A
5. Lagha AB, Haas B, Gottschalk M, Grenier D (2017) Antimicrobial potential of bacteriocins in poultry and swine production. BMC Vet Res 48(1):22. https://doi.org/10.1186/s13567-017-0425-6
6. Cavera VL, Arthur TD, Kashtanov D, Chikindas ML (2015) Bacteriocins and their position in the next wave of conventional antibiotics. Int J Antimicrob Agents 46:494–501. https://doi.org/10.1016/j.ijantimicag.2015.07.011
7. Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, Drider D (2019) Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Front Microbiol 10:57. https://doi.org/10.3389/fmicb.2019.00057
8. Chrastinová Ľ, Lauková A, Chrenková M, Poláčiková M, Formelová Z, Kandričáková A, Glatzová E, Ščerbová J, Bučko O, Ondruška Ľ, Szabóová R, Jurčík R (2018) Effects of enterocin M and durancin ED26E/7 substances applied in drinking water on the selected carcass characteristics and meat quality of broiler rabbits. Arch Zootech 21:5–17
9. Lauková A, Chrastinová Ľ, Pogány Simonová M, Strompfová V, Plachá I, Čobanová K, Formelová Z, Chrenková M, Ondruška Ľ (2012) *Enterococcus faecium* AL 41:its enterocin M and their beneficial use in rabbits husbandry. Probiotics Antimicrob Proteins 4:243–249. https://doi.org/10.1007/s12602-012-9118-7
10. Lauková A, Chrastinová Ľ, Plachá I, Kandričáková A, Szabóová R, Strompfová V, Chrenková M, Čobanová K, Žitňan R (2014) Beneficial effect of lantibiotic nisin in rabbit husbandry. Probiotics Antimicrob Proteins 6:41–46. https://doi.org/10.1007/s12602-014-9156-4
11. Lauková A, Pogány Simonová M, Chrastinová Ľ, Gancarčíková S, Kandričáková A, Plachá I, Chrenková M, Formelová Z, Ondruška Ľ, Ščerbová J, Strompfová V (2018) Assessment of Lantibiotic type bacteriocin—gallidermin application in model experiment with broiler rabbits. Int J Anim Sci 2(4):1028
12. Pogány Simonová M, Lauková A, Chrastinová Ľ, Strompfová V, Faix Š, Vasilková Z, Ondruška Ľ, Jurčík R, Rafay J (2009) *Enterococcus faecium* CCM7420, bacteriocin PPB CCM7420 and their effect in the digestive tract of rabbits. Czech J Anim Sci 54:376–386. https://doi.org/10.17221/1659-CJAS
13. Pogány Simonová M, Lauková A, Plachá I, Čobanová K, Strompfová V, Szabóová R, Chrastinová Ľ (2013) Can enterocins affect phagocytosis and glutathione peroxidase in rabbits? Cent Eur J Biol 8:730–734. https://doi.org/10.2478/s11535-013-0198-x
14. Pogány Simonová M, Lauková A, Žitňan R, Chrastinová Ľ (2015) Effect of rabbit origin enterocin-producing strain *Enterococcus faecium* CCM7420 application on growth performance and gut morphometry in rabbits. Czech J Anim Sci 60:509–512. https://doi.org/10.17221/8559-CJAS
15. Pogány Simonová M, Chrastinová Ľ, Lauková A (2019) Lantibiotic nisin applied in broiler rabbits and its effect on the growth performance and carcass quality. Probiotics Antimicrob Proteins 11:1414–1417. https://doi.org/10.1007/s12602-019-9525-0
16. Pogány Simonová M, Chrastinová Ľ, Kandričáková A, Gancarčíková S, Bino E, Plachá I, Ščerbová J, Strompfová V, Žitňan R, Lauková A (2020) Can have enterocin M in combination with sage beneficial effect on microbiota, blood biochemistry, phagocytic activity and jejunal morphometry in broiler rabbits? Animals 10:115. https://doi.org/10.3390/ani10010115
17. Pogány Simonová M, Chrastinová Ľ, Kandričáková A, Kubašová I, Formelová Z, Chrenková M, Miltko R, Belzecki G, Strompfová V, Lauková A (2020) Enterocin M and sage supplementation in post-weaning rabbits: effects on growth performance, caecal microbiota, fermentation and enzymatic activity. Probiotics Antimicrob Proteins 12:732–739. https://doi.org/10.1007/s12602-019-09584-z
18. Pogány Simonová M, Chrastinová Ľ, Chrenková M, Formelová Z, Kandričáková A, Bino E, Lauková A (2020) Benefits of enterocin

M and sage combination on the physico-chemical traits, fatty acid, amino acid and mineral content of rabbit meat. Probiotics Antimicrob Proteins 12:1235–1245. https://doi.org/10.1007/s12602-019-09627-5
19. Szabóová R, Lauková A, Chrastinová Ľ, Pogány Simonová M, Strompfová V, Plachá I, Čobanová K, Vasilková Z, Chrenková M (2011) Enterocin 4231 produced by *Enterococcus faecium* CCM4231 and its use in rabbits. Acta Vet Beograd 61(5–6):523–529. https://doi.org/10.2298/AVB1106523S
20. Abecia L, Fondevila M, Balcells J, Edwards JE, Newbold JC, McEwan NR (2005) Molecular profiling of bacterial species in the rabbit cecum. FEMS Microbiol Lett 244:111–115. https://doi.org/10.1016/j.femsle.2005.01.028
21. Combes S, Michelland RJ, Monteils V, Cauquil L, Soulié V, Tran NU, Gidenne T, Fortune-Lamothe L (2011) Postnatal development of the rabbit caecal microbiota composition and activity. FEMS Microbiol Ecol 77:680–689. https://doi.org/10.1111/j.1574-6941.2011.01148.x
