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Effect of Copper on Plasma Ceruloplasmin and Antioxidant Ability in Broiler Chickens Challenged by Lipopolysaccharide

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ABSTRACT : The effects of dietary copper (Cu) supplementation in broiler chickens challenged with a single injection of *Salmonella typhymurium* lipopolysaccharide (LPS) on the antioxidant capacity and plasma levels of ceruloplasmin (Cp) were evaluated. The broiler chickens were provided with a basal diet or diets supplemented with 8 and 50 mg/kg Cu from 1d of age. At 25d of age, 48 chickens with similar body weight were selected from each diet. Half of the chickens in each dietary treatment were injected intraperitoneally (i.p.) with LPS (1 mg/kg body weight). The other half was injected with saline, serving as the control. Body weight gain and feed consumption were significantly suppressed by LPS challenge during the first 12-h after injection, regardless of dietary Cu levels. Feed efficiency was reduced by LPS injection during the 72-h experimental period. Dietary Cu levels had no significant effect ($p>0.05$) on the plasma ceruloplasmin concentrations in chickens injected with saline. In contrast, high dietary level of Cu elevated plasma Cp levels in chickens with LPS challenge. Short-term LPS challenge had no significant effect on the antioxidant ability of broilers, as indicated by superoxide dismutase, ferric reducing/antioxidant power and the thiobarbituric acid reacting substances in the plasma. The result suggests that high dietary Cu level (as much as 50 mg/kg supplementation) is favorable for coping with short-term LPS challenge through upregulating plasma Cp levels. (**Key Words :** Copper, Ceruloplasmin, Lipopolysaccharide, Antioxidant Capacity, Broiler Chickens)

INTRODUCTION

Copper (Cu) is a component of a variety of intracellular and extracellular enzymes, including cytochrome oxidase, lysyl oxidase, superoxide dismutase, and tyrosinase (Klasing, 1998). Excess Cu beyond immediate requirements is stored as the complex form in metallothionein, mainly in the liver. Most plasma Cu is present as a component of ceruloplasmin (Cp). Ceruloplasmin is synthesized in the liver, where it receives six Cu atoms, and then is secreted into plasma. Most plasma Cu does not originate from recently consumed meals, but from the Cu consumed in prior weeks or months. Cu from the diet is directly incorporated into hepatic ceruloplasmin, or is slowly released during the normal turnover of Cu requiring enzymes, and then is bound to ceruloplasmin (Tom, 1993).

Trace elements respond rapidly and dramatically to infectious challenges by a wide variety of immunogens and pathogens (Beisel, 1977). Ceruloplasmin is necessary for iron transport and plays a role in the acute phase of immune response as well. During the acute phase response, plasma Cu concentrations decrease but hepatic Cu is unaffected (Laurin and Klasing, 1987). This change is related to increased hepatic synthesis and release of Cp (Cousins and Swerdel, 1985). The synthesis of Cp during an infection increases the Cu requirement (Baker and Ammerman, 1995; Koh et al., 1996). In normal conditions, plasma Cu levels or, better, Cp levels are considered as a clinical biomarker for Cu deficiency. However, plasma Cu and Cp levels of Cu-deficient chickens increase toward normal levels during the acute phase of an infection (Koh et al., 1996). The function of Cu in ceruloplasmin remains unclear.

Broiler chickens are continuously confronted with a multitude of stressors that can last for a few hours (e.g., catching, crating and transport) or for nearly the entire rearing period (e.g., heat stress, immune challenges) (Lin et al., 2004a). Young chickens have a higher Cu requirement than adults and are more likely to show a deficiency (NRC, 1994), especially when they are undergoing an immune challenge (Koh et al., 1996). Cu requirements are higher for chicks experiencing an acute phase response compared to

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non-challenged chickens (Koh et al., 1996). Plasma Cp and lipid perxodiation levels are significantly increased by stress mimicked by exogenous corticosterone administration (Lin et al., 2004b). Therefore, we hypothesized that dietary Cu level may have an effect on the redox status of LPS-challenged chickens, in which plasma Cp would be involved.

