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Superior growth performance in broiler chicks fed chelated compared to inorganic zinc in presence of elevated dietary copper

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Abstract

Background: The goal of this study was to compare the antagonism of elevated dietary Cu (250 mg/kg) from $CuSO_4$ on three different Zn sources ($ZnSO_4 \cdot H_2O$; [Zn bis(−2-hydroxy-4-(methylthio)butanoic acid)], $Zn(HMTBa)_2$, a chelated Zn methionine hydroxy analogue; and Zn-Methionine), as measured using multiple indices of animal performance in ROSS 308 broilers.

Methods: Three experiments were conducted in broiler chicks fed a semi-purified diet. All birds were fed a Zn-deficient diet (8.5 mg/kg diet) for 1 wk, and then provided with the experimental diets for 2 wks.

Results: Experiment 1 was a 2×2 factorial design with two levels of Cu (8 vs. 250 mg/kg diet from $CuSO_4$) and two Zn sources at 30 mg/kg [$ZnSO_4 \cdot H_2O$ vs. $Zn(HMTBa)_2$]. Elevated Cu impaired growth performance only in birds fed $ZnSO_4$. Compared to $ZnSO_4 \cdot H_2O$, $Zn(HMTBa)_2$ improved feed intake (12 %; $P < 0.001$) and weight gain (12 %, $P < 0.001$) and the benefits were more pronounced in the presence of 250 mg/kg diet Cu. Experiment 2 was a dose titration of $ZnSO_4 \cdot H_2O$ and $Zn(HMTBa)_2$ at 30, 45, 60, and 75 mg/kg diet in the presence of 250 mg/kg $CuSO_4$. Feed:gain was decreased and tibia Zn was increased with increasing Zn levels from 30 to 75 mg/kg. Birds fed $Zn(HMTBa)_2$ consumed more food and gained more weight compared to birds fed $ZnSO_4$, especially at lower supplementation levels (30 and 45 mg/kg; interaction $P < 0,05$). Experiment 3 compared two organic Zn sources ($Zn(HMTBa)_2$ vs. Zn-Methionine) at 30 mg/kg with or without 250 mg/kg $CuSO_4$. No interactions were observed between Zn sources and Cu levels on performance or tissue mineral concentrations. High dietary Cu decreased weight gain ($P < 0.01$). Tibia Cu and liver Cu were significantly increased with 250 mg/kg dietary Cu supplementation ($P < 0.01$). No difference was observed between the two Zn sources.

Conclusions: Dietary 250 mg/kg Cu significantly impaired feed intake and weight gain in birds fed $ZnSO_4 \cdot H_2O$, but had less impact in birds fed $Zn(HMTBa)_2$. No difference was observed between the two organic zinc sources. These results are consistent with the hypothesis that chelated organic Zn is better utilized than inorganic zinc in the presence of elevated Cu.

Keywords: Antagonism, Broilers, Chelates, Growth, Minerals, Organic, Performance

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Zhao *et al. Journal of Animal Science and Biotechnology* (2016) 7:13

Page 2 of 9

Background

Elevated dietary $CuSO_4$ (125–250 mg/kg diet) is often used in broiler and pig diets as a growth promoter and anti-bacterial agent, possibly by modulating the microbial population within the gastrointestinal tract [1, 2]. However, high dietary Cu can reduce the bioavailability of other important nutrients by forming insoluble complexes or by competing for absorption sites. The reciprocal antagonism between Zn and Cu is a prime example of competitive biological interactions between metals due to their similar chemical and physical properties [3, 4]. Excessive Zn supply has been shown to inhibit intestinal absorption, hepatic accumulation, and placental transfer of Cu, as well as to induce clinical and biochemical signs of Cu deficiency [5, 6]. The intestinal absorption of ^{65}Zn was decreased by approximately 20 % when the dietary Cu intake was increased from 3 to 24 mg/kg diet. The mechanism(s) of Zn and Cu interaction is not well understood, and the data are controversial to interpret. They compete for uptake sites in the mucosa, and are regulated by the same metallothionein protein. In addition, they might impact each other's solubility and binding behavior in gastrointestinal tract. Pang and Applegate indicated that high Cu supplementation (250 mg/kg in diet) increased the percentage of Zn associated with large complexes (>100,000 MW), and decreased the percentage of Zn associated with small complexes (<5,000 MW; $P < 0.05$), thereby suggesting an antagonism between Cu and Zn [7]. The association of Zn with smaller complexes might enhance the potential for absorption due to the larger surface area.

