Polish Journal of Veterinary Sciences Vol. 17, No. 4 (2014), 687–695

DOI 10.2478/pjvs-2014-0100

Original article

Immunological and biochemical indicators in turkeys fed diets with a different Methionine content

M. Kubińska[1], B. Tykałowski[2], J. Jankowski[1], A. Koncicki[2]

[1] Department of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury, Oczapowskiego 5, 10-719 Olsztyn, Poland
[2] Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland

Abstract

The objective of this study was to determine the effect of different levels of dietary methionine on selected immune parameters of young turkeys. A total of 357 one-day-old female Hybrid Converter turkeys were randomly divided into three groups with low, medium and high level of dietary methionine (LM, MM, HM) of seven replicates each. Methionine was added to the basal diet (LM) at 0.16% (MM) and 0.32% (HM). At 17 days of age, seven birds per group were vaccinated against *Ornithobacterium rhinotraheale* (ORT) infection, with ORNITIN (ABIC) vaccine. At 28 days of age, blood was sampled and the birds were euthanized. The serum concentrations and activity of selected biochemical parameters, total IgY and IgM, and vaccine-induced antibody titers (IgY) against ORT were determined. The percentages of $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ T cell subpopulations and IgM^+ B cell subpopulation were determined in blood and organs by flow cytometry.

Different supplementary levels of methionine had no significant effect on vaccine-induced antibody titers against ORT or total serum IgM and IgY levels, as well as on the percentages of peripheral blood T and B cell subsets. Increasing dietary methionine rates decreased the percentage of $CD4^+$ T cell subpopulation, however it has increased the percentage of IgM^+ B cell subpopulation in the spleen. Vaccination against ORT resulted in a significant decrease in the percentage of $CD4^+$ T cell subset and an increase in the percentage of $CD8^+$ T cell subset in the spleen. It could be concluded that MM turkeys have developed the most desirable values of immune parameters.

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

Introduction

Methionine is the first limiting amino acid in poultry diets, and methionine-deficient diets have a low biological value due to protein metabolism disorders. Additionally, methionine is required to initiate translation, i.e. the biosynthesis of all the proteins an organism needs. It is also involved in the maturation of ribonucleic acids. Methionine serves as a methyl group donor and a precursor of cysteine, cys-

Correspondence to: M. Kubińska, e-mail: magdalena.kubinska@uwm.edu.pl

tathionine, homocysteine, S-adenosylhomocysteine (SAH) and glutathione (Rubin et al. 2007, Elshorbagy et al. 2013, Jankowski et al. 2014). The S-adenosyl methionine (SAM) is a donor of methyl group for betaine, choline, phosphatidylcholine, creatine, epinephrine, melatonin, anserine and N-methyl amino acids. Fodder methionine levels should be adjusted to meet the specific nutritional requirements of birds since the optimum balance of amino acids is required to stimulate growth (protein synthesis), maximize carcass yield, reduce carcass fatness and improve feed conversion and therefore reduce production costs (Bunchasak 2009). A study investigating the role of methionine in fat metabolism in humans has shown that SAM is responsible for the synthesis of phosphatidylcholine – an integral component of lipoproteins involved in transporting lipids from the liver (Obeid and Hermann 2009). Reduced hepatic levels of SAM disrupt the synthesis of very low density lipoproteins (VLDLs), leading to the secretion of small, lipid-poor VLDL particles (Cano et al. 2011). Thus, all factors that contribute to the disruption of SAM and SAH metabolism or disruption of the transmethylation cycle can indirectly affect body fat percentage and fat mass (Elshorbagy et al. 2013).

Methionine, cysteine and their derivatives are also involved in functioning of the immune system (Rubin et al. 2007, Jankowski et al. 2014). Dietary methionine levels exceeding recommended values have been found to stimulate humoral and cellular immune response in broiler chickens (Tsiagbe et al. 1987, Swain and Johri 2000, Deng et al. 2007). One of the mechanisms proposed to explain methionine interference in the immune system is the proliferation of T cells that are sensitive to intracellular variations in glutathione and cysteine levels, compounds which also participate in methionine metabolism (Kinscherf et al. 1994, Grimble 2006).

