Relative bioavailability of copper in tribasic copper chloride to copper in copper sulfate for laying hens based on egg yolk and feather copper concentrations

J. W. Kim, J. H. Kim, J. E. Shin, and D. Y. Kil[1]

Department of Animal Science and Technology, Chung-Ang University, Anseong 456–756, Republic of Korea

ABSTRACT This experiment was conducted to determine the relative bioavailability (RBV) of Cu in tribasic copper chloride (TBCC) to Cu in copper sulfate (monohydrate form; $CuSO_4 \cdot H_2O$) for layer diets based on egg yolk and feather Cu concentrations. A total of 252, 72-wk-old Hy-Line Brown laying hens were allotted to 1 of 7 treatments with 6 replicates consisting of 6 hens per replicate in a completely randomized design. Hens were fed corn-soybean meal-based basal diets supplemented with 0 (basal), 100, 200, or 300 mg/kg Cu from $CuSO_4$ or TBCC for 4 wk. Results indicated that egg production, egg weight, and egg mass were not affected by dietary treatments. However, increasing inclusion levels of Cu in diets from $CuSO_4$ decreased ($P < 0.05$) feed conversion ratio (FCR), whereas increasing inclusion levels of Cu in diets from TBCC did not affect FCR, indicating significant interaction ($P < 0.05$). Increasing inclusion levels of Cu from TBCC or $CuSO_4$ increased ($P < 0.05$) Cu concentrations of egg yolk and feathers. Feather Cu concentrations were greater ($P < 0.01$) for hens fed diets containing $CuSO_4$ than for hens fed diets containing TBCC. The values for the RBV of Cu in TBCC to Cu in $CuSO_4$ based on $\log_{10}$ transformed egg yolk and feather Cu concentrations were 107.4% and 69.5%, respectively. These values for the RBV of Cu in TBCC did not differ from Cu in $CuSO_4$ (100%). The RBV measured in egg yolk did not differ from the RBV measured in feather. In conclusion, the RBV of Cu in TBCC to Cu in $CuSO_4$ can be determined using Cu concentrations of egg yolk and feathers although the values depend largely on target tissues of laying hens. For a practical application, however, the RBV value of Cu in TBCC to Cu in $CuSO_4$ could be 88.5% when the RBV values determined using egg yolk and feather Cu concentrations were averaged.

Key words: copper, egg yolk, feather, laying hen, relative bioavailability

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INTRODUCTION

Copper (**Cu**) is an essential trace element required for maintaining proper body functions and health for animals because the Cu plays a critical role as a cofactor in cytochrome oxidase, lysyl oxidase, superoxide dismutase, and ceruloplasmin (Davis and Mertz, 1987). In addition to the essentiality of Cu, inclusion of high amounts of Cu in diets has been practiced to improve productive performance of pigs and poultry, possibly due to its antimicrobial actions and improved nutrient digestibility in diets (Pettigrew, 2006; Leeson, 2009; Zhao et al., 2014). Currently, the NRC (1994) has recommended 4 to 5 ppm Cu for immature laying hens, but no such value has been specified for adult laying hens. It has been reported that Cu deficiency in laying hens resulted in anemia along with abnormal size and shape of eggs (Baumgartner et al., 1978). Thus, a sufficient amount of Cu should be included in diets fed to laying hens. However, the concentrations of Cu in feed ingredients and their bioavailability vary largely because of the differences in agronomic conditions and processing (NRC, 1994). Thus, various sources of supplemental Cu such as Cu oxide, Cu citrate, Cu sulfate (**$CuSO_4$**), and tribasic copper chloride (**TBCC**) have been additionally included in diets fed to laying hens (Leeson, 2009).

$CuSO_4$ is the most widely used Cu source in the poultry industry. However, $CuSO_4$ is reported to have some physiochemical problems owing to high hygroscopicity and chemical reactivity (Miles et al., 1998; Luo et al., 2005). Therefore, it is necessary to investigate alternative Cu sources to $CuSO_4$ and to determine their bioavailability. As compared to other Cu sources, TBCC has been considered a potential alternative to $CuSO_4$ because it is a less reactive and destructive form in animal diets compared with $CuSO_4$ (Cromwell et al., 1998; Liu et al., 2005; Kim and Kil, 2015). Several researchers have estimated the relative bioavailability (**RBV**) of Cu in TBCC to Cu in $CuSO_4$ (i.e., a

