THE EFFECT OF A PROBIOTIC CONTAINING *ENTEROCOCCUS FAECIUM* DSM 7134 ON REDOX AND BIOCHEMICAL PARAMETERS IN CHICKEN BLOOD*

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Abstract

It was postulated that administration of a probiotic to chickens can stimulate their antioxidant status while at the same time inhibiting oxidation processes. The objective of the study was to determine whether and how different levels and durations of application of a probiotic preparation containing live cultures of *Enterococcus faecium* and enriched with cholecalciferol and ascorbic acid influences indicators of lipid status and the redox reaction in the blood of broiler chickens. Four hundred day-old Ross 308 chickens were raised until their 42nd day of age. The animals were assigned to five experimental groups of 80 broilers each: a control group that did not receive the probiotic, T1 groups, which received the probiotic at 0.25 (E-0.25) or 0.1 g/l (E-0.1) throughout the rearing period, and T2 groups, which received the probiotic at the same levels, but during days 1–7, 15–21 and 29–35 of life. The probiotic preparation reduced the level of peroxides (LOOH), malondialdehyde (MDA), cholesterol (TC), low density (LDL) cholesterol, and non-esterified fatty acids (NEFA), and the activity of catalase (CAT), gamma-glutamyltransferase (GGT), acid phosphatase (AC), alkaline phosphatase (ALP) and 3-hydroxybutyrate dehydrogenase (HBDH), while ferric reducing ability of plasma (FRAP) and glutathione (GSH + GSSG) levels were increased in the blood plasma of the chickens. The results obtained indicate that administration of this probiotic to chickens, especially in the amount of 0.25 g/l for the entire rearing period, stimulates synthesis of low-molecular-weight antioxidants responsible for maintaining redox homeostasis, without exerting a negative effect on liver metabolism in chickens.

Key words: chicken, *Enterococcus faecium*, blood, lipid redox reaction

Clinical and experimental research indicates that probiotics have a beneficial effect on the physiology of the intestine (Sobczak and Kozłowski, 2015). Healthy gut microflora stimulates the immune system and may eliminate potentially negative effects of toxic products (ammonia or urea) of protein decomposition in the intestine

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(Juśkiewicz et al., 2007). As a result, there has been great interest in discovering probiotic strains that stimulate antioxidant potential and reduce oxidative damage (Russel et al., 2000). Oxidative stress can affect health and decrease the productivity of livestock, including broiler chickens (Douglas et al., 2016). Oxidative stress is associated with numerous chronic diseases, and lipid peroxidation contributes to hyperlipidaemia and hyperglycaemia, resulting in an increase in the level of proatherogenic lipoproteins (VLDL and LDL cholesterol), a decrease in the level of HDL cholesterol and impairment of its function (Tsimikas and Miller, 2011; Stancu et al., 2012). It is thought that probiotic properties are closely linked to the ability of the microorganisms to hydrolyse large protein molecules and thereby form biologically active peptides (such as glutathione). These data indicate that natural sources of antibiotics should be sought and tested for the purpose of protection against free radicals. Inclusion of probiotics in the diet may be a good strategy for supplying antioxidants, but further research is needed to evaluate the antioxidant properties of these supplements. The species *Enterococcus faecium* is already used as a probiotic supplement in poultry diets (Capcarova et al., 2010; Zhao et al., 2013). This bacterial species has been shown to enhance the immune response and improve the development of lymphatic organs in broiler chickens (Luo et al., 2013), beneficially modify the microbial population of the intestine (Samli et al., 2007), enhance resistance to infection (Audisio et al., 2000; Li et al., 2012), increase the absorptive surface of the intestines (Samli et al., 2007, 2010; Levkut et al., 2012) and exert a positive effect on the cellular proteome, thereby improving the meat quality and dressing percentage of broilers (Luo et al., 2013; Zheng et al., 2016). *E. faecium* further stimulates biosynthesis and metabolism of sulphur-containing amino acids and has a beneficial effect on metabolism of glycine, serine, threonine and tyrosine. Moreover, this species stimulates expression of proteins during inflammatory reactions (Zheng et al., 2016).

In the present study it was postulated that administration of a probiotic to chickens could improve their antioxidant status while at the same time inhibiting oxidation processes. Therefore, the objective of the study was to determine whether and how different doses and durations of application of a probiotic preparation containing live cultures of *Enterococcus faecium* and enriched with cholecalciferol and ascorbic acid influences indicators of lipid status and the redox reaction in the blood of broiler chickens.

Material and methods

Probiotic

The probiotic preparation contained live *Enterococcus faecium* DSM 7134 lactic acid bacteria and was 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 450,000 mg of ascorbic acid.

Animals

Four hundred one-day-old Ross 308 chickens were used in the study and were raised until their 42nd day of age. The experimental procedure was approved by the Second Local Ethics Committee for Experiments with Animals in Lublin (approval no. 30/2014). The chickens were kept in pens on straw litter and reared in standard hygiene conditions in a building with regulated temperature and humidity. They had permanent access to drinking water and received *ad libitum* complete feed mixtures appropriate for each rearing period in accordance with feeding standards for poultry (Nutrient Requirements for Poultry, 2005) (Table 1).

