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Original article

Biochemical and immunological responses of young turkeys to vaccination against *Ornithobacterium rhinotraheale* and different levels of dietary methionine

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Abstract

The objective of this study was to verify the hypothesis that increasing levels of dietary methionine can stimulate the mechanisms of cell-mediated and humoral immunity in young turkeys. The blood and organs involved in cell-mediated and humoral immune responses were analyzed in 8-week-old turkeys that had been vaccinated against *Ornithobacterium rhinotraheale* (ORT) infection (on days 17 and 48). The birds were fed diets with a low (LM), medium (MM) and high (HM) methionine content (0.45 and 0.40%, 0.60 and 0.51%, 0.71 and 0.57% in weeks 1 – 4 and 5 – 8, respectively).

Dietary methionine supplementation led to a significant increase in body weights of turkeys at 56 days of age, from 3532 g in group LM to 3720 g in group MM and 3760 g in group HM (p=0.001). A significant increase in vaccine-induced antibody titers against ORT was noted in group HM relative to group LM (p=0.006). Increasing levels of methionine had no significant effect on total serum IgG nor IgM levels and most serum biochemical parameters, TP, ALB, GLOB, GLU, AST, ALP, P and Ca. In comparison with group LM, group HM turkeys were characterized by a lower percentage of IgM^+ B cell subpopulation in the blood and bursa of Fabricius.

The percentages of $CD4^+$ and $CD8^+$ T cell subpopulations in the bursa of Fabricius in group HM were significantly different from those found in groups LM and MM.

The highest percentages of $CD4^+$ T cells and $CD8^+$ T cells in the spleen were observed in groups LM (p<0.001) and HM (p=0.04), respectively. The differences were statistically significant relative to the remaining groups. Turkeys of group LM were characterized by a lower $CD4^+$ T cell percentage in the thymus (p<0.001) and a lower $CD8^+$ T cell percentage in the cecal tonsils (CTs) (p<0.01). Vaccination against ORT resulted in a significant increase in the percentage of $CD4^+/CD8^+$ T cell subpopulation and a decrease in the percentage of $CD8^+$ T cell subset in the spleen.

Key words: methionine, turkeys, cell-mediated immunity, humoral response

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Introduction

Genetic progress and selection for growth rate, observed in poultry over recent decades, has resulted in reduced resistance to disease (Li et al. 1999b, Huff et al. 2005). Adequate nutrition, i.e. the supply of sufficient quantity and quality of nutrients, is required for optimum immune system function. In poultry, the intake of selected nutrients required for optimal immune responses may be higher than that recommended to meet the growth needs of birds, in particular as regards the dietary inclusion levels of amino acids (Świątkiewicz and Koreleski 2007).

Sulfur-containing amino acids, in particular methionine, are important for immune function. Methionine is the first limiting amino acid in poultry diets. Therefore, the methionine requirements of young and fast-growing turkeys should be precisely defined, as both methionine deficiency and excess can compromise the performance and immune system of birds (Tsiagbe et al. 1987, Swain and Johri 2000, Deng et al. 2007). According to Klasing et al. (1988) and Hosseini et al. (2012), dietary methionine levels required for immune system competence are higher than those needed to maximize the bird's potential for growth and productivity. The relationship between dietary methionine intake and immune system function has been investigated in numerous studies involving chickens (Tsiagbe et al. 1987, Zhang and Guo 2008, Bunchasak 2009) and in our previous experiment performed on 4-week-old turkeys (Kubińska et al. 2014).

The aim of this study was to evaluate the effect of different inclusion levels of dietary methionine fed to turkeys from 29 to 56 days of age on the growth rate, and selected biochemical and immunological parameters of birds.

Materials and Methods

Birds, management and diets

The experiment was carried out on 357 day-old female Hybrid Converter turkeys purchased at the „Grelavi" Hatchery (Ketrzyn, Poland) and raised at the Research Laboratory of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn (Poland) to 56 days of age. The birds were randomly divided into three experimental groups: LM – low methionine, MM – medium methionine, HM – high methionine. Each groups consisted of seven subgroups, with 17 birds per replicate. The animal protocol used in this study was approved by the Local Animal Commision for Animal Experiments (Olsztyn, Poland), and the study was carried out in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes (OJEU, 2010). The birds were kept in pens on litter in a building with a controlled environment. The temperature and lighting program were consistent with the recommendations of Hybrid Turkeys (2012). The birds had free access to feed and water throughout the growth period.