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[1]Corresponding author: dongyong@cau.ac.kr

reference Cu source) for broiler chickens and have reported no difference in the bioavailability between these sources (Miles et al., 1998; Luo et al., 2005; Kim and Kil, 2015). For laying hens, however, there have been limited data regarding the RBV of Cu in TBCC to Cu in $CuSO_4$. Moreover, because Cu is particularly accumulated in the liver, the concentrations of Cu in the liver have been widely used to determine the RBV of Cu among various Cu sources in animal diets (Ammerman, 1995; Leeson, 2009). However, other tissues such as the egg and feathers of poultry have also shown high responsiveness to different Cu sources and concentrations in diets (Vohra et al., 1968; Pekel and Alp, 2011; Kim and Kil, 2015). Moreover, as compared to the liver tissue, egg yolks and feathers can be collected using easier and less invasive techniques. Nevertheless, there has been a lack of data regarding the bioavailability of Cu among different Cu sources in diets as determined using egg yolk and feather of laying hens. Therefore, the current experiment was conducted to determine the RBV of Cu in TBCC to Cu in $CuSO_4$ based on egg yolk and feather Cu concentrations of laying hens.

MATERIALS AND METHODS

Birds, Diets, and Experimental Design

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

A total of 252, 72-wk-old Hy-Line Brown laying hens were used in this experiment. Prior to the experiment, all hens were fed a commercial corn-soybean meal-based diet for 4 wk. At the start of the experiment, hens were randomly allocated to 126 cages (30 cm × 37 cm × 40 cm, width × length × height, respectively) with 2 birds per cage in an environment-controlled layer house. Three adjoining cages were considered 1 replicate group, and the resulting 42 groups were allotted to 1 of 7 dietary treatments with 6 replicates per treatment in a completely randomized design. A commercial-type basal diet was formulated to contain adequate amounts of nutrients and energy for laying hens (NRC, 1994). The concentration of Cu in the basal diet was calculated to be 8.5 mg/kg, but the analyzed concentration of Cu in the basal diet was 15.0 mg/kg (as-is basis; Table 1). For 6 additional diets, Cu sources of either TBCC ($Cu_2[OH]_3Cl$; 59.5% Cu; DaOne Chemical Co., Ltd, Seoul, South Korea) or $CuSO_4$ monohydrate ($CuSO_4{\cdot}H_2O$; 35.8% Cu; DaOne Chemical Co., Ltd, Seoul, South Korea) were added to the basal diet at the levels of 100, 200, or 300 mg/kg Cu at the expense of cornstarch. Although most previous experiments have used the pentahydrate form of $CuSO_4$ ($CuSO_4{\cdot}5H_2O$) as a reference Cu source (Miles et al., 1998; Liu et al., 2005; Luo et al., 2005), the monohydrate form of $CuSO_4$ ($CuSO_4{\cdot}H_2O$) was chosen in this experiment as used in our previous experiment using broiler chickens (Kim and Kil, 2015). The reason is that the use of $CuSO_4{\cdot}H_2O$ has recently received increasing attention due to several possible benefits such as a reduced caking problem and better mixability in the mineral premix (Kim and Kil, 2015). In addition, there is currently a lack of information regarding the utilization of Cu in $CuSO_4{\cdot}H_2O$ for poultry diets. The analyzed concentrations of Cu in diets containing $CuSO_4$ were 104, 198, and 308 mg/kg (as-is basis), whereas those of Cu in diets containing TBCC were 107, 192, and 316 mg/kg (as-is basis). All diets were fed to hens in a mash form for 4 wk. During the experiment, hens were provided with feed and water ad libitum and were exposed to a lighting schedule of 16L:8D. The average room temperature in the layer house was 20°C during the experiment.

Table 1. Composition and nutrient content of basal diets (as-fed basis).

Item	Inclusion (%)
Ingredients	
Corn	53.42
Soybean meal, 46% CP	19.36
Wheat	5.00
DDGS[1]	5.00
Tallow	2.58
Dicalcium phosphate	1.05
Limestone	11.56
DL-Methionine	0.13
Salt	0.20
Vitamin premix[2]	0.20
Mineral premix[3]	0.10
Sodium bicarbonate	0.10
Cornstarch	0.20
Celite	1.00
Ethoxyquin	0.10
Total	100.00
Nutrient and energy content[4]	
AME_n, MJ/kg	11.72
Crude protein, %	15.20
Total lysine, %	0.76
Total methionine + cysteine, %	0.67
Calcium, %	4.50
Nonphytate phosphorus, %	0.34
Copper, mg/kg	8.5
Analyzed copper, mg/kg	15.0

