

Effects of Copper Sulfate on Productive, Reproductive Performance and Blood Constituents of Laying Japanese Quail Fed Optimal and Sub-Optimal Protein

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Abstract: A (3X3) factorial design experiment was conducted to study the effect of three levels of crude protein (16, 18 and 20%) and three levels of copper sulfate (0, 100 and 200 mg/kg diet) as a growth promoter on productive and reproductive performances, egg quality, blood serum constituents and economical efficiency of laying quail hens through 8 weeks. A total number of 270 hens and 135 males of Japanese quail at 8 weeks of age with nearly equal body weight and average rate of laying were randomly divided into 9 groups (30 hens and 15 males each). Each group of birds was sub divided into 3 replicates (10 hens and 5 males) and each replicate was housed in one wire cage. The results showed that the layer body weights at 12 or 16 weeks and weight gain at 12-16 and 8-16 weeks of age were significantly increased with increasing crude protein level from 16-18 or 20%, while there were no significant differences between the groups fed 18 and 20% crude protein throughout the experimental intervals and the whole period. Egg number, rate of laying and egg mass of laying quail hens increased with increasing crude protein at levels 16-18 or 20% (except at 12-16 week of age), while insignificant differences were found between the groups fed 18 and 20% crude protein throughout the experimental intervals and the whole period. Addition of copper sulfate at levels of 100 and 200 mg/kg to laying quail diets significantly improved egg number, rate of laying, egg mass and feed conversion ratio except at 8-12 weeks of age as compared with group non-supplemented with copper sulfate during the experimental period. The highest values of body weight and egg mass were recorded with 20 % protein plus 100 mg copper sulfate/kg diet, while, the best values of feed conversion and The highest values of egg number and rate of laying were recorded with 20 % protein plus 200 mg copper sulfate/kg diet from 8-16 weeks of age for quail layer as compared with other treatments. Laying quails hens fed diet contained copper sulfate levels significantly increased hatchability of fertile eggs percentage (except at 12 wks of age) as compared with those un-supplemented group. Various levels of crude protein or copper sulfate containing diets did not significantly affect on egg quality parameters, while egg yolk cholesterol was significantly decreased with increased copper sulfate levels. The highest values for total serum protein and serum albumin were recorded with experimental groups fed 18 or 20% protein levels compared with those received 16% protein diet, while serum cholesterol values were significantly decreased with increased crude protein. However, dietary copper sulfate supplementation reduced serum tri-glycerides, total cholesterol and low density lipoprotein cholesterol, but increased serum high density lipoprotein cholesterol. The best value of economic efficiency was recorded with laying quail hens fed 18% protein with 100 mg copper sulfate/kg diet compared with other treatment groups.

Key words: Protein, copper, feed supplement, performance, quails hens

INTRODUCTION

Recently, feeding cost in poultry production is considered the most expensive item (accounts for 65-70% of the total costs) particularly, dietary protein which is the most expensive in poultry diets. High-protein diets (20% crude protein CP) are recommended for Japanese quail layers for optimizing performance (NRC, 1994). (El-Hammady *et al.*, 1992) found that, feeding low protein diets had no adverse effects on laying hens compared to high protein diets. Also (Abdel-Azeem *et al.*, 2005) found that when dietary protein level reduced up to 16 %, the performance values of laying quail hens were decreased.

Copper sulfate is a naturally-occurring inorganic salt and copper is an essential trace element in poultry nutrition

(Davis and Mertz, 1987), copper deficiency in laying hens resulted in hypercholesterolemia (Kelvay *et al.*, 1984), proper bone growth and development as well as enzyme functions. Copper is often added to poultry diets at prophylactic concentrations for its growth promoting effects (Pesti and Bakalli, 1996). Traditionally, the source of copper has been copper sulfate pentahydrate due to cost and commercial availability. In recent years, additional copper sources have become available and the potential for commercial use as feed supplement has expanded. Copper is usually fed commercially at much higher pharmacological levels (100-300 mg/kg diet) because of its growth promoting properties (Bakalli *et al.*, 1995). (Pearce *et al.*, 1983) demonstrated that pharmacological levels of Cu (>250 mg/kg diet) caused

changes in 17 beta-estradiol and enzymes involved in carbohydrate, lipid and amino acid metabolism in mature laying hens and suggested that copper supplements can affect reproductive physiology and lipid metabolism. Egg production and feed conversion of laying hens were improved by addition of 300 mg Cu/kg of diet (Metwally, 2002). (Idowu *et al.*, 2006) clear that increasing the supplementation of copper sulfate pentahydrate, CuSO₄. 5H₂O and Copper Proteinates (CuP) from 0-250 mg Cu/kg of laying hens diet increased egg production and Haugh unit while the feed intake was decreased. (Arias and Koutsos, 2006) demonstrate that broiler performance and intestinal physiology can be positive effected by dietary copper source at level of 188 mg Cu/kg diet as well as microbial environment. Addition of different amounts of copper to laying hens diet have resulted in reductions in yolk cholesterol concentrations and blood plasma lipid concentrations (Pesti and Bakalli, 1998). Therefore, this study was conducted to evaluate the effect of dietary crude protein levels and copper sulfate levels as a growth promoter on productive and reproductive performance, egg quality, blood constituents and economical efficiency of laying quail hens.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Farm of Poultry Production, Faculty of Agriculture, Zagazig University, Egypt. A (3X3) factorial design experiment was conducted to study the effect of three levels of crude protein CP (16, 18 and 20%) and three levels of copper sulfate CuSO₄ (0, 100 and 200 mg/kg diet) on some productive and reproductive performance, egg quality, serum blood constituents and economical efficiency of Japanese quail layers. Diets were formulated to contain 16, 18 or 20% Crude Protein (CP) supplemented with methionine and lysine to meet the requirements recommended by (NRC, 1994) and 2670 k cal ME/kg (Table 1).

