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THE EFFECT OF ADDITION OF PROBIOTIC BACTERIA (*BACILLUS SUBTILIS* OR *ENTEROCOCCUS FAECIUM*) OR PHYTOBIOTIC CONTAINING CINNAMON OIL TO DRINKING WATER ON THE HEALTH AND PERFORMANCE OF BROILER CHICKENS

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Abstract

The aim of the study was to test whether the use of probiotic bacteria *Bacillus subtilis* or *Enterococcus faecium* or a phytobiotic containing cinnamon oil can improve the metabolic parameters, immune status, gut microbiota and histology, and growth performance of broiler chickens. The experiment was carried out on 560 one-day-old male Ross 308 broiler chickens raised until the age of 42 days. The broiler chickens were assigned to 4 experimental groups of 140 birds each (7 replications of 20 individuals each). The control group (Control) did not receive additives. A probiotic preparation containing live bacterial cultures of *Enterococcus faecium* (EF, in the amount of 0.25 g/l) or *Bacillus subtilis* (BS, 0.25 g/l) or a phytobiotic preparation containing cinnamon oil (OC, 0.25 ml/l) was administered to the broiler chickens with their drinking water throughout the rearing period. The most important results indicate that the use of BS and OC resulted in: a significant (P≤0.05) increase in the level of ferric reducing ability of plasma (FRAP), high-density cholesterol (HDL) and glutathione (GSH + GSSH) and a significant (P≤0.05) decrease in the level of malondialdehyde (MDA), lipid hydroperoxides (LOOH), total cholesterol (TC), triacylglycerols (TAG), nonesterified fatty acids (NEFA) and interleukin 6 (IL-6), a ratio of heterophils : leukocytes (H:L) and alkaline phosphatase (ALP) activity, alanine aminotransferase (ALT), acidic phosphatase (AC) and creatinine kinase (CK), relative to the C group. In the blood of broiler chickens from the OC treatment, aspartate aminotransferase (AST), lactate dehydrogenase activity and 3-hydroxybutyrate dehydrogenase (HBDH) significantly (P≤0.05) decreased in relation to the C group, and in broiler chickens from EF and BS treatments there was an increase (P≤0.05) in haemoglobin (Hb) content. Compared with group C, in the broiler chickens' nutritional content from EF, BS and OC treatments, the total number of coliforms and number of fungi significantly (P≤0.05) dropped and the number of aerobic bacteria increased (P≤0.05) in the length of the villus and the depth of the crypt. It has been found that *Bacillus subtilis*, *Enterococcus faecium* and phytobiotic containing cinnamon oil can improve the microbiological and histological appearance of broiler chicken intestine. The addition of probiotic bacteria *Bacillus subtilis* or phytobiotic containing cinnamon oil to drinking water is more preferable than *Enterococcus faecium* regarding stimulation of the immune system, blood redox status parameters, parameters of metabolic changes and the gut microbiome and morphometry.

Key words: *Bacillus subtilis*, *Enterococcus faecium*, cinnamon oil, chickens' blood parameters, microbiological indices

Probiotic microorganisms multiply rapidly in the gastrointestinal tract, improving intestinal morphometry and the composition of the intestinal microbiome, ensuring eubiosis, and determining the type of interactions occurring between microorganisms (Ajuwon, 2016; Chen et al., 2017; Sarangi et al., 2016). Adhering to the intestinal villi, they form a natural protective filter that protects against pathogens and strengthens immunity (Sikandar et al., 2017). Many species of probiotic bacteria have the ability to synthesize amino acids and enzymes that improve digestion and nutrient absorption (Ognik et al., 2017; Gadde et al., 2018). Phytobiotics stimulate the immune system and have antioxidant and antimicrobial effects. Cinnamon oil increases the production and secretion of digestive juices, improves carbohydrate and lipid metabolism, and exerts immunostimulatory, antioxidant and antibacterial effects (Arain et al., 2018). The results of studies by Sikandar et al. (2017) show that the use of *Bacillus subtilis* in broiler chicken rearing has a positive effect on animal performance and health through the development of gut microorganisms and immune organs. In addition, dietary supplementation with *Bacillus subtilis* bacteria may improve the growth performance and intestinal health of broiler chickens infected with Salmonella (Abudabo et al., 2019). Some probiotics, such as *Enterococcus faecium*, stimulate the biosynthesis and metabolism of sulfuric amino acids, which improves the metabolism of other amino acids: threonine, tyrosine, serine and glycine. According to Zheng et al. (2016), *E. faecium* stimulates protein expression during inflammatory reactions. According to Reis et al. (2018), cinnamon oil improves the viability of enterocytes and reduces oxidative damage to the intestinal epithelium, which improves utilization of nutrients and the overall condition of birds. Cinnamon oil reduces stress, positively regulates metabolism (Torki et al., 2015). Mehdipour and Afsharmanesh (2018) state that the phenolic components of cinnamon oil, can control and limit the growth and colonization of numerous pathogenic and non-pathogenic bacterial species in the intestines by disrupting and unsealing their cell membrane, ultimately causing disintegration of the cells. Cinnamaldehyde from cinnamon oil can selectively inhibit the growth and development of both pathogenic and commensal intestinal bacteria, which can be exploited to balance the microbial population in poultry to improve their intestinal health (Reis et al., 2018).

