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Original article

# Metabolic and immune response of young turkeys originating from parent flocks fed diets with inorganic or organic selenium

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## Abstract

The aim of this study was to verify the hypothesis that the health and growth of turkey poults may be improved by supplementing diets fed to parent flocks with available selenium. Experimental poults originated from parent flocks fed with diets containing 0.3 mg/kg inorganic selenium (control group Se<sub>M</sub>) and organic selenium (experimental group Se<sub>O</sub>). Egg yolk selenium content was comparable in both flocks (0.72 and 0.70 mg/kg d.m., respectively). Eggs from the Se<sub>O</sub> flock had a significantly lower content of thiobarbituric acid reactive substances – TBARS (31.13 vs. 53.10 nmol/g,  $p > 0.001$ ). Se<sub>O</sub> group poults were characterized by higher activity of glutathione peroxidase (7.54 vs. 5.92 U/mL,  $P = 0.001$ ) and superoxide dismutase (89.30 vs. 79.23 U/mL,  $P = 0.026$ ). The thigh muscles of Se<sub>O</sub> group birds had significantly higher selenium concentrations (0.74 vs. 0.57,  $p = 0.045$ ) and a significantly lower TBARS content (38.42 vs. 65.01,  $p = 0.001$ ).

No differences were found between the groups with respect to the content of total protein, albumins and uric acid, and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (DLH) in day-old poults.

On day 28, groups Se<sub>O</sub> and Se<sub>M</sub> differed in the activity of ALT (20.50 vs. 26.33,  $p = 0.05$ ) and SOD (87.29 vs. 100.02 U/mL,  $p = 0.035$ ).

There were no differences between the groups regarding the percentages of T lymphocyte subpopulations CD4<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>+</sup>CD8<sup>+</sup> and B lymphocyte subpopulations (IgM<sup>+</sup>) at 1 and 28 days of age.

Over the experimental period, mortality rates were similar in both groups (7.32 and 8.87%), and so were the final body weights of birds (1108 vs. 1135 g). The results of the study show that the dietary supplementation of organic selenium in turkey parent flocks reduces the rate of oxidation processes in the egg and in the tissues of newly-hatched poults, yet it has no effect on the analyzed parameters of cell-mediated immunity and the growth performance of birds during the first five weeks of their life.

**Key words:** turkey, selenium source, serum biochemical parameters, lymphocyte subpopulations, growth, feed utilization

## Introduction

Selenium, as an important constituent of glutathione peroxidase (GPx) and other selenium-dependent enzymes, is involved in the antioxidant defense mechanism of cells and tissues in vertebrates (Surai 2002). GPx helps protect the integrity of unsaturated bonds of cell membrane phospholipids through regeneration of reduced glutathione and neutralization of free radicals (Rayman 2004). Selenium is also involved in enzyme systems regulating energy metabolism, sperm function, prostaglandin synthesis, essential fatty acid metabolism, purine and pyrimidine synthesis, and immunity (Surai 2000).

The bioavailability of organic selenium is usually higher, which results in higher antioxidant effectiveness due to, among others, increased retention of selenium in the organs and muscles of broiler chickens, and in eggs (Mahmoud and Edens 2003, Daun and Åkesson 2004, Rayman 2004). It was also found that Se-yeasts were more effective in Se-enrichment of turkey breast muscles than selenite, while both Se sources had the same positive effect on the oxidative stability of meat (Mikulski et al. 2009).

It is known that chick embryo tissues contain large amounts of highly polyunsaturated fatty acids in the lipid fraction (Speake et al. 1998) and therefore they need antioxidant defense (Surai 2000). The results of many experiments indicate that selenium plays a similar role to vitamin E in protecting egg yolk lipids (Paton et al. 2002, Surai 2002). One of the experiments showed that the supplementation of hen diets with selenium decreased the concentrations of thiobarbituric acid-reactive substances (TBARS) and increased GPx activity in the egg yolk (Yaroshenko et al. 2003). Therefore, dietary supplementation with selenium is used to increase the egg laying capacity of hens and egg hatchability (Payne and Southern 2005).

Some research results suggest that the dietary source and the level of selenium affect the development of chicken embryos (Paton et al. 2002) as well as the development of chicks in the first decade of their life (Surai 2000). Similar experiments, but on a smaller scale, were carried out on turkeys which differ from chickens with regard to the rate of embryonic and poults development. In view of the above, a study was conducted to determine whether organic selenium fed to turkey parent flocks can improve the health of poults.

