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Evaluation of Mintrex® Copper as a Source of Copper in Broiler Diets[1,2]

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Abstract: This study was conducted to evaluate the bioavailability of an organic copper source, MINTREX® Cu, compared with reagent grade Cu sulfate as a source of Cu in broiler diets. Nutritionally complete basal diets were supplemented with either copper sulfate or MINTREX Cu to provide diets with 0, 10, 25, 50, 125, 250 and 500 mg kg^{-1} of supplemental Cu. Fifty commercial broiler strain (Cobb 500) male chicks were placed in each of 48 pens. Each diet (except for 500 mg kg^{-1}) was fed to four replicate pens. The 500 mg kg^{-1} level was fed to two replicate pens for each source. There were two feeding phases including starter (0-21 d) and grower (21-35 d). At the end of each phase, birds were weighed by pens and two birds per pen (four birds per pen for the 500 mg kg^{-1} levels) were killed to take liver and tibia samples for analysis of Cu concentration. Overall, there was no effect of Cu source or dietary Cu concentration on feed conversion or mortality. At 14 d the birds in the MINTREX treatment weighed significantly more than the birds in the Cu sulfate treatment. High Cu concentrations markedly decreased ($p<0.0001$) body weight regardless of Cu sources in both phases. Elevated dietary Cu concentration significantly increased ($p<0.01$) tibia ash Cu concentration for both Cu sources in both phases; however there was no good linear relationship between tibia Cu accumulation and non-zero Cu consumption. There were marked effects ($p<0.05$) of Cu source, concentration and their interaction on 14 d dry liver Cu concentration. Based on dry liver Cu concentration regressed on non-zero copper consumption, the relative bioavailability of MINTREX Cu was 111.63% for 14 d and 110.71% for 35 d when bioavailability of reagent grade Cu sulfate was set as 100%. This indicated that MINTREX Cu source has greater biological availability than reagent grade Cu sulfate for broilers.

Key words: Broilers, copper, organic trace minerals, liver concentrations

Introduction

Copper is an essential trace mineral for chicken development, growth and production. The common Cu sources used in poultry diets are inorganic Cu salts, primarily the sulfate form. With increasing concern of environmental pollution from minerals in the excreta, improving the biological availability by using organic trace minerals in broiler diet is one strategy to reduce their excretion (Ferket *et al.*, 2002). Most of the organic minerals presently marketed are classified as complexes, chelates or proteinates (Spears, 1996). An organic source of trace minerals, MINTREX, has recently been introduced[4]. It is a chelate of two 2-hydroxy-4 (methylthio) butanoic acid (HMTBa) ligands per atom of trace mineral, i.e. zinc, copper or manganese. HMTBa is an organic acid with a structure identical to methionine except for a hydroxyl group on the α-carbon instead of an amino group. MINTREX® Zn has been shown to be able to travel intact to the small intestine and to be equivalent to ALIMET®[4] as a methionine source for chicks (Richards *et al.*, 2005; Yi *et al.*, 2007). In a previous study from our laboratory, MINTREX® Mn was reported to have greater biological availability than inorganic forms of Mn (Yan and Waldroup, 2006). Thus, the objective of the present study was to evaluate the bioavailability of MINTREX Cu, compared to reagent grade Cu sulfate as a source of Cu in broiler diets.

Materials and Methods

Diets were formulated for starter (0-14 d) and grower (14-35 d) to meet or exceed nutrient standards of the average broiler producer in an agricultural survey (Agri-Stats, Fort Wayne IN). Corn and soybean meal of known moisture and protein content were used as the primary ingredients. For each age period, a basal diet was formulated with space allocated for addition of copper source, methionine source and inert filler (washed builders sand). One diet was then formulated using this basal diet using reagent grade copper sulfate heptahydrate to provide 500 mg kg^{-1} of supplemental copper and ALIMET 88% to provide supplemental methionine activity while the other diet was formulated using MINTREX Cu to provide 500 mg kg^{-1} of supplemental copper as well as methionine activity. A third diet (negative control) consisted of the basal diet void of added copper but with sufficient ALIMET to meet the methionine needs. All diets were supplemented with a complete vitamin mix obtained from a commercial poultry integrator. Iron, zinc and manganese needs were met by addition of sulfate forms of these trace minerals. For each age period, a large mix of the basal diet was prepared and aliquots used to prepare the three diets. After mixing these three diets, the negative control diet was blended in appropriate amounts with either the copper sulfate diet or with the MINTREX Cu diet to

