J. Animal and Poultry Prod., Mansoura Univ., Vol.9 (12): 489 – 495, 2018

Physiological and Antioxidant Responses of Japanese Quail to Dietary Copper Supplementation

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ABSTRACT

Two experiments were conducted to elucidate the effect of dietary copper supplementation on performance, physiological, hematological and antioxidant responses of day-old unsexed Japanese quail chicks (*Coturnix coturnix Japonica*). In each experiment, a total number of 250 quails were summited to five experimental treatments (50 chicks in five replicates each. The first one (control) was fed the basal diet while the other groups were fed the same diet supplemented with copper as cupric sulfate pentahydrate at levels of 50, 100, 150 and 200 mg/kg diet. In experiment 1 (Exp.1), chicks were fed the Cu-supplemented diets until 6 weeks of age whereas the supplementation period in experiment 2 (Exp.2) was for the first five weeks of age followed by a final period of one week during which birds fed the control basal diet. Results demonstrated that supplementation of copper sulfate to diets either or 6 (Exp. 1) or 5 weeks (Exp. 2) had significantly improved live body weight, weight gain, feed conversion ratio and low mortality rate of quail chicks. Hematological indices in terms of RBCs count, hemoglobin concentration, hematocrit, mean corpuscular volume, WBCs count and H /L ratio were significantly improves in quails fed Cu-supplemented diets up to 100 mg/Kg in Exp.1 and up to 150 mg/Kg in Exp. 2. Birds fed Cu – supplemented diets for six weeks (Exp. 1) up to 100mg/Kg had significantly higher plasma total protein, globulin, Immunoglobulins (Y , M , A) and high density lipoprotein (HDL) but lower plasma total cholesterol, triglycerides and low density lipoprotein (LDL) than the control ones. A similar trend was recorded for Exp. 2 quails, but plasma protein fractions were not significantly affected. In both experiments, blood reduced glutathione (GSH), superoxide dismutase (SOD) activity and total antioxidant capacity (TAC) were significantly increased with Cu-supplemented diets, while malondialdehyde (MDA) was significantly decreased in the experimental groups compared with the control. The obtained results indicate that dietary supplementation with cupric sulfate pentahydrate at levels up to 100 mg/kg could improve growth performance, antioxidant defense system, immunity and lower cholesterol content of growing Japanese quail.

Keywords: copper, performance, physiological response, antioxidant system, quail.

INTRODUCTION

Copper (Cu) was reported by many workers as an essential trace element required for survival of all organisms from bacterial cells to human (Linder 1991; Hamdi *et al*. 2018). Copper is involved in multiple extracellular and intracellular enzymes such as super-oxide dismutase, ceruloplasmin, cytochrome oxidase and lysyl oxidase (Klasing 1998; Wieleba and Pasternak 2001) Moreover, copper was nearly observed as a constituent element in all body cells, being particularly concentrated in the liver, which acts as the main copper storage organ of the body (Zhau, *et al*., 2010). It exerts a significant effect on the processes of vasculogenesis, angiogenesis, in the synthesis of hemoglobin and redox formation (Mroczek-Sosnowska *et al*., 2016).

Also, some researchers noted that supplementation of 125-250 ppm of Copper from cupric sulfate pentahydrate and cupric citrate reduced serum and breast muscle cholesterol levels (Pesti and Bakalli 1996; Scott *et al*. 2018). In another study, however, Cu-Met was found to exert a linear increase in serum cholesterol level, accompanied by a significant decrease in triglycerides level (Chowdhury *et al*. 2004). It was also observed that supplementation of high Cu levels resulted in high excretion rate of Cu through feces (Paik *et al*. 1999; Hamdi *et al*. 2018). Jenkins *et al*. (1970) have reported a 5% increase in growth response when Cu (250 mg/kg) was added to wheat-fish meal diet, but growth rate was depressed when the same level was added to a corn-soybean diet. A daily demand for Cu is low; however, it is necessary for normal body functioning. The requirement of poultry depends on species, age and production type, i.e. growing poultry; 4 mg kg−1, laying hens; 2.5 mg kg−1 broiler chickens; 8 mg kg−1 of feed mixture. In practice, feed mixtures for poultry are enriched with much higher levels of Cu, using mainly copper sulfate (CuSO4) due to its growth promoting effects (Leeson, 2009 and Karimi, *et al*., 2011). However, Zhao, *et al*. (2010) have observed that independent of the supplementation level only a small fraction (20%) is actually used for different body functions, while the rest is excreted in faeces which may cause pollution of soil, plants and aquatic environment.

