Effect of inorganic or organic selenium supplementation on productive performance, egg quality and some physiological traits of dual-purpose breeding hens

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ABSTRACT: One hundred and twenty (100♀ + 20♂) 30-weeks-old dual-purpose breeding hens of Gimmizah strain were housed in individual cages in a semi-open house. Birds were distributed randomly into five treatments of 20♀ + 4♂. The 1st treatment was fed a control (unsupplemented) diet (17.5% CP and 11.4 MJ per kg diet) containing 0.10 mg Se/kg (low level). The 2nd, 3rd, 4th and 5th treatments were fed the control diet supplemented with 0.15 and 0.30 mg Se/kg from inorganic (sodium selenite) and organic (selenomethionine, as Se-yeast Selplex® Alltech, Nicholasville, USA) sources, respectively. The total concentration of Se in experimental diets was 0.25 (medium level) and 0.40 ppm (high level). Feed and water were provided *ad libitum* throughout the experimental period (30–50 weeks of age). Different Se levels of the organic and inorganic form and their interaction did not significantly ($P > 0.05$) affect egg production percentage, and most of egg quality traits. Egg weight and egg mass significantly ($P < 0.002$) increased and the feed conversion ratio (FCR) significantly ($P < 0.04$) improved due to Se supplementation compared with hens fed the control diet. Piped embryos and spleen percentage significantly ($P < 0.05$) decreased due to Se supplementation. In addition, the level of organic and inorganic Se and their interaction significantly ($P < 0.0001$) decreased the plasma cholesterol concentration. Tibia Ca and P percentages and yolk selenium concentration significantly ($P < 0.03$; $P < 0.0001$ and $P < 0.0001$, respectively) increased due to Se supplementation and the greatest increase was recorded by a group fed diet with the high level (0.40) of organic Se. The duodenal and intestinal mucosa of the ileum was negatively affected by the high level of inorganic Se while chickens fed the organic form showed less toxic effects in hepatic and splenic tissues than those receiving the inorganic form. In conclusion, the organic and inorganic Se supplementation at 0.15 and 0.30 mg/kg diet, which corresponded to a dietary level of 0.25 and 0.40 mg/kg diet, improved the productive and reproductive performance of Gimmizah breeding hens. A decrease in plasma total cholesterol and an improvement in the bone mineralization were observed. The level of 0.25 mg/kg diet of organic Se was adequate to enrich eggs, which may be recommended for practical application and which would improve the consumer health benefit.

Keywords: selenium; egg production; fertility; hatchability; egg quality

The role of trace elements to increase poultry productivity is very important in the high-stress production environment. Nutritionists realize that both the level and the source of trace elements play an important role in ration formulations and optimizing production level, product quality, health status of birds and economic returns. Using chicken eggs in the human diet leads to additional benefits that can be derived from modifying the egg nutritional profile, particularly egg fats and antioxidants. In this regard, Kucharzewski et al. (2003) suggested that the low concentrations of zinc and selenium in the thyroid tissue confirm their participation in the carcinogenic process.

Nowadays, there is a considerable interest in replacing inorganic trace minerals by the metal amino acid complex that can meet the requirements of animals for both the 1st or the 2nd limiting amino acid and simultaneously improve the availability of the metal to animals. The aim behind the application of this new biotechnology in animal nutrition is to protect the metals from dietary antagonists such as phytate, non-starch polysaccharides, crude fibre and interaction among minerals as well as to reduce its negative effect on the availability of vitamins. It is clear that in some minerals, i.e. selenium, chromium and iron, organic forms were more efficient utilized than inorganic forms (Hallberg and Rossander-Hulthen, 1993; Skřivan et al., 2006).

Functional foods are nowadays of great interest and producing poultry product modified foods may be possible with the applications of the biotechnology in trace mineral nutrition (Surai, 2000, 2006; Bobcek et al., 2004; Skřivan et al., 2006; Gajčević et al., 2009). The aim of this work was to study the effect of different dietary levels and/or sources of Se on productive and reproductive performance, blood biochemical constituents and histopathology of breeding hens as well as a means of producing functional foods such as low cholesterol eggs and Se-enriched eggs.

MATERIAL AND METHODS

Birds, management and experimental design

A total number of 120, 30-weeks-old dual-purpose breeding chickens of Gimmizah (*Gallus gallus f. domestica*), a crossbred originated by crossing of Dokki$_4$ (Fayoumi × White Plymouth Rock) and

Table 1. The composition and analysis of experimental basal diet fed to Gimmizah laying hens during 30 to 50 weeks of age

Ingredient profiles	(%)
Yellow maize	63.14
Soybean meal 44% CP	27.10
Limestone	7.60
Dicalcium phosphate	1.50
Sodium chloride (NaCl)	0.30
Vitamin + mineral premix[1]	0.30
DL-methionine	0.06
Total	100.00
Calculated and determined values[2]	
ME MJ/kg diet	11.40
Lysine (%)	0.87
Calcium (%)	3.00
Av. phosphorus (%)	0.42
Chemical analyses	
Se (ppm)	0.10
CP (%)[3]	17.45

[1]vitamin-mineral premix supplied per 1 kg of diet: vitamin A 12 000 IU; vitamin D$_3$ 2 200 ICU; vitamin E10 mg; vitamin K$_3$ 2 mg; vitamin B$_1$ 1 mg; vitamin B$_2$ 4 mg; vitamin B$_6$ 1.5 mg; vitamin B$_{12}$ 10 Ug; nicotinic acid 20 mg; folic acid 1 mg; pantothenic acid 10 mg; biotin 50 Ug; choline chloride 500 mg; copper 10 mg; iron 30 mg; manganese 55 mg; zinc 50 mg; iodine 1 mg; selenium 0.1 mg supplemented as sodium selenite "Na$_2$SeO$_3$",
[2]calculated values (NRC, 1994);
[3]determined values (AOAC, 1995)

Barred Plymouth Rock) strain (100♀ + 20♂) were weighed individually and housed in individual wire laying cages until the end of the experiment (50 weeks of age). Birds were randomly divided into five groups, each consisting of 20 pullets and 4 cocks. Males were kept separately from females for artificial insemination and fed the crosspending female experimental diets. The first group was used as a control and fed the basal diet without Se supplementation containing 0.1% Se by analysis (Table 1). The second and the third treatment groups were fed the basal diet supplemented with 0.15 and 0.30 inorganic Se mg/kg diet as sodium

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selenite (Na_2SeO_3), respectively. The fourth and the fifth groups were fed the basal diet supplemented with 0.15 and 0.30 organic Se mg/kg diet as selenomethionine (Se-yeast Selplex® Alltech, Nicholasville, USA), respectively.

Feed and water were provided *ad libitum* throughout the experimental period (30–50 weeks of age). Birds were illuminated with a 15:9 h light-dark cycle. Vaccination and medical care were done according to common veterinary care under veterinarian's supervision.