Table 1: Composition (g/kg) and calculated analysis of experimental basal diets

	Starter 0-14 d			Grower 14-35 d		
	Cu source			Cu source		
Ingredients	None	Sulfate	Mintrex®	None	Sulfate	Mintrex®
Yellow corn	583.16	583.16	583.16	645.82	645.82	645.82
Poultry oil	19.34	19.34	19.34	20.62	20.62	20.62
Soybean meal	347.60	347.60	347.60	287.92	287.92	287.92
Ground limestone	13.76	13.76	13.76	11.66	11.66	11.66
Dicalcium phosphate	16.97	16.97	16.97	15.40	15.40	15.40
Sodium chloride	5.00	5.00	5.00	5.00	5.00	5.00
L-Threonine	0.73	0.73	0.73	0.40	0.40	0.40
L-Lysine HCl	1.79	1.79	1.79	1.60	1.60	1.60
Broiler vitamin mix[1]	5.00	5.00	5.00	5.00	5.00	5.00
Coban 60[2]	0.75	0.75	0.75	0.75	0.75	0.75
Ferrous sulfate	0.25	0.25	0.25	0.25	0.25	0.25
Manganese sulfate	0.31	0.31	0.31	0.31	0.31	0.31
Zinc sulfate	0.44	0.44	0.44	0.44	0.44	0.44
$CuSO_4 \cdot 5H_2O$	0.00	2.00	0.00	0.00	2.00	0.00
MINTREX® Cu	0.00	0.00	3.33	0.00	0.00	3.33
ALIMET® 88%	2.90	2.90	0.00	2.83	2.83	0.00
Washed sand	2.00	0.00	1.57	2.00	0.00	1.50
TOTAL	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
ME kcal/kg	3030.50	3030.50	3030.50	3107.00	3107.00	3107.00
Crude protein %	22.50	22.50	22.50	20.00	20.00	20.00
Calcium %	0.97	0.97	0.97	0.85	0.85	0.85
Nonphytate P %	0.44	0.44	0.44	0.40	0.40	0.40
Methionine %	0.58	0.58	0.58	0.55	0.55	0.56
Lysine %	1.36	1.36	1.36	1.18	1.18	1.18
Met+Cys %	0.97	0.97	0.97	0.90	0.90	0.91

[1]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.15 mg, [2]Elanco Animal Health division of Eli Lilly and Co., Indianapolis, IN 46825

Table 2: Analyzed copper concentration (mg kg^{-1}) of experimental diets

Added	Starter 0-14 d		Grower 14-35 d	
Cu mg kg^{-1}	Sulfate	MINTREX	Sulfate	MINTREX
0	16.998	16.998	9.950	9.950
10	55.418	21.895	23.363	22.050
25	34.383	38.115	33.605	31.705
50	50.400	58.475	37.948	65.530
125	150.925	129.425	131.100	125.950
250	215.525	288.825	222.867	260.275
500	472.100	505.525	485.150	515.300

provide diets with 0, 10, 25, 50, 125, 250 and 500 mg kg^{-1} of supplemental Cu. Composition of the basal experimental diets is shown in Table 1. Analyzed Cu concentration of each treatment diet is shown in Table 2. Each of the diets (except for 500 mg kg^{-1}) was fed to four replicate pens of chicks. The 500 mg kg^{-1} levels were fed to two replicate pens for each source. Diets were fed in mash form.

Male chicks of a commercial broiler strain[5] were obtained from a local hatchery where they had been vaccinated *in ovo* for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Fifty chicks were placed in each of 48 pens in a house of commercial design. New wood shavings over concrete floors served as litter. The Institutional Animal Care and Use Committee of University of Arkansas approved all procedures in this study. Chicks were managed according to guidelines approved by FASS (1999).

Mean body weight by pen was determined at 14 and 35 d of age. Feed consumption during each age period was determined. Birds were checked twice daily for mortality with the body weight of dead birds used to adjust feed conversion. At each weigh period, two replicate birds per pen (four replicate birds per pen for the 500 mg kg^{-1} levels) were killed by cervical dislocation and a 10 to 15 g sample of liver (fresh weight) uncontaminated by bile was collected from each bird and frozen for Cu analysis. Gizzards were evaluated for possible gizzard erosion. The right tibias were excised and frozen, then processed by methods described by Yan and Waldroup (2006) to determine tibia ash and copper concentration. The copper content of the final mixed feeds, copper sources and liver and tibia ash samples was determined in a commercial laboratory specializing in mineral analysis.