[1]Corn distillers dried grains with solubles.
[2]Provided per kilogram of the complete diet: vitamin A, 15,000 IU (retinly acetate); vitamin D_3, 3,000 IU; vitamin E, 15 IU (DL-α-tocopheryl acetate); vitamin K_3, 2 mg (menadione dimethpyrimidinol); vitamin B_1, 3 mg; vitamin B_2, 9 mg; vitamin B_6, 5 mg; vitamin B_{12}, 40 μg; folic acid, 2.9 mg; biotin, 260 μg; niacin, 40 mg.
[3]Provided per kilogram of the complete diet: zinc, 48 mg; manganese, 71 mg; iron, 80 mg; iodine, 1.5 mg; selenium, 180 μg.
[4]Calculated values from NRC (1994).

Sample Collection and Chemical Analysis

Laying performance factors including hen-day egg production rate, egg weight, and egg mass were recorded daily, whereas feed intake and feed conversion ratio (**FCR**) were calculated weekly over the 4-wk feeding period. The data for laying performance were then summarized for the entire experimental period. At the conclusion of the experiment, 3 eggs from each replicate were randomly collected. The egg yolk samples were separated from whole eggs, pooled, and freeze-dried for the later analysis of Cu concentrations. The clean feather samples were obtained from the back and belly area of hens (2 hens per replicate). The collected feather samples were rinsed with distilled water, oven-dried, and stored in the refrigerator at −20°C until further analysis.

The concentrations of Cu in diets, egg yolk, feathers, and Cu sources (i.e., TBCC and $CuSO_4 \cdot H_2O$) were determined using an inductively coupled plasma spectrometer (Optima 5300 DV, Perkin Elmer Inc., Shelton, CT) as described by Luo et al. (2005) with minor modifications (Kim and Kil, 2015). Briefly, 1 g of diet and egg yolk samples or 0.1 g of feather samples without the rachis or hollow portion was used for the analysis. The samples were weighed in duplicate and digested with 10 mL HNO_3 and 5 mL $HClO_4$ in a 500-mL Kjeldahl flask until the solutions were cleared. Afterwards, the solutions were carefully filtered through Whatman Grade 42 filtered paper (Whatman International Ltd., Maidstone, England) and transferred to 100 mL volume flasks after rinsing 5 times. The resulting filtrates were used to analyze Cu concentrations.

Statistical Analysis

Statistical analysis was conducted using the MIXED procedure (SAS Institute Inc., Cary, NC). All data were analyzed using a completely randomized design. The model included the main effects of Cu sources and concentrations in diets, and their interaction. The replicate was used as the experimental unit for all measurements. Means of response variables were separated using the PDIFF option of the LSMEANS procedure.

The RBV values were determined using $CuSO_4$ as the standard source (100%) by slope ratio comparisons using multiple linear regression analysis in the GLM procedure of SAS (Littell et al., 1997). However, hens fed diets containing 300 mg/kg added Cu from $CuSO_4$ showed the mild anorexic effects compared with hens fed the control diet, and therefore the data were excluded for the multiple regression analysis (Ammerman, 1995; Baker et al., 1991). In addition, egg yolk and feather Cu concentrations exhibited heterogeneous variance, and therefore, were subjected to $\log_{10}$ transformation before data analysis (Littell et al., 1997). Three assumptions of the linear responses, common intercept, and equality of the common intercept to the mean for the control group were tested as described by Littell et al. (1997). The responses for both sources were linear and the 2 regression lines shared a common intercept. In addition, the mean response of the control group did not differ from the common intercept, which indicates that Cu retention in egg yolks and feathers could be assumed to be linear down to the zero level of supplemental Cu in diets. Therefore, a multiple linear regression analysis was performed including the data from the zero level of supplemental Cu in diets (i.e., the control diet; Littell et al., 1997). Feed intake differed among dietary treatments, and therefore, daily added Cu intake rather than dietary Cu concentrations was used as the independent variable for the regression analysis (Ammerman, 1995; Kim and Kil, 2015). Log_{10} transformed egg yolk and feather Cu concentrations were regressed on added Cu intake calculated from average daily feed intake and added dietary Cu concentrations (Ammerman, 1995; Kim and Kil, 2015). Significance was considered at $P < 0.05$ for all analyses.

RESULTS

The analyzed concentrations of Cu in $CuSO_4$ and TBCC were 37.0 and 62.9%, respectively. These values were close to those presented by the $CuSO_4 \cdot H_2O$ or TBCC providers.

