

DOI 10.2478/pjvs-2014-0036

Original article

Effect of whole wheat feeding on selected immune parameters in growing male turkeys

B. Tykałowski¹, M. Śmiałek¹, D. Pestka¹, T. Stenzel¹, J. Jankowski²,
D. Mikulski², A. Koncicki¹

¹ Department of Poultry Diseases, Faculty of Veterinary Medicine,

University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland

² Department of Poultry Science, University of Warmia and Mazury, Oczapowskiego 5, 10-719 Olsztyn, Poland

Abstract

The objective of this study was to determine the effect of whole wheat feeding on selected parameters of humoral and cell-mediated immunity in growing turkeys. A total of 210 one-day-old heavy-type Hybrid Converter male turkeys were randomly divided into three different dietary treatment groups, each consisting of 7 replicate pens of 10 birds per pen. Until 4 wk of age, all birds were fed a commercial diet formulated to meet nutrient requirements. From 5 to 12 wk of age, diets were composed of wheat (ground-pelleted or whole grain) and protein-fat-mineral-vitamin concentrate. The total wheat concentration in diets was 500 or 600 g/kg in the feeding periods of 5-8 and 9-12 wk of birds' age, respectively. Whole grain wheat had a 0, 50 or 100% share of the total wheat amount in the daily ration in treatment groups W_0 , W_{50} or W_{100} , respectively. Commercial vaccines against ND (Newcastle disease) and TRT (Turkey rhinotracheitis) were administered to turkeys via the drinking water on days 20 and 30, respectively. Over the entire experiment, a significant linear decrease was observed in body weight gains (BWG) with increasing dietary levels of whole grain wheat. As a result the BWG of control turkeys (W_0) were significantly higher than the BWG of group W_{100} birds ($P = 0.002$). A significant linear increase in feed conversion ratio (FCR) was observed with increasing dietary levels of whole grain wheat ($P < 0.001$). The levels of antibodies against TRT and ND viruses after immunization were significantly higher in both the W_{50} and W_{100} group, in comparison to group W_0 ($P = 0.006$ and $P = 0.001$, respectively).

Turkeys from group W_{50} , in comparison to those from groups W_0 and W_{100} , had a significantly higher percentage of $CD4^+$ T cell subpopulation within the lymphocytes isolated from blood and ileal mucosa, as well as $CD4^+$ $CD8^+$ and $CD8^+$ T cell subpopulations within the blood immunocompetent cells ($P = 0.022$, $P = 0.029$, $P = 0.009$ and $P = 0.011$, respectively). In the cecal tonsils, the percentage of $CD8^+$ T cell subpopulation was significantly lower in group W_{50} than in groups W_0 and W_{100} ($P = 0.014$). The results of our study indicate that diluting diets with whole grain wheat stimulates the non-specific cell-mediated defense mechanisms of the gastrointestinal immune system in turkeys, thus positively affecting humoral response after vaccination.

Key words: whole wheat, growth performance, turkeys, cell-mediated immunity, humoral response

Introduction

The physiology of the avian digestive system is significantly affected by the type and form of diet. Diet composition influences the pH in the gastrointestinal tract and gastric acid secretion (production of proteolytic enzymes, lysozyme, mucus), thus affecting the composition of physiological gut microflora and the rate of gut peristalsis (Engberg et al. 2004, Taylor and Jones 2004). These untaught functions of the avian digestive system act as barriers and provide effective protection against harmful external factors including pathogens which enter the body via the digestive system. These barriers are supported by elements of mucosa-associated lymphoid tissue (MALT). MALT comprises the lymphatic tissue of the lamina propria of mucous membranes in different systems of the body, such as the respiratory and reproductive system, as well as the digestive system, where it is called gut-associated lymphoid tissue (GALT) (Lasek 2002, Smith and Beal 2008). The GALT consists of lymphoid cells residing in the epithelial lining and distributed in the underlying lamina propria as well as specialized lymphoid structures such as: Meckel's diverticulum, Peyer's patches (PP) and cecal tonsils (CT) (Smith and Beal 2008).