Most previous studies of dietary Zn-Cu antagonism have evaluated inorganic Zn and inorganic Cu. The availability of chelated organic trace minerals (OTM) in the marketplace now offers a potential solution to the inherent limitations associated with the widespread commercial practice of employing inorganic dietary $CuSO_4$, by reducing and possibly avoiding the antagonism between Cu and Zn. Results from recent studies indicate that OTM might be more available for absorption, likely due to reduced incidence of antagonistic reactions with other dietary constituents in the gastrointestinal tract [8–10]. The relative bioavailability of a chelated Zn methionine hydroxy analogue [Zn bis(−2-hydroxy-4-(methylthio)butanoic acid; $Zn(HMTBa)_2$] (compared to $ZnSO_4$) in broilers was reported to be 161 % based on tibia Zn, and 248 % based on metallothionein mRNA expression [11]. When dietary Ca and P were increased, a common practice in diets of layers and pets, the relative bioavailability of chelated Zn increased to 441 % (based on μg total tibia Zn). The authors suggested that the difference in relative bioavailability might be attributed to the fact that chelated Zn possesses stronger chemical (coordinate-covalent) bonds relative to $ZnSO_4$ and, thus, is more resistant to

antagonisms in gastrointestinal tract. However, published literature on relative bioavailability of different sources of Zn is mixed. The inconsistencies could be due to differences in the chemical characteristics (and thus bioavailability) between different organic Zn forms. Adding to the complexity of comparing results from different studies using various forms of Zn is the inconsistency of terminology (organic, chelates, complex, etc.) along with the difficulty of characterizing chemical structures.

The primary objectives of the experiments reported here were to investigate the potential antagonism imposed by high dietary $CuSO_4$ on Zn bioavailability in broilers, and to evaluate whether different Zn sources impact the Cu-mediated antagonism. We hypothesized that chelated Zn is more resistant to high Cu antagonism due to their stable structure and resistance to interactions with other nutrients in the gastrointestinal tract. Three Zn sources were used in the experiments: $ZnSO_4 \cdot H_2O$, $Zn(HMTBa)_2$, and Zn-methionine (Zn-MET). $Zn(HMTBa)_2$ is a chelated Zn methionine hydroxy analogue [Zn bis(−2-hydroxy-4-(methylthio)butanoic acid)] at 2:1 ratio. To the best of our knowledge, this is the first report of the evaluation of the impact of high Cu on the utilization of different Zn sources in broilers.

Methods

The animal protocols for all experiments were approved by the Novus International Institutional Animal Care and Use Committee, and complied with all federal and state statutes ensuring the humane and ethical treatment of experimental animals. Diet formulation and *in vivo* activities were performed at the Novus International Research Farm. $ZnSO_4 \cdot H_2O$ was purchased from Sigma-Aldrich (Saint Louis, MO USA). $Zn(HMTBa)_2$ (MINTREX® Zn, Novus International Inc, Saint Charles, MO USA) is a chelate of one Zn with two molecules of 2-hydroxy-4-(methylthio)butanoic acid (HMTBa), and was obtained from Novus International. Zinc methionine complex (Zn-MET) is the product resulting from complexing a soluble metal salt with methionine, and was purchased from Zinpro (Eden Prairie, MN USA) [12].

Three consecutive experiments were conducted. Experiment 1 was designed to determine whether 250 mg/kg dietary Cu from $CuSO_4$ antagonizes two different Zn sources [$ZnSO_4 \cdot H_2O$ vs. $Zn(HMTBa)_2$]. $ZnSO_4 \cdot H_2O$ and $Zn(HMTBa)_2$ were added at 21.5 mg/kg to achieve 30 mg/kg total Zn in the finished feed (the other 8.5 mg/kg came from feed ingredients). Similar calculations were used in Experiments 2 and 3, such that different Zn sources were added on top of the basal to achieve the targeted Zn concentration in the final feed. A total of 288 ROSS 308 male chicks were randomly divided into four experimental treatments with six replicate pens per

