Original article

Effect of combined administration of enterocin 4231 and sage in rabbits

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Abstract

Enterocin (*Ent*) 4231, produced by non-rabbit origin strain *Enterococcus faecium* CCM 4231 was used in combination with sage plant extract in rabbits with the aim to check their antimicrobial activity against microbiota, their effect on immunological, biochemical blood parameters, values of volatile fatty acids in caecum, *Eimeria* sp. oocysts occurrence and selected parameters of rabbits meat. The animals were divided into three experimental groups (EG1-*Ent* 4231; EG2- sage; EG3- *Ent* 4231 with sage) and control group (CG); 24 rabbits in each. Natural substances (NS) were administered for 21 days. The experiment lasted for 42 days. The reduction of microbiota in faeces was observed in EG3 at day 21 by a decrease in the numer of coagulase-positive staphylococci (P<0.01) in comparison with that determined in CG. The bacterial counts in the caecum were lower than those found in faeces. A decrease in the numer of *Pseudomonas*-like sp. in caeca of the experimental groups was observed at days 21 and 42 (difference in range 0.40-1.87 log cycles) comparing with that determined in CG. At day 21, a significant increase in phagocytic activity (PA, P<0.001) was found in blood of rabbits from EG2 comparing with that observed in CG. At day 42, a significant increase in PA (P<0.001) was determined in all experimental groups in comparison with CG. At day 21, in caecal content of EG3 significantly higher values of lactic acid were observed (P<0.05) in comparison with those found in CG. The reduction of *Eimeria* sp. oocysts was demonstrated after application of each of NS. Addition of NS did not influence biochemical parameters, meat quality of the animals and does not influence negatively the health status of rabbits.

Key words: enterocin, sage, rabbit, microbiota, effect

Introduction

Enterococci represent Gram-positive, facultative anaerobic lactic acid bacteria from the genus Enterococcus, division Firmicutes. They are also known to produce ribosomally synthesized, extracellularly released antimicrobial peptides – bacteriocins, mostly enterocins (Franz et al. 2007); the number of those characterized has been significantly increased. However, most of them are produced by the species *Enterococcus faecium* of different origin (Franz et al. 2007, Mareková et al. 2007). According to the classification scheme of Franz et al. (2007), enterocins belong to class I (lantibiotic enterocins), class II (small,

nonlantibiotic peptides), class III (cyclic enterocins) and class IV (large proteins). Class II is subdivided in three subclasses: II. 1, enterocins of the pediocin family; II. 2, enterocins synthesized without a leader peptide and II. 3, other linear, nonpediecin type enterocins.

Enterococcus faecium CCM 4231 (isolated in Laboratory of Animal Microbiology, Institute of Animal Physiology, Slovak Academy of Sciences) was found as the first bacteriocin-producing strain of ruminal origin with probiotic character (Lauková et al. 1993). Enterocin (*Ent*) 4231 is an antimicrobial termo-stable peptid (3-10 kDa) with optimum pH for its production between 4.0 and 7.5 in logarithmic phase of growth with activity 3200 AU/ml (Lauková et al. 1999b). Producer strain and its bacteriocin *Ent* 4231 have already been successfully applied into many ecosystems to reduce spoilage flora (Lauková et al. 1998a,b 1999a,b, 2001a,b). Producer strain *E. faecium* CCM 4231 was even tested in rabbits (Szabóová et al. 2008a).

Administration of natural substances (bacteriocins or phytoaditives and their essential oils) in rabbits ecosystem offer an acceptable way to prevent and/or overcome bacterial and viral infections as well as to improve welfare, health and meat quality of rabbits (Xu et al. 1999, Fichi et al. 2007, Szabóová et al. 2008a, 2008b). The use of secondary metabolites of plants containing essential oils with inhibitory effects against pathogens represents an alternative approach (Marcin et al. 2006). The genus *Salvia* (sage) encompasses about 900 species of plants belonging to the mint family *Lamiaceae* (Labiatae; Gali-Muhtasib et al. 2000). Different types of extracts of *Salvia officinalis* has possessed antioxidant, anti-inflammatory, hypoglycemic and anti-mutagenic bioactivities (Wang et al. 1998, Baricevic et al. 2001, Alarcon-Aquilar et al. 2002). Based on our previous results (Szabóová 2008b), in this study combined use of *Ent* 4231 and sage plant extract in rabbits was tested with the aim to detect their antimicrobial activity, immunological and biochemical blood parameters, the values of lactic acid and volatile fatty acids in the content of the caecum, reduction of *Eimeria* sp. oocysts and selected parameters of rabbit meat.

