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The Impact of Organic and Inorganic Selenium on the Immune System of Growing Broilers Submitted to Immune Stimulation and Heat Stress

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■ Keywords

Antibody, broiler, infectious bursal disease, performance, selenium.

ABSTRACT

One to 42-d-old (432) female broilers were fed different levels of inorganic selenium (ISe) and organic selenium (OSe), according to the following treatments: (1) 0.3 mg ISe; (2) 0.3 mg ISe + 0.2 mg OSe; (3) 0.5 mg ISe and (4) 0.3 mg OSe/ kg of feed. All birds were vaccinated against infectious bursal disease (IBD) at 19d of age and three birds/replicate (R) were inoculated with sheep red blood cells (SRBC) at 32d. Three other birds/R received Freund's adjuvant at 37d and avian tuberculin (AT) in the wattle at 47d of age. All birds were submitted to heat stress after 21d. Performance parameters, bursa and spleen weights, lymphocyte bursa depletion, antibody (Ab) production against IBD and SRBC, hematocrit, leukocytes, heterophil/lymphocyte ratio (H/L), and cellular reaction to AT were evaluated. The contrast analysis showed that OSe has improved feed intake (FI) between day 1 and 42 ($p<0.10$). Birds fed ISe presented worse H/L ratio ($p<0.10$), but higher Ab titers against IBD ($p<0.04$) and SRBC ($p<0.05$) than birds fed OSe, but OSe supplemented birds showed lower lymphocyte depletion scores in the bursa. The higher FI promoted by OSe may be beneficial when rearing broilers in hot weather. The use of ISe induced higher humoral immune response.

INTRODUCTION

Nutrient requirements for performance are well established in broilers, but not for efficient immune response. The additive effect of selenium (Se) and vitamin E deficiencies has been traditionally associated with muscular dystrophy and exudative diathesis in poultry due to their antioxidant action and capacity to protect the cell membrane (Finch & Turner, 1996; Edens, 2001; Payne & Southern, 2005). Selenium is involved in antibody production, and stimulates phagocytosis and chemotaxis of macrophages and neutrophils, depending on the pathogen and on the levels of vitamin E in the diet. It is an essential component of enzyme glutathione peroxidase (GPx), and plays a major role against diseases (Kidd, 2004). It was shown that, in E. tenella infections, Se was related to lower broiler mortality and less cecal lesions due to its capacity of protecting leukocytes against normal phagocytosis at the site of infection (Colnago et al.,1984). Selenium also acts in the metabolism of Cys and Met, and in the synthesis of thyroid hormones (Dahlke et al., 2005). In humans and animals, Se deficiency has been associated with liver necrosis, poor feathering, and cancer (Edens, 2001). Selenium is found in forages and grains, and its availability varies according to location and climate (Finch & Turner, 1996). Corn-soybean meal diets are usually supplemented with Se, and feeding recommendations for broilers range from 0.15 (NRC, 1994) to 0.33 mg/kg (Rostagno, 2005). Inorganic sources of selenium (ISe), particularly sodium selenite (Na_2SeO_3) were added to broilers feeds until recently, when organic Se sources became commercially available. When

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selenium is chelated to amino acids (Se-AA), such as methionine-associated Se (Se-Met), it is better absorbed and more available (Surai, 2000; Edens & Gowdy 2004) than ISe, and is considered an "organic" source (OSe). Although ISe can be used for the biosynthesis of selenoproteins, it is incorporated into body proteins only in the form of Se-AA because Met and Se-Met become analogs when sulfur is replaced by Se in the molecule, and therefore, are not differentiated by the genetic code that regulates this incorporation. Se-Met can also be used to synthesize body proteins (Daniels, 1996; Shcrauzer, 2000). Hydrogen selenide (H_2Se), a byproduct of Na_2SeO_3 (ISe) metabolism, is produced before Se is incorporated into body proteins, producing oxygen-reactive compounds in a fast recycling and diffusion process between the plasma and red blood cells. Se-AA sources are also converted into H_2Se, but their use in the synthesis of proteins other than body proteins reduces the damage from pro-oxidative effects (Leng et al., 2003).

The present experiment studied the effects of dietary Se level and source on the immune status of broilers challenged by heat stress (HS) and a vaccine against Infectious Bursal Disease by evaluating humoral and cell-mediated immune responses.

MATERIAL AND METHODS

Birds and treatments

In this experiment, 432 one-d-old female Ross 308 chicks were used. Birds were housed in 36 metal cages, in a climate-controlled room, and received 24 hours/day of light throughout the experiment. Bird performance was measured from 1 to 42 d. The experiment was divided in a starter (1 to 21 d) and a grower phase (22 to 42 d). However, in order to evaluate wattle-cell reaction (reaction to avian tuberculin), some birds were maintained until day 48.

