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Beneficial Effect of Bacteriocin-producing Strain *Enterococcus durans* ED 26E/7 in Model Experiment Using Broiler Rabbits

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ABSTRACT

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From the aspect of probiotic properties and bacteriocins, *Enterococcus faecium* belongs to the most frequently studied species among enterococci. This study deals with testing the strain of the species *Enterococcus durans* ED 26E/7 in broiler rabbits. The strain ED 26E/7 isolated from ewes lump cheese produces an antimicrobial substance durancin. Forty-eight post-weaned rabbits (aged 5 weeks) of both sexes were divided into experimental group (EG) and control group (CG) per 24 animals each, and kept in standard cages, two animals per cage. EG group rabbits were additionally administered the ED 26E/7 strain (500 µl/animal/day) into water for 21 days. CG group rabbits were fed a commercial feed. The experiment lasted 42 days. Faeces and blood samples were taken on days 0–1 (experiment onset), 21 (after a 3-week application), and 42 (3 weeks after ED 26E/7 strain cessation). On days 21 and 42, rabbits were slaughtered and caeca and appendix were sampled. The rabbits' digestive tract was found to be sufficiently colonized by the strain ED 26E/7; the antimicrobial effect was demonstrated in caecum and appendix (e.g. decrease in coliforms). Reduction of *Eimeria* sp. oocysts in EG compared to CG rabbits was detected on day 21, when also a significant ($P < 0.05$) increase of phagocytic activity in EG was registered. Values of glutathione-peroxidase were lower in EG than in CG rabbits on day 21 implying that the ED 26E/7 application had not evoked oxidative stress. Biochemical blood parameters and quality of meat were not negatively influenced. First time tested in animals, *E. durans* ED 26E/7 seems to be a new candidate for use in rabbits husbandry.

Keywords: enterococcal species; influence; rabbit; gut microbiota; physiological parameters; meat quality

Enterococci have constituted a unique taxonomic entity since the mid-90s when results of DNA–DNA hybridization experiments suggested their separation into a new bacterial genus *Enterococcus* from the former genus *Streptococcus* (Schleifer and Kilpper-Balz 1987; Werner et al. 2013). These

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Gram-positive bacteria form the third largest genus as lactic acid bacteria (LAB) following the genera *Lactobacillus* and *Streptococcus* (Franz et al. 2011). Some enterococci are also known to produce bacteriocins – proteinaceous substances with antimicrobial effect (Laukova et al. 1993; Franz et al. 2007) which can influence the intestinal microbiota (Pogany Simonova et al. 2009; Laukova et al. 2012a). Enterococci belong to the phylum Firmicutes, Class Cocci, Order Lactobacillales, Family Enterococcaceae, Genus *Enterococcus* (De Vos et al. 2009). Up to now, 54 enterococcal species have been validly described, most of them falling into seven species groups on the basis of 16S rRNA gene similarity (Franz et al. 2011). Following this classification, the species *Enterococcus durans* belongs to the *E. faecium* group (Tanasupawat et al. 2008). The species *E. durans* is most frequently detected in food products, e.g. cheese (Pieniz et al. 2014), but it can be isolated also from the digestive tract of animals, including food-producing animals and human (Devriese et al. 2002). Similarly as some *E. faecium* strains, also some *E. durans* strains are able to produce bacteriocins (Yanagida et al. 2005). Because our laboratory has been focused on bacteriocin-producing and probiotic enterococci and their use in protecting animal health for years, in this study we attempted to apply *E. durans* ED 26E/7 in broiler rabbits. ED 26E/7 was isolated from ewes cheese lump. Its identity was confirmed by MALDI-TOF spectrometry. It is a lactic acid and bacteriocin-producing strain possessing probiotic properties (Laukova et al. 2012b).

Rabbits represent not only a suitable animal model, but after weaning they have still problems with digestive disorders such as colibacillosis or clostridial enteritis. Therefore breeders search for different (innovative) ways to eliminate/prevent this problem. Following our previous studies, this study was focused on testing *E. durans* ED 26E/7 in broiler rabbits to find a new strain to protect husbandry, but also to compare its effect with other promising additives. Colonization of the strain was checked and its influence on microbiota, biochemical parameters, immunity, meat parameters (total water content, water holding capacity, energy value, total fat and proteins content, pH24) was analyzed. Moreover, the effect of ED 26E/7 on *Eimeria* oocysts in faeces of broiler rabbits was verified. To our knowledge, this is the first study on the effect of *E. durans* strain in animals.