It was assumed that by using of probiotics or phytobiotics in broiler chicken rearing, it would be possible to improve production results as well as immunological and antioxidant status. The aim of the study was to determine which supplement (*Bacillus subtilis* or *Enterococcus faecium* or a phytobiotic containing cinnamon oil) applied to drinking water more favourably affects the immune and antioxidant system and, consequently, the growth results of chickens.

Material and methods

Probiotic and phytobiotic preparation

The subject of the research was two probiotic preparations and a phytobiotic used commercially in broiler chicken rearing. The first preparation (EF) contains live *Enterococcus faecium* DSM 7134 lactic acid bacteria enriched with cholecalciferol and

ascorbic acid (Lohman Animal Health®, Germany). Dissolved in one litre of water, the probiotic contains *Enterococcus faecium* in the amount of 3.3×10^{12} CFU, 5 mg of cholecalciferol and 450 g of ascorbic acid. The second preparation (BS) contains live cultures of *Bacillus subtilis* PB6 bacteria and choline (Industries Inc., USA). Dissolved in one litre of water the product contains 2.0×10^{9} CFU *Bacillus subtilis* PB6 and 1500 mg of choline. The phytobiotic supplement contains 3000 mg/l cinnamon oil and citric acid (150,000 mg/l) (EW Nutrition, Germany).

Birds

The experiment was carried out on 560 one-day-old male Ross 308 broiler chickens raised until the age of 42 days. The birds were kept on straw litter in standard conditions in a building with regulated temperature and humidity. The size of the pens was in accordance with the Ross Broiler Management Manual (Aviagen, 2014). The broiler chickens had permanent access to drinking water and received *ad libitum* complete compound feeds, whose composition was established according to Smulikowska and Rutkowski (2005) (Table 1). The experimental procedure was approved by the Second Local Ethics Committee for Experiments with Animals in Lublin (approval no. 38/2018).

Table 1. Composition of diets for chickens (%)

Ingredients (g kg^{-1})	Starter 1–3 week	Grower 4–5 week	Finisher 6 week
1	2	3	4
Wheat	45.28	36.76	33.07
Maize	15.00	25.00	30.00
Soybean meal (46% protein)	272.2	22.79	17.81
Rapeseed meal (37% protein)	2.0	4.00	6.00
Soybean oil	2.0	4.00	6.00
DDGS[1] (26% protein)	4.01	4.36	4.69
Monocalcium phosphate	1.10	0.54	0.21
Coarse-ground limestone[2]	–	1.1	0.85
Fine-ground limestone	1.61	–	–
NaCl	0.36	0.32	0.28
DL-Met (99%)	0.36	0.24	0.20
L-Lys HCl	0.43	0.29	0.31
L-Thr (99%)	0.13	0.09	0.08
Premix[3, 4]	0.50	0.50	0.50
Calculated composition[5]			
ME (kcal kg^{-1})	3070	3140	3190
Crude protein (g kg^{-1})	210.0	198.5	187.5
Lys (g kg^{-1})	13.5	11.7	10.9
Met (g kg^{-1})	6.7	5.5	5.0

Table 1 – contd.

1	2	3	4
Met + Cys (g kg^{-1})	10.1	8.8	8.3
Ca (g kg^{-1})	9.8	7.3	6.0
P available (g kg^{-1})	3.9	2.8	2.1
Na (g kg^{-1})	1.6	1.5	1.4

[1]DDGS – maize distillers dried grains with solubles.

[2]Calcium carbonate.

