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THE EFFECT OF ADDING HESPERIDIN, DIOSMIN, QUERCETIN AND RESVERATROL EXTRACTS TO FEED FOR TURKEY HENS ON SELECTED IMMUNOLOGICAL AND BIOCHEMICAL BLOOD INDICES*

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Abstract

It was postulated that naturally occurring phenolic compounds obtained from various plant species may have potential use as feed additives for poultry. Therefore the aim of the study was to compare extracts of hesperidin, diosmin, quercetin and resveratrol in terms of their health-promoting (particularly immunostimulatory) effect on turkeys at different ages. The experiment was conducted on 720 Big 6 turkey hens assigned to 6 experimental groups of 120 individuals (6 repetitions with 20 birds each). The turkey hens in group G-C were the control, receiving a basal compound feed with no experimental additives. The turkey hens in the remaining groups, from the first to the 16th week of life, received a basal diet containing hesperidin (group G-H), diosmin (group G-D), quercetin (group G-Q) or resveratrol (group G-R) in the amount of 200 g per tonne of feed. Ht, Hb, RBC, WBC, lysozyme activity, %PC, IgA, IL-6, GLU, TP and minerals were determined in blood samples. The addition of quercetin or resveratrol in the amount of 200 g per tonne of feed was found to have a beneficial effect on haemoglobin synthesis and phosphorus availability, and may also modulate immunity in turkey hens.

Key words: turkey hens, polyphenol extracts, blood, immunity, haematological and biochemical indices

Naturally occurring herbs and bioactive compounds, including polyphenols obtained from various species of plants, can potentially be used as feed additives for poultry (Ognik and Sembratowicz, 2012; Ognik et al., 2013; Grela et al., 2014; Kwiecień et al., 2014; Ognik et al., 2015 a, b; Arczewska-Włosek and Świątkiewicz, 2015; Juśkiewicz et al., 2015; Jankowski et al., 2016; Ognik et al., 2016). As reported

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by Jankowski et al. (2016) a polyphenol supplement for turkeys increases plasma content of vitamin C and inhibits lipid peroxidation. According to Juśkiewicz et al. (2015), the addition of polyphenol extracts to turkey feed may improve growth performance. Polyphenols are a vast group of plant secondary metabolites produced by plants, for defence purposes, as a response to stress, or as attractants (Pietta, 2000). Among the large group of polyphenols we can distinguish four basic classes of plant phenols. These are phenolic acids, such as caffeic acid, chlorogenic acid and cinnamic acid; flavonoids, e.g. quercetin, kaempferol, hesperidin, naringenin, genistein, daidzein, luteolin and apigenin; stilbenes, e.g. resveratrol; and lignans, e.g. pinoresinol, sesaminol and sesamin (Grajek, 2007). The antioxidant properties of polyphenolic compounds are due to the presence of hydroxyl groups in their structure, and the more of these groups are in the compound, the higher their antioxidant potential (Rice-Evans et al., 1996). Apart from antioxidant activity, polyphenolic compounds exhibit a number of pharmacological and biological properties, confirmed mainly in laboratory animals (John et al., 2011; Jaccob and Hussain, 2012) and humans (Vauzour et al., 2010; Pereira Lima et al., 2014), but also in livestock animals (Kim et al., 2015). Literature data indicate that the flavonoids hesperidin, quercetin, daidzein, resveratrol and diosmin exhibit antioxidant and immunostimulatory properties (Chen et al., 1990; Frémont et al., 1999; Galati et al., 1994; Ognik et al., 2016).

We postulated that polyphenolic compounds include some which apart from their confirmed antioxidant activity (Ognik and Czech, 2010; Ognik, 2013; Ognik et al., 2015 a) may also have an immunomodulatory effect on the organism of turkeys. Therefore the aim of the study was to compare extracts of hesperidin, diosmin, quercetin and resveratrol in terms of their health-promoting (and in particular immunomodulatory) effect on the organism of turkeys at different ages.

Material and methods

Animals

The experimental procedure was approved by the Second Local Ethics Commission for Experiments with Animals in Lublin (approval no. 26/2011). The material for the experiment was 7-day-old Big 6 turkey hens. The birds were kept in pens measuring 2.5 × 4 m, on straw litter. The birds were reared in standard conditions in a room with regulated temperature and humidity.

During the experiment the birds in all groups had permanent access to drinking water and received *ad libitum* complete feed rations appropriate for each period of rearing (Table 1). The basal mixtures were balanced on the basis of wheat, maize meal, post-extraction soybean meal, and soybean oil. The basal mixtures were then differentiated by adding natural antioxidants, which were introduced to the mineral and vitamin premix (Table 2). The nutritional value of the feed mixtures is presented in Table 3. The feed mixtures contained dl-alpha-tocopheryl acetate in the amount of 50 mg kg^{-1} of feed (1–9 weeks of age) and 45 mg kg^{-1} (10–16 weeks of age).