Zhao *et al. Journal of Animal Science and Biotechnology* (2016) 7:13

Page 3 of 9

treatment and 12 birds per pen. The trial was a 2 × 2 factorial design with two levels of Cu (8 vs. 250 mg/kg diet from $CuSO_4$) and two Zn sources at 30 mg/kg [$ZnSO_4 \cdot H_2O$ vs $Zn(HMTBa)_2$]. Based on Experiment 1, Experiment 2 was designed to compare $ZnSO_4 \cdot H_2O$ and $Zn(HMTBa)_2$ in a dose titration in the presence of 250 mg/kg diet $CuSO_4$. A total of 576 ROSS 308 male chicks were allotted to eight experimental treatments with six replicate pens per treatment and 12 birds per pen. The dietary treatments included either $ZnSO_4 \cdot H_2O$ or $Zn(HMTBa)_2$ added to achieve the following dietary levels: 30, 45, 60, and 75 mg/kg Zn. All diets contained 250 mg/kg diet Cu from $CuSO_4$. Based on the results of the first two experiments, Experiment 3 was designed to compare two different organic Zn sources, $Zn(HMTBa)_2$ and Zn-MET, on performance in the presence of 8 vs. 250 mg/kg $CuSO_4$ dietary supplementation. A total of 480 day-old ROSS 308 male broilers were allotted to four treatments, with 8 replicate pens per treatment and 15 birds per pen. The trial was a 2 × 2 factorial design with two levels of Cu (8 and 250 mg/kg diet as $CuSO_4$) and two Zn sources [$Zn(HMTBa)_2$ vs. Zn-MET] at 30 mg/kg diet.

Birds were housed in stainless steel pens in a thermostatically controlled, electrically heated environment. The dimension of each battery pen was 51 × 69 × 35 cm (width, length, and height, respectively). Each pen was provided with water and an individual feeder. All birds were allowed to consume mash feed and water ad libitum. Room temperature was kept at 32 °C for the first two days, then reduced until a temperature of 23 °C was reached on d 17 of age. The light–dark cycle was as follows: on d 0 through d 7, there were 23 h of light and 1 h of darkness (lights off at 1200 h and on at 0100 h). On d 8 through d 22, there were 20 h of light and 4 h of darkness (lights off at 1200 h and on at 0400 h). All birds were fed a common semi-purified Zn-deficient diet (8.5 mg/kg diet) for the first week to reduce Zn stores and then were fed the experimental diets for about 2 wks (basal diet formula, see Table 1). A common semi-purified basal diet was used and formulated to meet National Research Council (1994) dietary recommendations for all nutrients except for Zn [13]. Each dietary treatment was then made by adding different Zn sources and Cu to the common basal. Methionine activity from $Zn(HMTBa)_2$ (80 % methionine activity) [14], and Zn-MET (20 % methionine activity) was accounted for, and all diets were adjusted to have isomethionine level.

Body weight and feed intake were recorded at beginning and the end of each experiment. On d 21 (d 19 for Experiment 3), one chick per cage (selected based on body weight approximating the pen mean) was chosen, euthanized with CO_2, and the left tibia (without cartilage cap) and liver (Experiment 3)were collected for Zn and Cu

(Experiment 3) analyses. Following the careful removal of muscle and connective tissue, the whole tibia was ashed. Tibia Zn and Cu were measured at the Novus International Analytical Services Laboratories (Saint Charles, MO USA) using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Perkin Elmer, Shelton, CT USA) following an internally validated method based on AOAC 985.01 [15]. Tibia Zn and Cu level were reported on a dry ash weight basis, and liver Zn and Cu levels reported on a dry matter basis.

Statistical analyses
In Experiments 1 and 3, data were analyzed with a 2-way ANOVA using the General Linear Models (GLM) procedure of SAS (version 9.1; Cary, NC USA). The model included the main effects of Zn sources, Cu levels (with and without high $CuSO_4$), and their interaction. In Experiment 2, data were analyzed with a 2-way ANOVA using the GLM procedure. The model included the main effect of two Zn sources [$Zn(HMTBa)_2$ vs. $ZnSO_4 \cdot H_2O$], and four different supplementation levels, and their 2-way interaction. Means were separated by Fisher's protected least significant difference method when the F test was significant. Mortality data were transformed to square root of mortality + 1 before analysis. Data are presented in natural numbers. Pen served as the experimental unit. Effects were considered significant at 95 % probability ($P \le 0.05$).

