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Effects of different levels of copper sulfate on small on intestinal physiology in japanese quail (*Coturnix coturnix japonica*)

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Abstract

This study was conducted to evaluate the effects of different Levels of copper sulfate on small intestine morphometry in Japanese quail. Four hundred and twenty, day old male Japanese quail randomly assigned into 5 treatments with 4 replicates (each replicates contain 20 birds). All experimental birds were received basal diet without copper sulfate from day 1 to 7. At day 8, experimental birds were received experimental diets: Control group (A) received basal diet, whereas B, C, D and E groups were received basal diet supplemented by 50, 100, 150 and 200 mg copper sulfate, respectively. At day 42, three birds from each group were randomly selected and slaughtered. Various sections of small intestine (10, 50 and 70 small intestine length) sampled, rinsed with phosphate to measure villi length and depth of liberkuhn crypts. According to the results, a significant increase was observed in depth of liberkuhn crypts (10% intestine length) and villi height (70% intestine length) in birds fed diet contain 200 mg copper sulfate ($p<0.05$). Furthermore, supplementation of various levels of copper sulfate significantly decreased depth of liberkuhn crypts and villi height in the other portion of small intestine in Japanese quail ($p>0.05$). It seems, different Levels of copper sulfate were not able to improve small intestine morphometry in Japanese quail.

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Introduction

The gastrointestinal (GI) tract is the primary location of entry for orally administered of diet. The functions of this system include digestion, absorption, protection and the structure of the gut is well adapted to perform these functions. The gut provides a barrier against the invasion by its microflora. The barrier consists of a layer of mucin overlying the gut epithelium, the epithelial cells themselves with their impermeable cell to cell tight junctions and also the innate and adaptive immune cells in the connective tissue below the epithelium (Antheony *et al.,* 1999; Shin *yet al.,* 2013). Utilization of energy by the chicken influenced by requirements for growth and maintenance of the GIT (Choct, 1999), intestinal epithelium surface and villus height and depth of liberkuhn crypts (Jin *et al.,* 1998; Iji, 1999). The relative opportunities for contact between ingested food, digestive enzymes, bile salts and the time available for contact between digested particles and absorptive surfaces are likely to influence by intestinal growth in poultry. Growth performance and nutrient absorption are influenced by rate of passage of digesta in birds (van der Klis and van Voorst, 1993; Uni *et al.,* 1995) and rats (Gohl and Gohl, 1977).

Virtually all poultry feeds contain supplemental minerals. The importance of the form of the supplemental minerals, however, is often overlooked. The advantage of organic trace minerals over inorganic salts is that the binding of the mineral to the organic ligand provides stability of the complex in the upper gastrointestinal system. Organic trace minerals resist dissociation in the crop, proventriculus and gizzard, thus allowing the intact complex to be delivered to the absorptive epithelium of the small intestine. The organic forms of many minerals, including zinc (Zn), Copper (Cu) and manganese (Mg) are widely used in animal agriculture (Ghodasara *et al.,* 2013). Copper is an essential trace mineral for poultry. Copper is plentiful in the environment and needed for the normal growth and metabolism of all living organisms. Both deficiency and excess of Cu sulphate lead to physiological disturbances and diseases. Copper sulfate is used as a fungicide in poultry feed and for the medication of drinking water (Iqbal *et al.,* 2012). Intestinal pH and solubility of Cu in the small intestine may eventually affect intestinal microbiota, mineral absorption, and bioavailability in birds (Pang and Applegate, 2007). It has been reported that intestine villi morphology and epithelial cell morphology are related to intestine function and growth rate of intestine (Ruttanavut *et al.,* 2008). Numerous epithelial cells were observed on the villous apex surface which suggested they are improves FCR and increases BWG in cockerels (Yamauchi *et al.,* 2006). Copper has received considerable attention due to its antimicrobial properties that improve performance in animals when fed over the minimum requirement (Barber *et al.,* 1955; Smith, 1969; Jenkins *et al.,* 1970; Miles *et al.,* 1998). Studies have shown that supplementation with various Cu sources (e.g., Cu sulfate, Cu citrate, or Cu chloride) increases growth rate and performance in broilers (Smith, 1969; Pesti and Bakalli, 1996; Miles *et al.,* 1998) and swine (Hill *et al.*, 2000; Arias and Koutsos, 2006). Little literatures have been published about the effects of different Levels of copper sulfate on blood biochemical traits in Japanese quail. The present study is a novel study to investigate the potential influence of different levels of Cu sulfate on intestinal villi length and depth of liberkuhn crypts in Japanese quail (*Coturnix coturnix japonica*).

