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Effects of Dietary Supplementation with Copper Sulfate or Tribasic Copper Chloride on Carcass Characteristics, Tissular Nutrients Deposition and Oxidation in Broilers

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Abstract: The experiment was conducted to compare the effects of dietary Tribasic Copper Chloride (TBCC) and Copper Sulfate ($CuSO_4$) on carcass characteristics, copper deposition and tissular nutrients oxidation in broilers. A 2×5 factorial (two copper sources: $CuSO_4$ or TBCC; five added copper levels as 0, 50, 150, 250 or 350 mg/kg) completely randomized design was conducted in experiment. 1,890, 1 day old, Cobb 48 commercial male chicks were randomly allotted into 63 floor pens of 30 birds each and fed to 40-days-old. Two birds from each pen were sacrificed. Their carcass characteristics and the contents of crude fat, copper, V_E or Malondialdehyde (MDA) in tissues were determined. Results indicated that: when added copper level was 150 mg/kg, half-evisceration yield and breast yield of broilers increased. The evisceration yield of broilers was raised by feeding TBCC and supplementation with 50 mg/kg copper from TBCC got the biggest evisceration yield; when added copper levels increased from 50-350 mg/kg, contents of copper or crude fat in liver or muscles and the level of V_E in heart were increased significantly, while, the levels of V_E or MDA in liver decreased; when added copper levels were less than or equal to 150 mg/kg, the copper levels in liver or muscles were similar between copper sources; while added copper levels were >150 mg/kg, the smaller quantities of copper in liver were gained by using TBCC comparing with feeding $CuSO_4$. It implicated that TBCC was a safe dietary copper source.

Key words: Broiler; tribasic copper chloride, carcass characteristics, copper deposition, prooxidant activity

INTRODUCTION

The essentiality of copper for poultry and livestock is well documented (Davis and Mertz, 1987). The high level copper from $CuSO_4$ has been added into swine diets as growth promoter generally, but it is still controversial for broiler diets. This dispute is enhanced by the shortcomings of supplementation with high level of $CuSO_4$, including its damages to nutrients by strengthening their oxidation (Miles *et al*., 1998; Hooge *et al*., 2000; Luo *et al*., 2005). Tribasic Copper Chloride has low hygroscopicity, very low chemical reactivity, less active in catalyzing the destruction of certain vitamins and other organic compounds when, concentrated in base mixes or when included in supplements and diets (Cromwell *et al*., 1998; Miles *et al*., 1998; Luo *et al*., 2005). And this copper source is as available and as safe as that in copper sulfate (Ammerman *et al*., 1995; Miles *et al*., 1998; Luo *et al*., 2005). However, paying more attention to the potential adverse effects of high level copper on the characteristics of animal products and ecological environment, we need think highly of the efficiency and safety of copper sources when adding them in manufacture.

The following study was conducted to investigate the effects of copper from TBCC and $CuSO_4$ on the carcass characteristics, tissular nutrients deposition and oxidation in broilers and to compare the prooxidation and product quality safety of the different copper sources for broilers.

MATERIALS AND METHODS

Experimental design and dietary treatments: A completely randomized design involving a 2×5 factorial arrangement of treatments was used in this study. Five supplemental levels including 0, 50, 150, 250 and 350 mg/kg copper from the two copper sources, TBCC and feed-grade Copper Sulfate ($CuSO_4.5H_2O$), were added into a basal diet. Because, treatments with no additions of both copper sources shared the same basal diet, there were a total of 9 treatments in this experiment. The basal corn-soybean meal diet (Table 1) was formulated to meet the requirements of starting and grower chicken (Ministry of Agriculture of the People's Republic, 2004), containing copper 10.18 or 9.52 mg/kg. All diets were made into pellets.