Table 1. Composition of mixtures for chicken (g/kg)

Components	Starter 1–3 week	Grower 4–5 week	Finisher 6 week
Wheat	452.8	367.63	330.70
Maize	150.0	250.0	300.0
Soybean meal 46% protein	272.21	227.90	178.09
Rapeseed meal 37% protein	20.0	40.0	60.0
Soybean oil	20.0	40.0	60.0
DDGS ¹ 26% protein	40.07	43.58	46.87
Monocalcium phosphate	11.03	5.42	2.05
Coarse-grained fodder chalk	-	10.93	8.52
Fine-grained fodder chalk	16.07	-	-
NaCl	3.63	3.23	2.83
DL-methionine 99%	3.61	2.40	2.00
L-lysine HCl	4.27	2.97	3.12
L-threonine 99%	1.31	0.94	0.82
Premix ²	5	5	5
Calculated nutrient composition of mixture (g/kg)			
Crude protein	210.0	198.5	187.5
Crude fibre	27.2	29.8	32.2
Crude fat	65.9	74.5	81.4
Lysine	13.5	11.7	10.9
Methionine	6.7	5.5	5.0
Methionine + Cysteine	10.1	8.8	8.3
Tryptophan	2.5	2.3	2.1
Arginine	13.1	12.1	11.1
Total calcium	9.8	7.3	6.0
Available phosphorus	3.9	2.8	2.1
Sodium	1.6	1.5	1.4
Metabolizable energy (kcal/kg)	3070	3140	3190

¹ DDGS – maize distillers dried grains with solubles.

² Premix – **1–3 week**: retinol – 1034 mg/kg; cholecalciferol – 25 mg/kg; tocopherol – 15 g/kg; menadione – 0.8 g/kg; thiamine – 0.6 g/kg; riboflavin – 1.6 g/kg; pyridoxine – 1 g/kg; cobalamin – 3.2 g/kg; folic acid – 0.4 g/kg; biotin – 40 mg/kg; nicotinic acid – 12 g/kg; calcium pantothenicum – 3.6 g/kg; choline – 360 g/kg; manganese – 20 g/kg; zinc – 16 g/kg; iron – 16 g/kg; copper v 1.6 g/kg; iodine – 0.2 g/kg; selenium – 30 mg/kg; coccidiostat – salinomycin; **4–5 week**: retinol – 827 mg/kg; cholecalciferol – 25 mg/kg; tocopherol – 10 g/kg; menadione – 0.6 g/kg; thiamine – 0.4 g/kg; riboflavin – 1.2 g/kg; pyridoxine – 0.8 g/kg; cobalamin – 3.2 g/kg; folic acid – 0.35 g/kg; biotin – 10 mg/kg; nicotinic acid – 12 g/kg; calcium pantothenicum – 3.6 g/kg; choline – 320 g/kg; manganese – 20 g/kg; zinc – 16 g/kg; iron – 16 g/kg; copper – 1.6 g/kg; iodine – 0.2 g/kg; selenium – 30 mg/kg; coccidiostat – salinomycin; **6 week**: retinol – 827 mg/kg; cholecalciferol – 25 mg/kg; tocopherol – 10 g/kg; menadione – 0.4 g/kg; thiamine – 0.4 g/kg; riboflavin – 1 g/kg; pyridoxine – 0.6 g/kg; cobalamin – 2.2 g/kg; folic acid – 0.3 g/kg; biotin – 10 mg/kg; nicotinic acid – 7 g/kg; calcium pantothenicum – 3.6 g/kg; choline – 320 g/kg; manganese – 20 g/kg; zinc – 16 g/kg; iron – 16 g/kg; copper – 1.6 g/kg; iodine – 0.2 g/kg; selenium – 30 mg/kg.

The experiment was carried out on 400 chickens assigned to five experimental groups of 80 birds each (4 replications of 20 individuals each). The control group (G-C) did not receive the probiotic. Groups E-0.25(T1) and E-0.1(T1) received the probiotic in their drinking water for the whole experimental period at levels of 0.25 and 0.1 g/l, respectively. The birds in groups E-0.25(T2) and E-0.1(T2) received the probiotic in the same amounts, but on days 1–7, 15–21 and 29–35 of life.

Laboratory analyses

Blood samples for further analyses were collected into two test tubes from the wing vein of eight birds from each group at the age of 42 days. One tube contained heparin as an anticoagulant (plasma) and the other did not (serum). Blood samples were then centrifuged at 3000 g for 10 min and the plasma was collected for further analysis. Kits developed by Cormay® were used to determine the following biochemical indices in the blood plasma samples: uric acid (UA), bilirubin (BIL), creatinine (CREAT), cholesterol (TC) and its high-density (HDL) and low-density (LDL) fractions, triacylglycerol (TAG), and activity of the enzymes alkaline phosphatase (ALP, EC 3.1.3.1), alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), lactate dehydrogenase (LDH, EC 1.1.1.27), γ -glutamyltransferase (GGT, EC 2.3.2.2), and creatine kinase (CK, EC 2.7.3.2). Spectrophotometric assays were used to test the blood plasma samples for activity of the antioxidant enzymes superoxide dismutase (SOD, EC 1.15.1.1), by the adrenaline method with a modification of the wavelength to 320 nm to increase the selectivity of transient reaction products (Misra and Fridovich, 1972), and catalase (CAT, EC 1.11.1.6) according to Aebi (1984). Total antioxidant capacity (ferric reducing ability of plasma – FRAP) was determined according to Benzie and Strain (1996), plasma concentrations of ascorbic acid (VIT. C) was determined according to Omaye et al. (1979) and glutathione (GSH + GSSG) was determined according to Akerboom and Sies (1981). In addition, levels of lipid peroxidation products were measured: peroxides (LOOH) according to Gay and Gębicki (2002) and malondialdehyde (MDA), as a final product of tissue lipid oxidation, according to Salih et al. (1987). The activity of acid phosphatase (AC, EC 3.1.3.2) and 3-hydroxybutyrate dehydrogenase (HBDH, EC:1.1.1.30) was determined in the blood serum using Cormay® kits. The level of nonesterified fatty acids (NEFA) was determined using reagents by Randox®.