Experiments performed by Hermiston and Gordon (1995) on mice, as well as by Smith and Peacock (1989) and by Uni et al. (1995, 2000) on chickens, revealed that enterocytes of intestinal villi and crypts, goblet cells and intraepithelial lymphocytes (IEL) play an important role in the gastrointestinal immune system. The development of the above structures is determined, among others, by the type and form of feed administered. The immune response in the avian digestive system (which leads to general MALT response) is primarily induced in PP and CT (Smith and Beal 2008). Thus, the immune response of MALT against pathogens may be initiated by local interactions occurring between the components of the gut-associated immune system and the lymphocytes which aggregate in the neighboring tissues in response to infection (Strober et al. 2002, Smith and Beal 2008).

Considering the pathogenesis of many infectious diseases in birds as well as the fact that the digestive system is one of the main entrances for pathogenic microorganisms (and live vaccines) into the body, the exploration of the phenomena that occur between GALT and the immune system in general is extremely important. The above assumption has been the stimulus for the present study, which aimed at investigating the effect of diet supplementation with whole grain wheat on the diversity of selected, specific (humoral) and non-specific (cell-mediated), immune parameters

in growing turkeys. It should be pointed out that previous studies (Jankowski et al. 2012, Jankowski et al. 2013b, Zduńczyk et al. 2013) have shown that the body weight gains of turkeys decrease with increasing dietary levels of whole grain wheat, the relative weights of gizzard and cecal digesta increase and the digesta pH decreases with increasing whole grain intake. In the cited studies, high levels of whole grain wheat in the feed had no significant effect on total bacteria count per g of cecal digesta and the morphology of intestinal mucosa, although it increased the length of villi and decreased crypt depth in the duodenum. Additionally, decreased length of villi was reported in the ileum and cecum, and decreased crypt depth was noted in the ileum.

The aim of our study was to determine the effect of different concentrations of whole grain wheat (W) addition among the total wheat content (whole grain and ground-pelleted (GW)) in the feed on selected parameters of humoral and cell-mediated immunity in growing turkeys.

Materials and Methods

Birds, management, and diets

The experiment was carried out at the Research Laboratories of the Department of Poultry Science and the Department of Poultry Diseases, University of Warmia and Mazury in Olsztyn (Poland), on 210 one-day-old heavy-type Hybrid Converter male turkeys randomly divided into three different dietary treatment groups. The poults were purchased from the "Grelavi" Hatchery, Ketrzyn, Poland. Each experimental group comprised 70 male turkeys (seven replicates of 10 birds each). The animal protocol used in this study was approved by the Local Animal Care and Use Committee (Olsztyn, Poland), and the study was carried out in accordance with EU Directive 2010/63/EU for animal experiments (OJEU, 2010). Over the entire rearing period of 1-12 weeks, 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 the Hybrid Turkeys Company (2012).

During the first 4 wks of rearing, all birds were fed a commercial diet formulated to meet nutrient requirements. From 5 to 12 wks of age, diets were composed with wheat (ground-pelleted or whole grain) and protein-fat-mineral-vitamin concentrate, whose composition is given in Table 1. The basal (control) diet was composed of ground-pelleted wheat and pelleted protein-fat-mineral-vitamin concentrate mixed at the proportion of 50:50 or 60:40 (w/w) in the feed-

Table 1. Dietary ingredients and the nutrient content of concentrate fed to turkeys from 5 to 8 and 9 to 12 wks of age.