Results
Experiment 1
As shown in Table 2, significant two-way interactions of Cu level and Zn source were observed on body weight ($P < 0.05$) and weight gain ($P < 0.05$). Compared to 8 mg/kg $CuSO_4$, weight gain was decreased 14 % with 250 mg/kg Cu supplementation in the $ZnSO_4 \cdot H_2O$ groups (382 vs. 328 g), but not in the $Zn(HMTBa)_2$ groups (404 vs. 390 g). Similar results were observed on final body weight, where 250 mg/kg dietary Cu reduced final body weight in $ZnSO_4 \cdot H_2O$ (465 vs. 407 g) but not in the $Zn(HMTBa)_2$ groups (481 vs. 467 g). No interaction was observed on feed intake ($P = 0.34$, Table 2). For main effect, dietary 250 mg/kg $CuSO_4$ decreased feed intake 10 % compared to the 8 mg/kg $CuSO_4$ control groups regardless of Zn source ($P < 0.001$, Table 2). Compared to $ZnSO_4 \cdot H_2O$, birds fed $Zn(HMTBa)_2$ consumed 12 % more feed ($P < 0.001$). Feed conversion, mortality, and tibia Zn were not affected by dietary treatments ($P > 0.50$). In summary, dietary Cu from $CuSO_4$ impaired feed intake across Zn sources, but decreased weight gain only in birds fed $ZnSO_4 \cdot H_2O$. Compared to $ZnSO_4 \cdot H_2O$, $Zn(HMTBa)_2$ improved weight gain and feed intake, and the benefits were more profound with 250 mg/kg dietary Cu supplementation.

Zhao *et al. Journal of Animal Science and Biotechnology* (2016) 7:13

Page 4 of 9

Table 1 Basal diet formula and nutrient profile (Experiments 1 through 3)

Ingredients	g/kg	Nutrients, g/kg	
Dextrose	317.5	Metabolize energy, MJ/kg	13.0
Corn starch	158.4	Crude protein	222.6
Soy concentrate, 84 %	152.8	Calcium	9.0
Cellulose	124.3	Total phosphorus	6.2
Soy protein concentrate	110.0	Available phosphorus	4.5
Soybean oil	50	Available amino acid	
Milo (grain sorghum)[a]	45	Lysine	11.5
Calcium phosphate	14.5	Methionine	6.8
MHA[b]	5.5	Methionine + cystine	8.3
Potassium phosphate	4.8		
Calcium carbonate	3.3		
Mineral premix[c]	2.5		
Choline, 70 %	2.3		
Potassium chloride	1.7		
Sodium chloride	1.6		
Magnesium sulfate heptahydrate	1.4		
Threonine	1.1		
Vitamin premix[d]	0.6		
L-lysine, 78 %	0.6		
Sodium bicarbonate	0.3		
Tryptophan	0.2		
Santoquin-Mix 6	0.1		
Silica[e]	1.5		

[a]Milo, a draught-tolerant crop also known as grain sorghum, is a food product for humans and livestock feed grain. In the US, the feed grain is mainly for poultry and cattle

[b]MHA is the calcium salt of 2-hydroxy-4 methylthio butanoic acid (Novus International), providing 84 % methionine activity. MHA inclusion rate was adjusted to reflect methionine activity provided from $Zn(HMTBa)_2$ (80 % methionine activity) and Zn-MET (20 % methionine activity) to maintain an equal methionine level across all treatments

[c]The mineral premix provided in the final diet (mg/kg): 60 Mn (MnO); 80 Fe ($FeSO_4 \cdot 7H_2O$); 8 Cu ($CuSO_4 \cdot 5H_2O$); 0.35 I (ED Iodide); 0.15 Se ($NaSeO_3$). Analyzed final basal diet Zn was 8.5 mg/kg

[d]Vitamin premix provided in the final diet (mg/kg): retinylacetate 10; cholecalciferol 6.25; dl-alpha tocopheryl acetate 90; menadione sodium bisulphate 7; calcium pantothenate 10; cyanocobalamin, 1.4; riboflavin 6.5; niacin 37; biotin 0.15, thiamin monoitrate 2.25; pyridoxine 4.25; and folic acid 0.9

[e]Silica was removed from the diet as different Zn sources were added

Experiment 2

As shown in Table 3, significant two-way interactions of Zn source and level were observed on body weight ($P = 0.02$), weight gain ($P = 0.005$), and feed intake ($P < 0.001$, Table 3). The superiority of $Zn(HMTBa)_2$ over $ZnSO_4 \cdot H_2O$ was more pronounced at lower levels of Zn supplementation. Birds achieved maximal performance at 45 mg/kg Zn supplementation from $Zn(HMTBa)_2$, and no further improvement was observed at levels above that (Table 3). In contrast, performance was linearly improved in birds fed Zn from $ZnSO_4 \cdot H_2O$ from 30 to 75 mg/kg, with the best performance at the highest level. Feed:gain was decreased and tibia Zn was increased with increased Zn level regardless of the source (main effect, $P < 0.05$). No differences were observed on tibia Zn or mortality between Zn sources ($P > 0.05$). In summary, birds fed $Zn(HMTBa)_2$ ate more and gained more compared to birds fed $ZnSO_4 \cdot H_2O$, especially at lower levels of Zn supplementation. Less $Zn(HMTBa)_2$ was needed to achieve similar performance compared to $ZnSO_4 \cdot H_2O$. Feed efficiency and tibia Zn increased with increased dietary Zn in the range of 30 to 75 mg/kg.

