INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 12, p. 221-226, 2013

RESEARCH PAPER OPEN ACCESS

# Effects of different levels of copper sulfate on blood biochemical traits in japanese quail (*Coturnix coturnix japonica*)

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**Key words:** Copper sulfate ,blood parameters, Japanese quail.

doi: http://dx.doi.org/10.12692/ijb/3.12.221-226 Article published on December 18, 2013

**Abstract**

The aim of the current study was to investigate effects of different Levels of copper sulfate on blood biochemical traits in Japanese quail. Four hundred, day old male Japanese quail were randomly assigned into 5 treatment groups with 4 replicates (each treatment included 20 replicate). All birds received basal diet up to 7 day of old. At day 8 until the end of the study birds were received experimental diets. Control group (A) received basal diet, whereas B, C, D and E groups were received basal diet supplemented by 50, 100, 150 and 200 mg copper sulfate, respectively. At day 42, birds were given 5h fasting period and 2 birds were randomly selected from each experimental group, slaughtered and blood samples taken for determining albumin, triglycerides, glucose, total protein, cholesterol, LDL and HDL levels. According to the obtained results there was no significant effect on blood albumin, triglycerides, glucose, total protein, cholesterol, LDL and HDL levels in Japanese quail fed supplemented diet by different levels of copper sulfate ($p>0.05$). It seems, different Levels of copper sulfate were not able to change blood parameters in Japanese quail.

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## Introduction

Copper is a needed trace mineral in poultry. Copper (Cu) is abundant in the environment and needed for the normal metabolism and growth in animals. Deficiency or excess of Cu sulphate lead to physiological disturbances and variety of diseases (Iqbal *et al.,* 2012) e.g. increases the Q-T interval in the electrocardiogram (ECG) of pigs (Onder *et al.,* 2006). Mineral bioavailability is influenced by variety of factors e.g. the form minerals in the feed stuffs (Chitithoti *et al.,* 2012). In recent years, the role of antimicrobial Cu growth, the addition of Cu in excess of the required drug concentrations (100 to 300 mg per kg) in poultry diets has become prevalent (Ewing *et al.,* 1998). Copper is findable in all tissues which is a critical components of the antioxidant system in the body. In the blood, plasma Cu is attached to its specific plasma transport protein, ceruloplasmin. Also, the portion of serum Cu is transported bound to albumin, transcuprein and copper-amino acid complexes (Megahed *et al.,* 2013). Copper sulfate is a naturally-occurring inorganic salt and has been added to poultry diets in excess of its nutritional needs as an antimicrobial and growth promoter (Iqbal *et al.,* 2012). Improvement in feed conversion and weight gain in broilers fed diets supplemented by 150 to 200 mg of Cu sulphate reported by Baker *et al.,* (1991) and Choi and Paik (1989). Furthermore, Paik (2001) observed that the addition of 125 to 250 mg Cu improved feed conversion and growth rate in broilers. Similar results have been found by adding Cu to poultry diets (Pesti and Bakalli, 1996). Many reports have been reported on effects of Cu on lipid metabolism in poultry (Jegede *et al.,* 2011; Mondal *et al.,* 2007; Skrivan *et al.,* 2000; Konjufca *et al.,* 1997; Pesti and Bakalli, 1996). In this regards, Mondal *et al.,* (2007) reported supplementation of diets by Cu improves cholesterol, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) metabolism in poultry. Also, it is reported serum Triglyceride (TG) levels diminish in broilers fed Cu supplemented diets (Bakilli *et al.,* 1995). Japanese quail (*Coturnix coturnix japonica*), a small-domesticated avian species and is commercially exploited for meat and egg production (Sangilimadan *et al.,* 2012). Little literatures have been published about the effects of different Levels of copper sulfate on blood biochemical traits in Japanese quail. According to previous studies on effects of Cu supplemented diets in poultry, as well as to understanding possible effects of Cu in Japanese quail, our hypothesis in present study was to clarify possible effects of Cu sulfate on Albumin (Alb), Triglycerides (TG), Glucose (Glu), Total Protein (TP), Cholesterol (Cho), LDL and HDL levels in Japanese quail.