Statistical analysis

The model assumptions of normality and homogeneity of variance were verified by the Shapiro-Wilk and Levene tests, respectively. The results were analysed by one-way ANOVA. Comparison of the control (G-C) vs. all other groups was performed by planned contrast analysis. In addition, Dunnett's two-tailed post-hoc test was used to compare each experimental group with the G-C group. In a model without the G-C group, two-way ANOVA was performed to examine the following effects: D – dose effect, T – time effect, and D $\times$ T – interaction between dose and time. When a significant interaction effect was noted, the Newman-Keuls test was used to determine the differences between the factors. The statistical analysis was performed according to the GLM procedure in Statistica 13 PL software (StatSoft Corp®). Treat-

ment effects were considered to be significant at $P \leq 0.05$. All data were expressed as mean values with pooled SE.

Results

The body weights of the chickens in the experimental groups at 42 days of age were 2.415 kg – group G-C; 2.515 kg – E-0.25(T1); 2.499 kg – E-0.1 (T1); 2.448 kg – E 0.1 (T1); and 2.392 kg – E 0.25(T2). The highest mean body weight (2.50 kg), accompanied by the lowest feed conversion rate (1.66) was attained by the chickens in the group receiving the probiotic supplement in the amount of 0.25 g/l for the entire rearing period. Mortality was 1% in the control and in the group receiving the preparation in the amount of 0.1 g/l for specific periods during rearing (T2), while the survival rate of the chickens in the remaining groups receiving the probiotic preparation was 100%.

Table 2. Effect of the level and duration of application of the probiotic (*Enterococcus faecium*) on redox status in the blood of the chickens¹

Item	LOOH μmol/l	MDA μmol/l	SOD U/ml	CAT U/ml	AST U/l	ALT U/l
Group ²						
G-C	3.852	0.407	28.16	2.638	219.3	4.112
E -0.25(T1)	1.803 c*	0.374 b*	32.92	2.514 b*	203.8	3.964
E -0.1(T1)	2.354 b*	0.386 ab	30.42	2.646 a	208.7	4.085
E -0.25(T2)	2.467 a*	0.396 ab	28.72	2.603 ab	221.6	4.231
E -0.1(T2)	2.426 a*	0.401 a	30.08	2.629 ab	216.4	4.252
SEM	0.111	0.027	0.476	0.082	12.32	0.196
Dosage effect (D) 0.25 g/l	2.135	0.385	30.82	2.272	212.7	4.097
0.1 g/l	2.390	0.393	30.25	2.637	212.5	4.168
Time effect (T) T1	1.692	0.380	31.67	2.580	206.2	4.024
T2	2.446	0.398	29.40	2.616	219.0	4.241
P-value						
G-C vs. all other	0.023	0.048	0.082	0.044	0.063	0.071
D effect	0.050	0.083	0.124	0.046	0.165	0.062
T effect	<0.001	0.064	0.746	0.064	0.628	0.047
DxT interaction	0.012	0.073	0.637	0.051	0.844	0.051

a–c – means within the same column differ significantly ($P \leq 0.05$) according to Newman-Keuls mean comparison (DxT interaction).

¹Data represent mean values of 4 replications per treatment. SEM = SD divided by the square root of the replication number, n=8.

²Group: G-C = water not supplemented with probiotic; E –0.25(T1) and E –0.25(T2) = G-C with probiotic – dose 0.25 g/l; E –0.1(T1) and E –0.1(T2) = G-C with probiotic – dose 0.1 g/l; E –0.25(T1), E –0.1(T1) supplemented with probiotic on days 1–42 of life; E –0.25(T2), E –0.1(T2) supplemented with probiotic on days 1–7, 15–21 and 29–35 of life.

*means within the same column differ significantly from the control (G-C) at $P \leq 0.05$ according to Dunnett's mean comparison.

LOOH – peroxides, MDA – malondialdehyde, SOD – superoxide dismutase, CAT – catalase, ALT – alanine aminotransferase, AST – aspartate aminotransferase.

The data in Table 3 indicate that administration of the probiotic to chickens via their drinking water in the amount of 0.25 g/l or 0.1 g/l for the entire rearing period or specific intervals during rearing caused a significant reduction in LOOH in the blood plasma as compared to the controls.

Table 3. Effect of the level and duration of application of the probiotic (*Enterococcus faecium*) on antioxidant status in the blood of the chickens¹

Item	FRAP μmol/l	GSH+GSSH μmol/l	VIT. C mg/l	UA μmol/l	BIL μmol/l	CREAT μmol/l
Group ²						
G-C	87.16	0.068	0.146	154.7	4.842	20.34
E -0.25(T1)	114.9 a*	0.189 a*	0.198 a*	156.3	4.769	21.41
E -0.1(T1)	97.47 ab*	0.179 ab*	0.184 ab*	149.4	4.791	19.68
E -0.25(T2)	109.7 ab*	0.181 a*	0.151 b	148.9	4.689	20.46
E -0.1(T2)	91.78 b*	0.164 b*	0.147 c	151.3	4.758	19.77
SEM	6.652	0.004	0.015	3.145	0.463	0.890
Dosage effect (D) 0.25 g/l	112.3	0.185	0.174	152.6	4.729	20.93
0.1 g/l	94.62	0.171	0.165	150.3	4.774	19.72
Time effect (T) T1	106.2	0.184	0.191	151.4	4.780	20.54
T2	100.7	0.172	0.149	150.1	4.723	20.11
P-value						
G-C vs. all other	0.021	0.026	0.038	0.115	0.095	0.088
D effect	0.014	0.067	0.073	0.125	0.082	0.074
T effect	0.062	0.086	0.031	0.082	0.074	0.067
D×T interaction	0.050	0.068	0.050	0.084	0.061	0.073

a-c – means within the same column differ significantly ($P \leq 0.05$) according to Newman-Keuls mean comparison (D×T interaction).

