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Effects of Dietary Copper on Growth Performance, Slaughter Performance and Nutrient Content of Fecal in Growing Goslings from 28 to 70 Days of Age

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ABSTRACT

A dose-response experiment with four dietary copper concentrations (4.17, 8.17, 12.17 and 16.17 mg/kg) was conducted to estimate the growth performance, slaughter performance, nutrient content of fecal and liver copper concentrations of growing Goslings from 28 to 70 d of age. Two hundred healthy male Yangzhou geese with similar body weight were randomized to four groups with five replicates per treatment and ten geese per replicate. Average daily feed intake, average daily gain and feed conversion ratio of geese for each pen were measured from 28 to 70 d of age. At 70 d of age, two geese were selected randomly from each pen and slaughtered to evaluate carcass quality. Metabolism experiment was conducted with five male geese from each group (one goose per pen) which body weight was close to the mean weight of the group from 64 to 70 d of age. Significant effects of dietary copper was found on body weight, feed conversion ratio, carcass yield, fecal copper concentrations and liver copper concentrations. Body weight, feed conversion ratio and carcass yield showed significant quadratic response to increase dietary copper concentration, while fecal copper concentration and liver copper concentration showed a significant linear response. The result showed that dietary Cu addition can improve growth by increasing the use of the feeding stuff and improving carcass yield in growing Goslings. Furthermore, taking into consideration, the optimal level of Gosling dietary copper was between 8.77 and 11.6 mg/kg from 28 to 70 days of age.

INTRODUCTION

With the development of poultry husbandry in China, goose production is becoming intensive and more widespread. The Yangzhou geese, which is a major breed in China, are medium in body size and have dual-purpose, for meat and egg production (Shi *et al*. 2007). However, owing to lack of experience of raising criterion for Goslings, husbandmen can only depend on personal experience or seek rearing criteria for chickens in determining the feeding allowances, thereby causing wasteful production. It is, therefore, imperative for geese intensive production to establish more accurate raising criterion.

Copper was an essential trace element for poultry and livestock (Davis & Mertz, 1987). And copper can maintain proper body functions and obtain the optimal growth performance of poultry (Banks *et al*. 2004). Also, copper is a cofactor for various enzymes such as cytochrome oxidase, lysyl oxidase and Cu-Zn superoxide dismutase (Davis & Mertz, 1987). Basing on the central role in various vital proteins, deficiency and toxicity, Leeson (2009) reported that copper deficiency could cause severe health problems including growth depression, disability of bones, poor feathering and anemia. Copper additive in the diet, proved that it has a beneficial effect on weight gain, feed conversion ratio and modification of the bacterial microflora in the alimentary tract (Ruiz *et al*. 2000; Nys, 2001). Moreover, Copper is also regarded as a growth promoter in the poultry aspect of breeding (Kim *et al*. 2011), taking into account that the use of antibiotic as growth promoters is prohibited by the European Union. However, an overdose of copper (100-250 mg/kg) can restrict the growth of chicks (Nys, 2001). Furthermore, a greater amount of Cu than its requirement, has often been added in the commercial geese diet due to lack of scientific data on geese. Excessive mineral supplementation is wasteful and leads to increased metals in the environment (Aksu *et al*. 2011). Therefore, the optimal level of Gosling dietary copper should be determined.

Currently, Copper requirement studies are mostly focused on chickens and turkeys (Yenice *et al*. 2015). However, relatively little research has been published describing the copper requirements of geese. It is advised by the NRC (1994) that copper is added at a rate of 2.5 mg/kg to laying hen diets and at a rate of 8 mg/kg to broiler chicken diets. However, the information for geese is missing.

The most common method used to determine copper responses in growing poultry, involves the addition of graded amounts of the copper under test to a basal diet deficient in that copper. Therefore, through the dose-response relationship for copper, the objective of our study was to determine the optimal copper concentration for growing Goslings from 28 to 70 d of age regarding growth performance, slaughter performance, nutrient content of fecal and liver copper concentration.

MATERIALS AND METHODS

Ethics statement

All bird-handing protocols were approved by the Yangzhou University Animal Experiments Ethics Committee, with the permit number: SYXK (Su) IACUC 2012-0029. All goose experimental procedures performed by the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of the People's Republic of China.