Birds and feeding: A total of 1890, 1 day old, Cobb 48 commercial male chicks were fed to 40 days in 63 floor pens of 30 birds each. Broilers were randomly allotted by weight to 1 of 9 treatments for 7 replicate pens. The broilers were assigned to floor pens (0.15 m^2 per bird) in a randomized complete block design, which had been vaccinated against Marek's disease and Infectious Bursal Disease. New wood shavings at a depth of

Table 1: Composition and nutrient levels of basal diet

Ingredient composition, % (on fed basis)	Starter (0-21 days)	Grower (22-40 days)
Corn	59.68	62.29
Soy-bean Meal	34.24	31.12
Soy-bean Oil	1.80	3.00
Lysine•hydrochloric	0.13	0.05
DL-Methionine	0.22	0.14
Ground Limestone	1.03	0.93
Calcium Phosphate	1.95	1.68
Salt	0.25	0.20
Micro-ingredient premix*, 2%	0.70	0.59
Nutrient compositions, % (Calculated, on dry mater basis)		
Crude protein	20.64	19.30
Ca	0.95	0.84
NPP	0.45	0.40
Lys	1.11	0.97
Met	0.49	0.39
Met+Cys	0.80	0.69
ME (MJ/kg)	12.04	12.50
Cu (mg/kg)	10.2	9.5

*Provided per kilogram of diet: Vitamine A 13500 IU, VD_3 3000 IU, VE 30 IU, VK_3 6 mg, VB_2 9 mg, VB_6 6 mg, VB_{12} 0.63 ug, Pantothenic acid 18 mg, Niacin 60 mg, Biotin 0.15 mg, Folic acid 1.5 mg, Choline (50%) 750 mg; Fe ($FeSO_4.7H_2O$) 100 mg, Zn ($ZnSO_4$. $7H_2O$) 100 mg, Mn ($MnSO_4.7H_2O$) 120 mg, I (KI) 0.7 mg, Se (Na2SeO3) 0.3 mg, Sodium Bicarbonate 100 mg, Salinomycin 60 mg

approximately 8 cm were used as litter material and each pen contained one hanging heat lamp, one tube feeder and one Plasson waterer. Light was provided 24 h to encourage feed intake. Feeds and water were available ad libitum.

Carcass traits test and sample collections: On day 40, after weighing the birds per pen, 2 birds from each replicate of treatments (close to mean body weight) were picked out. They were starved for 12 h (indeed drinking water was supplied ad libitum) and were sacrificed as per standard procedure for evaluation of carcass characteristics including the yield of evisceration weight, half-evisceration yield, breast yield and thigh yield. The various carcass traits recorded, were then expressed in terms of percentage of live weight. Liver samples, heart samples and pectoral muscles were collected and frozen individually in heat sealed plastic bags for vitamin E, MDA, copper and crude fat analysis.

Laboratory analysis: After homogenizing 0.2 g of liver tissue in 1.8 mL (wt/vol) of dehydrated alcohol (0.9%, wt/vol) and then extracting it with heptane, vitamin E levels of liver and heart were determined by a vitamin E detection kit (JianCheng Bioengineering Institute, Nanjing, China.) according to instructions provided by the manufacturer. The optical densities were read in a spectrophotometer (Shanghai Meipuda instrument co. Ltd., China; UV-1100) at 533 nm.

After homogenizing 0.2 g of liver tissue in 1.8 mL (wt/vol) of cold saline (0.9%, wt/vol), levels of MDA in liver, heart and breast were determined by a MDA detection kit (Nanjing JianCheng Bioengineering Institute, Nanjing, China) following the colorimetric method provided by the manufacturer. The optical densities were read in above spectrophotometer at 532 nm. Each sample was analyzed in duplicate and the results were averaged.

Liver and breast samples were predigested in 5 mL HNO_3 for 4 h on a hotplate, then all samples were dry ashed at 550°C for 12 h and solubilized in 4 mL 10% HNO_3, then metered volume to 10 mL. Copper concentrations were determined by a flame atomic absorption spectrophotometry (Analytik Jena AG, Germany; novAA 400) with the methods descripted by Zhang (2004). Validations of the mineral analysis were conducted using bovine liver powder as a standard reference. Standards were matched for acid and macroelement concentrations as needed and standard reference materials were included with samples.

After dried all liver or muscle samples at 65ºC, 2 g of sample were weighed accurately into the thimble. Then, samples in thimble were dried in an oven at 105ºC for 5 h and cooled subsequently in desiccators for 30 min. Constant weights of the samples were gain. Then the sample was placed directly into the distillation apparatus over a water bath for a 6 hours extraction. And then, constant dried weight of the sample without fat was gain. And the fat content in the samples can be concluded.