The experimental diets (Table 1) were based on a basal diet, which was formulated using the Brazilian Tables for Poultry and Swine (Rostagno, 2005) and to which Se levels and sources (inorganic or organic) were added to make up four treatments: 0.3 mg/kg ISe, 0.3 mg/kg ISe + 0.2 mg/kg OSe, 0.5 mg/kg ISe, or 0.3 mg/kg OSe. The inorganic selenium source contained 45% sodium selenite, and the organic selenium source was Sel-Plex®, a yeast-based commercial product containing at least 50% Se-Met (Edens, 2001). Both sodium selenite and Sel-Plex® were diluted in rice hulls, mixed into 3kg of the basal feed, and then added to the final feed batches. Vitamin E levels in the basal diet were 40 mg/kg (starter phase) and 25 mg/kg (grower phase). The commercial mineral premix used did not contain Se. Each of the four treatments had nine replicates (cages) of 12 birds each. Birds were fed the experimental diets and water ad libitum from 1 day of age. During the grower phase, birds were submitted daily to cyclic heat stress (CHS), consisting of 12 hours at 24ºC, three hours of a gradual increase from 24 up to 30-31ºC, six hours between 30 and 31ºC, and three hours of gradual decrease, from 30-31 down to 24ºC. Relative air humidity was kept at 74.1% ± 10.3%. All methods used in this experiment followed the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Immune stimulation

All birds were submitted to the following regime of immune stimulation:

1) vaccines administered at the hatchery, on day 1, against Marek's Disease, Fowl Pox and Infectious Bronchitis;
2) vaccination against Infectious Bursal Disease (IBD) at 19d of age;
3) i.v. inoculation of 10% SRBC at 32d of age; and
4) i.m. inoculation of complete Freund's adjuvant at 37d of age and avian tuberculin at 47d of age.

The vaccine against IBD (Winterfield strain, CEVAC IBD-L®, a live, freeze-dried vaccine) was added to the drinking water supplied to all birds, according to the manufacturer's instructions. Complete Freund's adjuvant (containing 4 mg/mL of killed Mycobacterium avium) was administered i.m at 0.5mL in three birds/replicate in order to sensitize them to a subsequent intradermal administration of 0.01mL of avian tuberculin in the wattle to measure cellular immunity.

Measurements

Birds were weekly weighed and their feed intake was measured to calculate feed conversion ratio (FCR), considering cage as replicate. At 19, 29, 35, and 42d, blood samples were collected from three birds per replicate for IBD antibody determination (not the same birds were collected at all ages). On day 42, blood samples from other three birds per replicate were collected for SRBC and hematological profile analysis, measuring hematocrit, total leukocytes and their subtypes: lymphocytes, heterophils, monocytes, eosinophils, basophils, and heterophil:lymphocyte (H/L) ratio. On the same day, three birds per replicate were euthanized by cervical dislocation, weighed, and

Table 1 - Composition of feedstuffs and nutritional levels of the experimental diets.

	Starter Phase				Grower Phase			
	Se Levels (mg/kg of diet) and Sources							
Feedstuffs, (%)	**0.3 ISe**	**0.3 ISe + 0.2 OSe**	**0.5ISe**	**0.3OSe**	**0.3 ISe**	**0.3 ISe + 0.2 OSe**	**0.5ISe**	**0.3OSe**
Ground corn	57.44	57.42	57.44	57.41	65.24	65.22	65.24	65.21
Soybean meal 45%	35.62	35.62	35.62	35.62	28.55	28.55	28.55	28.55
Phosphate	1.67	1.67	1.67	1.67	1.41	1.41	1.41	1.41
Limestone	1.12	1.12	1.12	1.12	1.00	1.00	1.00	1.00
Soybean oil	3.00	3.00	3.00	3.00	2.6	2.6	2.6	2.6
Salt	0.48	0.48	0.48	0.48	0.464	0.464	0.464	0.464
DL-Methionine	0.27	0.27	0.27	0.27	0.25	0.25	0.25	0.25
L-Lysine	0.16	0.16	0.16	0.16	0.27	0.27	0.27	0.27
L-Threonine	0.026	0.026	0.026	0.026	0.017	0.017	0.017	0.017
Choline Cl 60%	0.011	0.011	0.011	0.011	-	-	-	-
Monensin 20%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral Premix[1]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin Premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium selen 45 %[3]	0.000067	0.000067	0.000111	-	0. 000067	0. 000067	0.000111	-
Sel-Plex[4]	-	0.02	-	0.03	-	0.02	-	0.03
Total	100	100	100	100	100	100	100	100
Nutrient Calculation								
CP, %	21	21	21	21	18.5	18.5	18.5	18.5
ME, kcal/kg	3050	3050	3050	3050	3125	3125	3125	3125
Calcium, %	0.865	0.865	0.865	0.865	0.752	0.752	0.752	0.752
Available phosphorus, %	0.434	0.434	0.434	0.434	0.377	0.377	0.377	0.377
Sodium, %	0.21	0.21	0.21	0.21	0.203	0.203	0.203	0.203
Chlorine, %	0.33	0.33	0.33	0.33	0.323	0.323	0.323	0.323
Dig Lys, %	1.15	1.15	1.15	1.15	1.071	1.071	1.071	1.071
Dig Met., %	0.559	0.559	0.559	0.559	0.512	0.512	0.512	0.512
Dig Met+Cys %	0.85	0.85	0.85	0.85	0.778	0.778	0.778	0.778
Dig threonine%	0.74	0.74	0.74	0.74	0.642	0.642	0.642	0.642
Choline, mg/kg	1400	1400	1400	1400	1188	1188	1188	1188
Selenium, mg/kg	0.3	0.5	0.5	0.3	0.3	0.5	0.5	0.3