Table 1. The composition of basal mixtures

Components (%)	1–2 week	3–5 week	6–9 week	10–12 week	13–16 week
Maize (ground)	25.6	27.4	23.8	35.2	47.4
Wheat (ground)	20.0	25.0	30.0	25.0	25.0
Wheat bran	3.0	-	-	-	-
Soybean meal 46% CP	43.0	41.7	38.8	32.7	20.4
Fish meal 60% CP	3.5	-	-	-	-
Limestone	1.2	1.7	1.7	1.4	1.5
Soybean oil	0.5	1.0	2.5	3.0	3.0
Cytromix Plus[1]	0.2	0.2	0.2	0.2	0.2
Premix[2]	3.0	3.0	3.0	2.5	2.5
The calculated nutrient composition of 1 kg of mixture according to NRC (1994)					
M (kcal kg^{-1})	2736	2803	2913	3007	3129
Crude protein	27.1	25.5	24.5	22.0	17.5
Lysine	1.81	1.71	1.57	1.38	1.17
Methionine + Cysteine	0.98	0.90	0.88	0.79	0.70
Total calcium	1.39	1.23	1.17	1.06	0.94
Phosphorus available	0.77	0.67	0.59	0.57	0.47

[1]Cytromix Plus – citric acid, fumaric acid, phosphoric acid (62%).

[2] Mineral and vitamin premix: 1–2 week: Vitamin A: 150.0 mg kg^{-1}, Vitamin D_3: 4.166 mg kg^{-1}, Vitamin E: 2 333 mg kg^{-1}, Vitamin K_3: 133.3 mg kg^{-1}, Vitamin B_1: 166.7 mg kg^{-1}, Vitamin B_2: 336.3 mg kg^{-1}, Vitamin B_6: 200.0 mg kg^{-1}, Vitamin B_{12}: 1.0 mg kg^{-1}, Folic acid: 75.0 mg kg^{-1}, Biotin: 11.7 mg kg^{-1}, Nicotinic amid: 2 500 mg kg^{-1}, Calcium pantothenicum: 750 mg kg^{-1}, Choline: 20 000 mg kg^{-1}, Manganese: 5 000 mg kg^{-1}, Zinc: 3 333 mg kg^{-1}, Iron: 2 000 mg kg^{-1}, Copper: 500 mg kg^{-1}, Iodine: 66.7 mg kg^{-1}, Selenium: 10 mg kg^{-1}, Cobalt: 6.7 mg kg^{-1}, Calcium: 16%, Total phosphorus: 15.2%, Sodium: 2.0%, Lysine: 6.0%, Methionine: 4.8%, Coccidiostat-monensin (+); 3–9 week: Vitamin A: 129.9 mg kg^{-1}, Vitamin D_3: 3.333 mg kg^{-1}, Vitamin E: 1 833 mg kg^{-1}, Vitamin K_3: 100 mg kg^{-1}, Vitamin B_1: 116.0 mg kg^{-1}, Vitamin B_2: 300 mg kg^{-1}, Vitamin B_6: 166.7 mg kg^{-1}, Vitamin B_{12}: 0.9 mg kg^{-1}, Folic acid: 66.7 mg kg^{-1}, Biotin: 10 mg kg^{-1}, Nicotinic amid: 2 166 mg kg^{-1}, Calcium pantothenicum: 616 mg kg^{-1}, Choline: 13 333 mg kg^{-1}, Manganese: 4 000 mg kg^{-1}, Zinc: 3 000 mg kg^{-1}, Iron: 1 666 mg kg^{-1}, Copper: 666 mg kg^{-1}, Iodine: 58.3 mg kg^{-1}, Selenium: 10 mg kg^{-1}, Cobalt: 6.7 mg kg^{-1}, Calcium: 13.5%, Total phosphorus: 15.5%, Sodium: 3.5%, Lysine: 9.0%, Methionine: 5.3%, Threonine: 0.7%, Coccidiostat-monensin (+); 10–12 week: Vitamin A: 124.9 mg kg^{-1}, Vitamin D_3: 3.125 mg kg^{-1}, Vitamin E: 1 583 mg kg^{-1}, Vitamin K_3: 100 mg kg^{-1}, Vitamin B_1: 83.3 mg kg^{-1}, Vitamin B_2: 266 mg kg^{-1}, Vitamin B_6: 166.7 mg kg^{-1}, Vitamin B_{12}: 0.8 mg kg^{-1}, Folic acid: 66.7 mg kg^{-1}, Biotin: 9.2 mg kg^{-1}, Nicotinic amid: 2 083 mg kg^{-1}, Calcium pantothenicum: 583 mg kg^{-1}, Choline: 12 500 mg kg^{-1}, Manganese: 4 000 mg kg^{-1}, Zinc: 3 000 mg kg^{-1}, Iron: 1 667 mg kg^{-1}, Copper: 750 mg kg^{-1}, Iodine: 58.3 mg kg^{-1}, Selenium: 10 mg kg^{-1}, Cobalt: 6.7 mg kg^{-1}, Calcium: 12.5%, Total phosphorus: 14.5%, Sodium: 4.2%, Lysine: 10%, Methionine: 5.5%, Threonine: 1.5%, Coccidiostat-monensin (+); 13–16 week: Vitamin A: 135 mg kg^{-1}, Vitamin D_3: 3.400 mg kg^{-1}, Vitamin E: 1 800 mg kg^{-1}, Vitamin K_3: 90 mg kg^{-1}, Vitamin B_1: 90 mg kg^{-1}, Vitamin B_2: 300 mg kg^{-1}, Vitamin B_6: 166 mg kg^{-1}, Vitamin B_{12}: 0.8 mg kg^{-1}, Folic acid: 70 mg kg^{-1}, Biotin: 9.0 mg kg^{-1}, Nicotinic amid: 2 000 mg kg^{-1}, Calcium pantothenicum: 600 mg kg^{-1}, Choline: 12 000 mg kg^{-1}, Manganese: 4 800 mg kg^{-1}, Zinc: 3 200 mg kg^{-1}, Iron: 1 800 mg kg^{-1}, Copper: 900 mg kg^{-1}, Iodine: 60 mg kg^{-1}, Selenium: 12 mg kg^{-1}, Cobalt: 8.0 mg kg^{-1}, Calcium: 12%, Total phosphorus: 14.5%, Sodium: 5.2%, Lysine: 10%, Methionine: 5.2%, Threonine: 1.5%.