Experimental design and diets

The study was conducted using two hundred 28-d-old healthy male Yangzhou geese from a commercial hatchery (Yangzhou Goose Co. Ltd, Yangzhou, China). The geese were all of the similar body weight (BW; 1381±3.16 g) and were randomized to four groups that included five replicates per treatment and ten geese per replicate. A basal maize-soybean meal diet was formulated to provide an adequate concentration of all the nutrients required by the geese (NRC, 1994) except Cu (Table 1). The basal diet supplemented with four concentrations of Cu (0, 4, 8 and 12 mg Cu/kg dry matter-DM) from $CuSO_4.5H_2O$ (Chengdu Kangbeier Feed Co. Ltd., Chengdu, China). Calculated copper values for each group were A: 4.17 mg/kg, B: 8.17 mg/kg, C: 12.17 mg/kg and D: 16.17 mg/kg. Geese raised in separate wire-floor pens with 2 cm^2 square holes, 70 cm high from the floor. All manure was removed from underneath the wire-floor at the end of the experiment. Geese had free access to diets and water for 24 h. Water provided by the same half-open, plastic, cylindrical water tank, and pelleted feed provided in feed troughs on one side of each pen. Mortality and the body weight (BW) of dead geese were

Table 1 – Composition and nutrient levels of the basal diet.

Ingredients	Value(g/kg)	Nutrient Composition	Value
Maize	621	Metabolisable Energy[2], (MJ/kg)	11.06
Soybean meal	149	Crude Protein (%)	15.92
Corn gluten meal	41	Crude Fibre (%)	6.0
Alfalfa meal	159	Calcium (%)	0.81
Calcium hydrogen phosphate	11	Total Phosphorus (%)	0.56
Limestone	5	Methionine (%)	0.36
Salt	3	Lysine (%)	0.82
Vitamin and trace mineral premix[1]	10	Copper[3] (mg/kg)	4.17
Methionine	1		
Total	1000		

[1]One kilogram of premix contained: Vitamin A, 1, 200, 000 IU; Vitamin D, 400, 000 IU; Vitamin E, 1, 800 IU; Vitamin K, 150 mg; Vitamin B_1, 60 mg; Vitamin B_2, 600 mg; Vitamin B_6, 200 mg; Vitamin B_{12}, 1 mg; nicotinic acid, 3 g; pantothenic acid, 900 mg; folic acid, 50 mg; biotin, 4 mg; choline, 35 mg; Fe (as ferrous sulfate), 6 g; Mn (as manganese sulfate), 9.5 g; Zn (as zinc sulfate), 9 g; I (as potassium iodide), 50 mg; Se (as sodium selenite), 30 mg.

[2]The values are calculated from ingredient AME values for chickens.

[3]Analyzed values.

recorded when the death occurred. The geese were indoor rearing under similar environmental conditions (temperature: 26.0°C±3.0°C; RH: 65.5±5.0%; Lighting Period: 16 h; density: 0.5 m^2/gander).

Sample collection and analytical determination

The feed intake was measured by pen on a daily basis and BW recorded at 28 and 70 d of age. Average daily gain (ADG) and average daily feed intake (ADFI) of the Goslings were calculated at the end of the experiment, and the feed conversion ratio (FCR) values corrected for the BW of any goose that died during the experiment. FCR= ADFI (g) / ADG (g).

At 70 d of age, after being deprived of feed overnight, two Goslings were selected at random from each pen, and slaughtered by exsanguination. The geese eviscerated and live body weight, carcass weight, eviscerated carcass weight, half-eviscerated carcass weight, breast meat (including pectoralis major and pectoralis minor), leg meat (including thigh and drumstick) and abdominal fat measured.

A total excreta collection method was used (Matterson, 1965). At 64 d of age, five geese from each group (one goose per pen) with BW closest to the mean weight of the group selected and housed separately in wire floor metabolism cages (75cm×65cm×35cm) that were equipped with individual feeders and self-drinking system, and data recorded individually. The housing temperature maintained at 24°C, and geese were allowed *ad libitum* access to water and the study diets. Initial feed sample was stored at -20°C for laboratory analysis, and their manure was collected in plastic-covered trays without feathers from 67 to 70 d. Residual feed and excreta samples were frozen until required for the chemical analysis described by Zhang (2002).