Laying Performance

During the overall experimental period, egg production, egg weight, and egg mass were not influenced by dietary treatments (Table 2). However, hens fed diets containing $CuSO_4$ had less ($P < 0.01$) feed intake than those fed diets containing TBCC. Hens fed diets containing 300 mg/kg added Cu tended ($P = 0.05$) to have less feed intake than hens fed diets containing 100 or 200 mg/kg added Cu. No interaction for feed intake between Cu sources and Cu concentrations in diets was observed. Hens fed diets containing 300 mg/kg added Cu from $CuSO_4$ showed the least feed intake ($P < 0.05$) among the 7 dietary treatments. Likewise, hens fed diets containing 300 mg/kg added Cu from $CuSO_4$ showed the least FCR ($P < 0.05$) among the 7 dietary treatments. Main effects of Cu sources and Cu concentrations for FCR were not significant. However, the interaction for FCR between Cu sources and Cu concentrations in diets was identified ($P < 0.05$) because increasing inclusion levels of Cu in diets from TBCC had no effects on FCR, whereas increasing inclusion levels of Cu in diets from $CuSO_4$ decreased ($P < 0.05$) FCR.

Copper Concentrations of Egg Yolk and Feather

The different Cu sources had no effect on egg yolk Cu concentrations (Table 3). However, the concentrations of Cu in egg yolk for hens fed diets containing 300 mg/kg added Cu were greater ($P < 0.05$) than those for hens fed diets containing 100 or 200 mg/kg added Cu. No interaction was found between Cu source and concentration in the diets for egg yolk Cu concentrations. Hens fed diets containing 300 mg/kg added Cu from TBCC showed the greatest ($P < 0.05$) egg yolk Cu concentrations among the 7 dietary treatments.

For feather Cu concentrations, hens fed diets containing $CuSO_4$ had greater ($P < 0.01$) feather Cu concentrations than those fed diets containing TBCC. The

Table 2. Effects of different Cu sources and concentrations in diets on laying performance during a 4-wk feeding trial.[1]

Cu source	Added Cu (mg/kg)	Dietary Cu (mg/kg)[2]	Egg production (%)	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion ratio (g of feed/g of egg)
Control	0	15	85.0	66.1	56.3	117[a]	2.10[a]
$CuSO_4{\cdot}H_2O$	100	104	81.6	67.1	54.8	114[a]	2.11[a]
	200	198	83.3	66.6	55.5	110[a,b]	1.99[a,b]
	300	308	83.6	65.8	55.0	102[b]	1.87[b]
TBCC[3]	100	107	87.5	66.7	58.3	117[a]	2.02[a,b]
	200	192	87.4	66.8	58.3	118[a]	2.02[a]
	300	316	81.6	67.6	55.2	114[a]	2.07[a]
Pooled SEM (n = 6)			2.83	0.79	2.00	3.0	0.051
Added Cu source		$CuSO_4{\cdot}H_2O$	82.9	66.5	55.1	109	1.99
		TBCC	85.2	67.0	57.3	116	2.04
Pooled SEM (n = 18)			1.66	0.45	1.15	1.8	0.028
Added Cu level		100	84.6	66.9	56.6	116	2.06
		200	85.4	66.7	56.9	114	2.01
		300	82.6	66.7	55.1	108	1.97
Pooled SEM (n = 12)			2.03	0.55	1.41	2.2	0.035
P-value for main effects							
Cu source (S)			0.27	0.43	0.20	<0.01	0.28
Cu level (L)			0.62	0.95	0.63	0.05	0.17
S × L			0.37	0.35	0.68	0.39	0.02

[a,b]Means with different superscripts within a column differ ($P < 0.05$).
[1]Each value represents the mean of 6 replicates per each treatment.
[2]Analyzed Cu concentrations in diets (as-is basis).
[3]TBCC = tribasic copper chloride ($Cu_2[OH]_3Cl$; 59.5% Cu).