Table 2. Haematological indices in the blood of the turkeys receiving hesperidin, diosmin, quercetin and resveratrol

Item		Polyphenol (P)					Effect Age (A)	SEM	P -value		
		G-C (n=18)	G-H (n=18)	G-D (n=18)	G-Q (n=18)	G-R (n=18)			P	A	P×A
RBC	9	2.61	2.53	2.40	2.68	2.58	2.56	0.025			
10^{12} l^{-1}	12	2.27	2.30	2.35	2.46	2.30	2.91	0.029	0.068	0.145	0.097
	15	2.32	2.41	2.54	2.55	2.50	2.46	0.030			
Effect	(P)	2.40	2.41	2.43	2.56	2.46					
Ht	9	0.38	0.35	0.36	0.36	0.36	0.36	0.003			
l l^{-1}	12	0.36	0.36	0.37	0.36	0.37	0.36	0.002	0.074	0.183	0.245
	15	0.36	0.36	0.39	0.37	0.34	0.36	0.004			
Effect	(P)	0.36	0.35	0.37	0.36	0.35					
Hb	9	9.32	9.39	8.62	8.57	9.35	9.05	0.132			
g l^{-1}	12	10.6 b	10.9 b	10.6 b	11.4 a	11.2 ab	10.9	0.129	0.051	0.042	0.050
	15	11.4 b	12.4 ab	12.0 ab	13.0 a	12.4 ab	12.2	0.122			
Effect	(P)	10.4	10.9	10.4	10.9	10.9					
WBC	9	20.8	22.1	21.1	21.0	21.1	21.2	0.263			
10^9 l^{-1}	12	22.0	22.2	21.9	21.0	21.8	26.1	0.107	0.185	0.228	0.093
	15	23.2	23.0	23.1	22.8	22.5	22.9	0.171			
Effect	(P)	22.0	22.4	22.0	21.6	21.8					
HETERO%	9	48.8 a	36.8 c	37.1 bc	35.1 c	44.8 ba	40.5	0.874			
	12	32.3 b	30.8 b	31.0 b	28.0 b	40.1 a	32.4	0.997	0.005	0.044	0.174
	15	33.0 b	33.3 b	33.5 b	37.6 b	43.8 a	36.2	0.827			
Effect	(P)	38.0	33.6	33.8	33.5	42.9					
LYMPH	9	47.6 b	59.3 ab	58.5 ab	61.5 a	51.8 b	55.7	0.862			
%	12	61.6 ab	64.0 ab	64.6 ab	69.8 a	56.0 b	63.0	1.066	0.008	0.774	0.486
	15	62.8 a	63.5 a	63.0 a	58.8 ab	53.0 b	60.2	0.842			
Effect	(P)	57.3	62.2	62.0	63.3	53.6					
MONO	9	1.50	1.50	1.33	1.00	1.16	1.29	0.113			
%	12	3.16 a	2.50 b	2.83 ab	0.66 d	1.16 c	2.06	0.169	0.014	0.049	0.051
	15	1.16	1.00	1.33	1.16	1.00	1.13	0.085			
Effect	(P)	1.94	1.66	1.83	0.94	1.10					
EOSINO	9	1.16	1.00	1.83	1.00	1.16	1.23	0.120			
%	12	1.66	1.00	0.00	0.66	1.00	0.86	0.134	0.084	0.168	0.091
	15	1.33	1.00	1.16	1.16	1.16	1.16	0.115			
Effect	(P)	1.38	1.00	0.99	0.94	1.10					
BASO	9	0.83	1.33	1.16	1.33	1.00	1.13	0.114			
%	12	1.16	1.66	1.50	0.83	1.66	1.36	0.108	0.256	0.086	0.863
	15	1.66	1.16	1.00	1.16	1.00	1.19	0.111			
Effect	(P)	1.21	1.38	1.22	1.10	1.22					