The ninth experimental treatments were as follows:

- Diet 1) 16% CP + 0 mg CuSO₄ / kg diet.
- Diet 2) 16% CP + 100 mg CuSO₄ / kg diet.
- Diet 3) 16% CP + 200 mg CuSO₄ / kg diet.
- Diet 4) 18% CP + 0 mg CuSO₄ / kg diet.
- Diet 5) 18% CP + 100 mg CuSO₄ / kg diet.
- Diet 6) 18% CP + 200 mg CuSO₄ / kg diet.
- Diet 7) 20% CP + 0 mg CuSO₄ / kg diet.
- Diet 8) 20% CP + 100 mg CuSO₄ / kg diet.
- Diet 9) 20% CP + 200 mg CuSO₄ / kg diet.

A total number of 270 hens and 135 males of Japanese quail at 8 weeks of age with nearly equal body weight and average rate of laying were randomly divided into 9 treatment groups (30 hens and 15 males each). Each group of birds was sub divided into 3 replicates (10

Table 1: Composition and calculated analysis of experimental corn-soy diets fed during the laying quail period (8-16 wks of age)

Ingredients	Crude Protein level		
	16%	18%	20%
Yellow corn	62.24	59.80	55.86
Soybean meal	20.16	27.16	33.41
Wheat bran	9.32	5.00	3.00
Limestone (CaCO ₃)	5.05	5.05	5.25
Dicalcium phosphate	2.25	2.20	1.80
Vit. & Min. Premix*	0.30	0.30	0.30
Sodium chloride	0.25	0.25	0.25
DL-methionine	0.19	0.16	0.13
Lysine	0.24	0.08	0
Total	100	100	100
Chemical analysis:			
Calculated analysis **			
ME Kcal/kg	2688	2683	2660
Crude protein %***	15.91	17.93	19.85
Ether extract (EE) ***	2.61	2.44	2.25
Crude fiber %***	3.68	3.63	3.77
Calcium %	2.50	2.50	2.50
Available phosphorus %	0.53	0.52	0.47
Lysine %	1.00	1.00	1.05
Methionine %	0.45	0.45	0.45
Copper (mg)	10.05	11.17	12.27

*: Each 3 kg of Vit. And Min. premix contains 10000000 IU Vit. A; 2000000 IU Vit. D3; 10000 mg Vit. E; 1000 mg Vit. K3; 1000 mg Vit. B1; 5000 mg Vit. B2; 10 mg Vit B12; 1500 mg Vit. B6; 30000 mg Niacin; 10000 mg Pantothenic acid; 1000 mg Folic acid; 50 mg Biotin; 300000 mg Choline chloride; 4000 mg Copper; 300 mg Iodine; 30000 mg Iron; 50000 mg Zinc; 60000 mg Manganese; 100 mg Selenium and 00 mg Cobalt. **Calculated according to (NRC, 1994). ***Determined according to the methods of A.O.A.C (1990).

hens and 5 males each) and each replicate was housed in one wire cage. Birds were fed ad libitum and the fresh water was available all time during the experimental period. Artificial light was used giving a total of 16 h of light per day throughout the experimental period. Individual body weights were recorded at (8, 12 and 16 weeks of age) and body weight gain was calculated for intervals (8-12, 12-16 and 8-16 weeks of age). The productive performance of layers was estimated at (8-12, 12-16 and 8-16 weeks of age) For each replicate egg number and egg weight were recorded daily. Rate of laying (egg/hen/day), feed intake and egg mass were calculated weekly. Feed conversion was calculated (g feed intake/g egg mass/hen).

At the 12 and 16 weeks of age, 135 eggs from each treatment were collected and incubated till hatching. Percentages of fertility and hatchability of total fertile eggs were estimated. Egg quality traits were determined at 16 weeks of age on 5 eggs from each replicate. Egg shape index, yolk index, yolk, albumen and shell percentages, also haugh units score were calculated. At the end of experimental period, blood samples were withdrawn from three birds in each group from Jugular

vein in tubes. Blood was centrifuged for 20 min at 4000 g and blood serum samples were stored at -20°C until analysis of the total protein, albumin, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides and glucose. The analyses of crude protein in the diet were carried out according to A.O.A.C. (1990). Cholesterol was extracted from yolk samples by chloroform method (2:1) according to (Folch *et al.*, 1957). Total protein, albumin, total cholesterol, High Density Lipoprotein Cholesterol (HDL%), Low Density Lipoprotein Cholesterol (LDL%), triglycerides and glucose concentrations were determined in the serum. Yolk cholesterol by spectrophotometer method using commercial kits (Stanbio Laboratory, Boerne, Texas 78006, USA). Economical efficiency for egg production was calculated from the input/output analysis according to the price of experimental diets and eggs production. Values of economical efficiency were calculated as the net revenue per unit of total costs (Osman, 2003 and Soliman *et al.*, 2003). Data were subject to factorial design using general linear model of SAS software statistical analysis (SAS, 1998). The Significant means were separated by Duncan's New Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance: The data in Table 2 shows that layers fed CP at level of (20%) had the highest average of body weight and weight gain at both (12 and 16 wks of age) and through (12-16 and 8-16 wks of age) intervals. While those fed CP at level of (16%) had the lowest average of these traits while layers fed (18%) had intermediate values. The CP did not affect either body weight or weight gain at the early period of growth. These results are in agreement with the findings of (Soares *et al.*, 2003) who observed that laying was delayed and varied in body weight for quails fed lower protein level (16% CP). Also (Ali *et al.*, 2000) found that, high protein level in the quail diets, resulted in higher body weight and weight gain than the low protein level diet.

(Abdel-Azeem *et al.*, 2005) observed that the significant improvements in live body weight and weight gain for quail hens received high protein diets (20% CP).