The estimated methionine requirement of growing turkeys, in the first month of their life, vary widely from 0.55% (NRC 1994) to 0.70% (BUT 2012). The aim of this study was to investigate the hypothesis that increasing dietary levels of methionine could improve the growth performance and the immune system functioning of fast-growing turkeys.

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 28 days of age. The birds were randomly divided into three experimental groups: LM – low methionine, MM – medium methionine, HM – high methionine. Each group was further divided into seven replicates, with 17 birds per replicate. The animal protocol used in this study was approved by the Local Ethics Committee (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 programs were consistent with the recommendations for Hybrid Turkeys from 2012. The birds had free access to feed and water throughout the growth period.

The basal diet, composed of soybean meal, wheat and maize, contained 27% crude protein, 1.74% lysine, 0.42% methionine (0.86% met+cyst), 1.25% Ca, 0.65% available P and 11.5 MJ metabolizable energy (calculated values). Group LM turkeys received the basal diet, while in groups MM and HM the basal diet were supplemented with additional methionine of 0.16% and 0.32%, respectively. The methionine content of diets was determined at the analytical laboratory of Evonic Industries AG Feed Additives (Germany).

Vaccination

At 17 days of age, 147 turkeys (seven birds per replicate, marked individually) were vaccinated against *Ornithobacterium rhinotraheale* (ORT) infection by subcutaneous administration of 0,5 ml of ORT vaccine (Ornitin, ABIC, Poland).

Growth trial and sample collection

At 28 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 cytometric analysis or without the anticoagulant for biochemical and serological analysis. Selected birds from each group were euthanized and the spleen, thymus, bursa of Fabricius (BF) and cecal tonsils (CTs) were collected for mononuclear cell isolation and determination of the percentages of $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ T cell subpopulations and IgM^+ B cell subpopulation, by flow cytometry.

Biochemical analysis

Serum samples 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 on the Idexx VetTest Chemistry Analyzer, according to the manufacturer's instructions.

Serological analysis

Total serum IgY (IgG) and IgM concentration were determined using commercial immunoenzymatic kits, the IgG Turkey ELISA kit (KA2515, Abnova, Taiwan) 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 (Nr 99-43600). Particular stages of the ELISA assay were performed using the Eppendorf epMotion 5075 LH automated pippeting system, the BioTek ELx405 washer and the BioTek ELx800 absorbance microplate reader.

Isolation of mononuclear cells and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated according to a previously described procedure (Koncicki et al. 2012, Tykałowski et al. 2014). Collected organs: spleen, thymus, bursa of Fabricius and cecal tonsils were individually homogenized in 1 ml RPMI-1640 medium (Sigma-Aldrich, Germany) with 5% FCS and centrifuged, using an automated tissue homogenizer (TissueLyser II, Qiagen, Germany). The homogeneous suspension was passed through a sterile 70 µm mesh cell strainer (BD Falcon, USA). The tissue residues were discarded, and the isolated cells were washed with RPMI-1640 medium supplemented with 5% FCS and centrifuged at 450 x g for 10 min. Mononuclear cells were isolated by centrifugation (900 x g, 22°, 20 min, breaks off) on a Percoll density gradient prepared from 40% and 60% dilution of a isotonic Percoll stock solution in Hank's balanced salt solution (Sigma-Aldrich, Germany). After centrifugation, the mononuclear cell layer was washed twice with PBS supplemented with 5% FCS, and then cell pellets were resuspended in 1 ml of PBS. The cells were counted, and their viability was evaluated using the Vi-Cell XR Cell Viability Analyzer (Beckman Coulter, USA).

Flow cytometry: 1 x 10^6 of viable mononuclear cells were stained with FITC-conjugated Mouse Anti Chicken CD4 (1:10, MCA2164F, IgG2b, clone 2-35, Serotec, UK) and PE-conjugated Mouse Anti Chicken CD8a (1:5, MCA2166PE, IgG1, clone 11-39, Serotec, UK) or with FITC-conjugated Goat Anti Chicken IgM (AAI27F, polyclonal IgG, Serotec, UK). After 30 min of incubation (on ice and in the dark), the cells were washed twice in 3 mL PBS and further analyzed by flow cytometry, performed using the FACSCanto II flow cytometer (BD Biosciences, USA). Data were acquired and analyzed with FACSDiva version 6.1.3 software (BD Biosciences, USA) and with FlowJo V10 software (Tree Star Inc., Stanford, CA, USA), respectively. The cytometer setup and tracking beads (CST, BD Biosciences, USA) were used to initialize PMT settings. Unstained and single stained control cells for each fluorochrome were prepared and used to set up flow cytometry compensation.