a, b, c – values in rows with different denoted letters differ significantly at $P \leq 0.05$; 9th,12th and 15th days of age of the turkeys – blood was collected, G-C (control) received basal feed mixture with no experimental additives, G-H received basal feed mixture with hesperidin (200 g t^{-1}), G-D received basal feed mixture with diosmin (200 g t^{-1}), G-Q received basal feed mixture with quercetin (200 g t^{-1}), G-R received basal feed mixture with resveratrol (200 g t^{-1}), RBC – erythrocytes, Ht – haematocrit, Hb – haemoglobin, WBC – white blood cells, HETERO – heterophils, LYMPH – lymphocytes, MONO – monocytes, EOSINO – eosinophils, BASO – basophils.

Table 3. Immunological indices in the blood of the turkeys receiving hesperidin, diosmin, quercetin and resveratrol

Item		Polyphenol (P)					Effect Age (A)	SEM	P-value		
		G-C (n=18)	G-H (n=18)	G-D (n=18)	G-Q (n=18)	G-R (n=18)			P	A	P×A
LYSOSYME mg l^{-1}	9	1.93	2.03	2.23	2.12	2.13	2.08	0.073			
	12	2.21	2.12	2.22	2.41	2.22	2.23	0.034	0.04	0.042	0.068
	15	2.38 b	2.79 a	2.44 b	2.68 ab	2.82 a	2.62	0.088			
Effect	(P)	2.17	2.31	2.29	2.40	2.39					
%PC	9	37.45	39.90	37.80	39.20	40.65	39.0	0.009			
	12	42.70	44.60	44.70	44.60	45.67	44.4	0.012	0.03	0.014	0.042
	15	45.63 b	48.56 b	47.63 b	53.14 ab	58.21 a	50.6	0.121			
Effect	(P)	41.90	44.35	43.30	45.64	48.17					
IgA, ug ml^{-1}	9	1.87 ab	1.88 ab	1.79 b	2.19 a	2.18 a	1.98	0.769			
	12	2.12 ab	2.22 ab	1.65 b	2.34 a	2.66 a	2.19	0.345	0.048	0.896	0.238
	15	2.09 b	1.93 b	1.99 b	2.23 a	2.25 a	2.09	0.379			
Effect	(P)	2.02	2.01	1.81	2.25	2.36					
IL-6, pg ml^{-1}	9	20.6 a	17.5 b	19.5 ab	18.3 b	17.8 b	18.7	0.041			
	12	26.3 a	23.4 ab	22.2 ab	18.1 c	18.2 c	21.6	0.014	0.003	0.068	0.05
	15	21.6 ab	22.3 a	20.6 b	19.8 b	19.6 b	20.7	0.022			
Effect	(P)	22.8	21.0	20.7	18.7	18.5					

a, b, c – values in rows with different superscript letters differ significantly at $P \leq 0.05$; 9th,12th and 15th days of age of the turkeys – blood was collected, G-C (control) received basal feed mixture with no experimental additives, G-H received basal feed mixture with hesperidin (200 g t^{-1}), G-D received basal feed mixture with diosmin (200 g t^{-1}), G-Q received basal feed mixture with quercetin (200 g t^{-1}), G-R received basal feed mixture with resveratrol (200 g t^{-1}), %PC – percentage of phagocytic cells, IgA – Immunoglobulin A, IL-6 – interleukin 6.