The data in Tables 3 and 4 shows that egg number, rate of laying and egg mass were significantly ($p < 0.05$) increased by increasing CP level on laying Japanese quail hens during (8-12 wks of age) and for whole experimental period (8-16 wks of age). While, insignificant differences were observed during the period (12-16 wks of age). Similar trend was reported by (Zanaty *et al.*, 2001, Yakout *et al.*, 2004 and Moreira *et al.*, 2005) they observed that laying Japanese quail hens fed diets with 18 and 20% CP had higher egg production than those feed diets with 16% CP. (Abdel-Azeem *et al.*, 2005) reported that egg mass significantly increased

with increasing protein level in the laying quail hens diet. The results presented in Table 5 clear that there were no significant differences ($p < 0.05$) in feed intake (g/day) amount for laying quail hens fed various levels of CP at (16, 18 and 20% CP) during the experimental periods. While, feed conversion ratio during 8-12 and 8-16 weeks of age were significantly improved with increasing dietary protein level up to 18% in the laying Japanese quail hens diet. Similar trends were obtained by (Abdel-Azeem *et al.*, 2005) who noticed that no significant differences in daily feed consumption ratio was improved due to dietary protein. (Zanaty, 2006) found that feed conversion was significantly ($p < 0.05$) improved, respectively, by increasing dietary protein level from 14 -16 or 18% for Norfa hens. There was insignificant effect due to feeding different dietary protein levels on egg weight during the experimental period. The present results agree with those obtained by (Djouvinov and Mihailov, 2005) who reported that feeding low protein diets had no adverse effects on laying hens compared to high protein diets.

The results obtained in Tables 2, 4 and 5 pointed out that the added CuSO_4 at level of 100 and 200 mg/kg diet did not affect on body weight, weight gain, egg weight and daily feed intake. Similar results were obtained by (Bank *et al.*, 2004) and (Balevi and Coskun, 2004) who reported no significant effect on live body weight of laying hens fed diets supplemented with CuSO_4 during the experiment period, while (Ankari *et al.*, 1998) and (Metwally, 2002) showed no significant effect of CuSO_4 supplementation on either feed intake or egg weight. Addition of CuSO_4 (100 and 200 mg/kg) to laying quail diets significantly ($p \leq 0.05$) improved egg number, rate of laying, egg mass and feed conversion ratio (except at 8-12 weeks of age) as compared with un-supplemented of CuSO_4 during the experimental period. The present results agreed with those obtained by (Pesti and Bakalli, 1998) they reported that egg production was significantly increased ($p < 0.05$) in the second 4-wk period by supplemental 250 mg/Cu kg in laying hen diet. Also, they reported that feed conversion ratio was significantly improved by supplementation of 150 mg Cu/kg diet. (Metwally, 2002) showed that the addition of 300 mg Cu/kg diet improved the egg production and feed conversion compared with the control. (Awad *et al.*, 2008) who demonstrated that egg number, egg mass and laying rate of duck were significantly improved by approximately 13, 21 and 28% for groups fed diets supplemented with 100, 300 and 500 mg CuSO_4 / kg diet compared to the control group during the overall experimental period (22-45 wks).

The interaction between CP and CuSO_4 results indicated that the highest values of body weight at 12 and 16 wks of age and egg mass through 8-16 wks interval were recorded with 20 % CP plus 100 mg CuSO_4 / kg with respect to egg number, rate of laying and feed

Table 2: Means ± SE of body Weight and body Weight gain of laying Japanese quail as affected by dietary crude protein levels with or without copper sulfate supplementation during the experimental periods

Items	Body Weight (g)			Weight gain (g)		
	8 wks	12 wks	16 wks	8-12 wks	12-16 wks	8-16 wks
Protein levels (CP)						
16%	207.31±0.89	223.74±0.54 ^b	243.53±1.14 ^b	16.43±1.08	19.79±0.95 ^b	36.22±1.27 ^b
18%	209.52±0.51	225.27±0.47 ^{ab}	247.34±0.99 ^a	15.75±0.63	22.07±1.19 ^{ab}	37.82±1.12 ^{ab}
20%	209.14±1.05	226.56±0.95 ^a	250.39±1.21 ^a	17.42±0.56	23.83±0.57 ^a	41.25±0.83 ^a
Significant	NS	*	*	NS	*	*
CuSO₄ levels (mg/kg)						
0	208.36±0.87	225.16±0.48	245.88±1.50	16.80±0.72	20.72±1.09	37.52±1.26
100	209.22±0.96	226.27±0.88	248.76±1.43	17.05±0.69	22.48±0.76	39.54±0.78
200	208.39±0.87	224.14±0.78	246.63±1.35	15.75±0.97	22.49±1.28	38.24±1.66
Significant	NS	NS	NS	NS	NS	NS
Interaction effects (CP X CuSO₄ levels)						
16% CPX0 CuSO ₄	206.65±2.35	223.85±0.76 ^b	241.61±2.07 ^c	17.20±1.61	17.77±1.42	34.96±1.33
16% CPX100 CuSO ₄	206.57±0.58	223.67±1.28 ^b	244.32±1.05 ^{bc}	17.10±1.80	20.65±1.44	37.75±1.51
16% CPX200 CuSO ₄	208.70±1.46	223.70±1.14 ^b	244.66±2.72 ^{bc}	15.00±2.55	20.96±1.94	35.96±3.66
18% CPX0 CuSO ₄	208.96±0.47	225.19±0.45 ^b	245.71±0.95 ^{bc}	16.23±0.91	20.52±1.04	36.75±1.17
18% CPX100 CuSO ₄	209.57±1.44	226.17±0.47 ^{ab}	248.54±0.87 ^{ab}	16.60±1.12	22.38±0.63	38.97±0.58
18% CPX200 CuSO ₄	210.03±0.70	224.45±1.24 ^b	247.77±2.80 ^{ab}	14.42±1.18	23.32±3.65	37.75±3.49
20% CPX0 CuSO ₄	209.47±0.99	226.44±0.60 ^{ab}	250.31±1.73 ^{ab}	16.97±1.59	23.87±1.32	40.83±2.63
20% CPX100 CuSO ₄	211.52±1.58	228.99±0.62 ^a	253.41±1.43 ^a	17.47±1.00	24.42±0.98	41.89±0.50
20% CPX200 CuSO ₄	206.44±1.80	224.26±2.10 ^b	247.45±1.89 ^{abc}	17.82±0.31	23.19±0.90	41.02±0.88
Significant	NS	*	*	NS	NS	NS

