PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 15 (9): 878-882, 2016
ISSN 1680-5194

Effects of Dietary Copper Supplementation on Laying Performance, Egg Quality and Plasma Cholesterol Fractions in Laying Ducks

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Abstract: This study was designed to investigate the influences of dietary copper (Cu) on laying performance, egg quality, plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and low density lipoprotein-cholesterol (LDLC) concentrations in Shanma laying ducks. A total of 504 egg laying ducks aged 17 week were randomly allotted to seven groups (twelve ducks per replicate and six replicates each treatment). The control group was fed a corn-soybean meal diet, whereas the treatment groups were fed corn-soybean meal diets supplemented with 4, 8, 12, 16, 20, or 24 mg Cu-sulfate/kg. Egg production, egg weight, egg mass, feed conversion ratio, broken egg rate, abnormal egg rate, Haugh unit, yolk color, albumen height, eggshell weight, eggshell percent, eggshell breaking strength, eggshell thickness, plasma TC, HDLC and LDLC concentrations did not appear response to dietary Cu levels. Our results indicated that corn-soybean meal diet containing sufficient amount of Cu for laying performance and egg quality in laying ducks under the conditions of current experiment. Also, laying performance, plasma TC, HDLC, LDLC concentrations and egg quality were not sensitive traits to low levels of Cu.

Key words: Copper, laying ducks, laying performance, egg quality, cholesterol

INTRODUCTION

Copper (Cu) is a necessary mineral in poultry nutrition not only due to its role as a cofactor for many enzymes e.g., cytochrome oxidase, lysyl oxidase, tyrosinase, p-hydroxyphenyl pyruvate hydrolase, dopamine beta hydroxylase and copper-zinc superoxide dismutase (Leeson, 2009), but also due to its pivotal role in growth of blood vessel and muscle development during embryogenesis in chickens (Mroczek-Sosnowska *et al*., 2015). Deficiency of copper results in hypertriglceridaemia, hypercholesteraemia, anaemia, depigmentaion of feathers, abnormal bone and poor growth (Leeson, 2009). Cu is a growth enhancer and has antimicrobial properties (Skrivan *et al*., 2006). Dietary Cu supplementation improved egg production, egg weight, reduced percentage of abnormal egg (soft and broken egg), total cholesterol, triglycerides, low density-lipoprotein cholesterol and increased high density-lipoprotein cholesterol in laying hens (Lim and Paik, 2006; Guclu *et al*., 2008; Pekel and Alp, 2011; Jegede *et al*., 2012). Cu improved feed consumption, body weight gain, protein anabolism, relative weight of immune organs (thymus, spleen and Bursa of Fabricius, Complement C3 and C4, lysozyme, antibodies production (IgA, IgG and IgM), useful intestinal microflora (*Lactobacillus and Bifidobacterium*) and reduced harmful intestinal microflora such as *Coliforms* and *E-coli* in broiler chickens (Wang *et al*., 2011; Karimi *et al*., 2011). Cu enhanced the activity of digestive enzymes (trypsin, chemotrypsin, lipase and amylase), anti-superoxide anion, anti-hydroxy radical, antioxidant enzymes (superoxide dismutase, catalase, glutathione, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and declined malondialdehyde and protein carbonyl (Tang *et al*., 2013). However, influence of dietary Cu on laying performance, plasma cholesterol fractions and egg quality in laying ducks has not been investigated. Therefore, the main goals of this study were to investigate the influence of dietary Cu on laying performance, egg quality and plasma cholesterol fractions in laying ducks.

MATERIALS AND METHODS

Animals, diet and management: This study was approved by the Animal Care and Use Committee of Institute of Animal Science, Guangdong Academy of Agriculture Science. Five hundred and four Shanma female ducks (*Anas platyrhynchos*) aged 17 wk were randomly divided in the same room (with incandescent lighting of 10 lx, providing 15L: 9D) into 7 treatment

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groups, each of which included 6 replicates of 12 ducks for 28 wk (study period). Birds were housed in controlled cages (27.8 × 40 × 55 cm), separately. All ducks were given the same basal diet. The concentration of Cu in the basal diets was 4.6 mg/kg. The basal diet (Table 1) was formulated based on corn-soya bean meal to cover or exceed all nutrient requirements of Shanma laying ducks (Ruan *et al.*, 2015). Then graded levels of Cu (Cu-sulfate) were supplemented at 0 (Control), 4, 8, 12, 16, 20 and 24 mg/kg basal diet. All birds were given in average 150 g feed daily divided to two times, while water was allowed *ad libitum*.