1 - Composition per kg of diet: Mn, 150,000 mg; Zn, 140,000 mg; Fe, 100,000 mg; Copper, 16,000 mg; Iodine, 1,500 mg. 2 - Composition per kg of diet: Vit A, 8,000 KIU; Vit D3, 2,000 KIU; Vit K3, 1,800mg; Vit B1, 1,800 mg; Vit B2, 6,000 mg; Vit B6, 2,800 mg; Vit B12, 12,000 mcg; Pantothenic Acid, 10,000 mg; Niacin, 40,000 mg; Folic Acid, 1,000 mg; Biotin, 60,000 mcg. Vit E, 40,000 mg/kg, in the starting phase, and 25,000 mg/kg in the growing phase. 3 - Sodium selen 45 %= 45% sodium selenite. 4 - SelPlex, 0.1% Se; produced by Alltech Biotechnology, Lexington, Kentucky, USA

had their spleens and bursas collected and weighed. The bursas were also measured using a bursometer, and then identified and fixed in 10% formalin. The remaining birds in each replicated were reared until day 47, when three birds/replicate that had been previously inoculated with adjuvant (but not SRBC - these birds were not sampled for other measurements) were inoculated with avian tuberculin in one wattle and euthanized by electrocution 24 hours later. Inoculated and non-inoculated wattles from each bird were removed and weighed.

The immunological parameters measured were humoral immunity (ELISA for IBD and hemagglutination-HA for SRBC). The optical densities found in ELISA tests for IBD were converted into antibody titers by using a formula recommended by the kit manufacturer (IDEXX Corporation®). SRBC titers were determined according to a protocol adapted from Bartlett & Smith (2003). Cellular immunity was measured by avian tuberculin reaction, using the weight difference between inoculated vs. non-inoculated wattles in the same bird.

Lymphocyte depletion was detected in collected bursas stored in 10% buffered formalin, cut and then dehydrated, clarified, and embedded in paraffin. The tissue blocks were cut into 0.5 µm-thick sections and stained with hematoxylin-eosin. Lesions were examined under optical microscopy and classified according to Muskett's score, in a scale from one to five (Muskett, 1979).

Statistical analysis

A completely randomized experimental design was applied with four treatments of nine replicates per treatment, with 12 birds per replicate. Experimental data were submitted to analysis of variance (ANOVA) after their normality was verified, using SAS (2001). When significant F values ($p<0.10$) were obtained, means were compared by the LS-means test. SRBC antibody titers were submitted to square-root transformation. Orthogonal contrasts were also applied to compare the treatments containing inorganic vs. organic selenium (0.3 and 0.5 mg/kg ISe vs. 0.3 mg/kg OSe) as to their

effects on performance, antibody titers, quantitative and qualitative blood analyses (hematological profile), absolute and relative spleen and bursa weights, bursal lymphocyte depletion, and weight difference between inoculated and non-inoculated wattles. Except for performance (nine replicates/treatment), statistical analyses were conducted on the results obtained from 27 birds/treatment (three birds per replicate). The Chi-Square Test (χ^2) was also used to analyze the results for bursal lymphocyte depletion.

RESULTS AND DISCUSSION

No significant differences in mortality were found among treatments throughout the experiment. The performance results are shown in Table 2. ANOVA did not detect any significant effect of selenium levels and sources on performance, but the contrast analysis of mean values revealed that the use of OSe improved feed intake (FI) between days 1 and 21 ($p<0.09$) and 21 and 42 ($p<0.10$). However, the highest observed FI did not affect body weight gain (BWG) or feed conversion ratio (FCR) ($p>0.10$). The diets were formulated using a single basal diet, thereby reducing any potential errors in feed mixing, which supports any possible differences detected.