The experiment was conducted on 720 turkey hens assigned to 5 experimental groups of 120 individuals (6 repetitions with 20 birds each). Turkey hens from group G-C were the control, receiving the basal feed mixture with no experimental additives. The feed mixture for the turkey hens in groups G-H, G-D, G-Q and G-R contained a polyphenolic compound additive in the amount of 200 g t^{-1} of feed from the first to the 16th week of life. The turkey hens in group G-H received hesperidin (powdered 98% extract of the flavonoid from *Citrus aurantium L.*), group G-D received diosmin (powdered 98% extract of the flavonoid from *Citrus aurantium L.*), group G-Q received quercetin (powdered 98% extract of the flavonoid from *Citrus sinensis L.*), and group G-R received resveratrol (powdered 98% extract of the phytoalexin from *Polygonum cuspidatum*). The polyphenolic compounds were purchased from Chengdu Hawk Bio-Engineering Co, Ltd, China. The dosage for the additives tested was chosen on the basis of the most commonly recommended dosages of antioxidant supplements for livestock (particularly poultry) available on the market. The most frequently recommended amounts were found to range from 100 to 200 g t^{-1}. Testing of natural antioxidants in the diet of poultry was begun with a higher dosage because it was expected that a lower dosage might lead to less measurable results.

Laboratory analyses

Blood was collected into heparinized test tubes from the wing vein of 18 birds from each group (3 birds × 6 replications) at the age of 9, 12 and 15 weeks, following eight-hour fasting with unlimited access to drinking water. The following were determined in the blood samples: haematocrit (Ht) by the microhaematocrit method, haemoglobin (Hb) content by Drabkin's method, and erythrocytes (RBC) by the manual chamber technique, following dilution in Natt-Herrick solution. Haemoglobin concentration was determined spectrophotometrically following lysis of erythrocytes and release of haemoglobin (Feldman et al., 2000). Haematological tests included determination of white blood cells (WBC) and the percentage composition of white blood cells (leukogram) in stained blood smears, following Pappenheim's method (Bomski, 1989).

Immunological analyses included determination of the phagocytic activity of leukocytes against *Staphylococcus aureus* strain 209P, expressed as the percentage of phagocytic cells (PC) according to Park et al. (1968). The blood plasma level of lysozyme activity (currently classified as a protective barrier of the body) was determined by the turbidimetric method (Pinkiewicz, 1971; Siwicki et al., 1993). Test kits developed by Elabscience were used to determine the content of IgA and IL-6. Test kits by Cormay were used to determine the content of total protein (TP) and glucose (GLU).

Concentrations of phosphorus, calcium, magnesium, sodium and potassium in blood plasma were determined by the flame AAS technique with a UNICAM 939 spectrometer at the Central Apparatus Laboratory, University of Life Sciences, Lublin.

Statistical analysis

Statistical calculations were performed in SAS v. 9.4 (2013). For all analysed dependent variables in the blood, two-way (PA) analysis of variance (ANOVA) was performed with repetition of measurements of the dependent variable within the variable (A) according to the following model:

$$y_{ijk} = \mu + \alpha_i + \pi_{k(i)} + \beta_j + (\alpha\beta)_{ij} + (\beta\pi)_{jk(i)} + e_{ijk}$$

where:

μ – grand mean,

α_i – constant main effect of the i-th additive P, i =0,1.....4 (for control i = 0),

$\pi_{k(i)}$ – random characteristic reaction for the k-th turkey hen (for k = 1,2, …18) in the i-th group ($\pi_{k(i)}$ has a normal distribution $N(0,)$ for all levels of variable A),

β_j – constant main effect of the j-th age A, for j = 1, 2, 3,

$(\alpha\beta)_{ij}$ – constant effect of interaction of the i-th additive P and j-th age A,

$(\beta\pi)_{jk(i)}$ – random characteristic reaction for the k-th turkey hen for which variable A is at the i-th level, and the dependent variable is measured for the j-th time, has normal distribution $N(0,\sigma^2)$,

e_{ijk} – experimental error has normal distribution $N(0, \sigma^2)$.

Results

Administration of hesperidin, diosmin, quercetin or resveratrol was not found to affect the growth performance of the turkey hens. In the experimental groups the survival rate was 100%. The feed conversion ratio during days 7–12 of age was also very similar in all experimental groups, averaging 2.480 kg/kg. In the period from days 7 to 112 the turkey hens receiving the hesperidin supplement gained 9.548 kg/bird, the birds receiving diosmin gained 9.406 kg/bird, those receiving quercetin gained 9.269 kg/bird, and those receiving resveratrol gained 9.186 kg/bird (Ognik, 2013).