¹Data represent mean values of 4 replications per treatment. SEM = SD divided by the square root of the replication number, n=8.

²Group: G-C = water not supplemented with probiotic; E -0.25(T1) and E -0.25(T2) = G-C with probiotic – dose 0.25 g/l; E -0.1(T1) and E -0.1(T2) = G-C with probiotic – dose 0.1 g/l; E -0.25(T1), E -0.1(T1) supplemented with probiotic on days 1–42 of life; E -0.25(T2), E -0.1(T2) supplemented with probiotic on days 1–7, 15–21 and 29–35 of life.

*means within the same column differ significantly from the control (G-C) at $P \leq 0.05$ according to Dunnett's mean comparison.

FRAP – total antioxidant status, VIT C – ascorbic acid, GSH + GSSG – total glutathione, UA – uric acid, BIL – bilirubin, CREAT – creatinine.

It should be emphasized, however, that administration of the probiotic at the level of 0.25 g/l throughout the experimental period (T1) most favourably reduced the content of lipid peroxides and MDA, as well as CAT activity in the plasma of the chickens.

Analysis of antioxidant indicators in the blood (Table 4) showed that administration of the probiotic to chickens in their drinking water at 0.25 g/l or 0.1 g/l for the entire rearing period (T1) or specific intervals during rearing (T2) caused a significant increase in FRAP and GSH + GSSG levels. It should be emphasized that application of the probiotic in the amount of 0.25 g/l led to the greatest increase in FRAP and GSH + GSSG concentrations.

Table 4. Effect of the level and duration of application of probiotic (*Enterococcus faecium*) on lipid status in the blood of the chickens¹

Item	TC mmol/l	HDL mmol/l	LDL mmol/l	TAG mmol/l	NEFA μmol/l
Group ²					
G-C	3.852	2.322	1.495	0.462	48.26
E -0.25(T1)	3.554 b*	2.443 a*	0.874 c*	0.389*	36.65*
E -0.1(T1)	3.621 b*	2.382 ab	1.322 b*	0.461	36.49*
E -0.25(T2)	3.786 a*	2.364 ab	1.361 ab*	0.477	37.48*
E -0.1(T2)	3.884 a	2.331 b	1.412 a	0.472	36.15*
SEM	0.063	0.051	0.084	0.012	0.458
Dosage effect (D)					
0.25 g/l	3.670	2.403	1.117	0.433	37.06
0.1 g/l	3.752	2.356	1.367	0.466	36.32
Time effect (T)					
T1	3.587	2.412	1.098	0.399	36.57
T2	3.835	2.347	1.386	0.474	36.81
P-value					
G-C vs. all other	0.034	0.029	0.024	0.037	0.034
D effect	0.033	0.089	0.047	0.082	0.062
T effect	0.011	0.076	0.014	0.050	0.071
D×T interaction	0.048	0.077	< 0.001	0.047	0.089

a-c – means within the same column differ significantly ($P \leq 0.05$) as a result of Newman-Keuls mean comparison (D×T interaction).

¹Data represent mean values of 4 replications per treatment. SEM = SD divided by the square root of the replication number, $n=8$.

²Group: G-C = water not supplemented with probiotic; E -0.25(T1) and E -0.25(T2) = G-C with probiotic – dose 0.25 g/l; E -0.1(T1) and E -0.1(T2) = G-C with probiotic – dose 0.1 g/l; E -0.25(T1), E -0.1(T1) supplemented with probiotic on days 1–42 of life; E -0.25(T2), E -0.1(T2) supplemented with probiotic on days 1–7, 15–21 and 29–35 of life.

*means within the same column differ significantly from the control (G-C) at $P \leq 0.05$ according to Dunnett's mean comparison.

TC – cholesterol (TC), HDL – high-density cholesterol, LDL – low-density cholesterol, TAG – triacylglycerol, NEFA – nonesterified fatty acids.

Administration of the probiotic in the amount of 0.25 g/l or 0.1 g/l for the entire rearing period (T1) caused a significant increase in ascorbic acid concentration in the blood plasma of the chickens. Results concerning the level of blood lipid indicators are presented in Table 5. Administration of the probiotic in the amount of 0.25 g/l for the entire rearing period (T1) or periodically (T2) significantly reduced the content of TC and the LDL cholesterol fraction in the blood of the chickens. The dose of 0.1 g/l also reduced TC and LDL cholesterol in the blood, but only in the case of continuous application (T1). Irrespective of the period of administration and the dose, the addition of the probiotic to the chickens' drinking water also reduced the blood content of NEFA.

Administration of the probiotic in the amount of 0.25 g/l, for both the entire experimental period or during specific intervals, caused a significant decrease in ALP activity (Table 5). Furthermore, irrespective of the administration time and the dose, the addition of the probiotic to the chickens' drinking water reduced GGT activity in the blood. Administration of the probiotic in the amount of 0.25 g/l, for the entire rearing period (T1) or periodically (T2), significantly reduced HBDH activity in the

blood of the chickens. Adding the probiotic to drinking water in the amount of 0.1 g/l also reduced HBDH in the blood, but only in the case of continuous application (T1). Finally, supplementation with the probiotic also caused a significant reduction in AC activity in the blood of the chickens. Its inclusion at the level of 0.25 g/l for the entire experimental period (T1) resulted in the most favourable reduction in AC activity in the blood of the chickens (Table 5).