Performance, egg quality and plasma cholesterol fractions

Feed consumption and egg production were recorded daily. The average daily egg production of all ducks ranged from 85 to 100% for the peak-laying period. Eggs were collected and individually weighed. Feed conversion ratio (FCR) was calculated as grams of feed per gram of egg mass and then presented as the averages for the whole experimental period.

Four eggs were chosen at random from each replicate each month and the average of the 24 eggs per replicate was used to evaluate egg quality. Haugh units and yolk color were measured using Egg Analyzer (model EA-01, ORKA Food Technology Ltd., Ramat Hasharon, Israel). Eggshells were washed under running water, dried and weighed. Albumen height was determined by Caliper. Eggshell thickness was measured after removing shell membrane with a digital micrometer (model IT-014UT, Mitutoyo, Kawasaki, Japan). Eggshell thickness was a mean value of measurements at 3 locations on the eggs (air cell, equator and sharp end). Eggshell breaking strength of un-cracked eggs was measured by Egg Force Reader (ORKA Food Technology Ltd., Ramat Hasharon, Israel).

After 28 wk of feeding, 2 birds were chosen randomly from each replicate. Heparinized blood was collected from the wing vein, centrifuged (1200 × g) at 4°C for 10 min and plasma was kept at -20°C. The concentrations of plasma total cholesterol (TC), high density lipoprotein cholesterol (HDLC) and low density lipoprotein cholesterol (LDLC) were determined spectrophotometrically using commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis: One-way ANOVA using the GLM procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC, USA) was performed to analyze the data. Significant differences among treatments were declared at $p \leq 0.05$.

RESULTS AND DISCUSSION

Laying performance: Dietary Cu supplementation had no effect on egg production, egg weight, egg mass, FCR, percent of broken egg or abnormal egg (Table 2; $p > 0.05$). In agreement with our results, Al-Ankari *et al.* (1998), who showed that adding 50 mg Cu/kg diet had not positive effect on laying performance, but high level of Cu (250 mg) impaired laying rate and feed efficiency of Leghorn hens. Lim and Paik (2003) observed that egg weight, feed consumption and FCR did not improve by adding 100 mg Cu-methionine compared with the control (contained 20.5 mg Cu/kg diet). Balevi and Coskun (2004) reported that inclusion of Cu in laying hens diet at level of 50, 100, 150 or 200 mg Cu/kg for 3 months did not affect egg production, egg weight, FCR or percent of broken egg. Lien *et al.* (2004) elevating dietary Cu level from 0 to 250 mg did not modify egg laying rate or egg weight. Lim and Paik (2006) noted that inclusion of Cu in form of chitosan-chelate or yeast-chelate at level of 100 mg/kg diet for two months did not improve egg production, egg weight, feed intake, FCR or percent of broken egg compared with the basal diet that contained 20.5 mg Cu/kg. Lim *et al.* (2006) demonstrated that egg production, egg weight and feed intake did not change by supplementing Cu at 200 mg/kg diet compared with the control group. Attia *et al.* (2011) showed that feeding breeding hens diet contain different levels (0, 60 or 120) and sources (Cu-sulfate or Cu-lysine) of Cu for 20 wk did not alter their egg production, egg weight, egg mass, feed consumption, feed efficiency. Pekel and Alp (2011) found that adding Cu at level 250 mg/kg diet from different sources did not affect egg weight, egg mass or FCR. Results of Kaya and Macit (2012) study indicated that laying rate, egg weight, feed consumption

Table 1: Composition of the basal diet and nutrient levels

Ingredients	Value (%)
Maize	54.87
Soybean meal	16.57
Wheat by-product	12.43
Soy protein	5.00
Limestone	8.49
Calcium hydrogen phosphate	1.2
DL-Methionine	0.133
L-Lysine-HCl	0.007
Sodium chloride	0.3
Premix[1]	1.0
Total	100.0
Nutritional value	
AME (Kcal/kg)	2500
Crude protein	17.0
Calcium	3.6
Total P	0.63
Available phosphorus	0.35
Total lysine	0.86
Total methionine	0.40
Cu (mg/kg)	7.73 (4.6)[2]