## Materials and Methods

### Birds and diets

The experiment was carried out on an experimental poultry farm of the Department of Poultry Science at the University of Warmia and Mazury in Olsztyn.

The experimental materials comprised randomly selected 10 eggs, 10 day-old poults and 10 turkeys aged 28 days from parent flocks fed diets supplemented with 0.3 mg/kg inorganic selenium – sodium selenite (group Se<sub>M</sub>) or organic selenium – Sel-Plex<sup>®</sup> (group Se<sub>O</sub>). Older turkeys came from a growth trial conducted for five weeks, in which each group consisted of 120 BUT Big 6 turkeys kept in 24 cages, five birds per cage. During the rearing period, both groups received the same diet containing 0.3 mg/kg inorganic selenium.

### Blood and tissue sampling

On day 1 and 28, 10 birds were sacrificed by cervical dislocation, and blood samples were collected for biochemical analyses. On both days, samples of the liver and thigh muscles of turkeys were collected to determine the content of selenium and thiobarbituric acid reactive substances (TBARS).

### Biochemical analysis

Serum samples, obtained from blood collected in test tubes without anticoagulants, were assayed for the concentrations of total protein, albumin and uric acid, and for the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). The activities of AST, ALT and LDH were determined by the kinetic method, using commercially available BioSystems diagnostic kits and an A-25 biochemical analyzer. Serum lipid peroxidation was measured as thiobarbituric acid-reactive substances (TBARS) using the method of Mihara and Uchiyama (1978). Superoxide dismutase (EC 1.1.5.1, SOD) and glutathione peroxidase (EC 1.11.1.9, GPx) activities in erythrocyte lysates were assayed using kits from Randox Laboratories Ltd. (Crumlin, United Kingdom). Activity of SOD was measured using the methods of Woolliams et al. (1983), and activity of GPx – using the methods of Paglia and Valentine (1967).

### Lymphocyte analysis

The percentages of T lymphocyte subpopulations CD4<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>+</sup>CD8<sup>+</sup> and B lymphocyte subpopulations (IgM<sup>+</sup>) were determined in blood samples collected in anticoagulant tubes (EDTA-K<sub>2</sub>). One milliliter of blood from EDTA-K<sub>2</sub> was transferred to a microcentrifuge tube containing 1 ml PBS (Phosphate Buffered Saline) and 1% FCS (Fetal Calf Serum). The blood samples were placed onto 3 ml Histopaque – 1077 gradient (Sigma-Aldrich) and were

centrifuged in BD FALCON tubes at 400 x g, at room temperature, for 30 minutes. After centrifugation, the cloudy layer of mononuclear cells was delicately transferred into sterile tubes; it was rinsed twice in PBS with 1% FCS, and suspended in 1 ml PBS. A total of  $10^6$  cells from the suspension were placed in cytometry tubes (Beckman Coulter), and the following monoclonal antibodies (Southern Biotech) were added: Mouse Anti Chicken CD4-PE clone CT-4 (A0203-TH37U series, expiry date 2009-10), Mouse Anti Chicken CD8A-FITC clone 3-298 (D999-MF87B series, expiry date 2010-03), directed at the surface receptors of T lymphocytes, and Mouse Anti Chicken IgM-SPRD clone M-1 (L6203-VQ75V series, expiry date 2010-04) directed at the immunoglobulin receptor (BCR) of B lymphocytes ( $\text{IgM}^+$ ). The monoclonal antibodies were added as recommended by the manufacturer. The samples were incubated on ice for 30 minutes, in darkness. The cells were then rinsed twice in PBS with 1% FCS, and were centrifuged at 250 x g for 7 minutes at a temperature of 4°C, and the pellets obtained were suspended in 400  $\mu\text{l}$  PBS. The samples were analyzed in an Epics XL flow cytometer (Beckman Coulter). An immunophenotypic analysis of cells was performed using CXP software (Beckman Coulter).

### TBARS and selenium determination

The TBARS were determined by the method proposed by Draper and Hadley (1990). Sample absorbance was measured with a Specord 40 spectrophotometer (Analytik Jena AG), and TBARS levels were expressed in terms of nmol malondialdehyde in 1 g of meat. Selenium concentrations in liver, muscle and egg samples were determined based on calibration curves developed with the use of high-purity standards (ICP; Merck).