The basal diet fed to turkeys from 1 to 28 days of age was composed of soybean meal, wheat and maize, and it contained 27% crude protein, 1.74% lysine, 0.41% methionine (0.86% Met+Cys), 1.25% Ca, 0.65% available P and 11.5 MJ metabolizable energy (calculated values). The results obtained over that period have been described by Kubińska et al. (2014). The basal diet fed to turkeys from 29 to 56 days of age was composed of soybean meal, wheat and maize, and it contained 24.5% crude protein, 1.53% lysine, 0.36% methionine (0.78% Met+Cys), 1.09% Ca, 0.45% available P and 12.26 MJ metabolizable energy (calculated values). Group LM turkeys received only the basal diet, and in groups MM and HM the basal diet was supplemented with methionine at 0.14% and 0.28%, respectively.

Vaccination

At 17 and 48 days of age, 147 turkeys (seven birds per replicate, marked individually) were vaccinated against *Ornithobacterium rhinotraheale* (ORT) infection by injecting 0.5 ml of the inactivated vaccine Ornitin (ABIC, Poland) subcutaneously.

Growth trial and sample collection

At 56 days of age, all turkeys were weighed, and blood was sampled from 20 vaccinated and 20 unvaccinated birds selected randomly from each group (two or three birds per replicate). Blood samples were collected from the wing vein into sterile test tubes with the EDTA K anticoagulant for flow cytometry analysis or without the anticoagulant for biochemical and serological analysis. Selected turkeys (seven vaccinated and seven unvaccinated birds) were euthanized, and the spleen, thymus, bursa of Fabricius and cecal tonsils (CTs) were collected in order to determine the percentages of $CD4^+$, $CD8^+$, $CD4^+CD8^+$ T cell subpopulations and IgM^+ B cell subpopulation by flow cytometry.

Table 1. Methionine content of diets of the turkeys (%).

	Group					
	LM		MM		HM	
	1-28 d.	29-56 d.	1-28 d.	29-56 d.	1-28 d.	29-56 d.
Calculated	0.41	0.36	0.57	0.50	0.73	0.64
Analytical	0.45	0.40	0.60	0.51	0.71	0.57

LM – low methionine content, MM – medium methionine content, HM – high methionine content.

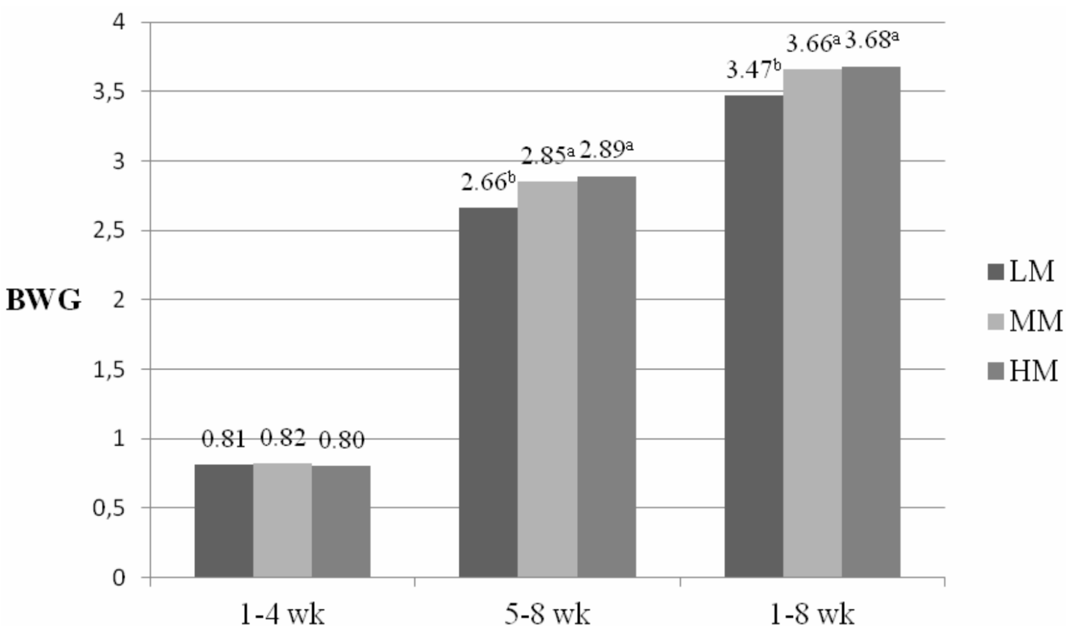

Fig. 1. Body weight gain in the turkeys fed diets with a different methionine content.