Copper concentration of feed, feces, and liver was analyzed by the methods of the Association of Official Analytical Chemists (AOAC, 2005). Approximately 0.5 g of each feed and feces sample and 0.4 g of each liver sample were weighed in triplicate and digested with ten mL of HNO_3 and 0.4 mL of $HClO_4$ at 200°C in a 50-mL calibrated flask until the solutions cleared. And then they were dried at 100 °C for 24 h and dry-ashed for ten h at 550°C. The ashed samples were dissolved in a nitric acid-perchloric acid mixture (1:1) and diluted with ddH_2O for mineral analysis. The content of Cu was measured using flame atomic absorption spectrophotometry (Analytik Jena novAA 400P, Jena, Germany).

Statistical analysis

Data compared in a completely random design by the GLM procedure of SAS software (SAS Institute, 1996). Correlation between variables was evaluated using Spearman's rank correlation coefficient analysis. After a simple regression analysis, stepwise multiple regression analysis conducted with Cu levels as the dependent variable. Threshold significance values for selection and retention of covariates in the stepwise selection procedure were 0.05. Duncan's multiple-range test was used to compare the differences among treatment means when probability values were significant ($P < 0.05$). The quadratic and linear relationships were measured using the REG procedure of SAS.

RESULTS AND DISCUSSION

Growth performance

From 28 to 70 d of age, copper supplementation significantly affected ADG, BW and FCR (Table 2). An increase in dietary copper resulted in an increase and

Table 2 – Effect of dietary copper on growth performance of growing goslings from 28 to 70 d of age1.

Copper level (mg/kg)	Body weight of 28 d (g)	Body weight of 70 d (g)	Average daily feed intake (g)	Average daily gain (g)	Feed conversion ratio (g/g)	Mortality (%)
4.17	1382	3669[b]	224.0	54.45	4.12[ab]	0
8.17	1381	3727[b]	220.6	55.86	3.96[a]	0
12.17	1381	3716[b]	221.1	55.59	3.98[a]	0
16.17	1381	3530[a]	220.4	51.16	4.32[b]	0
SEM[2]	0.7	27.9	0.860	0.659	0.049	0
Probabilities						
Copper	0.881	0.030	0.437	0.029	0.018	0
Copper linear	0.643	0.055	0.197	0.055	0.089	0
Copper quadratic	0.835	0.018	0.432	0.017	0.006	0

[1]Results are expressed as means, with n=5 per treatment. Values with different small letter superscripts (a,b) in the same column indicate a significant difference ($p<0.05$), whereas values with the same or no letter superscripts indicate no significant difference ($p>0.05$).

[2]Pooled standard error of mean (Residual df=16).

then a decrease in ADG, BW. No mortality found during the experiment. This pattern suggested that excess copper was not conducive to the growth of geese. The increased copper contents in the feeding stuff stimulate the weight gain of birds and improve the use of the feeding stuff; it stimulates tissue Protein Biosynthesis. Copper supplementation was also performed in other species. Kwiecien *et al*. (2014) did the experiment with 2, 4, or 8 mg/kg of copper supplemented by copper sulfate or copper glycinate and found beneficial effects on the growth and development of bones. Singh *et al*. (2015) reported that supplementation of broilers with methionine or yeast proteinate forms of Cu improved BW and FCR and markedly reduced excretion of the critical trace elements. However, throughout the rearing period, the replacement of $CuSO_4$ (8, 16 mg/kg) with Cu glycine chelate (4, 8 or 16 mg/kg) did not significantly alter the weight of chickens (Kwiecien *et al*. 2015).

Arias and Koutsos (2006) reported dietary Cu source and level could influence broiler performance and intestinal physiology as well as the microbial environment. Furthermore, Attia *et al*. (2012) found that supplementation with dietary 150 mg/kg Copper sulfate pentahydrate was commercially beneficial for the chickens. Another reason of growth stimulation was that copper could involve in pituitary growth hormone and secretion of several neuropeptides in the hypothalamus (Zhou *et al*. 1994).