Means having different letters at the same column are differ significantly. * = (p<0.05), NS = Not significant.

conversion through (8-16 weeks of age), the best values were observed with 20% CP plus 200 mg CuSO₄ / kg diet. On the other hand, layers fed diet with 18% CP plus 200 mg CuSO₄ / kg diet improved egg number through (8-16 wks of age) and feed conversion through (8-12 wks of age). These results may be indicating that the bird fed this combination of CP and CuSO₄ levels had the best production performance birds. This improvement could be due to ability of copper to improve the performance by improvement the activities of total proteases, amylase and lipase in small intestinal contents as reported by (Xia *et al.*, 2004). However, body weight, egg number; rate of laying, egg mass and feed conversion through (8-16 wks of age) were significantly (p≤0.05) difference.

Reproductive performance: The results in Table 6 shows that fertility, hatchability of total eggs and hatchability of fertile eggs percentages had numerically increased with increasing CP levels of quail hens diets during the experimental periods except the increase of hatchability of total eggs percentage was significant. Similarly (Shrivastav *et al.*, 1993) found that fertility and hatchability did not differ between treatments (16, 19, 22 or 25% CP). Also (Abdel-Azeem *et al.*, 2005) reported that fertility percentage had no effected significantly due to feeding dietary protein levels in the quail birds. Fertility and hatchability percentages of total eggs insignificantly affected by diets supplemented with CuSO₄ levels. Laying quails hens fed diet supplemented with CuSO₄ levels were significantly (p≤0.05) improved the

hatchability of fertile eggs % at (16 wks of age) as compared with those un-supplemented group. These results are in agreement with (Awad *et al.*, 2008) who found that hatchability of fertile eggs was significantly improved by 2.07% for the group fed diet supplemented with 100 mg CuSO₄ / kg compared to the control, respectively for duck. These results may be due to the reduction in total cholesterol and triglycerides contents in egg, as will as, Cu-egg content by supplementing copper sulfate to the diet because Cu decreased hepatic glutathione formation. The interaction between dietary protein and CuSO₄ supplementation levels showed insignificant effects on reproductive performance values.

Egg quality parameters: The results presented in Table 7 cleared that no significant effect of CP and CuSO₄ and the interaction between them on all egg quality traits which studied except that of egg yolk cholesterol content which was decreased significantly with CuSO₄ levels. Eggs produced from hens fed 100 or 200 mg CuSO₄ had significantly lower content of yolk cholesterol (428.65 and 423.44 mg, respectively compared that of the zero level of CuSO₄ (471.22 mg). These results are in agreement with those obtained by (Tarasewicz *et al.*, 2005) who found that yolk cholesterol and chemical compositions of egg yolk and white were at similar levels in Japanese quail fed 22, 20 or 18% CP. Also (Sehu *et al.*, 2005) noticed that no significant differences in egg quality of laying quail hens due to four dietary protein levels ranged from (16.45-19.75%). (Abdel-Rahman, 1993) found also, no effect of feeding different

Table 3: Means ± SE of egg number and rate of laying of laying Japanese quail as affected by dietary crude protein levels with or without copper sulfate supplementation during the experimental periods

Items	Egg number (egg/hen)			Rate of laying (egg/hen/day) %		
	8-12 wks	12-16 wks	8-16 wks	8-12 wks	12-16 wks	8-16 wks
Protein levels (CP)						
16%	19.33±0.23 ^b	22.56±0.34	41.32±0.60 ^b	69.05±0.84 ^b	80.55±1.22	74.80±0.86 ^b
18%	20.31±0.38 ^{ab}	23.00±0.32	43.54±0.68 ^a	72.54±1.36 ^{ab}	82.14±1.14	77.34±1.06 ^{ab}
20%	20.62±0.40 ^a	23.46±0.35	44.12±0.57 ^a	73.65±1.44 ^a	83.77±1.26	78.71±1.00 ^a
Significant	*	NS	*	*	NS	*
CuSO ₄ levels (mg/kg)						
0	19.60±0.29	22.22±0.33 ^b	41.38±0.57 ^b	70.00±1.03	79.37±1.17 ^b	74.68±0.78 ^b
100	20.17±0.47	23.34±0.34 ^a	43.54±0.71 ^a	72.02±1.66	83.37±1.20 ^a	77.70±1.28 ^a
200	20.50±0.35	23.44±0.34 ^a	44.07±0.59 ^a	73.21±1.25	83.73±0.87 ^a	78.47±0.79 ^a
Significant	NS	*	*	NS	*	*
Interaction effects (CP X CuSO ₄ levels):-						
16% CPX0 CuSO ₄	19.03±0.26	22.10±0.61	39.80±0.75 ^d	67.98±0.93	78.93±2.18	73.45±1.24 ^c
16% CPX100 CuSO ₄	19.13±0.52	22.63±0.71	41.77±1.23 ^{bcd}	68.34±1.87	80.83±2.54	74.58±2.20 ^{bc}
16% CPX200 CuSO ₄	19.83±0.35	22.93±0.59	42.40±0.64 ^{abcd}	70.83±1.26	81.91±2.10	76.37±0.62 ^{abc}
18% CPX0 CuSO ₄	19.33±0.29	22.03±0.62	41.37±0.88 ^{cd}	69.05±1.04	78.69±2.22	73.87±1.58 ^c
18% CPX100 Cu so ₄	20.73±0.73	23.53±0.34	44.37±0.35 ^{ab}	74.05±2.61	84.05±1.21	79.05±0.77 ^{ab}
18% CPX200 Cu so ₄	20.87±0.62	23.43±0.15	44.90±1.01 ^a	74.53±2.22	83.69±0.52	79.11±1.15 ^{ab}
20% CPX0 Cu so ₄	20.43±0.55	22.53±0.67	42.97±0.13 ^{abc}	72.98±1.96	80.48±2.39	76.72±0.24 ^{abc}
20% CPX100 Cu so ₄	20.63±0.99	23.87±0.58	44.50±1.45 ^{ab}	73.69±3.55	85.24±2.06	79.47±2.59 ^{ab}
20% CPX200 CuSO ₄	20.80±0.79	23.97±0.27	44.90±0.83 ^a	74.28±2.83	85.60±0.97	79.94±1.51 ^a
Significant	NS	NS	*	NS	NS	*