It is well known that copper deficiency induces hypercholesterolemia but the mechanism was not recognized, but Kim *et al*. (1992) demonstrated that copper regulates cholesterol biosynthesis by reducing hepatic glutathione concentration. Cholesterol bio-synthesis is regulated through the stimulation of 3-hydroxy-3 methylglutaryl coenzyme A (HMG-COA) reductase, which is the rate-limiting step of mevalonate production and, ultimately, cholesterol bio-synthesis in rats (Valsala and Kurup 1987). The peroxidative damage to cell membrane lipids considered the most negative effect of excess dietary copper. In a recent study, Ajuwon *et al*. (2011) reported that supplementation of dietary copper by level of 250 mg/kg diet caused a high lipid peroxidation and low concentrations of glutathione, SOD and catalase in the liver and erythrocytes of broiler chickens.

Kumar, *et al*. (2013) showed a significant cholesterol concentration after inclusion of Cu to poultry diets. In this respect Kim *et al*. (1992) demonstrated that copper regulates cholesterol biosynthesis by reducing hepatic glutathione concentration. They added that cholesterol biosynthesis is regulated through the stimulation of 3-hydroxy-3 methylglutaryl coenzyme A (HMG-COA) reductase, which is the rate-limiting step of mevalonate production and, ultimately, cholesterol bio-synthesis. Also, adding higher levels of Cu was reported to regulate cholesterol biosynthesis indirectly by decreasing the glutathione reductase formation and increasing the oxidized form of glutathione (Bakalli, *et al*., 1995; Kim, *et al*., 1992).

No work has been found concerning the productive and physiological responses of Japanese quail to feeding a high level of copper. Therefore, two experiments were conducted to recognize the impact of Cu supplementation to diets on enhancing performance, physiological, hematological and antioxidant responses of day-old unsexed Japanese quail chicks.

MATERIALS AND METHODS

Two experiments were conducted with day-old unsexed Japanese quail chicks (coturnix coturnix Japonica). In each experiment, a total number of 250 chicks were distributed into five experimental groups with 5 replicates of 10 birds each. The first (control) group was fed the basal diet and the other groups were fed the basal diet supplemented with copper as feed grade cupric sulfate pentahydrate at levels of 50, 100, 150 and 200 mg/kg diet. The basal diet was formulated to meet the NRC (1994) recommended requirements for growing quail including copper level (Table 1). The feed was offered ad-libitum and water was freely choice during the experimental period. In experiment 1 (Exp.1), chicks were fed the Cu-supplemented diets until 6 weeks of age whereas the supplementation period in experiment 2 (T2) was for the first five weeks of age followed by a final recovery period of one week during which birds fed the control basal diet.

Table 1. Ingredients and calculated analysis of the growing quail diet.

Ingredients	[%]
Corn (Yellow)	60.00
Soyabean meal [44%]	30.00
Fish meal [72%]	7.00
Bone meal	2.20
Sodium chloride	0.25
Ground limestone	0.20
Methionine	0.05
Premix[1]	0.30
Calculated analysis**	
Energy [kcal/kg]	2900
Protein	24.02
Fat	3.48
Fiber	3.28
Lysine	1.02
Methionine & cysteine	0.80
Calcium	0.96
Av. Phosphorus	0.48

***1 Vitamins and trace minerals premix provides [per kg]: Vit. A, 5,500 IU; cholecalciferol, 1,100 IU; vit. E, 11 IU; riboflavin, 4.4 mg; Ca pantothenate, 12 mg; nicotinic acid, 44 mg; choline Cl, 220 mg; vit. B12, 6.6 µg; vit. B6 2.2 mg; folic acid, 55 mg; d-biotin, 0.11 mg; thiamine, 1.1 mg; Mn, 60 mg; Zn, 50 mg; Fe, 30 mg; Cu, 5 mg; I, 105 mg.**

**** According to NRC 1994.**

Growth performance data:

Initial and final individual live body weight (LBW, g), weight gain (BWG, g) and feed consumption (FC, g) were recorded weekly for quails of both Exp.1 and Exp.2, then feed conversion ratio (FCR) was calculated according to the equation: FCR = FC (g) / BWG (g). Also, cumulative mortality was recorded and expressed as a percentage of live chicks.

Blood collection and biochemical analysis:

At six weeks of age, 10 birds / each treatment were randomly chosen and sacrificed for blood collection and analysis. Blood samples were collected from the sacrificed birds in clean-dry tubes ,with heparin as anticoagulant, and centrifuged (4000 rpm) for 10 minutes. Plasma was then decanted and stored at -20°C until assayed for total protein, albumin, cholesterol, triglyceride, low and high density lipoproteins (LDL ; HDL) by using biodiagnostic commercial kits purchased from (Bio-Med Diagnostics, Egypt. Co. for Biotechnology). A part from each blood sample was used to assess the hematological parameters including red (RBCs) and white (WBCs) blood cells count, then blood smears were prepared and stained with Wright,s stain for differential count of WBCs according to Feldman *et al*. (2000). Hemoglobin (Hb) concentration and the percentage of packed cells volume (PCV) were measured according to Drew *et al*. (2004). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also calculated according to Feldman *et al*. (2000). The lipid peroxidation was determined by measuring the biomarker malondialdehyde (MDA) in plasma, using the thiobarbituric acid reaction as described by Yoshoiko *et al*.(1979). The total antioxidant capacity (TAC) was determined according to Koracevic *et al*.(2001). The packed erythrocytes remaining after harvesting the plasma from the previous steps were taken to assay reduced glutathione (GSH) according to the method described by Beutler *et al*. (1963) and superoxide dismutase (SOD) according to Nishikimi *et al*. (1972).