## Materials and methods

*Birds and diets*

Four hundred and twenty, day old male Japanese quail (*Coturnix coturnix japonica*) were randomly assigned into 5 treatments with 4 replicates (each replicates contain 20 birds). Before experimental procedure, Japanese quail weighed and randomly assigned into experimental groups. All experimental birds were received basal diet without copper sulfate from day 1 to 7. At day 8, experimental birds were received experimental diets: Control group (A) received basal diet, whereas B, C, D and E groups were received basal diet supplemented by 50, 100, 150 and 200 mg copper sulfate, respectively. Diets were formulated using User Friendly Feed Formulation Done Again (UFFDA) (Pesti *et al.,* 1992), according to nutritional suggestions. Chemical composition of experimental diets is presented in table 1.

*Blood samples*

A 5-h prior the end of the study birds were deprived from food (FD5). Blood samples were collected using disposable syringes. Blood samples were centrifuged at 750× g for 10 minute and plasma was separated and stored at -20 °c until used. Serum Alb, TG, Glu, TP, Cho, LDL and HDL levels determined by calorimetric method using auto analyzer (Mindray-BS-200, Germany) at physiology laboratory at the Islamic Azad University, Shabestar branch. Shabestar, Iran.

*Statistical analysis*

Data were analyzed by one-way analysis of variance (ANOVA) in a completely randomized design and treatment means were tested for statistical significance by Duncan's multiple range tests using SAS Statistical software (9.1.3, 2007).

**Results**

Effects of different Levels of copper sulfate on blood parameters in Japanese quails in day 42 is presented in table 2. According to the obtained results, there was no significant effect on blood albumin, triglycerides, glucose, total protein, cholesterol, LDL and HDL levels in Japanese quail fed supplemented diet by different levels of copper sulfate ($p>0.05$).

**Table 1.** Feed composition and nutrient contents of experimental diets of Japanese quails at grower period.

| Treatments | | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Ingredients | | | | | |
| copper sulfate | 0 | 0/005 | 0/01 | 0/015 | 0/02 |
| Corn grain | 55 | 55 | 55 | 55 | 55 |
| Soybean meal | 33.34 | 33.35 | 33.35 | 33.35 | 33.35 |
| Gluten meal | 7 | 7 | 7 | 7 | 7 |
| Oyster shell | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| DCP | 0.84 | 0.32 | 0.32 | 0.32 | 0.32 |
| Sodium bicarbonate | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |
| Soybean oil | 0.11 | 0.12 | 0.12 | 0.12 | 0.12 |
| Vit. Premixes | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Min. Premixes | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| Nutrients (calculated) | | | | | |
| ME (Kcal/Kg) | 2900 | 2900 | 2900 | 2900 | 2900 |
| CP % [a] | 24 | 24 | 24 | 24 | 24 |
| Ca % | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Av. P % | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Met. % + Cys. % | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 |
| Lys. % | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 |
| Tryptophan % | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Na % | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| K % | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| Cl % | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |

DCP= Di calcium phosphate. ME = Metabolizable Energy. Lys: Lysine. Met: Methionine, Ca: Calcium, Cys: Cysteine.

**Table 2.** Effects of different Levels of copper sulfate on blood biochemical traits in Japanese quail.

| Treatments | TP (g/dl) | TG (mg/dl) | Cho (mg/dl) | LDL | HDL | Glu (mg/dl) |
|---|---|---|---|---|---|---|
| (A) Control | 1.24 | 92.37 | 176.63 | 84.50 | 59 [ab] | 258.75 |
| (B) copper sulfate 50mg | 1.18 | 93.37 | 181.88 | 96.37 | 63.62 [ab] | 284.88 |
| (C) copper sulfate 100mg | 1.04 | 89.25 | 189.50 | 95.87 | 63 [ab] | 279 |
| (D) copper sulfate 150mg | 1.06 | 93.62 | 183.88 | 94.75 | 69.12 [a] | 265.63 |
| (E) copper sulfate 200mg | 1.15 | 91.87 | 201.63 | 92.12 | 53.62 [b] | 271.63 |
| *P value* | 0.369 | 0.978 | 0.241 | 0.582 | 0.048 | 0.312 |
| SEM | 0.082 | 5.249 | 7.940 | 5.739 | 3.523 | 9.323 |

TP: Total Protein, TG: Triglycerides, Cho: Cholesterol, LDL: Low density lipoprotein, HDL: high density lipoprotein, Glu: Glucose. SEM: Standard error mean. There are significant differences between groups with different codes in a column (superscript letters a, b; $p < 0.05$).