Table 5. Effect of the level and duration of application of the probiotic (*Enterococcus faecium*) on enzymes activity in the blood of the chickens¹

Item	LDH U/l	ALP U/l	GGT U/l	CK U/l	HBDH U/l	AC U/l
Group ²						
G-C	503.8	928.8	17.33	2522.5	122.4	1.698
E -0.25(T1)	497.1	881.7 b*	13.62 b*	2499.2	93.71 b*	1.283 c*
E -0.1(T1)	493.4	911.3 a	15.30 ab*	2548.9	101.5 b*	1.450 b*
E -0.25(T2)	487.6	879.4 b*	15.29 ab*	2615.3	100.9 b*	1.533 a*
E -0.1(T2)	512.1	921.7 a	16.30 a*	2707.5	121.4 a	1.583 a*
SEM	13.82	20.31	0.347	32.12	9.952	0.124
Dosage effect (D)						
0.25 g/l	492.3	880.5	14.45	2557.2	97.30	1.408
0.1 g/l	502.7	916.5	15.80	2628.2	111.4	1.516
Time effect (T)						
T1	495.2	898.5	14.46	2550.9	97.60	1.366
T2	499.8	900.2	15.79	2661.4	111.1	1.558
P-value						
G-C vs. all other	0.072	0.046	0.020	0.061	0.021	0.032
D effect	0.066	0.050	0.075	0.087	0.050	0.048
T effect	0.073	0.071	0.063	0.061	0.053	0.041
D×T interaction	0.088	0.050	0.066	0.079	0.048	0.037

a-c – means within the same column differ significantly ($P \leq 0.05$) according to Newman-Keuls mean comparison (D×T interaction).

¹Data represent mean values of 4 replications per treatment. SEM = SD divided by the square root of the replication number, n=8.

²Group: G-C = water not supplemented with probiotic; E -0.25(T1) and E -0.25(T2) = G-C with probiotic – dose 0.25 g/l; E -0.1(T1) and E -0.1(T2) = G-C with probiotic – dose 0.1 g/l; E -0.25(T1), E -0.1(T1) supplemented with probiotic on days 1–42 of life; E -0.25(T2), E -0.1(T2) supplemented with probiotic on days 1–7, 15–21 and 29–35 of life.

*means within the same column differ significantly from the control (G-C) at $P \leq 0.05$ according to Dunnett's mean comparison.

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

Discussion

Organisms have developed an antioxidant defence system based on two mechanisms. The first involves the activity of antioxidant enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase and S-transferases) which neutralize free oxygen radicals. The other depends on the presence of low-molecular-weight antioxidants (such as ascorbic acid, tocopherol, glutathione) which directly interact

with oxidant molecules or react with intermediate redox metabolites, preventing the formation of lipid peroxides, hydroxyl radicals and alkoxyl radicals (Ognik and Wartecki, 2012; Ognik et al., 2016). These diverse molecules, performing varied physiological roles, terminate the free radical chain reaction by inhibiting the oxidation process through removal of free radicals or intermediate compounds. The body responds to factors modifying redox status with a change in the activity of antioxidant enzymes. During oxidative stress the activity of these enzymes, particularly catalase, usually increases (Ognik et al., 2016; Ognik and Krauze, 2016), as they are mobilized in the systemic defence mechanism.

According to Kumar et al. (2010), Rajput and Li (2012) and Li et al. (2012) probiotics may stimulate antioxidant defence mechanisms in animal tissues. In our study, administration of a probiotic preparation containing cultures of *Enterococcus faecium* at the level of 0.25 g/l of water to chickens during their entire rearing period caused a reduction in the content of lipid peroxidation products and catalase activity and an increase in total antioxidant potential and glutathione in the blood plasma. According to Rajput et al. (2013), probiotics increase the activity of CAT, SOD and GPx and the content of GSH+GSSG and reduce the concentration of MDA and uric acid (Ognik and Krauze, 2016). Zheng et al. (2016) demonstrate that *Enterococcus faecium* bacteria enhance resistance to oxidation of biological macromolecules and remove hydroxyl radicals, thereby increasing the body's antioxidant capacity. Rajput et al. (2013), in a study dealing with supplementation with *Saccharomyces boulardii* and *Bacillus subtilis*, found that these probiotics significantly increased SOD and CAT activity. Similar findings were also observed in chickens following administration of yeast-based probiotics by Milinkovic-Tur et al. (2007) and Kogan et al. (2008). According to Capcarova et al. (2010) and Rajput et al. (2013), it is possible that some probiotic microorganisms stimulate the natural antioxidant mechanisms of the body, and our results support this hypothesis. Kumar et al. (2010) also report that probiotics stimulate the circulation of molecules involved in systemic defence, such as glutathione. A favourable stimulatory effect of mannanoligosaccharides on antioxidant defence mechanisms and improvement of growth performance in turkeys were also observed by Ognik and Krauze (2012). Aluwong et al. (2013) also found a significant increase in CAT activity following the use of a probiotic prepared from *Saccharomyces cerevisiae*. In a study by Aluwong et al. (2013) however, the use of a probiotic did not increase SOD or CAT activity as compared to the control.

The increase in the glutathione level and total antioxidant capacity shown in this study following administration of the probiotic preparation is in accordance with the results shown by Kullisaar et al. (2002) and El-Sissi and Mohamed (2011). Glutathione is involved in the detoxification of free radicals and other endogenous or exogenous carcinogenic agents, as well as in the modulation of immune function, contributing to the protection of thiol groups of proteins against irreversible inactivation by free radicals (ROS). Specifically, by participating in the decomposition of lipid peroxides, glutathione functions as a reducing compound in the process of peroxide detoxification (Aw et al., 1992). Moreover, glutathione performs many functions not directly associated with antioxidant defence, assists in detoxification of electrophilic compounds and in the metabolism of leukotrienes and prostaglandins,

reduces dehydroascorbic acid to ascorbic acid, takes part in methemoglobin reduction, serves as a form of cysteine transport, and affects the activity of glycolysis enzymes. A reduction in the concentration of this tripeptide leads to a rapid increase in lipid peroxide and malondialdehyde levels in the cell (Łukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004). It is likely that the reduction in lipid peroxides was attained due to high content of glutathione, formed due to the involvement of probiotic microorganisms capable of hydrolysing large protein molecules and forming biologically active oligopeptides.

