



# Including copper sulphate or dicopper oxide in the diet of broiler chickens affects performance and copper content in the liver

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## ABSTRACT

Copper supplementation (125–250 ppm) in poultry diets is a common practice in many non-EU countries to enhance bird health, but high amounts of Cu may interact with phytate and affect animal performance. The effects could depend on the nature of the Cu source. Thus, the objective of this trial was to compare the effects of two sources of Cu, copper sulphate (CuSO<sub>4</sub>) and dicopper oxide (Cu<sub>2</sub>O, CoRouge<sup>®</sup>), at three levels of dietary Cu (15, 150, 300 ppm). A total of 576 one-day-old male broiler (Ross 308) were distributed into 6 experimental groups (8 pens/treatment, with 12 birds/pen). Body weight (BW) was individually monitored and feed disappearance was determined at 14, 28 and 35 d of age. On d 35 post-hatch, one bird per replicate was euthanized, the skin fat and breast muscle were sampled, and the liver and kidneys were collected. The two Cu sources were also evaluated *in vitro* to measure Cu and phytic phosphorus (PP) solubility, and PP hydrolysis by phytase at pH 2.5, 4.5 and 6.5. The use of 300 ppm of CuSO<sub>4</sub> decreased ( $P = 0.001$ ) BW on d 14, 28 and 35 and increased ( $P = 0.04$ ) liver Cu content in comparison with the use of 300 ppm of Cu<sub>2</sub>O. The feed-conversion ratio increased for broilers of the 300 ppm CuSO<sub>4</sub> group in comparison to the 300 ppm Cu<sub>2</sub>O group (2.19 vs. 1.84,  $P < 0.001$ ). The use of the highest level of Cu (300 ppm), either of Cu<sub>2</sub>O or CuSO<sub>4</sub>, also increased ( $P < 0.001$ ) Cu concentration in kidney and breast muscle in comparison to 15 and 150 ppm. In the *in vitro* trial, including a level of 300 ppm reduced PP solubility with CuSO<sub>4</sub> (68.66%) in comparison to Cu<sub>2</sub>O (97.41%), and reduced PP hydrolysis by phytase at pH 4.5 and 6.5 with both sources. It can be concluded that dietary levels of 150 and 300 ppm Cu of Cu<sub>2</sub>O are adequate to ensure broiler growth performance and limit organ accumulation in comparison to CuSO<sub>4</sub>.

## 1. Introduction

Broiler chickens need copper for iron transport and metabolism, red-blood-cell formation, enzyme-coenzyme catalytic reactions, immune and connective tissue maturation, especially in the cardiovascular system (Jegade et al., 2011) and bones (Banks et al., 2004a,b). Copper is also part of the linkage between elastin and collagen, which gives the bone its tensile strength (Carlton and Henderson, 1964). Copper requirements for broilers chickens at different ages were reported as being 5–8 mg/kg according to NRC

Abbreviations: CuSO<sub>4</sub>, copper sulphate; Cu<sub>2</sub>O, dicopper oxide; BW, body weight; ADFI, average daily feed intake; WG, average daily weight gain; FCR, feed conversion ratio

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(1994) and 3–10 mg/kg according to FEDNA (2008). In the European Union (EU), dietary copper is supplied for poultry up to a maximum of 25 mg/kg (EFSA, 2012). However, in other areas of the world, including USA, the poultry industry adds 125 ppm to 250 ppm Cu in the diets as growth promoters (Pesti and Bakalli, 1996). The mechanisms behind these effects are attributed to the bactericidal and bacteriostatic effects of Cu on the gastrointestinal tract's microbiota (Hawbaker et al., 1961; Bunch et al., 1965; Pang and Applegate, 2007) and growth-promoting effects (Pesti and Bakalli, 1996). Copper has become especially useful since the use of antibiotics as growth promoters has been prohibited over the last years. However, therapeutic doses of copper, which are usually included in poultry feeds as inorganic mineral salts (copper sulphate pentahydrate), are mostly excreted in the faeces and are a cause of environmental concerns. The high doses of Cu may also easily chelate phytate (Cheryan, 1980), the major storage form of phosphorus in plant seeds (Tamim and Angel, 2003). The solubility of these complexes depends on pH (Selle et al., 2000), the complexes being precipitated at pH 6.5 (approximate pH of intestine) and non-accessible for hydrolysis by phytase or absorption in the intestine (Pang and Applegate, 2006).

Copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) is the most commonly used source of Cu as a dietary supplement for poultry (Pesti and Bakalli, 1996; Pang and Applegate, 2006). It is very soluble in both water and acidic solvents, and has normally been used as a reference point to compare the bio-availability of various Cu sources (Pang and Applegate, 2006). Other Cu sources are being used and considered for use by poultry producers. They have different relative bio-availability and solubility, so they might differently affect intestinal microbiota (Pang et al., 2009) and PP hydrolysis (Banks et al., 2004a,b). The term “copper oxide” is used to refer to either cupric oxide ( $\text{CuO}$ ) or cuprous oxide ( $\text{Cu}_2\text{O}$ ), both oxides occur in nature, but the industrial production of  $\text{Cu}_2\text{O}$  requires an extra step of furnace reduction (The Merck Index, 1990; Aoyagi and Baker, 1993). Oxides of Cu are used to supply chicks feed because smaller inclusion rates are needed with  $\text{CuO}$  (80% Cu) and  $\text{Cu}_2\text{O}$  (89% Cu), as compared to  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (25% Cu, Baker et al., 1991). There is a difference in copper bio-availability due to its valence form. Cupric oxide ( $\text{CuO}$ ) has zero bio-availability, when compared with cuprous oxide (dicopper oxide;  $\text{Cu}_2\text{O}$ ), which is 100% available in animals (Baker, 1999).

In the present study, we have hypothesized that therapeutic doses of dicopper oxide can be included in the diet without affecting PP hydrolysis and broiler performance. Thus, the objective of the current work is to compare the effect of copper sulphate, the most commonly used Cu source for supplementation in poultry diet, and dicopper oxide ( $\text{Cu}_2\text{O}$ ; CoRouge®) on broiler chicken performance, mineral interactions in the digesta, and mineral accumulation in organs and tissues. The Cu sources were compared at three levels of dietary Cu in the diets, which ranged from a nutritional level (15 mg/kg) to an intermediate (150 mg/kg) or therapeutic level (300 mg/kg). An *in vitro* trial has also been designed to compare the solubility of both sources and to identify likely interactions with the phytic phosphorus (PP) and phytase hydrolysis.

## 2. Materials and methods

The experimental products under study were two different Cu sources: Copper Sulphate ( $\text{CuSO}_4$ ) containing 24.1% Cu, and Copper oxide ( $\text{Cu}_2\text{O}$ , CoRouge®, produced by ANIMINE) containing 75.4% Cu. All animal experimentation procedures used in the experiments were approved by the animal Ethics Committee of the Universitat Autònoma de Barcelona and were in compliance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010).

### 2.1. *In vivo* trial

#### 2.1.1. Bird management and husbandry

The study was carried out at a commercial growing poultry unit (Vila-rodona, Tarragona, Spain). The room was provided with 48 floor pens (4 lines of 12 pens each, divided by a central feeding aisle). A total of 576 one-day-old broiler male chickens (Ross 308) were randomly distributed into 6 experimental groups/treatments (8 pens/treatment, 12 birds/pen, 10.6 birds/m<sup>2</sup>) according to initial body weight and continuously controlled over a period of 35 days. Brooder temperature was maintained at 35 °C from d 1 to d 4 post-hatch, and was progressively reduced to 25 °C from d 14 to d 35. The light cycle was 24 h/d from d 1 to d 2, 23 h/d from d 3 to d 10, and 18 h/day from d 11 to d 35.

#### 2.1.2. Experimental design and diets

Three different diets (starter, grower and finisher) were formulated to meet the requirements for maintenance and growth (FEDNA, 2008) and offered to the broilers from d 1 to 14, from d 14 to 28, and from d 28 to d 35 (Table 1). The six experimental treatments were prepared according to two different Cu sources ( $\text{Cu}_2\text{O}$  and  $\text{CuSO}_4$ ) at 3 levels of inclusion (15 ppm, 150 ppm, 300 ppm Cu) (Table 2). Feed and water were offered *ad libitum*. All of the diets were presented in mash form. Diets were sampled and stored for their subsequent analysis.

Body weight (BW) was individually monitored and feed disappearance were determined at 14, 28 and 35 d of age. Mortality rate was also monitored. From these data, ADG, ADFI, and F:G corrected by mortality were determined. At the end of the experiment (d 35), one bird/pen (n = 8) was euthanized by cervical dislocation, tissue (fat and breast muscle) and organ samples (liver and kidney) were collected and weighed to determine Cu content. Ileal digesta were collected in the region from Meckel's diverticulum to about 2 cm anterior to the ileo-cecal junction and stored at −20 °C to determine mineral and micro-mineral content.

#### 2.1.3. Laboratory analyses

Representative samples of diets were analyzed. Analytical determinations of feeds were performed according to the methods of AOAC International (2005) for dry matter (Method 934.01), crude protein with the Dumas Method (Method 968.06), and EE was

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