Biochemical analysis

Serum samples, obtained from blood collected in test tubes without the anticoagulant, were assayed for the concentrations of phosphorus (P), calcium (Ca), total protein (TP), albumin (ALB), globulin (GLOB) and glucose (GLU), and for the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The tests were performed with the use of the Idexx VetTest Chemistry Analyzer, according to the manufacturer's instructions.

Serological analysis

Total serum IgG (IgY) and IgM levels were determined using commercial IgG Turkey ELISA kit and the IgM Turkey ELISA kit (KA2515, Abnova, Taiwan). Vaccine-induced antibody titers against ORT were determined with the use of the commercial Idexx ORT Ab ELISA test (INDEXX, USA). Particular stages of the ELISA assays were performed with the use of Eppendorf epMotion 5075 LH automated pipetting system, the BioTek ELx405 washer and the BioTek ELx800 microplate reader.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated according to a previously described procedure (Koncicki et al. 2012, Tykałowski et al. 2014). The isolation of mononuclear cells from the spleen, thymus, bursa of Fabricius and CTs, and the determination of the percentages of $CD4^+$,$CD4^+/CD8^+$, $CD8^+$ T cell and IgM+ B cell subpopulations in PBMCs and selected organs of the immune system were carried out as described by Kubińska et al. (2014).

Statistical analysis

One-way and/or two-way ANOVA was performed with the use of STATISTICA 10.0 software. The significance of differences between means in the groups was determined by Duncan's test.

Results

The calculated and analytical methionine content of experimental diets is presented in Table 1. In all the groups, dietary methionine content was close to the assumed levels.

Different dietary inclusion levels of methionine had no influence on the body weight gains (BWG) of turkeys in the first month of the experiment, whereas a significant effect of the experimental factor was noted in the second month and over the entire experimental period (Fig. 1). A decrease in BWG was observed in group LM relative to groups MM and HM ($P = 0.012$). As a result, at 56 days of age, turkeys from groups MM and HM were significantly heavier than group LM birds (3720 and 3742 vs. 3532 g, $P = 0.001$).

Biochemical parameters

The results of biochemical analysis are summerized in Table 2. Graded levels of supplemented methionine had no significant effect on the majority of the analyzed parameters. Statistically significant differences were noted only in group HM which was characterized by the lowest ALT activity ($p=0.02$) in comparison to LM and MM. Vaccination against ORT had no significant influence on the analyzed biochemical parameters.

Immunological parameters

The levels of serum IgM and IgG (IgY) as well as vaccine-induced antibody titers against ORT are presented in Table 3. No statistically significant differences in total serum IgM and IgG (IgY) concentrations were found between the groups. A significant ($p=0.006$) increase in vaccine-induced antibody titers against ORT, accompanied by a decrease in % CV (coefficients of variation) were observed in turkeys from groups MM and HM relative to group LM birds.

The percentages of $CD4^+$, $CD8^+$, $CD4^+CD8^+$ T cell subpopulations and IgM^+ B cell subpopulations witin PBMCs and mononuclear cells isolated from the spleen, thymus, bursa of Fabricius and CTs are summarized in Tables 4-8.

As shown in Table 4, diets with a different methionine content had no significant effect on the percentages of T lymphocyte subsets in PBMCs. Only the percentage of IgM^+ B cell subpopulation was significantly lower in groups MM and HM in comparison with the control group (LM). Vaccination against ORT had no influence on the percentages of the analyzed cells.

Significant changes in the percentages of the analyzed lymphocytes subpopulations were observed in the spleen (Table 5). Graded dietary methionine levels contributed to a significant decrease in the percentage of $CD4^+$ T cell subset and an increase in the percentage of $CD8^+$ T cell subset. Vaccinated turkeys, as compared with unvaccinated birds, were characterized by a significant ($p=0.04$) decrease in the percentage of $CD8^+$ T cell subpopulation and a significant ($p=0.01$) increase in the percentage of $CD4^+/CD8^+$ T cell subpopulation.