Table 3: Effect of source and level of copper on live performance of broilers on 14 d

Added Cu	Body weight (kg)			Feed conversion (kg feed:kg gain)			Mortality (%)		
mg kg^{-1}	Sulfate	MINTREX	Mean	Sulfate	MINTREX	Mean	Sulfate	MINTREX	Mean
0	0.384	0.384	0.384^{a}	1.480^{c}	1.480^{c}	1.480	1.50	1.50	1.50
10	0.375	0.390	0.382^{ab}	1.490^{c}	1.468^{c}	1.479	0.50	1.00	0.75
25	0.363	0.377	0.370^{b}	1.502^{c}	1.520^{c}	1.511	1.00	2.00	1.50
50	0.377	0.378	0.378^{ab}	1.504^{c}	1.562^{bc}	1.533	1.00	1.00	1.00
125	0.368	0.381	0.374^{ab}	1.684^{a}	1.462^{c}	1.573	1.50	0.50	1.00
250	0.372	0.370	0.371^{ab}	1.471^{c}	1.639^{ab}	1.555	1.50	0.00	0.75
500	0.325	0.349	0.337^{c}	1.752^{a}	1.264^{d}	1.508	1.00	4.00	2.50
Mean	0.366^{b}	0.375^{a}		1.554	1.485		1.14	1.43	
Variables		P diff	SEM		P diff	SEM		P diff	SEM
Source of Cu		0.0428	0.0028		0.0806	0.0172		0.8652	0.3277
Level of Cu		<.0001	0.0068		0.1500	0.0409		0.6021	0.8111
SourcexLevel		0.5608	0.0097		<.0001	0.0579		0.3610	1.1471

Table 4: Effect of source and level of copper on live performance of broilers on 35 d

Added Cu	Body weight (kg)			Feed conversion (kg feed:kg gain)			Mortality (%)		
mg kg^{-1}	Sulfate	MINTREX	Mean	Sulfate	MINTREX	Mean	Sulfate	MINTREX	Mean
0	2.012^{a}	2.012^{a}	2.012^{a}	1.635	1.635	1.635	3.00	3.00	3.00^{bc}
10	1.992^{ab}	2.013^{a}	2.002^{ab}	1.677	1.653	1.665	1.50	1.00	1.25^{c}
25	1.936^{abc}	1.945^{ab}	1.941^{c}	1.692	1.678	1.685	4.50	2.50	3.50^{ab}
50	1.972^{ab}	1.958^{ab}	1.965^{abc}	1.666	1.644	1.655	1.50	2.00	1.75^{bc}
125	1.973^{ab}	1.928^{abcd}	1.950^{bc}	1.687	1.651	1.669	3.00	1.00	2.00^{bc}
250	1.963^{ab}	1.924^{bcd}	1.943^{bc}	1.641	1.658	1.650	2.50	0.00	1.25^{c}
500	1.407^{e}	1.783^{cd}	1.595^{d}	1.778	1.573	1.676	5.00	9.00	7.00^{a}
Mean	1.894	1.937		1.682	1.642		3.00	2.64	
Variables		P diff	SEM		P diff	SEM		P diff	SEM
Source of Cu		0.2806	0.0122		0.1190	0.0129		0.2299	0.4133
Level of Cu		<.0001	0.0301		0.7843	0.0319		0.0009	1.0228
Source x Level		<.0001	0.0426		0.1640	0.0451		0.1843	1.4464

Growth performance data and tibia copper content were subject to two-way ANOVA analysis. The model included the main effects of copper source and dietary copper concentration and their interaction. Pen means were used as the experimental unit. All statements of significance were based on $p<0.05$. Liver copper concentrations exhibited variance heterogeneity and were subjected to log transformation prior to analysis. Ledoux *et al.* (1991) and Luo *et al.* (2005) have indicated that liver copper uptake in chicks could not be assumed to be linear down to the zero added copper level. Therefore, linear regressions were fitted over the non-zero consumption levels within each Cu source. Slope ratio analysis was performed by regressing tibia Cu concentration and log transformed liver Cu concentration on non-zero Cu intake to compare the bioavailability of the Cu sources tested in the trial. The slope ratio test followed the procedures given by Littell *et al.* (1997). All analyses used the General Linear Models procedure of SAS (SAS institute, 1991)