The reduction in MDA content observed in the present study can be considered a definitely positive effect of the probiotic. A similar result was obtained by El-Sissi and Mohamed (2011) in a study also conducted on chickens. This effect is possibly observed due to the probiotic's favourable influence on the function of biological membranes and its involvement in antioxidant mechanisms. MDA concentration is used as a biomarker in case of damage induced by free radicals. In case of failure of the enzymatic antioxidant defence system, the MDA concentration increases, whereas when stress is absent and enzymes function normally the concentration of this indicator remains unchanged or decreases. Thus an elevated concentration of MDA indicates impairment of antioxidant defence. According to previous studies, MDA plasma concentration is a sensitive marker of lipid peroxidation and elevated concentration of MDA is considered to be one of the main causes of secondary cell damage. MDA is a highly reactive compound with a much longer half-life than that of free radicals and is capable of diffusion to regions distant from the sites where it is formed. It can form covalent bonds with proteins, enzymes and nucleic acids, resulting in alteration of their structure and function. It also disturbs mitochondrial function and the electron transport chain, which initiates further oxidative chain reactions. As a result, inhibition of enzyme activity and changes in the fluidity and properties of biological membranes are observed (Liu et al., 2007). On the other hand, Aluwong et al. (2013) did not observe an effect of probiotic administration on the MDA level in broilers.

The potential effect of probiotics on lipid metabolism has recently attracted particular interest (Mikulski et al., 2012; Sobczak and Kozłowski, 2015). The reduction in the level of NEFA confirms the effect of the *Enterococcus faecium* supplement on reducing lipid oxidation processes and thereby limiting the stress response in the chickens. During stressful situations metabolic changes may occur and lipolysis is stimulated. This is accompanied by an increase in the level of fatty acids, as glycerol may be utilized to synthesize glucose (gluconeogenesis), leading to inhibition of triacylglycerol synthesis (Verago et al., 2001). It may be presumed that the reduction observed in blood lipid indicators – the concentration of cholesterol and triacylglycerols and the proportion of the LDL fraction of cholesterol – following administration of the probiotic at the level of 0.25 g/l for the entire rearing period (T1) may be due to the antioxidant activity of the probiotic, since it is well known that during oxidative processes the level of cholesterol, triacylglycerols and LDL cholesterol usually increases (Arslan, 2006). In the peroxidation process, in addition to markers considered to be specific for estimation of lipid oxidation, blood lipid status parameters, such as total cholesterol, its fractions, and the level of triacylglycerols may be

used as well. The content of cholesterol and phospholipids and the degree of fatty acid saturation significantly influence cell membrane fluidity. Lipid peroxidation may cause the phenomenon of hypercholesterolaemia (Fki et al., 2007). Mikulski et al. (2012) observed that probiotics decreased cholesterol level in egg yolk, probably owing to increased elimination of this sterol together with taurodeoxycholic and glycocholic acids, whose synthesis increases in the presence of a probiotic.

GGT is a marker of liver function and is located in hepatocyte membranes as well as in cells in the bile duct. This enzyme is involved in the degradation of glutathione by catalysing hydrolysis of the gamma-glutamyl bond between glutamate and cysteine. Moreover, it takes part in the transamination of glucogenic amino acids required for glucose production. The value for this indicator was lower in the groups receiving the probiotic, which can be considered a positive effect. A high GGT concentration in birds is used as an indicator of liver disease and bile flow disorders (Ognik and Krauze, 2016). When the bile ducts undergo damage the enzyme is released into the blood. GGT activity often increases in female birds during the laying period, evidently reflecting the increase in liver metabolism during egg laying (Alonso-Alvarez et al., 2007). The enzyme GGT is particularly important in the process of biodegradation of S-nitrosoglutathione, in the form of which endogenous nitric oxide can be stored. Harr (2002) reported that plasma GGT is more likely to be elevated in birds than in mammals during cholestasis or biliary epithelial disorders. Therefore, GGT is a useful tool to determine whether toxicants affect the bile ducts of chickens. A reduction in GGT activity in the blood of Japanese quail following administration of an *E. faecium* probiotic was noted by Babazadeh et al. (2011).

AC is an indicator enzyme of lysosomes, where surplus protein absorbed with food is degraded. High activity of this enzyme is observed in stress conditions and thus the decrease in this value observed in our study can be considered a positive effect. A strong increase in the activity of this enzyme indicates disintegration of liver cells, as the enzyme plays a key role in cellular bioenergetics and homeostasis of cellular ATP, catalysing a reversible transfer of a phosphate group. This makes it possible to control the level of ADP in the cytosol, next to the sites of ATP hydrolysis (Bessman and Geiger, 1981).

Similarly, the reduction in ALP and HBDH activity following administration of the probiotic in the amount of 0.25 g/l (T1 and T2) may indicate a positive effect of *Enterococcus faecium* on the function of the liver and other organs, since an increase in their activity appears in the case of impaired liver function and pathological conditions, including those not associated with the liver and bile ducts, such as bone or kidney disease, heart failure, or inflammatory states (Ognik and Krauze, 2016). A reduction in ALP activity in the blood of Japanese quails following administration of *E. faecium* was noted by Babazadeh et al. (2011).