Ingredient (g/kg as-fed basis)	Concentrate		Wheat grain
	5-8 wk	9-12 wk	
Soybean meal (46% CP)	776.0	750.0	
Rapeseed (20.5% CP)	80.0	75.0	
Soybean oil	55.6	70.7	
Limestone	31.4	36.7	
Monocalcium phosphate	32.8	35.8	
Choline chloride	1.4	1.7	
Salt	5.8	7.3	
DL-Methionine ¹	3.5	5.0	
L-Lysine HCL ²	7.6	8.2	
L-Threonine ²	0.9	3.4	
Vitamin- mineral premix ³	5.0	6.2	
<i>Calculated analysis⁴</i>			
AME, kcal/kg	2,670	2,740	3,070
CP, g/kg	380.0	370.0	120.0
Lys, g/kg	27.6	27.3	3.4
Met, g/kg	8.5	9.8	1.9
Met + Cys, g/kg	14.3	15.4	4.7
Thr, g/kg	15.0	17.0	3.4
Trp, g/kg	5.0	4.8	1.4
Arg, g/kg	25.8	24.9	5.7
Ca, g/kg	21.5	24.2	0.5
Available P, g/kg	9.1	9.7	1.3
Na, g/kg	2.6	3.2	0.1

¹ MetAMINO®, Evonik Degussa GmbH, Essen, Germany, 99% of methionine.

² Ajinomoto Eurolysine S.A.S, Amiens, France, 78% of lysine, 98.5% of threonine.

³ Provided the following per kilogram of diet in the successive (5-8 and 9-12 weeks) feeding periods: retinol 3.38 and 2.88 mg, cholecalciferol 0.12 and 0.10 mg, α -tocopheryl acetate 94 and 80 mg, vitamin K3 5.6 and 4.8 mg, thiamine 4.7 and 4.0 mg, riboflavin 7.5 and 6.4 mg, pyridoxine 5.6 and 4.8 mg, cobalamin 0.028 and 0.024 mg, biotin 0.28 and 0.24 mg, pantothenic acid 24 and 20 mg, nicotinic acid 75 and 64 mg, folic acid 2.8 and 2.4 mg, Fe 56 and 48 mg, Mn 112 and 96 mg, Zn 103 and 88 mg, Cu 19 and 16 mg, J 2.8 and 2.4 mg, Se 0.28 and 0.24 mg, choline chloride 376 and 320 mg, respectively.

⁴ Calculated from the analyses of feed ingredients provided by the manufacturer.

ing period of 5-8 and 9-12 wks of birds' age, respectively (W_0 diet). In the W_{50} and W_{100} treatment groups, whole grain was added at 50 or 100% concentration of the total dietary content of wheat, respectively.

Wheat grain from the same batch was used in all dietary treatments, and its chemical composition was estimated based on CP (crude protein) content (120.0 g/kg) and Polish feedstuff analysis tables (Smulikowska and Rutkowski, 2005). In order to obtain ground-pelleted wheat, grain was ground with a hammer mill at 3000 rpm and a screen diameter of 4 mm. In wks 5 to 8, the diameter of concentrate pellets was 3 mm, and the diameter and length of wheat pellets were 4 mm and approximately 2-3 mm, respectively. In wks 9-12, the diameter of concentrate pellets and wheat pellets was 4 mm. The composition and nutritional value of the concentrate ensured adequate nutrition of turkeys aged 5-8 and 9-12 wks with respect to CP, amino acids and AME (apparent metabolizable energy) as recommended by Hybrid

Turkeys (2012), and Ca and available P as recommended by the National Research Council (1994). The calculated nutritional value of daily diets was as follows: AME – 2,870 and 2,938 kcal/kg, crude protein – 250 and 220 g/kg, lysine – 15.5 and 13.0 g/kg, methionine – 5.2 and 5.1 g/kg, Ca – 11.0 and 10.0 g/kg, available P – 5.5 and 5.0 g/kg, in the feeding periods of 5 – 8 and 9-12 wks, respectively. Feed and water were given to birds ad libitum.

Growth trial

Commercial vaccines against ND (Nobilis ND Clone 30, Intervet) and TRT (Poulvac TRT, Pfizer) were administered to turkeys via the drinking water on days 20 and 30 respectively, in accordance with manufacturer's instructions. Body weight (BW) at 28 and 84 days of age and feed consumption from 28 to 84 days of age were recorded. Body weight gain (BWG) and feed conversion ratio (FCR) were cal-

culated. For performance indices, each replicate ($n = 7$) was considered as an experimental unit.