**Discussion**

Consuming diets containing 200 mg Cu kg decreased plasma Cho in broilers but the results was not significant. Many reports are presents on the effects of copper in lipid metabolism (Jegede *et al.,* 2011; Mondal *et al.,* 2007; Skrivan *et al.,* 2000; Konjufca *et*

*al.,* 1997; Pesti and Bakalli, 1996). It is reported that high levels of Cu in diets reduces plasma cholesterol levels in broiler (Idowu *et al.,* 2006). A decrease on Cho concentrations in plasma and breast muscle has been reported in broilers fed contacting Cu sulphate 250 mg (Bakalli *et al.,* 1995). High concentrations of Cu in the diet increased hepatic Cu concentration, decreased hepatic reduced glutathione (GSH) and oxidized glutathione to reduced glutathione ratio (GSH: GSSG) (Mondal *et al.,* 2007). Glutathione peroxidase impresses its effects through cholesterol biosynthesis on hydroxy methyl Glutaryl coenzyme A reductase (Vaisala and Kurup, 1987; Konjufca *et al.,* 1997). Liver reduced glutathione concentration decreased coenzyme A reductase enzyme activity Glvtaryl hydroxypropyl methylcellulose, which is a key enzyme in the biosynthesis of cholesterol and followed by reduced cholesterol synthesis (Mondal *et al.,* 2007). In addition, Konjufca *et al.,* (1997) showed that the use of Cu can affect 7-Alpha Hydroxylase levels. Bakalli *et al.,* (1995) was found that the use of 250 mg Cu sulfate source decreased serum TG concentrations in broiler chickens. In this regards, Paik (2001) reported that the addition of 125 mg Cu kg broiler diets reduced serum TG concentration of 250 mg per kg whereas Cu is has negligible effects on TG levels in chickens. In this regard, we cannot find previous research on the Japanese quail model to compare our results with. In this study supplementation of diets using Cu sulfate increased blood Cho levels in Japanese quail which are against to the previous results in broilers. It seems this result can because of variety of differences e.g. bird type, between strain differences and so on.

Moreover, Chowdhury *et al.,* (2004) revealed that serum TG levels decreased linearly with increasing dietary Cu and Cu methionine concentration. Use level of 200 mg Cu kg increased HDL in the blood serum of broiler chickens. Lien *et al.,* (2004) examined the effects of zero, 125 and 250 ppm Cu sulfate source and chrome surfaces (zero, 800 and 1600 mg kg of chromium picolinate) in laying hens. According to their results addition of Cu to the diet lowers Cho, Very Low Density Lipoprotein (VLDL) and increases HDL levels in laying hens. Similar results was observed in study by Abaza *et al.,* (2009), which addition of Cu sulfate at zero, 100 and 200 mg per kg in laying Japanese quail. In this study supplementation of diets using Cu sulfate increased blood Cho levels in Japanese quail which are against to the previous results in laying Japanese quail. It seems this result can because of strain differences.

In this experiment, blood Glo and TP levels were not significantly different compared to control group. However, it is noteworthy that the highest levels of blood Glu was belong to the group fed diet containing 200 mg per kg Cu sulfate. Abaza *et al.,* (2009), reported supplementation of Cu sulfate was not significantly increasing blood Glu (mg/100 mL) and TP (mg/100 mL) levels in laying Japanese quail. According to our results, supplementation of diets via different levels of Cu sulfate don't have significant effects on blood Glu and TP in Japanese quail which is in parallel to Abaza *et al.,* (2009). To our knowledge, there may be more mechanisms including in regulatory function of Cu sulfate on blood parameters in Japanese quail which do not clarified yet. Also we recommend further researches need to identify detail of mechanism.

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