[1]Supplied per kilogram of diet: retinyl palmitate, 12000 IU; cholecalciferol, 2000 IU; DL-α-tocopheryl acetate, 38 mg; menadione sodium bisulphite, 1.0 mg; thiamin mononitrate, 3.0 mg; riboflavin, 9.6 mg; pyridoxine hydrochloride, 6.0 mg cobalamin, 0.03 mg; chloride choline, 500 mg; nicotinic acid, 25 mg; calcium-D-pantothenate, 28.5 mg; folic acid, 0.6 mg; biotin, 0.15 mg; Fe, 50 mg; Mn, 90 mg; Zn, 90 mg; I, 0.5 mg; Se, 0.4 mg. [2]Measured values

Table 2: Effect of dietary copper on performance of laying ducks

	Dietary copper (mg/kg)								
Item	4.6	8.6	12.6	16.6	20.6	24.6	28.6	SEM[2]	p-value
Egg production (%)	90.1	90.6	92.3	92.3	91.9	93.3	92.8	1.4	0.6
Egg weight (g)	64.0	63.8	63.7	64.3	63.7	63.8	63.5	0.4	0.8
Egg mass (g/d/d)	57.6	57.9	58.8	59.3	58.5	59.5	59.0	0.8	0.6
FCR[1]	2.60	2.60	2.55	2.53	2.56	2.52	2.54	0.04	0.6
Broken rate (%)	2.1	1.5	1.8	1.8	1.6	1.9	1.5	0.6	0.9
Abnormal rate (%)	0.03	0.03	0.04	0.12	0.03	0.06	0.13	0.04	0.2

[1]Feed conversion ratio, g of feed/g of egg mass
[2]Standard error of means (n = 12 birds/replicate; n = 6 replicates/treatment)

Table 3: Effect of dietary copper on egg quality of laying ducks

	Dietary copper (mg/kg)								
Item	4.6	8.6	126	16.6	20.6	24.6	28.6	SEM[1]	p-value
Haugh unit	76.9	76.6	75.2	77.0	78.8	74.0	72.9	1.9	0.4
Yolk color	5.4	5.2	5.1	5.3	5.6	5.1	5.6	0.2	0.5
Albumen height, mm	6.4	6.2	6.1	6.4	6.5	5.9	5.9	0.2	0.3
Eggshell weight, g	5.9	6.1	6.2	6.3	6.1	6.1	5.9	0.1	0.5
Eggshell thickness, mm	0.36	0.36	0.36	0.38	0.37	0.37	0.37	0.007	0.5
Eggshell strength, N	4.2	4.4	4.2	4.3	4.1	4.2	4.1	0.2	0.7

[1]Standard error of means (n = 4 eggs/replicate; n 6 replicates/treatment)

Table 4: Effect of dietary copper on plasma cholesterol fractions in laying ducks

	Dietary copper (mg/kg)								
Item	4.6	8.6	12.6	16.6	20.6	24.6	28.6	SEM[1]	p-value
TC	2.05	2.13	2.30	1.77	1.68	1.88	2.07	0.2	0.1
HDL-C	1.42	1.19	1.60	1.41	1.54	1.47	1.20	0.2	0.08
LDL-C	1.10	1.40	1.36	1.05	1.00	1.12	1.43	0.1	0.2

TC = total cholesterol; HDLC= high-density lipoprotein cholesterol; LDLC = low density lipoprotein-cholesterol
[1]Standard error of means (n = 2 birds/replicate; n = 6 replicates/treatment)

declined and feed efficiency and broken egg rate were not influenced by adding 200 mg Cu in hens diet for 12 wk. Olgun *et al.* (2013) observed that supplemental Cu at level of 75 mg/kg diet did not enhance egg production, egg weight, egg mass, feed intake or FCR. Increasing Cu from organic or inorganic sources had no effect on egg production, egg weight, FCR and broken egg rate (Manangi *et al.*, 2015). Also, in broiler chickens, dietary Cu supplementation had no effect on body weight gain, feed intake or FCR (Song *et al.*, 2009). Increasing level of Cu from 0 to 250 mg or increasing the level and replacing inorganic-Cu with organic-Cu did not alter performance of broiler chickens in a study continued for 42 day compared with the control group (Karimi *et al.*, 2011; Liu *et al.*, 2012). On the other hand, Lim and Paik (2003) demonstrated that egg production improved by adding 100 mg Cu-methionine compared with the control (contained 20.5 mgCu/kg diet). Guclu *et al.* (2008) demonstrated that supplementation of Cu at 150 and 300 mg/kg diet improved egg production, but egg weight, feed intake and FCR did not change. In laying quails, increasing level of Cu from 0 to 200 mg enhanced egg production, egg mass and FCR (Abaza *et al.*, 2009). Pekel and Alp (2011) observed that adding Cu in form of sulfate at level 250 mg/kg diet significantly increased egg laying rate compared with the control diet that contained 11 mg Cu/kg. Olgun *et al.* (2013) recorded that diet supplemented with Cu at level of 150 mg/kg improved egg production, egg mass and FCR compared with the control or the level of 75 mg/kg.