[3]Vitamin provided per kilogram of diet: 1–3 week: vitamin A, 15,000 IU; vitamin D_3, 5,000 IU; vitamin E, 112 IU; vitamin K_3, 4 mg; vitamin B_1, 3 mg; vitamin B_2, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 16 mg; folic acid, 2 mg; biotin, 0.2 mg; nicotinic amid, 60 mg; calcium pantothenicum, 18 mg; choline,1.8 g; 4–5 week**:** vitamin A, 12,000 IU; vitamin D_3, 5,000 IU; vitamin E, 75 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_6, 4 mg; vitamin B_{12}, 16 mg; folic acid, 1.75 mg; biotin, 0.05 mg; nicotinic amid, 60 mg; calcium pantothenicum, 18 mg; choline, 1.6 g; 6 week: vitamin A, 12,000 IU; vitamin D_3, 5,000 IU; vitamin E, 75 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3 mg; vitamin B_{12}, 11 mg; folic acid, 1.5 mg; biotin, 0.05 mg; nicotinic amid, 35 mg; calcium pantothenicum, 18 mg; choline 1.6 g.

[4]Trace mineral provided per kilogram of diet: Mn, 100 mg; Zn, 80 mg; Fe, 80 mg; Cu, 8 mg; I, 1 mg; Se, 0.15 mg; coccidiostat – salinomycyne (except 6 week).

[5] Calculated according to the Polish Feedstuff Analysis Tables (Smulikowska and Rutkowski, 2005).

The broiler chickens from the control group (Control) received drinking water without additives. Broiler chickens in the EF treatment received a probiotic containing *Enterococcus faecium* in the amount of 0.25 g/l, while broiler chickens in the BS treatment received a probiotic with *Bacillus subtilis* at 0.25 g/l. Broiler chickens in the OC treatment were given a phytobiotic preparation containing cinnamon oil in the amount of 0.25 ml/l of water. The additives were administered from day 1 to day 42 of rearing (Table 2).

Table 2. The experimental scheme of applied probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) doses to chicken

Item	Groups			
	Control	EF	BS	OC
Cycle administration of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) [1]	–	6 × 7	6 × 7	6 × 7
Total intake of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) (g bird^{-1})	–	1.06	0.827	–
Total intake of phytobiotic containing cinnamon oil (ml bird^{-1})	–	–	–	0.56
Total intake of probiotic bacteria *Bacillus subtilis* (BS), PB6, 2.0 × 10^9 (CFU bird^{-1})	–	–	33.3	–
Total intake of probiotic bacteria *Enterococcus faecium* (EF) DSM 7134 CFU 3.3 × 10^{12} (CFU bird^{-1})	–	3.5	–	–
Total intake of phytobiotic containing cinnamon oil[1] (mg bird^{-1})	–	–	–	0.0017

[1]6 × 7 intake in 1–42 days of chicken life.

Body weight (BW) and feed intake were monitored at the end of each week and used to calculate the feed conversion ratio (FCR) (Mazanowski, 2011). Mortality was also monitored during the experiment. After six weeks of rearing, 21 birds from each group were slaughtered and dissected. At 42 days of age, 21 broilers per group (3 birds representing the average body weight in each pen) were slaughtered at a slaughterhouse. The birds (without being transported) were electrically stunned (400 mA, 350 Hz), hung on a shackle line, and exsanguinated by a unilateral neck cut severing the right carotid artery and jugular vein. After a 3-min bleeding period, the birds were scalded at 61°C for 60 s, defeathered in a rotary drum picker for 25 s, and manually eviscerated.

Laboratory analysis

At 42 days of age, 21 birds (3 each from each replicate group) representing the average body weight of each group were selected and fasted for 8 h. The blood samples were cooled and analysed within 4 hours of collection. They were centrifuged at 3000 g for 10 minutes to collect serum for further analysis. Kits developed by Cormay (Poland) were used to determine biochemical parameters in the plasma: glucose (GLU), total protein (TP), urea (UREA), total cholesterol (TC), uric acid (UA), bilirubin (BIL), creatinine (CREAT), high-density (HDL) and low-density (LDL) cholesterol, and triacylglycerols (TAG). The activity of the following enzymes was determined in the plasma: alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), creatinine kinase (CK; EC 2.3.7.2), lactate dehydrogenase (LDH; EC 1.1.1.27), γ-glutamyltransferase (GGT; EC 2.3.2.2), alkaline phosphatase (ALP; EC 3.1.3.1), acidic phosphatase (AC; EC 3.1.3.2) and 3-hydroxybutyrate dehydrogenase (HBDH; EC 1.1.1.30). The level of nonesterified fatty acids (NEFA) was determined using reagents by Randox (Germany). The activity of antioxidant enzymes in the plasma was analysed using spectrophotometric assays. To evaluate the activity of superoxide dismutase (SOD, EC 1.15.1.1), the adrenaline method was used with a modification of the wavelength to 320 nm proposed by Ognik and Wertelecki (2012). Catalase (CAT, EC 1.11.1.6) activity, concentrations of ascorbic acid (VIT. C) and the glutathione level (GSH + GSSG) were determined according to Ognik and Wertelecki (2012). The ferric reducing ability of plasma (FRAP), which represents total antioxidant capacity, was determined according to Benzie and Strain (1996). The level of lipid hydroperoxides (LOOH) was determined according to Gay and Gębicki (2002) and malondialdehyde (MDA) according to Salih et al. (1987).