These results partially disagree with those observed by Ribeiro et al. (2008), who observed that birds submitted to CHS and supplemented with 0.3 ppm OSe + 0.3 ppm ISe, in addition to vitamin E and C and organic Zn, presented lower FI and better FCR. In the study of Moreira et al. (2001), zero to 1.35 mg/kg OSe and ISe were tested and birds fed 1.05 mg/kg from 1 to 21 d presented higher FI, with no effect of Se source. Those authors mentioned that that response was unexpected, and was not observed in the period from 22 to 42 d. On the other hand, Dahlke et al. (2005) did not find any influence of Se levels or sources on performance responses, including FI, of broilers submitted to different temperatures. Yoon et al. (2007), comparing ISe levels from zero to 0.3 mg/kg and two sources of OSe, did not find any effect of Se levels or sources on performance. However, Se retention in the body was higher with OSe and also more efficient as compared to ISe as the birds aged and as the levels of Se in the diet were reduced. Those authors suggested that differences in the bioavailability of OSe sources based on the results of Se in the blood and the activity of GPx, may explain that result. Similarly, Payne & Southern (2005) also did not find any differences in performance, carcass traits, or GPx activity in broilers fed 0 or 0.3 mg/kg of Se, regardless of the source; however, Se retention in the muscle was higher when OSe was fed. Edens (2001) showed that the combined use of both Se sources did not improve body weight as compared to OSe alone, whereas FCR enhanced with OSe+ISe as compared to ISe alone.

The birds in the present study were submitted to CHS, and feed intake may be limited under hot conditions, according previous reports (Teeter et al., 1985; Dahlke et al., 2005; Ribeiro et al., 2005). In that sense, the increase in FI is a positive response, although weight gain and feed conversion were not enhanced.

No effect of the combined use of OSe and ISe (0.3 mg/kg ISe + 0.2 mg/kg OSe treatment) on performance was observed, which supports previous studies (Edens, 2001). Moreover, the level of 0.5 mg/kg Se (ISe) did not cause positive effects either, and this may be due to the fact that the birds do not require more than 0.3 mg/kg Se and any excess intake is promptly methylated and eliminated via urine and feces (Edens, 2001) and lungs (Rutz et al., 2005).

There were no significant effects of Se levels or sources on absolute or relative spleen and bursa weights (Table 3), which is consistent with the findings of Laganá et al. (2005) and Ribeiro et al. (2008), in which the supplementation of ISe or OSe, as well as of Zn, with and without vitamins C and E, had no impact on these parameters. According to Guimarães et al. (2003), broilers exposed to heat showed, at three weeks of age,

Table 2 - Performance of female broilers fed different Se levels and sources from 1 to 21 days, 22 to 42, and 1 to 42 days of age.

	1 to 21 days			22 to 42 days			1 to 42 days		
[1]Treatments	FI (g)	WG(g)	FC(g)	FI(g)	WG(g)	FC(g)	FI(g)	WG(g)	FC(g)
0.3 ISe	1182	841	1.40	2644	1276	2.08	3826	2116	1.81
0.3 ISe + 0.2 OSe	1172	846	1.39	2640	1273	2.08	3812	2118	1.80
0.5 ISe	1171	845	1.39	2632	1268	2.08	3803	2112	1.80
0.3 OSe	1201	860	1.40	2705	1290	2.10	3906	2150	1.82
P	0.26	0.43	0.56	0.39	0.89	0.94	0.26	0.62	0.86
CV	2.95	3.0	2.3	3.7	5.0	4.4	3.1	3.1	2.6
[2]Contrasts									
ISe x OSe[1]	1176 vs 1201	-	-	2638 vs 2705	-	-	3814 vs 3906	-	-
P	0.09	0.11	0.86	0.10	0.48	0.59	0.08	0.19	0.51

1- 0.3 mg/kg ISe, 0.3 mg/kg ISe +0.2 mg/kg OSe, 0.5 mg/kg ISe and 0.3 mg/kg OSe. 2 - Contrasts 0.3 mg/kg ISe + 0.5 mg/kg ISe vs 0.3 mg/kg OSe.

Table 3 - Absolute and relative weights of spleens and bursas, and bursal diameter of 42-d-old female broilers fed different levels and sources of Se in the diet and vaccinated against Infectious Bursal Disease.