MATERIAL AND METHODS

Enterococcus durans ED 26/7 (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 Laukova et al. (2012a). Rif^R variant (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 and a 21-day treatment were prepared and stored at 4°C.

The experiment was performed in co-operation with our colleagues from the National Agricultural and Food Centre, Nitra, Slovakia. Forty-eight post-weaned rabbits (aged 5 weeks) of both sexes were divided into the experimental group (EG) and the control group (CG) (n = 24 animals per each group). Animals involved in the experiment 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). Rabbits were kept in standard cages sizing 0.61 m × 0.34 m × 0.33 m, type D-KV-72 (Kovobel, Czech Republic), two animals per cage. The cages allowed faeces separation. A lighting cycle of 16 h light/8 h darkness was used throughout the experiment. The temperature and humidity were recorded continuously with a digital thermograph positioned at the same level as the cages. The heating and forced ventilation systems allowed the building air temperature to be maintained within 16 ± 4°C throughout the experiment. Relative humidity was about 70 ± 5%. 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 Slovak Veterinary and Food Administration and by the Ethic Commissions of both institutes. The rabbits were fed a commercial feed mixture for broiler rabbits (pelets 3.5 mm in average size) containing crude protein in the volume 177.99 g/kg of feed mixture/diet, fibre content was 146.97 g/kg,

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fat content was 36.08 g/kg, starch 129.05 g/kg, ash content 97.32 g/kg, organic matter 847.49 g/kg. Samples of individual feeds and complete granulated mixture were analyzed for the content of nutrients according to the Slovak Technical Norm (STN 46 7092 from 2010). The rabbits had *ad libitum* access to daily checked feed and water consumption. The EG group rabbits were moreover administered *Enterococcus durans* ED 26E/7 strain (marked by rifampicin 10^9 CFU/ml) at a dose of 500 µl/animal/day in water for 21 days. The CG rabbits were fed commercial feed. The duration of the experiment was 42 days.

Faecal sampling was carried out on day 0–1 (the start of experiment; ten mixture samples from 48 rabbits – initial microbial background), on day 21 (the end of *E. durans* ED 26E/7 application; five mixture samples from each group), and on day 42 (the end of experiment, 3 weeks after ED 26E/7 cessation; five mixture samples from each group). *E. durans* ED 26E/7 counts were checked to follow its colonization and its effect on the other microbiota using the ISO standard microbiological methods. The appropriate dilutions of samples in Ringer solution (pH 7.0; Oxoid Ltd., UK) were plated onto media according to ISO: M-Enterococcus agar was used for enterococci (ISO-7889; Difco), M-Enterococcus agar enriched with rifampicin (100 µg/ml) for *E.durans* ED 26E/7, Baird-Parker agar supplemented with egg yolk tellurite solution (ISO 21527-1; Becton and Dickinson, USA) for determining coagulase-positive staphylococci (CoPs), Mannitol Salt agar, *Clostridium difficile* agar with the supplement SR0096E and 7% (v/v) defibrinated horse blood (SR0050) (ISO 15883; Oxoid) for enumerating coagulase-negative staphylococci (CoNS), and *Clostridium* spp. Mac Conkey agar (Oxoid) for coliform bacteria. *Pseudomonads* were isolated on Pseudomonas agar (Biomark, India). The plates were incubated at 30°C/37°C for 24h/48 h depending on bacteria. The bacterial counts were expressed in colony-forming units (log10) CFU per gram ± SD. On days 21 and 42, four animals (n = 4) from each group were slaughtered. They were electrically stunned and killed by cutting the carotidis and jugular veins (Chrastinova et al. 2016). One gram of caecal content and appendix was treated according to the standard microbiological dilution method mentioned above. Caecal and appendix samples were plated on the media as already mentioned.

Blood samples (n = 6) were taken from rabbits' marginal ear vein (*vena auricularis*). On day 0–1 mixed blood samples including all groups were taken, on days 21 and 42 we took mixed samples separately for each group. Phagocytic activity (PA) was measured using the direct counting procedure with microspheric hydrophilic particles (MSHP). Ingestion of MSHP by polymorphonuclear cells (PMNs) was determined using a modified test described by Vetvicka et al. (1982): 50 ml of MSH particle suspension (ARTIM, Czech Republic) was mixed with 100 ml of blood in an Eppendorf-type test tube and incubated at 37°C for 1 h. Blood smears were then prepared and stained by May-Grünwald and Giemsa-Romanowski (Merck). In each smear, 100 cells were observed to determine the relative number of white cells containing at least three engulfed particles (phagocytic activity) and the index of phagocytic activity (IPA), calculated as the number of engulfed particles to the total number of neutrophils and monocytes observed. The percentage of phagocytic cells was evaluated using an optical microscope (Motic BA 400 Biological Microscope, Motic China Group Co., Ltd., China) by counting PMN up to 100.