Levels of immunoglobulin A (IgA) and interleukin 6 (IL-6) were determined by an immunoenzymatic ELISA assay using kits from Elabscience Biotechnology Co., Ltd (Houston, USA). To determine the phagocytic activity of leukocytes against *Staphylococcus aureus* strain 209P, the percentage of phagocytic cells, the phagocytic index, and serum lysozyme activity were assessed by the turbidimetric method according to Siwicki and Anderson (1993). The ability to kill phagocytized bacteria was assessed based on the capability of neutrophils to produce oxygen radicals, using the nitroblue tetrazolium test (Park et al., 1968).

The haematological tests: haemoglobin level (Hb), haematocrit (Ht), leukocyte count (WBC), erythrocyte count (RBC), were done with a Cell-Dyn 3500 Abbott

Company apparatus. Leukocytes and heterophils were calculated with chamber method. The smears to calculate the leukocyte and heterophils content were stained using the Pappenheim method (Bomski, 1989). After dissection, samples of the jejunum were collected and placed in sterile containers. This material was then subjected to microbiological analysis to determine the number of aerobic bacteria on nutrient agar, the number of coliform bacteria on Violet Red Bile Lactose Agar (VRBL) (incubation for 24 hours at 37°C), and the total number of yeasts and moulds on DG18 medium (incubation for 5–7 days at 25°C). After incubation, the total bacterial cell count was determined, and the values obtained were expressed as CFU/g. Microbiological testing included macroscopic and microscopic evaluations as well as Gram staining. Final identification of the bacterial colonies was performed using API tests (bioMérieux, Poland), according to PN-ISO 4832, PN-EN ISO 7218 and PN ISO 4832. Intestinal samples collected during dissection were subjected to histological evaluation. From each intestinal sample, 20 intestinal villi were selected. A representative section of 2 cm in length, cut 1 cm behind the Meckel's diverticulum towards the cecum, was collected for histological examination. Their length was measured from the tip to the base, and then they were cut into slices. The depth of 20 crypts was measured as well, after which they were cut in two lengthwise. All intestinal segments were fixed in a 4% buffered formalin (Sigma-Aldrich Corporation, St. Louis, Missouri, USA) solution with pH 7.2 for 24 hours and then stored in 70% ethanol. After fixation of fragments of the intestine, they were placed in increasing concentrations of alcohol solutions (70%, 95%, absolute) for dehydration. Then samples were cleaned with xylene as a cleaning agent and embedded in paraffin blocks in a tissue processor (Leica TP-20). Each intestinal histomorphologic tissue sample was prepared and stained with haematoxylin and eosin solution using standard paraffin-embedding methods (Xu et al., 2003) A computerized microscopic image analysis system was used to estimate villus length and crypt depth. A light microscope (Nikon Eclipse E600) with a digital camera (Nikon DS-Fi1) and a PC with image analysis software (NIS-Elements BR-2.20, laboratory imaging) were used.

Statistical analysis

The results of the experiment were verified by one-way ANOVA, and significant differences between groups were determined by Duncan's multiple range test. Data variability was expressed as the pooled standard error of the mean (SEM). The differences were considered significant at $P \leq 0.05$. The Statistica software package version 10 (StatSoft Inc., 2011) was used for statistical calculations.

Results

The results of the biochemical tests of the broiler chicken blood are presented in Tables 3–6. In the plasma of broiler chickens from treatments BS and OC, there was a reduction in TC (P=0.026), TAG, (P=0.013), NEFA (P=0.006) and the proportion of LDL cholesterol (P=0.006) and an increase in that of HDL cholesterol (P=0.022)