### Statistical analysis

The results obtained were validated statistically by a one-factorial analysis of variance (ANOVA) and Duncan's multiple range test with the use of Statistica 6.0 PL software. The effect of experimental factors was regarded as significant at  $P \leq 0.05$ .

### Results

Different selenium sources in diets fed to parent flocks had no significant effect on yolk selenium content (Table 1). Eggs from the  $\text{Se}_O$  flock had a significantly lower TBARS content (31.13 vs. 53.10 nmol/g,  $P > 0.001$ ).

Table 1. Selenium and TBARS content of the egg yolk in turkey parent flocks fed diets supplemented with inorganic ( $\text{Se}_M$ ) or organic selenium ( $\text{Se}_O$ ).

	Experimental group		SEM	p value
	$\text{Se}_M$	$\text{Se}_O$		
Selenium content, mg/kg dry matter	0.718	0.700	0.024	0.611
TBARS content, nmol/g	53.10 <sup>A</sup>	31.13 <sup>B</sup>	2.474	>0.001

No significant differences were found between the groups with respect to serum biochemical parameters of day-old birds (Table 2).  $\text{Se}_O$  group poult were characterized by higher whole blood glutathione peroxidase (7.54 vs. 5.92 U/mL,  $P = 0.001$ ) and erythrocyte superoxide dismutase activities (89.30 vs. 79.23 U/mL,  $P = 0.026$ ). There were no significant differences in the selenium and TBARS content of liver samples, while such differences were noted with regard to thigh muscles. The thigh muscles of  $\text{Se}_M$  group birds had significantly lower selenium concentrations (0.572 vs. 0.74,  $p = 0.045$ ) and a significantly higher TBARS content (65.01 vs. 38.42,  $p = 0.001$ ).

On day 28, differences between groups  $\text{Se}_M$  and  $\text{Se}_O$  were much smaller (Table 3), and concerned the activity of ALT (20.50 vs. 26.33,  $p = 0.05$ ) and SOD (87.29 vs. 100.02 U/mL,  $p = 0.005$ ).

No significant differences were observed between the groups regarding the percentages of T and B ( $\text{BCR IgM}^+$ ) lymphocyte subpopulations (Table 4). In comparison with younger birds, older turkeys were marked by similar levels of biochemical parameters and enzyme activities (Table 3), a lower percentage of T lymphocyte subpopulations, and a lower TBARS content of the liver.

Over the five-week experimental period, the final body weights of birds were comparable in both groups (1108 vs. 1135 g), and so were the feed conversion ratios (Table 5).

### Discussion

An experiment on laying hens showed that an increase in the selenium content of the diet from 0.2 to 0.6 mg/kg resulted in an increase in the selenium content of the egg yolk from < 400 to > 800 ng/g (Surai and Dvorska 2001). The results of other studies on laying hens also demonstrated that the selenium content of eggs is determined by the dietary level and source of this nutrient, and that organic selenium is more efficiently deposited in the egg yolk (Surai 2000, Paton et al. 2002). In the present experiment, yolk selenium content was similar in both groups. This may indicate that the acceptable content of Se in the diet (0.3 mg/kg) did not affect the utilization of this nutrient by turkeys.

Table 2. Metabolic indices of day-old turkeys.

	Experimental group		SEM	p value
	Se <sub>M</sub>	Se <sub>O</sub>		
Serum biochemical parameters				
Total protein, g/L	34.04	33.77	1.04	0.863
Albumins, g/L	10.86	11.71	0.80	0.471
Alanine aminotransferase (ALT), IU/L	17.71	19.29	1.17	0.367
Aspartate aminotransferase (AST), IU/L	1207	1256	36	0.362
Lactate dehydrogenase (LDH), IU/L	1140	1111	67	0.771
Uric acid, mg/dL	4.84	5.15	0.78	0.784
Glutathione peroxidase (GPx), U/mL	5.92 <sup>B</sup>	7.54 <sup>A</sup>	0.31	0.001
Superoxide dismutase (SOD), U/mL	79.23 <sup>B</sup>	89.30 <sup>A</sup>	2.95	0.026
Selected parameters of liver				
Selenium content, µg/g DM	1.310	1.620	0.078	0.111
TBARS content, nmol/g	80.19	79.37	11.01	0.959
Selected parameters of thigh muscles				
Selenium content, µg/g DM	0.572 <sup>b</sup>	0.740 <sup>a</sup>	0.031	0.045
TBARS content, nmol/g	65.91 <sup>A</sup>	38.42 <sup>B</sup>	5.05	0.001

Table 3. Metabolic indices of 28-day-old turkeys.