Materials and Methods

Animals and experimental design

Ninety six rabbits, 5-weeks old (male sex, Hy-Plus breed), were used in this experiment. All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals which was accepted by Slovak Governmental Veterinary and Food Institute.

Rabbits were divided into three experimental groups (EG1, EG2, EG3) and control group (CG) of 24 animals in each group. The animals were kept in the standard cages, two animals per cage. All the animals were fed a commercial diet for growing rabbits (ANPRO.FEED, VKZ Bučany, Slovakia) during the entire experiment with free access to water. Rabbits in EG1 received every day (from day 0-1 to day 21) *Ent* 4231 (prepared according to Lauková et al. 1997) in drinking water at a dose of 50 μl/animal/day (1600 AU/ml); rabbits in EG2 received (from day 0-1 to day 21) sage plant extract (*Salvia officinalis* extract contained 24% of thujone, 18% of borneol, 15% of cineole; Calendula company, Nová L'ubovňa, Slovakia) in drinking water and at a dose of 10 μl/animal/day. This dose was decided to use according to our previous *in vitro* studies testing an inhibitory activity of sage extract against target of bacteria (Szabóová et al. 2008b) as well as this dose was well tolerated by the animals. The animals in EG3 received (from day 0-1 to day 21) the combination of *Ent* 4231 (50 μl/animal/day, 1600 AU/ml) strain and sage plant extract (10 μl/animal/day). The experiment lasted for 42 days. The doses of additives and their application form were decided developing from our previous experiments (Szabóová et al. 2008a,b, Pogány Simonová et al. 2009, Lauková et al. 2009). Moreover, developing from our previous experiments, the volume of water drunk by rabbits is known.

Bacterial counts

Faeces (faecal mixture from 96 animals, n=10) were sampled at the beginning of the experiment, day 0-1), then the samples were taken at day 21 [3 weeks of natural substances application (NS), faecal mixture of each group, 24 rabbits in each group, n=5]; at day 42 (3 weeks after cessation of NS administration, faecal mixture of each group, 24 rabbits in each group n=5) to monitor an inhibitory effect of *Ent* 4231 and sage extract as well separately and in their combination. The samples (1g) were collected into sterile polyvinylchlorid (PVC) bags, treated by the standard microbiological method (dilutions in Ringer solution, pH 7.0; Oxoid Ltd., Basingstoke, Hampshire, England) plating appropriate dilutions on the selective media according to ISO (International Organization for Standardization). To enumerate enterococci, M-Enterococcus agar (Difco, Detroit, USA) was used. Baird-Parker agar supplemented with egg yolk tellurite solution (Becton & Dickinson, Cockeysville, USA), Mannitol Salt Agar (Difco, Detroit, USA), *Clostridium difficile* agar with the supplement SR0096E and 7% (v/v) defibrinated horse blood (SR0050, Oxoid Ltd., Basingstoke, Hampshire, England) were used to enumerate coagulase-positive

staphylococci (CPS) including *Staphylococcus aureus,* coagulase-negative staphylococci (CNS) and *Clostridium*-like sp. Mac Conkey agar and Cetrimide agar (Becton and Dickinson) were used to count coliform bacteria and *Pseudomonas*-like sp. The plates were incubated at 30ºC and/or 37ºC for 24-48 h depending on the bacterial species. Bacterial counts were expressed in colony forming units (log10 CFU) per gram.

