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The Effect of Organic Selenium Supplementation on the Broilers' Immune Response

Research Article

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ABSTRACT

The aim of this study was to evaluate the effect of organic selenium supplementation on the immune response of broilers vaccinated against coccidiosis, Infection Bursal Disease Virus (IBDV) and Newcastle Diseases Virus (NDV). The study included three treatments (T), T1= control (inclusion of inorganic selenium in a basal diet), T2= basal diet + 0.2 ppm of organic selenium as selenomethionine and selenocysteine, T3= basal diet + 0.4 ppm of organic selenium as selenomethionine and selenocysteine. Birds supplemented with organic selenium showed higher numbers of $CD3^+$ cells in duodenum and less severe ileal lesions compared to birds in the control group. Organic selenium supplementation in the diet had no significant, except for 14 to 21 days of age, effect on broiler weight gain.

KEY WORDS broiler, $CD3^+$ cells, immune system, organic selenium, performance.

INTRODUCTION

The animal nutrition industry is searching for more suitable diet formulations and dietary supplements to provide better animal nutrition at lower production costs. Most nutrients required for normal metabolic functions are either not endogenously synthesized or their synthesis is insufficient, and therefore must be continuously supplied in the diets. Standard feed supplements have been used over the years, but they do not properly supply the requirements of many nutrients when immune competence and not only animal performance is considered (Karadas and Surai, 2004). Considering essential nutrients, trace minerals (particularly selenium) are critical to maintain poultry health and performance.

Selenium is essential for animals due to its roles as metabolism regulator, adequate body development and reproductive performance, and aiding the immune system to neutralize free radicals and protect the body against infections (Surai, 2000; Rayman, 2000; Surai, 2002).

Despite being essential for animal metabolism, selenium levels in almost all feedstuffs are not sufficient to supply animal requirements.

Selenium is usually supplemented in broiler diets in its inorganic form (sodium selenite). However, this salt is very toxic, and needs to be solubilized in its ionic form in order to be absorbed in gastrointestinal tract.

In addition, the electric charges of this ionic form may interact with other diet components (minerals, proteins and carbohydrates), rendering them partially unavailable to animals (Rutz *et al.* 2004).

This has stimulated research on the use of organic selenium in animal diets to improve its bioavailability. This study aimed at evaluating the effect of organic selenium dietary supplementation on the immune response of broilers vaccinated against viral diseases and coccidiosis.

MATERIALS AND METHODS

A total number of 150 broiler chickens distributed in a completely randomized experimental design into 3 treatments with 50 replicates each, and one bird per replicate. The following treatments were applied: T1= control diet (inorganic selenium added to the basal diet), T2= diet supplemented with 0.2 ppm of organic selenium as selenomethionine and selenocysteine, T3= diet supplemented with 0.4 ppm of organic selenium as selenomethionine and selenocysteine.

Birds were individually identified by a wing band and housed in experimental pens from 01 to 25 days of age. Birds received feed and water *ad libitum* during the entire experimental period. The nutritional levels of diets (Table 1) were analyzed according to the methodology of the Brazilian Compendium of Animal Feeding (2005).

Broilers were weighed at one, seven, 14, 21 and 25 days of age. On days 7 and 14, each birds received by eye drop 0.03 mL of commercial vaccines against Newcastle Disease Virus (NDV) HB1 strain (New-Vaxin®-Schering-Plough), containing $10^{5.5}$ infective doses, and against Infection Bursal Virus Disease (IBVD) HVT-FC 126 strain (Gumborek®-Schering-Plough), containing $10^{3.0}$ infective doses. On day 7, broilers were also orally vaccinated against coccidiosis with a commercial vaccine (Bio-CoccivetR®-Biovet) containing approximately 400 viable oocysts of *E. acervulina*, *E.brunetti*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. tenella* and *E. mitis*.

Blood was weekly collected from the jugular vein and placed in duly identified tubes with anticoagulant (heparin) for serum analysis.