Statistical analyses

Data were statistically analyzed by one way ANOVA using the General Linear Models (GLM) procedure of SAS (SAS 2001), with copper level as the main effect. Duncan's new multiple range test (Duncan 1955) was used to separate means when significant differences are revealant.

RESULTS

Inspection of the data revealed that incorporation of copper in the diet improved growth and feed conversion ratio or not depending on the dietary copper levels either at 3 or 6 weeks of age (Table 2). Birds fed 50 and 100 mg-supplemented diets for six weeks (Exp.1) gained about 4.5 or 7.7 % in their body weight and improved their feed conversion ratio by about 6.7 and 7.5 % than the control group, respectively. While feeding 50 and 100 mg Cu/kg for 35 days (Exp.2) followed by a final period of 7 days without Cu-supplemented diets, increased quails body weight gains by 4.5 and 7.9% and enhanced FCR by 8.9 and 10 %, respectively (Table 3). Nonetheless, when copper was fed above 100 mg/kg, the benefit achieved in weight gain and feed conversion was lost in all experiments.

Birds fed Cu-supplemented diet up to 100 mg/kg had normal rates of mortality (4.2 %) at the period from 1 -6 weeks of age. Meanwhile, birds fed 150 and 200 mg Cu/kg diet had a slightly higher mortality rates, even it was not significantly differed. Inspection of the mortality rate data in parallel with the previous data of body weight gain and feed conversion ratio elucidated that supplementation up to 100 mg Cu could strongly be within the growth-promoting range of Cu while supplementation of 150 mg Cu/kg could be the minimum toxic level of Cu in Japanese quail.

Table 2. Effect of six weeks feeding of different Cu sulfate levels on productive traits of Japanese quail chicks (Exp. 1).

Copper Level	LBW		BWG	Feed conversion	Mortality[1]
	1 day	6 weeks	1-6weeks	1-6weeks	1-6weeks
[mg/kg]	[g]		[g]	[g:g]	[%]
0	8.5±0.60	199.5±4.54^{b}	191.1±5.22^{b}	2.67±0.018^{a}	2.63
50	8.4±0.50	208.1±8.07^{a}	199.7±6.29^{c}	2.49±0.023^{b}	3.12
100	8.6±0.58	214.5±9.24^{a}	205.8±8.30^{c}	2.47±0.010^{b}	3.20
150	8.6±0.46	188.9±6.58^{c}	180.3±9.31^{a}	2.83±0.032^{a}	5.68
200	8.5±0.55	192.3±8.18^{c}	183.6±9.42^{a}	2.87±0.028^{a}	7.24

[1] Cumulative mortality rate from 1- 6 weeks of age. *,c Means within columns with different letters differ significantly (p<0.05)

Table 3. Influence of five weeks feeding different copper sulfate levels on productive performance of old Japanese quail chicks (Exp. 2)

Copper Level	LBW		BWG	Feed conversion	Mortality[1]
	1 day	6 weeks	1-6weeks	1-6weeks	1-6weeks
[mg/kg]	[g]		[g]	[g:g]	[%]
0	8.6±0.66	201.5±9.75^{b}	192.8±7.52^{b}	2.69±0.06^{a}	2.26
50	8.4±0.58	209.8±7.26^{a}	201.4±6.85^{a}	2.45±0.03^{b}	2.34
100	8.5±0.74	216.6±9.44^{a}	208.1±9.70a	2.42±0.02^{b}	2.61
150	8.7±0.62	201.1±8.54^{b}	192.3±9.91^{b}	2.86±0.005^{a}	4.16
200	8.8±0.45	197.2±8.78^{b}	188.5±8.96^{b}	2.93±0.08^{a}	4.92

[1] Cumulative mortality rate from 1- 6 weeks of age. [a-c] Means with different letters in columns differ significantly ($p<0.05$)

Data concerning the hematological parameters showed that feeding diet with different Cu supplementation levels had significantly increased RBCs count, hemoglobin concentration, PCV (%), and MCV- but not MCH and MCHC- in Exp. 1 compared to control group (Tables 4) with higher values ($p\leq0.05$) recorded for birds fed 50 and 100 mg/Kg Cu –supplemented diets. However, in Exp.2, all blood indices (except, MCHC) were significantly influenced by Cu- supplementation levels up to 150 mg/Kg diet (Exp. 2). Moreover, the differential count of WBCs showed interesting results, where lymphocytes (%) were significantly increased and the heterophils (%) decreased. The consequences were significant decreases in the H / L ratio, especially in quails fed Cu- supplemented diets up to 150 mg/Kg in both experiments (Tables 4 and 5). In addition, the percentages other types of WBCs did not significantly affected by treatments.