Table 3. Effects of different Cu sources and concentrations in diets on egg yolk and feather Cu concentrations of laying hens during a 4-wk feeding trial.[1]

Cu source	Added Cu (mg/kg)	Dietary Cu (mg/kg)[2]	Egg yolk Cu (mg/kg)[2]	Feather Cu (mg/kg)[2]
Control	0	15	3.8[c]	25.3[c]
$CuSO_4{\cdot}H_2O$	100	104	4.4[b,c]	40.8[b,c]
	200	198	4.5[b]	53.6[b]
	300	308	4.9[a,b]	94.5[a]
TBCC[3]	100	107	4.4[b,c]	34.0[b,c]
	200	192	4.5[b]	43.9[b,c]
	300	316	5.2[a]	47.9[b]
Pooled SEM (n = 6)			0.22	6.83
Added Cu source		$CuSO_4{\cdot}H_2O$	4.6	62.9
		TBCC	4.7	41.9
Pooled SEM (n = 18)			0.14	4.24
Added Cu level		100	4.4[b]	37.4[b]
		200	4.5[b]	48.8[b]
		300	5.0[a]	71.2[a]
Pooled SEM (n = 12)			0.17	5.20
P-value for main effects				
Cu source (S)			0.47	<0.01
Cu level (L)			0.02	<0.01
S × L			0.66	0.02

[a–c]Means with different superscripts within a column differ ($P < 0.05$).
[1]Each value represents the mean of 6 replicates per each treatment.
[2]The analyzed concentrations of Cu in diets were expressed as-is basis, whereas those of Cu in egg yolk and feather were expressed as DM basis.
[3]TBCC = tribasic copper chloride ($Cu_2[OH]_3Cl$; 59.5% Cu).

feather Cu concentrations for hens fed diets containing 300 mg/kg added Cu were greater ($P < 0.01$) than those for hens fed diets containing 100 or 200 mg/kg added Cu. The hens fed diets containing 300 mg/kg added Cu from $CuSO_4$ had greater ($P < 0.05$) feather Cu concentrations than those fed diets containing 100 or 200 mg/kg added Cu from $CuSO_4$. In contrast, feather Cu concentrations did not differ among hens fed diets containing 100, 200, or 300 mg/kg added Cu from TBCC, which lead to a significant interaction ($P < 0.05$) between Cu sources and Cu concentrations in diets.

Relative Bioavailability of Cu in TBCC

The multiple linear regression analysis was performed excluding the data from hens fed diets containing 300 mg/kg added Cu from $CuSO_4$ because of its mild anorexic effects. The values for the RBV of Cu in

Table 4. Multiple linear regression analyses of $\log_{10}$ transformed Cu concentrations of egg yolk and feather of laying hens on added Cu intake from Cu sulfate ($CuSO_4 \cdot H_2O$) and TBCC (tribasic copper chloride; $Cu_2[OH]_3Cl$).[1]

Independent variables	Intercept	Slope ± SE ($\times 10^{-3}$)	R^2	Root MSE[2]	*P*-value[3]	RBV[4] ± SE, (%)	Confidence intervals, (%)
Log_{10} transformed egg yolk Cu concentration							
Added Cu intake (μg/d)	0.590	3.11 ± 1.09 $CuSO_4$	0.41	0.046	0.809	100	
		3.34 ± 0.70 TBCC				107.4 ± 30.9	45.1 to 169.7
Log_{10} transformed feather Cu concentration							
Added Cu intake (μg/d)	1.433	10.62 ± 2.30 $CuSO_4$	0.50	0.092	0.101	100	
		7.38 ± 1.40 TBCC				69.5 ± 13.3	42.7 to 96.3

[1]Multiple linear regression analysis was performed excluding the data from hens fed diets containing 300 mg/kg added Cu from $CuSO_4 \cdot H_2O$ due to its mild anorexic effects.
[2]Root MSE = root mean square error of the model.
[3]*P*-value for interaction (i.e., regression slopes) between Cu sources as a fixed variable and added Cu intake as a covariate.
[4]RBV = relative bioavailability; it is the slope ratio.

TBCC to Cu in $CuSO_4$ based on $\log_{10}$ transformed egg yolk and feather Cu concentrations are presented in Table 4. When added Cu intake was used as the independent variable, the values for the RBV of Cu in TBCC based on $\log_{10}$ transformed egg yolk and feather Cu concentrations were 107.4 ± 30.9% and 69.5 ± 13.3%, respectively. These RBV values did not differ from 100% of Cu in $CuSO_4$ as the standard source, indicating no difference in the bioavailability of Cu in TBCC relative to $CuSO_4$ as determined in both egg yolks and feathers. Based on the confidence intervals of each RBV value, the RBV value determined using egg yolk Cu concentrations did not differ from the RBV value determined using feather Cu concentrations. Relatively low R^2 values for the regression equation using egg yolk (0.41) and feather Cu concentrations (0.50) were observed, which is likely due to inherently large variation in Cu dynamics of these tissues.