Measurements

Daily egg production and egg weight were recorded in each hen, and egg mass and hen day egg production percentage were calculated. Feed intake (FI) was recorded weekly. Egg production criteria were calculated for monthly intervals during the production period, then FCR was calculated as g of feed required per each g of egg.

Eggs were collected for a 7-day period at 38, 42 and 46 weeks of age and were incubated in automatic incubators. The removed eggs and eggs not hatched on day 21 were broken to differentiate infertile eggs from those containing dead embryos. Fertility was calculated as the number of fertile eggs as relative to the total number of eggs set while hatchability was calculated as the number of hatched chicks as relative to the total number of egg set.

Eggs laid on two successive days, from each treatment at 40 and 48 weeks of age, were used for measuring egg quality traits. Egg shell (SW), yolk and albumen were weighed to the nearest 0.1 g (egg shells were washed, the inner egg shell membrane was separated and air-dried for 72 h before weighing). The following parameters were also measured: egg shape index (ESI) according to Ramanoff and Ramanoff (1949); yolk index (YI%) as reported by Funk (1948); albumen height (AH); Haugh unit score (HU) according to Haugh (1937); shell weight per unit of surface area (SWUSA) according to Attia et al. (1995); egg surface area according to Carter (1975). SWUSA was calculated by dividing the shell weight by the egg surface area and presented as mg/cm² of egg.

The content of Se in the basal diet and in egg yolk was determined as follows: two grams of basal diet or yolk mixture were digested by H_2SO_4 and hydrogen peroxide H_2O_2 according to Black (1982). Se was measured by the inductively coupled argon

plasma spectroscopy (ICP) in Agriculture Research Center, Giza, Laboratories according to the method of Cottenie et al. (1982). Feeds samples of experimental diets were chemically analyzed according to the official methods of AOAC (1995).

At the end of the experiment, blood samples (3 ml) were collected from the brachial vein into heparinized tubes of three birds/treatment. Plasma was immediately separated by centrifugation for 10 minutes at 3 200 rpm. Plasma triglyceride (TG) and total cholesterol (TC) were determined by colorimetric methods using available commercial kits (Diamond Diagnostics).

Two grams of yolk mixture (*n* = 5 per treatment) was extracted with a mixture of chloroform: methanol (2:1 v/v) using the procedure described by Folch et al. (1957). Cholesterol determination was done in triplicate using a commercial test kit for cholesterol analysis (Sigma diagnostic cholesterol reagent procedure No. 352, Sigma Chemical Co., St Louis, MO).

At the end of experiment, 5 females (50 weeks of age) were randomly chosen, weighed after 12 h fasting, slaughtered, feather picked and the total inedible parts (head, legs and inedible viscera) were taken aside and the remaining carcass (dressed weight) was weighed. Liver, spleen, abdominal fat, pancreas, spleen, intestine and ovary were separated and individually weighed. Percentages of internal organs to live body weight were calculated. The right tibia of slaughtered hens was removed, cleaned from tissues, set in hexane for 48 h to remove fat and dried in an oven for 24 h until constant weight (g), then ashed. Tibia Ca and phosphorus contents were determined according to the methods of AOAC (1995).

Histopathological study

Tissue specimens were collected from the liver, spleen and small intestine (duodenum, jejunum and ileum) of both treated and control hens. Fixation was done in bovine solution for specimens of the intestine as well as in 10% neutral buffered formalin solution for the other specimens. After washing in tap water, specimens passed through the steps of a routine paraffin embedding technique (dehydration in series of alcohol, clearing in xylol and embedding in melted paraffin wax). After blocking, paraffin sections of 3–5 microns in thickness were obtained with a microtome and later they were stained with haematoxylin and eosin according to Bancroft and

Table 2. Effects of different Se sources and/or levels on egg production traits in Gimmizah hens from 30 to 50 weeks of age (mean ± SD)

Level/source of of selenium	Egg production (%)	Egg weight (g)	Egg mass (g)	Feed intake (g/hen/day)	FCR (g feed/g egg)
Se level (ppm)					
0.10	56.4 ± 8.94	54.3[b] ± 2.61	30.6[b] ± 4.72	134.5[a] ± 6.39	4.52[a] ± 0.78
0.25	61.8 ± 4.39	54.7[b] ± 2.30	33.8[a] ± 2.79	128.9[c] ± 6.71	3.84[b] ± 0.37
0.40	62.4 ± 6.33	56.0[a] ± 1.35	34.9[a] ± 3.71	131.9[b] ± 7.29	3.82[b] ± 0.48
P-value	NS	0.002	0.002	0.008	0.04
Se source					
Control	56.4 ± 8.94	54.3[b] ± 2.61	30.6[b] ± 4.72	134.5[a] ± 6.39	4.52[a] ± 0.78
Inorg	62.1 ± 6.86	55.4[a] ± 2.56	34.4[a] ± 4.23	126.1[b] ± 4.96	3.73[b] ± 0.51
Org	62.1 ± 3.53	55.4[a] ± 1.91	34.4[a] ± 2.10	134.7[a] ± 6.35	3.93[b] ± 0.29
P-value	NS	0.002	0.002	0.0001	0.04
Se level × Se source					
Control (0.10)	56.4 ± 8.94	54.3[c] ± 2.61	30.6[c] ± 4.72	134.5 ± 6.39	4.52[a]+ 0.78
Inorg (0.25)	62.9 ± 4.85	53.9[c] ± 2.82	33.9[b] ± 3.23	124.4 ± 3.53	3.70[b] ± 0.37
Inorg (0.40)	61.3 ± 8.47	56.8[a] ± 1.11	34.8[ab] ± 5.08	127.8 ± 5.63	3.76[b] ± 0.63
Org (0.25)	60.7 ± 3.68	55.5[b] ± 1.28	33.7[b] ± 2.36	133.4 ± 6.08	3.98[b] ± 0.33
Org (0.40)	63.6 ± 2.76	55.3[b] ± 1.12	35.1[a] ± 1.55	135.9 ± 6.50	3.88[b] ± 0.25
P-value	NS	0.0003	0.002	NS	0.04

[a,b,c]means in the same column within similar treatments bearing different superscripts are significantly different at *P* ≤ 0.05; NS = not significant

Stevens (1990), and subjected to light microscopy to record histopathological changes in the various examined organs.

Statistical analysis

Data were analyzed by ANOVA using a two-way model (SAS, 1990). The main effects were Se levels and sources. The interaction between the two main factors was tested. A 0.05 level of significance of Student-Newman-Keuls test was used to test mean differences. All the percentages were converted as log 10 to normalize data distribution.