The activity of glutathione-peroxidase (GPx; U/gHb) in blood (n = 6) was determined using a specific commercial kit RANSEL (Randox, UK) according to the spetrophotometric assay procedure of Paglia and Valentine (1967).

Other biochemical analyses (total proteins (g/l), triglycerides, glucose, cholesterol, calcium (mmol/l), alanine-aminotranspherase (U/l)) were performed using commercial kits from Randox. Blood (n = 6, from each group) was sampled into dry non-heparinized Eppendorf tubes; blood serum was separated by centrifugation at 3000 *g* for 10 min, then stored frozen in plastic vials until analysis.

To detect *Eimeria* sp. oocysts, the quantitative McMaster (1986) method was used; oocysts counts were expressed in OPG/g (detected oocysts per gram of faecal sample).

At the end of the experiment, *longissimus dorsi* (MLD) muscles were separated (n = 4) by removing skin, fat, and connective tissue, chilled and stored 24 h at 4°C until analyses.

The pH was determined 24 h *post mortem* with a Radelkis OP-109 (Jenway, UK) with combined penetrating 3 mm into MLD. Water holding capacity, total water, protein, and fat contents were

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estimated using an Infratec 1265 Meat Analyzer (Tecator, AB, Sweden) and expressed in g/100 g; from this value, the energy value was calculated as previously described by Pogany Simonova et al. (2016):

$$EC\ (kJ/100\ g) = 16.75 \times protein\ content + 37.68 \times \times\ fat\ content$$

Statistical evaluation of the results was performed using one-way ANOVA test withTukey's *post hoc* test (the level of significance was set at $P < 0.05$ ± standard deviation).

RESULTS

E. durans ED 26E/7 sufficiently colonized the digestive tract of broiler rabbits; on day 21 the count in faeces reached up to 10^3 CFU/g (log10 2.75 ± 0.42 CFU/g) (Table 1). On day 42, the count of ED 26E/7 strain reached up to 10^2 CFU/g (log10 1.21 ± 0.45 CFU/g) (Table 1). Moreover, on day 21, a significantly higher count of ED 26E/7 strain in EG group was found compared to day 42 (a : b, $P < 0.001$) (Table 1). Similarly, on day 21 the total counts of enterococci in EG group were significantly higher compared to their counts in this group on day 42 (a : b, $P < 0.01$) (Table 1). In caecum and appendix, ED 26E/7 strain reached a sufficient amount, too (log 10 1.02 ± 0.16 CFU/g, 1.12 ± 0.22 CFU/g) (Table 1). When compared to the total enterococcal counts, in caecum and appendix the ED 26E/7 strain seemingly formed the greatest part of total enterococci. Although,

Table 1. Counts of *Enterococcus durans* ED 26E/7 and total count of enterococci in faeces, caecum, and appendix of broiler rabbits (in colony forming unit per gram, log10 CFU/g ± SD)

	EG ED26E/7	Enterococci
Faeces (n = 5)		
Day 0–1	nd	2.90 (0.51)
Day 21	2.75 (0.42)^{aA}	3.22 (0.54)^{aA}
Day 42	1.21 (0.45)^{b}	1.95 (0.65)^{b}
Caecum (n = 4)		
Day 21	1.02 (0.16)	1.39 (0.59)
Day 42	0.90 (0.00)	0.97 (0.03)
Appendix (n = 4)		
Day 21	1.12 (0.22)	1.10 (0.20)
Day 42	< 1.0	1.20 (0.52)

EG = experimental group (*Enterococcus durans* ED 26E/7), nd = not detected

Faeces ED 26E/7 strain Day 21 : ED 26E/7 strain Day 42 (a : b, $P < 0.001$), Faeces Enterococci Day 21 : Enterococci Day 42 (a : b, $P < 0.01$), Faeces ED 26E/7 strain Day 21 : Enterococci Day 21 (A : A, difference 0.47 log cycle)