Material and methods

Birds and diets

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

Intestinal morphology

At day 42, 5-h prior the start of the experiments, animals were deprived from food (FD5). Three birds from each group were randomly selected and slaughtered. Animals necropsied, and the entire gastrointestinal tract was quickly removed for further studies. Various sections of small intestine (10, 50 and 70 small intestine length) sampled (Mouwen, 1971), rinsed with phosphate to measure villi length and depth of liberkuhn crypts. Each sample was divided into 2 parts. One part for measuring villus dimensions and second one for determining depth of liberkuhn crypts. Each sample was prepared for microscopically study after staining with Periodic Acid schiff Solution (PAS), and stabilized by Clark stabilizer solution (Clarke, 1977), separating muscular layer and preparing lamella. In each sample, villi height was measured from the tip to the bottom of villus. Mean villi heights and depth of liberkuhn crypts from 2 birds (40 villi from different sections in each sample per bird) were attributed as a mean villi height in each group using the second sample per birds using an image Analyzer (Nikon Cosmozone 1S; Nikon Co., Tokyo, Japan) in the physiology laboratory of Islamic Azad University, Shabestar branch, Iran.

Statistical analysis

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

Results and discussion

Effects of different Levels of copper sulfate on average villi height, depth of liberkuhn crypts in Japanese quails in day 42 is presented in table 2. According to the results, supplementation of copper sulfate (200mg) significantly increased depth of liberkuhn crypts in 10 % portion of small intestine in Japanese quail ($p<0.05$). Also, an increase but not significant was observed on villi height in 10 % portion of small intestine in birds fed diet contain copper sulfate (200mg) ($p>0.05$).

Previous researchers have reported that dietary Cu enhances performance in poultry when fed at prophylactic dosages over the minimum nutritional requirement (Pesti and Bakalli, 1996; Zhang *et al.,* 2009). The feed intakes and body weights of 21- day-old chickens fed 300 or 450 mg Cu/kg diets from Tri-basic Cu chloride were increased compared to 300 or 450 mg Cu/kg levels from Cu sulfate but were similar at dietary concentrations of 150 mg/kg (Miles *et al.,* 1998). Source of Cu sulfate may increase efficiency through affecting the proximal small intestine due to the decomposition of Cu sulfate, hydrogen can lead to improved digestion and absorption in the intestinal wall and are linked (Iji *et al.,* 2001). The intestine as the major interface between an organism and its nutritional environment plays a critical role in postnatal development of a newborn animal (Noy and Sklan, 1995). In chicken, profound growth, morphological changes and functional maturation occur in the intestine during the early post-hatching period (Bayer *et al.,* 1975). The physiological intestinal functions develop along with the morphological changes in mucosal structure especially villus height to increase absorptive efficiency (Hill, 1971). Indices of tissue activity, ribosomal capacity, and cell size decreased with age but at differing rates in these intestinal sections. In this study because lack or researches, we could not find researches which have done prior this study to compare our results with.

In this experiment, a significant diminish were detected on depth of liberkuhn crypts and villi height in 50 % portion of small intestine in birds received all experimental diets supplemented using different levels of copper sulfate (50, 100, 150 and 200 mg) ($p<0.05$). Copper seems to have different sources of influence are different parts of the intestine (Iji *et al.,* 2001). Pharmacological levels of Cu may be beneficial for altering intestinal microbiota, increasing digestibility of other nutrients or improving gastrointestinal physiology. Pang *et al.* (2009) found that supplementation with Tri-basic Cu chloride at 187.5 mg Cu/kg dosage significantly increased the similarity coefficients of microbiota in the ileal mucosa compared with a similar level of Cu from Cu sulfate which suggested that Tri-basic Cu chloride may alter the microbial community associated with the ileal mucosa in broiler chickens yet did not significantly influence live performance or ileal phosphorus digestibility in their trial. Arias and Koutsos (2006) reported that chicks fed Tri-basic Cu chloride or positive control (antibiotic) diets had better crypt depth in the ileum compared with those fed control or Cu sulfate, and a greater quantity of duodenum intraepithelial lymphocytes compared with the negative control. In this study because lack or researches, we could not find researches which have done prior this study to compare our results with.