Data pertaining to haematological indices in the turkey hens are presented in Table 2. The addition of quercetin (G-Q) or resveratrol (G-R) to the feed led to an increase in haemoglobin content in the blood of the turkey hens. The haemoglobin content in the blood of turkey hens was also found to increase with age. The leukogram showed the greatest increase in the percentage of heterophils and the greatest decrease in the percentage of lymphocytes and monocytes in group G-R. In the 12th week of life a significant decrease in the percentage of monocytes was noted in the blood of the turkeys receiving hesperidin, quercetin and resveratrol in comparison with the control. The content of heterophils was found to decrease with age. The immunological indices of the blood are presented in Table 3. The blood of turkey hens in groups G-Q and G-R had the significantly highest lysozyme activity, the significantly highest percentage of phagocytic cells and IgA content, and the significantly lowest content of IL-6. Lysozyme activity and the percentage of phagocytic cells were found to increase with the age of the turkey hens.

Table 4. Biochemical indices in the blood plasma of the turkeys receiving hesperidin, diosmin, quercetin and resveratrol

Item		Polyphenol (P)					Effect Age (A)	SEM	P-value		
		G-C (n=18)	G-H (n=18)	G-D (n=18)	G-Q (n=18)	G-R (n=18)			P	A	P×A
1		2	3	4	5	6	7	8	9	10	11
GLU mmol l^{-1}	9	13.21	14.79	15.75	14.14	13.19	14.2	0.226			
	12	13.93	14.46	14.10	12.76	11.54	13.3	0.302	0.124	0.652	0.955
	15	13.14	13.28	14.95	14.91	13.89	13.8	0.196			
Effect	(P)	13.42	14.17	14.9	13.9	12.8					
TP g l^{-1}	9	33.88	31.88	30.26	34.70	31.21	32.3	0.455			
	12	33.00	30.69	33.93	32.73	32.56	32.6	0.403	0.066	0.709	0.841
	15	32.69	30.69	30.18	32.41	30.32	31.2	0.343			
Effect	(P)	33.19	31.08	31.45	33.28	31.36					
P mmol l^{-1}	9	2.06 b	2.19 b	2.18 b	2.56 a	2.46 a	2.29	0.042			
	12	1.67 b	1.77 b	2.04 ab	2.32 a	2.28 a	2.01	0.075	0.048	0.063	0.264
	15	1.68 b	1.93 b	2.12 ab	2.21 a	2.22 a	2.03	0.027			
Effect	(P)	1.80	1.96	2.11	2.36	2.32					

Table 4 – contd.

1	2	3	4	5	6	7	8	9	10	11	12
Ca mmol l^{-1}	9	2.98	2.90	2.95	2.96	2.79	2.91	0.041			
	12	2.81	2.90	3.03	2.89	2.72	2.87	0.041	0.081	0.882	0.601
	15	2.61	2.55	2.47	2.51	2.28	2.48	0.034			
Effect	(P)	2.80	2.78	2.81	2.78	2.59					
Mg mmol l^{-1}	9	0.88	0.72	0.79	0.87	0.75	0.80	0.016			
	12	0.87	1.07	1.05	0.96	1.06	1.00	0.024	0.263	0.806	0.155
	15	1.01	0.95	0.93	0.98	1.02	0.97	0.016			
Effect	(P)	0.92	0.91	0.92	0.93	0.94					
Na mmol l^{-1}	9	141.2	143.6	142.7	142.0	142.8	142.4	0.447			
	12	141.2	142.3	141.1	142.5	143.3	142.1	0.418	0.132	0.264	0.945
	15	141.6	142.3	142.4	142.0	140.6	141.7	0.432			
Effect	(P)	141.3	142.7	142.0	142.1	142.2					
K mmol l^{-1}	9	3.99	4.04	4.07	4.13	4.18	4.08	0.043			
	12	4.07	4.09	4.22	4.23	4.13	4.15	0.047	0.632	0.087	0.091
	15	4.48	4.15	4.29	4.14	4.38	4.28	0.042			
Effect	(P)	4.18	4.09	4.19	4.16	4.23					

a, b, c – values in rows with different denoted letters differ significantly at $P \leq 0.05$; 9th,12th and15th days of age of the turkeys – blood was collected, G-C (control) received basal feed mixture with no experimental additives, G-H received basal feed mixture with hesperidin (200 g t^{-1}), G-D received basal feed mixture with diosmin (200 g t^{-1}), G-Q received basal feed mixture with quercetin (200 g t^{-1}), G-R received basal feed mixture with resveratrol (200 g t^{-1}), GLU – glucose, TP – total protein, P – phosphorus, Ca – calcium, Mg – magnesium, Na – sodium, K – potassium.

Analysis of the biochemical blood indicators (Table 4) showed that the supplementation of quercetin or resveratrol to the basal feed significantly increased the content of phosphorus in the blood of the turkey hens. The addition of polyphenol extracts to the turkey hen diets was not found to influence the content of glucose, protein, calcium, magnesium, sodium or potassium in the blood.