	Experimental group		SEM	p value
	Se <sub>M</sub>	Se <sub>O</sub>		
Serum biochemical parameters				
Total protein, g/L	31.33	31.45	1.53	0.962
Albumins, g/L	9.67	11.00	0.54	0.112
Alanine aminotransferase (ALT), IU/L	20.50 <sup>b</sup>	26.33 <sup>a</sup>	1.83	0.054
Aspartate aminotransferase (AST), IU/L	1124	1165	41	0.493
Lactate dehydrogenase (LDH), IU/L	1861	1909	80	0.682
Uric acid, mg/dL	6.53	7.22	0.49	0.357
Glutathione peroxidase (GPx), U/mL	6.63	6.33	0.581	0.719
Superoxide dismutase (SOD), U/mL	87.29 <sup>B</sup>	100.02 <sup>A</sup>	3.799	0.035
Selected parameters of liver				
Selenium content, µg/g DM	2.147	1.578	0.098	0.139
TBARS content, nmol/g	23.36	26.58	3.56	0.534
Selected parameters of thigh muscles				
Selenium content, µg/g DM	0.534	0.508	0.039	0.056
TBARS content, nmol/g	69.03	64.51	9.96	0.753

Table 4. Cellular immunity parameters in blood of turkeys hatched from eggs laid by hens fed diets supplemented with various selenium sources.

Age of turkeys, days	Parameters	Experimental group		SEM	p value
		Se <sub>M</sub>	Se <sub>O</sub>		
1	CD4 <sup>+</sup>	23.93	22.37	2.01	0.593
	CD8 <sup>+</sup>	2.629	2.457	0.271	0.662
	CD4 <sup>+</sup> CD8 <sup>+</sup>	0.686	0.600	0.075	0.436
	BCR IgM <sup>+</sup>	0.049	0.049	0.008	1.00
28	CD4 <sup>+</sup>	12.70	18.17	2.32	0.134
	CD8 <sup>+</sup>	0.98	1.47	0.30	0.287
	CD4 <sup>+</sup> CD8 <sup>+</sup>	0.052	0.073	0.011	0.202
	BCR IgM <sup>+</sup>	0.100	0.090	0.023	0.773

Table 5. The growth performance of turkeys originating from parent flocks fed diets supplemented with inorganic (Se<sub>M</sub>) or organic selenium (Se<sub>O</sub>).

	Experimental group		SEM	p value
	Se <sub>M</sub>	Se <sub>O</sub>		
Body weight, g				
14 days	317	323	4	0.268
28 days	1108	1135	14	0.165
35 days	1787	1830	19	0.124
Feed conversion ratio, kg/kg				
1 – 14	1.564	1.535	0.023	0.367
15 – 28	1.758	1.773	0.030	0.723
29 – 35	1.712	1.686	0.026	0.475
1 – 35	0.689	0.685	0.010	0.806

Despite comparable selenium content of eggs, significant differences were found in the TBARS values determined in the yolk, pointing to lower levels of lipid oxidation products in eggs from hens fed diets supplemented with organic selenium. The above was most probably due to a higher content of vitamin E – the most important antioxidant found in eggs. As demonstrated by other authors, vitamin E accumulation in the egg yolk reflected its level in the breeder diet and varied with selenium supplementation, whereas dietary organic selenium significantly increased vitamin E levels in the yolk. In view of the above findings, it seems that in our study we observed a sparing effect of organic selenium on vitamin E, as suggested by Surai (2000).