The present study was conducted to determine the effects of dietary Cu supplementation on the antioxidant capacity and plasma ceruloplasmin concentrations in broiler chickens challenged with lipopolysaccharide (LPS).

MATERIALS AND METHODS

Chickens and diets

Broiler chickens (Arbor Acres, male) were obtained from a local hatchery at 1 d of age and assigned randomly to twenty four cages, with 10 chickens per cage. All the chickens were reared in an environmentally controlled room and the temperature was maintained at 34.8°C for the first 2 d and then decreased gradually to 21.8°C (45% RH) until 28 d of age. The experiment was conducted in accordance with laws and regulations that control experiments and procedures with live animals in China, as laid down by the China Animal Research Authority.

The chickens were divided into three groups of 60 chickens and randomly subjected to one of the following dietary treatments: basal diet, basal diet supplemented with 8 or 50 mg Cu/kg as $CuSO_4 \cdot 5H_2O$. The basal corn-soybean diet was formulated to meet the requirements recommended by NRC (1994) for broiler chickens, except that Cu was omitted from the trace mineral premix. The basal diet contained 6.5 mg/kg Cu from the raw materials. For a detailed description of the diet, see Lin et al. (2006). The light regime was 23 Light: 1 Dark. The chickens had free access to feed and water during the entire rearing period.

At 25-d of age, 48 chickens with similar body weight was selected from each diet treatment. Half of the chickens in each dietary treatment were injected intraperitoneal (i.p.) with 1 mg/kg body weight of *Salmonella typhymurium* lipopolysaccharide (LPS) (Sigma, St. Louis, Mo). The LPS was reconstituted in saline (9 g NaCl/100 ml) at 100 μg/kg and sterilized by passing through a 0.45-μm filter. The other halves of the chickens in each dietary treatment were injected i.p. with the same volume of sterilized saline. A 3-ml blood sample was respectively obtained from six chickens per treatment before LPS injection and at 12, 24 and 72 h after injection. At each bleeding time, six chickens were randomly selected from each treatment and were bled only for one time to avoid the severe stress from injection. Blood was drawn from the wing vein using a heparinized syringe within 30 s and collected in iced tubes. Total blood was frozen instantly by liquid nitrogen and stored under -20°C for the analysis of the erythrocyte CuZn-superoxide dismutase activity. Plasma was obtained after centrifugation at 3,000 rpm for 10 min at 4°C and stored at -20°C until analysis. No chicken was dead in 3 days after the injection. Feed intake (FI) and body weight (BW) gain were recorded at 0, 12, 24 and 72 h after the LPS injection, and feed efficiency (feed/gain) was calculated.

Plasma parameters

Erythrocyte activity of superoxide dismutase (SOD) was measured with a commercial kit (Jiancheng Bioengineering Institute, Nanjing). Total plasma antioxidant activity was determined by the ferric reducing/antioxidant power (FRAP) assay, as described by Benzie and Strain (1999). The measurement was conducted at room temperature and a 5-min time window was used. Ceruloplasmin (Cp) was measured with the o-dianisidine dihydrochloride end-point method, as described by Sugiyama et al. (2000). Plasma lipid peroxidation was estimated by spectrophotometric determination of thiobarbituric acid reacting substances (TBARS) with the method of Lin et al. (2004a).

Cu analysis

Diets were wet ashed in capped vials using nitric acid before measurement. Acid soluble ash and plasma Cu serum levels were analyzed by graphite furnace atomic absorption as described by Anderson et al. (1997), and are expressed on a dry weight basis.

Statistical analysis

Data on growth performance at each time point were analyzed with a two-factor ANOVA with immunogen, dietary Cu levels and their interaction as the main effects (SAS, version 8e, SAS Institute, 1998). Data on the plasma parameters were analyzed by a three way ANOVA model with immunogen, dietary Cu levels and time point as main effects and for interactions. When the main effects of immunogen, Cu levels and time point were significant ($p<0.05$), differences between means were discerned by the method of least significant differences (Koh et al., 1996).

