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Copper bioavailability, mineral utilization, and lipid metabolism in broilers

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Abstract: The study was conducted to investigate the effects of copper (Cu) sources and levels on mineral utilization, tissue copper residues, and lipid metabolism in Arbor Acres broilers. A total of 640 male broilers were randomly divided into 5 groups with 8 replicates per group and 16 broilers per replicate. The experiment was used in a 2 × 2 + 1 factorial experiment design. Broilers in the control group were fed a basal diet, and animals in the other four groups were fed basal diets supplemented with Cu from copper sulphate and copper methionine. Copper concentrations of the experimental diets were 20 and 40 mg Cu/kg dry matter. A metabolism trial of 4 days was conducted during the last week of experimental feeding. Neither Cu source nor Cu level affected average daily gain, average daily feed intake or feed gain ratio ($P > 0.05$). Broilers fed 40 mg Cu/kg diets had lower plasma cholesterol than those in the control group ($P < 0.05$). Copper supplementation decreased ($P < 0.05$) plasma low-density lipoprotein cholesterol but did not alter plasma high-density lipoprotein cholesterol concentrations or plasma triglyceride concentrations. Copper sulphate supplementation increased ($P < 0.05$) liver Cu but did not alter pectorals Cu, heart Cu, tibia Cu and tibia P. Broilers fed 40 mg/kg Cu from copper sulphate had a lower ($P < 0.05$) tibia Ca level. The concentration of liver Cu in the broilers fed copper methionine diets was higher ($P < 0.05$) than that in those fed copper sulphate. Compared with copper sulphate (100%), the relative bioavailability value of copper methionine was 117%. In conclusion, the relative bioavailability of copper methionine obtained in this study was greater than that of copper sulphate. Copper plays an important role in plasma lipids and in the digestion of dietary Ca in broiler chickens.

Keywords: copper sources; lipid profiles; mineral balance; relative bioavailability

Copper is one of the essential trace elements in animals that plays an important role in the physiological processes of haematopoiesis, reproduction, antioxidant and immune functions (Mayer et al. 2018). However, the copper content of feed that is too high or too low is not conducive to health (Li et al. 2017). Braude (1945) found that adding high-dose copper to piglet feed can significantly increase feed intake and promote growth. Since then, many scholars have confirmed that the high-copper diet has growth-promoting effects (Liu et al. 2016; Olukosi et al. 2018) and enhances antioxidant functions (Ognik et al. 2018) and immune function (Echeverry et al. 2016). However, excessive use of copper in the breeding process not only affects the health of livestock and poultry (Hu et al. 2017; Minervino et al. 2018), but also results in the residues of copper in livestock and poultry products (Zhao et al. 2016) and poses a risk to human health (Cresswell et al. 1990). In order to ensure the safety of meat, the Ministry of Agriculture and Rural Affairs of China has

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specified that the maximum amount of copper in broiler feed should be less than 35 mg/kg, and the copper content in meat products should be less than 10 mg/kg.

Copper sulphate is the most commonly used copper feed additive in livestock and poultry feed (Nicholson et al. 1999). However, copper sulphate has the characteristics of easily absorbing water and agglomeration, and catalyzing the oxidation of unsaturated fats in feed (Lu et al. 2013; Olukosi et al. 2018). As an organic Cu source, amino acid chelated Cu can reduce the enthalpy of Cu and other nutrients, promote the absorption and utilization of Cu and other mineral elements, and reduce Cu emissions (Palenikova et al. 2014; Flis et al. 2019).

Little work has been done evaluating Cu tissue retention in broilers. Therefore, the main objective of this study was to estimate the Cu residues and Cu excretion from copper sulphate and copper methionine in broilers. An additional digestibility experiment was conducted to investigate the influence of dietary Cu source and level on mineral utilization of broilers.

MATERIAL AND METHODS

The protocol for experimental use of animals was approved by the Institutional Animal Care Committee at The Anhui Science and Technology University (Bengbu, China).