Egg quality: Huagh units, yolk color, albumen height, eggshell weight, eggshell percent, eggshell eggshell thickness or breaking strength did not enhance by dietary Cu supplementation (Table 3; p>0.05). Similar results have reported by Al-Ankari *et al.* (1998), who recorded that Huagh unit and eggshell quality were not improved by increasing dietary Cu. In laying hens, Lim and Paik (2003) reported that Huagh unit, albumen height and eggshell thickens did not enhance by dietary Cu supplementation. Lim *et al.* (2006) observed that yolk color, eggshell breaking strength and eggshell thickness did not improve as a result to add Cu in laying hens diet. Lim and Paik (2006) demonstrated that adding Cu from different sources for 8 wks did not improve eggshell breaking strength. In dual-purpose breeder hens, level and source of Cu had no effect on egg quality (Attia *et al.*, 2011). Pekel and Alp (2011) and Pekel *et al.* (2012) reported that Cu at level of 250 mg/kg diet from different sources (Cu-sulfate, Cu-proteinate or Cu-lysine) did not enhance eggshell quality (eggshell weight and eggshell thickness) in study continued for 6 months. Sun *et al.* (20012) found that eggshell thickness and eggshell breaking strength did not alter by replacing inorganic Cu with organic Cu or increasing its level from organic source. Kaya and Macit

(2012) observed that adding 200 mg Cu in hens diet for 12 wk did not improve Haugh unit, yolk color, eggshell weight or eggshell thickness. On the other hand, Olgun *et al.* (2012) observed that eggshell weight relative to egg weight, eggshell thickness and eggshell breaking strength improved due to add Cu at level of 50,150 or 300 mg/kg diet in their study that continued for 16 wk in laying hens. Lim and Paik (2003) observed that eggshell strength improved by adding 100 mg Cu (organic form) compared with the control (contained 20.5 mg Cu/kg diet). In laying quails, supplemental diets with Cu did not affect Haugh unit or eggshell quality (Abaza *et al.*, 2009).

Plasma cholesterol fractions: No differences were found in concentrations of plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and low density lipoprotein-cholesterol (LDLC) (Table 4; $p>0.05$). Our results agree with the results of Lim and Paik (2006), Pekel and Alp (2011) and Guclu *et al.* (2011). Lim and Paik (2006) found that adding 100 mg Cu/kg diet from different organic sources (Cu-methionine, Cu-chitosan or Cu-yeast) for 8 wks did not change egg yolk TC. Pekel and Alp (2011) reported that dietary Cu supplementation at level of 250 mg/kg feed for 14 wk did not exhibit significant effects on TC or HDLC levels in plasma and yolk of laying hens. Feeding laying hens for 6 wks diets supplemented with high level (800 mg/kg feed) of inorganic Cu did not reduce TC in the serum (Guclu *et al.*, 2008). Also, results of Kaya and Macit (2012) in laying hens, showed that adding 200 mg Cu/kg diet for 12 wk had no effect on concentration of TC in plasma or egg yolk. Moreover, in white pekin ducks, inclusion low levels of Cu (4, 8 or 12 mg/kg diet) did not alter concentrations of TC in the liver or meat, while inclusion high levels of Cu (150 mg/kg diet) declined significantly concentration of TC in the liver and meat (Attia *et al.*, 2012). On contrary, Lim *et al.* (2006) demonstrated that dietary Cu supplementation at level of 200 mg/kg feed for 5 wks declined TC in serum and egg yolk of laying hens aged 54 wk without effect on HDLC. Also, Jegede *et al.* (2012) observed that adding 150 mg Cu/diet from organic or inorganic source for 12 wk reduced TC, HDLC and improved HDLC in plasma of growing pullets. Attia *et al.* (2011) found that adding 60 mg Cu/kg die for 20 wk decreased significantly TC, LDLC and improved significantly HDLC concentrations in hen breeders compared with control diet that was contained 10 mg Cu/kg. Also, early study by Ankari *et al.* (1998) recommended with adding 250 mg Cu/kg feed to reduce TC in plasma and egg yolk of laying hens. In our experiment, we added low level of Cu in the diets because the studies that have been carried out to investigate the effects of dietary Cu supplementation with low level in poultry are limited. Previous studies that have been shown improvements in laying performance and egg quality and a reduction in plasma TC level in hens or quails, added Cu with high level compared with our experiment. This may clarify why dietary Cu supplementation did not exhibit enhancements in laying performance, egg quality or a reduction in plasma TC concentration without taken in our consideration animal model.