[1]Treatments	Weight, spleen, g	[3]Relative weight, spleen, %	Weight, bursa, g	[3]Relative weight, bursa,%	[2]Diameter, bursa, mm
0.3 ISe	2.01	0.099	0.978	0.045	12.7
0.3 ISe + 0.2 OSe	2.25	0.108	0.922	0.044	12.1
0.5 ISe	2.09	0.097	0.844	0.039	12.1
0.3 OSe	1.90	0.089	0.870	0.041	12.0
P	0.34	0.22	0.44	0.5	0.53
CV	34.9	33.63	35.3	35.8	15.53

1 - 0.3 mg//kg ISe, 0.3 mg/kg ISe +0.2 mg/kg OSe, 0.5 mg/kg ISe and 0.3 mg/kg OSe. 2 - Data obtained with bursometer, with corresponding values in mm. 3 - Weight (relative to bird body weight).

higher cell death rates and hypotrophy in the bursa. In the present study, bursa weights were much lower than those reported before by Laganá et al. (2005) and Ribeiro et al. (2008), despite the fact that bursas were weighed in the present experiment one week later than the mentioned studies. This effect may be partially explained by the use of female birds, and was certainly caused by vaccination against IBD. A similar finding was observed by Rubin et al. (2007). who vaccinated birds against IBD on day 14.

Bursal diameter was not affected by treatments (Table 3). According to FORT DODGE's Technical Manual (2008), the lesion score of the birds assessed at 35-40 days of age, if vaccinated on d 21, varies according to the invasion power of the viral strain. Highly pathogenic viruses cause massive destruction of the bursal cortical-medullary layer, which is followed by its replacement by fibrous tissue and atrophy, leading to a considerable decrease in diameter. Under these conditions, bursal diameters should range from 9.5 to 15.9 mm. The average diameter of the bursas found in this experiment was 12 mm, therefore showing efficient vaccination.

Table 4 presents bursal lymphocyte depletion results, which were significantly affected by the treatments ($p<0.06$). The contrast of mean values (not shown) reveals the lowest depletion score ($p<0.01$) in the bursas of birds fed with 0.3 mg/kg of OSe as compared to birds fed ISe. The results of Moraes et al. (2004) showed an association between "strong" vaccine strains and higher lymphocyte depletion: the more pathogenic the vaccine strain, the smaller the bursa. Our study suggests that the use of OSe in the diet may be a good alternative to attenuate this condition, possibly through a protective effect on that organ. Leng et al. (2003) reported that the use of a GPx enzyme complex improves the antioxidant and protective action of adequate Se levels on cells. Other researchers (Schrauzer, 2000; Edens, 2001; Yoon et al., 2007) found the same effect when OSe was used, therefore explaining why the cell damage caused by the vaccine in the bursal tissue was minimized in this experiment. Finally, reported findings of studies in nutrigenomics (Dawson, 2006) showed the positive effect of OSe and ISe supplementation on gene transcription in intestinal and reproductive cells of rats, probably due to its capacity to enhance the activity of antioxidant mechanisms in these tissues.

Table 4 - Frequency of bursal lymphocyte depletion of 42-d-old broilers vaccinated against Infectious Bursal Disease at 19 days of age and fed different selenium levels and sources in the diet.

Treatments	[1]Scores				
	2	3	4	5	Total
0.3 ISe	10	13	4	0	27
0.3 ISe + 0.2 OSe	11	14	1	1	27
0.5 ISe	6	16	2	3	27
0.3 OSe	14	13	0	0	27
Total	41	56	7	4	108
χ^2				p<0.06	

1 - Scores: 2, 31 to 50% depletion; 3, 51 to 69%; 4, 70 to 80% and 5, > 90% (Muskett, 1979).

No significant differences were found in inoculated and non-inoculated wattles weights among treatments (data not shown). The use of female birds, a single AT inoculation, and the wide variability in the results (CV= 72%), contrarily to Rubin et al. (2007), may explain the lack of response.

Table 5 shows the results of the hematological profile analysis. Although the ANOVA showed no difference among Se levels or sources, in the contrast analysis, 0.3 mg/kg of ISe produced higher hematocrit levels ($p<0.07$) as compared to 0.3 mg/of de OSe. However, these values are not outside the acceptable ranges for such parameters (Feldmann et al., 2000). According to Yahav et al. (1997), high hematocrit values indicate dehydration. A higher H:L ratio ($p<0.10$) was also observed in birds fed ISe. This parameter is mentioned in literature as an important sign of stress, particularly heat stress. Under these conditions, the corticoids released in the blood decrease the number of lymphocytes (Gross & Siegel, 1983). On the other hand, immune challenges increase the number of heterophils in the first 6 to 12 hours of the immune response, and this

Table 5 - Hematological profile of 42-d-old broilers fed different Se levels and sources in the diet.