Experiment 3

No interactions between Cu levels and Zn sources were observed on performance ($P > 0.25$; Table 4). No differences were observed on feed intake, feed efficiency, and mortality among treatments ($P > 0.25$, Table 4). Similar to experiment 1, birds fed 250 mg/kg $CuSO_4$ gained less weight and were lighter compared to birds fed 8 mg/kg $CuSO_4$ (main Cu effect, $P < 0.05$). Tibia Zn and liver Zn were not different among treatments ($P > 0.50$, Table 5). Dietary Cu supplementation significantly increased bone and hepatic Cu level, regardless of Zn sources ($P < 0.001$,

Zhao *et al. Journal of Animal Science and Biotechnology* (2016) 7:13

Page 5 of 9

Table 2 Effects of Zn sources and CuSO$_4$ on growth performance and tibia Zn in broilers (Experiment 1)[1]

Cu levels, mg/kg	Zn Source, 30 mg/kg	D7 BW, g/bird	D21 BW, g/bird	Gain, g/bird	Feed Intake, g/bird	Feed:gain	Mortality, %	Ash based tibia Zn, mg/kg[2]
8	ZnSO$_4$	83	465[a]	382[b]	523	1.372	2.78	148
250	ZnSO$_4$	78	407[b]	328[c]	456	1.389	1.39	158
8	Zn(HMTBa)$_2$	77	481[a]	404[a]	569	1.407	5.55	170
250	Zn(HMTBa)$_2$	78	467[a]	390[ab]	528	1.355	2.78	150
SEM		2	8	7	13	0.027	2.26	14
Main effect means								
Cu level								
	8	79	473	393	546[a]	1.390	4.167	159
	250	78	437	359	492[b]	1.372	2.083	154
Zn source								
	ZnSO$_4$	80	436	355	489[b]	1.381	2.083	153
	Zn(HMTBa)$_2$	77	474	397	548[a]	1.381	4.165	160
2x2 factorial arrangement, *P* value								
Zn sources		–	<0.001	<0.001	<0.001	0.980	0.368	0.597
Cu level		–	<0.001	<0.001	<0.001	0.515	0.367	0.722
Zn Source * Cu level		–	0.012	0.011	0.334	0.208	0.762	0.298

[1]A total of 288 ROSS 308 male birds were used, with six cages per treatment and 12 birds per pen
[2]Tibias were collected from one bird per pen, total of six birds per treatment
[a,b,c]Means within a column with no common superscripts differ ($P < 0.05$)

Table 5). Liver Cu was increased greater than 10 fold with 250 mg/kg Cu supplementation. In summary, 250 mg/kg Cu decreased weight gain and significantly increased liver and tibia Cu concentration. No difference was observed between the two Zn sources.

Discussion

The data reported here are consistent with the hypothesis that chelated organic Zn forms are more efficient, compared to an inorganic zinc salt, in their abilities to overcome the antagonism mediated by elevated dietary Cu. Collectively, the data from our study indicate that a chelated form of Zn [Zn(HMTBa)$_2$] outperforms the inorganic ZnSO$_4 \cdot$H$_2$O, in the context of high dietary Cu-mediated antagonism in broiler chicks. Similarly, Richards et al. recently reported that Zn(HMTBa)$_2$ is more bioavailable than ZnSO$_4$, and the difference is greater under conditions of elevated dietary Ca and P supplementation [11].

To the best of our knowledge, this is the first report demonstrating that high dietary Cu in broilers antagonizes, to differing degrees, different dietary sources of Zn. We used a soy isolate/soy concentrate diet to assess the relative performance differences between zinc sources in chicks. The use of such a diet is an accepted model to assess relative bioavailability or performance differences between Zn sources [8]. The presence of phytate and fiber (similar to corn-soybean meal diets) makes this semi-purified diet more relevant to commercial diets compared to a crystalline amino acid, casein-dextrose, or egg white diet which are devoid of phytate.