DISCUSSION

In the current experiment, hens fed diets containing increasing Cu concentrations from $CuSO_4$ had less feed intake than those fed diets containing increasing Cu concentrations from TBCC. None of these variables affected egg production and egg weight. In addition, the feed intake was the least for hens fed diets containing 300 mg/kg added Cu from $CuSO_4$ than for those fed other diets. This observation may suggest that supplementation of 300 mg/kg Cu from $CuSO_4$ to the diets may have a mild anorexic effect on laying hens. This result is likely the reason for the lower FCR observed in hens fed diets containing 300 mg/kg added Cu from $CuSO_4$, and this also led to the significant interaction for FCR between Cu sources and Cu concentrations in diets. This observation was contradictory to the findings of Liu et al. (2005) who reported that dietary supplementation of Cu from TBCC or $CuSO_4$ at the levels of 0 to 390 mg/kg had no effects on feed intake of laying hens. However, our results were in agreement with previous observations, indicating that $CuSO_4$ may have an anorexic effect on broiler chickens as compared to TBCC if it was included at high levels (Miles et al., 1998; Luo et al., 2005; Kim and Kil, 2015). The reason for the conflicting results among previous experiments may be associated with variations in animals (e.g., age and genotype), experimental design, and environmental conditions among experiments. However, it has been suggested that $CuSO_4$ has finer particle size and greater water solubility than TBCC, and therefore, Cu in $CuSO_4$ may promote greater oxidative stress than Cu in TBCC, especially when diets containing high amounts of Cu were provided (Miles et al., 1998). In addition, Liu et al. (2005) used $CuSO_4$ pentahydrate ($CuSO_4 \cdot 5H_2O$), however we used $CuSO_4$ monohydrate ($CuSO_4 \cdot H_2O$), which may also be the reason for the different responses observed between experiments. However, to our knowledge, no experiments using poultry have been performed to compare the bioavailability of $CuSO_4$ sources with different extents of hydration. A similar reduction in feed intake was observed in our previous experiment, where we fed broiler chickens diets containing 300 mg/kg Cu from $CuSO_4$ monohydrate (Kim and Kil, 2015). As a result, the data from hens fed diets containing 300 mg/kg added Cu from $CuSO_4$ were excluded for the multiple linear regression analysis (Ammerman, 1995; Baker et al., 1991).

Feather Cu concentrations were greater than egg yolk Cu concentrations across all dietary treatments. As expected from previous experiments (Pesti and Bakalli, 1998; Pekel and Alp, 2011), increasing concentrations of Cu in diets increased egg yolk Cu concentrations. Likewise, increasing concentrations of Cu in diets increased feather Cu concentrations in this experiment. A similar dose-dependent increase in feather Cu concentrations was observed in our previous broiler experiment (Kim and Kil, 2015). Interestingly, increasing concentrations of Cu in diets resulted in a greater increase in feather Cu concentrations than in egg yolk Cu concentrations. In addition, the Cu source had no effect on egg yolk Cu concentrations, but diets containing increasing concentrations of Cu from $CuSO_4$ increased feather Cu concentrations to a greater extent than those containing increasing concentrations of Cu from TBCC, resulting in a significant interaction. These observations may suggest that the determination of the RBV of Cu among various Cu sources depends largely on the target body tissues of hens.

To date, very limited information is available regarding the bioavailability (i.e., RBV) of different Cu sources in diets fed to laying hens although a relatively large number of experiments have been conducted with broiler chickens. In previous experiments using broiler chickens, the bioavailability of Cu in TBCC ranged from 92.6 to 112.0% relative to 100% for Cu in $CuSO_4$ as a standard when liver Cu concentrations were used as a dependent variable (Miles et al., 1998; Luo et al., 2005; Kim and Kil, 2015). The suggested RBV values for Cu in TBCC to Cu in $CuSO_4$ for poultry were 105% (Baker and Ammerman, 1995) and 103% (Sauvant et al., 2004) when the RBV values from previous literature were summarized. Those values were close to the RBV value determined using egg yolk Cu concentrations of laying hens in this experiment. For the layer experiment, however, Liu et al. (2005) determined the RBV of Cu in TBCC to Cu in $CuSO_4$ based on various egg production parameters, and reported that the RBV of Cu in TBCC to Cu in $CuSO_4$ was 134.0% based specifically on egg weight. This difference was significant. A greater bioavailability of Cu in TBCC than Cu in $CuSO_4$ was also observed in the present experiment using log_{10} transformed egg yolk Cu concentrations as the dependent variable; however, the RBV of Cu in TBCC to Cu in $CuSO_4$ was not significantly different. In addition, the difference in the bioavailability of Cu in TBCC to Cu in $CuSO_4$ was smaller than the reported difference of 34.0% (Liu et al., 2005).