Statistical analysis: One-way ANOVA with post hoc multiple comparisons test (Duncan test) was performed to determine significant differences among treatment groups ($p<0.05$). Data were also analyzed for potential effects of copper source, copper levels and interactions between copper source and copper level using the general linear model procedure of SPSS 11.0 software (SPSS Inc., Chicago, IL). Data were expressed as means±SEM. A value of $p<0.05$ (or $p<0.01$) was considered statistically significant and denoted by different letter superscripts.

RESULTS

Body weight and carcass characteristics: There was no significant difference on initial body weight. Copper source, added copper level or an interaction between copper source and level had no significant effects on body weight of 40 days. Chicks consuming 150 mg/kg copper as $CuSO_4$ had a higher BW than those of chicks fed 350 mg/kg copper as TBCC ($p<0.05$).

Copper source affected evisceration yield ($p<0.01$) and chicks fed TBCC had a higher evisceration yield. Added copper level and an interaction between copper source and level did not affect ($p<0.05$) evisceration yield. Copper source, added copper level or an interaction between copper source and level had no significant effects on half-evisceration yield, breast yield and thigh yield. Chicks fed 150 mg/kg copper had the highest half-evisceration yield and breast yield ($p<0.05$).

Table 2: Effects of dietary copper sources and levels on body weight carcass characteristics of broilers*

Copper source	Copper level (mg/kg)	Initial body weight (g)	Body weight on 40 days (g)	Evisceration yield (% of live weight)	Half-evisceration Yield (% of live weight)	Breast yield (% of live weight)	Thigh yield (% of live weight)
Control	0	38.7	2470^{ab}	71.1^{abc}	86.3	21.9	23.0
$CuSO_4$	50	38.6	2425^{ab}	70.9^{abc}	86.2	22.1	23.0
	150	38.8	2493^{b}	72.0^{abc}	87.3	22.9	22.7
	250	38.8	2482^{ab}	70.4^{ab}	86.6	22.3	23.2
	350	38.9	2424^{ab}	70.1^{a}	86.7	21.8	22.5
TBCC	50	38.9	2474^{ab}	72.5^{c}	86.1	21.9	23.0
	150	38.8	2467^{ab}	71.9^{abc}	87.1	22.7	23.1
	250	38.7	2475^{ab}	71.3^{abc}	86.9	21.8	22.8
	350	38.8	2403^{a}	72.2^{bc}	86.9	22.2	22.9
S.E.M.		0.1	9.0	0.20	0.14	0.11	0.11
Copper source	$CuSO_4$	38.8	2459	70.9^{A}	86.7	22.2	22.8
	TBCC	38.8	2458	72.0^{B}	86.7	22.1	23.0
Copper level	0	38.7	2470^{b}	71.1	86.3^{ab}	21.9^{Aa}	23.0
	50	38.8	2449^{ab}	71.7	86.1^{a}	22.0^{ABa}	23.0
	150	38.8	2480^{b}	72.0	87.2^{b}	22.8^{Bb}	22.9
	250	38.8	2479^{b}	70.8	86.7^{ab}	22.1^{ABa}	23.0
	350	38.8	2413^{a}	71.2	86.8^{ab}	22.0^{ABa}	22.7
ANOVA				p-value			
Copper source		0.952	0.939	0.015	0.891	0.667	0.671
Copper level		0.999	0.084	0.272	0.084	0.051	0.848
Copper source×level		0.995	0.659	0.244	0.958	0.689	0.709

*n = 14; Within a column, values with different small letter superscripts mean significant difference ($p<0.05$), differ rent capital letter superscripts mean significant difference ($p<0.01$)

Table 3: Effects of dietary copper sources and levels on concentration of V_E or MDA in tissues of broilers*