The following assays were performed in the serum: total protein level by the biuret method, albumin level by Bromocresol Green method, and globulin level was calculated as the mathematical difference between total protein and albumin. These assays were performed using Automatic Biochemical System CELM SBA-200. Total erythrocytes, hematocrit, hemoglobin, and plasma total protein were determined in total blood, as well as the white series, in which total leukocytes were determined and differentiated in heterophils, lymphocytes, monocytes, eosinophils and basophils. Antibody titers were determined using a commercial ELISA (Enzyme Linked Immuno Sorbent Assay) kit (Idexx®) and read in Biotek EL800® micro plate reader. NDV antibody titers were determined by hemagglutination-inhibition (HI) test performed according to Cunnigham (1971). On days 21 and 25, five birds per treatment were euthanized using barbiturates, and necropsied to evaluate coccidiosis by lesion scoring, according to Johnson and Reid (1970). Liver, duodenum, and cecum samples were collected in buffered formalin solution (10%) for histological, morphometric and immunohistochemistry (only duodenum and cecum) analyses.

Duodenum and cecum samples were processed for histology and stained with hematoxylin-eosin. Villi height and crypt depth were measured according to Maiorka *et al.* (2000), using an image-analyzing system (Motic Images Plus 2.0) coupled to a microscope (Olympus BH2).

Liver samples were analyzed for normal morphology and presence of lymphoid clusters. Sections with lymphoid clusters were quantified with a microscope (Olympus BH2) at 10X magnification. Sections for immunohistochemistry were dewaxed and re-hydrated. Antigen was retrieved using citrate buffer at pH 6.0 and blocking of endogenous peroxidase with hydrogen peroxide at 3% and blocking protein, Anti-CD3, diluted at 1:300, was the primary antibody used. Anti-mice and anti-rabbit secondary antibodies were combined in the same amplification system and used to detect the reaction. Intra-epithelial $CD3^+$ cells in duodenum villi tips were counted and expressed as number of $CD3^+$ per field (100X). The presence lymphoid clusters were analyzed in cecal tissues.

Results were analyzed using statistical program Statistix for Windows Copyright (C) 1985. Antibody titers measured by ELISA and hemaglutination-inhibition tests were Log10 transformed. Data were submitted to analysis of variance (ANOVA) at $P<0.05$ and means were compared by the test of Tukey.

RESULTS AND DISCUSSION

There were no significant differences in weight gain among the treatments in first week of experimental week (Table 2). However, between 14 and 21 days, weight gains were significantly different (P=0.02), showing a positive association between weight gain and increased concentrations of organic selenium in diet, but again no differences were observed on day 25. On day 25, Inflammatory lesions scores of the intestine were linearly reduced in birds supplemented with organic selenium (Table 3). Ileal lesions were more frequent and severe in birds not supplemented with organic selenium (Table 4).

In the cecum, no gross inflammatory lesions were observed in birds necropsied at 21 and 25 days of age. No statistical differences were observed in hematological parameters and in vaccine titers against NDV and IBDV between the different treatments (Tables 5 and 6). The titration of vaccine antibodies against NDV obtained with hemaglutination-inhibition test was similar to the results obtained with ELISA.

The presence of lymphoid clusters was significantly higher (P=0.002) in the liver of birds supplemented with organic selenium than the control group (Table 7).