Table 4. Hematological indices response of quail chicks to dietary copper sulfate supplementation (Exp.1).

Parameter	Copper level (mg/Kg) 0	50	100	1 50	200	SEM	Sig.
RBC's (10^{6}/mm^{3})	2.56^{b}	3.24^{a}	3.42^{a}	3.12^{a}	2.95^{a}	0.083	*
Hb (g/100ml)	9.15^{b}	10.69^{a}	11.28^{a}	10.35^{a}	10.13^{a}	1.684	*
PCV %	36.6^{b}	39.8^{a}	41.9^{a}	38.4^{a}	35.5^{b}	3.261	*
MCVum3	142.82^{a}	122.78^{b}	122.53^{b}	123.11^{b}	120.28^{b}	8.954	*
MCH (pg)	35.58	32.97	32.94	33.14	34.32	2.635	NS
MCHC (g/dl)	25.12	26.92	26.88	26.94	28.46	2.046	NS
WBC's (10^{3}/mm^{3})	23.63^{a}	20.94^{b}	19.83^{b}	21.91^{a}	22.17^{a}	3.148	*
Lymphocytes, L (%)	56.44^{b}	62.45^{a}	60.89^{a}	5 9.62^{ab}	61.21^{a}	7.932	*
Heterophils, H (%)	34.81^{a}	31.78^{b}	30.22^{b}	32.83^{ab}	37.2^{a}	2.451	*
Monocytes (%)	3.22	2.89	3.15	3.78	3.68	0.068	NS
Eosinophils, (%)	3.62	3.45	3.25	4.21	3.82	0.146	NS
Basophils, (%)	1.38	1.32	1.17	1.34	1.29	0.054	NS
H / L ratio	0.62^{a}	0.51^{b}	0.50^{b}	0.55^{b}	0.61^{b}	0.043	*

[1] n= 10 birds / each supplemental copper level. [a-d] Means having different letters are significantly different with respect to the row ($p<0.05$)

Table 5. Hematological indices response of quail chicks to dietary copper sulfate supplementation (Exp. 2). Copper level (mg / Kg)

Parameter	0	50	100	150	200	SEM	Sig.
RBC's (10^{6}/mm^{3})	2.74^{b}	2.93^{a}	3.35^{a}	3.24^{a}	3.17^{a}	0.153	*
Hb (g/100ml)	10.23^{b}	10.87^{a}	11.24^{a}	11.26^{a}	10.05^{b}	1.845	*
PCV %	38.2^{b}	40.1^{a}	41.3^{a}	41.2^{a}	39.5^{b}	2.460	*
MCVum3	139.44^{a}	136.85^{a}	123.28^{b}	127.14^{b}	124.58^{b}	10.135	*
MCH (pg)	37.32^{a}	37.07^{a}	33.54^{b}	34.75^{ab}	31.72^{b}	3.271	*
MCHC (g/dl)	26.76	27.12	27.24	27.34	25.43	2.543	NS
WBC's (10^{3}/mm^{3})	21.42^{a}	18.87^{b}	18.36^{b}	19.15^{a}	20.32^{a}	2.086	*
Lymphocytes L (%)	61.82^{a}	58.65^{a}	61.29^{a}	63.23^{ab}	62.42^{a}	5.342	*
Heterophils, H (%)	32.56^{a}	31.78^{b}	28.45^{b}	32.44^{ab}	36.22^{a}	4.362	*
Monocytes (%)	3.34	3.29	3.02	3.12	3.08	0.103	NS
Eosinophils, (%)	3.57	3.38	3.20	4.01	3.22	0.469	NS
Basophils, (%)	1.32	1.27	1.14	1.54	1.39	0.385	NS
H / L ratio	0.53^{b}	0.54^{b}	0.47^{b}	0.51^{b}	0.61^{b}	0.036	*

[1] n= 10 birds / supplemental copper level. [a-d] Means with different superscripts are significantly different with respect to the row ($p<0.05$)

It is worthy to notice that, all supplementations of Cu had no significant effects on serum total protein and albumin, but increased the blood levels of globulin, IgG, IgM and IgA. All Cu supplementations decreased serum total lipids, cholesterol and LDL, while HDL level was significantly increased compared with the control group (Tables 6 and 7).