relative to Control group (Table 3). Treatments BS and OC resulted in a reduction in blood GLU (Table 3), MDA (P=0.024) and IL-6 (P=0.047) in the blood of the broiler chickens, and an increase in the Ig-A (P=0.024) and FRAP level (P=0.042) and glutathione level (P=0.035) (Tables 5, 6 and 7). The blood of broiler chickens from the BS and OC treatments showed a decrease in ALP (P=0.041), ALT (P=0.047), AC (P=0.026) and CK (P=0.047) activity, and in all treatments there was a decrease in GGT (P=0.045) activity and an increase in LOOH (P=0.23) relative to Control group (Tables 4 and 6). In the broiler chicken plasma from the OC treatment, CREAT (P=0.026) level and AST (P=0.047), LDH (P=0.039), HBDH (P=0.037) activity decreased relative to Control group, while the phagocytosis index increased (P=0.048) (Tables 4, 5 and 6). In the whole blood of broiler chickens from treatments EF and BS, there was an increase in Hb (P=0.021), while in the BS and OC groups there was a decrease in the H:L ratio (P=0.045) (Table 8). Compared to Control group (Table 9), in the digesta of the broiler chickens from treatments EF, BS and OC there was a decrease in the total number of coliform bacteria (P=0.013) and fungal count (P=0.023) and an increase in the number of aerobic bacteria (P=0.018) (Table 7) and in villus length and crypt depth (P=0.038 and P=0.044, respectively).

Table 3. Effect of the level and duration of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on biochemical parameters in the blood of the chickens

Groups	TC (mmol l^{-1})	HDL (mmol l^{-1})	LDL (mmol l^{-1})	TAG (mmol l^{-1})	NEFA (µmol l^{-1})	GLU (mmol l^{-1})	TP (mmol l^{-1})	UREA (mmol l^{-1})
Control	1.99 a	2.09 b	1.49 a	0.89 a	33.78 a	12.58 a	45.67	0.749
EF	1.92 a	2.15 b	1.44 a	0.53 ab	30.14 ab	10.89 a	46.19	0.747
BS	1.67 b	2.83 a	0.82 b	0.46 b	27.87 b	9.87 b	45.39	0.746
OC	1.57 c	2.74 a	0.87 b	0.44 b	24.98 b	8.99 b	44.28	0.744
SEM	0.049	0.049	0.055	0.025	14.3	0.049	0.098	0.025
P-value	0.026	0.022	0.045	0.013	0.006	0.043	0.061	0.056

Means within the same column differ significantly at P≤0.05 according to Dunnett's mean comparison.

TC – total cholesterol, HDL – high-density cholesterol, LDL – low-density cholesterol, TAG – triacylglycerols, NEFA – nonesterified fatty acids, GLU – glucose, TP – total protein, UREA – urea.

Table 4. Effect of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on the enzymes activity of chickens' blood plasma

Groups	AC (U l^{-1})	LDH (U l^{-1})	HBDH (U l^{-1})	ALP (U l^{-1})	GGT (U l^{-1})	CK (U l^{-1})
Control	1.49 a	549.6 a	147.36 ab	986.0 a	18.71 a	411.36 ab
EF	1.42 ab	497.0 ab	176.31 a	724.9 ab	16.12 b	479.36 a
BS	1.38 b	593.4 a	154.25 ab	558.4 b	16.04 b	369.12 b
OC	1.37 b	376.6 b	134.15 b	541.2 b	15.95 b	327.28 b
SEM	0.174	8.45	3.99	40.32	0.25	6.98
P-value	0.026	0.039	0.037	0.041	0.045	0.047

Means within the same column differ significantly at P≤0.05 according to Dunnett's mean comparison.

AC – acidic phosphatase, LDH – lactate dehydrogenase, HBDH – 3-hydroxybutyrate dehydrogenase, ALP – alkaline phosphatase, GGT – γ-glutamyltransferase, CK creatinine kinase.

Table 5. Effect of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on antioxidant status of chickens' blood serum

Groups	FRAP (μmol l^{-1})	GSH+GSSH (μmol l^{-1})	VIT. C (mg l^{-1})	UA (μmol l^{-1})	BIL (μmol l^{-1})	CREAT (μmol l^{-1})
Control	84.24 b	0.065 b	0.575	194.36	7.74	23.47 a
ES	90.41 ab	0.078 ab	0.574	141.25	5.48	18.47 b
BS	102.47 a	0.0899 a	0.625	189.69	6.79	21.71 a
OC	105.36 a	0.097 a	0.607	127.57	4.37	17.78 b
SEM	7.04	0.05	0.014	8.158	0.25	18.51
P-value	0.042	0.035	0.507	0.325	0.135	0.026

Means within the same column differ significantly at P≤0.05 according to Dunnett's mean comparison.
FRAP – ferric reducing ability of plasma, GSH+GSSH – glutathione level, VIT.C – ascorbic acid, UA – uric acid, BIL – bilirubine, CREAT – creatinine.