Table 1 The chemical compositions of the experimental diets

Composition %	Starter		Grower	
	Bromatological	Estimated	Bromatological	Estimated
Protein	23.94	22.989	23.46	21.037
Lipid	7.57	-	6.95	-
Mineral residue	5.44	-	5.62	-
Fiber	3.04	-	2.69	-
Calcium	0.97	0.909	0.93	0.889
Total Phosphorous	0.68	0.744	0.64	0.665
Available Phosphorous	-	0.459	-	0.397
Sodium	0.13	0.186	0.13	0.180
Potassium	-	0.929	-	0.848
chloride	-	0.305	-	0.298
Triptophane	-	0.252	-	0.228
Lysine	-	1.166	-	1.051
Metionine	-	0.542	-	0.469
Met. + Cys.	-	0.870	-	0.774
Treonine	-	0.796	-	0.728
Arginine	-	1.512	-	1.373
Leucine	-	1.830	-	1.698
Isoleucine	-	0.930	-	0.846
Histidine	-	0.568	-	0.522
Valine	-	1.007	-	0.924
Fenilalanine	-	1.054	-	0.965
ME kcal/kg	-	3001.7	-	3192.3
Vitamin premix[1]	-	0.1	-	0.09
Mineral premix[2]	-	0.05	-	0.045
Selenium level of basal diet (SLBD)	0.06 mg/kg	-	0.20 mg/kg	-
SLBD + 0.2 ppm Se org	0.23 mg/kg	0.2 mg/kg	0.27 mg/kg	0.2 mg/kg
SLBD + 0.4 ppm Se org	0.39 mg/kg			

[1] Vitamin premix (1 kg/ton): vitamin A: 8000000 IU; vitamin D3: 2000000 IU; vitamin E: 16000 IU; folic acid: 800 mg; phantotenic acid: 10000 mg; biotine: 60 mg; vitamin B_2: 4000 mg; vitamin B_6: 2000 mg; BHT: 100 mg; vitamin B_1: 1500 mg; vitamin B_3: 2000 mg; vitamin B_{12}: 10000 mg and niacine: 30000 mg.
[2] Mineral premix (0.5 kg/ton): Zinc: 110000 mg; Selenium: 360 mg; Iodo: 1400 mg; Copper: 20000 mg; Manganese: 156000 mg and Iron: 96000 mg.

Table 2 Effect of supplementing a basal feed with organic selenium during different periods of growth on body weight gain (g) of broiler chicks

Treatments	Weight gain (g)				
	1 to 7 days	7 to 14 days	14 to 21 days	21 to 25 days	1 to 25 days
Control	116.2±19.3	305.5±49.0	398.5±51.3[b]	272.8±46.8	1079.9±133.4
Selenium 0.2 ppm	114.4±17.3	308.6±40.6	411.1±49.7[ab]	272.2±74.2	1101.0±126.5
Selenium 0.4 ppm	111.1±17.5	317.5±49.2	428.3±51.8[a]	272.8±52.7	1132.6±103.0
P-value	0.374	0.411	0.020	0.990	0.156

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effect of supplementing a basal feed with organic selenium during different periods of growth on scores of macroscopic inflammatory lesions in the duodenum and jejunum of broiler chickens (Mean±SD)

Age	21 days		25 days	
Treatments	Duodenum	Jejunum	Duodenum	Jejunum
Control	1.40±1.34	0.200±0.44	1.00±0.71	0.60±0.55
Selenium 0.2 ppm	0.60±1.34	0.200±0.44	0.60±0.55	0.20±0.45
Selenium 0.4 ppm	1.00±1.22	0.00±0.00	0.40±0.55	0.20±0.45
P-value	0.635	0.618	0.315	0.351

Table 4 Effect of supplementing a basal feed with organic selenium during different periods of growth on percentage (%) of macroscopic alterations in ileum of broiler chickens

Treatments			ILEUM (%)			
	Food undigested		Muco hemorrhagic digesta		Inflammation of Peyer's patches	
	21 days	25 days	21 days	25 days	21 days	25 days
Control	-	80	80	20	80	20
Selenium 0.2 ppm	20	-	-	-	40	80
Selenium 0.4 ppm	20	20	-	-	20	60

In the duodenum, villi height and crypt depth showed statistical differences (P<0.05) among the different treatments (Table 8).