Means having different letters at the same column are differ significantly. * = (P<0.05), NS = Not significant.

protein levels on egg yolk index. Similarly (Metwally, 2002) and (Bank *et al.*, 2004) reported that layer diet supplemented with copper sulfate had no significant effects on egg albumen%, shell%, shape index, yolk index, Haugh units and specific gravity. (Lien *et al.*, 2004) observed that egg yolk cholesterol was significantly reduced by Copper (Cu) and chromium supplementation.

Blood constituents: The results in Table 8 indicated that the highest significant values of total serum protein and albumin with experimental groups fed diet containing 18 or 20% of CP compared with those received 16% CP diets. There was no significant ($p \leq 0.05$) effect on serum total protein, albumin, globulin and glucose of laying quail hens fed with the different levels of CuSO₄. These results are in agreement with the findings of (Abdel-Gawad *et al.*, 2004) who found that, using recommended levels of CP increased significantly total plasma protein and its fractions when compared with those fed low CP level. The values of serum globulin, triglycerides, HDL%, LDL% and glucose were not significantly ($p > 0.05$) influenced by different dietary CP levels. These results are in agreement with the findings of (Abdel-Hady and Abdel-Ghan, 2003) they indicated that, there were no significant differences in blood parameters due to protein level. Serum cholesterol values were significantly ($p > 0.05$) decreased with increased CP levels. These results are in agreement with (El-Naggar *et al.*, 1997) who showed that total serum cholesterol was significantly ($p < 0.05$) decreased linearly with increasing protein intake. In contrast (Sherif,

1989) showed that plasma cholesterol significantly decreased for chicks fed optimum protein diet compared to that fed low protein diet. However, dietary CuSO₄ supplementation reduced ($p < 0.05$) serum tri-glycerides, total cholesterol and LDL %, but increased ($p < 0.05$) serum HDL %. These results may be due to the change of lipid metabolism by Cu supplementation to the diet which was resulted in decreasing plasma lipid, 17 Beta-estradiol and hepatic lipogenic enzyme activity as reported by (Pearce *et al.*, 1983). Moreover (Konjufca *et al.*, 1997) indicated that the addition of Cu decreased fatty acid synthesis activity. Also, the higher copper concentration resulted in decreasing the formation of hepatic glutathione and ultimately cholesterol formation (Kirn *et al.*, 1992). (Bakalli *et al.*, 1995) demonstrated that feeding 250 mg copper/kg diet reduced total cholesterol (26%) and plasma triglycerides (43%). (Konjufca *et al.*, 1997) stated that plasma cholesterol level was reduced in male broilers fed 250 mg copper sulfate/kg diet. (Pearce *et al.*, 1983), (Ankari *et al.*, 1998) and (Pesti and Bakalli, 1998) reported that plasma cholesterol and triglycerides were significant reduction by feeding hens fed supplemented with 250 mg Cu/kg (20 and 24%). The interaction between dietary protein and CuSO₄ levels supplementation showed insignificant effects on serum globulin, HDL%, LDL% and glucose values, While, total protein, albumin, tri-glycerides and cholesterol were significantly ($p > 0.05$) effected.

Economic Efficiency (EEF): Data presented in Table 9 shows that, laying quail hens fed diet contained 18% CP recorded the highest value of Economic Efficiency (EEF)

Table 4: Means ± SE of egg weight and egg mass of laying Japanese quail as affected by dietary crude protein levels with or without copper sulfate supplementation during the experimental periods

Items	Egg weight (g)			Egg mass (g/hen)		
	8-12 wks	12-16 wks	8-16 wks	8-12 wks	12-16 wks	8-16 wks
Protein levels (CP)						
16%	10.88±0.03	10.88±0.02	10.88±0.02	210.27±2.49 ^b	245.49±3.79	455.76±5.24 ^b
18%	10.88±0.02	10.79±0.10	10.83±0.06	220.97±4.17 ^{ab}	248.04±3.78	469.01±6.60 ^{ab}
20%	10.87±0.04	10.90±0.02	10.89±0.03	224.12±4.02 ^a	255.60±3.78	479.72±5.61 ^a
Significant	NS	NS	NS	*	NS	*
CuSO₄ levels (mg/kg)						
0	10.92±0.02	10.89±0.01	10.91±0.01	214.03±3.11	242.09±3.73 ^b	456.12±4.85 ^b
100	10.86±0.02	10.92±0.01	10.89±0.01	219.10±5.21	254.88±3.80 ^a	473.98±9.94 ^a
200	10.84±0.04	10.76±0.10	10.80±0.06	222.23±3.43	252.15±3.18 ^{ab}	474.38±4.97 ^a
Significant	NS	NS	NS	NS	*	*
Interaction effects (CP X CuSO₄ levels)						
16% CPX0 CuSO ₄	10.96±0.04	10.88±0.04	10.92±0.02	208.55±3.16	240.39±7.04	448.94±7.03
16% CPX100 CuSO ₄	10.82±0.05	10.92±0.02	10.87±0.03	206.98±6.04	247.25±8.02	454.22±14.06
16% CPX200 CuSO ₄	10.86±0.06	10.85±0.02	10.86±0.03	215.29±2.88	248.82±6.23	464.11±4.77
18% CP X 0 CuSO ₄	10.88±0.02	10.89±0.03	10.88±0.01	210.27±2.81	239.91±7.47	450.18±10.10
18% CPX100 CuSO ₄	10.89±0.03	10.90±0.02	10.89±0.02	225.74±8.23	256.45±4.17	482.18±4.27
18% CPX200 CuSO ₄	10.87±0.05	10.58±0.29	10.73±0.17	226.89±6.89	247.77±5.43	474.66±11.08
20% CPX0 CuSO ₄	10.93±0.04	10.92±0.01	10.92±0.02	223.26±5.80	245.98±7.10	469.23±1.36
20% CPX100 CuSO ₄	10.89±0.03	10.93±0.01	10.91±0.02	224.60±10.56	260.95±6.52	485.55±15.66
20% CPX200 CuSO ₄	10.80±0.10	10.84±0.05	10.82±0.07	224.51±6.91	259.87±2.90	484.38±6.94
Significant	NS	NS	NS	NS	NS	NS

Means having different letters at the same column are differ significantly. * = (p<0.05), NS = Not significant.

