Polish Journal of Veterinary Sciences Vol. 16, No. 4 (2013), 619–627

DOI 10.2478/pjvs-2013-0089

Original article

Combined administration of bacteriocin--producing, probiotic strain *Enterococcus faecium* CCM7420 with *Eleutherococcus senticosus* and their effect in rabbits

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Abstract

The effect of *Enterococcus faecium* CCM7420 (EF) – enterocin-producing and probiotic strain of rabbit origin, *Eleutherococcus senticosus* extract (ES) and their combination (ES+EF) was determined on selected bacteria in faeces and caecum content, leukocytes phagocytosis, blood biochemistry and growth performance. Ninety-six weaned rabbits were divided into 3 experimental (ES, EF, ES+EF) and control group (CG). The rabbits in the groups ES and EF+ES were fed commercial diet enriched with *E. senticosus* extract (30 g/100 kg feed), rabbits in groups EF and CG were fed untreated diet. The rabbits in the EF and ES+EF groups were administered with an overnight culture of *E. faecium* CCM7420 strain (500 µl/animal/day into water, 109 CFU/ml). The treatment period lasted 21 days. The microbiological examinations in faecal samples confirmed the presence of *E. faecium* CCM7420 strain. In groups EF and ES+EF, the reduction of faecal coliforms, *Pseudomonas*-like sp., *Clostridium*-like sp. and *S. aureus* was recorded. Leucocyte phagocytosis significantly increased in all experimental groups ($P<0.0001$) compared to CG. The lowest GPx values were measured in the ES+EF group. Higher total protein, triglycerides and calcium concentrations were detected in experimental groups compared to CG. The cholesterol concentration decreased in the ES group. The highest average daily gain was recorded in EF group; in ES+EF the better feed conversion ratio and no mortality was recorded. These results indicated that the dietary supplementation with the *E. faecium* CCM7420 and *E. senticosus* extract stimulate the leukocytes phagocytosis and reduces the potential pathogens in rabbits digestive tract without oxidative stress and improve the growth performance.

Key words: *Eleutherococcus senticosus*, microflora, phagocytic activity, probiotic, rabbit

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Introduction

In rabbits, weaning is a critical period due to the nutritional (substitution of milk with solid feed) and environmental changes (separation from mother, manipulation, transfer) which often leads to stress and induce zootechnical, physiological and digestive disorders. To keep the health status of rabbits, the control and improvement of environmental conditions, welfare and feeding strategies are needed.

There is an increasing global interest in using natural substances as alternatives to synthetic drugs and growth promoters. They represent a well-tried tool for disease prevention and/or *post*-therapy in various animal species, including the rabbits, mainly through the formerly mentioned stressfull weaning period often associated with microbial disturbances, mortality and/or morbidity. These nonantibiotic compounds with bacteriostatic or bactericidal activity – probiotics, prebiotics, bacteriocins, plant extracts, organic acids have a broad range of effects in the animals, from health promoting, beneficial for animal production to gastrointestinal microflora and metabolism changes and immune system stimulating as well (Falcão-e-Cunha et al. 2007).

The positive effect of pro- and/or prebiotics, bacteriocins, plant extracts and/or their combinations on the control of pathogens and parasites, nutrient utilization, oxidative stress, immunomodulation, growth performance and meat quality has been shown in several studies (Kritas et al. 2008, Pogány Simonová et al. 2009, Szabóová et al. 2011, 2012). Plant bioactivity has been known and used for centuries, but it is only recently that they have been studied in more detail to understand their production, mode of action, biological effects on livestock production. However, rabbit feed enrichment with phytoadditives is documented (Chrastinová et al. 2007, Simonová et al. 2008a, Szabóová et al. 2008, 2011, 2012) and to study new herbal species it is required to obtain and widen knowledge of their metabolism and biological effect on the animal in order to evaluate their further use in rabbit breeding. The use of bioactive plants as feed additives alone or in combination with probiotics could be helpful in prevention of impaired immune function and also enhance health status and production in rabbits.