RESULTS

Growing performance

LPS injection had a significant ($p<0.05$) effect on BW gain and feed consumption during the first 12 h after administration (Table 1). However, this significant effect was only detected in chickens supplemented 8 mg Cu/kg diet. Dietary Cu treatment had no significant effect on BW gain, feed consumption or feed efficiency during the 48-h experimental period. In contrast, feed efficiency was significantly decreased by LPS treatment in chickens

Table 1. Effect of Cu on growing performance of broiler chickens with lipopolysaccharide challenge (i.p., 1 mg/kg body weight)

Time (h)	Level of dietary Cu supplementation					
	0 mg/kg		8 mg/kg		50 mg/kg	
	LPS	Saline	LPS	Saline	LPS	Saline
BW gain (g/per bird)						
0-12	56.3±3.4[a]	57.7±10.9[a]	14.3±3.3[b]	53.0±19.5[a]	40.7±5.0[ab]	55.7±4.7[a]
12-24	4.7±8.9	10.0±2.6	16.7±9.9	20.7±14.7	9.0±3.8	20.0±6.5
24-72	182.7±12.7	167.7±10.9	175.7±32.5	172.7±36.0	187.0±11.5	146.0±3.2
0-72	243.3±16.3	235.3±22.2	206.3±22.6	245.7±31.2	236.3±9.8	221.7±7.8
Feed intake (g/per bird)						
0-12	70.2±4.2[ab]	84.2±15.9[ab]	49.4±11.3[b]	74.7±27.6[ab]	57.8±7.2[ab]	99.6±8.3[a]
12-24	49.4±7.1	54.3±14.4	47.7±28.3	53.0±37.7	60.9±25.7	55.8±18.1
24-72	288.4±20.0	266.4±17.2	260.2±48.1	246.7±51.4	294.4±18.1	260.5±5.7
0-72	408.0±20.5	404.9±24.9	385.0±39.5	402.2±65.2	413.1±28.2	392.0±20.0
Feed:gain (g/g)						
0-72	1.68±0.03[ab]	1.74±0.09[ab]	1.87±0.05[a]	1.63±0.09[b]	1.75±0.05[ab]	1.77±0.03[ab]

	p value		
Main effect	BW gain	Feed intake	Feed:gain
LPS	0.0401	0.0426	NS
Cu	NS*	NS	NS
LPS×Cu	NS	NS	NS

[a, b] Values (mean±SEM, n = 4 pens of 24 chickens) within the same row with different superscript differ significantly ($p<0.05$).
* NS means no significant effect.

supplemented with 8 mg/kg Cu compared to other groups. The significantly elevated rectal temperature was observed only at 12 h after LPS injection (Sailine 40.42±0.09°C vs. LPS 40.89±0.10°C, $p = 0.0067$) compared to other time points (Zhao, 2005; data not shown).

Plasma Cu and ceruloplasmin (Cp) concentrations

When the diet was supplemented with 8 mg/kg Cu, LPS administration significantly ($p<0.05$) decreased plasma Cu concentration at 24 h after injection (Table 2). In contrast, a significantly ($p<0.05$) decreased plasma Cu was observed in chickens with basal diet. Dietary Cu levels had no significant effect on plasma Cp level. After injected with LPS, plasma Cp was significantly ($p<0.05$) increased at the 12 and 24 h time points, regardless of dietary Cu level. No significant differences were observed at 72 h time point (Table 2).

