



# 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

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## ABSTRACT

This experiment was conducted to determine the relative bioavailability (RBV) of copper (Cu) in tribasic copper chloride (TBCC) to Cu in copper sulfate (monohydrate form;  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ ) as a reference Cu source for broiler diets based on liver and feather Cu concentrations. A total of 588 1-day-old broiler chicks were randomly allotted to 1 of 7 dietary treatments with 7 replicate cages of 12 birds each (6 males and 6 females). Birds were fed the corn-soybean meal-based basal diet (8.76 mg/kg Cu), or basal diets supplemented with 0, 100, 200, or 300 mg/kg Cu from either  $\text{CuSO}_4$  or TBCC for 21 d. Results indicated that birds fed diets containing 300 mg/kg of Cu from  $\text{CuSO}_4$  had the least ( $P < 0.05$ ) average daily gain (ADG) and average daily feed intake (ADFI) among 7 dietary treatments. The ADG and ADFI were less ( $P < 0.01$ ) for birds fed diets containing  $\text{CuSO}_4$  than for those fed diets containing TBCC. Birds fed diets containing 300 mg/kg of Cu had greater ( $P < 0.01$ ) liver and feather Cu concentrations than those fed diets containing 100 or 200 mg/kg of Cu. The RBV of Cu in TBCC to Cu in  $\text{CuSO}_4$ , when it was calculated based on  $\log_{10}$  transformed liver Cu concentrations and added Cu intake, was  $92.6 \pm 7.9\%$ , whereas the RBV of Cu in TBCC to Cu in  $\text{CuSO}_4$  determined using feather Cu concentrations was  $84.3 \pm 8.4\%$ . However, these two values were not significantly different. In conclusion, the RBV of Cu in TBCC to Cu in  $\text{CuSO}_4$  varies with target tissues, but the values are not significantly different. Thus, the RBV of Cu in TBCC to Cu in  $\text{CuSO}_4$  can also be determined using feather Cu concentrations in broiler chickens. From a practical standpoint, the RBV is likely close to 88.5% when values determined from liver and feather Cu concentrations are averaged.

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## 1. Introduction

Copper (Cu) is an essential trace element for maintaining proper body functions and for obtaining the optimal growth performance of poultry (Banks et al., 2004). One of the main functions of Cu is as a cofactor for various enzymes such as cytochrome oxidase, lysyl oxidase, and Cu-Zn superoxide dismutase (Davis and Mertz, 1987). The Cu is also a constituent of ceruloplasmin in plasma and is involved in converting ferrous iron to ferric iron through ferroxidase activity (Cater and

**Abbreviations:** ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; BWG, body weight gain; Cu, copper;  $\text{CuSO}_4$ , copper sulfate; FI, feed intake; RBV, relative bioavailability; TBCC, tribasic copper chloride.

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Mercer, 2006). Due to its central role in various pivotal enzymes and proteins, deficiency and toxicity of this element can cause severe health problems including growth depression, disability of bones, poor feathering, and anemia (Leeson, 2009).

The Cu requirement has been established to be 8 mg/kg of diets for broiler chickens (McNaughton and Day, 1979; NRC, 1994), but a greater amount of Cu than its requirement has been often included in the commercial broiler diet (Leeson, 2009). The reason is that the differences in the concentrations of Cu and its bioavailability in various feed ingredients result from the variation in the processing procedure and cultivating conditions for feed ingredients (NRC, 1994). Therefore, several supplemental Cu sources such as Cu chloride, Cu oxide, Cu citrate, Cu sulfate ( $\text{CuSO}_4$ ), and tribasic copper chloride (TBCC) have been added to broiler diets.