[1]Treatments	Hematocr	Tot Leu	Heter	Eosin	Basop	Monoc	Lymphoc	H:L
0.3 ISe	29.19	7736	3105	187	231	597	3613	0.96
0.3 ISe +0.2 OSe	28.11	8192	3036	210	261	692	3993	0.81
0.5 ISe	28.04	8400	2959	305	264	899	3972	0.80
0.3 OSe.	28.00	7461	2610	211	187	596	3858	0.73
P	0.23	0.23	0.52	0.12	0.40	0.13	0.47	0.39
CV (%)	8.8	21.7	42.0	81.4	74.0	73	25.0	58.0
ISe vs OSe[2]	28.6 vs 28	8068 vs 7461	3032 vs 2610	246 vs 211	248 vs 187	645 vs 596	3793 vs 3852	0.88 vs 0.73
P	0.27	0.17	0.18	0.46	0.17	0.24	0.79	0.21
0.3 ISe vs 0.3 OSe[3]	29.2 vs 28	7736 vs 7461	3036 vs 2610	187 vs 211	231 vs 187	597 vs 596	3613 vs 3858	0.96 vs 0.73
P	0.07	0.58	0.17	0.65	0.38	0.99	0.37	0.10

Mean values followed the same letter are not statistically different by the test of Duncan. Hematocr=hematocrit (%); Hb= hemoglobin (g/dL); TotLeu= total leukocytes; Heter= heterophils; Eosin= eosinophils; basop= basophils; Monoc=monocytes; Lymphoc= lymphocytes (number of cells/µL blood). H:L=heterophil/lymphocyte ratio.1- 0.3 mg/kg ISe, 0.3 mg/kg ISe +0.2 mg/kg OSe, 0.5 mg/kg ISe and 0.3 mg/kg OSe. 2 - Contrasts 0.3 mg/kg ISe + 0.5 mg/kg ISe vs 0.3 mg/kg OSe; 3 - Contrast 0.3 mg/kg ISe vs 0.3 mg/kg OSe.

cells are the first line of defense in broilers (Harmon, 1998). Values of 0.91 and 0.60 in broilers submitted to CHS and thermoneutral environments, respectively were previously reported by Laganá et al. (2005), and the supplementation of organic Zn and Se was not sufficient to reverse this condition. In our experiment, the use of OSe seemed to partially attenuate the effects of CHS, lowering the H:L ratio to 0.73. No data were found in the literature challenging these findings. However, the explanation may lie in the influence of Se in maintaining the functionality of neutrophils, which are the heterophil counterparts in broilers (Harmon, 1998), as postulated by Arthur et al. (2003).

An important finding was that total leukocytes and lymphocytes were well below the normal standards, according Feldmann (2000), characterizing leukopenia and lymphopenia. Both were reported as partial responses to corticosteroids in some poultry species (Davison & Flack, 1981) and in birds under HS (Borges et al., 2003; Aengwanich et al., 2003). Lymphopenia was related to some viral diseases (Campbell, 2007). Oladele et al. (2005) reported lymphopenia in 4-week-old broilers and turkeys, 24h after intra-ocular inoculation with IBD virus. However, 72h after inoculation, the population of lymphocytes was already within normal ranges. In the present experiment, blood was collected 23 days after vaccination, allowing values to go back to normal prior to sampling. In addition, there are no reports associating vaccine strains, or even field strains, to leukopenia or lymphopenia in broilers.

Table 6 shows the results of the assessment of Ab titers against IBD and SRBC. There were no treatment effects on Ab response to IBD. Statistical differences were found for collection dates, and the highest titers

Table 6 - Antibody titers against Infectious Bursal Disease1 (at 19, 29, 35 and 42 days of age) and SRBC 2 (at 42 days of age) in broilers fed different selenium levels and sources in the diet.

[1]Treatments	[2]Antibody Titers Against Infectious Bursal Disease	[3]Antibody Titers Against SRBC
0.3 ISe	2347	18.2a
0.3 ISe +0.2 OSe	2398	9.0b
0.5 ISe	2425	8.8b
0.3 OSe	2072	9.7b
P	0.49	0.003
[4]Collections		
1	Negative	-
2	2667c	-
3	3516a	-
4	3059b	-
P	<0.0001	-
CV (%)	43.1	62
P	0.0357	-
[5]0.3 ISe + 0.5 ISe vs 0.3 OSe	3181 vs 2763	
P	0.0357	-
[6]0.3 ISe vs 0.3 OSe	-	18.2 vs 8.8
P	-	0.0175

Means followed by the same letter in the same column are not different (p>0.05) by LSmeans.1 - 0.3 mg/kg ISe, 0.3 mg/kg ISe +0.2 mg/kg OSe, 0.5 mg/kg ISe and 0.3 mg/kg OSe. 2 - Results obtained by ELISA (Flock Check IBD Kit, IDEXX Inc.). Results are considered negative when values are lower than 396. 3 - Results obtained by hemagglutination and represented as square root. 4 - Collection 1: pre-vaccination, at 19 days of age; collection 2: 10 days after vaccination; collection 3: 14 days after vaccination, and collection 4: 23 days after vaccination. 5 - Contrast 0.3 mg/kg ISe + 0.5 mg/kg ISe vs 0,3 mg/kg OSe; 6 - Contrast 0.3 mg/kg ISe vs 0.3 mg/kg OSe.

were obtained in the third collection, that is, 14d after vaccination. However, the contrast analysis revealed that ISe resulted higher titers ($p<0.04$) as compared to OSe. The level of 0.3 mg/kg ISe promoted the highest production of Ab against SRBC ($p<0.05$), with significant differences as compared to the other treatments.