Plasma level of erythrocyte CuZn-SOD, ferric reducing/ antioxidant power and thiobarbituric acid reacting substances

Neither dietary Cu levels nor LPS injection had any significant influence on CuZn-SOD activity (Table 3). LPS administration had no detectable influence on plasma level of FRAP (Table 4). In contrast, for the chickens treated with saline, the FRAP in 50 mg/kg Cu group was higher than that of the 0 mg/kg group. The interaction effect of dietary Cu and LPS injection was significant for FRAP ($p<0.05$). At 12 h or 72 h after LPS injection, the FRAP in 50 mg/kg group was significantly lower than that of the 0 mg/kg group. Plasma uric acid levels were significantly increased after the injection of LPS in 50 mg/kg Cu group compared to that of the other Cu supplementation levels (Table 4). At 72 h after LPS injection, TBARS concentration was significantly lower ($p<0.05$) than their counterparts at 0 h and 12 h, but no significant difference was detected between 0 h and 12 h (Table 4). Dietary Cu level had no significant influence on plasma levels of TBARS ($p>0.05$).

DISCUSSION

Cu has been extensively used as growth-promoting additive in pigs (Hu et al., 2004; Xia et al., 2004), steers (Engle and Johnson, 2003) and broilers (Lim and Paik, 2006). As a component of Cp, the role of Cu during the acute stress was investigated in the present study.

As a bacterial inflammation, LPS can cause acute stress (Zhao et al., 2005). In agreement with previous reports (Koh et al., 1996; Xie et al., 2000; Bouchon et al., 2001), LPS injection in our experiment induced a short time immunological stress, which was reflected by the depressed feed intake and BW gain. Furthermore, this effect was consolidated by the observation of the elevated rectal temperature at 12 h after the injection and the augmented concentration of plasma Cp. The adverse effect of LPS challenge on BW gain seemed to be related to dietary Cu

Table 2. Effect of dietary Cu on plasma Cu and ceruloplasmin (Cp) concentrations of broiler chickens with lipopolysaccharide challenge (i.p., 1 mg/kg body weight)

Time (h)	Level of dietary Cu supplementation					
	0 mg/kg		8 mg/kg		50 mg/kg	
	LPS	Saline	LPS	Saline	LPS	Saline
Cu (mg/L)						
0 h	1.80±0.25	1.80±0.25^{x}	1.95±0.42^{x}	1.95±0.42	2.73±0.46	2.73±0.46
24 h	1.43±0.10^{ab}	0.91±0.08^{by}	0.80±0.09^{by}	1.22±0.16^{ab}	1.51±0.34^{ab}	1.82±0.50^{a}
Means	1.61	1.35	1.59	1.43	2.23	2.12
Cp (μg/L)						
0 h	1,026±78^{y}	977±403	1,073±143^{y}	1050±81	1,054±83^{y}	1,047±102
12 h	2,058±123^{ax}	950±92^{b}	1,685±235^{ax}	968±84^{b}	1,584±168^{ay}	1,035±141^{b}
24 h	1,772±156^{bx}	1,117±111^{c}	1,733±150^{bx}	1,040±85^{c}	2,287±323^{ax}	1,114±96^{c}
72 h	1,141±103^{y}	1,456±111	1,294±191^{xy}	1,236±182	1,434±148^{y}	1,334±174
Means	1,687^{m}	1,174^{n}	1,561^{m}	1,099^{n}	1,738^{m}	1,164^{n}

	p value						
	LPS	Cu	Time	Cu×LPS	LPS×Time	Cu×Time	Cu×LPS×Time
Cu	NS*	0.0043	<0.0001	NS	NS	NS	NS
Cp	<0.0001	NS	0.1149	NS	<0.0001	NS	0.1033

$^{a-c}$ Values (means±SEM, n = 6) with different superscript within the same row differ significantly ($p<0.05$).
$^{x-z}$ Values (means±SEM, n = 6) with different superscript within the same column differ significantly ($p<0.05$).
$^{m, n}$ Values with different superscript in the same dietary Cu supplemental level differ significantly ($p<0.05$).
* NS means no significant effect.