Discussion

According to the latest research published by Tako et al. (2014), polyphenolic compounds, despite their documented antioxidant properties, can create the risk of lowering haemoglobin levels by inhibiting absorption of iron from food. Iron is a component of haemoglobin that enables oxygen transport. Polyphenols bind with iron ions to form complexes which are unable to enter the blood from the digestive system. Our study on turkey hens did not confirm a negative effect of polyphenolic compounds on haemoglobin content in the blood of these birds. In fact, the addition of quercetin or resveratrol to the feed caused an increase in the haemoglobin level in the blood of turkey hens. Christev et al. (2011), administering a dry extract of *Tribulus terrestris* to helmeted guinea fowl in their feed for 12 weeks in the amount

of 10 mg/kg b.w./day, noted a significant increase in haemoglobin in comparison with the control. The authors emphasize that this increase may have been caused by the main polyphenolic compound present in *Tribulus terrestris*, i.e. protodioscin. Unigwe (2011) administered roselle (*Hibiscus sabdariffa*) to chickens in their drinking water for 56 days in the amount of 1.2 g/l or 3 g/l, and found no significant differences in haemoglobin content between the control and the groups receiving the extract. However, the author of the study emphasized that the haemoglobin content in the blood of the chickens increased with the dosage of roselle (which contains the polyphenols gossypetin, hibiscetin and anthocyanins). Abdulkarimi and Daneshyar (2012) administered an alcohol extract of thyme to broiler chickens in their drinking water from days 1 to 42 of life in the amount of 0.2%, 0.4% or 0.6%, but noted no effect of this additive on haemoglobin content in the blood of chickens. Kehinde et al. (2011) supplemented ground ginger to broiler chickens for 5 weeks in the amount of 1.5, 3.0, or 4.5% of their basal feed, and also found no significant effect of this additive on haemoglobin content in the blood of the chickens.

Our study also showed that the haemoglobin content in the blood of turkey hens increased with the age of the birds. Similar observations, i.e. an increase in haemoglobin with the age of birds, were made by Addass et al. (2012), who measured this indicator in the blood of chickens during a 150-day rearing period.

Our study showed that the addition of resveratrol to the feed caused an increase in the percentage of heterophils and a decrease in the percentage of lymphocytes and monocytes. Administration of the aloe preparation (containing resveratrol) to the turkey hens also resulted in a considerable increase in the percentage of heterophils, which confirms the immunostimulatory properties of the components of resveratrol. Heterophils play a key role in phagocytic reactions (Ognik et al., 2015 b). Dougnon et al. (2014), during a 56-day experiment on chickens receiving 0.5 or 1% cayenne pepper in their basal feed for one or two months, noted a significant decrease in the percentage of lymphocytes as compared with the control. Literature data indicate that cayenne pepper, apart from capsaicin, also contains quercetin and luteolin, which are polyphenolic compounds (Zimmer et al., 2012). According to Koncicki and Krasnodębska-Depta (2005), either an increase or a decrease in heterophils or monocytes may be observed during various pathological conditions in poultry, and the direction of these changes depends on whether the course of the disease is chronic or acute. In general, in acute infections (coccidiosis in chickens, acute mycoplasmosis, colibacteriosis, staphylococcosis, histomoniasis, or infection with *Campylobacter jejuni* or *Clostridium perfringens*), as well as during severe stress, an increase in the number of heterophils and a decrease in the number of lymphocytes is observed, usually accompanied by leukocytosis (elevated leukocyte count) and monocytopenia (decreased monocyte count) (Lloyd and Gibson, 2006; Gheith et al., 2011; Adamu et al., 2013). Although the percentage of heterophils and lymphocytes in the leukogram of the turkey hens receiving resveratrol in their feed suggests acute infection in these birds, this condition cannot be definitively confirmed due to the low leukocyte level (the WBC count did not differ between groups). Hence we may suppose that the increase in the content of heterophils was the result of stimulation of the immune system by the use of resveratrol. The birds receiving resveratrol dis-

played no pathological symptoms and were in good health, which is also indicated by the low level of interleukin IL-6. Interleukin IL-6 is an early, sensitive indicator of inflammatory reactions in the organism, and its content during inflammatory states can increase even 100-fold (Kasapis and Thompson, 2005). An increase in heterophils, decrease in lymphocytes and lack of change in leukocyte count, as well as an increase in indicators of non-specific immunity, were noted in the blood of turkey hens receiving linseed oil with a natural form of vitamin E (RRR-d-alpha tocopherol) in their feed (Ognik and Czech, 2014). Another study found an increase in leukocyte count with no changes in the leukogram in chickens receiving feed with liquorice extract in the amount of 0.5 or 1 g/kg for 49 days (Sedghi et al., 2010). The authors of the study explained the leukocytosis as the effect of stimulation of the immune system. Liquorice extract contains many polyphenolic compounds with confirmed antioxidant and immunostimulatory properties, such as liquiritin, isoliquiritin, isoflavones, glabridin, hispaglabridins (Omar et al., 2012) and licochalcone A (Song et al., 2015). In our study, the content of heterophils decreased with the age of the birds. A similar association was noted by Talebi et al. (2005).