Table 6. Effect of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on redox status of chickens' blood plasma

Groups	LOOH (μmol l^{-1})	MDA (μmol l^{-1})	SOD (U ml^{-1})	CAT (U ml^{-1})	AST (U l^{-1})	ALT (U l^{-1})
Control	3.75 a	0.49 a	29.47	2.76	279.3 a	4.95 a
ES	2.86 b	0.42 ab	28.44	2.98	226.6 ab	4.45 ab
BS	1.89 c	0.36 b	29.41	2.77	239.6 ab	3.82 b
OC	1.74 bc	0.39 b	28.97	3.21	213.9 b	3.58 b
SEM	0.108	0.12	0.383	0.05	3.68	0.13
P-value	0.023	0.024	0.126	0.067	0.047	0.045

Means within the same column differ significantly at P≤0.05 according to Dunnett's mean comparison.
LOOH – lipid hydroperoxides, MDA – malondialdehyde SOD – superoxide dismutase, CAT – catalase AST – aspartate aminotransferase ALT – alanine aminotransferase.

Table 7. Effect of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on the immune status of chickens' blood serum

Groups	Lysozyme (mg l^{-1})	Phagocytic cells (%)	Phagocytic index	Nitroblue tetrazolium test (%)	Interleukin 6 (mg ml^{-1})	Immunoglobulin A (mg ml^{-1})
Control	1.17	38.47	4.78 b	21.87	2.23 a	0.478 b
ES	2.67	40.22	5.44 ab	22.77	1.74 ab	0.504 ab
BS	2.54	40.57	5.76 ab	25.58	1.25 b	0.678 a
OC	2.87	41.08	6.78 a	24.87	1.23 b	0.622 a
SEM	0.04	0.24	0.08	0.12	0.07	0.11
P-value	0.339	0.225	0.048	0.324	0.047	0.024

Means within the same column differ significantly at P≤0.05 according to Dunnett's mean comparison.

In the blood of broiler chickens from group EF, an increase in the count of RBC (P=0.039) was noted and in the OC IF (P=0.048) was increased, in relation to the control group. The lowest mortality (P=0.03) was recorded in the OC group. No sig-

nificant effects of the additives on the growth performance of broiler chickens were observed (Table 10).

Table 8. Effect of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on hematological parameters of broiler chickens' blood

Groups	Ht ($l\ l^{-1}$)	Hb ($g\ l^{-1}$)	RBC ($10^{12}\ l^{-1}$)	WBC ($10^{9}\ l^{-1}$)	H:L
Control	27.80	4.37 b	1.7 b	15.95	1.77 a
ES	28.10	5.64 a	1.95 a	15.85	1.31 b
BS	31.4	5.27 ab	1.8 ab	15.96	1.24 b
OC	29.8	4.95 ab	1.84 ab	15.99	1.32 b
SEM	0.111	0.041	0.186	0.69	0.175
P-value	0.36	0.021	0.039	0.44	0.045

Means within the same column differ significantly at P≤0.05 according to Dunnett's mean comparison.
Ht – haematocrit, Hb – haemoglobin, RBC – erythrocytes, WBC – leukocytes, H:L – heterophils to leukocytes ratio.

Table 9. Effect of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on the microbiological analysis of the jejunal contents and on measurements of jejunal villi and crypts in the broiler chickens

Groups	Total number of fungi (CFU g^{-1})	Total number of aerobic bacteria (CFU g^{-1})	Total number of coliform bacteria (CFU g^{-1})	Mean length of jejunal villi (μm)	Mean depth of jejunal crypts (μm)
Control	487 a	216874 c	587258 a	1087.99 b	207.47 c
ES	198 b	769789 b	287598 b	1264.55 a	277.57 b
BS	156 c	848998 a	187447 c	1339.87 a	288.57 a
OC	112 d	878224 a	115198 d	1358.87 a	285.44 a
SEM	16.21	75.42	14.23	48.28	13.98
P-value	0.023	0.018	0.013	0.038	0.044

Means within the same column differ significantly at P≤0.05 according to Dunnett's mean comparison.

Table 10. Effect of application of probiotic bacteria *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) on the effects of rearing of the broiler chickens

Groups	BW (kg)					BWG 1–42 day	DFI (g $bird^{-1}$)	FCR 1–42 day	Mortality rate (%)
	1day	14 day	21 day	35 day	42 day				
Control	0.048	0.39	0.899	2.00	2.75	2.702	112	1.75	0.71
ES	0.045	0.47	0.908	2.079	2.76	2.715	112	1.74	0.18
BS	0.046	0.47	0.911	2.098	2.77	2.724	111	1.72	0.18
OC	0.047	0.47	0.913	2.11	2.77	2.723	111	1.72	0.18
SEM	0.02	0.06	0.04	0.08	0.36	0.24	2.34	0.12	NA
P-value	0.84	0.51	0.054	0.052	0.56	0.051	0.24	0.054	NA

BW – body weight, FCR – feed conversion ratio.
NA – not analysed.