RESULTS AND DISCUSSION

Selenium concentration in basal diet

The chemical composition of basal diet (control) showed that the Se concentration was 0.10 ppm

(Table 1). This value is within the acceptable range of the recommendation for laying hens cited by NRC (1994). Standing this result, the total concentration of selenium in experimental diets was 0.10, 0.25 and 0.40 mg/kg diet both for either organic or inorganic source.

Productive, quality and reproductive traits

Table 2 shows the effect of Se level and/or source on egg production traits. The egg production percentage for the whole experimental period was not significantly affected by the level and source of Se and the interaction between these variables was not significant either. The increasing Se level up to 0.40 ppm significantly increased both egg weight and egg mass compared with those in hens fed the control diet. A similar response was observed due to Se supplementation when the inorganic or organic form was compared with the control diet. The results of the interaction indicated that the high

Table 3. Effects of different Se sources and/or levels on the quality of egg and shell in Gimmizah hens from 30 to 50 weeks of age (mean ± SD)

Level/source of selenium	Yolk (%)	Yolk index	Albumen (%)	HU	Shell (%)	SWUSA (mg/cm²)	Shell thickness (mm)	Shape index
Se level								
0.10	33.94 ± 1.69	41.74 ± 3.12	53.42 ± 1.63	74.16 ± 7.11	12.63 ± 0.90	105.5 ± 8.79	0.334 ± 2.88	76.76 ± 5.88
0.25	37.31 ± 3.72	42.22 ± 2.70	50.13 ± 4.17	70.98 ± 11.22	12.56 ± 1.30	103.9 ± 9.96	0.319 ± 3.81	78.88 ± 5.85
0.40	35.84 ± 3.76	42.07 ± 3.11	52.14 ± 3.93	68.14 ± 8.90	12.04 ± 0.91	100.4 ± 7.34	0.325 ± 2.66	77.11 ± 2.95
P-value	NS	NS	NS	NS	NS	NS	NS	NS
Se source								
Control	33.94 ± 1.69	41.74 ± 3.12	53.42 ± 1.63	74.16 ± 7.11	12.63 ± 0.90	105.5 ± 8.79	0.334 ± 2.88	76.76[b] ± 5.88
Inorg	36.44 ± 3.08	41.62 ± 2.58	51.15 ± 3.31	67.65 ± 11.03	12.43 ± 1.21	103.9 ± 9.96	0.305 ± 3.81	79.58[a] ± 5.60
Org	36.71 ± 4.43	42.68 ± 3.12	51.12 ± 4.89	71.46 ± 8.96	12.17 ± 1.08	100.4 ± 7.34	0.323 ± 2.66	76.41[b] ± 2.82
P-value	NS	NS	NS	NS	NS	NS	NS	0.02
Se level × Se source								
Control (0.10)	33.94 ± 1.69	41.74 ± 3.12	53.42 ± 1.63	74.16 ± 7.11	12.63 ± 0.90	105.5 ± 8.79	0.334 ± 2.88	76.76[b] ± 5.88
Inorg (0.25)	36.91 ± 2.73	41.75 ± 2.38	50.09 ± 2.28	68.82 ± 13.08	13.00 ± 1.23	107.4 ± 9.91	0.317 ± 3.61	82.14[a] ± 6.42
Inorg (0.40)	35.96 ± 3.46	41.49 ± 2.87	52.20 ± 3.91	66.47 ± 9.01	11.85 ± 0.91	99.4 ± 7.22	0.324 ± 2.80	77.02[b] ± 3.20
Org (0.25)	37.72 ± 4.61	42.70 ± 3.01	50.16 ± 5.59	73.13 ± 9.12	12.13 ± 1.27	100.4 ± 9.13	0.292 ± 3.74	75.63[b] ± 2.72
Org (0.40)	35.71 ± 4.20	42.65 ± 3.37	52.08 ± 4.13	69.80 ± 8.89	12.22 ± 0.91	101.5 ± 7.65	0.320 ± 2.66	77.19[b] ± 2.82
P-value	NS	NS	NS	NS	NS	NS	NS	0.01

[a,b,c]means in the same column within similar treatments bearing different superscripts are significantly different at $P \leq 0.05$; NS = not significant; con = control; inorg = inorganic; org = organic

level (0.40 ppm) of inorganic Se or the medium level (0.25) and the high level (0.40 ppm) of organic Se significantly increased egg weight and egg mass compared with those in hens fed the control diet (Table 2). However, the high level (0.40 ppm) of inorganic Se had the best effect on egg weight and both the inorganic and organic form at the high level (0.40 ppm) of Se positively affected egg mass.

The increasing Se level to 0.25 and 0.40 ppm significantly decreased FI and significantly improved the FCR by 15 and 15.5%, respectively, compared with those in hens fed the control diet, showing no differences between the medium and the high level. However, the inclusion of inorganic Se significantly decreased FI by 6.7% and 6.8%, respectively, compared with FI in hens fed the control diet and organic Se. The FCR was significantly improved by 17.5 and 13.1%, respectively, in hens receiving organic or inorganic Se supplementations com-

pared with those fed the control diet. The interaction between the Se level and source did not affect FI, while it significantly affected the FCR. The latter indicates that Se supplementation at 0.25 or 0.40 ppm of inorganic or organic Se significantly improved the FCR by 22.2, 16.8, 11.9 and 14.2%, respectively, compared to the control diet.

In the literature, the effect of Se supplementation on egg production traits is not clear and it is based on Se content of the basal diet. Results reported by Jiakui and Xialong (2004), Utterback et al. (2005) and Leeson et al. (2008) indicated no differences in egg production, egg weight and FI of laying hens due to inorganic and organic Se supplementation. Ganpule and Manjunatha (2003) demonstrated that selenium yeast supplementation significantly improved the FCR of laying hens compared with that of hens fed the control diet. On the other hand, Ševčíková et al. (2006) reported that the FCR was not affected by Se supplementation. The above-

Table 4. Effects of different Se sources and/or levels on reproductive traits and tibia characteristics of Gimmizah hens from 30 to 50 weeks of age (mean ± SD)