Statistical analysis

Two-way ANOVA was performed with the use of STATISTICA 10.0 software. The significance of differences between means in groups was determined by Duncan's test at a significance level of $p=0.001$.

Results

A laboratory analysis of feed amino acids concentration revealed a relatively high consistency between the expected and actual methionine content of experimental diets (Table 1). At 28 days of age, the average body weight of turkeys was similar in all groups, ranging from 869 to 871 g ($p=0.88$).

Biochemical parameters

Selected serum biochemical parameters in 28-day-old turkeys are shown in Table 2. No significant differences in the concentrations of the analyzed substances and enzyme activities were noted between groups of birds fed diets with graded levels of supplemental methionine. Vaccination against ORT had no effect on the analyzed biochemical parameters.

Table 1. Calculated and analytical content of methionine in turkey diets (g/kg).

	Group		
	LM	MM	HM
Calculated	4.15	5.75	7.35
Analytical	4.49	5.98	7.10

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

Table 2. Serum biochemical parameters of 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	7.66	11.98	3.83	0.98	2.75	284.50	28.67	287.17	316.33
	MM	7.68	11.15	3.63	1.00	2.63	273.83	24.33	275.83	324.33
	HM	7.77	11.03	3.49	0.90	2.59	282.14	22.43	268.14	300.14
Vac	LM	7.80	11.04	3.83	1.03	2.77	287.00	26.43	269.57	301.00
	MM	8.41	11.19	3.54	0.97	2.60	274.57	28.14	295.43	305.57
	HM	7.61	11.37	3.76	1.04	2.74	284.57	21.43	281.71	310.57
	SEM	0.12	0.04	0.06	0.02	0.04	2.21	1.45	6.18	11.44
Dosage	LM	7.73	11.02	3.80	1.01	2.76	285.85	27.46	277.69	308.08
	MM	8.08	11.17	3.58	0.98	2.61	274.23	26.38	286.38	314.23
	HM	7.69	11.20	3.62	0.97	2.66	283.36	21.93	274.93	305.36
Vaccination	Unv	7.71	11.05	3.62	0.96	2.65	280.26	25.00	276.58	312.89
	Vac	7.94	11.20	3.70	1.01	2.70	282.05	25.33	282.24	305.71
P-value	D	0.43	0.18	0.41	0.85	0.44	0.09	0.28	0.09	0.95
	V	0.34	0.10	0.55	0.31	0.59	0.67	0.95	0.67	0.75
	Interaction	0.32	0.28	0.55	0.40	0.68	0.98	0.69	0.98	0.86

Data represent mean values for 20 unvaccinated birds (Unv) and 20 vaccinated birds (Vac) per group LM – low methionine, MM – medium methionine, HM – high methionine; D – Dossage, V – Vaccination, P – phosphorus, Ca – calcium, TP – total protein, ALB – albumin, GLOB – globulin, GLU – glucose, ALT – alanine aminotransferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase

Table 3. Serum immunological parameters of turkeys.

	Group	Total IgM ng/ml	Total IgG ng/ml	ORT Titer	ORT %CV
Unv	LM	67.50	10.96	38.50	114.6
	MM	85.64	15.70	35.40	180.7
	HM	68.45	19.55	55.30	119.1
Vac	LM	55.00	11.16	2778.00	75.2
	MM	77.93	20.89	2311.50	64.3
	HM	70.80	11.93	1566.70	39.6
	SEM	4.23	1.60	208.74	–
Dossage	LM	61.25	11.06	2331.50	
	MM	81.79	18.29	1552.80	
	HM	69.62	15.74	1062.90	
Vaccination	Vac	67.91	14.65	2452.07[a]	
	Unv	73.86	15.40	43.07[b]	
P-value	D – effect	0.15	0.07	0.18	
	V – effect	0.49	0.77	<0.001	
	Interaction	0.77	0.12	0.17	

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group
LM – low methionine, MM – medium methionine, HM – high methionine; D – Dossage, V – Vaccination, CV – coefficient of variation
[a,b] – means with different superscript in the same column differ significantly ($p<0,01$)

Table 4. Percentages of peripheral blood T cell and B cell subpopulations.