Animals, treatments and diets. In a 42-day trial, a total of 640 1-day-old broiler chicks (Arbor Acres) with an average body weight of 46.68 ± 0.28 g were allotted to 5 treatments (8 replicates per treatment and 16 broilers per replicate) in a 2 × 2 + 1 factorial arrangement in such a way that the mean body weight was similar ($P > 0.10$) amongst the treatments. Factors in the model were two sources of Cu (copper sulphate and copper methionine) fed at two dietary Cu levels (20 and 40 mg/kg) and the control. Dietary treatments were as follows: (1) basal diet without supplemental Cu (control); (2) basal diet + 20 mg Cu/kg dry matter (DM) as copper sulphate (CuS20); (3) basal diet + 40 mg Cu/kg DM as copper sulphate (CuS40); (4) basal diet + 20 mg Cu/kg DM as copper methionine (CuM20) and (5) basal diet + 40 mg Cu/kg DM as copper methionine (CuM40). Ingredients and chemical composition of the basal diets are presented in Table 1. The final Cu content of the diets is presented in Table 2.

Table 1. Ingredient and chemical composition of basal diets

Item	Starter	Finisher
Ingredient (%)		
Corn	59.83	65.00
Soybean meal	31.21	27.50
Fish meal	2.20	
Soybean oil	2.00	3.00
Dicalcium phosphate	2.00	1.80
Ground limestone	0.80	0.70
Salt	0.40	0.40
Methionine[2]	0.21	0.18
Lysine	0.35	0.42
Premix[1]	1.00	1.00
Analysed composition (g/kg)		
Metabolisable energy[3] (MJ/kg)	12.33	12.72
Dry matter	913.30	893.80
Crude protein	209.90	182.40
Crude fat	38.66	39.96
Contents of animo acid (%)		
Lysine	1.53	1.39
Methionine	0.53	0.49
Methionine + cysteine	0.88	0.76
Contents of mineral elements (mg/kg)		
Calcium	9381	8818
Phosphorus	4868	4396
Zinc	99.38	96.36
Copper	7.89	7.36
Iron	135.66	126.18

[1]provided per kg of diet: 12 000 IU vitamin A; 2700 IU vitamin D3; 35 mg vitamin E; 2.25 mg vitamin K3; 3 mg vitamin B1; 8 mg riboflavin; 4.5 mg vitamin B6; 24 mg vitamin B12; 60 mg niacin; 1.5 mg folic acid; 0.2 mg biotin; 18 mg pantothenic acid; 76 mg Fe (as FeSO4·7H2O); 65 mg Zn (as ZnSO4); 85 mg Mn (as MnO2); 0.7 mg I (as KI); and 0.2 mg Se (as Na2SeO3·5H2O)

[2]additive amount of methionine: Starter: Cu15 2.03 g; Cu30 1.96 g; Cu60 1.82 g; Cu120 1.53 g; Cu240 0.97 g. Finisher: Cu15 2.43 g; Cu30 2.36 g; Cu60 2.22 g; Cu120 1.93 g; Cu240 1.37 g

[3]calculated values, others were values analysed based on dry matter samples

There were 8 replicate cages per treatment with 16 birds per cage. All birds were housed in stainless steel cages (200 cm long × 100 cm wide × 40 cm high) with concrete floors of 0.125 m^2/bird. The di-

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Table 2. Final Cu content of the diets (mg/kg)

Additive Cu dose		Actual Cu dose	
		in starter	in finisher
Control	0	7.89	7.36
Copper sulphate	20	27.63	27.56
	40	48.11	47.68
Copper methionine	20	27.69	27.63
	40	47.76	47.16

ets were fed in 2 phases consisting of a starter phase (days 0–21), and a finisher phase (days 22–42).

Experimental procedure and sample collection. The broilers were weighed by cage and feed intake was recorded on days 0, 21, and 42 to calculate the average daily gain (ADG), the average daily feed intake (ADFI), and the feed/gain ratio (F/G). Anytime when a bird died, the body weight of the bird was promptly weighed and the pen-feed intake at that time was recorded to correct the feed conversion rate.

The collection period for excreta started at 08:00 h on day 39 and lasted until 08:00 h on day 42. All excreta were collected and pooled across days for each replicate. Excreta were frozen (–4°C) between collections. Pooled excreta were mixed in an industrial mixer, sub-sampled, freeze-dried, and ground. Excreta were analysed for mineral concentrations. Mineral digestibility was determined using the quantitative feed and excreta method described by Anwar and Ravindran (2016).