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

Treatments	A	B	C	D	E
Ingredients					
copper sulfate	0	50	100	150	200
Corn grain	55	55	55	55	55
Soybean meal	33.34	33.35	33.35	33.35	33.35
Gluten meal	7	7	7	7	7
Oyster shell	1.5	1.5	1.5	1.5	1.5
DCP	0.84	0.32	0.32	0.32	0.32
Sodium bicarbonate	0.26	0.26	0.26	0.26	0.26
Soybean oil	0.11	0.12	0.12	0.12	0.12
Vit. Premixes	0.25	0.25	0.25	0.25	0.25
Min. Premixes	0.25	0.25	0.25	0.25	0.25
Salt	0.16	0.16	0.16	0.16	0.16
Nutrients (calculated)					
ME (Kcal/Kg)	2900	2900	2900	2900	2900
CP % [a]	24	24	24	24	24
Ca %	0.80	0.80	0.80	0.80	0.80
Av. P %	0.30	0.30	0.30	0.30	0.30
Met. % + Cys. %	0.81	0.81	0.81	0.81	0.81
Lys. %	1.35	1.35	1.35	1.35	1.35
Tryptophan %	0.30	0.30	0.30	0.30	0.30
Na %	0.15	0.15	0.15	0.15	0.15
K %	0.85	0.85	0.85	0.85	0.85
Cl %	0.14	0.14	0.14	0.14	0.14

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

Table 2. Effects of different Levels of copper sulfate on average villi height, depth of liberkuhn crypts in Japanese quail.

Treatments	10% depth of liberkuhn crypts	10% villi height	50% depth of liberkuhn crypts	50% villi height	70% depth of liberkuhn crypts	70% villi height
(A) Control	65.539 ^{b}	290.41 ^{a}	60.918 ^{a}	221.954 ^{a}	52.735 ^{a}	130.558 ^{b}
(B) copper sulfate 50mg	63.781 ^{b}	298.08 ^{b}	42.761 ^{b}	156309 ^{b}	37.086 ^{b}	139.124 ^{b}
(C) copper sulfate 100mg	64.666 ^{b}	250.82 ^{b}	48.098 ^{b}	158.953 ^{b}	37.590 ^{b}	159.631 ^{a}
(D) copper sulfate 150mg	66.606 ^{b}	243.17 ^{b}	47.444 ^{b}	162.669 ^{b}	42.294 ^{b}	138.556 ^{b}
(E) copper sulfate 200mg	74.991 ^{a}	290.81 ^{a}	47.174 ^{b}	165.779 ^{b}	49.221 ^{b}	164.445 ^{a}
P value	0.0156	0.0001	0.0001	0.0001	0.0001	0.0001
SEM	2.404	8.388	2.207	4.543	1.949	3.347

SEM: Standard error mean. There are significant differences between groups with different codes in a column (superscript letters a, b; $p < 0.05$).

Supplementation of diets using different levels of Cu sulfate (50, 100, 150 and 200 mg) significantly decreased depth of liberkuhn crypts in 70 % small intestine length, but a significant increase was detected in villi height in 70 % small intestine length in Japanese quail fed diet contain 200mg copper sulfate ($p<0.05$). In general, the development of villi of small intestine by feed intake increased. The result is consistent with the rapid expansion in size and complexity of plica (internal folds) of villi during the development period of chicks (Uni *et al.,* 1998). Crypt depth which reflects enterocyte-differentiating activity increased linearly in all intestinal segments. The impact of reduced cell turnover on nutrient digestibility in the small intestine of quail remains unknown and requires further investigation. In this study because lack or researches, we could not find researches which have done prior this study to compare our results with. These results indicate that presumably these effects occur because of levels of Cu sulphate. It seems, further researches needs to elucidate the direct effects of Cu sulfate on villi height and depth of liberkuhn crypts in quail.

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