Eleutherococcus senticosus, better known as the Siberian ginseng, belong to the Araliaceae family; liquid or dry extrats of *E. senticosus* are used all over the world to stimulate the immune system in human, to strengthen resistance against infection, to increase physical and mental performance levels in humans. In general, it has been studied and has shown antibacterial, antioxidant, anticancer, antipyretic, antiinflammatory, choleretic, immunostimulatory, antihyperglygemic, hypocholesterolemic and radioprotectant effects (Davydov and Krikorian 2000) in human. Roots and rhizome of *E. senticosus* contain characteristicals eleutheroside B (0.50%), derivates of phenyl propane (e.g. syringin, caffeic acid, sinapyl alcohol, coniferyl aldehyde) and eleutheroside E (0.13%), lignases (e.g. sesamin, syringoresinol and its glucoside) in addition to traces of coumarins (e.g. isofraxidin and its glucoside), vitamin E, provitamins (β-carotene) and considerable amounts of polysaccharides (2-6%; Davydov and Krikorian 2000, Steinmann et al. 2001).

The objective of our work was to study the effect of *E. senticosus* extract alone and in combination with rabbit-derived bacteriocin-producing probiotic strain *Enterococcus faecium* CCM7420 on the strain colonization, microbiological (bacteria in faeces) and immunological parameters, biochemical blood parameters and on growth performance of rabbits.

Materials and Methods

In vitro assay

For *in vivo* test, by rifampicin-marked variant of *E. faecium* CCM7420 (EF2019, our isolate from rabbit faeces; Simonová and Lauková 2007; deponed to Czech Collection of Microorganisms, number CCM7420) was used to differ it from other enterococci. Briefly, an 18 hours culture of strain CCM7420 was plated onto obliqued TH agar (Todd-Hewitt agar, Difco) enriched with rifampicin (100 μg/ml) and cultivated at 37°C. Colonies with the highest concentration of rifampicin were checked; the inoculation and cultivation were continually repeated to have the rifampicin-marked variant.

The effect of *E. senticosus* extract (ethanolic extraction, dry matter 95±1%, ash 7.3±0.5%, purchased from Calendula, a.s., Nová L'ubovňa, Slovakia) on the growth of bacteriocin-producing strain CCM7420 was tested *in vitro* in order to be sure that the extract and the strain can be combined in the *in vivo* experiment. The inhibitory activity was assayed by the agar spot test (De Vuyst et al. 1996) and no inhibition of CCM7420 strain by *E. senticosus* extract was recorded.

Experiment schedule

Ninety-six weaned rabbits of both sex (Hyplus breed), in age of 5 weeks were randomly divided to 3 experimental (ES – *E. senticosus*, EF – *E. faecium* CCM7420, ES+EF – *E. senticosus* in combination

Table 1. Ingredients and determined chemical composition of the commercial diet.

Feed ingredients (%)	Diet A	Diet B	Chemical analysis, minerals and vitamins** (g[a], mg[b]/kg feed)	Diet A	Diet B
Dehydrated lucerne meal	25	25	Dry matter[a]	900.0	904.8
Extracted sunflower meal	17.4	17.4	Crude protein[a]	172.1	171.4
Oats	5	5	Crude fibre[a]	165.2	173.6
Wheat bran	8.7	8.7	Fat[a]	43.2	41.4
Dry malting sprouts	17	17	Ash[a]	65.4	70.4
Apple pomace	7	7	Nitrogen free extract[a]	454.1	448.1
Barley	4.7	4.5	Organic compounds[a]	834.7	834.4
Olive-seed meal	5	6	Acid detergent fibre (ADF)[a]	196.2	210.3
DDGS	5	5	Neutral detergent fibre(NDF)[a]	313.5	365.1
Rape oil	1.7	1.7	Starch[a]	174	157.4
Carob meal (*Ceratonia siliqua*)	0.2	0	Calcium[a]	8.4	6.7
Wheat meal+*E. senticosus* (30 g)	1	0	Phosphorus[a]	3.6	4.1
Premix minerals*	2.5	2.5	Magnesium[a]	2.1	1.9
			Sodium[a]	1.5	1.4
			Potassium[a]	10.9	11.2
			Iron[b]	397.8	359.9
			Zinc[b]	146.1	132.2
			Cuprum[b]	34.4	31.2
			Digestible energy (MJ/kg)	11.6	11.4
			Metabolic energy (MJ/kg)	11.0	10.8