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	21.09	2.45	0.46	14.38
	MM	23.34	2.89	0.58	10.07
	HM	24.98	2.98	0.69	15.48
Vac	LM	22.06	2.84	0.66	17.34
	MM	20.27	2.78	0.61	16.15
	HM	21.15	2.79	0.48	12.68
	SEM	1.04	0.22	0.07	0.97
Dosage	LM	21.58	2.64	0.56	15.86
	MM	21.81	2.84	0.60	13.11
	HM	23.07	2.88	0.59	14.08
Vaccination	Unv	23.14	2.77	0.58	13.31
	Vac	21.16	2.80	0.58	15.39
P-value	D	0.84	0.92	0.98	0.49
	V	0.38	0.95	0.98	0.28
	Interaction	0.65	0.88	0.54	0.18

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group
LM – low methionine, MM – medium methionine, HM – high methionine; D – Dossage, V – Vaccination

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

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	38.79	25.51	2.68	16.01
	MM	34.35	20.89^b	2.55	25.64
	HM	28.78	26.92^a	4.89	21.16
Vac	LM	30.69	25.27	3.45	22.19
	MM	27.73	29.65^a	3.38	24.25
	HM	23.96	27.32^a	3.46	25.56
	SEM	1.48	0.81	0.25	1.02
Dosage	LM	34.74a	25.39	3.06	19.09^b
	MM	31.04^{ab}	25.27	2.97	24.94^a
	HM	26.37^b	27.12	4.17	23.36^{ab}
Vaccination	Unv	33.97^a	24.44^b	3.37	20.93
	Vac	27.46^b	27.41^a	3.43	23.99
P-value	D	0.04	0.46	0.06	0.03
	V	0.02	0.04	0.90	0.09
	Interaction	0.86	0.02	0.08	0.19

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group
LM – low methionine, MM – medium methionine, HM – high methionine; D – Dossage, V – Vaccination
^{a,b} – means with different superscript in the same column differ significantly ($p<0.01$)

Immunological parameters

The serum IgM and IgY concentration and vaccine-induced antibody titers against ORT are presented in Table 3. Total serum IgM concentration was higher in both vaccinated and unvaccinated birds from groups MM and HM in comparison to corresponding birds from the control group (LM), but these differences were not statistically significant. Total serum IgY concentration was also higher in groups MM and HM, in comparison to the control group, but again no significant differences were recorded. Graded levels of dietary methionine had no significant effect on the titers of anti – ORT specific antibodies in turkeys from groups LM, MM and HM, subjected to a single vaccination against ORT at 17 days of age.

Tables 4-8 show the percentages of $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ T cell subpopulations and IgM^+ B cell subpopulation within the PBMCs and mononuclear cells isolated from the spleen, thymus, BF and CTs of 28-day-old both vaccinated (Vac) and unvaccinated (Unv) turkeys from groups LM, MM and HM, respectively. Table 4 shows that neither diets with a different methionine content nor vaccination against

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

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	1.61	5.41	81.11	1.38
	MM	1.54	4.97	81.45	1.26
	HM	2.11[a]	5.72	78.15	1.43
Vac	LM	1.21[bc]	5.49	81.72	1.29
	MM	1.84[a]	5.86	80.85	1.50
	HM	1.27[c]	4.75	84.57	2.12
	SEM	0.09	0.28	1.14	0.15
Dosage	LM	1.41	5.45	81.41	1.34
	MM	1.69	5.41	81.15	1.38
	HM	1.69	5.23	81.36	1.77
Vaccination	Unv	1.75[a]	5.36	80.24	1.36
	Vac	1.44[b]	5.37	82.38	1.64
P-value	D	0.21	0.95	0.99	0.47
	V	0.04	0.99	0.39	0.38
	Interaction	0.02	0.48	0.47	0.60

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group
LM – low methionine, MM – medium methionine, HM – high methionine; D – Dossage, V – Vaccination
[a,b] – means with different superscript in the same column differ significantly ($p<0,01$)