Results and Discussion

Gizzards were in good condition and the linings were not affected by high Cu concentration and different Cu sources. The effects of diets containing different Cu sources and levels on body weight, feed conversion and mortality at 14 d are shown in Table 3. Both source and level of Cu had a significant effect on 14 d body weight. Body weight was maintained relatively constant until reaching a level of 500 mg kg^{-1} added Cu at which time body weights were significantly reduced. Birds fed MINTREX Cu weighed significantly more than those fed copper sulfate as a source of copper. Overall, there was no effect of Cu source or dietary Cu concentration on feed conversion or mortality. However, the use of MINTREX Cu tended to improve ($P = 0.08$) 0-14 d feed conversion.

At 35 d, there was a significant effect of dietary Cu level on body weight (Table 4) with a significant source × level interaction. As the dietary copper level increased body weight declined at the higher levels of supplemental copper, but the decline was less severe in chickens fed diets supplemented with MINTREX Cu. There were no significant effects of source or level of copper on feed conversion. Mortality was significantly influenced by dietary copper levels; this was associated primarily with higher mortality at the 500 mg kg^{-1} level of supplemental copper. There were no significant differences in mortality between chicks fed the two different sources of copper. There were significant source x level interactions on feed consumption in both phases of production; however they were not consistent. In both phases, feed consumption

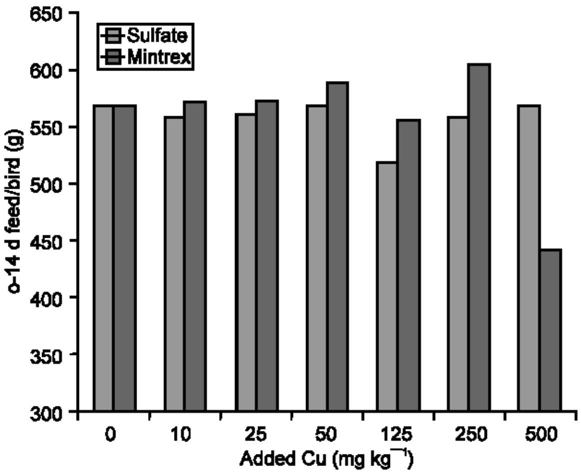

Fig. 1: Effect of copper source and level on 0 to 14 d feed consumption

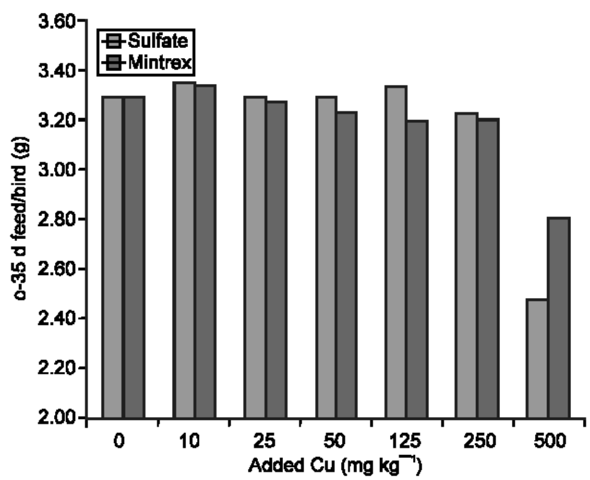

Fig. 2: Effect of source and level of copper on 0 to 35 d feed consumption

was not significantly affected by dietary copper levels until the level of 500 mg kg^{-1} was reached. Consumption of diets supplemented by the two sources was relatively equal within a given level of copper supplementation except for the 500 mg kg^{-1} level. At 14 d (Fig. 1) birds fed the diet supplemented with 500 mg kg^{-1} copper from MINTREX Cu had significantly lower feed consumption than did birds fed the same level of supplemental Cu from copper sulfate. However, at 35 d the reverse was true with birds fed the diet supplemented with 500 mg kg^{-1} copper from copper sulfate consuming significantly less feed than chicks fed the same level of supplemental Cu from MINTREX Cu (Fig. 2).