On day 42, 60 birds (2 chicks per replicate) were randomly selected and weighed after feed deprivation for 12 h. Blood samples were collected from the wing vein and centrifuged at 3000 *g* for 15 min. Serum was separated and stored at –80°C for further analyses. After blood sampling, birds were electrically stunned and killed by exsanguination, whereupon the pectorals, liver, heart, and tibia were excised. Soft tissues were freeze-dried, ground, and analysed for mineral content, whereas tibias were cut into small pieces, defatted, dried, and analysed for mineral content.

Chemical analysis. Feed and excreta samples were analysed following AOAC (2005) for DM (method 930.15) and crude protein (method 984.13). Amino acids in the diets were analysed using an HPLC AA analyzer (Chrom Tech, USA) using ninhydrin for post column derivatization and norleucine as the internal standard. Samples were hydrolysed with 6 N HCl at 110°C for 24 h (method 994.12). Methionine and cystine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 994.12, alternative 3). Copper, zinc, iron, Ca and P concentrations of feed, tissue, excreta and plasma were analysed by flame atomic absorption spectroscopy (Shimadzu Scientific Instruments, Japan) (method 999.10 or method 985.01). The concentrations of plasma cholesterol (CHO), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides (TG) (the assay kits for the above were supplied by Nanjing Jiancheng Bioengineering Institute, China) were analysed by an automatic biochemistry analyzer (Hitachi 7020; Hitachi High Technologies, Inc., Japan).

Statistical analysis. The retention coefficient for each nutrient was calculated as follows:

Retention coefficient = (nutrient intake – nutrient excretion)/nutrient intake

Data were analysed as a 2 × 2 + 1 factorial experiment based on a completely randomised design using the GLM procedure (SAS Institute Inc., USA). The following model was used to analyse growth performance, mineral utilisation and tissue mineral concentrations:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

where:

μ = overall mean
A_i = effect of the i^{th} Cu source
B_j = effect of the j^{th} level of Cu supplement
AB_{ij} = interaction of A_i and B_j
e_{ijk} = residual effects

Liver Cu data were used to estimate relative Cu bioavailability from copper methionine, using copper sulphate as the standard source, by multiple linear regression and a slope ratio method (Littell et al. 1997; Cui et al. 2018). Means were compared using Duncan's multiple range test and $P < 0.05$ was considered as the significant level, and $P > 0.05$ and < 0.10 was considered a trend.

RESULTS

Growth performance. Neither the Cu source nor the Cu level affected ADG, ADFI or F/G in any period (Table 3). Nevertheless, the highest ADG was seen in the CuM40 group. The Cu source ×

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Table 3. Effect of Cu source and level on growth performance of broilers

Items	Treatment					SEM	*P*-values			
	control	CuS20	CuS40	CuM20	CuM40		treat	sources	level	sources × level
Days 1–21										
ADG (g/day)	36.54	36.97	36.59	37.08	37.31	0.40	0.679	0.359	0.868	0.495
ADFI (g/day)	49.92	50.21	49.71	50.56	50.94	0.80	0.859	0.392	0.949	0.632
F/G	1.37	1.36	1.36	1.37	1.37	0.02	0.997	0.762	0.930	0.994
Days 22–42										
ADG (g/day)	87.10	88.33	88.62	87.68	88.94	0.74	0.489	0.816	0.279	0.493
ADFI (g/day)	173.61	169.59	172.57	172.17	169.11	2.01	0.514	0.819	0.982	0.125
F/G	1.99	1.92	1.95	1.96	1.90	0.03	0.148	0.959	0.483	0.075
Days 1–42										
ADG (g/day)	61.82	62.65	62.60	62.38	63.13	0.41	0.345	0.770	0.417	0.357
ADFI (g/day)	111.77	109.90	111.14	111.36	110.02	1.12	0.749	0.883	0.966	0.276
F/G	1.81	1.75	1.78	1.79	1.74	0.02	0.219	0.990	0.622	0.135

ADG = average daily gain, ADFI = average daily feed intake, F/G = feed gain ratio, CuS = copper sulphate, CuM = copper methionine, treat = treatment

[a,b]means in a row without common superscripts differ ($P < 0.05$)

level interactions slightly improved the feed conversion rate during days 22 to 42 ($P = 0.075$).

