

# Effects of High Dietary Levels of Selenium and Copper on Growth Performance, Selected Blood Biochemical Parameters and Antibody Production Against Newcastle Disease Vaccine Virus in Broiler Chickens

## Research Article

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## ABSTRACT

An experiment was conducted to evaluate the effects of high dietary levels of selenium (as sodium selenite) and copper (as copper sulphate) on growth performance, serum biochemical parameters and antibody production against Newcastle disease vaccine (NDV) virus in broiler chickens. A total of 160 Ross 308 unsexed day old broiler chickens were randomly allocated into four treatment groups (n=40 per group) each comprising four replicates (n=10 per replicate). Group 1 was served as control and the chickens in this group were fed a standard diet without any selenium or copper supplementation. Chickens in Group 2 received a diet with selenium supplementation at the rate of 1 mg/kg of feed; Group 3 received a diet with copper supplementation at the rate of 200 mg/kg of feed; Group 4 received a diet supplemented with a combination of 1 mg/kg selenium and 200 mg/kg copper. Combined supplementation of selenium and copper decreased feed intake and body weight gain and increased feed conversion ratio ( $P<0.05$ ). These effects were not observed when selenium or copper was supplemented alone ( $P>0.05$ ). Serum uric acid and triglycerides concentrations decreased ( $P<0.05$ ) and tended to be increased ( $P=0.11$ ), respectively, by feeding copper and selenium. Moreover, serum albumin concentration was significantly increased in chickens given copper supplementation alone ( $P<0.05$ ), but not in those supplemented with copper and selenium ( $P>0.05$ ). Dietary treatments had no effects on antibody titres against NDV at day 28 of age ( $P>0.05$ ); antibody titres against NDV, however, decreased at day 42 of age in broilers receiving a combination of copper and selenium ( $P<0.05$ ). The results clearly indicate a negative interaction between the usage of sodium selenite and copper sulphate on growth performance in broiler chickens. More studies should be performed to clarify the action modes of such effects.

**KEY WORDS** blood parameters, broilers, copper, Newcastle disease, performance, selenium.

## INTRODUCTION

Selenium and copper are essential trace elements through their functions as cofactors in numerous biochemical reactions. Selenium plays an important role in the regulation of various metabolic processes in the body, and is an integral

part of at least 25 selenoproteins (Pappas *et al.* 2008; Gladyshev and Hatfield, 2010). However, the physiological role of selenium in organisms is chiefly concentrated on the activity of glutathione peroxidases. It has been suggested that selenium might enhance immunity, growth, reproductive performance, and the ability to resist disease (Ghazi *et*

*al.* 2012; Habibian *et al.* 2014). In chickens, it was reported that selenium deficiency is linked to a number of diseases, which include exudative diathesis, pancreatic dystrophy, nutritional muscular dystrophy and suppression of immunity (Cantor *et al.* 1982).

Copper is essential for erythropoiesis, the transport and utilization of iron in the biosynthesis of haemoglobin, and the normal growth and osteogenesis (Harris *et al.* 1980). It participates in avian feather pigmentation; it is also required as a specific cofactor in the antioxidant enzyme Cu/Zn-superoxide dismutase (Aydemir *et al.* 2000) and is incorporated into ceruloplasmin (Twomey *et al.* 2005) and cytochrome C oxidase (Engelking, 2004). Copper deficiency is known to cause anaemia, cardiovascular disorders and impaired glucose and lipid metabolism. Copper supplementation was found to reduce plasma cholesterol in chickens (Skrivan *et al.* 2000), as well as to significantly reduce broiler meat (Skrivan *et al.* 2000; Shahzad *et al.* 2012) and egg yolk (Lien *et al.* 2004) cholesterol contents. Moreover, Hosseini *et al.* (2011) reported a significantly higher immune response in terms of antibody titres against Newcastle disease virus (NDV) and infectious bronchitis when broilers fed a diet supplemented with 105 mg copper/kg.