Level/source of selenium	Fertility (%)	Hatchability (%)	Embryonic mortality (%)	Piped embryos (%)	Tibia		
					tibia (%)	Ca (%)	iP (%)
Se level							
0.10	93.02 ± 2.77	76.02[b] ± 6.8	2.92 ± 3.56	14.09[a] ± 6.6	0.52 ± 0.03	13.55[b] ± 1.5	7.80 ± 0.73
0.25	92.81 ± 3.80	89.12[a] ± 2.5	0.00 ± 0.00	3.69[b] ± 4.5	0.50 ± 0.06	21.06[a] ± 4.3	6.90 ± 2.42
0.40	95.04 ± 4.40	88.94[a] ± 6.2	1.08 ± 2.76	5.02[b] ± 4.4	0.50 ± 0.03	21.23[a] ± 5.9	7.01 ± 1.13
P-value	NS	0.02	NS	0.05	NS	0.03	NS
Se source							
Control	93.02 ± 2.77	7 6.02[c] ± 6.8	2.92 ± 3.56	14.09[a] ± 6.6	0.52 ± 0.03	13.55[c] ± 1.5	7.80[b] ± 0.73
Inorg	94.05 ± 4.39	91.09[a] ± 4.1	0.00 ± 0.00	2.96[b] ± 3.1	0.50 ± 0.05	20.30[a] ± 4.9	8.58[a] ± 0.63
Org	93.80 ± 4.16	86.97[b] ± 4.4	1.08 ± 2.76	5.74[b] ± 5.1	0.50 ± 0.05	21.99[a] ± 5.4	5.33[c] ± 0.85
P-value	NS	0.02	NS	0.05	NS	0.03	0.0001
Se level × Se source							
Control (0.10)	93.02[ab] ± 2.77	76.02[c] ± 6.8	2.92 ± 3.56	14.09[a] ± 6.6	0.52 ± 0.03	13.55[c] ± 1.5	7.80[b] ± 0.73
Inorg (0.25)	90.66[b] ± 2.71	89.44[ab] ± 1.2	0.00 ± 0.00	1.22[c] ± 2.2	0.51 ± 0.05	24.90[a] ± 0.1	9.15[a] ± 0.23
Inorg (0.40)	97.44[a] ± 2.70	92.74[a] ± 5.4	0.00 ± 0.00	4.70[bc] ± 3.1	0.49 ± 0.05	15.70[b] ± 0.5	8.00[b] ± 0.08
Org (0.25)	94.96[ab] ± 3.68	88.80[b] ± 3.6	0.00 ± 0.01	6.16[b] ± 5.0	0.49 ± 0.07	17.22[b] ± 2.2	4.66[d] ± 0.37
Org (0.40)	92.64[ab] + 4.69	85.14[b] ± 4.7	2.17 ± 3.77	5.33[b] ± 5.7	0.51 ± 0.03	26.76[a] ± 1.1	6.01[c] ± 0.58
P-value	0.007	0.02	NS	0.05	NS	0.0001	0.0001

[a,b,c]means in the same column within similar treatments bearing different superscripts are significantly different at $P \leq 0.05$; NS = not significantl; con = control; inorg = inorganic; org = organic

mentioned literature may support the results of the present study regarding the positive effect of Se on egg weight, egg mass and FCR and the lack of effect on egg production percentage.

The level and source of Se and their interaction had no significant effect on any traits of egg quality (Table 3), showing that Se content of the basal diet was adequate to support egg production of good quality. However, recent evidences by Payne et al. (2005) and Gajčević et al. (2009) indicated that eggs produced by hens fed a diet with organic Se had higher HU values than eggs of hens fed the recommended level of Se. Furthermore, Gajčević et al. (2009) reported that the dietary supplementation of a higher amount of Se resulted in increased GSH-Px activity in the hens' blood ($P < 0.05$).

The level and source of Se had no significant effect on fertility and embryonic mortality percentage compared with those in hens fed the control diet. On the other hand, the hatchability percentage improved significantly and the piped embryos significantly decreased. The improvement in the hatchability percentage was ~ 17% for the medium (0.25) and high (0.40 ppm) level (Table 4). The results of the interaction indicated a significant effect with the greatest fertility percentage (97.4%) in hens fed the high level (0.40 ppm) of inorganic Se and the least significant one (90.7%) in hens fed the medium (0.25 ppm) level of inorganic Se. The organic Se groups exhibited intermediate values. In addition, the medium (0.25) and the high (0.40 ppm) levels of inorganic and organic Se in layer diets significantly improved the hatchability percentage while the embryonic mortality percentages significantly decreased compared with those in hens fed the basal diet (0.10 ppm of Se). On the other hand, the interaction between the level and the source of Se did not significantly affect the embryonic mortality percentage compared with that in hens fed the control diet. Surai (2006) indicated that Se has an important role in improved fertility, embryonic development and hatchability of poultry. Moreover, Se is deposited in the egg and distributed among the developing tissues during embryogenesis (Surai, 2000; Paton et al., 2002). However, the present results showed that sodium selenite had better hatchability than the Selplex®, which needs further verification. The improvement quoted herein could be due to the ability of Se to remove oxygen free radicals and lipid peroxidase and to alleviate the degree of tissue damage caused by the oxygen and the free radicals caused by hatching stress (Surai, 2006).

Tibia characteristics

Selenium level, source and the interaction between the level and the source of Se had no significant effect on the tibia weight percentage compared with that in hens fed the control diet (Table 4). The increasing level of Se in the inorganic and organic source significantly increased the calcium percentage in tibia compared with that in hens fed the control diet. These results indicated that Se supplementation improved the bone mineralization of laying hens during 30–50 weeks of age (Table 4). The Se level in layer diets had no significant effect on the inorganic phosphorus percentage in tibia. The phosphorus percentages in tibia significantly decreased by organic Se but significantly increased by inorganic Se compared with those in hens fed the control diet. Although inorganic Se increased tibia phosphorus, tibia Ca percentage and tibia weight percentage did not differ between the two sources.

It is worth noting that the medium level (0.25 ppm) of inorganic Se resulted in the greatest Ca and phosphorus in tibia, nonetheless, the relative weight of tibia was not changed. The mechanism by which Se affects the bone formation is not known at present, for example the Se presence in human bones is about 16% of total body Se (Zachara et al., 2001). A relationship between active vitamin D metabolites and the Se-dependent enzyme thioredoxin reductase was established (Schutze et al., 1999). An effect of thioredoxin reductase activity on 1.25 $(OH)_2D_3$ would be expected and this could be an important link between Se and bone metabolism. On the other hand, antioxidant properties of various selenoproteins could also be of importance in maintaining the antioxidant protection of the oviduct during eggshell formation (Surai, 2006).