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

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	0.41	0.69	0.11	79.68
	MM	0.36	0.60	0.12	76.89
	HM	0.66	0.99	0.22	75.43
Vac	LM	0.47	0.20	0.18	77.27
	MM	0.32	0.46	0.11	76.52
	HM	0.40	0.60	0.15	79.75
	SEM	0.07	0.06	0.02	0.73
Dosage	LM	0.44	0.59	0.14	78.48
	MM	0.34	0.53	0.12	76.70
	HM	0.53	0.79	0.18	77.59
Vaccination	Unv	0.47	0.76[a]	0.15	77.33
	Vac	0.39	0.52[b]	0.15	77.85
P-value	D	0.54	0.17	0.31	0.62
	V	0.57	0.05	0.89	0.73
	Interaction	0.66	0.63	0.29	0.18

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group
LM – low methionine, MM – medium methionine, HM – high methionine; D – Dossage, V – Vaccination
[a,b] – means with different superscript in the same column differ significantly ($p<0,01$)

ORT had a significant effect on the percentages of analyzed T and B subpopulations lymphocyte in PBMCs. No significant differences in the percentages of the analyzed lymphocyte subpopulations in CTs were recorded between vaccinated and unvaccinated birds from groups LM, MM and HM (Table 8). The percentages of the double positive ($CD4^+/CD8^+$) T cell subpopulation in CTs were significantly increasing by methionine supplementation.

The most dynamic changes in the percentages of the analyzed lymphocyte subpopulations were observed in the spleen (Table 5). Graded dietary methionine levels had a significant influence on the percentages of $CD4^+$ T cell and IgM^+ B cell subpopulations. The percentages of $CD4^+$ and $CD8^+$ T cell subpopulations were significantly affected by vaccination against ORT. A significant decrease ($p=0.016$) in the percentage of $CD4^+$ T cell subset and an increase

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

	Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	IgM^+
Unv	LM	40.28	18.29	3.46	26.27
	MM	40.74	12.41	3.39	27.18
	HM	36.63	16.42	4.23	27.52
Vac	LM	36.26	13.00	2.78	31.60
	MM	36.16	14.33	3.76	28.12
	HM	39.43	16.65	4.66	26.55
	SEM	0.79	1.06	0.22	0.80
Dosage	LM	38.27	15.64	3.12^b	28.94
	MM	38.45	13.37	3.57^{ab}	27.65
	HM	38.03	16.54	4.44^a	27.03
Vaccination	Unv	39.22	15.71	3.69	26.99
	Vac	37.28	14.66	3.73	28.76
P-value	D	0.98	0.49	0.04	0.62
	V	0.22	0.63	0.92	0.29
	Interaction	0.12	0.39	0.46	0.29

Data represent mean values for 20 unvaccinated (Unv) turkeys and 20 vaccinated (Vac) turkeys per group
LM – low methionine, MM – medium methionine, HM – high methionine; D – Dossage, V – Vaccination
^{a,b} – means with different superscript in the same column differ significantly ($p<0,01$)

($p=0.037$) in the percentage of $CD8^+$ T cell subset were noted in vaccinated turkeys, as compared with unvaccinated birds.

Different levels of dietary methionine had no significant effect on the percentages of the analyzed lymphocyte subpopulations in the central organs of the immune system in turkeys. Significant differences between vaccinated and unvaccinated birds were found with regard to the percentages of $CD4^+$ T cell subset in the thymus (Table 6) and $CD8^+$ T cell subset in the bursa of Fabricius (Table 7).

Discussion

Relatively few studies have investigated the effect of methionine-supplemented diets on the growth rate of turkeys in the first three or four weeks of rearing. Waldroup and England (2002) demonstrated that the methionine content of diets higher than 0.52% had no beneficial influence on the body weights of 21-day old turkeys. On the other hand, Hoehler et al. (2005) reported that turkeys fed supplemental methionine had higher body weights at 21 days of age than control birds fed a basal diet. Lemme et al. (2005) also noted a positive effect of graded methionine levels on body weight gains in turkey toms. In our study, dietary methionine had no influence on the body weight gains of female turkeys raised to 28 days of age.