treatment. Chickens with 8 mg/kg Cu supplementation showed a stronger response to LPS challenge during the first 12 h, compared to 0 and 50 mg/kg Cu supplementation. It was noteworthy to state that the feed intake in the same time was also significantly decreased in chickens with 8 mg/kg Cu. The simultaneously suppressed feed consumption should be responsible at least partially for the retarded BW gain. The previous works in sheep (McMahon et al., 1999) and fish (Volkoffa and Peter, 2004) shows that the suppressed expressions of appetite-regulating peptides are involved in the reduced feed consumption of LPS challenged animals. But it was confusing why diet with 8 mg/kg Cu supplementation resulted in this decreasement rather than basal diet. Further research is needed to uncover the underlying mechanism. The injection of saline also decreased the feed intake and body weight gain in the current study, which was believed to come from acute stress induced by the catching and the injecting. It is well known that the acute stress will result in decreased feed intake (reviewed by Ferket and Gernat, 2006).

In previous study, LPS treatment could increase plasma level of Cu (Koh et al., 1996). In the present study, however, no significant change of plasma Cu level was observed at 24-h after LPS administration at any dietary Cu treatment.

Table 3. Effect of dietary Cu on plasma erythrocyte CuZn-SOD (U/ml) of broiler chickens with lipopolysaccharide challenge (i.p., 1 mg/kg body weight)

Time (h)	Level of dietary Cu supplementation					
	0 mg/kg		8 mg/kg		50 mg/kg	
	LPS	Saline	LPS	Saline	LPS	Saline
0	5,409±668^{y}	5,393±577	6,228±600	6,245±552	5,148±844	5,247±963
12	6,542±404^{xy}	7,475±1,584	6,005± 957	7,250±482	7,033±1,093	10,232±3,461
24	7,695±869^{xy}	7,183±649	5,634±1,037	7,560±1,031	5,867±744	6,521±796
72	8,170±820^{x}	7,925±533	7,421±510	11,553±333	7,878±636	8,967±933
Means	7,469	7,531	6,354	8,879	6,926	8,573

	p value						
	LPS	Cu	Time	Cu×LPS	LPS×Time	Cu×Time	Cu×LPS×Time
CuZn-SOD	NS*	NS	0.0610	NS	NS	NS	NS

CuZn-SOD = CuZn-superoxide dismutase.
$^{x, y}$ Values (mean±SEM, n = 6) within the same column with different superscript differ significantly ($p<0.05$). * NS means no significant effect.

Table 4. Effect of dietary Cu on the plasma ferric reducing/antioxidant power (FRAP) and thiobarbituric acid reacting substances (TBARS) of broiler chickens with lipopolysaccharide challenge (i.p., 1 mg/kg body weight)

Time (h)	Level of dietary Cu supplementation					
	0 mg/kg		8 mg/kg		50 mg/kg	
	LPS	Saline	LPS	Saline	LPS	Saline
FRAP (μmol/L)						
0	460±24^{by}	460±24^{by}	537±63^{ab}	537±63^{ab}	623±52^{a}	623±52^{a}
12	852±32^{ax}	922±36^{ax}	565±54^{c}	714±47^{b}	634±34^{bc}	574±36^{c}
72	919±51^{ax}	854±28^{ax}	569±20^{bc}	652±68^{b}	569±41^{bc}	485±56^{c}
Means	886	888	567^{n}	680^{m}	601	526
UA (mg/L)						
0	36.3±5.3^{y}	35.6±9.0^{z}	37.9±6.2	37.1±5.5^{xy}	36.6±5.4	37.0±11.3^{x}
12	105.8±14.6^{ax}	114.7±18.6^{ax}	45.3±9.8^{b}	33.2±5.8^{bxy}	31.1±4.8^{b}	18.9±3.6^{by}
72	107.3±11.2^{ax}	87.3±15.8^{abxy}	38.2±9.8^{c}	57.2±19.3^{bcx}	36.2±4.1^{c}	21.6±5.2^{cy}
Means	88.5	88.4	43.9	36.8	27.8^{m}	20.5^{n}
TBARS (μmol/L)						
0	3.99±0.45^{x}	3.99±0.45^{x}	4.45±0.30^{x}	4.45±0.30^{x}	3.89±1.17^{x}	3.89±1.17^{x}
12	4.54±1.01^{x}	3.87±0.77^{x}	5.92±0.99^{x}	4.65±0.46^{x}	4.63±0.49^{x}	5.45±0.23^{x}
72	1.52±0.49^{y}	0.82±0.21^{y}	0.97±0.14^{y}	1.01±0.27^{y}	1.39±0.51^{y}	1.11±0.23^{y}
Means	2.89	2.48	3.45	2.46	3.01	3.28