Table 5: Means ± SE of feed intake and feed conversion of laying Japanese quail as affected by dietary crude protein levels with or without copper sulfate supplementation during the experimental periods

Items	Feed intake (g/hen/day)			Feed conversion (g feed intake/g egg mass)		
	8-12 wks	12-16 wks	8-16 wks	8-12 wks	12-16 wks	8-16 wks
Protein levels (CP)						
16%	37.18±0.32	42.71±0.24	39.94±0.21	4.96±0.07 ^a	4.88±0.07	4.91±0.06 ^a
18%	36.98±0.26	42.33±0.29	39.66±0.13	4.70±0.10 ^b	4.79±0.09	4.74±0.07 ^b
20%	37.05±0.31	42.49±0.28	39.77±0.18	4.64±0.05 ^b	4.66±0.07	4.65±0.05 ^b
Significant	NS	NS	NS	*	NS	*
CuSO₄ levels (mg/kg)						
0	37.41±0.30	42.71±0.29	40.06±0.16	4.90±0.07	4.95±0.07 ^a	4.92±0.06 ^a
100	36.99±0.28	42.49±0.30	39.74±0.15	4.75±0.11	4.68±0.08 ^b	4.71±0.07 ^b
200	36.81±0.27	42.32±0.23	39.57±0.19	4.65±0.06	4.71±0.07 ^b	4.67±0.05 ^b
Significant	NS	NS	NS	NS	*	**
Interaction effects (CP X CuSO₄ levels):-						
16% CPX0 CuSO ₄	37.65±0.45	42.89±0.47	40.27±0.38	5.06±0.10 ^a	5.00±0.16	5.03±0.12 ^a
16% CPX100 CuSO ₄	37.00±0.70	42.70±0.58	39.85±0.26	5.01±0.14 ^{ab}	4.8±0.14	4.92±0.12 ^{ab}
16% CPX200 CuSO ₄	36.89±0.61	42.54±0.36	39.72±0.48	4.80±0.11 ^{abcd}	4.79±0.10	4.79±0.01 ^{abc}
18% CPX0 CuSO ₄	37.31±0.62	42.53±0.52	39.92±0.18	4.97±0.05 ^{abc}	4.97±0.16	4.97±0.09 ^a
18% CPX100 CuSO ₄	36.91±0.35	42.27±0.69	39.59±0.22	4.59±0.21 ^{cd}	4.62±0.15	4.60±0.02 ^c
18% CPX200 CuSO ₄	36.73±0.46	42.17±0.47	39.45±0.23	4.54±0.09 ^d	4.77±0.16	4.66±0.12 ^{bc}
20% CPX0 CuSO ₄	37.26±0.67	42.72±0.70	39.99±0.28	4.67±0.04 ^{abcd}	4.87±0.11	4.77±0.04 ^{abc}
20% CPX100 CuSO ₄	37.07±0.59	42.50±0.44	39.79±0.36	4.64±0.16 ^{bcd}	4.57±0.12	4.60±0.13 ^c
20% CPX200 CuSO ₄	36.82±0.54	42.25±0.48	39.54±0.35	4.60±0.08 ^{cd}	4.56±0.10	4.57±0.05 ^c
Significant	NS	NS	NS	*	NS	**

Means having different letters at the same column are differ significantly. * = (p<0.05), ** = (p<0.01); NS = Not significant.

compared with 16 and 20% CP. These results are in agreement with the findings of (Abdel-Hady and Abdel-Ghany, 2003) who found that decreasing crude protein level in broiler chick diets increased EEF. Addition of CuSO₄ to diets at levels of (200 and 100 mg/kg diet)

recorded the highest EEF value compared with un-supplemented. These results are agreed with that reported by (Awad *et al.*, 2008) who reported that the addition of CuSO₄ to diet at levels of 100, 300 and 500 mg/kg diet improved EEF compared with control group

Table 6: Means ± SE of reproductive performance of laying Japanese quail as affected by dietary crude protein levels with or without copper sulfate supplementation at 12 and 16 wks of age