The effect of source and level of copper on copper content of tibia and liver are shown in Table 5. Level of copper had a significant effect on tibia copper content at both 14 and 35 d of age; tibia levels remained fairly constant until a 500 mg kg^{-1} was fed. There were no significant effects of dietary copper source on tibia ash; a significant source x level interaction on 14 d tibia copper content was observed but showed no consistent pattern.

Liver copper accumulation has been shown to be sensitive to dietary copper sources and levels. At both 14 and 35 d, there was a significant effect of copper level on liver copper concentration; there tended to be a gradual increase in liver copper concentration as dietary copper level increased, with a marked increase in chicks fed 250 and 500 mg kg^{-1} of copper. Birds fed supplemental copper from MINTREX Cu had significantly higher overall liver copper levels at 14 d than did birds fed copper sulfate and numerically higher ($P = 0.10$) levels at 35 d. At both 14 and 35 d there was a source x level interaction for liver copper content. The liver copper content increased as the level of copper from either source increased, but the increase was markedly greater for chicks fed the diets supplemented with MINTREX Cu.

Ledoux *et al.* (1991) indicated that liver was a sensitive organ for dietary Cu and the only tissue in which log of Cu uptake was related linearly to dietary Cu. The common intercept and equality of the basal diet mean to the common intercept were satisfied for the slope-ratio assay. Results of the linear regression and slope-ratio analysis are shown in Table 6. There was little relationship between dietary nonzero copper consumption and tibia copper concentration at either 14 or 35 d of age. Transformed log liver Cu concentration had a very high linear relationship with Cu consumption at both 14 and 35 d of age. When bioavailability of reagent grade Cu sulfate was set as 100%, the relative bioavailability of MINTREX Cu was 111.63% (0.0192/0.0172) for 14 d and 110.71% (0.0031/0.0028) for 35 d.

The broiler chick's nutritional requirement for Cu is approximately 8 mg kg^{-1} (NRC, 1994). Because of its growth promoting properties, Cu is usually fed at much higher pharmacological levels (Bakalli *et al.*, 1995; Pesti and Bakalli, 1996). However, dietary Cu concentration over 250 mg kg^{-1} results in reduced feed intake and growth of chicks (Ledoux *et al.*, 1989). In another experiment (Ledoux *et al.*, 1991), addition of 0.4% methionine to the diet alleviated the problems with feed intake and growth reduction.

Results differ regarding the biological availability of different organic complexes of copper for broiler chicks. Baker *et al.* (1991) reported that a copper-lysine complex provided Cu only as efficiently as copper sulfate. Pott *et al.* (1994) reported a relative Cu bioavailability of 99 for a copper-lysine complex compared to 100 for copper sulfate. Guo *et al.* (2001a) compared five organic copper products to reagent grade copper sulfate in two separate

Table 5: Effect of source and level of copper on copper content (mg kg^{-1}) of various tissues of broiler chicks

	Tibia ash						Dry liver					
	14 d			35 d			14 d			35 d		
Added Cu mg kg^{-1}	Sulfate	MINTREX	Mean	Sulfate	MINTREX	Mean	Sulfate	MINTREX	Mean	Sulfate	MINTREX	Mean
0	6.00^{cdef}	6.00^{cdef}	6.00^{b}	2.33	2.33	2.33^{bc}	11.93^{d}	11.93^{d}	11.93^{c}	11.43^{d}	11.43^{d}	11.43^{c}
10	7.52^{abcde}	4.38^{f}	5.95^{b}	1.78	1.97	1.87^{c}	13.17^{d}	15.33^{d}	14.25^{c}	10.19^{d}	13.52^{d}	11.86^{c}
25	7.62^{abcd}	4.90^{ef}	6.26^{b}	2.48	2.82	2.65^{b}	10.37^{d}	14.61^{d}	12.49^{c}	13.01^{d}	13.68^{d}	13.34^{c}
50	5.84^{cdef}	5.35^{def}	5.60^{b}	2.49	3.25	2.87^{b}	12.84^{d}	12.40^{d}	12.62^{c}	13.00^{d}	11.61^{d}	12.30^{c}
125	6.34^{bcdef}	8.46^{abc}	7.41^{ab}	2.35	2.53	2.44^{b}	13.92^{d}	12.92^{d}	13.42^{c}	14.44^{d}	13.35^{d}	13.90^{c}
250	6.84^{bcdef}	10.74^{a}	8.79^{a}	2.94	2.45	2.70^{b}	91.21^{c}	114.94^{c}	103.07^{b}	50.64^{cd}	88.09^{c}	69.36^{b}
500	9.30^{ab}	9.68^{ab}	9.42^{a}	4.06	4.86	4.46^{a}	366.73^{b}	589.33^{a}	478.03^{a}	383.89^{b}	617.62^{a}	500.75^{a}
Mean	7.07	7.08		2.633	2.886		74.31^{b}	110.21^{a}		70.94	109.90	
Variables		P diff	SEM		P diff	SEM		P diff	SEM		P diff	SEM
Source of Cu		0.8805	0.3918		0.2752	0.1274		0.0074	5.551		0.1061	10.458
Level of Cu		0.0014	0.9501		<.0001	0.3089		<.0001	13.617		<.0001	25.653
SourcexLevel		0.0047	1.3436		0.5292	0.4369		<.0001	19.258		0.0074	36.279