The reduction observed in these parameters is paralleled with the dietary Cu level increase. Birds fed 200 mg/kg diet showed the lowest cholesterol, and triglycerides at 6 weeks of age. Plasma total cholesterol and triglycerides of birds fed Cu-supplemented diets for 5 weeks of age had the same trend observed in Exp.1 (Table 7). It appears that withdrawal of copper from the feed for the final 7 days (Exp.2) allowed for an increase in cholesterol and triglycerides (Table 7) at six weeks of age. At 6 weeks of age, data revealed significant increases ($P<0.05$) in the HDL levels especially in quails fed 50 and 100mg/kg diet, which recorded 49.94 and 54.83 mg/dl (Exp.1), 59.26 and 54.95 in Exp. 2, respectively.

Results in Tables 8 and 9 showed also that the antioxidant defense system against different oxidative stressors was significantly activated by Cu supplementation. In this concern SOD, GSH, and TAC were significantly increased ($p<0.05$), while MDA concentration was significantly decreased. The most improvement was clearly recorded for quails fed Cu-supplemented diets at 50 and 100 mg/kg.

Table 6. Effect of copper sulfate levels on some blood parameters of quail chicks (Exp.1).

	Copper level (mg/Kg)						
Items	**0**	**50**	**100**	**150**	**200**	**SEM**	**Sig.**
PTP (g/dl)	3.95[b]	4.22[a]	4.52[a]	3.86[b]	4.35[a]	0.123	*
Albumin,A(g/dl)	2.32	2.16	2.25	2.05	2.46	0.84	NS
Globulin,G(g/dl)	1.63[b]	2.06[a]	2.27[a]	1.81[b]	1.89[b]	0.06	*
A / G ratio	1.42[a]	1.05[b]	0.99[b]	1.13[b]	1.30[a]	0.04	*
Cholesterol (mg/dl)	146.38[a]	125.48[b]	134.53[a]	106.11[c]	110.28[c]	10.65	*
Tri.g (mg/dl)	99.12[a]	72.92[b]	66.84[bc]	75.94[b]	59.46[c]	8.46	*
HDL (mg/dl)	44.63[b]	49.94[a]	54.83[a]	45.91[b]	58.17[a]	6.14	*
LDL (mg/dl)	82.24[a]	48.45[b]	55.59[b]	44.86[b]	40.32[b]	5.92	*
IgG (ug/ml)	414.8[b]	431.8[a]	443.2[a]	459.8[a]	419.2[b]	24.251	*
IgM (ug/ml)	95.22[b]	127.89[a]	145.15[a]	113.78[b]	118.68[b]	15.152	*
IgA (ug/ml)	110.62[b]	143.45[a]	152.25[a]	134.21[ab]	123.82[b]	12.347	*

[1] **n= 10 birds / supplemental copper level.**
[a-d] **Means within rows with different superscripts are significantly different ($p<0.05$)**

Table 7. Effect of copper sulfate levels on some blood parameters of quail chicks (Exp.2).

	Copper level (mg/Kg)						
Items	**0**	**50**	**100**	**150**	**200**	**SEM**	**Sig.**
PTP (g/dl)	4.82	4.73	4.85	4.62	4.66	1.042	NS
Albumin,A(g/dl)	2.64	2.28	2.46	2.25	2.49	0.526	NS
Globulin,G(g/dl)	2.18	2.45	2.39	2.37	2.15	0.183	NS
A / G ratio	1.22[a]	0.93[b]	0.72[c]	0.95[b]	1.16[a]	0.045	*
Cholesterol (mg/dl)	162.24[a]	148.53[b]	150.72[a]	148.46[c]	139.54[c]	12.327	*
Tri.g (mg/dl)	118.57[a]	89.76[b]	95.63[bc]	105.51[b]	109.38[c]	9.858	*
HDL (mg/dl)	43.82[b]	59.26[a]	54.95[a]	48.87[b]	52.46[a]	7.530	*
LDL (mg/dl)	94.12[a]	71.18[b]	76.27[b]	78.34[b]	65.54[b]	8.974	*
IgG (ug/ml)	384.5[b]	422.4[a]	415.9[a]	398.2[ab]	408.6[a]	32.145	*
IgM (ug/ml)	98.37[b]	133.72[a]	149.64[a]	145.78[a]	126.54[a]	11.234	*
IgA (ug/ml)	110.62[b]	145.34[a]	136.49[a]	156.48[a]	152.35[a]	18.437	*

[1] **n= 10 birds / supplemental copper level.**
[a-d] **Means with different superscripts are significantly different with respect to the row ($p<0.05$)**

Table 8. Effect of different levels of copper sulfate on antioxidant status of quail chicks (Exp.1).