Aaseth and Ringstad (1991) have speculated that interacting constituents in the feed can influence the bioavailability or absorption of selenium and copper and that the interaction between selenium and copper in an organism can be synergistic or antagonistic. It has been shown that selenium can counteract the toxic effect of copper (Tatum *et al.* 2000) by converting it into a metabolically inactive form (Dougherty and Hoekstra, 1982). On the other hand, copper can affect the metabolism of selenium and reduce selenium toxicity by interfering with selenium absorption and by causing accumulation of nontoxic compounds in tissue of chickens (Tatum *et al.* 2000).

Therefore, an optimal balance between selenium and copper in feed and organisms is essential for normal performance and health of poultry. National Research Council (NRC, 1994) permits a level of supplemental selenium in diets at 0.1 mg/kg. The maximum allowed selenium inclusion level in the United States is currently 0.3 mg/kg (AAFCO, 2003) and the current total maximum European Union authorized level of selenium in complete feed is 0.5 mg/kg (Council Directive, 2004). Toxicity occurs when the concentration of selenium in chicken feed is 5.0 mg/kg or higher (Apsite *et al.* 2012); the oral LD<sub>50</sub> of selenium (in the form of sodium-selenite) for chickens was reported to be 9.7 mg/kg of body weight (Surai *et al.* 2002).

The content of copper in poultry diet needs to be 5 to 10 mg/kg (Stef *et al.* 2006). In recent years the copper level in avian feed sometimes is supplemented up to 100 to 150 mg/kg (Lien *et al.* 2004; Lu *et al.* 2010). The maximum

tolerable copper level for chickens in the diet is considered to be 250 mg/kg, as limited by the NRC (1994). Addition of high copper dose to the diet is used to stimulate the growth of broiler chickens (Skrivan *et al.* 2000). However, knowledge on the influence of enhanced levels of selenium and copper on chicken health is still fragmentary.

The aim of this work was to investigate the effect of high doses of selenium, copper, and their combination in the diet on growth performance, selected blood biochemical parameters, and antibody response against NDV in broiler chickens.

## MATERIALS AND METHODS

### Broiler chickens and experimental design

All experimental protocols were approved by the Animal Care and Use Committee of Razi University (Kermanshah, Iran). A total of 160 unsexed one-day-old Ross 308 broiler chickens were purchased from a local hatchery and used in the present study. Broilers were assigned to four treatment groups in a completely randomized design; each group comprising 40 chickens. Each group was further divided into four sub-groups of 10 broilers each (as replicates):

Group 1: served as control and broilers in this group were fed a standard diet without any supplementation of selenium and / or copper.

Group 2: received a diet with selenium supplementation (as sodium selenite, Na<sub>2</sub>SeO<sub>3</sub>) at the rate of 1 mg/kg of feed.

Group 3: was composed of broilers that received a diet with copper supplementation (as copper sulphate, CuSO<sub>4</sub>.5H<sub>2</sub>O) at the rate of 200 mg/kg of feed.

Group 4: were provided with a diet supplemented with combination of 1 mg/kg selenium and 200 mg/kg copper. Broilers were provided *ad libitum* access to feed and water according to a two-phase feeding program on a starter and grower diet during the 1 to 21 and 22 to 42 days of ages, respectively. The basal diets were formulated to meet or exceed NRC (1994) nutrient requirements (Table 1). The broiler chickens were kept in battery cages (100×100×35 cm), in an environmentally controlled house from 1 to 42 days. The temperature was maintained at 33 ± 1 °C in the first week and reduced by 2 to 3 °C per week to 21 °C. The lighting program during the experimental period consisted of a period of 23 hours light and 1 hours of darkness.

### Growth performance

All broilers were weighed individually after their arrival from the hatchery to the experimental farm (initial weight) and on days 21 and 42. Daily weight gain for each dietary treatment was calculated. Feed intake was recorded in the course of the whole experiment for each treatment, and the feed conversion ratio was calculated subsequently.