* Provided per kg diet: 45% Extracted soybean meal, 22% Dicalcium phosphate, 13% Dicalcium carbonate, 10% Natrium chloride, 5% Natrium hydrocarbonate, 2% Methionine, 3% Mineral and vitamin mixture**

with *E. faecium* CCM7420) groups and control group (CG) of 24 rabbits in each. Rabbits were housed in standard cages (0.61 m x 0.34 m x 0.33 m, of the type D-KV-72 supplied by Kovobel company, Czech Republic), 2 animals per cage. The cages allowed the faeces separation. A cycle of 16 h of light and 8 h of dark was used through the experiment. Temperature and humidity were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed the building air temperature to be maintained within 16 ± 4ºC through the experiment. Relative humidity was about 70 ± 5%. Animals of the ES and ES+EF groups were fed commercial pellet diet A enriched with dry root extract of *E. senticosus* (30 g/100 kg feed), rabbits in groups EF and CG were fed untreated pellet diet B (Table 1); all animals had access to water *ad libitum*. The samples of individual feeds and complete granulated mixture were analyzed for the content of nutrients according to STN 46 7092 (Table 1). Every day, at the same time in the morning, the rabbits in the EF and ES+EF groups were administered an overnight culture of *E. faecium* CCM7420 strain (1.0 x 109 CFU/ml, 500 µl/animal/day into water). *E. faecium* CCM7420 strain doses as well as the diet A (with *E. senticosus* extract) were administered to rabbits during 21 days (treatment period). From day 22 all animals were fed only the untreated diet B. The experiment lasted for 42 days. All care and experimental procedures involving animals followed the guidelines stated in the *Guide for the Care and Use of Laboratory Animals* (1996) and the trials were accepted by the Ethical Commission of Institute of Animal Physiology in Košice and by Slovak Veterinary and Food Administration.

Body weight and feed consumption of rabbits were measured every week during the experiment. Mortality and morbidity were also recorded in groups daily. Sampling of faeces was done on days 0/1 (the start of the experiment; 12 mixture samples from all rabbits – initial microbial background), on day 21 (the end of CCM7420 and *E. senticosus* application; 5 mixture samples from each group) and on day 42 (the end of the trial, 21 days after the strain cessation; 5 mixture samples from each group). Blood was sampled on days 0/1, 21 and 42.

Bacterial enumeration

Bacteria from faecal samples were isolated by the standard microbiological method using the appropriate dilutions in Ringer solution (pH 7.0; Oxoid Ltd.,UK). Dilutions were plated onto following media: M-Enterococcus agar (Becton & Dickinson, USA) for enterococci, Todd-Hewitt agar (Imuna) enriched with rifampicin (100 µg/ml) for *E. faecium* CCM7420, Mannitol Salt agar for coagulase-negative staphylococci, Baird-Parker agar enriched with Egg Yolk Tellurite supplement (Becton & Dickinson) for

Table 2. The effect of *E. senticosus* (ES), *E. faecium* CCM7420 (EF) and their combinative (ES+EF) application on the bacterial counts (log_{10} CFU/g ± SD) in faeces of rabbits.