	Copper level (mg/Kg)						
Parameter	**0**	**50**	**100**	**150**	**200**	**SEM**	**Sig.**
SOD (U/g Hb)	22.84[b]	42.96[a]	38.65[a]	33.18[b]	30.89[b]	3.583	*
GSH µmol /gHb	48.54[b]	68.45[a]	62.78[a]	54.43[b]	52.88[b]	4.357	*
MDA(µmol/L)	28.36[a]	19.45[b]	21.65[b]	18.39[b]	15.63[b]	1.572	*
TAC(µmol/L)	1.62[b]	1.98[a]	2.03[a]	2.14[a]	2.23[a]	0.626	*

[1] **n= 10 birds / supplemental copper level.**
[a-d] **Means within rows with different superscripts are significantly different ($p<0.05$)**

Table 9. Effect of different levels of copper sulfate on antioxidant status of quail chicks (Exp.2).

	Copper level (mg/Kg)						
Parameter	**0**	**50**	**100**	**150**	**200**	**SEM**	**Sig.**
SOD (U/g Hb)	24.28[b]	38.67[a]	35.74[a]	27.45[b]	29.64[b]	4.347	*
GSH µmol /gHb	49.64[b]	63.16[a]	61.85[a]	53.44[b]	50.96[b]	6.468	*
MDA(µmol/L)	20.56[a]	16.47[b]	11.39[b]	15.35[b]	12.68[b]	1.742	*
TAC(µmol/L)	1.34[b]	1.86[a]	1.75[a]	1.90[a]	2.05[a]	0.463	*

[1] **n= 10 birds / supplemental copper level.**
[a-d] **Means having different letters are significantly different with respect to the row ($p<0.05$)**

DISCUSSION

Growth performance:

In this study, the significant influence of copper on productive traits of quails may be due to its growth promoting effect and / or antibiotic properties, the latter acting in some ways to reduce bacterial toxins. It appears also that the supplementation levels of 50 and 100 mg/Kg diet were the best inclusion levels in both experiments. The positive effects occurred on the productive performance of quails in response to Cu supplementations are in harmony with those obtained in broilers (Arias and Koutsos 2006; Świątkiewicz *et al*. 2014; Scott *et al*. 2018; Yang *et al*. 2018). This improvement that clearly observed in both weight gain and feed conversion ratio with feeding copper up to 100 mg/kg indicates that this level of copper could be used as a growth-promoting level in the quail diets. This is in close agreement with the findings of many authors who found that copper supplementation at the levels of 125-250 ppm improved growth rate and feed conversion ratio (FCR) in broilers (Świątkiewicz *et al*. 2014; Scott *et al*. 2018; Yang *et al*. 2018) and in pigs (Cromwell *et al*. 1989). It is well known that copper ions serve as important catalytic cofactors in redox chemistry for proteins that carry out fundamental biological functions that are required for growth and development. Also, the ingested Cu is readily absorbed and distributed to copper-requiring proteins with apparently little storage of excess Cu in the body (Pena *et al*. 1999). It seems that the former pathway of Cu within the body cells to stimulate the production of a certain kind of proteins is more active and potent during the growth-promoting level of copper. Zhou *et al*. (1994) proposed several mechanisms by which copper can promote growth. Such mechanisms are: 1. Release into the gut to affect microflora populations; 2. Increased serum mitogenic activity; 3. Increased pituitary growth hormone expression (LaBella *et al*. 1973); 4. Increased neuropeptide secretion (Tsou *et al*. 1977); 5. Post-translational modification of regulatory peptides (Eipper and Mains 1988); 6. As a component of the growth factor lamin (Parkart, 1987).

Hematological parameters:

The observed changes in blood hematology in response to Cu- supplementation could be explained by the relationship between copper and iron metabolism. Although Cu is not actually present as a main constituent of hemoglobin, it is found in certain other blood plasma proteins, i.e. ceruloplasmin, which is concerned with the release of iron from the cells into the plasma. In this regard, Fox (2003) and Mullally *et al*. (2004) reported that copper deficiency impairs the animal's ability to absorb iron, mobilize it from the tissues and utilize it in hemoglobin synthesis. This element was shown to directly stimulate erythrocytes synthesis, as it determines iron absorption into the body and its incorporation to hemoglobin as well as Cu is a component of other proteins in blood, i.e. erythrocuprein which occurs in erythrocytes, where it plays a role in oxygen metabolism. Our results are in line with the findings obtained by Winnica (2008) who reported that the higher number of RBCs recorded with Cu-supplements to diet is due to the participation of Cu in the process of hemoglobin synthesis. Also, studies by Makaraski and Zdura (2006) revealed that Cu lysine inclusion to turkey diet had significantly affected the hematological indices which in close agreement with the present results. The low H / L ratio by Cu- supplementation up to 150 mg /Kg in both experiments may reflect an ameliorative effect on different stressors including oxidative stress on blood homeostasis.