Copper source	Copper level (mg/kg)	Liver V_E (ug/g)	Heart V_E (ug/g)	Liver MDA (nmoL/mg)	Muscle MDA (nmoL / mg)	Heart MDA (nmoL/mg)
Control	0	192.9	84.5^{A}	2.15^{ab}	0.88	1.62
$CuSO_4$	50	166.0	100.3^{AB}	1.90^{ab}	0.90	1.57
	150	172.4	95.5^{AB}	1.90^{ab}	1.00	1.59
	250	181.9	112.4^{AB}	1.77^{ab}	1.04	1.33
	350	159.0	90.28^{A}	1.55^{a}	1.06	1.57
TBCC	50	178.9	96.6^{AB}	1.85^{ab}	0.86	1.29
	150	177.0	135.7^{B}	2.31^{b}	1.05	1.28
	250	158.1	108.6^{AB}	2.05^{ab}	1.01	1.53
	350	158.5	97.9^{AB}	1.55^{a}	0.97	1.37
S.E.M.		3.64	3.51	0.07	0.04	0.06
Copper source	$CuSO_4$	174.4	96.6	1.85	0.98	1.53
	TBCC	173.1	104.6	1.98	0.95	1.42
Copper level	0	192.9^{B}	84.5^{A}	2.15^{B}	0.88	1.62
(mg/kg)	50	172.5^{AB}	98.4^{AB}	1.87^{AB}	0.88	1.43
	150	174.7^{AB}	115.6^{B}	2.11^{B}	1.02	1.44
	250	170.0^{AB}	110.5^{AB}	1.91^{AB}	1.03	1.42
	350	158.7^{A}	94.1^{AB}	1.55^{A}	1.01	1.47
ANOVA		---------- p-value ----------				
Copper source		0.844	0.200	0.281	0.743	0.351
Copper level		0.041	0.015	0.016	0.453	0.833
Source×level		0.537	0.143	0.683	0.976	0.657

*n = 14; on fresh basis, by analysis; Within a column, values with different small letter superscripts mean significant difference ($p<0.05$), different capital letter superscripts mean significant difference ($p<0.01$)

Tissue V_E and MDA content: Added copper level affected vitamin E contents in livers and hearts of broilers ($p<0.05$). Liver vitamin E content decreased with added copper level increasing and liver vitamin E contents of broilers fed 350 mg/kg copper were less than the control group sharply ($p<0.01$). The heart vitamin E increased and subsequently decreased following added copper level. Heart vitamin E contents of broilers fed 150 mg/kg copper were higher than the control group significantly ($p<0.01$). Copper source and an interaction between copper source and level had no significant effects on liver and heart vitamin E content of broilers.

Added copper level affected liver MDA significantly ($p<0.05$). Copper source, added copper level and an

Table 4: Effects of dietary copper sources and levels on tissular contents of copper and fat in broilers*

Copper source	Copper level (mg/kg)	Liver crude fat (%)	Muscle crude fat (%)	Liver copper (mg/kg)	Muscle copper (mg/kg)
Control	0	10.80^{a}	4.16^{a}	15.51^{A}	6.19^{A}
$CuSO_4$	50	10.48^{a}	4.26^{a}	27.67^{B}	8.29^{AB}
	150	13.71^{ab}	5.14^{ab}	49.23^{C}	16.22^{BC}
	250	15.92^{b}	4.84^{ab}	141.62^{D}	17.83^{C}
	350	13.25^{ab}	4.90^{ab}	245.93^{E}	16.87^{C}
TBCC	50	12.38^{ab}	4.09^{a}	31.18^{BC}	13.80^{BC}
	150	11.52^{ab}	4.49^{ab}	41.82^{BC}	11.27^{BC}
	250	13.33^{ab}	4.61^{ab}	43.87^{BC}	11.66^{BC}
	350	11.73^{ab}	5.60^{b}	130.19^{D}	15.51^{BC}
S.E.M.		0.48	0.13	8.96	0.73
Copper source	$CuSO_4$	12.83	4.66	95.99^{A}	13.08^{b}
	TBCC	11.95	4.59	52.51^{B}	11.68^{a}
Copper level	0	10.80^{a}	4.16^{A}	15.51^{A}	6.19^{A}
(mg/kg)	50	11.43^{a}	4.17^{A}	29.42^{B}	11.04^{B}
	150	12.62^{ab}	4.82^{AB}	45.52^{C}	13.74^{BC}
	250	14.62^{b}	4.73^{AB}	92.75^{D}	14.74^{BC}
	350	12.49^{ab}	5.25^{B}	188.06^{E}	16.19^{C}
ANOVA		---------- p-value ----------			
Copper source		0.312	0.766	0.000	0.706
Copper level		0.066	0.021	0.000	0.000
Source×level		0.465	0.494	0.000	0.077

*n = 14; on air-dried basis, by analysis; General linear model of log10 transformed copper concentration; Within a column, values with different small letter superscripts mean significant difference ($p<0.05$), differ rent capital letter superscripts mean significant difference ($p<0.01$)

interaction between copper source and level had no significant effects on heart and breast MDA content of broilers.