Plasma lipid profiles. There were no level × source interactions for any of the analysed plasma lipid profiles (Table 4). Plasma LDL cholesterol ($P < 0.01$) was decreased by Cu addition. Broilers in the CuM40 and CuS40 groups had lower plasma cholesterol than those in the control group ($P < 0.05$). In addition, Cu source had a slight effect on plasma cholesterol ($P = 0.083$) and LDL cholesterol ($P = 0.072$) with copper methionine treatment concentration less than copper sulphate treatments. In contrast, plasma HDL cholesterol concentrations or plasma TG concentrations were not affected by Cu source or level.

Mineral balance. There were no level × source interactions for any trace mineral balance values (Table 5). Intake of Cu, Cu retention and excreta Cu increased ($P < 0.01$) with the dose of Cu, which was expected. In addition, the copper sulphate inclusion resulted in higher ($P < 0.05$) excreta Cu than when copper methionine was in the diet, thus resulting in a reduced Cu retention coefficient ($P < 0.05$). Calcium and P balance were not affected ($P > 0.10$) by the dose of Cu. Nevertheless, excreta Ca of broilers fed copper sulphate supplemented diets was higher ($P < 0.05$) than that of the copper methionine supplemented group, thus resulting in a reduced Ca retention coefficient ($P < 0.05$).

Tissue mineral composition. Copper source had a significant effect on liver Cu with copper methionine treatment concentrations more than with copper sulphate treatments ($P < 0.05$, Table 6). In addition, liver Cu and plasma Cu increased ($P < 0.01$) with the dose of Cu, which was expected. In contrast, pectorals Cu, heart Cu, tibia Cu and

Table 4. Effect of Cu source and level on plasma lipid profiles in broilers

Items (mmol/l)	Treatment					SEM	*P*-values			
	Control	CuS20	CuS40	CuM20	CuM40		treat	sources	level	sources × level
Cholesterol	3.72[a]	3.57[ab]	3.46[bc]	3.48[abc]	3.27[c]	0.07	0.007	0.083	0.046	0.530
HDL cholesterol	2.36	2.29	2.26	2.26	2.25	0.03	0.185	0.602	0.466	0.796
LDL cholesterol	0.84[a]	0.73[b]	0.62[c]	0.70[b]	0.61[c]	0.01	0.001	0.072	0.001	0.669
TG	0.78	0.78	0.78	0.77	0.77	0.01	0.967	0.530	0.827	0.846

TG = triglycerides, HDL = high-density lipoprotein, LDL = low-density lipoprotein, treat = treatment

[a–c]means in a row without common superscripts differ ($P < 0.05$)

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Table 5. Effect of Cu source and level on trace mineral balance in broilers

Items	Treatment					SEM	*P*-values			
	control	CuS20	CuS40	CuM20	CuM40		treat	sources	level	sources × level
Cu (mg/day)										
Intake	1.07^{c}	4.08^{b}	6.99^{b}	4.04^{a}	6.93^{a}	0.027	0.001	0.134	0.001	0.950
Excreta	0.76^{d}	3.06^{c}	5.33^{a}	2.91^{c}	5.16^{b}	0.051	0.001	0.016	0.001	0.894
Retention	0.31^{c}	1.03^{b}	1.66^{b}	1.12^{a}	1.77^{a}	0.054	0.001	0.108	0.001	0.924
Retention coefficient	0.29^{a}	0.25^{bc}	0.24^{c}	0.28^{ab}	0.26^{abc}	0.011	0.051	0.043	0.096	0.653
Ca (g/day)										
Intake	1.19	1.21	1.20	1.20	1.20	0.007	0.317	0.339	0.632	0.123
Excreta	0.62^{b}	0.67^{ab}	0.69^{a}	0.63^{b}	0.64^{b}	0.014	0.017	0.011	0.257	0.624
Retention	0.57^{a}	0.55^{ab}	0.51^{b}	0.56^{ab}	0.56^{ab}	0.016	0.129	0.062	0.245	0.301
Retention coefficient	0.48	0.45	0.42	0.47	0.46	0.012	0.055	0.028	0.248	0.421
P (g/day)										
Intake	0.64	0.65	0.64	0.64	0.65	0.004	0.317	0.339	0.632	0.123
Excreta	0.33	0.34	0.34	0.33	0.34	0.007	0.671	0.330	0.710	0.610
Retention	0.31	0.31	0.30	0.31	0.31	0.008	0.955	0.620	0.571	0.823
Retention coefficient	0.48	0.47	0.47	0.48	0.47	0.011	0.886	0.461	0.623	0.896

treat = treatment; $^{a–d}$means in a row without common superscripts differ ($P < 0.05$)

tibia P were not affected ($P > 0.10$) by the dose and source of Cu. Furthermore, Cu source had a significant effect on tibia Ca concentration ($P < 0.05$) with copper methionine treatment concentrations more than with copper sulphate treatments.