Conclusion: Corn-soybean diet that contained 4.6 mg Cu is sufficient for optimizing laying performance and egg quality in Shanma laying ducks under the conditions of our experiment, which has been reported in broiler chickens, white pekin ducks, laying hens and hen breeders (Liu *et al.*, 2012; Winiarska-Mieczan and Kwiecien, 2015; Attia *et al.*, 2012; Balevi and Coskun, 2004; Attia *et al.*, 2011). Body weight gain, feed consumption, FCR and carcass composition did not affect by increasing dietary Cu level from 5.7 to 250 or reducing it from 16 to 4.0 mg/kg (Liu *et al.*, 2012; Winiarska-Mieczan and Kwiecien, 2015). In white pekin ducks, growth performance and mortality percentage did not change by increasing dietary Cu concentration from 7.0 to 11 mg/kg (Attia *et al.*, 2012), while increasing dietary Cu concentration from 10 to 120 mg/kg in hen breeders (Attia *et al.*, 2011) and from 5 to 50 mg/kg in laying hens did not modify laying performance or egg quality (Balevi and Coskun, 2004).

ACKNOWLEDGMENTS

This work was supported by the Fund for China Agricultural Research System (CARS-43-13), the Science and Technology Program of Guangdong Province (2011A020102009) and 2016A020210043 and Operating Funds for Guangdong Provincial Key Laboratory of Animal Breeding (2014B030301054) .

REFERENCES

Abaza, I.M., W. Ezzat, M.S. Shoeib, A.A. Eizaiat and I.I. Hassan, 2009. Effects of copper sulfate on productive, reproductive performance and blood constituents of laying Japanese quail fed optimal and sub-optimal protein. Int. J. Poult. Sci., 1: 80-89.

Attia, Y.A., A.A. Abdalah, H.S. Zeweil, F. Bovera, A.T. El-Din and M.A. Araft, 2011. Effect of inorganic or organic copper additions on reproductive performance, lipid metabolism and morphology of organs of dual-purpose breeding hens. Arch. Geflugelk, 3: 169-178.

Al-Ankari, A., H. Najib and A. Al-Hozab, 1998. Yolk and serum cholesterol and production traits, as affected by incorporatinga supraoptimal amount of copper in the diet of the leghorn hen. Br. Poult. Sci., 39: 393-397.

Balevi, T. and B. Coskun, 2004. Effects of dietary copper on production and egg cholesterol content in laying hens. Br. Poult. Sci., 45: 530-534.

Guclu, B.K., K. Kara, L. Beyaz, F. Uyanik, M. Eren and A. Atasever, 2008. Influence of dietary copper proteinate on performance, selected biochemical parameters, lipid peroxidation, liver and egg copper content in laying hens. Biol. Trace. Elem. Res., 125: 160-169.

Jegede, A.V., O.O. Oduguwa, A.O. Oso, A.O. Fafiolu, O.M.O. Idowu and L. Nollet, 2012. Growth performance, blood characteristics and plasma lipids of growing pullet fed dietary concentrations of organic and inorganic copper sources. Livest. Sci., 145: 298-302.

Karimi, A., G. Sadeghi and A. Vaziry, 2011. The effect of copper in excess of the requirement during the starter period on subsequent performance of broiler chicks. J. Appl. Poult. Res., 20: 203-209.

Kaya, H. and M. Macit, 2012. Effect of inclusion of garlic (Allium sativum) powder at different levels and copper into diets of hens on performance, egg quality traits and yolk cholesterol content. Int. J. Poult. Sci., 11: 114-119.

Leeson, S., 2009. Copper metabolism and dietary needs. World's Poult. Sci. J., 65: 353-366.

Lim, H.S. and I.K. Paik, 2006. Effects of dietary supplementation of copper chelates in the form of methionine, chitosan and yeast in laying hens. Asian-Aust. J. Anim. Sci., 19: 1174-1179.

Lim, H.S. and I.K. Paik, 2003. Effects of supplementary mineral methionine chelates (Zn, Cu, Mn) on the performance and eggshell quality of laying hens. Asian-Aust. J. Anim. Sci., 16: 1804-1808.