Table 2. Counts of selected bacterial group in faeces of broiler rabbits (in colony forming unit per gram, log10 CFU/g ± SD)

	EG	CG
Enterococci		
Day 0–1	2.90 (0.51)	2.90 (0.51)
Day 21	3.22 (0.54)^{a}	3.27 (0.89)
Day 42	1.95 (0.65)^{b}	3.51 (1.09)
LAB		
Day 0–1	4.14 (0.52)	4.14 (0.52)
Day 21	4.17 (1.07)	3.68 (0.75)
Day 42	4.19 (0.26)	4.02 (0.21)
CoPS		
Day 0–1	3.22 (0.68)	3.22 (0.68)
Day 21	3.24 (0.42)	3.66 (0.49)
Day 42	2.72 (0.44)	3.17 (0.36)
CoNS		
Day 0–1	3.90 (0.48)	3.90 (0.48)
Day 21	3.49 (0.39)	3.84 (0.71)
Day 42	3.61 (0.14)	3.66 (0.10)
***Clostridium* spp.**		
Day 0–1	4.80 (1.14)	4.80 (1.14)
Day 21	4.67 (0.86)	4.74 (0.13)
Day 42	4.10 (0.62)	3.83 (0.63)
***Pseudomonas* spp.**		
Day 0–1	4.50 (0.78)	4.50 (0.78)
Day 21	4.94 (0.97)	3.68 (0.75)
Day 42	4.14 (0.45	4.02 (0.21)
Coliforms		
Day 0–1	3.33 (0.77)	3.33 (0.77)
Day 21	3.61 (1.18)	2.82 (1.77)
Day 42	2.59 (0.64)	2.59 (0.46)

EG = experimental group (*Enterococcus durans* ED 26E/7), CG = control group, Day 0–1 = start of experiment, Day 21 = 3 weeks after ED 26E/7 strain application, Day 42 = 3 weeks after ED 26E/7 cessation, LAB = lactic acid bacteria, CoPS = coagulase-positive staphylococci, CoNS = coagulase-negative staphylococci

Enterococci Day 21 : Enterococci Day 42 (a : b, difference 0.47 cycle)

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Table 3. Counts of selected bacterial group in caecum of broiler rabbits (in colony forming unit per gram, log10 CFU/g ± SD)

	EG	CG
Enterococci		
Day 21	1.39 (0.59)^{A}	0.95 (0.05)^{B}
Day 42	0.97 (0.03)	0.90 (0.00)
LAB		
Day 21	3.47 (0.97)	3.59 (0.33)
Day 42	4.03 (0.60)	4.00 (0.09)
CoPS		
Day 21	2.47 (0.83)	1.75 (0.37)
Day 42	2.32 (0.30)	2.03 (0.17)
CoNS		
Day 21	2.87 (0.22)	2.75 (0.08)
Day 42	3.62 (0.26)	3.72 (0.16)
***Clostridium* spp.**		
Day 21	5.46 (0.67)^{A}	6.14 (1.18)^{B}
Day 42	4.38 (1.20)^{A}	5.37 (0.27)^{B}
***Pseudomonas* spp.**		
Day 21	3.55 (0.23)	3.86 (0.35)
Day 42	3.07 (0.87)	3.13 (0.28)
Coliforms		
Day 21	0.93 (0.04)^{A}	3.41 (0.80)^{B}
Day 42	1.94 (0.66)^{A}	2.70 (1.34)^{B}

EG = experimental group (*Enterococcus durans* ED 26E/7), CG = control group, Day 0–1 = start of experiment, Day 21 = 3 weeks after ED 26E/7 strain application, Day 42 = 3 weeks after ED 26E/7 cessation, LAB = lactic acid bacteria, CoPS = coagulase-positive staphylococci, CoNS = coagulase-negative staphylococci

Coliforms Day 21EG : 21CG (A : B, difference 2.48 log cycle), Coliforms Day 42EG : 42CG (A : B, difference 0.76 log cycle), *Clostridium* spp. Day 21EG : 21CG (A : B, difference 0.68 log cycle), *Clostridium* spp. Day 42EG : 42CG (A : B, difference 0.99 log cycle), Enterococci Day 21EG : 21CG (A : B, difference 0.44 log cycle)