Sample collection and ELISA tests

At 12 wks of age, blood samples were collected into sterile test tubes from the vena basilica of 23 turkeys from each group (3-4 birds selected randomly per replicate). Vaccine-induced serum antibody level against ND and TRT viruses were determined with the use of commercial Idexx ELISA kits. Particular stages of the ELISA assays were performed with the use of the following additional equipment: Eppendorf epMotion 5075 LH automated pipetting system, BioTek ELx405 washer and BioTek ELx800 absorbance microplate reader.

At 12 weeks of age, seven birds representing the average BW of each group (one bird per pen as a replicate) were sacrificed by cervical dislocation. Before slaughter, 1.5-2 ml blood samples for cytometric analyses were collected from the vena basilica of each bird into sterile test tubes containing Potassium EDTA at 1.6 mg/ml (Sarstedt, Germany), and mixed thoroughly with a roller mixer for 5 min. After slaughter, the CT and 10-cm terminal sections of ileum were collected in order to isolate mononuclear cells for further cytometric analysis.

Isolation of mononuclear cells from peripheral blood

1.5 ml blood samples were diluted 1:1 (v/v) in sterile PBS supplemented with 1% FCS (Sigma-Aldrich, Germany), and the total volume of these solutions were then layered onto 3 ml of Histopaque-1077 (Sigma-Aldrich, Germany) and centrifuged at 400 x g at room temperature for 30 min., with the break off. After centrifugation, the mononuclear cell layer was carefully transferred to sterile test tubes and then washed twice with PBS supplemented with 5% FCS at 350 x g for 7 min. Finally, cell pellets were resuspended in 1 ml of PBS. The mononuclear cells were counted and their viability was evaluated using a Vi-Cell XR automated cell counter (Beckman Coulter, USA).

Isolation of mononuclear cells from the CT

CTs were homogenized in 1 ml RPMI-1640 medium (Sigma-Aldrich, Germany) with 5% FCS, using an automated tissue homogenizer (TissueLyser II, Qiagen, Germany). The homogeneous suspension was

passed through a sterile, 70 μ m 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 at 450 x g for 10 min. Mononuclear cells were isolated by centrifugation (900 x g, 22°C, 20 min., with the break off) on a Percoll density gradient prepared from 40% and 60% dilution of an isotonic Percoll stock solution in Hank's balanced salt solution (Sigma-Aldrich, Germany). After centrifugation, the mononuclear cell layer was carefully transferred to sterile test tubes and washed twice with PBS supplemented with 5% FCS, and finally cell pellets were resuspended in 1 ml of PBS. The cells were counted and their viability was evaluated using the Vi-Cell XR.

Isolation of mononuclear cells from the ileal mucosa

Each ileum section was cut into 1-2 mm pieces which were transferred to 100 ml glass flasks with 25 ml collagenase type IV solution (200 units/ml; Sigma-Aldrich, Germany) in an incomplete medium (RPMI-1640, 10 mM HEPES, Penicillin/Streptomycin; Sigma-Aldrich, Germany). The samples were incubated for 30 min. at 38°C and vortexed at 250 rpm for the whole time of incubation. After incubation, the suspension was passed through a sterile, 70 μ m mesh cell strainer (BD Falcon, USA), and then washed twice with RPMI-1640 supplemented with 5% FCS at 450 x g for 10 min. Further steps of cell isolation were identical to that applied to CTs.

Determination of percentages of CD4⁺, CD4⁺CD8⁺ and CD8⁺ T cell subpopulations in peripheral blood, cecal tonsils and ileal mucosa

1 x 10⁶ of viable mononuclear cells isolated from peripheral blood, CT and ileal mucosa, were transferred to test tubes and 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). 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 cytometer. Flow cytometry analysis was performed using a FACSCanto II cytometer (BD Biosciences, USA). Data were acquired by FACSDiva version 6.1.3 software (BD Biosciences, USA) and analyzed by FlowJo V10 software (Tree Star Inc., Stanford, CA, USA). Cytometry setup and tracking beads (CST, BD Biosciences, USA) were used to initialize PMT settings.