	ES	EF	ES+EF	CG	P value
			Day 0		
E. faecium CCM7420			–		–
Enterococcus sp.			2.80 ± 0.76		–
LAB			2.54 ± 0.72		–
CoNS			3.43 ± 0.58		–
CoPS			2.57 ± 0.64		–
S. aureus – like			2.81 ± 0.67		–
Coliform bacteria			2.43 ± 1.18		–
Pseudomonas-like sp.			3.07 ± 1.07		–
Clostridium-like sp.			2.25 ± 0.42		–
			Day 21		
E. faecium CCM7420	–	1.59 ± 0.49	1.05 ± 0.30	–	
Enterococcus sp.	3.36 ± 0.49	3.31 ± 0.43	2.98 ± 0.10	3.82 ± 0.26	0.0148
LAB	3.35 ± 0.77	3.58 ± 0.43	2.64 ± 0.95	3.67 ± 0.48	0.1186
CoNS	3.80 ± 0.53^{ac}	2.40 ± 0.47^{b}	3.13 ± 0.65^{abc}	3.91 ± 0.49^{ac}	0.0014
CoPS	2.75 ± 0.87	3.56 ± 0.25	2.11 ± 1.36	3.29 ± 0.63	0.0821
S. aureus	1.20 ± 0.36^{a}	1.20 ± 0.36^{a}	1.10 ± 0.00^{a}	2.35 ± 0.71^{b}	0.0009
Coliform bacteria	2.63 ± 0.46^{a}	1.30 ± 0.61^{bc}	1.02 ± 0.10^{bc}	2.90 ± 0.93^{a}	0.0002
Pseudomonas-like sp.	4.12 ± 0.17^{a}	3.53 ± 0.63^{ac}	2.88 ± 0.43^{bc}	4.40 ± 0.37^{ad}	0.0002
Clostridium-like sp.	1.27 ± 0.26^{a}	2.27 ± 0.76^{ab}	1.17 ± 0.19^{a}	2.65 ± 1.23^{b}	0.0132
			Day 42		
E. faecium CCM7420	–	1.37 ± 0.60	< 1.0	–	
Enterococcus sp.	4.43 ± 0.56	4.60 ± 0.42	4.34 ± 0.45	4.53 ± 0.09	0.4081
LAB	4.61 ± 0.50	3.82 ± 1.43	4.35 ± 0.38	4.37 ± 0.09	0.9668
CoNS	4.60 ± 0.33	2.85 ± 0.68	4.10 ± 0.41	3.66 ± 0.31	0.5237
CoPS	4.41 ± 0.38	4.56 ± 0.45	3.86 ± 0.82	4.52 ± 0.08	0.9529
S. aureus	4.35 ± 0.38	3.60 ± 0.00	3.30 ± 0.47	ND	0.8329
Coliform bacteria	3.77 ± 1.05	1.27 ± 0.74	1.70 ± 1.61	4.62 ± 1.49	0.1758
Pseudomonas-like sp.	4.74 ± 0.32	3.77 ± 0.47	4.59 ± 0.28	4.89 ± 0.27	0.3590
Clostridium-like sp.	2.26 ± 0.28	1.63 ± 0.15	2.35 ± 0.85	2.79 ± 0.28	0.4884

CoNS – coagulase-negative staphylococci, CoPS – coagulase-positive staphylococci, LAB – lactic acid bacteria, ND – not detected.

[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).

coagulase-positive staphylococci and *S. aureus*, De-Mann-Rogosa-Sharpe agar (Merck, Germany) for lactic acid bacteria, *Clostridium* difficile agar (Oxoid) for *Clostridium*-like species (anaerobic cultivation), Mac Conkey agar for coliforms and incubated at 37°C for 24-48 h, and Pseudomonas agar (Biomark, India) for *Pseudomonas*-like species, incubated at 25°C for 24-48 h. The bacterial counts were expressed in log 10 of colony forming units per gram (log10 CFU/g ± SD).

Blood analysis

Blood (n=6) was sampled from the marginal ear vein (*Vena auricularis*) into dry non-heparinized Ependorf tube on days 0/1, 21 and 42 for biochemical analyses. Biochemical parameters in blood serum were determined by colorimetric methods (Spectrophotometer UV-2500 Shimadzu, Japan) using kits (Randox, UK) for the following parameters: total protein (TP245), triglyceride (TR210), cholesterol (CH200), glucose (GL2623), calcium (CA590), glutathione peroxidase (RS504). Phagocytic activity (PA) was tested microscopically after Pappenheim staining according to the method of Šteruská (1981) and expressed as percentage of phagocyte ingested yeast cells to the total number of phagocytes (100 PMNs were counted per sample).

Statistical analysis

Statistical analysis of the results was performed by one-way analysis of variance (ANOVA) with the *post hoc* Tukey post-test with the level of significance set at $P<0.05$. The results are quoted as means ± SD (in the case of microbiological, biochemical and performance parameters) and means ± SEM (in the case of GPx,

Table 3. The effect of *E. senticosus* (ES), *E. faecium* CCM7420 (EF) and their combinative (ES+EF) application on phagocytic activity of leukocytes and blood biochemistry of rabbits.