In the present experiment, maternal organic selenium supplementation affected selected physiological parameters in the offspring, including an increase in the serum levels of GPx and SOD, a statistically non-significant increase in the selenium content of the liver, a significant increase in the selenium content of thigh muscles ( $p = 0.045$ ), and a significant decrease in the TBARS content of thigh muscles. The above results are consistent with those reported for chickens (Mahmound and Edens 2003). Payne and Southern (2005) demonstrated that most of the absorbed selenium (89% organic Se vs. 77% inorganic Se) is hustled through the liver, thus leading to increased selenium retention in the liver (Surai 2000) and muscles of chickens and turkeys (Yoon et al. 2007, Mikulski et al. 2009). In a study by Karadas et al. (2004), selenium levels in the maternal diet affected selenium concentrations in the tissues of postnatal quails. Newly-hatched quails from selenium-enriched eggs contained 3.5-fold and two-fold more selenium in the liver and in other tissues, respectively, compared with quails hatched from normal eggs. Similar trends were also observed in other experiments on chickens hatched from eggs with different selenium content (Pappas et al. 2005). Elevated selenium concentrations in tissues increase the antioxidant pool which in turn enhances the activity of antioxidant enzymes and reduces the levels of lipid oxidation products (TBARS). In an experiment by Yoon et al. (2007), selenium concentrations in diets were increased from 0.1 to 0.3 mg/kg, thus significantly ( $P < 0.05$ ) increasing blood GPx activity.

In our study, more pronounced differences in selenium and TBARS content were noted in thigh muscles, in comparison with the liver. As suggested by other authors, leg muscles subjected to excess load most probably need more potent antioxidant protection than the liver. Daun and Åkesson (2004) reported that red thigh muscles are characterized by a higher selenium content and higher GPx activity than white breast muscles. Mahmound and Edens (2003) demonstrated that organic selenium was more

effective than inorganic selenium in increasing GPx activity in the blood, but not in the liver of chickens. Another experiment showed that MDA accumulation in the liver of day-old and 5-day-old chickens from antioxidant-supplemented hens was significantly reduced, thus indicating that liver susceptibility to lipid peroxidation substantially decreased in postnatal development (Surai 2000). This could be the case also in our study, as suggested by a nearly four-fold lower TBARS content of the liver in 28-day-old turkeys, compared with day-old birds.

No significant differences were noted in the values of the analyzed biochemical parameters of the blood, liver and thigh muscles of turkeys aged 28 days. In previous studies on chickens, the positive effect of Se and vitamin E supplementation of the maternal diet was observed at 5 and 10 days of age when vitamin E concentrations in the liver and plasma were significantly elevated, compared with those of the control group (Surai 2000). This study showed that one of the important features of chick postnatal development is vitamin E depletion in the liver. Selenium supplementation of the maternal diet increased vitamin E levels in the liver and plasma of day-old chicks, and the difference was maintained through 10 days of postnatal development.

It is known that the first two weeks post-hatch represent the most important period of immune system development, and the maternal diet has been shown (Klasing 1998) to have a profound effect on this process. In the avian immune system, a central role is played by CD4<sup>+</sup> cells which, similarly as in mammals, are involved in the initiation and maintenance of the immune response of birds. CD8<sup>+</sup> cells are known to be effector cells in cytotoxic response, by killing infected target cells (Arstila et al. 1994, Sharma 1997, Rumińska et al. 2008). Selenium has been shown to stimulate the transformation of T lymphocytes to cytotoxic cells (Kiremidjian-Schumacher et al. 1994). Vitamin E supplementation exerts a similar immunomodulatory effect on CD4<sup>+</sup>CD8<sup>+</sup> T cells (Erf et al. 1998). In the present study, different selenium sources in the maternal diet had no effect on the analyzed parameters of cell-mediated immunity in poults, as shown by small differences in the percentages of T lymphocyte subpopulations CD4<sup>+</sup>, CD8<sup>+</sup> and CD4<sup>+</sup>CD8<sup>+</sup> and B lymphocyte subpopulations (BCR IgM<sup>+</sup>). The levels of T lymphocyte subpopulations were considerably higher in day-old poults than in turkeys aged 28 days. Also in chickens, the first week of life is a period of rapid expansion of leukocyte populations (Klasing 1998).

In our study, the source of selenium in diets fed to parent flocks had no effect on the growth performance of poults and feed conversion ratio during the first five weeks post-hatch. This is consistent with the results reported for broiler chickens fed diets supple-

mented with 0.1 – 0.3 mg/kg selenium (Ryu et al. 2005, Yoon et al. 2007).

The results obtained show that the dietary supplementation of organic selenium in turkey parent flocks reduces the rate of oxidation processes in the egg and in the tissues of newly-hatched poults, yet it has no effect on the percentages of T and B lymphocyte subpopulations and the growth performance of birds during the first five weeks of their life.

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