Table 6: Multiple linear regression of tibia ash and log transformed liver Cu concentration

		14 d			35 d		
	Parameter	Intercept	Sulfate slope	MINTREX slope	Intercept	Sulfate slope	MINTREX slope
Tibia ash							
	Estimate	5.9071	0.0145	0.0311	2.2346	0.0013	0.0013
	Standard error	0.5134	0.0083	0.0080	0.1810	0.0005	0.0004
	P-value	<0.0001	0.0888	0.0003	<0.0001	0.0126	0.0041
	R square		0.0639	0.3341		0.3316	0.1500
Log liver							
	Estimate	2.2060	0.0172	0.0192	2.1111	0.0028	0.0031
	Standard error	0.1073	0.0017	0.0017	0.0954	0.0003	0.0002
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	R square		0.8129	0.8015		0.7909	0.8413

experiments. When copper sulfate was assigned a value of 100%, linear regression slope ratios of $\log_{10}$ liver copper regressed on added dietary copper gave estimated relative bioavailability values of 124±5.1, 122±5.3 and 111±6.0 for copper lysine, copper amino acid and copper proteinate C, respectively in experiment 1. In the second experiment the estimated relative bioavailability values were 111±7.6, 109±8.4 and 105±7.5 for copper lysine, copper proteinate A and copper proteinate B, respectively. Guo *et al.* (2001b) reported relative bioavailability of 127.7% and 99.3% for a copper amino acid chelate and a copper proteinate, respectively, in comparison to copper sulfate at 100. Miles *et al.* (2003) reported a relative Cu bioavailability for a copper-amino acid chelate (amino acid ligand not identified) of 96±14 versus reagent grade copper sulfate. Feeding supplemental copper as sulfate or tribasic sources increased liver copper concentrations in the chick (Luo *et al.*, 2005). Guo *et al.* (2001b) reported the estimated relative bioavailability values for Cu Lys, Cu AA and Cu ProC were 124, 122 and 111% for 20 d chickens compared to Cu sulfate, respectively. Metal-amino acid chelates and complexes of Cu at low levels (60 mg kg^{-1}) were not different from high levels (120 mg kg^{-1}) from copper sulfate when maintaining growth performance and serum concentration, but resulted in greatly reduced fecal excretion of Cu (Lee *et al.*, 2001).

Utilization of inorganic trace minerals is dependent on the ability of the animal to convert them to organic biologically active forms (Spears, 1996). Organic sources of trace minerals should maintain their structural integrity in the relatively low pH environment of the upper digestive tract, thereby preventing mineral loss to dietary antagonisms. Once they arrive at absorptive sites in the small intestine, the minerals can be absorbed by their corresponding mineral receptors (Eide, 2004; Petris, 2004). In addition to serving as the chelating ligands, the HMTBa in MINTREX organic trace minerals are also absorbed and serve as a source of methionine activity (Richards *et al.*, 2005; Yi *et al.*, 2007). In summary, the results of the present study indicate MINTREX Cu source has approximately 10-11% greater biological availability than reagent grade Cu sulfate for broilers. Use of this product at adjusted dietary Cu levels should reduce fecal excretion of Cu in broilers.

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