Items	Fertility %		Hatchability of total eggs %		Hatchability of fertile eggs%	
	12 wks	16 wks	12 wks	16 wks	12 wks	16 wks
Protein levels (CP)						
16%	82.87±1.93	79.35±0.48	68.04±1.87	65.37±0.33 ^b	82.07±1.00	82.41±0.60
18%	83.13±0.86	80.90±1.12	68.57±0.94	67.01±1.02 ^{ab}	82.49±0.83	82.84±0.86
20%	85.55±0.98	82.59±0.89	70.62±1.36	68.03±0.57 ^a	82.51±0.91	82.42±0.78
Significant	NS	NS	NS	*	NS	NS
CuSO₄ levels (mg/kg)						
0	82.30±1.18	81.16±0.99	66.64±1.38	65.90±0.84	80.97±1.22	81.19±0.50 ^b
100	84.17±1.15	80.81±1.06	69.81±1.08	67.76±0.67	82.95±0.74	83.90±0.86 ^a
200	85.09±1.66	80.86±0.90	70.78±1.57	66.76±0.74	83.15±0.33	82.58±0.53 ^{ab}
Significant	NS	NS	NS	NS	NS	*
Interaction effects (CP X CuSO₄ levels)						
16% CPX0 CuSO ₄	81.26±3.77	79.95±1.37	65.71±4.13	64.64±0.45	80.78±2.35	80.88±1.00
16% CPX100 CuSO ₄	84.64±2.53	78.65±0.33	69.74±1.82	66.16±0.26	82.46±2.16	84.12±0.18
16% CP X 200 CuSO ₄	82.71±4.60	79.46±0.54	68.67±4.16	65.32±0.68	82.98±0.59	82.22±0.84
18% CPX0 CuSO ₄	82.19±1.13	79.90±1.59	67.26±1.44	64.46±0.52	81.90±2.61	80.72±1.15
18% CPX100 CuSO ₄	82.43±1.68	81.56±2.48	68.06±1.71	68.71±1.76	82.56±0.99	84.30±1.61
18% CPX200 CuSO ₄	84.79±1.66	81.25±2.37	70.38±1.73	67.84±2.00	83.00±0.42	83.52±1.22
20% CPX0 CuSO ₄	83.44±0.30	83.65±1.67	66.93±1.69	68.59±1.59	80.23±2.21	81.98±0.52
20% CPX100 CuSO ₄	85.45±2.06	82.23±1.88	71.65±2.10	68.40±0.51	83.83±0.58	83.29±2.45
20% CPX200 CuSO ₄	87.76±1.60	81.88±1.53	73.27±2.00	67.12±0.70	83.46±0.85	82.00±0.68
Significant	NS	NS	NS	NS	NS	NS

Means having different letters at the same column are differ significantly. * = (p<0.05), NS = Not significant.

Table 7: Means± SE of egg quality of laying Japanese quail as affected by dietary crude protein levels with or Without copper sulfate supplementation during the experimental periods

Items	Egg weight (g)	Egg shape index	Yolk Index	Yolk (%)	Albumin (%)	Shell (%)	Haugh Unit %	Yolk cholesterol (mg/gm yolk)
Protein level(CP)								
16%	11.52±0.20	76.97±1.30	46.06±0.52	26.96±0.13	58.54±0.59	14.53±0.50	91.46±0.16	445.05±11.60
18%	11.83±0.18	79.49±0.50	46.07±0.33	26.49±0.47	58.04±0.81	15.17±0.54	91.64±0.15	438.93±12.53
20%	12.03±0.10	79.67±1.21	45.93±0.71	26.26±0.37	58.96±0.86	15.48±0.55	91.72±0.14	439.32±12.08
Significant	NS	NS	NS	NS	NS	NS	NS	NS
CuSO₄ levels (mg/kg)								
0	11.66±0.18	77.94±1.64	45.55±0.63	26.50±0.45	59.01±0.74	14.49±0.46	91.51±0.13	471.22±9.39 ^a
100	11.93±0.18	79.40±0.85	46.48±0.51	26.63±0.28	57.64±0.80	15.73±0.62	91.53±0.17	428.65±8.84 ^b
200	11.79±0.16	78.79±0.89	46.04±0.43	26.58±0.33	58.89±0.71	14.96±0.47	91.80±0.13	423.44±10.11 ^b
Significant	NS	NS	NS	NS	NS	NS	NS	*
Interaction effects(CP X CuSO₄ levels):								
16% CPX0 CuSO ₄	11.46±0.46	77.39±4.64	45.11±1.24	27.04±0.39	58.65±1.22	14.31±1.01	91.51±0.23	476.95±15.50
16% CPX100 CuSO ₄	11.58±0.51	76.37±1.29	47.02±1.05	26.87±0.28	58.41±1.66	14.72±1.42	91.16±0.54	429.30±16.71
16% CPX200 CuSO ₄	11.52±0.46	77.15±2.48	46.05±1.11	26.97±0.25	58.56±1.35	14.55±1.15	91.71±0.14	428.91±18.21
18% CPX0 CuSO ₄	11.36±0.36	78.33±0.74	46.47±0.50	26.41±1.55	59.46±2.12	14.13±0.70	91.20±0.27	469.14±18.28
18% CPX100 CuSO ₄	12.50±0.36	80.61±1.29	45.62±0.99	26.55±0.65	56.91±1.60	16.55±1.29	91.96±0.23	424.61±17.61
18% CPX200 CuSO ₄	11.83±0.34	79.54±1.03	46.13±0.76	26.51±1.02	57.77±1.71	14.84±1.29	91.78±0.35	423.05±23.53
20% CPX0 CuSO ₄	12.15±0.30	78.09±3.90	45.07±2.17	26.05±0.89	58.92±1.84	15.03±1.45	91.81±0.26	467.58±21.38
20% CPX100 CuSO ₄	11.90±0.22	81.24±1.85	46.79±1.44	26.47±0.91	57.62±2.33	15.91±1.52	91.45±0.21	432.03±18.27
20% CPX200 CuSO ₄	12.03±0.20	79.67±2.25	45.95±1.21	26.27±0.84	60.35±1.59	15.48±0.85	91.91±0.40	418.36±17.69
Significant	NS	NS	NS	NS	NS	NS	NS	NS

Means having different letters at the same column are differ significantly. * = (p<0.05), NS = Not significant.

in pekin duck. The interaction between dietary protein levels and CuSO₄ levels supplementation showed that the best EEF value was recorded with laying quail hens fed diet contained 18% protein supplemented with 100 mg CuSO₄ / kg diet compared with other groups. It is concluded that, laying quail hens fed diet contained 18% CP supplemented with 100 mg CuSO₄ / kg diet resulted improvement of most of the productive and reproductive

performances, without any deleterious effects. Diet containing 18% protein and 100 mg CuSO₄ / kg was more economic than the un-supplemented diet under Egyptian commercial condition. It could be recommended to use protein and CuSO₄ at a level of 18% and 100 mg/kg diet in quail hen diet without detrimental effects on body weight, egg production, egg quality and fertility and hatchability percentages.