The reciprocal antagonisms between dietary Zn and Cu are well known. For example, high dietary Cu (204 mg Cu/kg for 10 d) retarded growth performance in turkey poults [16]. Hall et al. reported a 20 % decrease in ^{65}Zn absorption when dietary Cu was raised from 3 to 24 mg/kg in rats [5]. The mechanism(s) by which Cu antagonizes Zn is not well understood, although it has been postulated that these antagonisms are due to their similar chemical and physical properties [3]. It seems unlikely that Cu-Zn interaction occurs at a systemic level, because a) the absorption of these two metals occurs via different transporters, b) their functions and metabolism are quite different, and c) they do not share common storage proteins except metallothionein [3].

Dietary 250 mg/kg Cu significantly impaired feed intake and weight gain in birds fed ZnSO$_4 \cdot$H$_2$O but had little or no impact in birds fed Zn(HMTBa)$_2$. Computer modeling analyses suggest that the interaction between HMTBa and Zn (at a 2:1 molar ratio) is more stable over a range of physiological pH values compared to the interaction between methionine and Zn (at a 1:1 molar ratio) (unpublished observations). In turn, Zn(HMTBa)$_2$ would be predicted to be more stable in solution than Zn-MET, and less susceptible to dietary antagonisms over the variable pH range found in the gastrointestinal tract. Thus, the inherent physicochemical features of Zn(HMTBa)$_2$ might contribute to greater overall bioavailability and

Zhao *et al. Journal of Animal Science and Biotechnology* (2016) 7:13

Page 6 of 9

Table 3 Effects of Zn sources with high dietary CuSO$_4$ on performance and tibia Zn in broilers (Experiment 2)[1]

Zn level, mg/kg	Zn Source	CuSO$_4$, mg/kg	D7 BW, g/bird	D21 BW, g/bird	Gain, g/bird	Feed Intake, g/bird	Feed:gain	Mortality, %	Ash based tibia Zn, mg/kg[2]
30	ZnSO$_4$	250	78	406[d]	328[d]	455[c]	1.388	1.54	158
45	ZnSO$_4$	250	78	488[bc]	411[bc]	550[a]	1.341	1.39	219
60	ZnSO$_4$	250	76	501[ab]	425[ab]	557[a]	1.315	0.00	256
75	ZnSO$_4$	250	78	515[a]	438[a]	571[a]	1.307	0.00	263
30	Zn(HMTBa)$_2$	250	78	468[c]	390[c]	527[b]	1.355	2.78	150
45	Zn(HMTBa)$_2$	250	80	519[a]	440[a]	571[a]	1.302	0.00	228
60	Zn(HMTBa)$_2$	250	80	514[ab]	434[a]	566[a]	1.304	1.24	271
75	Zn(HMTBa)$_2$	250	77	519[a]	441[a]	572[a]	1.300	1.24	267
SEM			2	9	8	8	0.020	1.16	12
Main effect means									
Zn source									
	ZnSO$_4$		78	475	396	531	1.345	1.111	208
	Zn(HMTBa)$_2$		78	500	422	561	1.333	2.220	217
Zn level									
	30		78	455	376	519	1.381[a]	3.125	157[c]
	45		79	504	425	561	1.321[b]	0.694	224[b]
	60		78	507	429	561	1.309[b]	0.694	263[a]
	75		77	517	439	572	1.303[b]	0.694	265[a]
2 × 4 factorial arrangement, *P* value									
Zn level		–	<0.001	<0.001	<0.001	0.004	0.549	<0.001	
Zn Source		–	<0.001	<0.001	<0.001	0.102	0.403	0.569	
Zn source * level		–	0.021	0.005	<0.001	0.827	0.549	0.833	

[1]A total of 576 ROSS 308 male chicks were allotted to eight treatments with six replicate cages per treatment and 12 birds per cage.
[2]Tibias were collected from one bird per pen, total six birds per treatment
[a-d]Means within a column with no common superscripts differ (*P* < 0.05)

superior performance compared to the ZnSO$_4 \cdot$ H$_2$O evaluated here. Collectively, the data reported here indicate that Zn(HMTBa)$_2$ was less susceptible to Cu-mediated antagonism compared to ZnSO$_4 \cdot$ H$_2$O. Furthermore, bioavailability experiments using tissue zinc levels and zinc-responsive gene expression as indicators of bio-availability have shown that Zn(HMTBa)$_2$ exhibits greater relative bioavailability than these other sources [11, 17, 18].