The use of OSe decreased Ab production against SRBC and IBD. The action of this source of Se may be more related to tissue and organ integrity, and to the enhancement of metabolic systems that are key to the organic balance. Opposite results in terms of immunity are commonly reported in the literature (Lessard et al., 1997). Due to the wide range and specificity of actions of cells and molecules that regulate the immune system and their interactions, the decision for a given nutritional level or source will not always and equally affect all types of immune response.

CONCLUSIONS

Organic selenium improved the feed intake of broilers submitted to cyclic heat stress, an effect that can be useful during high temperatures commonly experienced in Brazilian practical conditions. Neither cellular immunity, nor spleen and bursa weight or bursal diameter were affected by Se levels or sources. However, broilers fed OSe presented lower scores of bursal lymphocyte depletion after vaccination against IBD, suggesting a protective effect on this tissue. Broilers receiving OSe also had the best H/L ratio. On the other hand, broilers fed diets with ISe were more efficient in the production of Ab against SRBC and IBD.

REFERENCES

Aengwanich W, Sridama P, Phasuk Y, Vongpralab T, Pakdee P, Katawatin S, Simaraks S. Effects of ascorbic acid in cell mediated, humoral immune response, and pathophisiology of white blood cell in broiler under heat stress. The Songklanakarin Journal of Science and Technology 2003; 25(3):297-305.

Arthur JR, McKenzie RC, Beckett GJ. Selenium in the immune system. Journal of Nutrition 2003; 133:1457S-1459S.

Bartlett JR, Smith MO. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poultry Science 2003; 82:1580-1588.

Boa-Amponsem, K, Picard M, Blair ME, Meldrum B, Siegel PBI. Memory antibody responses of broiler and leghorn chickens as influenced by dietary vitamin E and route of red blood cell administration. Poultry Science 2006; 85:173-177.

Borges SA, Maiorka A, Silva, AVF. Fisiologia do estresse calórico e a utilização de eletrólitos em frangos de corte. Ciência Rural 2003; 33(5):975-981.

Campbell, TW. Hematology in birds. In: Campbell TW, Ellis CK. Avian and exotic animal hematology and cytology. Ames: Blackwell; 2007. p.10-34.

Colnago GL, Jensen LS, Long PL. Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. Poultry Science 1984; 63(6):1136-1143.

Dahlke F, Gonzales E, Furlan RL, Gadelha AC, Maiorka A, Almeida JG. Avaliação de diferentes fontes e níveis de selênio para frangos de corte em diferentes temperaturas. Archives of Veterinary Science 2005; 10(1):21-26.

Daniels LA. Selenium metabolism and bioavailability. Biological Trace Element Research 1996; 54:185-199.

Davison TF, Flack IH. Changes in the peripheral blood leucocyte populations following an injection of corticotrophin in immature chicken. Research in Veterinary Science 1981; 30:79-82.

Dawson KA. Nutrigenomics: feeding the genes for improved fertility. Animal Reproduction Science 2006; 96:312-322.

Edens FW. Involvement of Sel-Plex in physiological stability and performance of broiler chickens. Proceedings of 17th Alltech´s Annual Symposium; 2001; Lexington, KY. p.349.

Edens FW, Gowdy KYM. Selenium sources and selenoproteins in pratical poultry production. Proceedings of 20th Alltech´s Annual Symposium; 2004; Lexington, KY. p.35

FASS. Guide for the care and use of agricultural animals in agricultural research and teaching. Savoy: Federation of Animal Science Societies; 1999.

Feldman BN, Jain NC, Zinkl JG. Schalm´s veterinary hemathology. 5thed. Philadelphia: Blacwell Publishing; 2000.

Finch JM, Turner RJ. Effects of selenium and vitamin E on the immune responses of domestic animals. Research in Veterinary Science 1996; 60:97-106.

Fort Dodge. Programa de Monitoria da Bolsa de Fabrício [cited 2008 out]. Available from: http://www.fortdodge.com.br/fd/aves/manuais/aves01/folheto_bf/index.htm.

Gross WB, Siegel HS. Evaluation of heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 1983; 27:972-979.