in general, the counts of *E. durans* ED 26E/7 were lower in caecum than in faeces, on day 21 in caecum of EG compared to CG rabbits a decrease in coliform bacteria (difference 2.48 log cycle, A : B) was noted (Table 3). On the other hand, coliforms in faeces were not influenced (Table 2). But their occurrence was reduced in appendix of EG compared to CG rabbits (difference 2.81 log cycle, A : B) (Table 4). In general, bacteria in faeces were not influenced by ED 26E/7 application except for enterococci which were slightly increased on day 21 compared to day 42 (difference 0.47 log cycle, a : b) (Table 2). Enterococci in caecum and appendix of EG rabbits were also slightly increased compared to CG group (A : B, differences 0.20; A : B, 0.44 log cycle) (Tables 3, 4). LAB were counted in high numbers in faeces, caecum, and appendix (up to around log10 4.0 CFU/g) and their counts were not negatively influenced by ED 26E/7 strain application. Staphylococci were not influenced by ED 26E/7 strain and their counts were quite high but balanced in the digestive tract. On day 21

Table 4. Counts of selected bacterial group in appendix of broiler rabbits (in colony forming unit per gram, log10 CFU/g ± SD)

	EG	CG
Enterococci		
Day 21	1.10 (0.20)^{A}	0.90 (0.00)^{B}
Day 42	1.05 (0.17)	1.10 (0.14)
LAB		
Day 21	4.00 (0.75)	4.75 (0.39)
Day 42	4.44 (0.37)	3.84 (0.47)
CoPS		
Day 21	2.03 (0.44)	2.33 (0.38)
Day 42	2.10 (0.03)	2.29 (0.38)
CoNS		
Day 21	2.26 (0.31)	2.55 (0.25)
Day 42	3.61 (0.20)	2.89 (0.45)
***Clostridium* spp.**		
Day 21	5.67 (1.06)^{A}	6.59 (0.22)^{B}
Day 42	5.06 (0.8)	4.38 (0.61)
***Pseudomonas* spp.**		
Day 21	4.33 (0.48)	4.96 (0.28)
Day 42	3.29 (0.23)	3.80 (0.32)
Coliforms		
Day 21	2.23 (0.61)^{A}	5.04 (0.59)^{B}
Day 42	2.89 (1.20)	1.45 (0.35)

EG = experimental group (*Enterococcus durans* ED 26E/7), CG = control group, Day 0–1 = start of experiment, Day 21 = 3 weeks after ED 26E/7 strain application, Day 42 = 3 weeks after ED 26E/7 cessation, LAB = lactic acid bacteria, CoPS = coagulase-positive staphylococci, CoNS = coagulase-negative staphylococci

Coliforms Day 21EG : 21CG (A : B, difference 2.81 log cycle), *Clostridium* spp. Day 21EG : 21CG (A : B, difference 1.12 log cycle), Enterococci Day 21EG : 21CG (A : B, difference 0.20 log cycle)

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Table 5. Phagocytic activity (PA) (in %), index of phagocytic activity (IPA), and glutathione-peroxidase (GPx) values (in U/g Hb ± SD)

	EG	CG
PA (n = 6)		
Day 0–1	30.83 (1.45)[b]	30.80 (1.45)
Day 21	32.17 (1.33)[a]	30.67 (1.97)[b]
Day 42	32.67 (1.51)[a]	30.17 (1.72)[b]
IPA		
Day 0–1	1.47 (0.10)	1.47 (0.10)
Day 21	1.40 (0.13)	1.43 (0.14)
Day 42	1.45 (0.05)	1.42 (0.08)
GPx (n = 6)		
Day 0–1	155.14 (40.47)	155.14 (40.47)
Day 21	155.91 (35.02)[a]	163.28 (49.72)[b]
Day 42	168.33 (35.87)[a]	191.70 (14.43)[b]

EG = experimental group (*Enterococcus durans* ED 26E/7), CG = control group, Day 0–1 = start of experiment, Day 21 = 3 weeks after ED 26E/7 strain application, Day 42 = 3 weeks after ED 26E/7 cessation

PA – Day 21EG : 21CG (a : b, $P < 0.05$), Day 42EG : 42CG (a : b, $P < 0.05$); IPA – EG Day 0–1 : Day 21 (a : b, $P < 0.05$); GPx – Day 21EG : Day 21CG (a : b, difference 7.37), Day 42EG : Day 42CG (a : b, difference 23.37)

Clostridium spp. bacteria were decreased in caecum and appendix in EG rabbits compared to CG group. Their counts were high and reduction with a difference of 0.68 log cycle in caecum (A : B, Table 3) and 1.12 log cycle in appendix (A : B, Table 4) was noted. The count of *Pseudomonas* spp. bacteria was not influenced.