In the experiment, the BW of 70 d of age geese and FCR provided a significant fit to quadratic models, respectively: $Y=3450.61+67.02X-3.82X^2$; $Y=4.587-0.144X+0.00787X^2$. Accordingly, the optimal copper concentration for geese for maximum FCR and maximum BW were 9.15 and 8.77 mg/kg, respectively, which was just as the supplementation for NRC (1994) recommended copper amount (absence for goose). Despite that there was still some differences between our results and other experiments, the reason may be associated with the diversities in the particle size of $CuSO_4$ between previous and present experiments. In addition, this would also be due to the fact that Yangzhou geese are a typical dual-purpose poultry. Nevertheless, the difference in the effects of Cu levels on growth performance of growing poultry indicates that there is a possible difference in the bioavailability of Cu. Clearly, more research is needed to test this hypothesis.

Slaughter performance

The effects of dietary copper on carcass measurements of growing Goslings from 28 to 70 d of age are shown in Table 3. There was no significant effect of dietary copper on half-eviscerated carcass proportion, eviscerated carcass proportion, breast muscle proportion, leg muscle proportion and abdominal fat proportion. However, the dietary copper increased the carcass yield. Carcass yield was significantly affected by the dietary copper concentration and the quadratic model was $Y=85.38+0.673X-0.029X^2$. According to the model, the optimal copper concentration, for growing Goslings from 28 to 70 d of age for maximum carcass yield was 11.6 mg/kg. Which may be related to antimicrobial effects of Cu against the pathogenic bacteria (Pang & Applegate, 2007), which leads to an increase in nutrient absorption that can be used for protein synthesis. Despite Wang *et al*. (2014) who reported that no significant effect on fat, breast, thigh, drumstick and carcass in broiler when the diet was supplemented with copper (5 and 200 mg/kg). However, similar results have been reported by Payvastegan *et al*. (2013), who found that Cu addition at 250 mg/kg significantly increased carcass yield compared to those fed the diets without Cu-supplemented in broilers.

Table 3 – Effect of dietary copper on slaughter performance of geese at 70 days of age[1].

Copper level (mg/kg)	Carcass yield (%)	Half-eviscerated carcass proportion (%)	Eviscerated carcass proportion (%)	Breast muscle proportion (%)	Leg muscle proportion (%)	Abdominal fat proportion (%)
4.17	87.99[a]	81.23	73.71	9.98	14.73	3.10
8.17	88.02[a]	81.48	73.78	9.81	13.87	2.82
12.17	90.17[b]	81.75	73.61	9.86	14.84	3.54
16.17	88.34[a]	81.39	73.74	9.12	14.30	3.11
SEM[2]	0.260	0.259	0.259	0.219	0.246	0.117
Probabilities						
Copper	0.004	0.914	0.997	0.522	0.510	0.213
Copper linear	0.121	0.753	0.978	0.204	0.888	0.456
Copper quadratic	0.045	0.565	0.958	0.529	0.746	0.730

[1]Results are expressed as means, with n=5 per treatment. Values with different small letter superscripts (a,b) in the same column indicate a significant difference ($p<0.05$), whereas values with the same or no letter superscripts indicate no significant difference ($p>0.05$).

[2]Pooled standard error of mean (Residual df=16).

Nutrient content of fecal and liver copper concentrations

The effect of dietary copper on liver copper concentration is present in Table 4. As observed in the study, increasing dietary copper concentration by increasing $CuSO_4$ significantly increased liver copper levels. In a similar experiment, Luo *et al*. (2005) observed that feeding supplemented copper increased liver copper concentrations in chicks when the basal corn-soybean meal diet (11.45 mg/kg copper) supplemented with 0, 150, 300 and 450 mg/kg copper from $CuSO_4$. Guclu *et al*. (2008) recorded that the liver copper concentration increased when the diet supplemented with copper protein amounting to 300 and 450 mg/kg. In the study, increasing Cu concentrations in diets linearly increased log10 transformed liver Cu concentrations. Liver copper concentration provided significant fit to logarithmic function models: $Y=1.6062\ln(x)+1.0111$ ($r^2=0.9772$). This was similar to the previous experiments (Luo *et al*. 2005; Kim & Kill 2015). The result may be due to the fact that the liver is the main metabolic site for some trace elements, including Cu from poultry when the intake of these trace elements exceeds its required levels (Arnold *et al*. 1973). Therefore, livers can be highly sensible tissues that respond to different concentrations of dietary trace elements, and thus, can be used as one of the indicators to evaluate whether copper is excessive. It might also be that organic forms were better assimilated by metals than inorganic forms in vivo, which depend on how easily these compounds convert into adequate organic combinations which were biologically active.