Relative bioavailability values. Regression equations for liver Cu on supplemental Cu intake are in Figure 1. Bioavailability of copper methionine relative to copper sulphate was estimated from liver Cu concentration using multiple linear regressions and a slope ratio method. Copper methionine was more bioavailable than copper sulphate, based on linear regression of liver Cu. Compared with copper sulphate (100%), relative bioavailability values of copper methionine were 117%.

DISCUSSION

Results obtained in this study demonstrate that body weight and feed intake did not differ in birds fed up to 40 mg/kg Cu from copper methionine

Table 6. Effect of Cu source and level on tissue mineral profile in broilers

Items	Treatment					SEM	*P*-values			
	control	CuS20	CuS40	CuM20	CuM40		treat	sources	level	sources × level
Liver Cu (mg/kg)	11.28^{d}	15.04^{c}	16.84^{ab}	15.88^{bc}	17.88^{a}	0.37	0.001	0.020	0.001	0.794
Pectorales Cu (mg/kg)	4.86	4.91	4.97	4.93	5.03	0.07	0.552	0.598	0.290	0.722
Heart Cu (mg/kg)	3.65	3.68	3.72	3.70	3.76	0.07	0.862	0.711	0.533	0.923
Plasma Cu (mg/l)	0.19^{c}	0.20^{bc}	0.206^{ab}	0.207^{ab}	0.213^{a}	0.004	0.003	0.066	0.094	0.743
Tibia										
Cu (mg/kg)	4.25	4.36	4.46	4.49	4.43	0.11	0.643	0.689	0.856	0.555
Ca (g/kg),	175.99^{a}	168.25^{ab}	164.08^{b}	174.46^{a}	170.33^{ab}	2.39	0.016	0.021	0.115	0.994
P (g/kg)	80.09	78.31	75.79	79.66	77.93	1.95	0.625	0.393	0.299	0.845

treat = treatment; $^{a–d}$means in a row without common superscripts differ ($P < 0.05$)

Trace elements are related to fat-free DM. The analysed concentrations of Cu in liver, pectorals and heart were expressed as DM basis, whereas those of trace elements in tibia are related to fat-free DM

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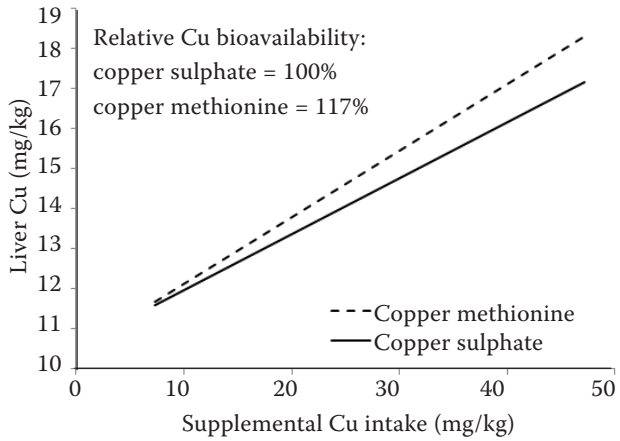

Figure 1. Linear regression of liver Cu on supplemental Cu intake and relative bioavailability of Cu

