EFFECT OF DIET SUPPLEMENTATION WITH *ENTEROCOCCUS DURANS* ED26E/7 AND ITS DURANCIN ED26E/7 ON GROWTH PERFORMANCE, CAECAL ENZYMATIC ACTIVITY, JEJUNAL MORPHOLOGY AND MEAT PROPERTIES OF BROILER RABBITS*

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Abstract

The present study investigates the effects of *Enterococcus durans* ED26E/7 beneficial strain and its enterocin – durancin (Ent) ED26E/7 on selected parameters in rabbits: growth performance, caecal enzymatic activity, jejunal morphometry and meat physico-chemical characteristics. Seventy-two rabbits (aged five weeks, M91 meat line, both sexes) were divided into experimental groups E1 (*E. durans* ED26E/7 strain; dose 500 µL/animal/day, concentration 10⁹ CFU/mL) and E2 (durancin EntED26E/7; dose 50 µL/animal/day, with activity 12 800 AU/mL) and control group (C). The additives were administered in drinking water for a period of 21 days. All animals remained in good health during the experiment. The highest body weight gain (increase by 1.5% compared to C) was noted in E1 group during ED26E/7 strain application (P<0.001). Both bioactive compounds positively influenced (reduced) the feed conversion ratio (P<0.001). The ED26E/7 strain and its EntED26E/7 application stimulated the activity of most enzymes tested in the caecum; only the amylolytic and inulolytic activity in E2 group decreased during durancin ED26E/7 addition. Both additives, but mainly the ED26E/7 strain, showed a tendency to improve the jejunal morphological parameters till the end of the experiment (day 42). The meat physico-chemical parameters were not negatively influenced by the application of *E. durans* ED26E/7 strain and its durancin ED26E/7. The diet supplementation with bacteriocinogenic and probiotic *E. durans* ED26E/7 strain and its EntED26E/7 may improve the growth performance, caecal enzymatic activity and jejunal morphometry of rabbits, without any negative effect on rabbit meat quality.

Key words: *Enterococcus durans*, durancin, rabbit, morphometry, meat quality

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Rabbits are fragile animals with complex and unique digestive processes and as a result they are very sensitive to digestive disturbances and enteric diseases, particularly around weaning time (Gidenne and Fortun-Lamothe, 2002). For this reason antibiotics used to be widely used as therapeutic agents and growth promoters, but were finally banned in the European Union (European Commission, 2003) because of the development of multidrug resistant bacteria and antibiotic chemical residues in animal products (van den Bogaard and Stobberingh, 1999). There are continuing efforts to develop safe and natural feed additives to improve rabbit gut health and productivity. These non-antibiotic alternatives are probiotics, prebiotics, synbiotics, bacteriocins and organic acids, and herbs and their extracts, are also successfully used in rabbit breeding (Pogány Simonová et al., 2009, 2020 a, b; Lauková et al., 2012 a, b, 2014, 2017 a; Dalle Zotte et al., 2016; Kalma et al., 2016). Several studies concerning the probiotic application in rabbits such as Lact-A-Bac, Lactosacc, Acid-Pak-4-Way, Biosal, Lactina and Toyocerin, composed mainly of bacilli, lactobacilli and yeasts alone or in combination have been reported (Amber et al., 2004; Matuševičius et al., 2004, 2011). There are still only a few declared probiotic preparations in the EU based on the genera *Enterococcus*: Lactina (EFSA, 2019), Toyocerin (Matuševičius et al., 2011) and ProRabbit (Slovak Republic; Pogány Simonová et al., 2009, 2020 a), despite the abundance of caecal and faecal enterococci (Simonová and Lauková, 2004; Linaje et al., 2004). Most studies present improved health, growth performance, immunity, blood parameters and intestinal microbiota of rabbits after feed supplementation with beneficial enterococci, mainly with *Enterococcus faecium* as probiotic and/or bacteriocinogenic strains (CCM7420/EF2019 – Pogány Simonová et al., 2009, 2020 a; EK13, CCM8558/AL41 – Lauková et al., 2006, 2012 a; NCIMB30183 – Benato et al., 2014). Besides beneficial *E. faecium* strains, several *E. durans* strains with probiotic properties have also been tested under *in vitro* conditions (*E. durans* LAB18s; Pieniz et al., 2014) and also experimentally *in vivo* in mice (*E. durans* EP1; Carasi et al., 2017) and in rabbits (*E. durans* ED26E/7; Lauková et al., 2017 a). During these experiments, the immunostimulatory, anti-inflammatory, antimicrobial and antioxidant activities of tested *E. durans* strains were recorded in addition to the animals' jejunal morphometry, meat quality and composition changes (Lauková et al., 2012 a, 2014; Pogány Simonová et al., 2015, 2016, 2019, 2020 a, b, c; Chrastinová et al., 2018).