Our study showed that the use of quercetin or resveratrol as a feed additive can stimulate aspects of specific and non-specific immunity in turkey hens. The immunostimulatory effect of quercetin and resveratrol is indicated by the high activity of lysozyme, the significantly high percentage of phagocytic cells and the significantly high content of IgA in the blood of these birds. Ognik and Sembratowicz (2012) periodically administered aloe extract with trans-resveratrol to turkey hens in their drinking water in the amount of 70 ml/kg b.w./day, and observed stimulation of non-specific immunity (increased percentage of phagocytic cells, phagocytic index and lysozyme activity). Rusinek-Prystupa and Tatara (2014) administered grapefruit extract to turkey hens for slaughter in their drinking water from their 6th to 9th week of life in the amount of 0.021 ml/kg b.w. and noted a significant increase in lysozyme activity and the percentage of phagocytic cells in the blood of the birds. Pourhossein et al. (2015) administering extract of sweet orange peel to broiler chickens for 42 days in their drinking water in the amount of 1,250 ppm, noted an increase in the blood content of IgG and IgM. Rasouli and Jahanian (2015) supplemented genistein to broiler chicken diets for 42 days in the amount of 10, 20, 40, 80, 160 and 180 mg/kg of basal feed, and found that the addition of just 10 mg/kg caused an increase in the weight of lymphatic organs such as the thymus and the bursa of Fabricius. The authors explain the increased weight of the lymphatic organs as the effect of stimulation of the immune system. Alagawany and Abd El-Hack (2015) administered powdered rosemary to 36-week-old laying hens up to their 52nd week of life in the amount of 3, 6 and 9 g/kg of feed, but found no effect of this additive on IgA content in the blood of the birds. It should be emphasized that rosemary contains numerous polyphenolic compounds, such as carnosol, carnosic acid, ursolic acid, rosmarinic acid and rosmanol, which may stimulate the immune system (Petivala et al., 2013). Hager-Theodorides et al. (2014) also found an increase in the production of IgY and IgA in response to SRBC challenge in broilers fed with quercetin. The results of a study by Kongkathip et al. (2010) showed that the addition of 0.05% turmeric extract to the diet of broilers reduces the stress level in the birds and modulates the im-

mune response to a vaccine against Newcastle disease. The anti-inflammatory effect of quercetin and resveratrol is evidenced by the decrease in the content of IL-6 noted in the present study. The anti-inflammatory properties of quercetin and resveratrol have been well documented in the literature (Kang et al., 2009; Wung et al., 2005; Min et al., 2007). Although the immunomodulatory properties of hesperidin and diosmin have been well documented in the literature (Sezer et al., 2011), our study did not confirm this effect of the use of these polyphenols for turkeys.

Our study found that adding quercetin or resveratrol to feed for turkey hens increased the phosphorus content in the blood. Rafiee et al. (2013), after administering ginger (containing quercetin, rutin, catechin, epicatechin and naringenin) to chickens in their feed, also noted an increase in phosphorus in the blood. Kwiecień et al. (2014) administered a herb mixture of common nettle and pansy in the amount of 1% to chickens diets and observed an increase in phosphorus content in the bones. Phosphate ions generated as a result of hydrolysis of phytates can react with transitional metals such as iron or copper to form insoluble salts. Polyphenolic compounds present in feed may prevent this process by forming complex compounds with these metals, resulting in better bioavailability of phosphorus for the organism. It is postulated that iron or copper complexes with polyphenols, e.g. in feed are absorbed into the bloodstream from the digestive tract. Metallothioneins present in the blood, having very high affinity for divalent heavy metals, may pick up the iron or copper from these complexes, which causes the iron to become available for haemoglobin synthesis, as indicated by the results of the study.

To sum up, quercetin or resveratrol in the amount of 200 g/tonne of feed has a beneficial effect on haemoglobin synthesis and availability of phosphorus, and also modulates immunity in turkey hens. Further research is needed to determine possibilities for using quercetin or resveratrol as a factor enhancing immunoprophylaxis, e.g. during vaccinations of poultry against coccidiosis, Newcastle disease, and other diseases.

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