At days 21 and 42, three animals from each group were slaughtered. Caeca were separated from the other parts of the gastrointestinal tract and caecal contents (1g) were collected into sterile PVC sample tubes (Falcon, USA) to count microbiota. They were serially diluted in Ringer solution (according to the standard microbiological method) and plated on the media mentioned above. Lactic acid (g/100g) and volatile fatty acid values (acetic, propionic and butyric acids- mmol/l) were determined using gas chromatography from the samples of caecal content (15 g) at days 21 and 42.

Biochemical parameters and phagocytic activity

Biochemical parameters were examined at days 0-1, 21 and 42: serum levels of proteins and lipids (g/l), cholesterol (mmol/l), glucose (mmol/l), calcium (mmol/l), glutathion peroxidase (GPx; U/ml) using commercial kit Randox (England).

Phagocytic activity was measured by direct counting procedure using microspheric hydrophilic particles (MSHP). Ingestion of MSHP by polymorphonuclear cells (PMN) was determined using a modified test described by Vetvicka et al. (1982): 50 µl of MSHP suspension (ARTIM, Prague, Czech Republic) was mixed with 100 µl of blood in an Eppendorf-type test tube and incubated at 37ºC for 1 h. Blood smears were then prepared and stained in accordance with May-Grunwald and Giemsa--Romanowski (Pappenheim stain – May-Grünwald solution was added on a slide with blood smear and worked for 3 minutes. Then 5 ml of distilled water was added and worked for 1 minute. After removal of the solution were added 75 drops of Giemsa-Romanowski solution and this solution worked for 20 minutes. Blood smears were examined microscopically after rinsing with distilled water). Phagocytic activity (PA) was calculated as number of white cells containing at least three engulfed particles/100 white cells (neutrophils and monocytes) and the index of PA was calculated as number of engulfed particles/total number of neutrophils and monocytes observed. The percentage of phagocytic cells was evaluated using an optical microscope, by counting PMN up to 100.

Eimeria sp. oocysts counting

Eimeria sp. oocysts were enumerated in the faecal samples microscopically at days 0-1, 21 and 42 of the experiment and expressed as counts of oocysts per 1 g of faeces (OPG). The samples were stored at 4ºC and then evaluated by the quantitative flotation technique – McMaster method (Ministry of Agriculture, Fisheries and Food, UK, 1986).

Evaluation of meat

The following parameters were tested 24 h *post mortem*: total lipids and proteins, energetic value and the value of pH. The ultimative pH was determined 48 h *post mortem* by a Radelkis OP-109 with a combined electrode penetrating 3 mm into the *Musculus longissimus dorsi* (MLD). 100 g of MLD was sampled and after cooling at 5ºC analysed using the spectroskop Infratec 1265 (STN 570185) – Meat Analyser (FOOS North America, Inc.; Minnesota; USA). Total proteins and lipids were expressed in g/100 g.

Statistical Analysis

The results are expressed as mean ± standard deviation (SD), statistical evaluation of the results was performed by the one-way ANOVA and the Tukey test.

Results

At day 21 (3 weeks after NS application), in faeces of EG3 (*Ent* 4231 and sage extract), a significant decrease in CPS (P<0.01; Table 1) was detected in comparison with CG. At days 21 and 42, a decrease in *Clostridium*-like sp. in faeces of EG1 was detected in comparison with CG (day 21, difference 1.53 logarithmic cycle (log); day 42, difference 1.57 log cycle, Table 1). The counts of the other microbiota in faeces were not influenced by the administration of both NS. In general, the bacterial counts in the caecum were lower than those found in faeces; the reduction of *Pseudomonas*-like sp. in caecal contents of the experimental groups was observed at days 21 and 42 (day 21, EG1: difference 0.40 log cycle; EG2: 1.59 log cycle; EG3: 1.87 log cycle; day 42, EG1: 1.16 log cycle; EG2: 1.05 log cycle; EG3: 1.56 log cycle) comparing with CG (differences in log cycle between EG and CG are calculated from their counts; Table 2).