Organs

The level and/or the source of selenium had no significant effect on most of the organs except the relative weight of spleen which significantly decreased compared with that in hens fed the control diet. In addition, the relative weight of pancreas significantly increased at both the medium (0.25) and high (0.40 ppm) level of Se in respect of the control group (Table 5). In agreement with the present results, EL-Sebai (2000) and Ševčíková et al. (2006) found that no significant effect due to

Table 5. Effects of different Se sources and/or levels on the percentage of some organs in Gimmizah hens at 50 weeks of age (mean ± SD)

Level/source of selenium	Spleen (%)	Abdominal fat (%)	Ovary (%)	Oviduct (%)	Liver (%)	Pancreas (%)	Intestinal weight (%)
Se level							
0.10	$0.162^a \pm 0.03$	3.21 ± 1.82	1.72 ± 0.68	2.98 ± 0.34	0.52 ± 0.03	$13.55^b \pm 1.5$	7.80 ± 0.73
0.25	$0.107^b \pm 0.03$	5.09 ± 1.28	2.41 ± 0.83	2.58 ± 0.25	0.50 ± 0.06	$21.06^a \pm 4.3$	6.90 ± 2.42
0.40	$0.081^b \pm 0.03$	7.27 ± 1.69	2.13 ± 0.75	2.61 ± 0.18	0.50 ± 0.03	$21.23^a \pm 5.9$	7.01 ± 1.13
P-value	0.05	NS	NS	NS	NS	0.03	NS
Se source							
Control	$0.162^a \pm 0.03$	3.21 ± 1.82	1.72 ± 0.68	2.98 ± 0.34	2.20 ± 0.37	0.21 ± 0.04	2.02 ± 0.55
Inorg	$0.090^b \pm 0.03$	5.77 ± 1.97	1.96 ± 0.69	2.55 ± 0.20	1.86 ± 0.44	0.22 ± 0.02	2.15 ± 0.33
Org	$0.098^b \pm 0.04$	6.59 ± 1.71	2.57 ± 0.79	2.64 ± 0.23	2.09 ± 0.12	0.21 ± 0.01	2.44 ± 0.54
P-value	0.05	NS	NS	NS	NS	NS	NS
Se level × Se source							
Control (0.10)	$0.162^a \pm 0.03$	3.21 ± 1.82	1.72 ± 0.68	2.98 ± 0.34	2.20 ± 0.37	0.21 ± 0.04	2.02 ± 0.55
Inorg (0.25)	$0.088^c \pm 0.01$	4.66 ± 1.45	1.92 ± 0.02	2.52 ± 0.22	1.80 ± 0.22	0.21 ± 0.02	2.15 ± 0.11
Inorg (0.40)	$0.092^c \pm 0.04$	6.89 ± 1.91	2.01 ± 1.05	2.57 ± 0.20	1.93 ± 0.62	0.23 ± 0.02	2.16 ± 0.50
Org (0.25)	$0.126^b \pm 0.03$	5.52 ± 1.11	2.90 ± 0.99	2.64 ± 0.30	2.08 ± 0.17	0.21 ± 0.01	2.38 ± 0.82
Org (0.40)	$0.070^c \pm 0.01$	7.65 ± 1.61	2.24 ± 0.41	2.65 ± 0.18	2.10 ± 0.08	0.20 ± 0.02	2.50 ± 0.08
P-value	0.05	NS	NS	NS	NS	NS	NS

[a,b,c]means in the same column within similar treatments bearing different superscripts are significantly different at $P \leq 0.05$; NS = not significant; con = control; inorg = inorganic; org = organic

supplementation of Se on liver weight of broiler chickens.

Biochemical constituents

The level and the source of Se did not significantly affect the albumin and triglyceride concentration of the plasma compared with the control treatment (Table 6). Similarly, Abaza (2002) found that the plasma triglyceride was insignificantly affected by Se or by the combination of Se and vitamin E. However, Attia et al. (2006) found that vitamin E and/or Se supplementation significantly decreased triglycerides. The low dosage of inorganic Se and the high dosage of organic Se significantly decreased the plasma triglyceride concentration compared with the other Se treatments showing a significant interaction between the level and the source of Se.

Both the level and source of Se significantly decreased the plasma cholesterol concentration compared with that in hens fed the control diet (Table 6). In accordance with the present observations Abd-El-Latif et al. (2004) reported that additions of Zn, Se and vitamin E significantly alleviated the concentration of cholesterol in quails fed dietary ochratoxin compared with quails fed dietary ochratoxin without such feed additives. On the other hand, Abaza (2002) observed that the plasma cholesterol concentration was significantly increased by Se or by the combination of Se and vitamin E. However, Ljubic et al. (2006) suggested that the organic Se supplementation influences cholesterol metabolism in adipose tissue by decreasing the total cholesterol concentration during the fattening period and increasing the free cholesterol concentration after 48 h feed deprivation. Changes in enzymes responsible for regulating cholesterol synthesis, oxidation or elimination may be

Table 6. Effects of different Se sources and/or levels on plasma total protein, albumin, globulin, Alb/Glb, triglyceride, cholesterol, HDL and selenium yolk concentrations of Gimmizah hens from 30 to 50 week of age (mean ± SD)

Level/source of selenium	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb/Glb	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	Yolk Se (ppm fresh yolk)
Se level								
0.10	5.47 ± 0.4	4.06 ± 0.1	1.41 ± 0.4	3.25 ± 1.3	537.2 ± 39.5	195.0[a] ± 8.6	403.8[b] ± 71.0	0.209[c] ± 0.01
0.25	5.24 ± 0.7	3.73 ± 0.6	1.51 ± 0.6	3.03 ± 1.6	457.1 ± 99.9	131.6[c] ± 44.9	573.2[a] ± 45.1	0.223[b] ± 0.01
0.40	5.97 ± 1.4	3.81 ± 0.4	2.16 ± 1.7	3.62 ± 2.8	464.8 ± 79.9	163.1[b] ± 12.4	426.4[b] ± 43.2	0.356[a] ± 0.06
P-value	NS	NS	NS	NS	NS	0.0001	0.0001	0.0001
Se source								
Control	5.47[b] ± 0.4	4.06 ± 0.1	1.41[b] ± 0.4	3.25[ab] ± 1.3	537.2 ± 39.5	195.0[a] ± 8.6	403.8[b] ± 71.0	0.209[c] ± 0.01
Inorg	6.17[a] ± 1.2	3.58 ± 0.3	2.60[a] ± 1.3	1.83[b] ± 0.9	448.3 ± 97.5	169.6[b] ± 12.1	403.8[b] ± 170.9	0.261[b] ± 0.05
Org	5.03[b] ± 0.7	3.96 ± 0.6	1.07[b] ± 0.5	4.82[a] ± 2.2	473.6 ± 80.9	125.1[c] ± 38.2	595.9[a] ± 36.6	0.318[a] ± 0.09
P-value	0.02	NS	0.003	0.003	NS	0.0001	0.0001	0.0001
Se level × Se source								
Control (0.10)	5.47[b] ± 0.4	4.06 ± 0.1	1.41[b] ± 0.4	3.25 ± 1.3	537.2[a] ± 39.5	195.0[a] ± 8.6	403.8[b] ± 71.0	0.209[e] ± 0.01
Inorg (0.25)	5.47[b] ± 0.8	3.60 ± 0.4	1.87[b] ± 0.5	2.03 ± 0.5	373.1[b] ± 63.8	173.5[b] ± 2.5	561.3[a] ± 44.7	0.219[d] ± 0.01
Inorg (0.40)	6.87[a] ± 1.3	3.55 ± 0.3	3.32[a] ± 1.6	1.63 ± 1.2	523.5[a] ± 20.9	165.8[b] ± 17.1	246.2[c] ± 1.8	0.303[b] ± 0.01
Org (0.25)	5.00[b] ± 0.6	3.86 ± 0.9	1.15[b] ± 0.5	4.03 ± 1.8	541.1[a] ± 20.9	89.7[c] ± 5.2	585.2[a] ± 48.7	0.227[c] ± 0.01
Org (0.40)	5.06[b] ± 0.9	4.07 ± 0.4	0.99[b] ± 0.6	5.61 ± 2.5	406.0[b] ± 51.7	160.5[b] ± 6.7	606.7[a] ± 20.9	0.408[a] ± 0.01
P-value	0.02	NS	0.003	NS	0.0001	0.0001	0.0001	0.0001

[a,b] means in the same column within similar treatments bearing different superscripts are significantly different at *P* ≤ 0.05; NS = not significant; con = control; inorg = inorganic; org = organic

responsible for lowering the cholesterol synthesis in mature as well as immature chickens (Konjufca et al., 1997).