The objectives of this study were to determine the effects of *Enterococcus durans* ED26E/7 beneficial strain and its enterocin, durancin (Ent) ED26E/7, on growth performance, caecal enzymatic activity, jejunal morphometry and meat physico-chemical characteristics of rabbits.

Material and methods

Animals and housing

The experiment was conducted at the National Agricultural and Food Centre (NAFC) at Nitra-Lužianky in Slovakia. Experimental procedures followed the

guidelines approved by the State Slovak Veterinary and Food Administration and the Ethics Committees of both institutions (permission code: SK CH 17016 and SK U 18016). Seventy-two post-weaned rabbits (aged 35 days, both sexes, equal male to female ratio per treatment) were randomly divided into three experimental groups (E1 – *E. durans* ED26E/7 strain, E2 – durancin ED26E/7 and C – control) with 24 animals in each. Animals represented maternal albinotic line (crossbreed New Zealand White × Buskat Rabbit × French Silver) and paternal acromalictic line (crossbreed Nitra's Rabbit × Californian Rabbit × Big Light Silver). The rabbits were kept in standard cages (size 0.61 m × 0.34 m × 0.33 m, type D-KV-72 supplied by the Kovobel company, Domažlice, Czech Republic), two animals per cage. The cages allowed feces separation. A cycle of 16 h light and 8 h dark was used throughout the experiment. Temperature $20 \pm 4^\circ\text{C}$ and humidity $70\% \pm 5\%$ were maintained throughout the experiment by means of heating and ventilation systems, and data were recorded continuously with a digital thermograph positioned at the same level as the cages.

Experimental design, slaughtering and sampling

The animals were fed an untreated diet (pellets of 3.5 mm in average size) containing crude protein 178.0, crude fiber 147.0, crude fat 36.1, starch 129.1, total ash content 97.3 and organic matter 847.5 g per kg of diet. The ingredients and chemical composition of this diet is presented in Table 1. The metabolizable energy content was calculated using the equation of Wiseman et al. (1992). Chemical analyses were conducted according to AOAC (1995) and Gidenne et al. (2001) for dry matter (DM), crude protein (CP), crude fiber (CF), crude fat, nitrogen free extract, ash and organic matter. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analysed sequentially (Van Soest et al., 1991) with a thermo-stable amylase pre-treatment and starch according to the alpha-amylglucosidase method.

Enterococcus durans ED26E/7 (marked by rifampicin, concentration 10^9 CFU/mL) at a dose of 500 µL per animal per day was used in our experiment prepared as previously described by Lauková et al. (2012 b). Rifampicin variant (Rif^R; to distinguish it from the other enterococci) was prepared as follows: 0.1% inoculum in MRS broth (Merck, Germany) was grown for 18 h at 37°C . Overnight culture was centrifuged at 10 000 g for 30 min. Supernatant was removed and the cells were resuspended in Ringer solution (Merck) to reach the final cell concentration 10^9 CFU/mL. The counts were checked by the spreading of the dilutions in Ringer solution onto M-Enterococcus agar (Difco, USA) and incubated at 37°C for 24–48 h. Application doses (500 µL) for each animal in E1 group treatment were prepared and stored at 4°C and added for 21 days.