Blood biochemical responses:

Regarding the effect of copper on blood serum constituents, it is clear that the most obvious changes were recorded for total cholesterol, triglycerides, HDL and LDL levels. Also, all supplementations of Cu had no consistent trend on serum total protein and albumin and globulin levels but IgG, IgM and IgA were significantly increased.

The increase and /or decrease in plasma protein concentration may suggest an acceleration in the biosynthesis

of tissue protein, or decelerated the degradation processes of protein. Complexes of elements with amino acids or proteins may be reabsorbed in an different form by the intestinal mucosal membrane via the use of the system of amino acids transportation, owing to which they are assimilated by organism better (Ashmead 1993, Noy *et al*. 1994, Koreleski 1997). On the other hand, Lebaq-Verheyden *et al*. (1974) have observed that Ig G constitutes the largest portion of chicken blood immunoglobulin, followed by Ig M, and they concluded that treatment of broilers with Copper at 50-100 ppm increased the levels of blood Ig G and Ig M, which in accordance with our results. The increased immunoglobulins may reflect better immunity of quails fed different Copper diets. This confirms the findings of latter authors.

The hypocholesterolemic effect of copper observed in this study is well documented. Since the addition of Cu to the feed ingredients decreased the level of total cholesterol, in comparing to the control treatment. In this respect, Sevcikova *et al*. (2003) showed that cholesterol concentration was significantly decreased about 24.9% when Cu-glycine chelate was added to diets as compared to the control group. Also, Aksu *et al*. (2010) observed a drop in the cholesterol and LDL fraction levels with an increase in the HDL level in the plasma of chickens fed diets enriched with organic forms of Zn, Cu and Mn. Moreover, Mondal *et al*. (2007) mentioned that cholesterol level in chickens' blood decreased when Cu level was 200 mg/kg diet, while supplementing of 400 mg/kg diet resulted in a higher plasma HDL. Konjifca *et al*. (1997) reported also that organic Cu supplementation to broiler diet leads to an increase in cholesterol and HDL fractions concentration.

The cholesterol-lowering mechanism of copper sulfate was firstly established in rats (Valsala and Kurup 1987; Kim *et al*. 1992). They demonstrated that liver copper results in reducing hepatic reduced glutathione concentration which inhibits the activity of HMG-COA reductase, the rate-limiting step in the synthesis of mevalonate, and finally reduced the biosynthesis of cholesterol. Bakalli *et al*. (1995) verified that such mechanism is also operative in the chicken where they observed that a high dietary copper level (250 mg/kg) fed to broiler chicks decreased blood reduced glutathione concentration and subsequently reduced plasma total cholesterol.

Similarly, the observed increase in HDL level with levels of 50 and 100mg/kg diet are in close agreement with Lien *et al*. (2004) who found dietary supplemental copper to reduce serum VLDL-cholesterol and increase HDL-cholesterol of laying hens. These increases in plasma HDL-cholesterol might be resulted from the accelerated rate of cholesterol degradation, concomitant with a process of esterification in which the long chain fatty acids changed their moiety by transformation from lecithin (Bakalli *et al*., 1995 and Lien *et al*., 2004).

Plasma triglyceride concentration of the quail chicks fed Cu-supplemented diets were significantly ($p<0.05$) decreased as supplemental level of Cu increased (Tables 6 and 7). This result is consistent with those observed in pigs (Elliot and Bowland 1968 and Amer and Elliot 1973), egg-laying hens (Pearce *et al*. 1983), and in broiler chicks (Bakalli *et al*. 1995; Hamdi *et al*. 2018). Triglycerides-lowering effect of Cu supplementation could be through its reducing impact on the activity of fatty acid synthetase (FAS) which in turn resulted in a reduction in fatty acid synthesis from Acetyl COA and finally decreasing triglycerides biosynthesis. This mechanism of Cu to reduce plasma triglycerides level was evidenced by the findings of Qureshi *et al*. (1983) and Konjufca *et al*. (1997), they reported that FAS activity was significantly suppressed by copper feeding.

Antioxidants response

It is well known that the peroxidative damage to cell membrane lipids considered the most negative effect of excess dietary copper. In a recent study, Ajuwon *et al*. (2011) reported that supplementation of dietary copper by level of 250 mg/kg diet caused a high lipid peroxidation and low concentrations of glutathione, SOD and catalase in the liver and erythrocytes of broiler chickens.

The reduction observed of MDA levels in birds fed Cu-supplemented diets is consistent with the findings of Kumar *et al*. (2013), who found that plasma MDA of broiler chicken was reduced when fed on diets supplemented with Copper sulphate pentahydrate. Although the ability of free Copper ions exaggerate the elaboration of reactive oxygen species (Gaetke and Chow 2003), however the lipid peroxidation (MDA) significantly reduced in quails fed up to 150mg/kg.