**Table 1** Ingredients and nutrients composition of the experimental basal diets

Item	Starter (1-21 days)	Grower (22-42 days)
Ingredients	(%)	(%)
Maize	61.96	67.73
Soya bean meal	34.16	28.34
Soya bean oil	0.04	0.47
Limestone	1.25	1.33
Dicalcium phosphate	1.39	1.03
Salt	0.40	0.29
Sand	0.08	0.11
Vitamin-mineral premix <sup>1</sup>	0.5	0.50
Lysine-HCL	0.10	0.14
DL-Methionine	0.13	0.06
Nutrients composition		
Metabolizable energy (kcal/kg)	2850	2950
Crude protein	20.49	18.44
Lysine	1.32	1.18
Methionine	0.49	0.39
Methionine + cysteine	0.83	0.69
Calcium	0.87	0.81
Available phosphorus	0.48	0.40
Copper (mg/kg)	24.17	22.26
Selenium (mg/kg)	0.26	0.26

<sup>1</sup>The vitamin-mineral premix provided the following quantities per kg of diet: vitamin A: 10000 IU (all-trans-retinal); cholecalciferol: 2000 IU; vitamin E: 20 IU ( $\alpha$ -tocopherol); vitamin K<sub>3</sub>: 3.0 mg; riboflavin: 18.0 mg; niacin: 50 mg; D-calcium pantothenic acid: 24 mg; choline chloride: 450 mg; vitamin B<sub>12</sub>: 0.02 mg; folic acid: 3.0 mg; manganese: 110 mg; iron: 60 mg; copper: 10 mg; iodine: 0.46 mg; selenium: 0.2 mg; antioxidant: 250 mg.

### Antibody response to newcastle disease virus

All chicks were intramuscularly immunized with the killed vaccine of Newcastle and Avian Influenza (H9N2) viruses at day 16 of age. Live NDV (Nobilis ND Lasota) was administered orally (drinking water) at day 28. The vaccination schedule was based on instructions for growing Ross 308 broiler chickens (Aviagen, 2002) and local status of health and disease.

On days 28 and 42, four broilers were randomly selected from each treatment and blood samples were collected from the wing vein into a 5-ml syringe. The same broiler chickens were used for each blood sampling time. Blood samples were centrifuged at 3000×g for 15 minutes and the sera collected and stored at -20 °C until further analyses. The NDV specific antibodies in sera of broiler chickens were determined by haemagglutination inhibition (HI) test (Marquardt *et al.* 1985). The Lasota strain was used as antigen in the tests.

### Serum biochemical parameters

Individual serum samples on day 42 were analysed colorimetrically for glucose, triglycerides, total cholesterol, albumin, and uric acid concentrations using the commercial kit package according to the manufacturer's instruction (Pars Azmun, Tehran, Iran).

### Statistical analyses

Data were subjected to ANOVA in a completely randomized design using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The mean values were compared by Duncan's multiple-range test. A level of  $P < 0.05$  was considered statistically significant and  $P < 0.1$  was considered to indicate tendencies.

## RESULTS AND DISCUSSION

### Growth performance

The effects of dietary supplementation of selenium and copper separately or in combination on the growth performance of broiler chickens are shown in Table 2. Combined supplementation of selenium and copper caused a decreased feed intake and body weight gain and an increased feed conversion ratio compared to the control diet throughout the experimental period ( $P < 0.05$ ). However, these effects were not observed when selenium or copper was supplemented alone ( $P > 0.05$ ).

### Serum biochemical parameters

The effects of the experimental diets on blood biochemical parameters of broilers at day 42 of age are presented in Table 3. No significant effect of dietary treatments was detected on serum glucose and cholesterol concentrations ( $P > 0.05$ ). However, serum uric acid and triglycerides concentrations decreased ( $P < 0.05$ ) and tended to be increased ( $P = 0.11$ ), respectively, by feeding copper and selenium. The serum concentration of albumin was significantly increased in broilers given copper supplementation alone ( $P < 0.05$ ), but not in those given supplementation with copper and selenium ( $P > 0.05$ ).

### Antibody titre against NDV

The effects of dietary supplementation of selenium and copper separately or in combination on the antibody titres against NDV at days 28 and 42 of the experiment are given in Table 4. Dietary treatments had no effects on antibody titres against NDV at day 28 of age ( $P > 0.05$ ); however, it decreased considerably at day 42 of age in broilers receiving combination of copper and selenium ( $P < 0.05$ ).