The rabbits in group E2 were administered durancin ED26E/7 (prepared according to Mareková et al. (2007); dose 50 µL/animal/day, with activity 12 800 AU/mL, at concentration 0.8 g/L, from day zero to day 21) in their drinking water. Activity of EntED26E/7 was tested with the agar spot test according to De Vuyst et al. (1996) against the principal indicator strain *E. avium* EA5 (isolated from piglet faeces in our laboratory). The doses of additives and their manner of application were decided on the basis of our previous *in vitro* studies testing the inhibitory activity of

EntED26E/7 against target bacteria (Lauková et al., 2012 b) and an experiment with rabbit-derived bacteriocin-producing strain *E. faecium* EF2019 (CCM7420; Pogány Simonová et al., 2009). Based on our previous experiments, these additives can be dissolved in distilled water and/or phosphate buffer (Mareková et al., 2007) and also, we had information about the volume of water drunk by rabbits; the additives were applied firstly to 100 mL of drinking water 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. Drinking water was provided through nipple drinkers. The experiment lasted for 42 days.

Table 1. Ingredients and chemical composition of granulated diet

Feed ingredients (%)		Chemical composition, minerals and vitamins (g ^a , mg ^b /kg feed)	
Dehydrated lucerne meal	36.0	Crude protein ^a	178.0
Extracted sunflower meal	5.5	Crude fiber ^a	147.0
Oats	13.0	Fat ^a	36.1
Wheat bran	9.0	Ash ^a	97.3
Dry malting sprouts	15.0	Organic compounds ^a	847.5
Extracted rapeseed meal	5.5	Acid detergent fiber (ADF) ^a	191.1
Barley	8.0	Neutral detergent fiber (NDF) ^a	339.5
DDGS	5.0	Starch ^a	129.1
Sodium chloride	0.3	Calcium ^a	6.0
Premix minerals ¹	1.7	Phosphorus ^a	5.9
Limestone	1.0	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
		Metabolizable energy (MJ/kg)	11.0

DDGS: Dried distillers grains with solubles. ¹Premix 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.

The metabolizable energy content was calculated using the equation of Wiseman et al. (1992). Dry matter, crude protein, crude fiber, crude fat, nitrogen free extract, ash, organic matter, neutral and acid detergent fiber were analysed according to AOAC (1995) and Gidenne et al. (2001).

Freshly-voided faeces were collected using nets mounted under the cages (two to three nets/group), for confirmation of the *E. durans* ED26E/7 strain. At the start of the experiment (day zero, without additives), ten samples from all experimental and control groups were collected and mixed together to establish the initial microbial background. After three weeks of additives application (day 21) and three more weeks after additives cessation (day 42, the end of the experiment), we collected eight samples per group. At days 21 and 42, rabbits were randomly selected for slaughter ($n = 8$), stunned with electronarcosis (50 Hz, 0.3 A/rabbit/4s), immediately hung by the hind legs on the processing line and quickly bled by cutting the carotid arteries and jugular veins. Caecum and jejunum were sampled; caecum for microbiota analyses and enzymatic activity and jejunum for morphometric testing.

Performance traits, enzymatic activity and morphometry testing

Body weight and feed consumption were measured every week to calculate average daily weight gain and feed conversion. Mortality and morbidity were also recorded during the experiment.

The degradation of plant carbohydrates (cellulose, starch, inulin, pectin, xylan) was determined using the method previously described by Miltko et al. (2016).