Table 2. Body weight gains (BWG) and feed conversion ratio (FCR) of turkeys from 5 to 12 wks of age¹.

	Initial body weight (kg)	BWG (kg/bird)	FCR (kg/kg)
Treatment ²			
W ₀	0.96	8.53 ^a ± 0.12	2.12 ^b ± 0.02
W ₅₀	0.96	8.21 ^a ± 0.09	2.23 ^a ± 0.02
W ₁₀₀	0.96	7.84 ^b ± 0.12	2.27 ^a ± 0.01
Pooled SEM	0.008	0.088	0.018
<i>P</i> – value			
group	–	0.002	<0.001
linear contrast	–	<0.001	<0.001
quadratic contrast	–	0.860	0.254

¹ Data represent mean values of 7 replications per treatment.

² W₀, W₅₀, W₁₀₀; 0, 50, 100% whole grain wheat share of the total wheat amount in the daily ration, respectively.

^{a-b} Means within the same column with no common superscripts differ significantly (*P* < 0.05) as a result of Newman-Keuls mean comparison.

Table 3. Antibody titers against TRT and ND viruses in blood serum of vaccinated turkeys¹.

	TRT	ND
Treatment ²		
W ₀	628.4 ^b ± 110.0	118.6 ^b ± 12.0
W ₅₀	1303.3 ^a ± 135.3	256.9 ^a ± 43.2
W ₁₀₀	1156.4 ^a ± 145.6	229.8 ^a ± 28.0
Pooled SEM	86.39	18.82
<i>P</i> – value		
group	0.006	0.001
linear contrast	0.014	0.002
quadratic contrast	0.017	0.059

¹ Data represent mean values of 23 turkeys per treatment.

² W₀, W₅₀, W₁₀₀; 0, 50, 100% whole grain wheat share of the total wheat amount in the daily ration, respectively.

^{a-b} Means within the same column with no common superscripts differ significantly (*P* < 0.05) as a result of Newman-Keuls mean comparison.

Unstained control cells and single stain control for each fluorochrome were prepared and used to set up flow cytometric compensation.

Statistical analysis

For growth performance parameters, each replicate pen (*n* = 7) was considered as an experimental unit. Other results were analyzed with each bird as a replicate. The Box-Cox transformation (Box and Cox, 1964) was applied to antibody titers against TRT and ND viruses. The percentages of T cell subpopulations, except for CD4⁺ T cells in the cecal tonsils, were transformed to arcsine of the square root before analysis to achieve homogeneity of variance. The statistical analysis was performed according to the GLM procedure for Statistica 10.0PL software (StatSoft Inc., 2007). Growth performance data were subjected to one-way ANCOVA with initial body weight at 28 days of age as the dependent variable (covariate).

The other datasets were analyzed by one-way ANOVA. When a significant treatment effect was noted, the post-hoc Newman-Keuls test was used to determine differences between the treatment groups. In addition, the data were subjected to linear and quadratic polynomial contrasts to evaluate the effect of different dietary levels of wheat. The effects were considered to be significant at *P* ≤ 0.05 and were expressed as mean values with pooled SEM.

Results

The BWG and FCR of turkeys aged from 5 to 12 wks are presented in Table 2. Over the entire experiment, the BWG of control turkeys (W₀) were significantly higher than the BWG of group W₁₀₀ birds (*P* = 0.002). A significant linear decrease (*P* < 0.001) was observed in BWG with increasing dietary levels of whole grain wheat. During the entire experiment, FCR was significantly lower in group W₀ than in

Table 4. Percentages of T cell subpopulations in blood, ileal mucosa and cecal tonsils¹.