Tissue fat and copper concentrations: Copper source, added copper level and an interaction between copper source and level had no significant effects on the content of crude fat in liver. But the content of crude fat in livers increased with the copper level increasing. The contents of crude fat in livers of broilers fed 250 mg/kg copper were higher than the control group and the birds fed 50 mg/kg copper ($p<0.05$). Copper source and an interaction between copper source and level did not affect the content of crude fat in breast muscle. Added copper level affected the content of crude fat in breast ($p<0.05$). The content of crude fat in breast increased with added copper level increasing. The content of crude fat in breast muscle of broilers fed 350 mg/kg copper were higher than the control and the birds fed 50 mg/kg copper ($p<0.01$).

Copper source, added copper level, or an interaction between copper source and level affected ($p<0.01$) liver copper significantly (Table 4). Liver copper concentrations in chicks were increased by supplementation with copper ($p<0.01$). Chicks consuming diets with 350 mg/kg copper from $CuSO_4$ had the highest liver copper concentration and the control group had the lowest one. When added level of copper exceeded 150 mg/kg, liver copper of broilers fed with TBCC was less than that of the broilers fed $CuSO_4$.

Added copper level affected copper concentrations of breast muscle in chicks ($p<0.01$). Copper concentrations of breast muscle in chicks were increased by supplementation with copper too ($p<0.01$). Copper source and an interaction between copper source and level had no significant effects on copper concentrations of breast muscle.

DISCUSSION

It was reported by Arias and Koutsos (2006), that carcass weight (45 days posthatch) of chicks fed diet with copper 188 mg/kg from $CuSO_4$ or carcass weight (47 days posthatch) of chicks fed diet with copper 188 mg/kg from TBCC were heavier than those of chicks fed diet without supplemental copper ($p<0.05$); while, these diets did not affect breast muscle mass. In the present study, results indicated that diets with high levels of copper from the two sources had no improvement on the bird bodyweight of d40 and 350 mg/kg copper in diets even reduced the bodyweight. But chicks fed 150 mg/kg copper as $CuSO_4$ had a bigger evisceration yield, a bigger half-evisceration yield and a greatest breast yield. And half-evisceration yield, breast yield and thigh yield of the broilers fed with 150 mg/kg copper as TBCC were non-significantly more than those of the control group.

Otherwise, copper sources in this study affected the evisceration yield of broilers significantly ($p<0.01$). The evisceration yield of broilers fed $CuSO_4$ decreased while, those of broilers fed TBCC increased. Chicks fed 50 mg/kg copper as TBCC had the greatest evisceration

yield, which was more than those of birds consuming 250 and 350 mg/kg copper as $CuSO_4$ significantly ($p<0.05$). The difference between the half-evisceration yield and the evisceration yield represented the percentage of heart, liver, proventriculus, gizzard, abdominal fat, head, neck and shank (Yang *et al*., 2003). This part reduced 11% while, the group fed with 50 mg/kg copper as TBCC compared with the control group. A smaller percentage of this part implicates a better feed convert rate.

In the present study, the crude fat in liver and breast increased when broilers fed with high level copper. Liver crude fat of broilers fed with 250 mg/kg copper as $CuSO_4$ increased significantly comparing with the control ($p<0.05$). Crude fat in muscle of broilers consuming 350 mg/kg copper as TBCC increased notably ($p<0.05$). These increments of crude fat content might be due to promoting fat absorption by dietary supplementation with copper (Luo *et al*., 1996). However, Chowdhury *et al*. (2004) reported that dietary Cu-M*et al*though accumulated more fat in liver at high levels of supplementation (150 and 200 g/kg), but had no effect on excreta fat as well as digestibility of fat.