Table 1. Microbiota in faeces of rabbits expressed in CFU/g.

	EG1	EG2	EG3	CG
Day 21[A]				
Enterococcus sp.	4.40 ± 1.00	5.28 ± 1.29	3.30 ± 0.91	5.33 ± 0.57
CNS	3.70 ± 0.56	3.61 ± 0.80	3.98 ± 1.27	3.89 ± 1.33
CPS	3.90 ± 0.63	5.30 ± 1.01	2.31 ± 0.35[a]	4.80 ± 0.20[b]
Clostridium-like sp.	3.47 ± 0.72	4.41 ± 1.08	4.39 ± 1.52	5.00 ± 0.74
Pseudomonas-like sp.	3.50 ± 0.46	5.15 ± 0.88	3.98 ± 0.68	4.42 ± 0.40
Coliform bacteria	2.70 ± 1.48	3.69 ± 1.55	< 1.00	3.64 ± 0.78
Day 42[B]				
Enterococcus sp.	3.35 ± 0.47	3.72 ± 0.39	3.56 ± 0.35	2.61 ± 0.45
CNS	3.29 ± 0.11	3.34 ± 0.18	3.29 ± 0.10	3.45 ± 0.10
CPS	3.68 ± 0.64	3.28 ± 0.48	3.62 ± 0.23	2.87 ± 0.34
Clostridium-like sp.	3.40 ± 1.54	5.11 ± 0.91	4.63 ± 1.39	4.97 ± 0.91
Pseudomonas-like sp.	3.79 ± 0.47	4.03 ± 0.34	3.52 ± 0.32	3.18 ± 0.26
Coliform bacteria	< 1.00	2.65 ± 0.73	1.30 ± 0.42	1.83 ± 0.53

EG1 – experimental group (*Ent* 4231; n=5); EG2 – experimental group (sage; n=5); EG3 – experimental group (*Ent* 4231 with combination of sage; n=5); CG – control group (n=5); [A] 3 weeks of *Ent* 4231 strain and sage administration; [B] 3 weeks after cessation of *Ent* 4231 and sage application; [a,b] P<0.01; Day 0-1: *Enterococcus* sp.: 2.64 ± 1.04 CFU/g; CNS: 2.54 ± 0.14 CFU/g; CPS: 2.47 ± 0.93 CFU/g; *Clostridium*-like sp.: 2.82 ± 1.17 CFU/g; *Pseudomonas*-like sp.: 1.56 ± 0.58 CFU/g; Coliform bacteria: 1.71 ± 0.57 CFU/g; decrease of *Clostridium*-like sp. in EG1 at days 21 and 42 in comparison with CG (day 21, difference 1.53 log cycle; day 42, difference 1.57 log cycle)

Table 2. Microbiota in caecum content in rabbits expressed in CFU/g.

	EG1	EG2	EG3	CG
Day 21[A]				
Enterococcus sp.	1.00 ± 0.00	1.00 ± 0.00	< 1.00	1.00 ± 0.00
CNS	2.94 ± 0.09	2.87 ± 0.11	2.63 ± 0.39	3.53 ± 0.51
CPS	1.24 ± 0.24	1.56 ± 0.66	1.55 ± 0.40	2.24 ± 0.30
Clostridium-like sp.	3.10 ± 0.14	4.29 ± 0.91	3.05 ± 0.18	3.49 ± 0.30
Pseudomonas-like sp.	3.65 ± 0.44	2.46 ± 0.13	2.18 ± 0.08	4.05 ± 0.54
Coliform bacteria	1.30 ± 0.00	2.04 ± 0.00	< 1.00	2.68 ± 1.24
Day 42[B]				
Enterococcus sp.	< 1.00	< 1.00	< 1.00	< 1.00
CNS	3.33 ± 0.30	3.34 ± 0.19	2.98 ± 0.26	3.19 ± 0.15
CPS	2.02 ± 0.20	2.03 ± 0.16	1.79 ± 0.16	2.10 ± 0.04
Clostridium-like sp.	2.15 ± 0.93	2.63 ± 0.48	2.18 ± 0.59	2.47 ± 0.38
Pseudomonas-like sp.	2.30 ± 0.49	2.41 ± 0.14	1.90 ± 0.30	3.46 ± 0.12
Coliform bacteria	2.15 ± 1.15	1.00 ± 0.00	< 1.00	1.79 ± 0.79