Specification	T cell subpopulations								
	CD4 ⁺			CD4 ⁺ CD8 ⁺			CD8 ⁺		
	Blood	Ileal mucosa	Cecal tonsils	Blood	Ileal mucosa	Cecal tonsils	Blood	Ileal mucosa	Cecal tonsils
Treatment ²									
W ₀	12.70 ^b ±0.95	19.59 ^b ±1.62	33.70±2.41	0.22 ^b ±0.03	5.73±1.30	4.14±0.84	3.95 ^b ±0.46	15.04±2.01	20.67 ^a ±0.74
W ₅₀	20.79 ^a ±3.14	28.07 ^a ±3.32	33.66±0.69	0.42 ^a ±0.08	7.87±1.89	4.31±0.31	7.69 ^a ±1.55	19.76±1.63	15.50 ^b ±1.63
W ₁₀₀	13.48 ^b ±1.32	20.46 ^b ±1.21	33.17±1.54	0.18 ^b ±0.02	9.41±2.21	6.17±0.87	3.52 ^b ±0.24	19.99±0.94	19.16 ^a ±0.82
Pooled SEM	1.383	1.496	0.932	0.037	1.062	0.445	0.665	1.008	0.791
P – value									
group	0.022	0.029	0.971	0.009	0.385	0.116	0.011	0.071	0.014
linear contrast	0.791	0.792	0.828	0.595	0.175	0.062	0.749	0.041	0.358
quadratic contrast	0.006	0.009	0.916	0.003	0.896	0.354	0.003	0.263	0.005

¹ Data represent mean values of 7 turkeys per treatment.

² W₀, W₅₀, W₁₀₀; 0, 50, 100% whole grain wheat share of the total wheat amount in the daily ration, respectively.

^{a-b} Means within the same column with no common superscripts differ significantly ($P < 0.05$) as a result of Newman-Keuls mean comparison.

groups W₅₀ and W₁₀₀ ($P < 0.001$), and a significant linear increase ($P < 0.001$) in FCR was noted with increasing dietary levels of whole grain wheat.

Antibody levels in the serum of turkeys vaccinated against TRT and ND are shown in Table 3. The level of antibodies against TRT and ND viruses after immunization were significantly higher in groups W₅₀ and W₁₀₀, in comparison with group W₀ ($P = 0.006$ and $P = 0.001$, respectively).

The percentages of T cell subpopulations in the blood, ileal mucosa and CTs of turkeys fed diets with different levels of whole grain wheat are presented in Table 4. Turkeys from group W₅₀, in comparison to those of groups W₀ and W₁₀₀, had a significantly higher percentage of CD4⁺ T cell subpopulation within the lymphocytes isolated from blood and ileal mucosa, as well as CD4⁺ CD8⁺ and CD8⁺ T cell subpopulations within the blood immunocompetent cells ($P = 0.022$, $P = 0.029$, $P = 0.009$ and $P = 0.011$, respectively). In the CTs, the percentage of CD8⁺ T cell subpopulation was significantly lower in group W₅₀ than in groups W₀ and W₁₀₀ ($P = 0.014$).

Discussion

A significant linear decrease (linear contrast $P < 0.001$) was observed in the body weight gains of turkeys with increasing dietary levels of whole grain wheat used as a substitute for ground and pelleted wheat. As a result, the BWG of birds from group W₁₀₀ were significantly ($P < 0.05$) lower than in those from group W₀ and W₅₀. Additionally, increasing levels of whole grain in the diet reduced the feed conversion

ratios (linear contrast $P < 0.001$). These results corroborate the findings of Jankowski et al. (2013). Bennett and Classen (2003) demonstrated that increased levels of whole wheat in commercial turkey rations (21% on average over the entire experiment) resulted in significant reductions in the final body weights of birds, but this has not been related to the physical form of the wheat. The physical form of wheat in turkey diets affects the morphology of intestinal mucosa (Jankowski et al. 2013b, Zduńczyk et al. 2013) i.e. the depth of duodenal crypts and the length of cecal villi decrease with increasing dietary levels of whole wheat grain. The authors cited (Jankowski et al. 2013b, Zduńczyk et al. 2013) also reported that increased concentration of whole wheat in turkey diets caused an increase in the relative weight of gizzard tissue and the viscosity of small intestinal and cecal digesta, which was accompanied by a decrease in the concentrations of ammonia and total short-chain fatty acids, a drop in the digesta pH, and a reduction in total *Escherichia coli* and *Clostridium perfringens* counts in the cecal digesta.