In order to perform morphometric testing (villus cut surface, villus circumference, villus height (VH), crypt depth (CD), and villus height to crypt depth ratio VH:CD), intestinal tissue of the proximal jejunum was sampled and treated as previously described by Žitňan et al. (2008). Briefly, the intestinal tissue samples (1 cm² each) were fixed in 4% neutral formaldehyde solution. After rinsing with water, the samples were dehydrated in a graded ethanol series of absolute ethanol (30%, 50%, 70%, 90%), cleared in benzene, then saturated with and embedded in paraffin. Sections of 5 µm thickness (ten slices of each sample) were stained with haematoxylin/eosin and observed under a light microscope. The circumference, cut surface area and length of 30 villi and depth of 30 crypts were determined by the computer-operated Image C picture analysis system (Imtronic GmbH, Berlin, Germany) and the interactive measurement (IMES) analysis program, by using a colour video camera (Sony 3 CCD, Sony Electronics Ltd., Tokyo, Japan) and a light microscope (Axiolab, Carl Zeiss AG, Jena, Germany).

Physico-chemical analysis of rabbit meat (*Longissimus thoracis and lumborum*)

After the bleeding, the *Longissimus thoracis and lumborum* (LTL) muscles were separated by removing the skin, fat and connective tissue, chilled and stored 24 h at 4°C until physico-chemical analysis started. The ultimate pH was determined 24 h *post mortem* with a Radelkis OP-109 (Jenway, England) with a combined electrode penetrating 3 mm into the MLD. The electrical conductivity (µS.cm⁻¹) defined as locations of muscles were evaluated using PMV 51 (Tecpro Metall GmbH, Neuss, Germany). Colour measurements were taken on MLD surface of the carcass at 24 h after bleeding. Colour characteristics were expressed using the CIE L*a*b* system (lightness-L*, 0: black and 100: white; redness and greenness-a*; yellowness and blueness-b*) using a Lab Miniscan (HunterLab, Reston, VA, USA). Lightness measurements at room temperature were also taken. Total water, protein and fat

contents were estimated using an INFRATEC 1265 spectroscope (FOSS, Tecator AB, Höganäs, Sweden) and expressed in g/100 g. The near infrared transmission (NIT) principle is based on the fact that the measured sample absorbs the near infrared light at different wavelengths according to different characteristics such as fat or protein content (Burns and Ciurczak, 2007). From these values, the energy value was calculated [$EC \text{ (kJ/100 g)} = 16.75 \times \text{protein content (g/100 g)} + 37.68 \times \text{fat content (g/100 g)}$; Strmiska et al., 1988]. Water holding capacity was determined by compression method at constant pressure (Hašek and Palanská, 1976). The analysed samples (0.3 g in weight) were placed on filter papers (Schleicher and Schuell No. 2040B, Dassel, Germany) with tweezers previously weighed. Together with the papers, samples were sandwiched between Plexiglas plates and then subjected to a pressure of 5 kg for 5 min. The results were calculated from the difference in weight between the slips with aspirating spot and the pure filter paper.

Statistical analysis

Treatment effects on the growth performance, enzymatic activity and jejunal morphometry were analysed 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 effects of period and treatment and their interactions. Differences between the mean values of the different dietary treatments were considered statistically significant at $P < 0.05$. Data are expressed as means and pooled standard deviations of the mean (SD). Statistical analysis of meat physico-chemical properties was performed with one-way analysis of variance (ANOVA), with the Tukey's post hoc test. The results are quoted as means $\pm$ 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 (small letters a, b). All statistical analyses were performed using GraphPad Prism statistical software (GraphPad Prism version 6.0, GraphPad Software, San Diego, CA, USA).

Results

Growth performance

Throughout the trial, all animals remained in good health and no mortality was noted (Table 2). The growth performance, average daily weight gain (ADWG) and feed conversion ratio (FCR) of the rabbits were influenced by period, treatment and treatment $\times$ period interaction (Table 2). The highest body weight gain was noted in the E1 group during ED26E/7 strain application (day 21; E1 vs. E2: $P < 0.001$) and ADWG during the trial increased by 1.5% compared to C. Lower feed conversion was recorded in both experimental groups compared to C (day 21; E1, E2 vs. C: $P < 0.001$; day 42: E2 vs. C: day 21; E1 vs. C: $P < 0.05$), with the lowest value detected in E1.