On day 21 a significant increase of PA values in EG ($P < 0.05$, Table 5) compared to CG rabbits was found. PA activity was also increased compared to that on day 0–1 (the start of experiment). In EG and CG rabbits, PA values at the end of experiment (day 42, 3 weeks after ED 26E/7 strain cessation) were almost at the same level as on day 21. On day 42 a significant increase in PA in EG compared to CG rabbits was noted ($P < 0.05$) (Table 5).

On day 21 values of GPx were at the same level as on day 0–1 in EG rabbits. Positive result is that they were lower than in CG rabbits. Although on day 42 the GPx value was slightly increased in EG rabbits, still lower value was measured in EG compared to CG rabbits; it means that the ED 26E/7 application did not evoke oxidative stress (Table 5).

Blood serum parameters indicated no influence, only a slight effect was noted in relation to the reference values for individual parameters (Table 6).

Eimeria oocysts were counted in the faecal samples in the average amount 1763.0 OPG/g at the start of the experiment. However, on day 21 (3 weeks after *E. durans* ED 26E/7 application) *Eimeria* oocysts were reduced in EG group, the absence of oocysts was confirmed; although *Eimeria* oocysts were decreased in CG rabbits either, there was still a difference of 50.0 oocysts if compared EG to CG (Table 7). On day 42 (3 weeks after ED 26E/7 cessation) *Eimeria* oocysts occurred also in EG; however, the count was still lower if

Table 6. Selected blood parameters (n = 6) (means ± SD)

	TP (g/l)	Trig	Chol	Glu	P	Ca	ALT (μkat/l)
		(mmol/l)					
EG/CG							
Day 0–1	53.5 (5.20)	2.08 (0.21)	3.53 (0.41)	6.28 (0.21)	2.02 (0.09)	2.82 (0.51)	0.169 (0.05)
Day 21							
EG	59.8 (2.60)	1.93 (0.36)	3.23 (0.32)	6.20 (0.28	1.92 (0.05)	3.03 (0.41)	0.115 (0.05)
CG	58.6 (5.10)	1.85 (0.20)	3.33 (0.53)	6.12 (0.22)	1.85 (0.08)	3.10 (0.16)	0.108 (0.04)
Day 42							
EG	62.2 (3.70)	1.79 (0.08)	3.09 (0.48)	6.39 (0.41)	1.91 (0.08)	3.15 (0.06)	0.153 (0.03)
CG	61.5 (3.60)	1.90 (0.22)	3.10 (0.36)	6.38 (0.24)	1.93 (0.04)	3.10 (0.12)	0.152 (0.04)

EG = experimental group (*Enterococcus durans* ED 26E/7), CG = control group, Day 0–1 = start of experiment, Day 21 = 3 weeks after ED 26E/7 strain application, Day 42 = 3 weeks after ED 26E/7 cessation, TP = total proteins, Trig = triglycerides, Chol = cholesterol, Glu = glucose, P = phosphorus, Ca = calcium, ALT = alaninaminotranspherase, SD = standard deviation reference values (Kerr 1989): TP 40–85 g/l, Trig 1.40–1.76 mmol/l, Glu 5.5–8.6 mmol/l, Chol 0.55–4.44 mmol/l, Ca 2.2 to 4.2 mmol/l, P 0.55–2.13 mmol/l, ALT 0.166–0.75 μkat/l

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Table 7. Reductive effect of *Enterococcus durans* ED 26E/7 strain against *Eimeria* sp. (in OPG/g), counts of *Eimeria* oocysts per gram of faecal sample ± SD (n = 8)

	EG	CG
Day 21	negative	50.0 (7.07)
Day 42	381.25 (0.19)	1687.50 (41.07)

EG = experimental group (*Enterococcus durans* ED 26E/7), CG = control group, Day 21 = 3 weeks after ED 26E/7 strain application, Day 42 = 3 weeks after ED 26E/7 cessation count of *Eimeria* oocysts at the start of experiment on Day 0–1 (n = 10) was 1763 OPG/g

compared to CG (difference 1306.25 oocysts/g) (Table 7).