	p value						
	LPS	Cu	Time	Cu×LPS	LPS×Time	Cu×Time	Cu×LPS×Time
FRAP	NS*	<0.0001	0.1737	0.0147	0.1520	NS	NS
UA	NS	<0.0001	0.0001	NS	NS	0.0030	0.0554
TBARS	NS	NS	<0.0001	NS	NS	NS	NS

[a-c] Values (Means±SEM, n = 6) within the same row with different superscript differ significantly (p<0.05).

[x-z] Values (Means±SEM, n = 6) within the same column with different superscript differ significantly (p<0.05).

[m, n] Values represent the effect of the LPS injection within a dietary Cu supplemental level and different superscript differ significantly (p<0.05).

* NS means no significant effect.

The LPS-injection induced difference could possibly have happened at other time point. Moreover, the chickens fed diet supplemented with 8 mg/kg Cu had the lowest level of plasma Cu compared to other chickens, suggesting that feed consumption as well as dietary Cu level were all associated with plasma Cu level.

After injection of LPS, the level of the plasma acute phase proteins, including hemopexin and α1-acid glycoprotein, increase at 12 h, reach the highest level at 24 h and decline thereafter (Kevyn et al., 2001). The dynamic change of Cp in the present study showed a similar trend. The level of Cp increased at 12 h after LPS injection, reached the highest level at 24 h, and restored to normal level at 72 h with no difference between treatments. In the same line with previous work (Koh et al., 1996), higher dietary Cu level improved plasma Cp in LPS-challenged chickens compared to chickens injected with saline, suggesting that high dietary Cu level (as much as 50 mg/kg supplementation) would be favorable for the coping with LPS challenge. It was interesting to note that the increased plasma Cp level in high dietary Cu treatment was not in line with the plasma Cu concentration. This observation was in accordance with results in mammals. In mammals, there are two kinds of Cp in the plasma, one including Cu (holo-Cp) and the other without Cu (apo-Cu). In humans, dietary Cu is not the key factor that affects the Cp synthesis, but the ratio of holo-Cp and apo-Cp (Gitlin et al., 1992) does. In this study, plasma Cp level was measured with the p-phenoldiamine method (Sugiyama et al., 2000), which only detects the total Cp level rather than the level of Cp containing Cu. Further studies are needed to establish the relationship between dietary Cu and plasma Cp levels. Nevertheless, our results indicated that high dietary level of Cu (as much as 50 mg/kg supplementation) could promote synthesis of Cp during LPS challenge.

The activity of CuZn-SOD was significantly increased in rats treated with LPS (Ponukalina et al., 2001). In the present study, however, the injection of LPS and the dietary Cu levels had no significant effect on erythrocyte CuZn-SOD activity, which is in agreement with the results of Koh et al. (1996). The result indicated that, unlike mammals, erythrocytes' CuZn-SOD activity in broiler chickens was not a sensitive indicator to the stored Cu level.

The plasma total antioxidant ability was estimated by

the measurement of FRAP in the present study. The FRAP assay is a novel method for assessing "antioxidant power" (Benzie and Strain, 1996). This noninhibition method, the FRAP assay, was a direct test of the total antioxidant power (Benzie and Strain, 1999). It was defined as "ferric reducing ability of plasma" (Benzie and Strain, 1996) and now has been defined to mean "ferric reducing/antioxidant power" (Benzie and Strain, 1999). The FRAP assay gives fast, reproducible results with plasma, with single antioxidants in pure solution and with mixtures of antioxidants in aqueous solution and added to plasma (Benzie and Strain, 1996). The injection of LPS had no significant effect on plasma FRAP. Confusingly, chicken with basal diet showed high plasma FRAP. The significant elevated FRAP level was related to the increased concentration of plasma uric acid, which has been proven to be an effective antioxidant in both mammals (Hellsten et al., 1997) and chickens (Simoyi et al., 2002; Simoyi et al., 2003). We cannot give a reasonable explanation for the dramatic elevation of plasma UA concentration in chickens fed with diet of 0 Cu mg/kg observed and further studies are needed.