GALT comprises T cells, B cells, natural killer (NK) cells, dendritic cells, goblet cells, plasmocytes, macrophages and heterophils, as well as specialized lymphoid structures such as: Meckel's diverticulum, PPs and CTs (Smith and Beal 2008). These organized lymphoid structures facilitate the cooperation between immunocompetent cells, and therefore enable induction of the mucosal immune response. Additionally, the above lymphoid structures in the intestinal mucosa are functionally related to the bone marrow and spleen (Jeurissen et al. 1994, Bar-Shira et al. 2003, Smith and Beal 2008) which indicates the possibility of general immune response being induced lo-

cally in the GALT. Functional and morphological changes in the gastrointestinal tract of turkeys, which have been described previously, raise the question if supplementation with whole grain wheat could also affect the gut-associated immune system and therefore the activation of general immune response (Strober et al. 2002, Smith and Beal 2008). The above assumptions prompted us to investigate the effect of different dietary levels of whole grain wheat on the humoral immune response in turkeys vaccinated against ND and TRT and on the cell-mediated immune response based on the percentages of selected T cell subpopulations in the blood, ileal mucosa and CTs.

In our study, the level of vaccine-induced antibodies against TRT and ND viruses were significantly ($P < 0.05$) higher in both the W_{50} and W_{100} group, in comparison to group W_0 . Turkeys from group W_{50} , in comparison to those of groups W_0 and W_{100} , had a significantly higher percentage of $CD4^+$ T cell subpopulation within the lymphocytes isolated from blood and ileal mucosa, as well as $CD4^+$ $CD8^+$ and $CD8^+$ T cell subpopulations within the blood immunocompetent cells ($P = 0.022$, $P = 0.029$, $P = 0.009$ and $P = 0.011$, respectively). In the CT, the percentage of $CD8^+$ T cell subpopulation was significantly lower in group W_{50} than in groups W_0 and W_{100} ($P = 0.014$). Our findings show that the type and form of turkey diet can affect both the local gastrointestinal immune system and the general (humoral) immune response after vaccination. Weaker immune reaction in turkeys from group W_{100} in comparison to those from group W_{50} could be the result of increasing the whole wheat content which caused an increase of viscosity of the intestinal digesta (Jankowski et al. 2013b) and therefore weaker digestion (Jankowski et al. 2013a) and availability of nutritional ingredients. This also caused the lower body weights of turkeys from group W_{100} .

The flow cytometry method was used to determine selected parameters of the immune system in turkeys. CD4 and CD8 T cell receptors were stained with monoclonal antibodies intended for use in chickens, which cross react with turkey lymphocytes receptors, as shown previously in numerous studies (Van Nerom et al. 1997, Koskinen et al. 1999, Li et al. 1999, Li et al. 2000, Powell et al. 2009).

The results of our study indicate that whole grain wheat diluted diets affect gastrointestinal function and intestinal mucosa morphology, and also stimulates the non-specific cell-mediated defense mechanisms of the gastrointestinal immune system, thus positively affecting humoral antibody responses after per os vaccination against TRT and ND.

Acknowledgements

This work was supported by the Polish Committee for Scientific Research, Project No. NN311 400 339.