Biochemical parameters

The values of all biochemical parameters analyzed in the study remained within the normal ranges for turkeys (Krasnodębska-Depta and Koncicki 2011). The results of our study indicate that methionine added to experimental diets at 0.16% and 0.32% had no significant effect on the analyzed metabolic pathways in turkeys. In the absence of published research investigating the effect of dietary methionine on the analyzed parameters in turkeys, we had to compare our findings with the results of experiments involving chickens. The serum concentrations of total protein, albumins and globulins, determined in our study, are consistent with those reported by Al-Mayah (2006) who noted no significant differences in the values of serum total protein, albumin and globulin between 28-day-old broiler chickens that received different amounts of DL-methionine powder with feed or drinking water. Zhang and Guo (2008) demonstrated that liquid DL-2-hydroxy-4-methylthio butanoic acid (LMA), used as a dietary methionine source, had no effect on the serum levels of total protein and albumins in chickens aged 21 days. In the cited study, different doses of LMA affected total serum globulin levels, but the noted changes were not linear. In this study, vaccination against ORT had no influence on the values of the examined biochemical parameters.

Immunological parameters

Vaccine-induced antibody titers against ORT were lower in groups MM and HM than in the control group, but% CV (coefficients of variation) tended to decrease in those groups, which indicates that vaccine-induced antibody titers were more uniform across turkeys within groups MM and HM, compared to the LM group (Table 3). In similar study Swain and Johri (2000) found no significant differences in serum antibody titers 10 days after vaccination against Newcastle disease virus (NDV) in broiler chickens fed diets with higher methionine levels. Chickens that received 6.86 g DL-methionine/kg were characterized by significantly lower antibody titers, in comparison with the remaining groups.

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.

Commercially available fluorochrome-labeled monoclonal and polyclonal antibodies that recognize specific receptors and molecules on the surface and inside the immunocompetent cells are designed for flow cytometry detection of quantitative and qualitative changes in lymphocyte populations and subpopulations in the blood and organs of birds during the ongoing disease process (Koncicki et al. 2012). Flow cytometry also allows the assessment of the functional status of various cell subpopulations in response to feed additives, drugs, immunosuppressants and immunostimulants (Tykałowski et al. 2014).

In our study, quantitative changes in the subpopulations of lymphocytes isolated from the blood and organs of turkeys, determined by flow cytometry, were expressed as percentages. 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. 1999, Koncicki et al. 2012, 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 the 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 effect 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). Cited authors reported that 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 a proliferative indices of thymocytes and bursal cells in those chickens were significantly decreased together with an increase in the percentage of apoptotic cells in those organs.

In our study, the most desirable values of immune parameters were noted in the group MM turkeys that received a diet supplemented with methionine at 5.98 g/kg, i.e. only slightly (by 8.7%) above the NCR (1994) recommendations. Thus, the hypothesis that immune system function in fast-growing turkeys can be modulated by increasing dietary methionine rates has been positively verified. Yet further research is needed to define the optimal dietary levels of methionine at successive stages of turkey production and to determine their effects on ultimate performance indicators.

References

Al-Mayah AA (**2006**) Immune response of broiler chicks to DL-methionine supplementation at different ages. Int J Poult Sci 5: 169-172.

Bouyeh M (**2012**) Effect of excess lysine and methionine on immune system and performance of broilers. Ann Biol Res 3: 3218-3224.

Bunchasak C (**2009**) Role of dietary methionine in poultry production. J Poult Sci 46: 169-179.

BUT (2012) Commercial Performance Goals. http://www.aviagen.com/home.aspx?siteId=8

Cano A, Buqué X, Martínez-Uña M, Aurrekoetxea I, Menor A, García-Rodríguez JL, Lu SC, Martínez-Chantar ML, Mato JM, Ochoa B, Aspichueta P (**2011**) Methionine adenosyltransferase 1A gene deletion disrupts hepatic very low-density lipoprotein assembly in mice. Hepatology 54: 1975-1986.

Deng K, Wong CW, Nolan JV (**2007**) Carry-over effects of early-life supplementary methionine on lymphoid organs and immune responses in egg-lying strain chickens. Anim Feed Sci Tech 134: 66-76.

Elshorbagy AK, Nijpels G, Valdivia-Garcia M, Stehouwer CD, Ocke M, Refsum H, Dekker JM (**2013**) S-adenosylmethionine is associated with fat mass and truncal adiposity in older adults. J Nutr 143: 1982-1988.