Regardless of Se source and the interaction between Se level and source, the Se level of 0.25 ppm significantly increased the plasma HDL concentration compared with the other levels (Table 6). A decrease in plasma cholesterol is associated with an increase in plasma HDL. Organic Se significantly increased plasma HDL compared with the other treatments, regardless of the Se level and the interaction between Se level and source. Indeed, the decrease in plasma cholesterol of the organic Se group may be a reflection of the increase in plasma HDL. The interaction between Se level and source significantly affected HDL that was increased due to supplementation of 0.25 ppm of the inorganic and organic Se source as well as the high dosage of organic Se (0.40 ppm).

Yolk selenium

Table 6 shows the effect of Se level and/or source on yolk selenium content. The increasing dietary Se level significantly increased the yolk selenium content linearly. The increases were 6.7 and 70.3% due to diets containing the medium (0.25) and the high (0.40 ppm) level of Se, respectively. The inclusion of inorganic and organic Se significantly increased the yolk selenium content compared with that in hens fed the control diet. However, hens fed organic Se deposited significantly more Se in yolks than those fed the inorganic source. This result indicated that organic Se was more potent than the inorganic source. Payne et al. (2005), Skřivan et al. (2006) and Leeson et al. (2008) reported similar results. The interaction results indicated that all supplemented levels and both sources significantly increased yolk Se content compared to the control group. Moreover, there was a linear increase within each source with increasing Se level. Furthermore, both levels of organic Se had greater values than the corresponding levels of inorganic source.

Research to produce Se enriched eggs for human health benefits shows that organic Se supplementation (e.g. selenomethionine or Se-yeast) from 0.1 mg to 0.5 ppm increases the concentration of Se in whole egg from ~ 0.1 to 0.4 mg Se/kg (Paton et al., 2002) and in other animal tissues (Bobček et al., 2004). Payne et al. (2005), Utterback et al. (2005) and Skřivan et al. (2006) stated that the use of Se

yeast in laying hen diets was very effective from the aspect of increasing the Se content of eggs. As a result of dietary Se supplementation, the Se concentration in egg yolk was significantly increased (Surai, 2006) included that in the albumen and yolk (Gajčević et al., 2009). The reported Se yeast (selenomethionine) improved product quality (reduced carcass drip loss, Se enrichment of meat and eggs) and decreased intensity of lipid peroxidation in yolks as shown by thiobarbituric acid reactive substances (Gajčević et al., 2009) may be due to a significant increase in GSH-Px activity in the blood of hens (Gajčević et al., 2009). Paton et al. (2002) concluded that a possible reason for the elevated level of Se in yolk, albumen and egg contents after supplementation of dietary Se-yeast can be due to the fact that hens have additional metabolic pathways by which Se is transferred into the egg. For example, increasing Se level in the egg albumen of hens fed Se-yeast may be due to the incorporation of greater amounts of Se as selenomethionine during albumen synthesis, selenomethionine could replace methionine, thereby providing additional Se. Surai (2006) reported that the Se-enriched quail eggs (865.2 ng/g Se/egg yolk DM) could supply 50% of the recommended daily allowance (RDA) of Se. Previous studies showed that the production of Se-enriched chicken eggs were a valuable option for improving the Se status of the general population in various countries (Yaroshenko et al., 2003). This technology was successfully applied commercially and Se-enriched eggs found their way to the supermarket shelves in more than 25 countries worldwide (Surai, 2006). In addition, two eggs from the group fed a diet containing 0.40 ppm of Se could supply 100% of RDA according FAO/WHO (2002) requirements.

Histopathological study

The administration of various levels of Se in different forms, organic or inorganic ones, led to nearly similar hepatic as well as splenic changes of the treated chickens but with dose dependent effects that were smaller or milder in the low levels of especially organic forms. The hepatic changes in severe cases were characterized by fatty vacuolations with some variable degrees that may be advanced and somewhat serious and may led to the necrosis of hepatic tissue in the case of administration at high levels (Figures 1, 2, 3, 4 and 5).

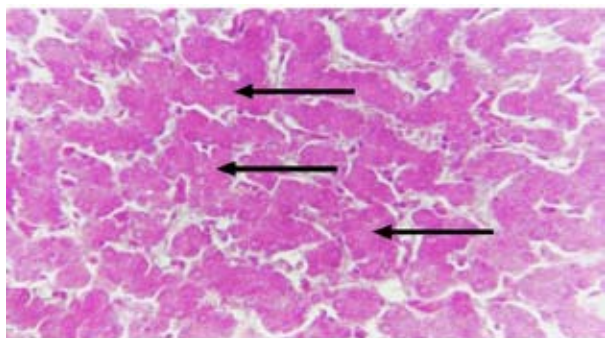*Czech J. Anim. Sci., 55, 2010 (11): 505–519*

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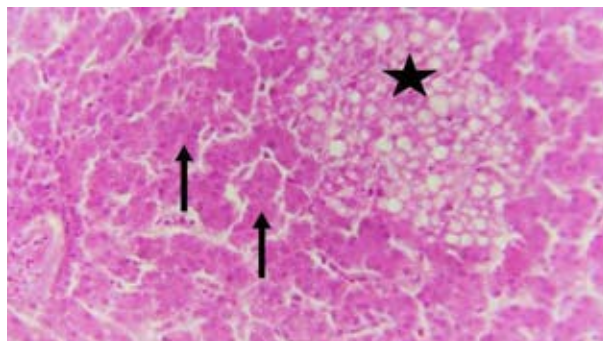Figure 1. Liver of a control chicken shows normal hepatocytic acini (arrows); H and EX400