References

- Bar-Shira E, Sklan D, Friedman A (2003) Establishment of immune competence in the avian GALT during the immediate post-hatch period. *Dev Comp Immunol* 27: 147-157.
- Bennett CD, Classen HL (2003) Effect of whole wheat dilution on performance and carcass characteristics of male turkeys. *J Appl Poult Res* 12: 468-475.
- Box GE, Cox DR (1964) An analysis of transformations. *J R Stat Soc* 26: 211-253.
- Engberg RM, Hedemann MS, Steinfeldt S, Jensen BB (2004) Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poult Sci* 83: 925-938.
- Hermiston ML, Gordon JI (1995) Organization of the crypt-villus axis and evolution of its stem cell hierarchy during intestinal development. *Am J Physiol* 268: 813-822.
- Hybrid Turkeys (2012) Commercial management guide. Nutritional guidelines. Accessed Sept. 22, 2012. <http://www.hybridturkeys.com>.
- Jankowski J, Mikulski D, Zduńczyk Z, Mikulska M, Juśkiewicz J (2012) The effect of diluting diets with ground and pelleted or with whole wheat on the performance of growing turkeys. *J Anim Feed Sci* 21: 735-747.
- Jankowski J, Mikulski D, Zduńczyk Z, Juśkiewicz J (2013a) A note on the particle size distribution of intestinal digesta and nutrient digestibility in growing turkeys fed diets with different whole-grain wheat contents. *J Anim Feed Sci* 22: 366-370.
- Jankowski J, Zduńczyk Z, Mikulski D, Przybylska-Gornowicz B, Sosnowska E, Juśkiewicz J (2013b) Effect of whole wheat feeding on gastrointestinal tract development and performance of growing turkeys. *Anim Feed Sci Technol* 185: 150-159.
- Jeurissen SH, Vervelde L, Janse M (1994) Structure and function of lymphoid tissue in the chicken. *Poult Sci Rev* 5: 183-207.
- Koskinen R, Lamminmäki U, Tregaskes CA, Salomonsen J, Young JR, Vainio O (1999) Cloning and modeling of the first nonmammalian CD4. *J Immunol* 162: 4115-4121.
- Lasek W (2002) The mucosal immune system. In: Gołąb J, Jakóbsiak M, Lasek W (eds) *Immunology*. PWN Warszawa, pp 288-299.
- 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 CD8 α molecule. *Poult Sci* 78: 1526-1531.
- Li Z, Nestor KE, Saif YM, Luhtala M (2000) Flow cytometric analysis of T lymphocyte subpopulations in large-bodied turkey lines and a random bred control population. *Poult Sci* 79: 219-223.
- NRC (National Research Council) (1994) *Nutrient requirements of poultry*. 9th revised ed., National Academic Press, Washington, DC.

- OJEU (2010) Official Journal of the European Union. Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Series L 276: 33-79.
- Powell F, Lawson M, Rothwell L, Kaiser P (2009) Development of reagents to study the turkey's immune response: identification and molecular cloning of turkey CD4, CD8 α and CD28. *Dev Comp Immunol* 33: 540-546.
- Smith MW, Peacock MA (1989) Comparative aspects of microvillus development in avian and mammalian enterocytes. *Comp Biochem Physiol A* 93: 617-622.
- Smith AL, Beal R (2008) The avian enteric immune system in health and disease. In: Davison F, Kaspers B, Schat KA (eds) *Avian Immunology*. Elsevier Academic Press, pp 243-271.
- Smulikowska S, Rutkowski A (2005) Recommended Allowances and Nutritive Value of Feedstuffs. In: *Poultry Feeding Standards* 4th ed. The Kielanowski Institute of Animal Physiology and Nutrition of the PAS, Jablonna, Poland, pp117-119.
- StatSoft Inc (2007) Statistica (data analysis software system) version 8.0. Accessed Jan 16, 2011. <http://www.statsoft.com>
- Strober W, Fuss IJ, Blumberg RS (2002) The immunology of mucosal models of inflammation. *Annu Rev Immunol* 20: 495-549.
- Taylor RD, Jones GPD (2004) The incorporation of whole grain into pelleted broiler chicken diets. II. Gastrointestinal and digesta characteristics. *Br Poult Sci* 45: 237-246.
- Uni Z, Noy Y, Sklan D (1995) Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. *Poult Sci* 74: 1622-1629.
- Uni Z, Geyra A, Ben-Hur H, Sklan D (2000) Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. *Br Poult Sci* 41: 544-551.
- 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.
- Zduńczyk Z, Jankowski J, Mikulski D, Przybylska-Gornowicz B, Sosnowska E, Juśkiewicz J (2013) Gastrointestinal morphology and function in turkeys fed diets diluted with whole grain wheat. *Poult Sci* 92: 1799-1811.