Significant differences were also found between groups with regard to the percentage of $CD4^+$ T cell subset in the thymus (Table 6), and the percentages of $CD4^+$ and $CD4^+/CD8^+$ T cell subsets and IgM^+ B cell subpopulation in the bursa of Fabricius (Table 7). Increased methionine levels in experimental diets led to a significant increase in the percentage of $CD4^+$ T cell subpopulation in the above organs, and to a decrease in the percentage of IgM^+ B cell subpopulation in the bursa of Fabricius in group HM relative to group LM.

Table 8 data indicate that an increase in dietary methionine levels contributed to a significant increase in the percentage of $CD8^+$ T cell subset in CTs.

Discussion

In the first month of the study, no differences in body weight gains were found between turkeys fed methionine-deficient diet LM and diet HM with methionine content above the NRC recommendations (1994). Similar results were reported in an experiment with broiler chickens (Swain and Johri 2000) where dietary methionine levels varied over a wider range of 0.37% to 0.87%. In other studies (Deng et al. 2007, Rubin et al. 2007), reduced body weight gains were noted in chickens fed diets with a twice greater methionine deficiency, relative to the NRC recommendations (1994), than in our experiment with turkeys.

Lemme et al. (2005) analyzed six graded dietary methionine+cysteine levels and found that increased methionine concentrations contributed to body weight gains in turkey toms between 5 and 9 weeks of the rearing period. We evaluated three graded dietary methionine levels: a methionine-deficient diet, relative to the NRC recommendations (1994), decreased

Table 2. Serum biochemical parameters in the turkeys.

	Group	P (mg/dL)	Ca (mg/dL)	TP (g/dL)	ALB (g/dL)	GLOB (g/dL)	GLU (mg/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)
Unv	LM	7.42	10.51	3.47	0.96	2.51	272.86	28.29	250.86	554.29
	MM	7.94	10.44	3.61	1.06	2.57	318.43	24.00	244.57	529.43
	HM	7.40	10.46	3.73	1.10	2.63	235.00	18.29	233.86	543.57
Vac	LM	7.87	10.47	3.59	1.00	2.59	264.71	24.29	243.57	552.71
	MM	8.01	10.54	3.73	1.09	2.70	272.00	27.28	264.57	561.57
	HM	7.31	10.46	3.83	1.07	2.74	271.29	21.00	264.85	575.29
	SEM	0.11	0.05	0.04	0.02	0.03	10.47	1.08	4.28	16.99
Dosage	LM	7.65	10.49	3.53	0.98	2.55	268.79	26.29[a]	247.21	553.50
	MM	7.97	10.49	3.67	1.07	2.63	295.21	25.64[a]	254.57	545.50
	HM	7.35	10.46	3.78	1.09	2.69	253.14	19.64[b]	249.36	559.43
Vaccination	Vac	7.73	10.49	3.71	1.05	2.68	269.33	24.19	257.67	563.19
	Unv	7.59	10.47	3.60	1.03	2.57	275.43	23.52	243.09	542.42
P-value	D	0.06	0.95	0.08	0.06	0.21	0.26	0.02	0.76	0.95
	V	0.49	0.86	0.22	0.71	0.09	0.77	0.74	0.09	0.57
	DxV	0.57	0.85	0.99	0.73	0.92	0.28	0.27	0.17	0.91

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group: LM – low methionine content, MM – medium methionine content, HM – high methionine content; D – Dosage, V – Vaccination, P – phosphorus, Ca – calcium, TP – total protein, ALB – albumin, GLOB – globulin, GLU – glucose, ALT – alanine aminotransferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase; [a,b] – means with different superscripts in the same column differ significantly ($p<0.05$).

Table 3. Serum immunological parameters in the turkeys.

	Group	Total IgM (ng/ml)	Total IgG (ng/ml)	ORT Titer	ORT %CV
Unv	LM	47.37	10.91	324.30	60.3
	MM	39.93	11.21	233.20	79.4
	HM	40.12	8.52	260.90	81.6
	SEM	4.48	1.11	37.47	–
	P-value	0.76	0.58	0.61	–
Vac	LM	45.18	13.63	25367.90[b]	19.3
	MM	39.28	17.78	28772.00[a]	13.1
	HM	38.20	17.31	29283.60[a]	9.5
	SEM	2.53	1.12	557.32	–
	P-value	0.50	0.27	0.006	–

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group; LM – low methionine content, MM – medium methionine content, HM – high methionine content; CV – coefficient of variation; [a,b] – means with different superscripts in the same column differ significantly ($p<0.05$).