Figure 2. Liver of a chicken treated with low levels of inorganic selenium shows one focus of severe hepatocytic fatty vacuolations (asterisk) surrounded by mild or less degenerated hepatocytic acini (arrows); H and EX250
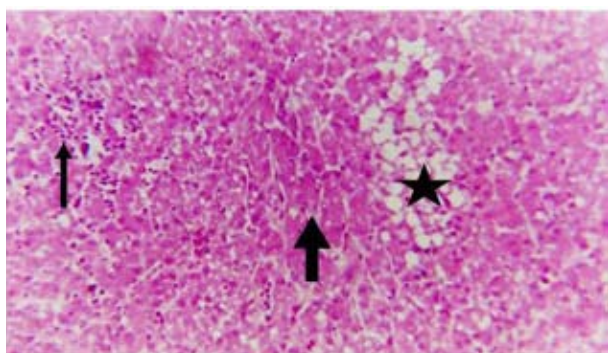

Figure 3. Liver of a chicken treated with high levels of inorganic selenium shows an area of severe fatty vacuolations (asterisk) surrounded by either small hepatocytic fatty vacuolations or necrosis (thick arrow) and another area of lymphocytic infiltration (thin arrow); H and EX250
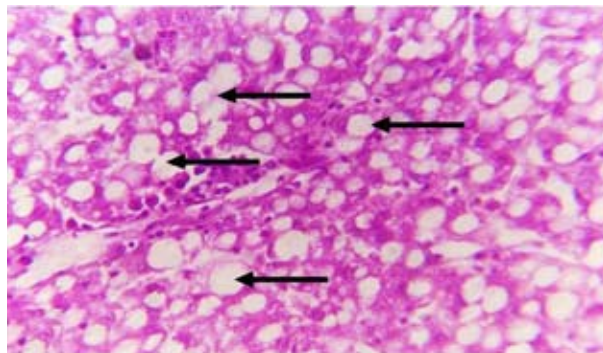
Figure 4. Liver of a chicken treated with low levels of organic selenium shows an advanced degree of fatty vacuolations in excess numbers of hepatocytic (arrows); H and EX400
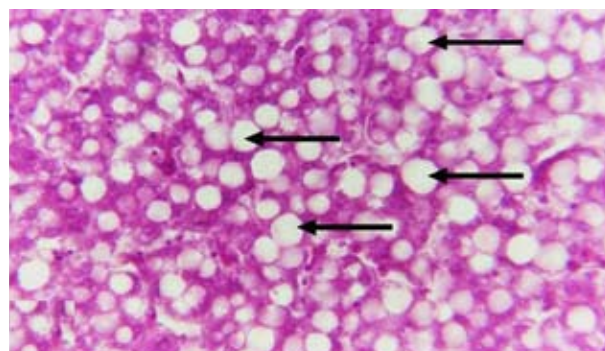
Figure 5. Liver of a chicken treated with high levels of organic selenium shows diffuse and severe sharply edged fatty vacuolations (arrows); H and EX400
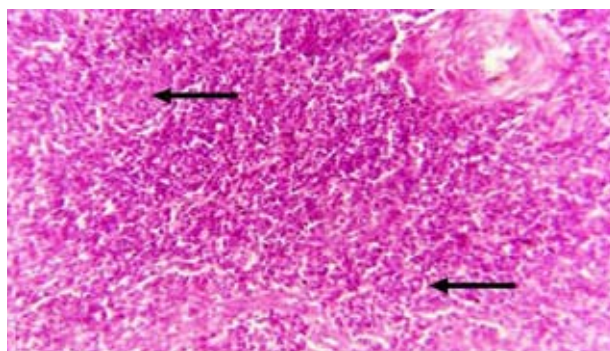

Figure 6. Spleen of a control chicken: normal splenic tissue with small-sized germinal centres (arrows) surrounded by various numbers of small lymphocytes; H and EX250

The spleen of the control group showed a normal histological structure where some small-sized germinal centres appeared normally surrounded by variable numbers of small lymphocytes (Figure 6). On the other hand, microscopic splenic changes were detected due to treatment with both levels and sources of selenium (Figures 7, 8. 9 and 10).

The microscopic changes in the small intestine were variable from part to another and from the administered form to another. The duodenal mucosa
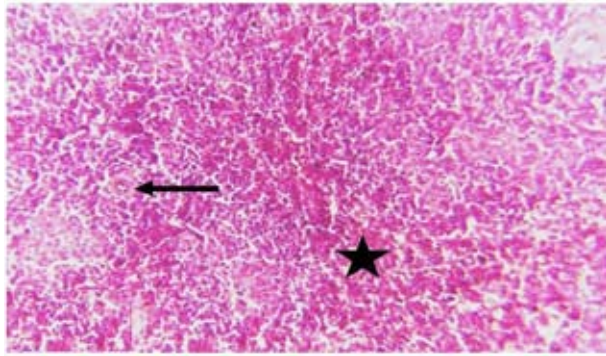
Figure 7. Spleen of a chicken treated with low levels of inorganic selenium: small-sized follicular arterioles (arrow) surrounded by areas of hyperplasia and congestion (asterisk); H and EX400
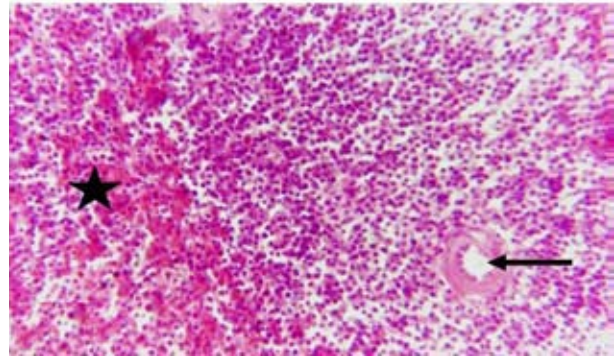

Figure 8. Spleen of a chicken treated with high levels of inorganic selenium: area of severe congestion (asterisk), medium-sized follicular artery (arrow) surrounded by an excess of mature small lymphocytes H and EX400
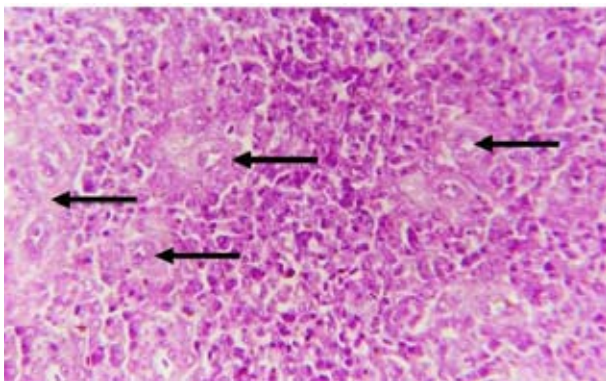