Table 2. The effect of *E. durans* ED26E/7 and its enterocin-duracin ED26E/7 application on the growth performance of rabbits

Parameter	Day	E1	E2	C	SD	Significance of effects		
						treatment	period	treatment $\times$ period
Initial live weight (35 d of age, g)	0	1272.9	1256.7	1321.7	109.5			
Intermediate live weight (56 d of age, g)	21	2150.6	1997.0	2120.0	113.5	<0.0001	<0.0001	0.0045
Final live weight (77 d of age, g)	42	2804.5 a	2617.5 b	2830.5 a	163.7			
Average daily weight gain (35–56 days of age, g/d)	21	41.80 a	35.25 b	38.01 ab				
Average daily weight gain (35–56 days of age, g/d)	42	31.14	29.55	33.83		<0.0001	<0.0001	<0.0001
Average daily weight gain (g/d)		36.47	32.40	35.92				
Feed conversion ratio (35–56 days of age, g/g)	21	2.42 a	2.48 a	2.76 b	0.19			
Feed conversion ratio (56–77 days of age, g/g)	42	3.39 ab	3.34 a	3.49 b	0.18	<0.0001	<0.0001	0.0087
Feed conversion ratio per kg gain		2.91	2.91	3.13	0.19			
Mortality (n)		0	0	0				

35–56 days of age – application of *E. durans* ED26E/7 and its enterocin-duracin ED26E/7.56–77 days of age – after the *E. durans* ED26E/7 and its enterocin-duracin ED26E/7 cessation.

The values in the columns E1, E2 and C are means.

a, b – mean values within lines with different letters are significantly different ($P < 0.05$) using Bonferroni's post hoc test.

Table 3. The effect of *E. durans* ED26E/7 and its enterocin-durancin ED26E/7 application on the caecal enzymatic substrates of rabbits

Parameter	Day	E1	E2	C	SD	Significance of effects		
						treatment	period	treatment $\times$ period
Cellulose (μmol glucose/g DM of caecum digesta/min)	21	8.40 a	6.57 b	6.46 b	1.69	0.0208	0.4470	0.1673
	42	7.12	6.42	6.99	1.44			
Xylan (μmol xylose/g DM of caecum digesta/min)	21	14.12	12.71	12.43	2.52	0.1615	<0.0001	0.0088
	42	9.23 a	8.84 a	11.92 b	1.74			
Pectin (μmol glucuronic acid/g DM of caecum digesta/min)	21	6.56	5.83	5.96	0.44	0.0112	<0.0001	<0.0001
	42	4.10 a	4.76 b	5.88 c	0.51			
Starch (μmol glucose/g DM of caecum digesta/min)	21	15.62 a	8.47 b	12.48 ab	2.87	0.0291	0.4352	0.1628
	42	11.0	9.62	12.89	3.13			
Inulin (μmol fructose/g DM of caecum digesta/min)	21	3.05	2.71	3.01	0.30	<0.0001	<0.0001	<0.0001
	42	2.33 a	2.73 a	3.49 b	0.63			

The values in the columns E1, E2 and C are means.

a, b – mean values within lines with different letters are significantly different ($P < 0.05$) using Bonferroni's post hoc test.