		ES	EF	ES+EF	CG	P value
Total proteins (40-85 g/l)	Day 0			51.06 ± 1.95		
	Day 21	53.40 ± 1.71	53.22 ± 3.65	54.16 ± 1.46	52.73 ± 3.09	0.8230
	Day 42	57.60 ± 3.45	58.29 ± 1.25	60.75 ± 3.17	58.51 ± 2.37	0.2365
Triglycerides (6.89-8.67 mmol/l)	Day 0			1.84 ± 1.16		
	Day 21	1.67 ± 0.38^{ab}	1.25 ± 0.21^{a}	2.03 ± 0.64^{b}	1.48 ± 0.42^{ab}	0.0388
	Day 42	1.06 ± 0.27	1.15 ± 0.11	1.21 ± 0.23	1.19 ± 0.45	0.8165
Cholesterol (0.55-4.44 mmol/l)	Day 0			2.97 ± 0.39		
	Day 21	2.61 ± 0.63^{a}	4.43 ± 0.16^{b}	3.40 ± 0.81^{a}	2.84 ± 0.42^{a}	<0.0001
	Day 42	2.94 ± 0.69	2.69 ± 0.49	2.51 ± 0.35	2.29 ± 0.27	0.1467
Glucose (3-8 mmol/l)	Day 0			7.69 ± 0.70		
	Day 21	8.15 ± 1.02^{a}	7.52 ± 0.96^{ab}	7.54 ± 1.28^{ac}	9.68 ± 1.48^{ad}	0.0175
	Day 42	6.86 ± 0.85	6.91 ± 0.89	5.74 ± 1.02	6.17 ± 0.49	0.0701
GPx	Day 0/1					
(U/g Hb)	Day 21	141.25 ± 40.21	167.72 ± 49.65	130.92 ± 21.08	131.50 ± 31.09	0.8837
	Day 42	143.87 ± 23.13	141.70 ± 57.86	133.71 ± 40.44	166.24 ± 58.50	0.9664
Phagocytic activity (%)	Day 0			40.90 ± 0.55		
	Day 21	48.83 ± 0.87^{a}	44.33 ± 1.43^{b}	47.33 ± 0.60^{ab}	39.00 ± 0.73^{c}	<0.0001
	Day 42	51.83 ± 0.48^{a}	50.33 ± 1.02^{ab}	54.83 ± 0.87^{ac}	38.50 ± 0.85^{d}	<0.0001
IPA	Day 0			2.55 ± 0.08		
	Day 21	2.92 ± 0.05^{a}	2.47 ± 0.05^{b}	2.90 ± 0.06^{ac}	2.15 ± 0.08^{d}	<0.0001
	Day 42	3.00 ± 0.06^{a}	2.42 ± 0.05^{b}	2.88 ± 0.06^{ac}	2.32 ± 0.06^{b}	<0.0001

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different (P<0.05).

Table 4. The effect of *E. senticosus* (ES), *E. faecium* CCM7420 (EF) and their combinative (ES+EF) application on the growth performance of rabbits.

	ES	EF	ES+EF	CG	P value
Number of rabbits	(n=24)	(n=24)	(n=24)	(n=24)	
Initial live weight (35 d), g	1121.7 ± 138.3	1136.7 ± 137.6	1077.5 ± 102.2	1109.6 ± 169.4	0.5042
Intermediate live weight (56 d), g	1994.4 ± 251.4	2067.0 ± 250.2	1943.3 ± 222.2	1933.3 ± 229.5	0.2005
Final weight (77 d), g	2712.0 ± 283.9	2813.5 ± 214.3	2723.3 ± 204.7	2627.0 ± 301.5	0.0987
Average daily gain (g/d)	37.86	39.92	39.19	36.13	
Feed conversation ratio between 35-56 days of age (g/g)	2.93	2.92	2.59	2.91	
Feed conversation ratio between 56-77 days of age (g/g)	4.24	4.21	3.97	4.40	
Feed conversation ratio per kg gain	3.59	3.57	3.28	3.66	
Mortality between 35-56 days of age (n)	1	1	0	3	
Mortality between 56-77 days of age (n)	0	0	0	1	
Mortality (n)	1	1	0	4	

35-56 days of age – application of *E. senticosus* (ES), *E. faecium* CCM7420 (EF) and their combination (ES+EF)
56-77 days of age – after the *E. senticosus* extract and *E. faecium* CCM7420 strain cessation

PA, IPA). 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, c, d**).