Figure 9. Spleen of a chicken treated with low levels of organic selenium shows hyperplastic splenic tissue with an excess of newly formed hyperplastic germinal centres (arrows); H and EX400
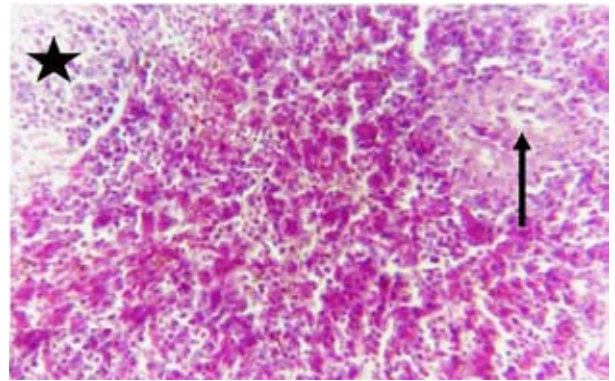

Figure 10. Spleen of a chicken treated with high levels of organic selenium shows well formed lymphoid follicles (asterisk) and expanded germinal centre (artery); H and EX400
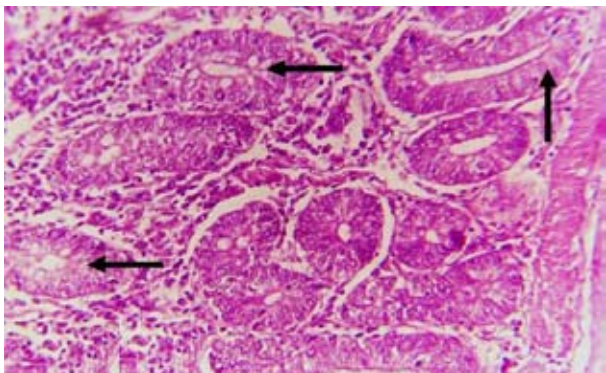

Figure 11. Intestine of a control chicken shows the normal duodenal wall with normal crypts of intestinal glands (arrows); H and EX250
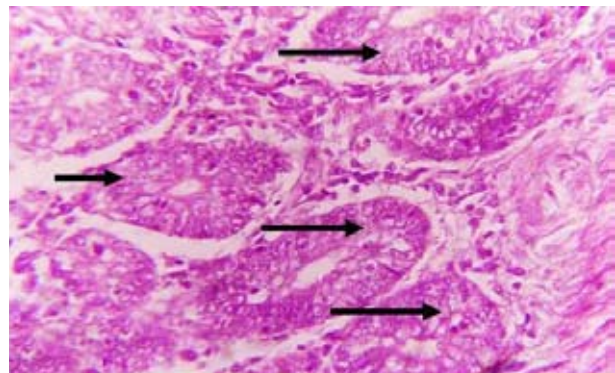

Figure 12. Duodenum of a chicken treated with a high level of inorganic selenium shows vacuolar and hydropic degeneration of the epithelial cells lining the intestinal crypts (arrows); H and EX400

appeared to be affected only by the high levels of treatment with the inorganic form of Se. It was noticeable that the intestinal mucosa of the ileum was affected by administration of the high levels of Se. The microscopic ileal changes were similarly and obviously seen

at both high levels of organic and inorganic forms of Se but with somewhat small differences in their degrees (Figures 11, 12, 13, 14 and 15).

Although the nutritional supplements of Se are beneficial for feed performance and digestion, the
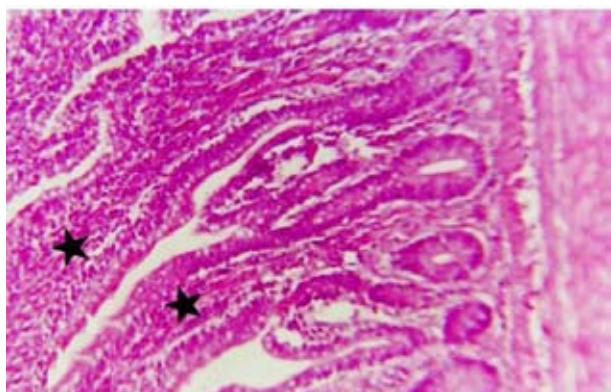
Figure 13. Ileum of a control chicken shows normal thickened villi with lymphocytic elements (asterisk); H and E. X160
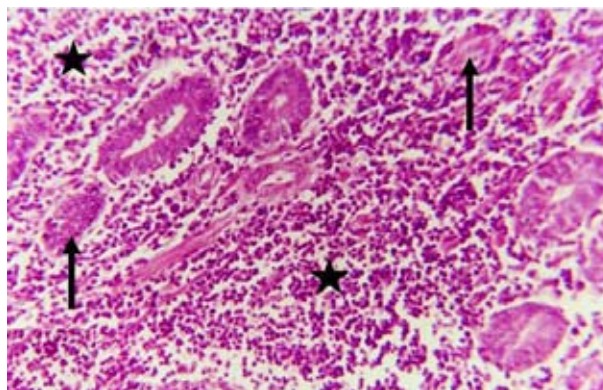

Figure 14. Ileum of a chicken treated with high levels of inorganic selenium shows an excess of mononuclear cell infiltration and aggregation (asterisk) in between degenerated and necrotic intestinal glands (arrows); H and E X250
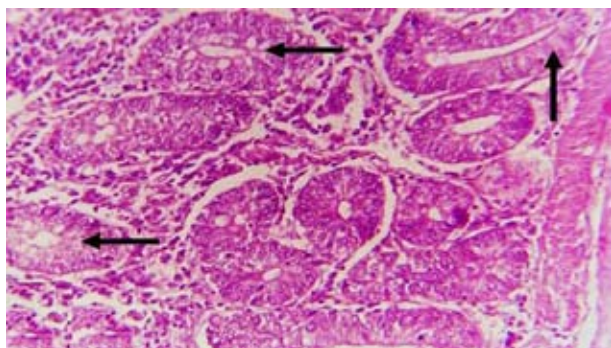

Figure 15. Ileum of a chicken treated with high levels of organic selenium shows periglandular oedema and glandular atrophy (arrows); H and E X250

high levels of administration of especially inorganic forms may lead to some adverse effects on the liver functions as well as the absorption and FCR in treated chickens.

CONCLUSIONS

It is possible to produce Se enriched eggs of crossbred breeding hens by feeding a diet supplemented with organic Se at a medium (0.25) and high (0.40 ppm) level with expected improvement in the consumer health benefit. In addition, organic Se improved the productive and reproductive performance of laying hens, decreased the cholesterol concentration, improved the tibia mineralization and was less toxic than the inorganic form when the histopathology of liver, spleen and intestine was considered. Furthermore, the level and source of Se in layer diets decreased total plasma cholesterol. Moreover, Se increased percentage of Ca and

P in tibia compared to that in hens fed the control diet. However, the medium level (0.25 ppm) of organic Se was adequate and can be recommended to enrich eggs with less harmful effects on tissues than the high level (0.40 ppm).

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