Discussion

The results of studies by other authors indicate the beneficial effect of probiotics and phytobiotics on the metabolism, immune status, histological parameters and microflora composition of the intestines of broiler chickens (Karimi-Kivi et al., 2015; Ognik et al., 2017; Sobczak and Kozłowski, 2015). At the same time, numerous literature data indicate that the use of probiotics or phytobiotics in poultry rearing does not affect growth performance (Samanya and Yamauchi, 2002; Bai et al., 2018; Gheisar et al., 2016; Symeon et al., 2014). However, comparative research on the effectiveness of various doses of a preparation containing *Enterococcus faecium* or cinnamon in broiler chicken rearing has shown that the phytobiotic increased final body weight and reduced FCR to a greater extent than the probiotic (Capcarova et al., 2008; Symeon et al., 2014).

In our study, the reduction in TC, TAG, NEFA and the proportion of LDL cholesterol in the plasma, with a corresponding increase in HDL, following administration of the *Bacillus subtilis* or the cinnamon oil preparation should be considered a positive effect. Decreased TAG and the proportion of LDL to HDL cholesterol in the plasma of ostrich chickens receiving a commercial preparation containing a mixture of *Bacillus subtilis* and *Bacillus licheniformis* have also been reported by Karimi-Kivi et al. (2015). Decreased plasma levels of TC and TAG in broiler chickens have been observed by Pourakbari et al. (2016) following administration of a probiotic preparation containing *Enterococcus faecium*. Koochaksaraie et al. (2011) and Reis et al. (2018) have demonstrated a beneficial effect of cinnamon oil on broiler chicken metabolism, in the form of decreased plasma levels of TC, TAG and NEFA. Sobczak and Kozłowski (2015), on the other hand, found no clear influence of *Bacillus subtilis* on the plasma level of TC and TAG in laying hens, but observed a marked increase in TC in the egg yolk fat. The reduction in TAG and NEFA levels confirms that *Bacillus subtilis* has a beneficial effect by reducing lipolysis (Karimi-Kivi et al., 2015), which is physiologically accompanied by an increase in NEFA, resulting from the use of glycerol for glucose synthesis in gluconeogenesis and not for the synthesis of TAG (Karimi-Kivi et al., 2015). The increased NEFA level in turn limits the ability of cells to store TAG or to use them as a source of energy, which leads to lipotoxicity and activation of inflammatory processes and oxidative stress. Probiotic bacteria reduce the plasma concentration of TC and TAG by increasing the capacity of cholesterol hydroxylase for enzymatic deconjugation and dehydration of primary bile acids during their biosynthesis (Karimi-Kivi et al., 2015). Probiotics also increase the rate of bile salt hydrolysis, which leads to increased excretion of released bile acids from the gastrointestinal tract. The result is the stimulation of biosynthesis of new bile acids, which is the main pathway of cholesterol catabolism and its removal from the body. Probiotic microorganisms also inhibit the activity of hydroxymethyl-glutaryl-coenzyme-A reductase, which is involved in cholesterol synthesis, thereby slowing down synthesis of this steroid from acetyl-CoA (Karimi-Kivi et al., 2015). According to Koochaksaraie et al. (2011), cinnamon derivatives stimulating the excretion of cholesterol from the body are responsible for the hypocholesterolemic effect of cinnamon oil. Comparison of the effect of *Enterococcus faecium* and

cinnamon (*Cinnamomum*) on the lipid profile of broiler chicken blood indicates that cinnamon has a more beneficial effect, which is manifested as a decrease in TAG, TC and the proportion of LDL cholesterol in the blood (Gheisar et al., 2016).

The decrease in AST, ALT, GGT, ALP and LDH activity in the plasma of broiler chickens receiving *Bacillus subtilis* or cinnamon oil in our study indicates the beneficial effect of these additives on metabolism in the liver and heart cells, as increased activity of these enzymes in the blood suggests cell damage in these organs (Ognik and Krauze, 2016). The reduction of AC activity observed in the plasma of broiler chickens receiving the preparation containing live cultures of *Bacillus subtilis* and the phytobiotic preparation with cinnamon oil should also be considered beneficial. Increased AC activity in the blood indicates degradation of liver cells and the release of this enzyme from them. This is an undesirable phenomenon because AC plays a key role in cellular bioenergetics and ATP homeostasis. As in our study, Reis et al. (2018) have reported a decrease in the activity of these enzymes following administration of cinnamon oil to broiler chickens. A reduction in GGT activity in broiler chicken blood after administration of *Enterococcus faecium* has also been demonstrated by Ognik et al. (2017).