Table 5 Effect of supplementing a basal feed with organic selenium during different periods of growth on titration of vaccine antibodies for Newcastle disease in broilers by ELISA test (Mean±SD)

Treatments	7 days	14 days	21 days
	Titer (log10)		
Control	2.8±0.9	2.4±0.2	2.3±0.5
Selenium 0.2 ppm	2.9±0.5	2.6±0.4	2.5±0.9
Selenium 0.4 ppm	3.2±0.5	2.3±0.5	2.9±0.3
P-value	0.650	0.772	0.406

Table 6 Effect of supplementing a basal feed with organic selenium during different periods of growth on titration of vaccine antibodies for Gumboro disease in broilers by ELISA test (Mean±SD)

Treatments	7 days	14 days	21 days
	Titer (log10)		
Control	3.60±0.10	3.10±0.20	2.60±0.30
Selenium 0.2ppm	3.60±0.00	3.10±0.10	2.40±0.10
Selenium 0.4ppm	3.50±0.00	2.80±0.10	2.40±0.20
P-value	0.132	0.118	0.488

Table 7 Effect of supplementing a basal feed with organic selenium during different periods of growth on lymphoid clusters in broilers' liver (Mean±SD)

Treatments	Lymphoid clusters[1]	
	21 days	25 days
Control	4.0±2.2	2.6±1.5[b]
Selenium 0.2 ppm	3.0±1.9	6.8±1.7[a]
Selenium 0.4 ppm	6.0±3.6	6.4±1.5[a]
P-value	0.425	0.002

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Villi were higher in the control group than in the supplemented groups and decreased as dietary selenium increased. Crypts were only different when collected at 21 days of age. In the cecum (Table 9), villi were higher in birds fed the diets containing organic selenium, with the deepest crypt depth obtained in the treatment with 0.2 ppm organic selenium.

The number of $CD3^+$ cells in the duodenum was higher in broilers fed diets containing organic selenium. On day 25, the number of these defensive cells was proportionately higher when the concentration of selenium in the diet increased (Table 10). In the cecum, the number of $CD3^+$ cells were not significantly different; so lymphoid clusters only tended to be higher in birds supplemented with organic selenium.

Change in body weight of broiler chicks, except for period from 14 to 21 days when broilers chicks were in challenge with viral and coccidiosis vaccines, was not statistically influenced by the dietary treatments.

This suggests that, when there is homeostasis, the amount of selenium in the basal diet is usually sufficient to allow good live performance, but when there is stress or inflammatory response, the body requires higher amounts of nutrients to supply their requirements, as suggested by Koutsos and Klasing (2001).

Experiments performed by Rutz *et al*. (2003) and Anciuti *et al*. (2004) demonstrated that the inclusion of organic selenium in the diet of broiler chickens also promoted higher weight gain.

Duodenal villi heights were significantly lower in birds supplemented with organic selenium compared to those fed inorganic selenium.

Also, crypts of broilers fed organic selenium were shallower at 21 days and deeper at 25 days than those fed the control diet. According to Maiorka *et al*. (2003), birds with higher turnover of intestinal mucosa cells present deeper crypts because of their high mitotic activity. However, when the rate of mitosis increases and the rate of extrusion is absent, reduced or maintained, the number of cells increases, and therefore villi become higher (Maiorka *et al*. 2002). Santin *et al*. (2001) also suggested that the villi height is directly related to better nutrient digestion and absorption capacity and bird development. In the present study, increase in villi height and crypt depth may also be related to the higher presence of $CD3^+$ cells observed in the duodenal mucosa of birds supplemented with organic selenium. CD3 is expressed by all lymphocytes and is the main surface antigen of T lymphocytes during their life cycle (Barua and Yoshimura, 2004). The accumulation of immune cells in the submucosa may change villi morphology, and therefore their height may not be directly related to better nutrient absorption. It was also verified that birds supplemented with organic selenium also presented less lesions and undigested food in ileum compared to the control group.