22. Gidenne T (1994) Estimation of volattile fatty acids and of their energetic supply in the rabbit caecum: effect of the dietary fibre level. Vleme Journees de la Recherche Cunicole, Paris, pp 293–299
23. Combes S, Fortune-Lamothe L, Cauqui L, Gidenne T (2013) Engineering the rabbit digestive ecosystem to improve digestive health and efficacy. Animal 7(9):1429–1439. https://doi.org/10.1017/s1751731113001079
24. Padilha MTS, Licois D, Gidenne T, Carre B, Fonty G (1995) Relationships between microflora and caecal fermenation in rabbits before and after weaning. Reprod Nutr Dev 35:375–386. https://doi.org/10.1051/rnd:19950403
25. Gidenne T, Fortun-Lamothe L (2002) Feeding strategy for young rabbits around weaning. A review of digestive capacity and nutritional needs. Anim Sci 75:169–184. https://doi.org/10.1017/S1357729800052942
26. Gidenne T (2015) Dietary fibres in the nutrition of the growing rabbit and recommendations to preserve digestive health: a review. Animal 9(2):227–242. https://doi.org/10.1017/S1751731114002729
27. Mareková M, Lauková A, Skaugen M, Nes FI (2007) Isolation and characterization of a new bacteriocins produced by enviromental isolate *Enterococcus faecium* AL41. J Ind Microbiol Biotechnol 34:533–537. https://doi.org/10.1007/s10295-007-0226-4
28. Lauková A, Pogány Simonová M, Chrastinová Ľ, Kandričáková A, Ščerbová J, Plachá I, Čobanová K, Formelová Z, Ondruška Ľ, Štrkolcová G, Strompfová V (2017) Beneficial effect of bacteriocin-producing strain *Enterococcus durans* ED 26E/7 in model experiment using broiler rabbits. Czech J Anim Sci 62:168–177. https://doi.org/10.17221/21/2016-CJAS
29. De Vuyst L, Callewaert R, Pot B (1996) Characterization of the antagonistic activity of *Lactobacillus amylovorus* DCE471 and large scale isolation of its bacteriocin amylovorin L471. Syst Appl Microbiol 9:9–20. https://doi.org/10.1016/S0723-2020(96)80003-8
30. Miltko R, Bełżecki G, Kowalik B, Skomiał J (2016) Presence of carbohydrate-digesting enzymes throughout the digestive tract of sheep. Turk J Vet Anim Sci 40:271–277. https://doi.org/10.3906/vet-1507-70
31. Pierozan MK, Pauletti GF, Rota L, dos Santos ACA, Lerin LA, Di Luccio M, Mossi AJ, Atti Serafini L, Cansian RL, Oliveira JV (2009) Chemical characterization and antimicrobial activity of essential oils of salvia L. species. Ciênc Tecnol Aliment 29:764–770. https://doi.org/10.1590/S0101-20612009000400010
32. Kritas SK, Petridou EI, Fortomaris P, Tzika E, Arsenos G, Koptopoulos G (2008) The effect of probiotics on microbiology, health and performance of fattening rabbits. Asian Australas J Anim Sci 21:1312–2131. https://doi.org/10.5713/AJAS.2008.70186
33. Hanson NB, Lanning DK (2008) Microbial induction of B and T cell areas in rabbit appendix. Dev Comp Immunol 32(8):980–991. https://doi.org/10.1016/j.dci.2008.01.013
34. Monteils V, Cauquil L, Combes S, Godon J-J, Gidenne T (2008) Potential core species and staellite species in the bacterial community within the rabbit caecum. FEMS Microbiol Ecol 66:626–629. https://doi.org/10.1111/j.1574-6941.2008.00611.x
35. Sirotek K, Marounek M, Suchorská O (2006) Activity and cellular localization of amylases of rabbit caecal bacteria. Folia Microbiol 51(4):309–312. https://doi.org/10.1007/BF02931821
36. Gupta P, Samant K, Sahu A (2012) Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Int J Microbiol Article ID 578925:1–5. https://doi.org/10.1155/2012/578925
37. Sirotek K, Marounek M, Rada V, Benda V (2001) Isolation and characterization of rabbit caecal pectinolytic bacteria. Folia Microbiol 46:79–82. https://doi.org/10.1007/BF02825893
38. Lavrenčič A (2007) The effect of rabbit age on vitro caecal fermentation of starch, pectin, xylan, cellulose, compound feed and its fibre. Animal 1:241–248. https://doi.org/10.1017/S1751731107303467
39. Marounek M, Vovk SJ, Skřivanová V (1995) Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. Br J Nutr 73:463–469. https://doi.org/10.1079/bjn19950048
40. Bovera F, Iannaccone F, Mastellone V, Nizza S, Lestingi A, De Martino L, Lombard P, Mallardo K, Ferrara M, Nizza A (2012) Effect of spray application of *Lactobacillus plantarum* on *in vivo* performance, caecal fermentations and haematological traits of suckling rabbits. Ital J Anim Sci 11:145–149. https://doi.org/10.4081/ijas.2012.e27
41. Phuoc TL, Jamikorn U (2017) Effects of probiotic supplement (*Bacillus subtilis* and *Lactobacillus acidophilus*) on feed efficiency, growth performance, and microbial population of weaning rabbits. Asian Australas J Anim Sci 30(2):198–205. https://doi.org/10.5713/ajas.15.0823