In experiments such as these, careful consideration must be given to the response range of the outcome measures (eg weight gain, tissue Zn, etc.). Differences between Zn sources can only be determined at low intakes (below the requirement or inflection point). Homeostatic physio-logical mechanisms preclude the demonstration of differences between Zn sources, once the supplementation level is above the inflection point [8, 11, 19]. At deficient intake, Zn absorption is maximized. Bioavailability is significantly dependent on the degree of absorption. In purified diets, in the absence of phytate, antagonisms, or a challenge (lipopolysaccharides, etc.), there is little difference in utilization between organic and inorganic sources. In commercial diets or phytate-containing diets, absorption of organic sources of minerals are depressed, but to a lesser extent than inorganic sources. For example, it was demonstrated by Wedekind et al. that the inflection point or breakpoint varies among Zn sources [8]. The break-point or inflection point for bone Zn was determined to be 54, 60 and 65 mg total Zn per kg/diet for Zn-MET, ZnSO$_4$ and ZnO, respectively for birds fed a corn-SBM diet. Their studies also showed an inflection point for weight gain for ZnSO$_4$ for chicks fed a soy isolate diet to occur at 33 mg Zn/kg diet [20]. Comparison of Zn sources above the inflection point would result in an underestima-tion of the bioavailability or performance difference that may truly exist between Zn sources. These findings were confirmed in our studies. Marked differences between Zn(HMTBa)$_2$ and ZnSO$_4 \cdot$ H$_2$O were observed at 30 and 45 mg Zn/kg diet, but not at higher levels. In the presence of excess Cu (250 mg Cu/kg diet), the requirement or in-flection point for weight gain, although not defined in this study, is likely higher than the 33 mg Zn/kg determined by Wedekind et al., wherein no excesses of Ca or Cu were present [8].

Table 4 Effects of organic Zn sources and high dietary $CuSO_4$ on growth performance in broilers (Experiment 3)[1]

$CuSO_4$, mg/kg	Zn source, 30 mg/kg	D8 BW, g/bird	D19 BW, g/bird	Gain, g/bird	Feed Intake, g/bird	Feed:gain	Mortality, %
8	Zn(HMTBa)$_2$	83	375	336	457	1.362	1.67
250	Zn(HMTBa)$_2$	82	367	328	438	1.336	0.83
8	Zn-MET	81	374	335	457	1.364	1.67
250	Zn-MET	80	356	317	447	1.408	2.50
SEM		1	4	4	12	0.030	1.39
Main effect means							
Cu level							
	8	82	375[a]	335[a]	457	1.363	1.77
	250	82	361[b]	332[b]	442	1.372	1.77
Zn source							
	Zn(HMTBa)$_2$	82	371	332	447	1.349	1.25
	Zn-MET	81	365	326	452	1.385	2.18
2 × 2 factorial arrangement, *P* value							
Zn source		–	0.187	0.198	0.703	0.217	0.553
Cu level		–	0.004	0.005	0.227	0.759	1.000
Zn Source* Cu level		–	0.292	0.271	0.696	0.245	0.554

[1]A total of 288 ROSS 308 male birds were used, with six cages per treatment, and 12 birds per pen
[a,b]Means within a column with no common superscripts differ (*P* < 0.05)

Our study had some limitations and strengths. A limitation of this study was the amount of dietary Cu selected for the elevated Cu condition. For environmental reasons, the European Union and China restrict the maximum amount of Cu in animal feed. The 250 mg/kg diet $CuSO_4$ is above the maximum allowed levels in these geographic areas, so the level used in this study might not be relevant for these regions. However, the mineral antagonism demonstrated in our studies is still important, since antagonism could potentially happen at lower levels of Cu supplementation, and 250 mg/kg dietary Cu is a level used commercially in the United States.