Based on log_{10} transformed feather Cu concentrations, the RBV of Cu in TBCC to Cu in $CuSO_4$ was 69.5% in this experiment. This RBV value did not differ from 100% for Cu in $CuSO_4$, which was also observed when it was determined using egg yolk Cu concentrations. This result indicates that feather Cu concentrations of laying hens can be used to determine the RBV of Cu among various Cu sources, which is also supported by our previous broiler experiment (Kim and Kil, 2015). To our knowledge, however, there are no data pertaining to the RBV of Cu in TBCC to Cu in $CuSO_4$ based on feather Cu concentrations in laying hens. Therefore, it was difficult to compare the values from this experiment with previous data.

A numerical difference in the RBV values determined between egg yolks and feathers was observed. This result was expected because differences in the sensitivity or responsiveness of different body tissues to different Cu sources and concentrations in diets exist (Fairweather-Tait, 1987). Similar difference in the RBV values of Cu in TBCC to Cu in $CuSO_4$ was also observed in our previous experiment using broiler chickens based on the liver and feather Cu concentrations (Kim and Kil, 2015). This is also supported by Aoyagi and Baker (1993) who reported that the RBV values of Cu in Cu-methionine complex to Cu in $CuSO_4$ were 96% and 88% based on bile and liver Cu retention, respectively. For other trace mineral, Henry et al. (1986) also reported that the RBV values of manganese (**Mn**) in MnO to Mn in $MnSO_4$ differed among target tissues (81%, 46%, and 70% for bones, kidney, and liver Cu retention, respectively). Therefore, researchers should avoid a belief in one constant value for the RBV of a mineral because the bioavailability of a mineral is highly dependent on a target tissue (Henry et al., 1986; Fairweather-Tait, 1987; Kim and Kil, 2015). Despite a large tissue-specific variation in the RBV of a mineral, from a practical aspect, the presentation of one representative RBV value is preferable if RBV values measured in different tissues are not significantly different. In this sense, many researchers have proposed the representative RBV value of a mineral by averaging the values obtained from different tissues (Henry et al., 1986; Kim and Kil, 2015) or from different experiments (Baker and Ammerman, 1995; Sauvant et al., 2004). Based on the result for no difference in the RBV values measured between egg yolks and feathers in this experiment, we suggest that the RBV of Cu in TBCC to Cu in $CuSO_4$ for laying hens is approximately 88.5% by averaging the RBV values determined using egg yolk or feather Cu concentrations. Surprisingly, this average value was identical to the average RBV value of Cu in TBCC to Cu in $CuSO_4$ when the values obtained from liver Cu concentrations (92.6%) and feather Cu concentrations (84.3%) were averaged in our previous experiments using broiler chickens in the same experiment design (Kim and Kil, 2015).

There were, however, possible limitations in the current experiment. As the goodness of fit for the regression model, the R^2 values for the regression equation using egg yolk (0.41) and feather Cu concentrations (0.50) were relatively low, possibly due to inherent characteristics (i.e., high variability) of Cu dynamics in those tissues of laying hens. The reason for the high variability in egg yolk and feather Cu concentrations is not clear; however, it is likely associated with variations in daily egg production and feather turnover rate among hens, and relatively low Cu transfer from diets into egg yolks and feathers. In addition, relatively aged laying hens (72-wk-old) were used in this experiment, which may also be the reason for high variability in egg yolk and feather Cu concentrations. However, the lack of information regarding RBV determination of minerals for laying hens leads to a difficulty in comparing our R^2 values with previous ones. It has also been reported that R^2 values for RBV determination with various Cu sources varied from 0.62 to 0.99 for different body tissues in broiler chickens (Baker and Ammerman, 1995). Thus, R^2 values in this kind of regression analysis are not always an unbiased measure of a goodness of fit for the model, but the types of response variables and different experimental conditions can induce a large range of R^2 values (Littell et al., 1995).

In conclusion, the RBV of Cu in TBCC to Cu in $CuSO_4$ for laying hens can be determined based on the concentrations of Cu in egg yolks and feathers that are collected using an easier and more animal-friendly procedure. However, the RBV values measured between these tissues were highly variable, which indicates that the bioavailability of Cu in various Cu sources depends largely on the target tissue. For a practical standpoint, however, an average value of 88.5% bioavailability for Cu in TBCC to Cu in $CuSO_4$ could be reasonably used for a practical layer diet.