Grimble RF (**2006**) The effects of sulfur amino acid intake

on immune function in humans. J Nutr 13 (Suppl 6): 1660S-1665S.

Hoehler D, Lemme A, Roberson K, Turner K (**2005**) Impact of methionine sources on performance in turkeys. J Appl Poult Res 14: 296-305.

Hybrid Turkeys (**2012**) A Hendrix Genetix. www.hybridturkeys.com

Jankowski J, Kubińska M, Zduńczyk Z (**2014**) Nutritional and immunomodulatory function of methionine in poultry diets – a review. Ann Anim Sci 14: 17-31.

Kinscherf R, Fischbach T, Mihm S, Roth S, Hohenhaus-Sievert E, Weiss C, Edler L, Bärtsch P, Dröge W (**1994**) Effect of glutathione depletion and oral N-acetyl-cysteine treatment on $CD4^+$ and $CD8^+$ cells. FASEB J 8: 448-451.

Koncicki A, Tykałowski B, Stenzel T, Śmiałek M, Pestka D (**2012**) Effect of infection of turkeys with haemorrhagic enteritis adenovirus isolate on the selected parameters of cellular immunity and the course of colibacillosis. Pol J Vet Sci 15: 215-220.

Krasnodębdska-Depta A, Koncicki A (**2011**) Biochemical diagnosis. In: Mazurkiewicz M (ed) Poultry diseases. University of Life Sciences, Wrocław, pp 792-806.

Lemme A, Kozłowski K, Jankowski J, Petri A, Zduńczyk Z (**2005**) Responses of 36- to 63-day-old BUT Big 6 turkey toms to graded dietary methionine+cysteine levels. J Anim Feed Sci 14 (Suppl 1): 467-470.

Li Z, Nestor KE, Saif YM, Fan Z, Luhtala M, Vainio O (**1999**) Cross-reactive anti-chicken CD4 and CD8 monoclonal antibodies suggest polymorphism of the turkey CD8h molecule. Poult Sci 78: 1526-1531.

NRC (National Research Council) (**1994**) Nutrient Requirements of Poultry. 9th ed., National Academy Press, Washington.

Obeid R, Herrmann W (**2009**) Homocysteine and lipids: S-adenosyl methionine as a key intermediate. FEBS Lett 583: 1215-1225.

Rubin LL, Canal CW, Ribeiro AL, Kessler A, Silva I, Trevizan L, Viola T, Raber M, Goncalves TA, Kras R (**2007**) Effects of methionine and arginine dietary levels on the immunity of broiler chickens submitted to immunological stimuli. Rev Bras Cienc Avic 9: 241-247.

Swain BK, Johri TS (**2000**) Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. Br Poult Sci 41: 83-88.

Tsiagbe VK, Cook ME, Harper AE, Sunde ML (**1987**) Enhanced immune responses in broiler chicks fed methionine-supplemented diets. Poult Sci 66: 1147-1154.

Tykałowski B, Śmiałek M, Pestka D, Stenzel T, Jankowski J, Mikulski D, Koncicki A (**2014**) Effect of whole wheat feeding on selected immune parameters in growing male turkeys. Pol J Vet Sci 17: 255-262 .

Van Nerom A, Ducatelle R, Haesebrouck F, Arnouts S, Goddeeris B, Davison TF, Kaspers B (**1997**) Monoclonal and polyclonal antibodies to chicken immunoglobulin isotypes specifically detect turkey immunoglobulin isotypes. Vet Immunol Immunophatol 57: 305-314.

Waldroup PW, England JA (**2002**) Evaluation of amino acid needs of large white male turkeys on at age-adjusted basis. Int J Poult Sci 1: 59-62.

Wu B, Cui H, Peng X, Fang J, Cui W, Liu X (**2012**) Effect of methionine deficiency on the thymus and the subsets and proliferation of peripheral blood T-cell, and serum IL-2 contents in broilers. J Integr Agr 11: 1009-1019.

Wu B, Cui H, Peng X, Fang J, Cui W, Liu X (**2013**) Pathology of bursae of Fabricius in methionine-deficient broiler chickens. Nutrients 5: 877-886.

Zhang LB, Guo YM (**2008**) Effects of liquid DL-2-hydroxy-4-methylthio butanoic acid on growth performance and immune responses in broiler chickens. Poult Sci 87: 1370-1376.