Nathan et al. (2002) reported that when the body's interior antioxidant/reduce balance is disturbed, the Cu in the plasma or tissue would tend to show its oxidant property. In contrast, the result of TBARS in the present study indicated that short-time LPS challenge had no meaningful effect on the broiler's oxidative injury. However, this finding should be interpreted with caution. Our measurement was conducted only at several time points. The short-term change in other time points could have existed. Moreover, dietary Cu level had no significant effect on the broiler's antioxidant ability under short-time LPS challenge as well. A long-term experiment should be done to study whether the Cu reserves in the broilers body had concealed the interaction between the short-time LPS challenge and the dietary Cu supplementation.

In conclusion, the present study showed that short-time LPS challenge had little effect on the broiler's antioxidant ability as indicated by SOD, FRAP and TBARS. High dietary level of Cu (as much as 50 mg/kg supplementation) promoted the elevation of circulating Cp concentration under LPS challenge. Considering the important roles that Cp plays, this result suggests that high dietary Cu level may be favorable for coping with short-term LPS challenge.

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REFERENCES

Anderson, R. A., N. A. Bryden, C. M. Evock-Clover and N. C. Steele. 1997. Beneficial effects of chromium on glucose and lipid variables in control and somatotropin-treated pigs are associated with increased tissue chromium and altered tissue copper, iron and zinc. J. Anim. Sci. 75:657-661.

Baker, D. H. and C. B. Ammerman. 1995. Copper bioavailability. In: Bioavailability of Nutrients for Animals. Academic Press, San Diego. pp. 127-156.

Beisel, W. R. 1977. Magnitude of the host nutritional responses to infection. Am. J. Clin. Nutr. 30:1236-1247.

Benzie, I. F. and J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239:70-76.

Benzie, I. F. and J. J. Strain. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth. Enzymol. 299:15-27.

Bouchon, A., F. Facchetti, M. A. Weigand and M. Colonna. 2001. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 410:1103-1107.

Cousins, R. J. and M. R. Swerdel. 1985. Ceruloplasmin and metallothionein induction by zinc and 13-cis-retinoic acid in rats with adjuvant inflammation. Proc. Soc. Exp. Biol. Med. 179:168-172.

Engle, T. E. and L. R. Johnson. 2003. The effects of copper source and concentration on lipid metabolism in growing and finishing angus steers. Asian-Aust. J. Anim. Sci. 16:1131-1136.

Ferket, P. R. and A. G. Gernat. 2006. Factors that affect feed intake of meat birds: a review. Int. J. Poult. Sci. 5:905-911.

Gitlin, J. D., J. J. Schroeder, L. M. Lee-Ambrose and R. J. Cousins. 1992. Mechanisms of caeruloplasmin biosynthesis in normal and copper-deficient rats. Biochem. J. 282:835-839.

Hellsten, Y., P. C. Tullson, E. A. Richter and J. Bangsbo. 1997. Oxidation of urate in human skeletal muscle during exercise. Free Radic. Biol. Med. 22:169-174.

Hu, C. H., M. S. Xia, Z. R. Xu and L. Xiong. 2004. Effects of copper-bearing montmorillonite on growth performance and digestive function of growing pigs. Asian-Aust. J. Anim. Sci. 17:1575-1581.

Kevyn, L., P. H. Adler, R. K. Peng and K. C. Klasing. 2001. The kinetics of hemopexin and a1-acid glycoprotein levels induced by injection of inflammatory agents in chickens. Avian Dis. 45:289-296.