Lien, T.F., K.L. Chen, C.P. Wu and J.J. Lu, 2004. Effects of supplemental copper and chromium on the serum and egg traits of laying hens. Br. Poult. Sci., 45: 535-539.

Lim, H.S. and I.K. Paik, 2006. Effects of dietary supplementation of copper chelates in the form of methionine, chitosan and yeast in laying hens. Asian-Aust. J. Anim. Sci., 19: 1174.

Lim, K.S., S.J. You, H.K. An and C.W. Kang, 2006. Effects of dietary garlic powder and copper on cholesterol content and quality characteristics of chicken eggs. Asian-Aust. J. Anim. Sci., 19: 582-586.

Liu, S., L. Lu, S. Li, J. Xie, L. Zhang, R. Wang and X. Luo, 2012. Copper in organic proteinate or inorganic sulfate form is equally bioavailable for broiler chicks fed a conventional corn-soybean meal diet. Biol. Trace. Elem. Res., 147: 142-148.

Mroczek-Sosnowska, N., E. Sawosz, P. Vadalasetty, M. Lukasiewicz, J. Niemiec, M. Wierzbicki and A. Chwalibog, 2015. Nanoparticles of Copper Stimulate Angiogenesis at Systemic and Molecular Level. Int. J. Mol. Sci., 16: 4838-4849.

Manangi, M.K., M. Vazques-Anon, J.D. Richards, S. Carter and C.D. Knight, 2015. The impact of feeding supplemental chelated trace minerals on shell quality, tibia breaking strength and immune response in laying hens1. J. Appl. Poult. Res. http://dx.doi.org/10.3382/japr/pfv029.

Olgun, O., O. Yazgan and Y. Cufadar, 2012. Effects of boron and copper dietary supplementation in laying hens on egg shell quality, plasma and tibia mineral concentrations and bone biomechanical properties. Rev. Med. Vet., 163: 335-342.

Olgun, O., O. Yazgan and Y. Cufadar, 2013. Effect of supplementation of different boron and copper levels to layer diets on performance, egg yolk and plasma cholesterol. J. Trace. Elem. Med. Biol., 27: 132-136.

Pekel, A.Y. and M. Alp, 2011. Effects of different dietary copper sources on laying hen performance and egg yolk cholesterol. J. Appl. Poult. Res., 20: 506-513.

Pekel, A.Y., G. Demirel, M. Alp, N. Kocabagli and N. Acar, 2012. Influence of different dietary copper sources on eggshell quality and phosphorus retention in laying hens. J. Appl. Poult. Res., 21: 460-466.

Ruan, D., Y.C. Lin, W. Chen, S. Wang, W.G. Xia, A.M. Fouad and C.T. Zheng, 2015. Effects of rice bran on performance, egg quality, oxidative status, yolk fatty acid composition and fatty acid metabolism-related gene expression in laying ducks. Poult. Sci, pp: 286.

Skrivan, M., V. Skrivanova and M. Marounek, 2006. Effect of various copper supplements to feed of laying hens on Cu content in eggs, liver, excreta, soil and herbage. Arch. Environ. Contam. Toxicol., 50: 280-283.

Song, Z., L. Zhu, T. Zhao, H. Jiao and H. Lin, 2009. Effect of copper on plasma ceruloplasmin and antioxidant ability in broiler chickens challenged by lipopolysaccharide. Asian-Aust. J. Anim. Sci., 22: 1400-1406.

Sun, Q., Y. Guo, J. Li, T. Zhang and J. Wen, 2012. Effects of methionine hydroxy analog chelated Cu/Mn/Zn on laying performance, egg quality, enzyme activity and mineral retention of laying hens. J. Poult. Sci., 49: 20-25.

Tang, Q.Q., L. Feng, W.D. Jiang, Y. Liu, J. Jiang, S.H. Li and X.Q. Zhou, 2013. Effects of dietary copper on growth, digestive and brush border enzyme activities and antioxidant defense of hepatopancreas and intestine for young grass carp (Ctenopharyngodon idella). Biol. Trace Elem. Res., 155: 370-380.

Wang, C., M.Q. Wang, S.S. Ye, W.J. Tao and Y.J. Du, 2011. Effects of copper-loaded chitosan nanoparticles on growth and immunity in broilers. Poult. Sci., 90: 2223-2228.

Winiarska-Mieczan, A. and M. Kwiecien, 2015. The effects of copper-glycine complexes on chemical composition and sensory attributes of raw, cooked and grilled chicken meat. J. Food Sci. Technol., 52: 4226-4235.