Table 4. Percentages of T and B cell subpopulations in peripheral blood mononuclear cells of the turkeys.

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	16.14	1.60	0.24	10.81
	MM	13.73	1.63	0.24	6.60
	HM	19.27	2.48	0.41	7.47
Vac	LM	15.77	1.51	0.34	11.83
	MM	12.88	1.77	0.31	8.91
	HM	15.79	2.08	0.34	8.09
	SEM	1.06	0.16	0.03	0.64
Dosage	LM	15.96	1.56	0.29	11.32^a
	MM	13.31	1.71	0.27	7.76^b
	HM	17.53	2.29	0.38	7.78^b
Vaccination	Vac	16.38	1.91	0.30	8.29
	Unv	14.82	1.79	0.33	9.61
P-value	D	0.31	0.18	0.30	0.03
	V	0.49	0.72	0.54	0.27
	DxV	0.83	0.80	0.45	0.82

Data represent mean values for 7 unvaccinated (Unv) turkeys and 7 vaccinated (Vac) turkeys per group; LM – low methionine content, MM – medium methionine content, HM – high methionine content; D – Dosage, V – Vaccination; ^{a,b} – means with different superscripts in the same column differ significantly ($p<0.05$)

Table 5. Percentages of T and B cell subpopulations in the spleen of the turkeys.

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	31.91	37.10	2.88	10.63
	MM	27.90	40.71	3.07	12.21
	HM	25.52	40.21	3.14	13.05
Vac	LM	33.97	32.15	3.46	10.15
	MM	24.05	36.64	3.82	15.92
	HM	26.66	38.73	3.53	12.69
	SEM	0.99	0.91	0.11	0.70
Dosage	LM	32.94^a	34.62^b	3.17	10.39
	MM	25.98^b	38.68^{ab}	2.45	14.07
	HM	26.09^b	39.47^a	4.33	12.87
Vaccination	Vac	28.45	39.34^a	3.02^b	11.97
	Unv	28.23	35.84^b	3.60^a	12.92
P-value	D	<0.001	0.04	0.53	0.09
	V	0.89	0.04	0.01	0.47
	DxV	0.25	0.65	0.75	0.35

Explanations: see Table 4

Table 6. Percentages of T and B cell subpopulations in the thymus of the turkeys.

	Group	CD4⁺	CD8⁺	CD4⁺/CD8⁺	IgM⁺
Unv	LM	2.55	6.60	76.17	1.19
	MM	4.00	8.58	73.01	0.70
	HM	3.71	7.46	76.79	0.99
Vac	LM	2.56	8.40	74.26	1.02
	MM	2.88	7.45	73.29	1.01
	HM	3.49	9.49	73.36	0.99
	SEM	0.15	0.49	1.08	0.06
Dosage	LM	2.56[b]	7.50	75.22	1.10
	MM	3.44[a]	8.01	73.15	0.85
	HM	3.61[a]	8.48	75.08	0.99
Vaccination	Vac	3.42[a]	7.55	75.33	0.96
	Unv	2.98[b]	8.45	73.64	1.00
P-value	D	<0.001	0.73	0.73	0.24
	V	0.05	0.39	0.48	0.70
	DxV	0.09	0.38	0.81	0.25

Explanations: see Table 4

Table 7. Percentages of T and B cell subpopulations in the bursa of Fabricius of the turkeys.

	Group	CD4⁺	CD8⁺	CD4⁺/CD8⁺	IgM⁺
Unv	LM	0.16[b]	0.46	0.16[b]	89.15
	MM	0.20[b]	0.51	0.19[b]	86.32
	HM	0.41[a]	0.76	0.38[a]	82.39
Vac	LM	0.22[b]	0.55	0.27[b]	87.12
	MM	0.28[b]	0.56	0.23[b]	84.78
	HM	0.23[b]	0.52	0.22[b]	84.40
	SEM	0.03	0.03	0.02	0.66
Dosage	LM	0.19[b]	0.50	0.21[b]	88.14[a]
	MM	0.23[b]	0.53	0.21[b]	85.55
	HM	0.32[a]	0.64	0.30[a]	83.39[b]
Vaccination	Vac	0.26	0.57	0.24	85.96
	Unv	0.24	0.54	0.24	85.43
P-value	D	0.17	0.01	0.03	0.01
	V	0.79	0.38	0.91	0.64
	DxV	0.13	>0.001	>0.001	0.29

Explanations: see Table 4

Table 8. Percentages of T and B cell subpopulations in the cecal tonsils of the turkeys.