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REFERENCES

Ammerman, C. B. 1995. Methods for estimation of mineral bioavailability. Pages 83–94 in Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, Inc., San Diego, CA.

Aoyagi, S., and D. H. Baker. 1993. Nutritional evaluation of a copper-methionine complex for chicks. Poult. Sci. 72:2309–2315.

Baker, D. H., and C. B. Ammerman. 1995. Copper bioavailability. Pages 127–156 in: Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, Inc., San Diego, CA.

Baker, D. H., J. Odle, M. A. Funk, and T. M. Wieland. 1991. Research note: Bioavailability of copper in cupric oxide, cuprous oxide, and in a copper-lysine complex. Poult. Sci. 70:177–179.

Baumgartner, S., D. J. Brown, E. Salevsky, and R. M. Leach. 1978. Copper deficiency in the laying hen. J. Nutr. 108:804–811.

Cromwell, G. L., M. D. Lindemann, H. J. Monegue, D. D. Hall, and D. E. Orr, Jr. 1998. Tribasic copper chloride and copper sulfate as copper sources for weanling pigs. J. Anim. Sci. 76:118–123.

Davis, G. K., and W. Mertz. 1987. Copper. Pages 301–364 in Trace Elements in Human and Animal Nutrition. 5th ed. Vol. 1. W. Mertz, ed. Academic Press, New York.

Fairweather-Tait, S. J. 1987. The concept of bioavailability as it relates to iron nutrition. Nutr. Res. 7:319–325.

Henry, P. R., C. B. Ammerman, and R. D. Miles. 1986. Bioavailability of manganese sulfate and manganese monoxide in chicks as measured by tissue uptake of manganese from conventional dietary levels. Poult. Sci. 65:983–986.

Kim, J. W., and D. Y. Kil. 2015. Determination of relative bioavailability of copper in tribasic copper chloride to copper in copper sulfate for broiler chickens based on liver and feather copper concentrations. Anim. Feed Sci. Tech. 210:138–143.

Leeson, S. 2009. Copper metabolism and dietary needs. World's Poult. Sci. J. 65:353–366.

Littell, R. C., A. J. Lewis, and P. R. Henry. 1995. Statistical evaluation of bioavailability assays. Pages 5–33 in Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, Inc., San Diego, CA.

Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimate of relative bioavailability of nutrients using SAS procedures. J. Anim. Sci. 75:2672–2683.

Liu, Z., M. M. Bryant, and D. A. Roland. 2005. Layer performance and phytase retention as influenced by copper sulfate pentahydrate and tribasic copper chloride. J. Appl. Poult. Res. 14:499–505.

Luo, X. G., F. Ji, Y. X. Lin, F. A. Steward, L. Lu, B. Liu, and S. X. Yu. 2005. Effects of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and oxidation stability of vitamin E in feed. Poult. Sci. 84:888–893.

Miles, R. D., S. F. O'keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability and dietary prooxidant activity. Poult. Sci. 77:416–425.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Pekel, A. Y., and M. Alp. 2011. Effects of different dietary copper sources on laying hen performance and egg yolk cholesterol. J. Appl. Poult. Res. 20:506–513.

Pesti, G. M., and R. I. Bakalli. 1998. Studies on the effect of feeding cupric sulfate pentahydrate to laying hens on egg cholesterol content. Poult. Sci. 77:1540–1545.

Pettigrew, J. E. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, Part 1. Anim. Biotechnol. 17:207–215.

Sauvant, D., J. M. Perez, and G. Tran. 2004. Relative biological values of mineral sources Pages 298–299 in: Tables of Composition and Nutritional Value of Feed Materials: Pigs, Poultry, Cattle, Sheep, Goats, Rabbits, Horses and Fish. D. Sauvant, J. M. Perez, and G. Tran, ed. Wageningen Academic Publishers, Wageningen, the Netherlands, and INRA, Paris, France.

Vohra, P., G. D. Gottfredson, and F. H. Kratzer. 1968. The effects of high levels of dietary EDTA, zinc or copper on mineral contents of some tissue of turkey poults. Poult. Sci. 47: 1334–1343.

Zhao, J., G. Allee, G. Gerlemann, L. Ma, M. I. Gracia, D. Parker, M. Vazquez-Anon, and R. J. Harrell. 2014. Effects of a chelated copper as growth promoter on performance and carcass traits in pigs. Asian-Australas. J. Anim. Sci. 27:965–973.