In the present study, diet supplementation with sodium selenite had no significant effect on feed intake, body weight gain or feed conversion ratio during the overall period of the experiment. A similar pattern was observed in the broilers given copper sulphate supplemented diets. These findings favourably compare with the earlier report of Ryu *et al.* (2005) who reported that diet inclusion of selenium at 0, 1, 2, 4 or 8 mg/kg (as sodium selenite) had no effect on body weight and feed conversion ratio of broilers from 3 to 6 weeks of age. Similarly, Lu *et al.* (2010) obser-

ved no differences in body weight or feed conversion ratio when broilers fed diets containing 0, 100, 150 and 200 mg copper/kg (from copper sulphate) from 1 to 3 weeks of age. However, in contrast, Swain *et al.* (2000) reported an improvement in the efficiency of feed utilization when broiler chickens given a diet with 0.5 or 1.0 mg/kg added selenium from sodium selenite. Also, Samanta *et al.* (2011) reported that an increase in supplemental copper (75, 150 or 250 mg/kg as copper sulphate) resulted in an increase in body weight and feed intake in broilers from 1 to 42 days of age. Otherwise, Payvastegan *et al.* (2013) reported that dietary supplements of 125 or 250 mg copper/kg as copper sulphate markedly improved the weight gain of broiler chicken between days 21 to 42 and 1 to 42, but not at 1 to 21 days. Arias and Koutsos (2006) reported that supplementing the broiler diet with extra copper (188 mg/kg diet) was more effective under immune-challenging conditions. Similarly, Kumar *et al.* (2003) indicated that the beneficial effects of selenium (0.5 mg/L of drinking water) on body weight of broilers are more pronounced during immunological stress. Therefore, the extent of the microbial challenge may influence the response of broilers to dietary selenium and copper supplementation.

Combined supplementation of selenium and copper caused a decreased feed intake and body weight gain and an increased feed conversion ratio in comparison to the other treatment groups. Apsite *et al.* (2012) reported that the dietary addition of selenium (1 mg/kg diet from sodium selenite), copper (100 mg/kg diet from copper sulphate) or their combination stimulated growth of chicken live weight, although broilers fed combined supplementary level of selenium and copper gained less body weight and had higher feed conversion ratio when compared with those given only selenium or copper supplement. The reason for the growth-depressing effects of a higher amount of copper (200 mg/kg diet) in the present study is not clear with certainty and is in disagreement with studies reporting that selenium can counteract the toxic effect of copper (Tatum *et al.* 2000) by converting it to a metabolically inactive form (Dougherty and Hoekstra, 1982).

Copper absorbed in the intestine is transported to the liver bound primarily to serum albumin, and to some extent transcuprein (Valko *et al.* 2005). During the process of cellular copper uptake, Cu (II) is reduced to Cu (I) and copper enters the cell through various transmembrane transporters (Wang and Guo, 2006).

**Table 2** Effects of dietary treatments on body weight gain, feed intake and feed conversion ratio in broiler chickens

Item	Dietary treatments				SEM
	Group 1	Group 2	Group 3	Group 4	
	Feed intake (g/chicken/day)				
0-21 days	39.14 <sup>a</sup>	37.31 <sup>a</sup>	36.75 <sup>a</sup>	31.27 <sup>b</sup>	0.947
22-42 days	109.59 <sup>a</sup>	104.47 <sup>a</sup>	104.70 <sup>a</sup>	87.56 <sup>b</sup>	2.590
0-42 days	74.37 <sup>a</sup>	70.89 <sup>a</sup>	70.73 <sup>a</sup>	59.41 <sup>b</sup>	1.760
	Body weight gain (g/chicken/day)				
0-21 days	20.47 <sup>a</sup>	20.54 <sup>a</sup>	20.98 <sup>a</sup>	10.69 <sup>b</sup>	1.152
22-42 days	62.30 <sup>a</sup>	68.66 <sup>a</sup>	61.86 <sup>a</sup>	35.73 <sup>b</sup>	3.695
0-42 days	41.39 <sup>a</sup>	44.60 <sup>a</sup>	41.42 <sup>a</sup>	23.21 <sup>b</sup>	2.393
	Feed conversion ratio (g gain/g feed)				
0-21 days	1.91 <sup>b</sup>	1.81 <sup>b</sup>	1.77 <sup>b</sup>	2.92 <sup>a</sup>	0.130
22-42 days	1.75 <sup>b</sup>	1.52 <sup>b</sup>	1.76 <sup>b</sup>	2.45 <sup>a</sup>	0.102
0-42 days	1.79 <sup>b</sup>	1.59 <sup>b</sup>	1.75 <sup>b</sup>	2.56 <sup>a</sup>	0.106