EG1 – experimental group (*Ent* 4231; n=3); EG2 – experimental group (sage; n=3); EG3 – experimental group (*Ent* 4231 with combination of sage; n=3); CG – control group (n=3); [A] 3 weeks of *Ent* 4231 strain and sage administration; [B] 3 weeks after cessation of *Ent* 4231 and sage application; the reduction of *Pseudomonas*-like sp. in the experimental groups at days 21 and 42 (day 21, EG1: difference 0.40 log cycle; EG2: 1.59 log cycle; EG3: 1.87 log cycle; day 42, EG1: 1.16 log cycle; EG2: 1.05 log cycle; EG3: 1.56 log cycle) comparing with CG

At day 21, a significant increase in PA (P<0.001; Table 3) was determined in blood of rabbits from EG2 comparing with CG. At day 42, a significant increase in PA (P<0.001; Table 3) in all experimental groups (EG1; EG2; EG3) was found in comparison with CG. PA at day 21 in EG1 was 18.67% ± 0.42; in EG2 was 27.67% ± 0.42; in EG3 20.50% ± 0.43 in comparison with CG (22.50% ± 0.85); while at day

Table 3. Phagocytic activity (in %) during experiment in rabbits.

	Start of the experiment; day 0-1	Day 21[A]	Day 42[B]
EG1	21.60 ± 4.22	18.67 ± 0.42	25.20 ± 0.80[a]
EG2	21.60 ± 4.22	27.67 ± 0.42[a]	30.50 ± 0.96[a]
EG3	21.60 ± 4.22	20.50 ± 0.43	28.60 ± 0.51[a]
CG	21.60 ± 4.22	22.50 ± 0.85[b]	20.40 ± 0.51[b]

EG1 – experimental group (*Ent* 4231; n=5); EG2 – experimental group (sage; n=5); EG3 – experimental group (*Ent* 4231 with combination of sage; n=5); CG – control group (n=5); [A] 3 weeks of *Ent* 4231 strain and sage administration; [B] 3 weeks after cessation of *Ent* 4231 and sage application; [a,b] P<0.001

Table 4. The values of lactic acid and volatile fatty acids in ceacum content at days 21 and 42.

			Day 21[A]	Day 42[B]
EG1	lactic acid	g/100g	0.044 ± 0.006	0.034 ± 0.006
	acetic acid	mmol/l	5.540 ± 0.418	3.876 ± 0.436
	propionic acid	mmol/l	0.353 ± 0.069	0.353 ± 0.048
	butyric acid	mmol/l	1.007 ± 0.115	0.755 ± 0.214
EG2	lactic acid	g/100g	0.042 ± 0.006	0.036 ± 0.003
	acetic acid	mmol/l	6.221 ± 0.998	3.929 ± 0.623
	propionic acid	mmol/l	0.555 ± 0.163	0.264 ± 0.012
	butyric acid	mmol/l	1.243 ± 0.480	0.991 ± 0.310
EG3	lactic acid	g/100g	0.045 ± 0.004[a]	0.029 ± 0.001
	acetic acid	mmol/l	5.595 ± 0.658	3.450 ± 0.510
	propionic acid	mmol/l	0.372 ± 0.025	0.276 ± 0.082
	butyric acid	mmol/l	1.381 ± 0.145	0.810 ± 0.190
CG	lactic acid	g/100g	0.028 ± 0.008[b]	0.031 ± 0.001
	acetic acid	mmol/l	5.767 ± 0.769	4.671 ± 0.807
	propionic acid	mmol/l	0.417 ± 0.049	0.438 ± 0.148
	butyric acid	mmol/l	1.296 ± 0.267	1.189 ± 0.259

EG1 – experimental group (*Ent* 4231; n=3); EG2- experimental group (sage; n=3); EG3 – experimental group (*Ent* 4231 with combination of sage; n=3); CG- control group (n=3); [a,b] P<0.05; [A] 3 weeks of *Ent* 4231 strain and sage administration; [B] 3 weeks after cessation of *Ent* 4231 and sage application

42, PA in rabbits of EG1 was 25.20% ± 0.80; EG2 30.50% ± 0.96 and EG3 28.60% ± 0.51 in comparison with CG (20.40% ± 0.51; Table 3).