Table 8: Means ± SE of serum biochemical traits for laying Japanese quail as affected by dietary crude protein levels with or without copper sulfate supplementation at the end of the experimental period

Items	Total protein (mg/100 mL)	Albumin (mg/100 mL)	Globulin (mg/100 mL)	Tri-glycerides (mg/dl)	Cholesterol (mg/dl)	HDL%	LDL%	Glucose (mg/100 mL)
Protein levels (CP)								
16%	5.18±0.15 ^b	2.16±0.06 ^b	3.02±0.14	204.93±2.04	167.84±1.73 ^a	30.98±0.84	69.02±0.84	86.93±0.88
18%	5.87±0.13 ^a	2.64±0.10 ^a	3.22±0.20	202.96±2.44	163.28±2.24 ^{ab}	31.86±0.93	68.14±0.93	86.57±1.18
20%	6.05±0.16 ^a	2.83±0.07 ^a	3.21±0.18	205.17±1.93	160.42±2.30 ^b	31.57±0.99	68.43±0.99	87.20±1.31
Significant	**	**	NS	NS	*	NS	NS	NS
CuSO ₄ levels (mg/kg)								
0	5.50±0.21	2.52±0.12	2.98±0.12	210.10±0.88 ^a	168.62±1.59 ^a	29.32±0.61 ^b	70.68±0.61 ^a	87.35±0.98
100	5.77±0.20	2.57±0.14	3.20±0.19	201.66±2.24 ^b	162.89±1.83 ^b	32.52±0.70 ^a	67.48±0.70 ^b	86.29±1.12
200	5.82±0.16	2.55±0.12	3.27±0.19	201.29±1.48 ^b	160.03±2.47 ^b	32.57±0.90 ^a	67.43±0.93 ^b	87.06±1.26
Significant	NS	NS	NS	**	*	*	*	NS
Interaction effects(CP X CuSO ₄ levels):								
16% CPX0 CuSO ₄	4.90±0.34 ^b	2.16±0.15 ^b	2.74±0.26	209.24±2.13 ^{ab}	170.70±2.07 ^a	28.99±0.40	71.01±0.40	87.04±1.96
16% CPX100 CuSO ₄	5.24±0.24 ^{ab}	2.16±0.13 ^b	3.08±0.14	201.11±4.62 ^{abc}	165.24±3.58 ^{abc}	32.32±1.85	67.68±1.85	86.23±1.74
16% CPX200 CuSO ₄	5.39±0.22 ^{ab}	2.17±0.08 ^b	3.23±0.30	204.44±2.68 ^{abc}	167.58±3.38 ^{abc}	31.63±1.37	68.37±1.37	87.52±1.44
18% CPX0 CuSO ₄	5.61±0.13 ^{ab}	2.61±0.18 ^{ab}	3.00±0.10	210.17±1.69 ^a	168.75±3.77 ^{ab}	29.00±0.82	71.00±0.82	87.23±1.98
18% CPX100 CuSO ₄	5.96±0.28 ^a	2.69±0.20 ^a	3.27±0.48	199.07±5.24 ^c	163.28±3.41 ^{abcd}	32.81±1.00	67.19±1.00	85.87±2.10
18% CPX200 CuSO ₄	6.03±0.23 ^a	2.64±0.19 ^{ab}	3.40±0.42	199.63±1.47 ^{bc}	157.81±2.07 ^{cd}	33.78±1.55	66.22±1.55	86.60±2.80
20% CPX0 CuSO ₄	5.98±0.30 ^a	2.79±0.12 ^a	3.19±0.23	210.91±1.12 ^a	166.41±2.70 ^{bc}	29.98±1.80	70.02±1.80	87.77±1.93
20% CPX100 CuSO ₄	6.12±0.36 ^a	2.87±0.17 ^a	3.25±0.43	204.80±1.76 ^{abc}	160.16±3.05 ^{bcd}	32.44±1.22	67.56±1.22	86.76±2.75
20% CPX200 CuSO ₄	6.03±0.27 ^a	2.84±0.14 ^a	3.19±0.36	199.81±3.11 ^{bc}	154.69±3.52 ^d	32.30±2.24	67.70±2.24	87.07±3.01
Significant	*	*	NS	*	*	NS	NS	NS

Means having different letters at the same column are differ significantly. * = (p<0.05), ** = (P<0.01); NS = Not significant.

Table 9: Input-output analysis and economic efficiency of Japanese quails as affected by dietary crude protein levels with or without copper sulfate supplementation during the experimental period (8-16 weeks of age)

Items	Interaction effects (CP x CuSO ₄ levels)								
	16% CP			18% CP			20% CP		
	0	100	200	0	100	200	0	100	200
	----- CuSO ₄ levels (mg/kg) -----								
Egg number	39.80	41.77	42.40	41.37	44.37	44.90	42.97	44.50	44.90
Price/egg (LE)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total revenue hen (LE)	5.97	6.27	6.36	6.21	6.66	6.74	6.45	6.68	6.74
Total feed intake/hen (kg)	2.26	2.23	2.22	2.24	2.22	2.21	2.24	2.23	2.21
Price/Kg feed (LE) ¹	1.83	1.87	1.91	1.9	1.94	1.98	1.97	2.01	2.05
Total feed cost/ hen (LE)	4.14	4.17	4.24	4.26	4.31	4.38	4.41	4.48	4.53
Fixed hen (LE)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total cost hen (LE)	4.64	4.67	4.74	4.76	4.81	4.88	4.91	4.98	5.03
Net revenue/hen (LE)	1.33	1.60	1.62	1.45	1.85	1.86	1.53	1.69	1.70
Economic efficiency (EEF)	28.78	34.16	34.17	30.48	38.46	38.13	31.20	33.97	33.88

LE= Egyptian pound, Cost at one kg feed 1.97, 1.90 and 1.83 LE for 20,18 and 16% CP, respectively at time of experiment. Cost of 500 gm copper sulfate =20.0 LE., Economic efficiency = {Net revenue (LE) / Total cost (LE) X 100}.

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