Means within row with different superscripts are significantly different, Duncan's multiple-range test were applied to compare means.

SEM: standard error of the means.

<sup>1</sup> Group 1: control group without any supplementation, Group 2: group given a diet with selenium supplementation at the rate of 1 mg/kg of feed, Group 3: group given a diet with copper supplementation at the rate of 200 mg/kg of feed, Group 4: group given a diet supplemented with combination of 1 mg/kg selenium and 200 mg/kg copper.

**Table 3** Effects of dietary treatments on serum biochemical parameters at day 42 of age

Item	Dietary treatments <sup>1</sup>				SEM
	Group 1	Group 2	Group 3	Group 4	
Glucose (mg/dL)	234.25	231.50	232.25	207.50	7.493
Triglycerides (mg/dL)	140.62 <sup>b</sup>	187.23 <sup>a</sup>	139.25 <sup>b</sup>	185.25 <sup>a</sup>	12.036
Cholesterol (mg/dL)	149.50	149.00	146.00	158.50	6.631
Uric acid (mg/dL)	3.34 <sup>a</sup>	3.46 <sup>a</sup>	2.42 <sup>b</sup>	2.51 <sup>b</sup>	0.168
Albumin (g/dL)	2.89 <sup>b</sup>	4.66 <sup>a</sup>	3.87 <sup>ab</sup>	3.77 <sup>ab</sup>	0.273

<sup>a,b</sup> Means within row with different superscripts are significantly different, Duncan's multiple-range test were applied to compare means.

SEM: standard error of the means.

Group 1: control group without any supplementation; Group 2: group given a diet with selenium supplementation at the rate of 1 mg/kg of feed; Group 3: group given a diet with copper supplementation at the rate of 200 mg/kg of feed; Group 4: group given a diet supplemented with combination of 1 mg/kg selenium and 200 mg/kg copper.

**Table 4** Effects of dietary treatments on the titres anti-Newcastle disease virus HI in broiler chickens at day 28 and 42 of age

Item	Dietary treatments <sup>1</sup>				SEM
	Group 1	Group 2	Group 3	Group 4	
28 day	1.25	1.66	0.50	1.25	0.411
42 day	3.00 <sup>a</sup>	2.75 <sup>ab</sup>	1.75 <sup>bc</sup>	1.00 <sup>c</sup>	0.375

Means within row with different superscripts are significantly different, Duncan's multiple-range test were applied to compare means.

SEM: standard error of the means.

Group 1: control group without any supplementation; Group 2: group given a diet with selenium supplementation at the rate of 1 mg/kg of feed; Group 3: group given a diet with copper supplementation at the rate of 200 mg/kg of feed; Group 4: group given a diet supplemented with combination of 1 mg/kg selenium and 200 mg/kg copper.

The intracellular transport of copper is performed with the aid of either glutathione as the Cu (I)-Glutathione complex or a class of small cytosolic proteins known as copper chaperons (Chmielnicka *et al.* 1983; Wang and Guo, 2006). The Cu (I)-Glutathione complex serves as a vehicle for delivering copper to metallothioneins, a family of proteins that serve the purpose of intracellular metal detoxification (Wang and Guo, 2006). Therefore, one explanation for our finding that selenium obviously induced an enhanced susceptibility to copper toxicity could be the inhibition of metallothionein synthesis as reported by Chmielnicka *et al.* (1983) after oral selenium treatment of rats.