At day 21 in caecal content of rabbits in EG3 significantly higher values of lactic acid were noted (P<0.05; Table 4) in comparison with CG. The values of other volatile fatty acids were not significantly changed.

The reduction of *Eimeria* sp. oocysts was demonstrated after application of each of NS (*Ent* 4231; sage; combination of both) in EG1 (not detected – nd); in EG2 (30.00 ± 2.74 OPG); in EG3 (20.00 ± 2.74 OPG) comparing with CG (1184.00 ± 45.83 OPG) at day 21. Moreover, at day 42 the reduction of *Eimeria* sp. oocysts was found in EG1 (2250.00 ± 161.79 OPG); in EG2 (20.00 ± 2.74 OPG); in EG3 (nd) comparing with CG (5640.00 ± 263.20 OPG).

The feeding of NS by rabbits did not influence biochemical parametres e.g. they did not evoke oxidative stress – not influence values of GPx during the entire experiment as well as does not influence negatively the health status of rabbits.

By the monitoring of meat quality parameters, slight changes between experimental (EG1, EG2, EG3) and control groups (CG) were noted – total proteins (EG1: 21.96 ± 0.35; EG2: 21.83 ± 0.30; EG3: 22.03 ± 0.15; CG: 22.20 ± 0.10 g/100g), pH (EG1: 5.93 ± 0.07; EG2: 5.97 ± 0.11; EG3: 5.83 ± 0.07; CG: 5.83 ± 0.07) and water content (EG1: 75.83 ± 0.25; EG2: 76.00 ± 0.36; EG3: 75.93 ± 0.11; CG: 75.50 ± 0.40 g/100g) which approves rabbits killing by considerable way. The value of total lipids in rabbits meat in all experimental groups was lower in comparison with CG (EG1: 1.16 ± 0.25; EG2: 1.16 ± 0.15; EG3: 0.63 ± 0.15; CG: 1.26 ± 0.37 g/100g).