Results

The microbiological examinations in faecal samples confirmed the presence of *E. faecium* CCM7420 strain (Table 2). The survival rate of CCM7420 strain after rabbit digestion was lower in rabbits administered additives combination (ES+EF) than in EF. Surprisingly, the decrease of enterococci, lactic acid bacteria and staphylococci was detected in the ES+EF group during the additives application (day 21, difference 0.84, 1.03 and 0.78 log log cycles) compared to CG. Significant reduction of faecal coliforms (EF: $P<0.001$; ES+EF: $P<0.0001$), *S. aureus* (ES, EF: $P<0.01$; ES+EF: $P<0.01$). *Pseudomonas*-like sp. (EF: $P<0.05$; ES+EF: $P<0.001$) and *Clostridium*-like sp. (ES: $P<0.05$; ES+EF:$P<0.05$, Table 2) was noted compared to CG. The lowest numbers of mentioned bacteria were recorded in the ES+EF group; this reduction was lasting till the end of the experiment, except *S. aureus* cells.

The significant increase of PA was noted after additives application (ES, ES+EF: $P<0.0001$; EF: $P<0.001$ on day 21) compared to CG (Table 3). The highest PA was measured in the ES group (48.83 ± 0.87 %), followed by the groups EF+ES (47.33 ± 0.60 %) and EF (44.33 ± 1.43 %, Table 3). Higher PA was noted in all experimental groups on day 42, compared to CG ($P<0.0001$) and also to data from day 21. The CCM7420 strain showed also stimulative effect on unspecific immunity parameter. The lowest values of GPx were measured in the ES+EF group (130.92 ± 21.08 U/g Hb on day 21; 133.71 ± 40.44 U/g Hb on day 42, Table 3). The treatment effects on the serum total protein and triglyceride concentration ($P<0.05$) were recorded (Table 3). In groups with *E. senticosus* application, the decrease of cholesterol concentrations was found (ES: $P<0.0001$; ES+EF: $P<0.05$, day 21). The glucose concentration was reduced in all EG ($P<0.05$, day 21), while the serum calcium concentrations increased during additives application (ES: $P<0.05$; EF: $P<0.01$; Table 3).

All animals were in good health conditions throughout the trial. During the *E. senticosus* and *E. faecium* CCM7420 applications, the minimal mortality was recorded in all EG compared to CG (Table 4). Better feed conversion was noted in ES+EF, compared to CG. No mortality in this group was also recorded. The body weight gains are the most frequently controlled parameters during additives application. Our results confirmed these observations; under our conditions, average daily gain increased by 10.4% in the EF, by 8.5% in the ES+EF and 4.8% in the ES compared to the CG.

Discussion

The gut microbiota is an important constituent in the intestine's mucosal barrier; the increase of the host defense had been already demonstrated by the application of potentially beneficial microorganisms and other natural antimicrobials (bacteriocins, organic acids, plant extracts; Fortun-Lamothe and Boullier 2007). The reduction of enterococci and lactic acid bacteria could be probably explained by the activity of the strain against other enterococci and/or LAB. Changes in bacterial composition – decrease of faecal coliforms, *Pseudomonas*-like sp., *Clostridium*-like sp. and *S. aureus* – during the additives supplementation confirm the antibacterial effect of CCM7420 strain, also in the combination (ES+EF) group. The inhibitory effect of the bacteriocin-producing and probiotic enterococci and/or plant extracts on rabbits intestinal microbiota was already reported in our previous studies (Simonová et al. 2008a,b, Szabóová et al. 2008, Pogány Simonová 2009, Szabóová et al. 2011, 2012). Kritas et al. (2008) also described the lower frequency of *E. coli* and *C. perfringens* in rabbits treated by probiotic. The dominancy in antimicrobial activity of CCM 7420 strain combined with *E. senticosus* can be confirmed by the fact, that of *E. senticosus* extract, possesses slight or no antimicrobial activity (Simonová et al. 2008a). We suppose that the dietary modulation of the gastrointestinal microbiota by natural antimicrobial substances can result in an enhancement of colonization resistance against potentially pathogenic bacteria.