The broilers given with copper supplementation alone had significantly higher serum concentration of albumin than the control broilers. However, when selenium and copper were supplemented simultaneously, the serum concentration of albumin was similar to those of the control group. Since serum albumin is the main carrier protein for copper in the circulation (Wang and Guo, 2006), this result seems to be in contradiction with our latter hypothesis. However, it has been reported that selenite is incorporated into the plasma proteins normally present (Vokal-Borek, 1979) and bound there to sulphhydryl groups. Selenium in such protein-selenium compounds has been shown to bind mercury (Burk *et al.* 1974) and similar bindings have been discussed for zinc (Chmielnicka *et al.* 1983). It may be speculated that such a binding to plasma proteins could be relevant also for other metals, e.g. copper.

Jegade *et al.* (2011) and Apsite *et al.* (2012) reported significantly higher uric acid concentrations in serum of broilers given high dietary copper levels. The elevated accumulation of uric acid in blood serum suggests a copper-induced disturbance in kidney excretion function. Although uric acid acts as an antioxidant and has a free-radical scavenging effect, when it accumulates to a high level in blood it can cause health problems (Jegade *et al.* 2011). Apsite *et al.* (2012) reported that dietary supplementation of selenium reduced the toxic effect of copper in broiler chickens as indicated by a normal level of uric acid in the blood serum of chickens fed combined dietary supplemental level of 1 mg selenium/kg and 100 mg copper/kg. In contrast, in the present study, the serum concentration of uric acid was lower for broilers given copper supplementation (either alone

or in combination with selenium) compared to the other groups. However, this observation does not necessarily mean that both diets have similar effect on the uric acid metabolism. The lower serum concentration of uric acid in broilers receiving combined supplementation of copper and selenium may be due in part to the lower feed intake of these chickens.

No significant effect of dietary treatments was detected on serum glucose and cholesterol concentrations. However, serum triglycerides concentrations tended to be increased by feeding selenium. These results are in contradiction with the results of Samanta *et al.* (2011) and Payvastegan *et al.* (2013) who observed the lowest concentration of plasma total cholesterol and triglycerides in the broiler chickens given a supplement of 250 mg copper/kg as copper sulphate. Likewise, Kanchana and Jeyanthi (2010) reported significantly lower serum triglycerides and total cholesterol concentrations in layer chickens given a diet supplemented with selenium at 0.2 or 0.4 mg/kg in the form of selenium yeast. As discussed earlier in this paper, the efficacy of mineral applications in chickens' diet would be affected not only by its source and administration level but also by several environmental factors. Habibian *et al.* (2014), in an experiment investigating the effects of different levels of selenium (0, 0.5 or 1 mg/kg as selenomethionine) on serum biochemical variables in broiler chickens, reported that selenium supplementation had no significant effect on serum parameters of broilers reared under normal condition. However, when the broilers were subjected to a high environmental temperature the serum concentrations of glucose, triglycerides, and total cholesterol significantly decreased due to dietary selenium supplementation.

Antibody titres against NDV were not significantly influenced by selenium supplementation, agreeing with the findings of Swain *et al.* (2000), in which the supplementation of selenium (0.5 or 1 mg/kg as sodium selenite) had no impact on this parameter. Similarly, copper supplementation had no effects on antibody titres against NDV at day 28 of age. However, the anti-NDV titre considerably decreased at day 42 of age by copper supplementation. These findings are in disagreement with the results of Saeedi (2010) and Hosseini *et al.* (2011). Saeedi (2010) found no significant differences in the antibody titres against NDV among broilers receiving

diets supplemented with 0, 8, 16 or 32 mg copper/kg (as either copper sulphate or copper methionine). However, Hosseini *et al.* (2011) reported that the antibody titres of NDV (day 12), increased by copper supplementation at 105 mg/kg diet as copper sulphate. This may be due to the relatively higher level of copper used in the present study.

## CONCLUSION

The results of the present study clearly indicated a negative interaction between the usage of sodium selenite and copper sulphate on growth performance and antibody production against NDV in broiler chickens. More studies should be performed to confirm and to clarify the action modes of such effects.

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