Discussion

At recent time, an increase in the number of studies testing enterocins *in vivo* was noted; there are more studies involving poultry, pigs, cows, food (Foulquié Moreno et al. 2006, Strompfová et al. 2006, Svetoch and Stern 2010). In rabbits husbandry, post-weaned period is problematic. The most frequent agens detected in this period are coliform bacteria, clostridiae, staphylococci (Isolauri et al. 1990, Chrastinová et al. 2010). Therefore, significant decrease in CPS, reduction of clostridiae in faeces and pseudomonads in the caecum after *Ent* 4231 application is important and promising result. Previously, *Ent* 4231 has been experimentally added to the rumen fluid, cattle dung water and food products and showed the inhibition of listeriae and staphylococci (Lauková et al. 1998a,b, 1999a,b, 2001a,b). Reduction of *Staphylococcus aureus* and *Clostridium*-like bacteria in the rabbits faeces after administration of *Ent* 2019 already reported Pogány Simonová et al. (2009). However, *Ent* 2019 is produced by the rabbits isolate *E. faecium* EF2019-CCM7420 and *Ent* 4231 is produced by non-rabbit origin strain. The other *Ent-Ent* M produced by non-rabbit origin strain *E. faecium* AL 41 (environmental isolate) was reported to reduce CPS (Lauková et al. 2009). It seems, that enterocins like proteinaceous substances can act beneficially in GIT, although their mode of action is not fully known. Mechanism for the interaction of plants including *Salvia* sp. with host organism may be releated to intestinal and extraintestinal effects. Intestinal effects may be explained by the effects on microbiota (Montagne et al. 2003) or intestinal mucosal system (Garcia et al. 2007). Microflora can influence activities of certain enzymes involved in the metabolism uptake, and incorporation by enterocytes of dietary nucleic acids components (Whitt and Savage 1988). The inhibitory mechanism is the damage of permeability of cell membrane integrity, pH homeostasis (Helander et al. 1998), the balance of inorganic ions (Sikkema et al. 1995) and also depends on the concentration of essential oils. The low concentration of plant essential oils inhibits the activity of enzymes involved in energy metabolism of the cells as well as it inhibits the activity of enzymes involved in the precipitation of proteins. Phenols, active components of many essential oils destabilize the cytoplasmic and outer cell membranes, reduce the pH gradient, cause the depletion of ATP, what induce the leakage of ions, ATP, amino acids, nucleic acids and in a consequence the cell death (Tranter et al. 1993, Ultee et al. 2002). Antimicrobial activity of sage plant extract has been tested mostly individually (Eidi et al. 2005, Szabóová et al. 2008b, Revajová et al. 2010); also in our experiment, in sage group of rabbits (EG2) higher PA value was measured at day 21 and 42 as compared with those found in EG1, EG3 and CG. On the other side, our experiment showed beneficial influence of sage plant extract also in combination with *Ent*. Moreover, higher percentage of PA in rabbits of the experimental groups at day 42 has shown prolonged immuno-stimulatory effect of NS. Plachá et al. (2010) have reported significantly increased values of PA (P<0.001) in hens administrating *Ent* M (produced by the strain *E. faecium* AL41) in the drinking water in comparison with hens fed by the standard diet. According to Lauková et al. (2008), administration of *Eleutherococcus senticosus* (as phytoadditive) separately showed stimulating effect on PA in rabbits. Also, Nofrarías et al. (2006) have reported immuno-modulatory effect of dietary plant extracts (carvacrol, cinnamaldehyd) which can affect the intestinal morphology and immune cell subsets of gut tissues and blood in weaned pigs. Neutrophil polymorphonuclear leucocytes (granulocytes) are responsible for the non-specific immune response and in the first line share for phagocytosis intro-defence of the host to infectious and inflammatory actions (Escribano et al. 2005).

A decrease in the numer of *Eimeria* sp. oocysts in rabbits after administration of *Ent* 4231 has been considered as very important result, although the same effect was previously observed after application of *Ent* 2019 (Poghny Simonová et al. 2009). It means, that in spite of not known mechanism of action yet, enterocins can cause a decrease in the numer of *Eimeria* oocysts. Moreover, Strompfová et al. (2010) reported a decrease in the numer of *Eimeria* oocysts under *in vitro* conditions using enterocin-producing strains. The chamomile essential oil administration influenced survival of *Eimeria* sp. oocysts in faeces of rabbits; there the reduction of *Eimeria* sp. oocysts was recorded through the entire experiment (Simonová et al. 2007). Anticoccidial effects of green tea-based diets were evaluated in chickens by Seung et al. (2007). The green tea-fed chickens produced significantly reduced number of faecal oocysts (P<0.05) when compared to that observed in the *E. maxima*-infected group fed standard diet. It is also important, that administration of such NS as enterocins or sage did not evoke oxidative stress; this is also one more beneficial point showing their further use in breedings. Similarly, administration of *Ent* 2019 for rabbits did not evoke oxidative stress (Lauková et al. 2008, Pogány Simonová et al. 2009).

In conclusion, it can be stated, that higher effectivity of *Ent* 4231 was observed in *Eimeria* oocysts and clostridiae reduction; sage extract administered individually was more effective in stimulation of unspecific immunity parameter (higher increrase of PA in this group). However, PA was also increased in rabbits administered the combination of both NS.

That is, they can be recommended for rabbits husbandry. Moreover, they did not influence negatively the quality of meat. Of course, further clinical testing is requested.

Acknowledgement

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