Bioactive plants and compounds also enhance immune function of animals, both humoral and cellular activity (Durmic and Blache 2012). The highest PA values in ES and EF+ES groups confirmed the stimulative effect of *E. senticosus* on immunity described in many reports concerning the humans (Coleman et al. 2003, Lee et al. 2008). The CCM7420 strain showed also stimulative effect on unspecific immunity parameter, similarly to our previous studies in rabbits (Simonová et al. 2008a, Lauková et al. 2012, Szabóová et al. 2012, Pogány Simonová et al., 2013) or in dogs (Strompfová et al. 2012). Bovera et al. (2007) also presented the increase of lymphocytes as signs of immunostimulatory effect in suckling rabbits after *Lactobacillus plantarum* spray application, but lower counts of neutrophils responsible for phagocytosis. The activation of innate immunity through phagocytic

cells by probiotic treatment was presented in chicken (Higgins et al. 2007), mice (Perdigon et al. 1986) and piglet model (Shu et al. 2001). While these effects are well studied in humans, there has been little research in production animals. That is why more research is required to understanding mechanisms.

The concentration of glutathione-peroxidase (GPx) is often monitored in meat and other organs – liver, kidney in rabbits (Dokoupilová et al. 2007), but not often in blood serum. The activity of GPx in blood can be marker of reactions after stress, induced by factors such as diet changes, manipulation. The evidently good health of rabbits indicated that oxidative stress was not evoked during the experiment. No negative influence of bacteriocins and probiotics application on GPx values was reported in our previous studies (Simonová et al., 2008a,b, Pogány Simonová et al., 2013). Papadomichelakis et al. (2011) also described no influence of dietary benzoic acid addition on oxidative stress (GSH-Px in liver tissues and blood erythrocytes) in fattening rabbits. Lee et al. (2008) showed the protective effect of dried *E. senticosus* tea against the oxidative DNA damage.

The tested serum parameters were in the range of normal values defined for these parameters by previous studies (Burnett et al., 2006, Özkan et al., 2012) in rabbits. The influence on serum protein level by probiotic addition is often depends on the period (length) and dose of its application, the initial protein concentration and animals age and weight. Although the digestibility of feed protein and fat was not measured, we supposed the better resorption and utilization of these nutrients from the gastrointestinal tract caused by *E. senticosus* and CCM7420 strain addition, similarly to other findings (Simonová et al. 2008a,b, Shrivastava and Jha 2010). The bioactive plants compounds may also affect blood parameters by maintaining (positive effect) or altering (negative effect) them (Durmic and Blache 2012). Antihyperlipidemic or hypocholesterolaemic effects of plants have also been reported (Kuda et al. 2004). The hypocholesterolaemic effect of *E. senticosus* was confirmed in our study, similarly to other observations (Lee et al. 2008, Strompfová et al. 2012). The most of reports presents the anti- and/or hypocholesterolaemic effect of probiotic supplementation in rabbits already during their application (Shrivastava and Jha 2010). Our results are contradictory to them (Table 3). The lower glucose concentrations in EG could be explained by increased H^+ concentration caused by higher concentrations of organic acids tested in caecum content (tested, but data not shown), which inhibited gluconeogenesis (Iles et al. 1977). The increased of H^+ (lactate accumulation in the organism) first stimulates the physicochemical mineral dissolution by increasing the osteoclasts and osteoblasts activity – bone resorption (Bushinsky and Frick 2000), and also the Ca reabsorption in renal tubules for pH neutralization. Although the lactate in blood was not measured, these facts are supporting our findings about increased serum calcium concentrations during additives application. Moreover, rabbits metabolize calcium very differently from other animals. The rabbits' blood calcium levels fluctuate widely, dependent upon the level of calcium in their diet and the intestinal absorption as well (Redrobe, 2002).

All animals were in good health conditions throughout the trial. The probiotics and plant extracts application is associated with their beneficial effect on body weight, morbidity and/or mortality in rabbit farms (Matusevičius et al. 2004, Chrastinová et al. 2007, Kritas et al. 2008). Low mortality, improved weight gain and feed conversion ratio are also in agreement with our previous findings (Simonová et al. 2008a,b, Pogány Simonová et al. 2009).

Conclusions

The dietary supplementation with *E. senticosus* extract and *E. faecium* CCM7420 enterocin-producing and probiotic strain alone and in combination could improve growth performance, stimulate digestive immunity and increase the host's defence capacities by increase of phagocytic activity and reduction/competition with potential pathogens, without induction of oxidative stress in rabbits. However, more research is required to spread and improve actual knowledge about these natural additives, to understand their mechanisms of action in animals and to achieve higher effectivity on the health and production of rabbits.

Acknowledgements

This work was partially supported by the project VEGA 2/0002/11. We are grateful to Mrs. Margita Bodnárová for their skilfull technical assistance.

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