Table 4. The effect of *E. durans* ED26E/7 and its enterocin-durancin ED26E/7 application on the jejunal morphometry of rabbits

Parameter	Day	E1	E2	C	SD	Significance of effects		
						treatment	period	treatment × period
Villus cut surface (μm^2)	21	83347.0	81473.0	81102.0	2194.0	0.5264	0.1891	0.6947
	42	82043.0	81288.0	81218.0	1176.0			
Villus circumference (μm)	21	1606.0	1571.0	1558.0	40.0	0.6162	0.2495	0.5957
	42	1595.0	1575.0	1585.0	34.0			
Villus height (μm)	21	702.0	688.0	681.0	14.0	0.8787	0.6242	0.9291
	42	693.0	689.0	683.0	12.0			
Crypt depth (μm)	21	166.0 a	181.0 ab	187.0 b	10.0	0.3240	0.0050	0.5489
	42	168.0	175.0	180.0	6.0			
Villus height: crypt depth	21	4.25	3.83	3.65	0.30	0.9613	0.9309	0.9929
	42	4.13	3.95	3.80	0.22			

The values in the columns E1, E2 and C are means.

a, b – mean values within lines with different letters are significantly different ($P < 0.05$) using Bonferroni's post hoc test.

Enzymatic activity and jejunal morphometry

In general, increased enzymatic activity was observed in the caecum, except for amylolytic (day 21: E2 vs. E1, C: $P < 0.01$; Table 3) and inulolytic activity in E2 group after durancin ED26E/7 administration. This effect of treatment was observed in all enzymatic activity, except for the xylanolytic activity of caecal contents, while period and treatment $\times$ period effects were observed in xylanolytic, pectinolytic and inulolytic activity. Significant increase in cellulolytic (E1 vs. E2, C: $P < 0.01$) and amylolytic (E1 vs. E2: $P < 0.01$) activity was found in E1 after *E. facium* ED26E/7 strain application (day 21). At day 42, reduced xylanolytic (E1 vs. C: $P < 0.05$; E2 vs. C: $P < 0.001$), pectinolytic (E1 vs. E2: $P < 0.05$; E1, E2 vs. C: $P < 0.001$) and inulolytic (E1, E2 vs. C: $P < 0.001$) activity was recorded. There was lower enzymatic activity in experimental groups compared to control data after additives cessation.

Among the morphological parameters, significant period effect was recorded on crypt depth (CD; day 21; E1 vs. C: $P < 0.01$; Table 4).

Physico-chemical properties of rabbit meat (*Longissimus thoracis and lumborum*)

No significant differences were observed between the groups for rabbit meat pH₂₄, colour and chemical composition (Table 5).

Table 5. The effect of *E. durans* ED26E/7 and its enterocin-durancin ED26E/7 application on the physico-chemical composition of rabbits *Longissimus thoracis and lumborum* (LTL; means $\pm$ SD)

	E1	E2	C	SD	P-value
pH 24 h after killing	5.72	5.76	5.79	0.08	0.5597
Water content (g/100 g)	75.80	75.68	75.80	0.10	0.2801
Protein content (g/100 g)	21.64	21.81	21.73	0.11	0.1794
Fat content (g/100 g)	1.55	1.63	1.48	0.09	0.1478
Electrical conductivity (μ S)	2.57	1.97	2.18	1.04	0.9970
<i>L</i> * (lightness)	52.78	51.74	50.82	3.83	0.7080
<i>a</i> * (redness)	1.20	0.68	0.88	0.49	0.9829
<i>b</i> * (yellowness)	6.93	6.62	7.53	1.12	0.2878
Water holding capacity (g/100 g)	29.2	27.94	29.54	3.35	0.7819
Energy value (kJ/100 g)	421.50	423.13	419.30	4.29	0.5005

The values in the columns E1, E2 and C are means.

Discussion

Growth performance

Diet supplementation with bioactive compounds usually produces improved ADWG and FCR in rabbits (Falcão-e-Cunha et al., 2007; Kalma et al., 2016; Bhatt et al., 2017); these observations were also confirmed by our findings. In agreement

with the results on weight gain in this experiment, similar results were reported from this laboratory using bacteriocinogenic and probiotic *E. faecium* strains CCM4231, CCM7420(EF2019) and CCM8558(AL41) and their enterocins (Szabóová et al., 2008, 2011; Pogány Simonová et al., 2009, 2020 a; Lauková et al., 2012 a). Comparing the results achieved in trials with beneficial, bacteriocin-producing strains and their enterocins, more intensive effects on growth and ADWG of rabbits were recorded after application of probiotic strains than in rabbits receiving enterocins (Pogány Simonová et al., 2009; Lauková et al., 2012 a). FCR results accord with those reported by Szabóová et al. (2008, 2011), Pogány Simonová et al. (2009) and Lauková et al. (2012 a, b, 2017 b, 2018) for beneficial strains (CCM7420, CCM4231) and bacteriocins (Ent7420, EntM, EntCCM4231, EntEF55, gallidermin).