The  $\text{CuSO}_4$  has been most widely used as a Cu source in broiler diets. However,  $\text{CuSO}_4$  is relatively expensive and involves some physicochemical problems such as high hygroscopicity and chemical reactivity (Luo et al., 2005). Therefore, its alternative Cu sources and their relative bioavailability (RBV) have gained great attention. The TBCC has been appreciated as a potential alternative to  $\text{CuSO}_4$  because it appears a less reactive and destructive form of Cu in case of addition with certain vitamins in base mixtures and other supplements to diets (Cromwell et al., 1998). The RBV of Cu in TBCC to Cu in  $\text{CuSO}_4$  has been estimated from 92% to 109% for broiler chickens (Miles et al., 1998; Luo et al., 2005). In previous experiments, the values for the RBV were mostly determined based on liver Cu concentrations as affected by increasing concentrations of Cu originated from either TBCC or  $\text{CuSO}_4$  in diets (Miles et al., 1998; Luo et al., 2005). The concentrations of Cu in the liver can be a highly sensitive indicator for determining Cu bioavailability among various Cu sources (Ammerman, 1995). However, the procedure is tedious and should be performed by sacrificing animals, which is likely subjective to the blame regarding animal welfare issue. Therefore, an easier and more animal-friendly procedure should be developed. It can be hypothesized that feather Cu concentrations can be used as a potential measurement in determination of the RBV of Cu among Cu sources for poultry because feathers are also highly sensitive to various Cu sources and concentrations in poultry diets (Vohra et al., 1968). However, there has been a lack of data pertaining to the RBV of Cu in TBCC to Cu in  $\text{CuSO}_4$  when it is determined based on feather Cu concentrations in broiler chickens.

Therefore, the objective of this experiment was to determine the RBV of Cu in TBCC to Cu in  $\text{CuSO}_4$  in broiler diets based on liver and feather Cu concentrations and was to compare their RBV of Cu.

## 2. Materials and methods

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

### 2.1. Birds, diets, and experimental design

A total of 588 1-day-old Ross 308 broiler chickens were obtained from a commercial hatchery (Yangji hatchery, Pyeongtaek, South Korea) and were raised in battery cages (76 cm × 78 cm × 45 cm, width × length × height) placed in an environmentally controlled room. Birds were randomly allotted to 1 of 7 dietary treatments with 7 replicate cages of 12 birds each (6 males and 6 females). The corn–soybean meal-based basal diet containing 8.76 mg/kg of Cu (calculated value, as-is basis) was formulated to meet or exceed energy and nutrient requirements of broiler chickens (NRC, 1994; Table 1). The Cu-free mineral premix was used in the basal diet. The analyzed concentration of Cu in the basal diet was 13.0 mg/kg DM. Each of 2 feed-grade Cu sources of either TBCC ( $\text{Cu}_2(\text{OH})_3\text{Cl}$ ; 59.5% Cu) or  $\text{CuSO}_4$  monohydrate ( $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ ; 35.8% Cu) was then added to the basal diet at the level of 0, 100, 200, or 300 mg/kg Cu at the expense of cornstarch. Although pentahydrate form of  $\text{CuSO}_4$  ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) has been widely used in most of previous experiments, monohydrate form of  $\text{CuSO}_4$  ( $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ ) was chosen in this experiment because there is an increasing attention on the use of  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$  due to possible benefits such as less caking problems in the mineral premix, and the lack of information regarding the utilization of Cu in  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$  for poultry diets is available. The analyzed concentrations of Cu in diets containing  $\text{CuSO}_4$  were 116, 243, and 341 mg/kg DM, whereas those of Cu in diets containing TBCC were 117, 248, and 361 mg/kg DM. The experimental diets were in mash form and were fed to birds for 21 d. The diets and water were available at all times. The room temperature was maintained at 30 °C during the first week of the experiment and then gradually decreased to 24 °C at the end of the experiment. A 24-h lighting schedule was used during the entire experiment. Body weight gain (BWG) and feed intake (FI) were recorded at the end of experiment, and gain to feed ratio was also calculated as BWG divided by FI. At the conclusion of experiment, 2 birds (1 male and 1 female) with a body weight (BW) close to the average BW per replicated cage were sacrificed by cervical dislocation for collecting liver and feather samples. The feather samples were obtained from back and belly areas of birds. The collected liver and feather samples were then stored in the refrigerator at –20 °C before further analysis.

### 2.2. Chemical analysis

The Cu concentrations in the diet, liver, feather, and Cu sources (i.e., TBCC and  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ ) were measured by an inductively coupled plasma spectrometer (Optima 5300 DV, Perkin Elmer Inc., Shelton, CT) as described by Luo et al. (2005) with minor modification. In short, before analysis, liver and feather samples were dried overnight at 105 °C. Approximately 1 g of diets and dried liver samples or 0.1 g of dried feather samples (mixed feather from back and belly area) without rachis

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