It can be speculated that the higher amount of $CD3^+$ cells may protect the duodenal mucosa. According to Leng *et al*. (2003), selenium improves the immune status of chickens, enhancing the capacity of immune-competent cells to respond to antigenic stimuli. This positive correlation between quantity of immune cells in the gastrointestinal tract and dietary organic selenium level was also observed by Leng *et al*. (2003) and Arthur *et al*. (2003) in broiler chickens and mice, respectively.

The authors suggest that this effect is due to the antioxidant action of selenium, which has an important role in the immune response. During the process of phagocytosis, some immune cells, such as heterophils and macrophages, produce free radicals that may affect biological molecules and cause cell damage. Free radicals may also modify the function of phagocytes and consequently, adaptive immune

Table 8 Effect of supplementing a basal feed with organic selenium during different periods of growth on duodenum histology, villi and crypts' measurement (µm) in broiler chicks (Mean±SD)

Treatments	Duodenum[1]			
	Villi (µm)		Crypts (µm)	
	21 days	25 days	21 days	25 days
Control	947.0±219[a]	1046.7 ± 140[a]	122.3±23[a]	64.3±15[b]
Selenium 0.2 ppm	847.3±210[ab]	989.3±141[ab]	115.6±18[ab]	58.5±91[b]
Selenium 0.4 ppm	842.5±218[b]	949.8±112[b]	105.4±13[b]	74.6±11[a]
P-value	0.025	0.015	0.003	0.000

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 9 Effect of supplementing a basal feed with organic selenium during different periods of growth on cecum histology, villi and crypts' measurement (µm) in broiler chicks (Mean±SD)

Treatments	Cecum[1]			
	Villi (µm)		Crypts (µm)	
	21 days	25 days	21 days	25 days
Control	131.1±11[c]	70.2±28[b]	70.5±13	81.9±17[ab]
Selenium 0.2 ppm	148.5±15[b]	112.1±45[a]	76.4±14	91.2±35[a]
Selenium 0.4 ppm	161.7±27[a]	113.8±32[a]	74.2±15	74.0±16[b]
P-value	0.000	0.000	0.269	0.029

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 10 Effect of supplementing a basal feed with organic selenium during different periods of growth on quantification of CD3+ cells in duodenum and lymphoid clusters in cecum (µm) of broiler chicks (Mean±SD)

Treatments	Immunohistochemistry[1]			
	Duodenum		Cecum	
	21 days	25 days	21 days	25 days
Control	15.1±3.3[b]	17.2±3.6[b]	1.6±0.5	1.8±0.8
Selenium 0.2 ppm	23.0±4.9[a]	21.5±5.7[b]	2.8±0.8	2.6±0.5
Selenium 0.4 ppm	21.0±3.4[a]	306±36[a]	2.2±0.8	2.0±0.7
P-value	0.002	0.000	0.078	0.218

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

response, with reduced production of cytokines and proliferation of lymphocytes and antibodies, undermining the immune competence of animal in the absence of selenium (Karadas and Surai, 2004). There are several studies showing the association between selenium and immune system (Surai, 2002; Rayman, 2000). However, when evaluating selenium influence through hematological and serum levels, this correlation is not that clear. In the present study, the supplementation of diets with organic selenium did not significantly change vaccine antibody titers. The same results was observed in the experiment of Kindlein *et al.* (2007), when layers supplemented with 0.3 ppm of selenium and vaccinated against *Escherichia coli* and avian encephalomyelitis showed no significant difference in antibody titers compared to those not supplemented with this mineral. Similarly, Daza *et al.* (2000) reported that piglets supplemented with selenium (0.3 ppm) showed normal range of hematological parameters with no significant difference compared to the non-supplemented group, suggesting that these parameters may not be adequate for evaluating the influence of selenium on the immune response of animals.

CONCLUSION

Broiler chickens supplemented with organic selenium showed higher presence of $CD3^+$ cells in duodenum and reduced severity of intestinal lesions compared to the control group. Influence of organic selenium on body weight gain of broiler chicks was only positive when the birds were in challenge with viral and coccidiosis vaccines.

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