Caecal enzymatic activity and jejunal morphometry

Enzymatic activity in the caecum depends on the age of rabbits (Gidenne and Fortun-Lamothe, 2002). In general, higher enzymatic activity is detected in young than in adult rabbits; Marounek et al. (1995) found higher cellulolytic, xylanolytic and inulolytic activity in younger rabbits, in accordance with our results, but lower starch and pectin fermentation. Lavrenčič et al. (2007) recorded lower pectin, xylan and cellulose fermentation with increasing age, but higher intensity of starch degradation, which is similar to our results, showing a tendency to decrease till the end of the trial, except for the amylolytic activity in E2 group (EntED26E/7). On the other hand, EntM and sage application did not affect the caecal enzymatic fermentation (Pogány Simonová et al., 2020 d).

Increased enzymatic activity observed during additives application can be explained in terms of a more stable caecal ecosystem. with the capability of better microbial fermentation and nutrient utilization, also improving the growth rate and feed conversion. We expected an increase in beneficial microbiota due to the antimicrobial effect of *E. durans* ED26E/7 probiotic strain and its durancin ED26E/7 against spoilage and/or pathogenic bacteria.

Only a few studies have reported the dietary treatment effect of probiotics, prebiotics and bacteriocins on rabbit intestinal morphology (Chiou et al., 1994; Oso et al., 2013; Pogány Simonová et al., 2015, 2020 b). During our application of ED26E/7 strain and its EntED26E/7, all the tested morphological traits in the jejunum were increased compared to control data (except CD, which was decreased; E1 vs. C: $P < 0.01$), with the highest values in group E1. These results accord with those reported by Oso et al. (2013) and Pogány Simonová et al. (2015), and a study using enterocin EntM (Pogány Simonová et al., 2020 b).

Physico-chemical properties of rabbit meat (*Longissimus thoracis and lumborum*)

Application of the tested additives in this study did not significantly influence the physico-chemical properties of rabbit meat. The pH₂₄ values accord with those reported by Dalle Zotte (2002) and Polak et al. (2006), but the values were lower than pH₂₄ results achieved during enterocin EntM and sage administration to rabbits (Pogány Simonová et al., 2020 c). The negative correlation between pH value

and meat lightness (the lower the pH, the clearer the lightness; Dalle Zotte and Ou-hayoun, 1998) was also confirmed in our results. Increases in lightness (L^*) were found in E1 and E2 groups compared to C, which is similar to the results reported by Pogány Simonová et al. (2016) using CCM7420 strain, but contrary to Pogány Simonová et al. (2020 c, e) using EF9a strain and EntM. Decreased values of yellowness (b^*) accord with achievements based on EntM application. Regarding low yellowness data we assume there was no or only minimal free radicals formation. On the other hand, Bhatt et al. (2017) also demonstrated higher lipid concentrations in rabbit meat after *Lactobacillus acidophilus* and *Lactococcus lactis* probiotic strains application. Physico-chemical parameters and nutritional value of rabbit meat were not negatively influenced during bioactive compounds administration (Pogány Simonová et al., 2016, 2019, 2020 c, e).

Conclusion

We conclude that diet supplementation with bacteriocinogenic and probiotic *E. durans* ED26E/7 strain and its durancin ED26E/7 may improve the growth performance, caecal enzymatic activity and jejunal morphometry of rabbits, without any negative effect on rabbit meat quality.

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