Klasing, K. C. 1998. Comparative avian nutrition. CAB International, New York.

Koh, T. S., R. K. Peng and K. C. Klasing. 1996. Dietary copper level affects copper metabolism during lipopolysaccharide-induced immunological stress in chicks. Poult. Sci. 75:867-872.

Laurin, D. E. and K. C. Klasing. 1987. Effects of repetitive immunogen injections and fasting vs. feeding on iron, zinc, and copper metabolism. Biol. Trace Elem. Res. 14:153-165.

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. 19:1174-1178.

Lin, H., E. Decuypere and J. Buyse. 2004a. Oxidative stress induced by corticosterone administration in broiler chickens: 1. chronic exposure. Comp. Biochem. Physiol. B 139:737-744.

Lin, H., E. Decuypere and J. Buyse. 2004b. Oxidative stress induced by corticosterone administration in broiler chickens (Gallus gallus domesticus) 2. short-term effect. Comp. Biochem. Physiol. B 139:745-751.

Lin, H., S. J. Sui, H. C. Jiao, J. Buyse and E. Decuypere. 2006. Impaired development of broiler chickens by stress mimicked by corticosterone exposure. Comp. Biochem. Physiol. A 143: 400-405.

McMahon, C. D., D. F. Buxton, T. H. Elsasser, D. R. Gunter, L. G. Sanders, B. P. Steele and J. L. Sartin. 1999. Neuropeptide Y restores appetite and alters concentrations of GH after central administration to endotoxic sheep. J. Endocrinol. 161:333-339.

Nathan, E. H., K. Satoshi, M. M. Grazia, A. J. Hoogeboom, G. J. Jong and D. G. Jonathan. 2002. Mechanisms of copper incorporation into human ceruloplasmin. J. Biol. Chem. 277: 46632-46638.

National Research Council. 1994. Nutrition requirements of poultry. 9th Ed. National Academy Press, Washington, DC.

Ponukalina, E. V., G. A. Afanas'eva, N. P. Chesnokova and V. F. Kirichuk. 2001. Lipid peroxidation and the antioxidant blood system in dynamics of pestilential and choleraic intoxication. Patol. Fiziol. Eksp. Ter. 3:17-18.

SAS Institute. 1998. User's Guide: Statistic. SAS Institute Inc., Cary, North Carolina.

Simoyi, M. F., E. Falkenstein, K. V. Dyke, K. P. Blemings and H. Klandorf. 2003. Allantoin, the oxidation production of uric acid is present in chicken and turkey plasma. Comp. Biochem. Physiol. B 135:325-335.

Simoyi, M. F., K. V. Dyke and H. Klandorf. 2002. Manipulation of plasma uric acid in broiler chicks and its effect on leukocyte oxidative activity. Am. J. Physiol. 282:R791-R796.

Sugiyama, T., Y. Kawarada and F. Hirasawa. 2000. Experimental protocols for reactive oxygen and nitrogen species. Oxford University Press, New York.

Tom, B. 1993. Nutritional biochemistry. California: Academic Press.

Volkoffa, H. and R. E. Peter. 2004. Effects of lipopolysaccharide treatment on feeding of goldfish: role of appetite-regulating peptides. Brain Res. 998:139-147.

Xia, M. S., C. H. Hu, Z. R. Xu, Y. Ye, Y. H. Zhou and L. Xiong. 2004. Effects of copper-bearing montmorillonite (Cu-MMT) on escherichia coli and diarrhea on weanling pigs. Asian-Aust. J. Anim. Sci. 17:1712-1716.

Xie, H., N. C. Rath, G. R. Huff, W. E. Huff and J. M. Balog. 2000. Effects of salmonella typhimurium lipopolysaccharide on broiler chickens. Poult. Sci. 79:33-40.

Zhao, T. 2005. Effects of dietary copper and immunological stress on antioxidant stress activity of broilers. Master. Thesis, Shandong Agricultural University, Shandong, China.