Conclusions

In conclusion, the probiotic preparation used, containing live cultures of *Enterococcus faecium* and enriched with cholecalciferol and ascorbic acid, had a beneficial effect on indicators of lipid status and the redox reaction in the blood of broiler chickens. The results of the study indicate that the use of this probiotic for chick-

ens, particularly at the level of 0.25 g/l and for the entire rearing period stimulates synthesis of low-molecular-weight antioxidants responsible for maintaining redox homeostasis in broilers without exerting a negative effect on liver metabolism.

References

- Aebi H. (1984). Catalase *in vitro*. Meth. Enzymol., 105: 121–126.
- Akerboom T.P., Sies H. (1981). Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Meth. Enzymol., 77: 373–382.
- Alonso-Alvarez C., Perez C., Velando A. (2007). [Effects of acute exposure to heavy fuel oil from the prestige spill on a seabird](#). Aquat. Toxicol., 84: 103–110.
- Aluwong T., Kawu M., Raji M., Dzenda T., Govwang F., Sinkalu V., Ayo J. (2013). Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. Antioxidants, 2: 326–339.
- Arslan C. (2006). L-carnitine and its use as a feed additive in poultry feeding a review. Rev. Med. Vet., 157: 134–142.
- Audisio M.C., Oliver G., Apella M.C. (2000). Protective effect of *Enterococcus faecium* J96, a potential probiotic strain, on chicks infected with *Salmonella pullorum*. J. Food Protect., 63: 1333–1337.
- Aw T.Y., Williams M.W., Gray L. (1992). Absorption and lymphatic transport of peroxidized lipids by rat small intestine *in vivo*: role of mucosal GSH. Am. J. Physiol., 262: 99–106.
- Babazadeh D., Vahdatpour T., Nikpiran H., Jafargholipour M., Vahdatpour S. (2011). Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (*Coturnix japonica*). Indian J. Anim. Res., 81: 870–874.
- Benzie I.F.F., Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal. Biochem., 239: 70–76.
- Bessman S.P., Geiger P.J. (1981). Transport of energy in muscle: the phosphocreatine shuttle. Science, 211: 448–452.
- Capcarova M., Weiss J., Hrnecar C., Kolesarova A., Pal G. (2010). Effect of *Lactobacillus fermentum* and *Enterococcus faecium* strains on internal milieu, antioxidant status and body weight of broiler chickens. J. Anim. Physiol. Anim. Nutr., 94: 215–224.
- Douglas D.N., Pu C., Lewis J., Bhat R., Anwar-Mohamed A., Logan M., Lund G., Addison W.R., Lehner R., Kneteman N.M. (2016). Oxidative stress attenuates lipid synthesis and increases mitochondrial fatty acid oxidation in hepatoma cells infected with hepatitis C virus. J. Biol. Chem., 22: 1974–1990.
- El-Sissi A., Mohamed S. (2011). Impact of symbiotic on the immune response of broiler chickens against NDV and IBV vaccines. GJBRR, 6: 186–191.
- Fki I., Sahnoun Z., Sayadi S. (2007). Hypocholesterolemic effects of phenolic extracts and purified hydroxytyrosol recovered from olive mill wastewater in rats fed a cholesterol-rich diet. J. Agric. Food Chem., 7: 624–631.
- Gay C., Gębicki J.M. (2002). Perchloric acid enhances sensitivity and reproducibility of the ferric-xylenol orange peroxide assay. Anal. Biochem., 304: 42–46.
- Harr K.E. (2002). Clinical chemistry of companion avian species: A review. Vet. Clin. Pathol., 31: 140–151.
- Juśkiewicz J., Semaskaite A., Zduńczyk Z., Wróblewska M., Gruzauskas R., Juśkiewicz M. (2007). Minor effect of the dietary combination of probiotic *Pediococcus acidilactici* with fructooligosaccharides or polysaccharidases on beneficial changes in the cecum of rats. Nutr. Res., 27: 133–139.
- Kogan G., Pajtinka M., Babincova M., Miadokova E., Rauko P., Slamenova D. (2008). Yeast cell wall polysaccharides as antioxidants and antimutagens: can they fight cancer? Neoplasma, 55: 387–393.
- Kullisaar T., Zilmer M., Mikelsaar M. (2002). Two antioxidative lactobacilli strains as promising probiotics. Int. J. Food Microbiol., 72: 215–224.