Y = 10.034 + 0.1498 * X1 + 0.1751 * X2, where X1 = copper sulfate and X2 = copper methionine; R^2 = 0.72. Standard error was 0.18 and 0.18 for X1 and X2, respectively, and 0.60 for the intercept. Values on the x-axis are based on analysed values for Cu in the diets

or copper sulphate, which is consistent with the previous report in broiler chickens (Chowdhury et al. 2004). However, such growth-promoting effects of Cu (120 to approximately 250 mg/kg) have been commonly reported in fur-bearing animals (Wu et al. 2014b; Liu et al. 2015) and swine (Coble et al. 2017), but with little evidence in poultry. Different forms of Cu differ in their solubility, availability and effects on animal performance appear to be more promising in pigs than in broilers. The discrepancy in responses of growth performance to dietary supplemental Cu between these animals may be due to differences in feedstuffs, dietary Cu concentrations, and trial environment. In the present study, feed conversion was slightly improved by copper methionine at 40 mg/kg supplementation. Although the mechanism responsible for the feed utilization in broilers is still unclear, an increase in nutrient digestibility is likely to be the main contributing factor. Wu et al. (2018) found that dietary Cu supplementation increased the digestibility of energy and fat in broilers. Similar results were also reported in other species such as pigs and fur-bearing animals (Wu et al. 2014c). Therefore, it is likely that high dietary Cu concentrations promote the growth of broilers by increasing nutrient utilization.

Copper sulphate supplements decreased the Ca retention coefficient in the experiment, likely due to an antagonistic interaction between Cu and Ca. Therefore, we believe the decrease in tibia Ca was the result of reduced Ca absorption by copper sulphate addition. The mechanism by which copper sulphate reduces Ca retention in broilers is still unclear. Moreover, dietary copper sulphate supplementation can reduce the quantity of bacterial flora of the intestinal tract (Zhang et al. 2017; Di Giancamillo et al. 2018). Microbial activities degraded mineral complexes with phytic acid and produced short-chain fatty acids to improve mineral solubility (Ptak et al. 2015).

As more Cu is added to the diet, the amount excreted by the animal also increases, which was expected. Thus, the addition of high levels of Cu can have negative repercussions in the environment because of a large amount of Cu being excreted through the faeces, resulting in excessive accumulation of Cu in the soil when manure is used. It is reported that metal chelates of amino acids can enhance the bioavailability of trace elements (Star et al. 2012; Wang et al. 2016). Singh et al. (2015) reported that methionine chelate Cu was absorbed more readily than copper sulphate Cu. The data from this trial indicate that the utilization of copper methionine was better than that of copper sulphate in broilers. Manangi et al. (2012) found that broilers fed diets supplemented with copper methionine had reduced contents of Cu in excreta compared with those fed the basal diet with copper sulphate, achieving a reduction in trace mineral concentrations in the litter.

In the present study, liver Cu concentration was increased by dietary Cu supplementation. Similar results have been reported in broilers (Olukosi et al. 2018) and other animals (Wu et al. 2014a; Liu et al. 2015). High liver Cu concentration reduces hepatic glutathione concentration, which subsequently decreases the activity of HMG-CoA reductase, the key enzyme of CHO biosynthesis, and thus downregulates CHO biosynthesis (Kim et al. 1992; Bunce 1993). Moreover, Konjufca et al. (1997) reported that broilers fed Cu-supplemented diets had higher cholesterol 7a-hydroxylase activity, the rate-limiting enzyme in CHO catabolism, compared with those fed the basal diet without Cu addition. Mondal et al. (2007) found that Cu supplementation ranging from 200 to 400 mg/kg of DM reduced plasma CHO, LDL cholesterol and increased HDL cholesterol in broilers. In the present study, Cu supplementation decreased the concentration of plasma CHO and LDL cholesterol,

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which is consistent with the above finding. However, Wu et al. (2015) indicated that Cu supplementation reduced serum TG and CHO but did not affect HDL cholesterol and LDL cholesterol. The difference in the response of lipid metabolism to dietary Cu supplementation in these studies may be due to different animal species and dietary Cu concentration.

Copper absorbed by animals is mainly stored in liver (Zatulovskaia et al. 2015). Liver Cu concentration is a sensitive index for evaluating the relative bioavailability of Cu sources (Wu et al. 2015). Copper chelated to amino acids is more mobile to the cell membranes and is more readily absorbed than inorganic Cu (Singh et al. 2015). Thus, the use of copper methionine at a much lower concentration for animal diets may be more effective. Our results showed that the relative bioavailability value of copper methionine was greater than that of copper sulphate.

The data from this trial indicate that Cu plays an important role in plasma lipids and in the digestion of dietary Ca in broiler chickens. Copper methionine is more bioavailable than copper sulphate, which is evident from liver Cu and Cu balance study.

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