Guimarães EB, Vasconcelos AC, Martins NRR, Oliveira RFM, Moro L, Nunes JES, Santos FGA. Porcentagem de parênquima e índice apoptótico da bolsa cloacal em frangos de corte em ambiente de conforto e estresse térmico. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2003; 55(2):178-186.

Harmon BG. Avian heterophils and inflammation and disease resistance. Poultry Science 1998; 77:972-977.

Kidd MT. Nutritional modulation of immune function in broilers. Poultry Science 2004; 83:650-657.

Laganá C, Ribeiro AML, González FHD, Almeida LL, Terra SR, Barbosa PR. Suplementação de vitaminas e minerais orgânicos nos parâmetros bioquímicos e hematológicos de frangos de corte em estresse por calor. Boletim da Indústria Animal 2005; 62:157-165.

Leng L, Bobcek RS, Kuricová S, Boldizárová K, Grešáková L, Ševcíková Z, Révajová V, Levkutová M, Levkut, M. Comparative metabolic and immune responses of chickens fed diets containing inorganic selenium and Sel-PlexTM organic selenium. Proceedings of the 19th Alltech´s Annual Symposium; 2003; Lexington, KY.

Lessard M, Hutchings D, Cave N. Cell-mediated and humoral immune responses in broiler chickens maintained on diets containing different levels of vitamin A. Poultry Science 1997; 76:1368-1378.

Moraes HLS, Salle CTP, Padilha AP, Nascimento VP, Souza GF, Pereira RA, Artencio JA, Salle FO, Canal CW. Infectious bursal disease: Evaluation of pathogenicity of commercial vaccines in Brazil in specific pathogen free chickens. Brazilian Journal of Poultry Science 2004; 6(4):243-247.

Moreira J, Santos CD, Abreu CMP, Bertechini AG, Oliveira DF, Cardoso MG. Efeito de níveis e fontes de selênio na atividade enzimática da glutationa peroxidase e no desempenho de frangos de corte. Ciência e Agrotecnologia 2001; 25(3):645-649.

Muskett JC, Hopkins IG, Edwards KR, Thornton DH. Comparision of two Infectious Bursal Disease vaccine strains: Efficacy and potential hazards in susceptibility and maternally immune birds. Veterinary Record 1979; 104:332-334.

National Research Council. Nutrient requirements of poultry. 9th ed. Washington: National Academy Press; 1994.

Oladele DA, Adene DF, Obi TU, Nottidge HO, Aiyedun AI. Sequential hematological study of experimental infectious bursal disease virus infection in chickens, turkeys and ducks. Revue d´elevage et de Médecine Vétérinaire des Pays Tropicaux 2005; 58(4):211-215.

Payne RL, Southern LL. Changes in glutathione peroxidase and tissue selenium concentrations of broilers after consuming a diet adequate in selenium. Poultry Science 2005; 84:1268-1276.

Ribeiro AML, Vogt LK, Canal CW, Laganá C, Streck AF. Suplementação de vitaminas e minerais orgânicos e sua ação sobre a imunocompetência de frangos de corte submetidos a estresse por calor. Revista Brasileira de Zootecnia 2008; 37(4):636-644.

Rostagno HS. Composição dos alimentos e exigências nutricionais para aves: tabelas brasileiras para aves e suínos. Viçosa (MG): UFV; 2005.

Rubin LL, Canal CW, Ribeiro AML, Kessler A, Silva I, Trevizan L, Viola T, Raber M, Gonçalves TA, Krás R. Effects of methionine and arginine levels on the immunity of broiler chickens submitted to immunological stimuli. Brazilian Journal of Poultry Science 2007; 9(4):241-247.

Rutz F, Anciuti MA, Rech JL, Xavier EG. Following response to Sel-Plex® and other organic minerals trough the broiler breeder maze: case studies in Brazil. Proceedings of the 21th Alltech´s Annual Symposium; 2005; Lexington, KY. p.55.

Schrauzer GN. Selenomethionine: a review of its nutritional significance, metabolism and toxicity. Journal of Nutrition 2000; 130:1653-1656.

Surai PF. Organic selenium: benefits to animals and humans, a biochemist´s view. Proceedings of the 16th Alltech´s Annual Symposium; 2000; Lexington, KY. p.205.

Teeter RG, Smith MO, Owens FN, Arp SC, Sangiah S, Breazile JE. Chronic heat stress and respiratory alkalosis: occurrence and treatment in broiler chicks. Poultry Science 1985; 64:1060-1064.

Yahav S, Straschnow A, Plavnick J, Hurwitz S. Blood system response of chickens to changes in environmental temperatures. Poultry Science 1997; 76:627-633.

Yoon I, Werner TM, Butler JM. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poultry Science 2007; 86:727-730.