The use of *Enterococcus faecium*, *Bacillus subtilis* and cinnamon oil reduced the level of LOOH in the blood of the broiler chickens. The addition of *Bacillus subtilis* or cinnamon oil was more beneficial in reducing the concentration of MDA and increasing FRAP and GPx activity. Symeon et al. (2014) have also found that cinnamon oil reduces the MDA level in the blood of broiler chickens. According to these authors (Symeon et al., 2014), the antioxidant activity of the cinnamon components may result from suppression of lipid peroxidation and enhancement of GPx and GGT activity, which are responsible for antioxidant defence. Dietary supplementation with phytobiotics may be a good method of introducing a natural antioxidant into phospholipid membranes while at the same time inhibiting oxidative reactions. Furthermore, the addition of a phytobiotic improves liver metabolism, which leads to an increase in the level of polyunsaturated fatty acids and omega-6 fatty acids and a reduction in the amount of saturated fatty acids both in the liver cells and in the blood (Symeon et al., 2014; Ognik et al., 2016).

The results of the immunoassays performed in our study indicate that the use of *Bacillus subtilis* or cinnamon oil is much more beneficial than the use of *Enterococcus faecium*, due to the increased IgA level and reduced IL-6 content in the broiler chicken serum. A similar relationship was also noted by Waititu et al. (2014), who used *Bacillus subtilis* in broiler chicken rearing.

Villus length and crypt depth in the small intestine of broiler chickens were found to increase as a result of the addition of the *Bacillus subtilis* or *Enterococcus faecium* probiotic preparation or the cinnamon oil phytobiotic. Similar results in studies on broiler chickens have been reported by Bai et al. (2018) and Ognik et al. (2017). Appropriate villus height and crypt depth are conditions for the integrity of the small intestine. Longer intestinal villi increase the absorptive area, which improves nutrient absorption (Cui et al., 2018). Increased length of the villi of the intestine after the administration of *Enterococcus faecium* was found by Coskun et al. (2015) and Awad et al. (2008, 2009).

In turn, the greater depth of the intestinal crypts may indicate a faster regeneration and reconstruction of the villi, suggesting that the intestinal response mechanism of the host tries to compensate for the normal reduction or disappearance of the villi, for example due to inflammation caused by pathogens and their toxins. In addition, the higher the ratio of the length of the villus to the depth of the crypts, the more slowly the intestinal mucosa is used up. The slower rate of intestinal epithelial exchange affects the gut tissue, which may improve animal growth indicators (Gao et al., 2008). Probiotics used in poultry production favourably modulate the composition and quantity of intestinal microflora and protect against colonization of the digestive tract by pathogenic bacteria. The metabolism of probiotic bacteria leads to the production of short- and medium-chain fatty acids and bacteriocins and to a reduction in the pH and redox potential in cells. The inclusion of probiotics in the diet of broilers results in an increase in the number of aerobic bacteria in the intestinal contents, and bacteria of the genus *Bacillus* compete with potential enteropathogens for nutrients and binding sites on enterocytes, thereby reducing the population of intestinal pathogens (Karimi-Kivi et al., 2015; Gadde et al., 2018).

An increase in the RBC count in the blood of piglets receiving *Enterococcus faecium* was recorded by Strompfová et al. (2006), but Capcarova et al. (2008) found a reduction in the RBC count in the blood of turkeys receiving this species of probiotic bacteria. Increasing the phagocytosis index is in line with the results of Tamam et al. (2017). These authors clearly confirm the immunomodulatory effect of cinnamon ingredients, claiming that they stimulate unspecific immunity by increasing the number of macrophages and phagocytic activity. According to Mollazadeh and Hosseinzadeh (2016), the bioactive ingredients of cinnamon oil prevent mortality, help to protect against cardiovascular diseases and reduce the risk of metabolic syndrome. The metabolic syndrome is characterized by dyslipidemia, i.e. a simultaneous increase in TAG, LDL, TC and GLU levels and oxidative stress. Considering that in the blood of chickens from the OC procedure, these indicators were the most favourable, the reduction in mortality in this group may have been caused by the beneficial effects of cinnamon oil for inhibiting the metabolic syndrome.

Conclusion

It was established that the addition of probiotic bacteria *Bacillus subtilis* (BS) at a dose of 0.25 g/l and phytobiotic containing cinnamon oil (OC) 0.25 ml/l to drinking water more preferably than *Enterococcus faecium* (EF) stimulates the immune system, blood redox status parameters, parameters of metabolic changes and the gut microbiome and morphometry. It has been found that *Bacillus subtilis* (BS), *Enterococcus faecium* (EF) and phytobiotic containing cinnamon oil (OC) can improve the microbiological and histological appearance of broiler chicken intestines.

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