The present results revealed significant increases in SOD, GSH and TAC of quail chicks fed Cu-supplemented diets either for 5 or 6 weeks of age (Tables 8 and 9). This result comes in accordance with that obtained by Bakalli *et al*. (1995) and Konjufca *et al*. (1997). Also, Robbins and Baker (1980) sustained the second probability where they stated that Cu, which has a strong oxidizing ability associated with the presence of free SH-groups, may affect and / or limit the availability of GSH because the difficulty of Cu-SH bond to be easily dissociated. Supplementation of copper in the form of methionine or glycine decreased the level of MDA, as an indicator of cell membranes injury form free radicals oxidative stress, because the higher levels of MDA may be a diagnostic criteria for the antioxidant defense system damage due to oxidative stress (Farambi *et al*., 2004; Venkataraman *et al*., 2004; Kato *et al*., 2007).

The present results agreed with those reported by Jarosz *et al*. (2018) who reported that SOD activity increased by feeding broiler chickens on diets rich in copper sulphate. It is well-known that Cu has the ability to regulate SOD activity in the tissues and body fluids of both growing and aged animals (Surai 2016). Thus, excess of dietary copper might be the main reason of high SOD activity in quails fed Cu-supplemented diets. Moreover, Cu deficiency was reported by many investigators to reduce both Cu and Zn-SOD forms activity without inhibiting the biosynthesis or storage of these elements in the tissues (Dameron and Harris 1987; Oztürk and Tarhan 2001).

CONCLUSION

In conclusion, dietary supplementation with cupric sulfate pentahydrate (CuSO4. 5H2O) at level of 50 or100 mg/kg could improve productive performance, immunity and lowering blood cholesterol content of growing Japanese quail. Moreover, these positive impacts extended to internal antioxidants defense throughout reducing MDA levels and improving the antioxidant status in terms of SOD, GSH, and total antioxidant capacity.

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الإستجابات الفسيولوجية والضد تأكسدية لإضافة كبريتات النحاس لعلائق السمان الياباني

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أجريت هذه التجربة لتقييم التأثيرات الفسيولوجية الناتجة عن إضافة مستويات مختلفة من كبريتات النحاس إلي علائق السمان الياباني خلال فترة النمو. تم إجراء تجربتان : التجربة الأولي تم تغذية السمان علي العلائق المحتوية علي مستويات: صفر ، 50 ، 100 ، 150 ، 200 ملليجرام من كبريتات النحاس لمدة 6 أسابيع ، أما في التجربة الثانية فقد تمت التغذية لمدة خمسة أسابيع ثم الأسبوع الأخير علي عليقة المقارنة . وفي كل تجربة كان عدد السمان 250 سمانة موزعة علي خمسة مجاميع بكل منها 50 طائر في خمسة مكررات تحتوي علي 10 طيور/ مكرر. هذا وقد أخذت القياسات في عمر ستة اسابيع لكلا التجربتان. وتوضح النتائج أن أضافة كبريتات النحاس بمعدل 50 أو 100 ملليجرام / كجم من العليقة كان له تأثير معنوي علي زيادة وزن الجسم وتحسين كفاءة التحويل الغذائي دون زيادة معنوية في معدل النفوق ، وذلك سواء استمرت الإضافة لمدة ستة (التجربة الأولي) أو خمسة أسابيع (التجربة الثانية) . بالنسبة لصورة الدم .. كان للمعاملات تأثير معنوي علي زيادة عدد كرات الدم الحمراء ، تركيز الهيموجلوبين ، الهيماتوكريت ، ومعظم دلائل الدم مقارنة بالمجموعة الضابطة. لوحظ أيضا عدم وجود تأثير معنوي علي عدد كرات الدم البيضاء مع تحسن في نسبة الخلايا الليمفاوية وكذلك النسبة بينها وبين الخلايا المتعادلة. تشير النتائج ايضا الي حدوث انخفاض معنوي في مستوي كوليستيرول الدم ، الجليسريدات الثلاثية مع زيادة في الكوليستيرول عالي الكثافة وذلك نتيجة إضافة النحاس للعليقة. حدث أيضا تحسن معنوي في الجلوبيولينات المناعية والإنزيمات المسئولة عن حماية جدر خلايا الجسم من عمليات الأكسدة . وقد خلصت النتائج إلي أهمية إضافة كبريتات النحاس بمستوي 50 – 100 ملليجرام / كيلوجرام من عليقة السمان خلال مرحلة النمو (6 أسابيع) وذلك لتحسين النمو والوقاية من الإجهاد التأكسدي وتحسين مناعة الطيور مع خفض مستوي الكوليسترول في الدم .