Meat parameters in EG rabbits were not negatively influenced being similar to values measured in CG. On day 42, water holding capacity in EG was 29.21 ± 2.35 g/100 g; in CG 29.54 ± 4.53 g/1, total water content reached 75.8 ± 0.14 g/100 g and 75.8 ± 0.08 g/100 g respectively; total protein content was also almost the same in both groups (EG: 21.64 ± 0.09 g/100 g, CG: 21.73 ± 0.03 g/100 g). Fat content, energy value, and pH24 were not influenced (fat content in EG: 1.55 ± 0.13 g/100 g, in CG: 1.48 ± 0.09 g/100 g; energy value in EG: 421.5 ± 4.72 KJ/100 g, in CG: 419.33 ± 4.72 KJ/100 g; pH24 in EG: 5.72 ± 0.04, in CG: 5.79 ± 0.14).

DISCUSSION

E. durans ED 26E/7 colonized the digestive tract of broiler rabbits reaching up to 10^3 CFU/g (3.0 log10 CFU/g). In caecum and appendix lower counts of ED 26E/7 strain were found. Interesting is the presence of ED 26E/7 strain in appendix; there exist only few studies dealing with the occurrence of general microbiota in appendix as well as information related to colonization of appendix by probiotic strains. Our study demonstrated an antimicrobial effect of ED 26E/7 strain in appendix.

The fact that additive enterococci, probiotic and/or bacteriocin-producing, can sufficiently colonize the digestive tract of animals, including the rabbits, independently on the source they were isolated from, has already been confirmed in our previous experiments with *E. faecium* strains AL41, EF2019=CCM7419 or CCM4231 (Pogany Simonova et al. 2009; Szaboova et al. 2011; Laukova et al. 2012a, 2015a). Moreover, beneficial effect of e.g. AL41 strain was noted not only in rabbits but also in poultry or horses (Laukova et al. 2015b). It can be stated (in contrast to e.g. probiotic lactobacilli) that adhesion of enterococci or their colonization does not depend on the strain origin (Laukova et al. 2004). The enterococcal isolates did not preferentially bind to mucus originating from the same host (animal, human) rather than to that they were isolated from. So no differences in their adhesive ability were recorded when human, canine, and porcine mucus and isolates from these source-derived strains were tested (Laukova et al. 2004). This indicates that no host-specific binding exists. The conclusion of Rinkinen et al. (2000) was similar. Surprisingly the antimicrobial effect of ED 26E/7 strain was not noted in faeces; however, in caecum and appendix this effect of ED 26E/7 strain was shown via reduction of coliforms, *Clostridium* spp. Although the observed reduction was insignificant, mathematical differences (log cycles) up to almost 3.0 log cycle were calculated. In our previous studies with the application of *E. faecium* AL41=CCM8558, EF2019=CCM7420 or CCM7419=EK13 in broiler rabbits, staphylococci were significantly reduced, decrease of coliforms and *Clostridium* spp. was found as well (Pogany Simonova et al. 2009; Laukova et al. 2012a, 2015c), and the similar effect was also noted in poultry (Laukova et al. 2015c). The antimicrobial effect could be explained by lactic acid or bacteriocin production; ED 26E/7 strain produces a bacteriocin substance – durancin (Laukova et al. 2012b). Not so evident inhibition effect of ED 26E/7 strain as e.g. compared to *E. faecium* AL41=CCM8558 could be explained by lower production of lactic acid by this strain compared to some strains of the species *E. faecium* (Laukova and Kuncova 1991); but the effect of the bacteriocin produced by ED 26E/7 strain directly in the digestive tract could be influenced by environmental niche.

It is known that probiotic bacteria are capable of immunity stimulation by improving the intestinal barrier and mucosal immune system due to modulation of the intestinal microbiota, and by production of antibacterial compounds. Higgins et al. (2010) reported on the activation of innate immunity through phagocytic cells by Lactobacillus-based probiotic in chickens. In our previous experiments using *E. faecium* strains a stimulative prolonged effect of probiotic and