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	50.28^b	7.31^b	2.54	21.10
	MM	50.99^b	12.19^a	2.69	15.86
	HM	47.03	10.22	3.59	15.73
Vac	LM	51.17^b	9.76	2.06	20.08
	MM	41.45^a	10.05	3.36	22.15
	HM	50.14^b	12.36^a	3.26	18.67
	SEM	1.05	0.48	0.16	0.99
Dosage	LM	50.72	8.54^b	2.30	20.59
	MM	46.22	11.12^a	3.03	19.00
	HM	48.59	11.29^a	3.43	17.20
Vaccination	Vac	49.43	9.91	2.94	17.56
	Unv	47.59	10.72	2.89	20.30
P-value	D	0.13	0.01	0.01	0.38
	V	0.30	0.29	0.85	0.17
	DxV	0.02	0.04	0.15	0.33

Explanations: see Table 4

the body weight gains of female turkeys, whereas diets with methionine concentrations excessing of the recommended intake had no influence on the growth rate of birds. Also in experiments with chickens, diets with an increased methionine content, relative to the NRC guidelines (1994), did not stimulate the growth of birds (Deng et al. 2007, Rubin et al. 2007, Bouyek 2012).

In the first month of our study, when total body weight gains were low (0.8 kg/month), turkeys did nor respond to graded dietary methionine levels. In the second month, when total body weight gains were 3.5-fold higher, a methionine-deficient diet decreased the body weight gains of birds, and dietary methionine content 10% higher than the recommended intake (NRC 1994) had no effect on their growth rate.

Biochemical parameters

The values of all biochemical parameters analyzed in our study did not excess the standard values of these parameters as described Krasnodębska-Depta and Koncicki 2011. Our findings show that supplemental methionine added to experimental diets had no significant effect on the analyzed metabolic pathways in turkeys. Only ALT activity was significantly lower in group HM than in groups LM and MM, but it remained within the normal range. Due to the absence of published research investigating the effect of graded dietary methionine levels on the biochemical parameters in 8-week-old turkeys, we had to compare our findings with the results of experiments involving 15-week-old turkeys and broiler chickens. The serum concentrations of total protein and the activity of selected liver enzymes, determined in our study, are consistent with those reported by Øzsoy and Yalçin (2011) who observed no significant differences in the values of the above parameters in 15-week-old female turkeys. The serum levels of total protein, albumins and globulins, determined in our study, correspond with those reported by Al-Mayah (2006) who observed no significant differences in the values of the above parameters between 28-day-old broiler chickens that received different amounts of DL-methionine powder with feed or drinking water. Additionally, Zhang and Guo (2008) demonstrated that liquid DL-2-hydroxy-4-methylthio butanoic acid (LMA), used as a dietary methionine source, had no influence on the serum levels of total protein and albumins in chickens at 21 days of age.

Immunological parameters

Neither graded inclusion levels of methionine nor vaccination against ORT significantly affected total serum IgG levels. A considerable, but not statistically significant increase was observed in the total serum IgG levels of turkeys from groups MM and HM, vaccinated against ORT, compared with vaccinated turkeys birds from group LM. Our findings are partially consistent with those reported by Hosseini et al. (2012) who found no significant differences in serum IgM levels and antibody titers against Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV) in broiler breeder hens fed diets supplemented with methionine at 0.25 – 0.45%. The cited authors noted significant differences in the serum levels of IgG and antibody titers against sheep red blood cells (SRBC) and demonstrated that methionine requirements needed for the optimum immune responses were higher than those needed for good performance and productivity.

Increased dietary methionine concentrations led to a significant ($p=0.006$) increase in vaccine-induced antibody titers against ORT in groups MM and HM relative to group LM. Percental CV tended to decrease in those groups, which indicates that vaccine-induced antibody titers were more uniform across turkeys within groups MM and HM, compared with group LM. In a study by Zhang and Guo (2008), diets supplemented with methionine (LMA) had no effect on serum antibody titers against NDV in broiler chickens.