Table 5 Effects of Zn sources and dietary $CuSO_4$ on tissue mineral content (Experiment 3)[1]

Cu levels, mg/kg	Zn sources, 30 mg/kg	Tibia Zn (ash based), mg/kg	Tibia Cu (ash based), mg/kg	Liver Zn (dry wt. based), mg/kg	Liver Cu (dry wt. based), mg/kg
8	Zn(HMTBa)$_2$	143	6.5	104	13.5
250	Zn(HMTBa)$_2$	154	11.8	69	169.7
8	Zn-MET	140	6.0	93	13.3
250	Zn-MET	147	10.2	57	128.2
SEM		8	0.8	24	19.8
Main effect means					
Cu level					
	8	141	6.23[b]	98	13.4[b]
	250	150	11.0[a]	63	150[a]
Zn source					
	Zn(HMTBa)$_2$	148	9.14	86	91.6
	$ZnSO_4$	143	8.11	75	70.8
2 × 2 factorial arrangement, *P* value					
Zn source		0.543	0.229	0.653	0.296
Cu level		0.231	<0.001	0.168	<0.001
Zn Source* Cu level		0.780	0.513	0.981	0.301

[1] One bird per pen was killed for tissue collection, total of six birds per treatment
[a,b]Means within a column with no common superscripts differ (*P* < 0.05)

Zhao *et al. Journal of Animal Science and Biotechnology* (2016) 7:13

Page 8 of 9

Organic trace minerals, in general, and $Zn(HMTBa)_2$, specifically, offer an alternative solution to address the challenge of providing required dietary minerals, at lower levels, to meet animal nutritional requirements while avoiding potential antagonism with other nutrients. Furthermore, in Experiment 2, differences between two Zn sources were only observed at 30 and 45 mg/kg, not at 60 and 75 mg/kg. This suggests that the Zn requirement (in the presence of 250 mg/kg Cu) is between 45 and 60 mg/kg. In future studies, dietary Zn supplementation should be chosen below the inflection point, to more sensitively assess bioavailability differences among different Zn sources. Finally, in future studies, biological function parameters should be measured to understand how Cu antagonizes Zn, along with the physiological consequences of that antagonism. For example measurement of metallothionein, collagen, and immune function have served as reliable indices in previous studies. More research is needed to understand the biological consequences of Cu-Zn antagonism in addition to performance and mineral storage in tissues.

The statistical design of our studies and the use of an experimental basal diet containing phytate were some of the strengths of our studies. A factorial design offers advantages over one-way ANOVA studies. There is more power to measure main effects as well as the ability to measure interactions. Significant interactions or trends were observed in all three experiments which demonstrated the improved performance of chelated $Zn(HMTBa)_2$ vs $ZnSO_4 \cdot H_2O$ in the presence of elevated Cu. The presence of phytate, common in cereals and grains, is an important antagonist that reduces Zn bioavailability. In the presence of antagonisms (ie, phytate, fiber, elevated Cu, Ca, P, etc.), bioavailability differences between OTM and ITM are increased [20]. The presence of elevated Cu and phytate are conditions that are relevant to diets fed commercially to poultry and livestock. The advantages of the $Zn(HMTBa)_2$ chelate demonstrated in our study under conditions of elevated Cu has also been demonstrated under other antagonistic conditions (eg, elevated Ca and P) [11]. Bioavailability of $Zn(HMTBa)_2$, relative to $ZnSO_4 \cdot H_2O$ was 161 % (total bone Zn) and 248 % (metallothionein) in the presence of typical Ca and P (0.82 % Ca and 0.47 % available P). However, in the presence of elevated Ca and P (1.2 % Ca; 1 % available P) bioavailability of $Zn(HMTBa)_2$, relative to $ZnSO_4 \cdot H_2O$ was even greater: 441 % (total bone Zn) and 426 % (metallothionein) [11].

The use of high dietary $CuSO_4$ as a growth promoter is common practice in both the broiler and swine industries in North America and some other geographical areas. The bioavailability of other nutrients (Zn and P) should be considered when high $CuSO_4$ is used. One practical strategy is to increase the level of addition of these other nutrients as is often practiced in today's broiler industry. However, this could result in additional antagonisms along with excess nutrient excretion to the environment. Organic trace minerals have the potential advantage of providing dietary minerals that are more bioavailable. Consequently, less mineral is required to achieve a similar performance level compared to inorganic trace minerals [21, 22]. However, not all OTMs are equally capable of avoiding these antagonisms, and thus do not always provide equivalent bioavailability. More research is needed to fully decipher the mode of action of OTMs and their benefits, compared to inorganic sources of Zn, along with understanding the differences among different OTMs.

Conclusions

Dietary 250 mg/kg Cu significantly impaired feed intake and weight gain in birds fed $ZnSO_4 \cdot H_2O$ and had little or no impact in birds fed $Zn(HMTBa)_2$. No significant differences were observed between $Zn(HMTBa)_2$ and Zn-Met.

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Zhao *et al. Journal of Animal Science and Biotechnology* (2016) 7:13

Page 9 of 9

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