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bacteriocin-producing strains on PA was repeatedly noted (Pogany Simonova et al. 2013). This effect was noted not only in broiler rabbits, but similarly in poultry (Laukova et al. 2015c) or dogs (Strompfova et al., pers. comm.). Bobikova et al. (2015) reported beneficial effect of *E. faecium* AL41 on the mRNA expression of IgA and IgA+ cells in chickens treated with AL41 strain and infected with *Salmonella* Enteritidis. Levkut et al. (2012) reported that *E. faecium* EF55 strain applied to chickens resulted in significantly higher numbers of lymphocytes in peripheral blood and a tendency to increase CD3, CD4, CD8, and IgM cells. Chytilova (2013) confirmed the hypothesis that for example Toll-like receptors (TLR) can play a key role in the protective mode of action of probiotic strains. TLR represent evolutionary conserved pattern recognition receptors (PRR), which are important components for the defence against invading agents (microbiota). These cells are exprimed on the cells of the immune system, but also on the cells associated with the external environment (e.g. epithelial cells). Their expression rapidly changes in response to pathogens, cytogens or to environmental stress (Akira et al. 2006). Some TLR are exposed on the cell surface, and they can recognize different forms of infection from the environment. ED 26E/7 also significantly stimulated PA, although the values measured in our experiment were lower compared to those e.g. by *E. faecium* AL41 strain. Anyway, the stimulation of PA was confirmed while IPA was not influenced. Lower PA values after ED 26E/7 application if compared e.g. to *E. faecium* AL41 application could be explained by the fact that the species *E. faecium* belong to dominating microbiota in the digestive tract of animals; *E. durans* is not so frequently detected there, although it is able to colonize the digestive tract.

There exists a different view concerning the beneficial effect of probiotic and bacteriocin-producing strains on oxidative stress. Enzyme GPx is a component of the antioxidative defence system which protects the organism from the detrimental effect of oxygen radicals causing lipid peroxidation in cell membranes (Paglia and Valentine 1967). In our study, reduced value of GPx, although not significantly, was noted in broiler rabbits treated with ED 26E/7 strain compared to CG rabbits; it indicates that the strain application did not evoke oxidative stress. Kullisaar et al. (2011) noted reduction of oxidative stress in human with the antioxidant probiotic strain *Lactobacillus fermentum* LfME-3. In contrast, no influence on antioxidative defence system was noted after the probiotic and bacteriocin-producing *E. faecium* AL41 strain application (Laukova et al. 2015c).

Biochemical parameters such as total proteins, triglycerides, cholesterol, glucose, ALT, Ca, P were not influenced by the application of ED 26E/7 strain. On the other hand, a hypocholesterolemic effect of probiotic *L. fermentum* CCM 7421 applied to dogs was demonstrated by Strompfova et al. (pers. comm.).

We cannot explain the exact mode of action in the reduction of *Eimeria* oocysts by ED 26E/7 strain; it may be associated with immunity as PA was increased, or the counts of oocysts may be cut down by the antimicrobial substance (durancin) produced by ED 26E/7 strain. Similarly, decrease in *Eimeria* oocysts in rabbits was mentioned after the *E. faecium* CCM4231 application, which is an enterocin-producing and probiotic strain (Szaboova et al. 2011), and also after the application of *E. faecium* EF2019 (CCM7420), a rabbit-derived probiotic and bacteriocin-producing strain (Pogany Simonova et al. 2009, 2013). Buckova et al. (2015) even reported reduction of a parasitic infection (*Trichinella spiralis)* in a model mice experiment after the application of ED 26/7 strain, when a significant decrease in the number of muscle larvae was noted. Reproductive capacity index (RCT) of *T. spiralis* was also reduced in mice with ED 26E/7 strain application. Seemingly the parasitic burden in the host muscles might be reduced by immune mechanism stimulated by bacterial strains.

Lower values of water holding capacity do not mean an altered meat quality. In spite of the fact that no specific sensory analyses were conducted, it can be stated that the meat was juicy, tasty, with good consistency. Pogany Simonova et al. (2016) also reported no negative influence of the rabbit-derived strain *E. faecium* CCM7420 on rabbit meat parameters.

CONCLUSION

E. durans ED 26E/7 sufficiently colonized the digestive tract of broiler rabbits; the total enterococci were slightly increased. In faeces of rabbits no antimicrobial effect was noted; however, on

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day 21 a decrease of coliform bacteria (difference 2.48 log cycle) was noted in caecum and appendix of EG compared to CG rabbits. On day 21 also the count of *Clostridium* spp. was decreased in caecum and appendix in EG compared to CG rabbits. On day 21 a significant increase of PA was found. Application of ED 26E/7 strain did not evoke oxidative stress; the values of GPx in EG rabbits were lower than in CG rabbits. Blood serum parameters indicated no influence, only a slight effect was noted in relation to the reference values for individual parameters. On day 21 (3 weeks after *E. durans* ED 26E/7 application), reduction of *Eimeria* oocysts was noted in EG compared to CG rabbits.

To our knowledge, the present study is the first to check the beneficial effect of the species *E. durans* and the strain itself in broiler rabbits and generally in animals.

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