Tribasic copper chloride possesses several chemical characteristics that make it less oxidation as a copper source in production, such as small particle size, excellent flow and low water solubility (Ammerman *et al*., 1995; Miles *et al*., 1998; Luo *et al*., 2005). Miles *et al*. (1998) reported that copper source and copper concentration affected peroxide values of lipid extracted from stored diets significantly. TBCC promoted less oxidation than coarse $CuSO_4$ at 300 mg/kg added copper, as the modal particle size of TBCC was lower (67 mm) than $CuSO_4$ (455 mm). In current study, with the increasing of added copper level, the liver MDA of broilers fed $CuSO_4$ decreased, while, the liver MDA of broilers fed TBCC changed as an initial increasing and a subsequent decreasing. And supplementation with either $CuSO_4$ or TBCC into diets did not affected MDA contents in heart and muscle.

Hooge *et al*. (2000) reported that vitamin E is greater in TBCC diets than in copper sulfate diets at the same copper levels. It was also reported by Luo *et al*. (2005) that the vitamin E contents in liver and plasma of broilers access to TBCC were higher than those of birds fed copper sulfate ($p<0.01$). In the present study, dietary supplementation with copper influenced the VE contents in liver and heart of broilers ($p<0.05$). While, liver V_E of the broilers decreased with the copper level increasing, the heart V_E content changed as an initial notable increasing and a subsequent little decreasing. The above findings implicate it is a complex effect of dietary supplementation with high copper on fat and V_E, which dietary copper may get involved in absorption, deposition and oxidation of lipids.

Liver copper concentration increases linearly with the increasing of added copper level. It was reported by Miles *et al*. (1998) that liver copper increased significantly when, dietary copper level exceeded 300 mg/kg. And in the study of Bakalli *et al*. (1995), muscle copper (35 and 42 days) increased lightly (14.5%, $p>0.05$) when, supplemented 250 mg/kg copper as $CuSO_4$ into birds diets, but the copper content in the breast muscle was lower. Results obtained in the present study were in agreement with the above studies. Chicks consuming added copper diets had the higher liver copper and muscle copper contents. Liver copper increased significantly ($p<0.01$) when, broilers fed diets added copper from either sources. Chicks fed 350 mg/kg copper as $CuSO_4$ or TBCC had 15.9 and 8.4 times liver copper of the control, respectively. Liver copper of chicks fed with 150~350 mg/kg copper as $CuSO_4$ was higher than those of TBCC. Meanwhile, chicks consuming copper as $CuSO_4$ or TBCC had 1.3~2.9 or 1.8~2.5 times muscle copper of the control, respectively.

Results of this trail (data not shown) showed that high level copper promoted the performance in the starter not but the grower and resulted in chronic copper poisoning with a depressing performance in the grower period. Thus, it need a withdrawal time when we added copper into the broiler diets. Liver copper of birds fed TBCC was similar to those of birds fed $CuSO_4$ when, copper played a role as growth promotion factor (50~150 mg/kg copper, data not shown). Liver copper of chicks consuming 250 mg/kg copper as TBCC or $CuSO_4$ were 2.8 or 9.1 times of the control group. Chicks supplemented with 350 mg/kg copper as $CuSO_4$ had a higher liver copper than those fed TBCC ($p<0.01$). It implicated that chicks were more sensitive to copper as $CuSO_4$ than TBCC when copper levels >250 mg/kg. Thus, there was a higher toxic dose of copper as TBCC than $CuSO_4$. This is in agreement with the study of Miles *et al*. (1998), who reported that the Cu sulfate had a greater percentage of larger particles than TBCC and TBCC had a better uniformity of particle size. Luo *et al*. (2005) reported also that TBCC was a safer product and more available to broilers than copper sulfate.

Tolerance limit of copper in meat is 10 mg/kg (Ministry of Health of the People's Republic, 1994). In the present study, when muscle copper levels were converted into values on fresh basis, they were all <5 mg/kg and in the safety margin. While, added level of copper >50 mg/kg from either copper sources, levels of liver copper in birds are exceed the tolerance limit, even though liver copper of chicks consuming TBCC was lower than those fed $CuSO_4$.

Conclusion: In conclusion, due to its similar growth promotion effect with a less prooxidant activity to damage nutrients, a better carcass characteristics and a higher toxic dose in broilers, TBCC can be used as a safe dietary copper source.

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