As observed for liver copper concentrations in this experiment, increasing copper concentrations in diets also linearly increased log10 and transformed fecal copper levels (Table 4). And the fecal copper concentration provides significant fit to logarithmic function models: $Y=13.023\ln(x)-9.3558$ ($r^2=0.998$). This observation is consistent with Kwiecien *et al*. (2015), who suggested that the supply of copper decreasing from 16 to 4 mg/kg reduced the excretion of this element (Cu, Fe, Zn and Ca) into the environment. Furthermore, Nollet *et al*. (2008) found that the smallest loss of copper was recorded at the lowest doses (2.5 and 5 mg/kg) when the diet was supplemented with copper at five levels. Due to further Cu supplementation from inorganic, organic sources increases the Cu-excretion and may cause additional environmental problems. The results of literature-based experiments indicate that decreasing dietary copper is an effective method to reduce the excretion of copper of droppings, and, thus, may reduce the risk of environmental contamination.

Table 4 – Effect of dietary copper on nutrient content of fecal and liver copper concentrations of geese[1].

Copper level (mg/kg)	Fecal Crude protein (%)	Fecal Crude ash (%)	Fecal Crude fibre (%)	Fecal Crude fat (%)	Fecal copper (mg/kg)	Liver copper (mg/kg)
4.17	34.02	2.02	21.45	3.10	9.05[a]	3.40[a]
8.17	32.86	2.09	22.93	3.21	18.49[b]	4.23[ab]
12.17	36.11	2.17	22.23	3.02	22.92[c]	4.93[ab]
16.17	29.31	2.09	22.41	4.07	26.86[d]	5.63[b]
SEM[2]	1.239	0.039	0.569	0.213	2.028	0.317
Probabilities						
Copper	0.286	0.661	0.875	0.295	0.000	0.049
Copper linear	0.325	0.475	0.713	0.168	0.000	0.007
Copper quadratic	0.259	0.370	0.626	0.277	0.008	0.907

[1]Results are expressed as means, with n=5 per treatment. Values with different small letter superscripts (a,b) in the same column indicate a significant difference ($p<0.05$), whereas values with the same or no letter superscripts indicate no significant difference ($p>0.05$).

[2]Pooled standard error of mean (Residual df=16).

In the study, the correlations between BW, FCR, carcass yield, liver copper concentration, fecal copper concentration and dietary copper are present in Table 5. No significant correlation was observed between BW (r=-0.394; *P*=0.086), FCR (r=-0.315; *P*=0.176), carcass yield (r=0.220; *P*=0.172) and dietary copper. Nevertheless, there were positive association between liver copper concentration (r=0.664; *P*=0.004), fecal

Table 5 – The correlations between body weight, feed conversion ratio, carcass yield, liver copper, fecal copper and dietary copper.

Item	Body weight	Feed conversion ratio	Carcass yield	Liver copper	Fecal copper
Correlation	-0.394	0.333	0.220	0.664	0.962
Probabilities	NS	NS	NS	**	**

NS, not significant; *$p<0.05$; **$p<0.01$

copper concentration (r=0.962, *P*=0.000) and dietary copper. This model suggested that high levels of copper may cause Copper accumulates in the liver, and, consequently, contribute to the increased concentration of droppings, thereby leading to adverse effects on nutrient utilization and environmental pollution. Our results indicated that concentration of copper has species-specific and tissues-specific bioaccumulation.

CONCLUSIONS

The result of this feeding trial demonstrated that dietary Cu addition can improve growth by increasing the use of the feeding stuff and improving carcass yield in growing Goslings. Additionally, High levels of copper cause Copper accumulates in the liver, and, consequently, contributes to the increased concentration of droppings, thereby leading to adverse effects on nutrient utilization. Furthermore, taking into consideration BW, FCR, carcass yield, liver copper concentration and fecal copper concentration, the optimal level of Gosling dietary copper was between 8.77 and 11.6 mg/kg from 28 to 70 days of age.

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