In our study, the percentages of the subpopulations of T and B lymphocytes isolated from the blood, and central and peripheral immune organs of turkeys were determined by flow cytometry. The monoclonal (anti CD4 and CD8a) and polyclonal (anti IgM) antibodies used in the present experiment were originally developed to study the immune system of chickens, but they had been tested for cross-reactivity with turkeys, and the results of those tests were published by numerous authors (Van Nerom et al. 1997, Li et al. 1999a, Koncicki et al. 2012, Kubińska et al. 2014, Tykałowski et al. 2014).

The available literature provides no information on the effect of methionine supplementation above the recommended levels on the percentages of T cell and B cell subpopulations in the blood and organs of the immune system in turkeys, which made it difficult to compare our findings with previous research. The vast majority of experimental studies conducted to date have focused on the influence of methionine on selected immune mechanisms in chickens (Swain and Johri 2000, Deng et al. 2007, Rubin et al. 2007), and only a few authors have investigated the effect of methionine (mostly methionine deficiency) on lymphocytes in peripheral blood and lymphoid organs in broilers (Zhang and Guo 2008, Bouyeh 2012, Wu et al. 2012, 2013).

In recent studies, Wu et al. (2012, 2013) investigated immunological parameters in chickens fed a starter diet (from 1 to 21 days of age) followed by a grower diet (from 22 to 42 days of age), in which methionine content was reduced by 0.24% and 0.12%, respectively, relative to basal diets containing 0.5% and 0.4% methionine, as recommended by NRC (1994). The authors reported a significant decrease in the percentages of peripheral blood $CD3^+$, $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cell subsets, the relative weights of the thymus and bursa of Fabricius, and proliferative indices of thymocytes and bursal cells accompanied by an increase in the percentage of apoptotic cells in those organs.

In our previous study (Kubińska et al. 2014), diets with a different methionine content had no significant effect on the percentages of $CD4^+$ and $CD8^+$ T cell subpopulations and IgM^+ B subpopulation in PBMCs, the thymus, bursa of Fabricius and CTs in 28-day-old turkeys. Significant differences were found in the percentages of $CD4^+$ T cell subset in the thymus and $CD8^+$ T cell subset in the bursa of Fabricius between vaccinated and unvaccinated birds. In the present study, diets with increased methionine inclusion rates contributed to a significant decrease in the percentage of IgM^+ B cell subpopulation in the bursa of Fabricius and PBMCs. The percentages of $CD4^+$ and $CD8^+$ T cell subpopulations in the bursa of Fabricius in group HM were significantly higher than those noted in groups LM and MM. An increase in the percentages of $CD4^+$ and $CD8^+$ T cell subsets, resulting from increased dietary methionine concentrations, was also noted in the thymus and CTs, respectively.

Both in our previous study with turkeys raised to 28 days of age, and in the present experiment where the birds were raised to 56 days of age, dynamic changes in the percentages of the analyzed lymphocyte subpopulations were observed in the spleen, which provides an ideal environment for interactions between lymphoid cells and non-immune cells. The spleen is a lymphatic organ and, since most bird species do not have organized lymph nodes, it plays a key role in the generation of the immune response. In comparison with LM group birds, group HM turkeys were characterized by a significant increase in the percentage of $CD8^+$ T cell subpopulation and a decrease in the percentage of $CD4^+$ T cell subpopulation in the spleen. The percentage of IgM^+ B cells increased in response to dietary methionine supplementation, but the differences were not statistically significant. Vaccination against ORT resulted in a significant increase in the percentage of $CD4^+/CD8^+$ T cell subpopulation and a decrease in the percentage of $CD8^+$ T cell subset in the spleen.

The results of this study indicate that turkeys fed diets with increased methionine levels (groups MM and HM) had higher body weights at 56 days of age than group LM birds. Supplemental methionine had no adverse influence on the functions of internal organs, which was confirmed by biochemical analyses. The optimum values of immunological parameters, in particular vaccine-induced antibody titers against ORT, total serum IgG (IgY) levels and the percentages of the analyzed T cell and B cell subpopulations, were noted in group MM turkeys.

Our findings partially support the hypothesis that immune system function in fast-growing turkeys can be modulated by increasing dietary methionine concentrations. Further research is needed to define the optimal dietary levels of methionine at successive stages of turkey production.

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