- Kumar M., Kumar A., Nagpal R., Mohania D., Behare P., Verma V., Kumar P., Poddar D., Aggarwal P.K., Henry C.J., Jain S., Yadav H. (2010). Cancer-preventing attributes of probiotics: An update. *Int. J. Food Sci. Nutr.*, 61: 473–496.
- Levkut M., Revajová V., Lauková A., Ševčíková Z., Spišáková V., Faixová Z., Levkútová M., Stropfová V., Pistl J., Levkut M. (2012). Leukocytic responses and intestinal mucin dynamics of broilers protected with *Enterococcus faecium* EF 55 and challenged with *Salmonella enteritidis*. *Res. Vet. Sci.*, 93: 195–201.
- Li W.F., Huang Q., Li Y.L., Rajput I.R., Huang Y., Hu C.H. (2012). Induction of probiotic strain *Enterococcus faecium* EF1 on the production of cytokines, superoxide anion and prostaglandin E2 in a macrophage cell line. *Pak. Vet. J.*, 32: 530–534.
- Liu X., Zhang S., Shan X., Christie P. (2007). Combined toxicity of cadmium and arsenate to wheat seedlings and plant uptake and antioxidant enzyme responses to cadmium and arsenate co-contamination. *Ecotox. Environ. Safe.*, 68: 305–313.
- Luo J., Zheng A., Meng K., Chang W., Bai Y., Li K. (2013). Proteome changes in the intestinal mucosa of broiler (*Gallus gallus*) activated by probiotic *Enterococcus faecium*. *J. Proteomics*, 91: 226–241.
- Lukaszewicz-Hussain A., Moniuszko-Jakoniuk J. (2004). Liver catalase, glutathione peroxidase and reductase activity, reduced glutathione and hydrogen peroxide levels in acute intoxication with chlorfenvinphos, an organophosphate insecticide. *Pol. J. Environ. Stud.*, 13: 303–309.
- Mikulski D., Jankowski J., Naczmański J., Mikulska M., Demey V. (2012). Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. *Poultry Sci.*, 91: 2691–2700.
- Milinkovic-Tur S., Stojevic Z., Pirsljin J., Zdelar-Tuk M., Poljicak-Milas N., Beer Ljubic B., Gradinski-Vrbanac B. (2007). Effect of fasting and refeeding on the antioxidant system in cockerels and pullets. *Acta Vet. Hung.*, 55: 181–189.
- Misra H.P., Fridovich I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, 247: 3170–3175.
- Nutrient Requirements for Poultry (2005). Fourth edition revised and enlarged. Collective work (A. Rutkowski – co-editor), PAN IFiZZ, Jabłonna, Poland.
- Ognik K., Krauze M. (2012). Dietary supplementation of mannanoligosaccharides to turkey hens on their growth performance and antioxidant status in the blood. *S. Afr. J. Anim. Sci.*, 42: 379–388.
- Ognik K., Wiertelicki T. (2012). Effect of different vitamin E sources and levels on selected oxidative status indices in blood and tissues as well as on rearing performance of slaughter turkey hens. *Poultry Sci.*, 21: 259–271.
- Ognik K., Krauze M. (2016). The potential for using enzymatic assays to assess the health of turkeys. *Worlds Poultry Sci. J.*, 72: 535–550.
- Ognik K., Czech A., Stachyra K. (2013). Effect of a natural versus a synthetic antioxidant, and sex and age on the redox profile in the blood of growing turkeys. *S. Afr. J. Anim. Sci.*, 43: 473–481.
- Ognik K., Cholewińska E., Sembratowicz I., Grela E., Czech A. (2016). The potential of using plant antioxidants to stimulate antioxidant mechanisms in poultry. *Worlds Poultry Sci. J.*, 72: 291–298.
- Omaye S.T., Tumbull J.D.H., Sauberlich E. (1979). Selected methods for determination of ascorbic acid in animal cells, tissues and fluids. *Meth. Enzymol.*, 62: 3–11.
- Rajput I.R., Li W.F. (2012). Potential role of probiotics in mechanism of intestinal immunity. *Pak. Vet. J.*, 32: 303–308.
- Rajput I.R., Li Y.L., Xu X., Huang Y., Zhi W.C., Yu D.Y., Li W. (2013). Supplementary effects of *Saccharomyces boulardii* and *Bacillus subtilis* B10 on digestive enzyme activities, antioxidation capacity and blood homeostasis in broiler. *IJABE*, 15: 231–237.
- Russel J.R., Dun-Xian T., Carmen N., Gitto O.E. (2000). Actions of melatonin in the reduction of oxidative stress. *J. Biomed. Sci.*, 7: 444–458.
- Salih M., Smith D.M., Price J.F., Dawson L.E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Sci.*, 66: 1483–1488.
- Samli H.E., Senkoylu N., Koc F., Kanter M., Ağa A. (2007). Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and intestinal microbiota. *Arch. Anim. Nutr.*, 61: 42–49.

- Samli H.E., Dezcán S., Koc F., Özduven M.L., Okur A.A., Senkoğlu N. (2010). Effects of *Enterococcus faecium* supplementation and floor type on performance, morphology of erythrocytes and intestinal microbiota in broiler chickens. *Brit. Poultry Sci.*, 51: 564–568.
- Sobczak A., Kozłowski K. (2015). The effect of a probiotic preparation containing *Bacillus subtilis* ATCC PTA-6737 on egg production and physiological parameters of laying hens. *Ann. Anim. Sci.*, 15: 711–723.
- Stancu C.S., Toma L., Sima A.V. (2012). [Dual role of lipoproteins in endothelial cell dysfunction in atherosclerosis](#). *Cell Tissue. Res.*, 349: 433–446.
- Tsimikas S., Miller Y.I. (2011). Oxidative modification of lipoproteins: mechanisms, role in inflammation and potential clinical applications in cardiovascular disease. *Curr. Pharm. Des.*, 17: 27–37.
- Verago J.L., Grassi-Kassisse D.M., Spadari-Bratfisch R.C. (2001). Metabolic markers following beta-adrenoceptor agonist infusion in footshock-stressed rats. *Braz. J. Med. Biol. Res.*, 34: 1197–1207.
- Zhao X., Guo Y., Guo S., Tan J. (2013). Effects of *Clostridium butyricum* and *Enterococcus faecium* on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. *Appl. Microbiol. Biotechnol.*, 97: 6477–6488.
- Zheng A., Luo J., Meng K., Li J., Zhang S., Li K. (2014). Proteome changes underpin improved meat quality and yield of chickens (*Gallus gallus*) fed the probiotic *Enterococcus faecium*. *BMC Genomics*, 15: 1167–1181.
- Zheng A., Luo J., Meng K., Li J., Bryden W., Chang W., Zhang S., Wang L., Liu G., Yao B. (2016). Probiotic (*Enterococcus faecium*) induced responses of the hepatic proteome improves metabolic efficiency of broiler chickens (*Gallus gallus*). *BMC Genomics*, DOI: 10.1186/S12864-016-2371-5.

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