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Effect of copper nanoparticles and copper sulphate on metabolic rate and development of broiler embryos

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ABSTRACT

Copper (Cu) is regularly used as a growth promoter in poultry production. However, it has been demonstrated that the content of Cu inside eggs might not be sufficient to support the embryonic development. It is possible to supply the embryo with extra nutrients by *in-ovo* administration. Recently, it has been shown that *in-ovo* administration of copper nanoparticles (Cu-NP) and copper sulphate ($CuSO_4$) remarkably improved the body weights of growing chickens. Thus, the objective of the present experiment was to elucidate the potential effects of Cu-NP and $CuSO_4$ on the metabolic rate (oxygen consumption — O_2 and energy expenditure — EE) and development during embryogenesis.

Fertilised broiler eggs were divided into six groups: a non-injected control, a placebo injected with demineralised water, two groups injected, at day one of incubation, with $CuSO_4$ (50 and 100 mg/kg) and two groups injected with Cu-NP (50 and 100 mg/kg). Gaseous exchange was measured in an open-air-circuit respiration unit, and EE was estimated from day 10 to day 19 of embryogenesis. Body weight at 24 h after hatching and the relative organ weights were used as a measure of hatching development. *In-ovo* injection of 50 mg/kg Cu-NP and $CuSO_4$ significantly increased O_2 consumption and EE on the 16th and 19th day of incubation compared with the control group; Cu-NP had the largest effect on the metabolic rate. However, organ weights (intestine, heart, liver, and breast) relative to the yolk-free body weight were not affected in the injected groups. In addition, blood parameters did not show any changes among the groups. This result demonstrates that *in-ovo* injection of Cu-NP affects the metabolic rate of embryos, which might explain their improved performance after hatching.

1. Introduction

Copper (Cu) is a fundamental trace element required for several biochemical processes such as enzyme-coenzyme catalytic reactions, oxygen transport and haemoglobin synthesis. (Kim et al., 2008). It is also used as an efficient growth and health promoter for poultry (Richards et al., 2010). In poultry, there is substantial interest in using Cu as an alternative to antibiotics that can produce equivalent effects on chicken performance. In fact, feed mixtures are enhanced with high levels

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of Cu as growth promoters; however, increasing Cu in animal diet could result in low digestibility and absorption in pigs and poultry, causing more Cu to be excreted in faeces and leading to environmental pollution (Gonzales-Eguia et al., 2009; Zhao et al., 2010; Karimi et al., 2011).

Some authors have suggested that Cu salts are less bioavailable than organic Cu and nano-sized Cu (Creech et al., 2004; Gonzales-Eguia et al., 2009); however, the results regarding organic Cu are inconsistent and need to be supported by further studies. Chickens spend 40% of their entire lifespan inside the egg as embryo; thus embryogenesis is the most critical period of growth and development of chickens (Azaranova et al., 2012). During the last days of incubation, the amount of Cu in the yolk is low leading to mineral deficiency in the embryo; consequently, the embryo consumes less Cu during that period (Yair and Uni, 2011). Therefore, efforts had been made to supplement broiler eggs with Cu (*in-ovo* nutrition) to improve hatchability and chicken performance (Bakyaraj et al., 2012; Mroczek-Sosnowska et al., 2015a). Recently, it has been reported that *in-ovo* injection of copper nanoparticles (Cu-NP) might achieve more efficient penetration into embryonic tissue than copper sulphate ($CuSO_4$), consequently enhancing chicken performance (Mroczek-Sosnowska et al., 2015a). Moreover, the injection of Cu-NP does not harm the development of the embryos or affect chick mortality (Joshua et al., 2016).

Cu-NP can move across cellular and also nuclear membranes and can penetrate cells and intracellular structures, and move to defined target points within the body (Xia et al., 2010; Maojo et al., 2012). It has been demonstrated that 50 mg/kg of Cu-NP stimulates the development of embryonic blood vessels at the molecular and systemic level, more effectively than $CuSO_4$ (Mroczek-Sosnowska et al., 2015b). Considering that the mechanism of action of Cu-NP during embryogenesis is not clear, we hypothesised that *in-ovo* injection of Cu-NP may affect the metabolic rate of embryos. Therefore, determination of oxygen consumption (O_2) and energy expenditure (EE) might be a valuable parameter for predicting the metabolic rate during embryogenesis (Tona et al., 2004; Hamidu et al., 2010).

The objective of the present study was to evaluate whether *in-ovo* supplementation of Cu-NP or $CuSO_4$ would affect the metabolic rate and development of broiler embryos.

1.1. Material and methods

1.1.1. Experimental design

The experimental procedures followed the Danish National Legislation. Broiler eggs (n = 300) from commercial breeder Ross 308 chickens (37 weeks old) were obtained from a Danish hatchery, and were randomly distributed into six groups (45 eggs per group): a non-injected control, a placebo injected with demineralised water, two groups injected with Cu-NP (50 and 100 mg/kg) and two groups injected with $CuSO_4$ (50 and 100 mg/kg). On day 1 of incubation, the eggs were weighed then injected into the air sac with 0.3 mL (15 and 30 μg/egg) of the appropriate solution using a sterile 27 gauge, 20 mm needle. Before and directly after injection the hole was sanitised with an alcohol swab, and was sealed with hypoallergenic tape. The eggs were incubated for 21 days under standard conditions (37.8 °C, 60% humidity, turned once per hour during the first 18 days, and at 37 °C and 70% humidity from day 19 until hatching).

1.1.2. Colloids

Colloidal Cu solutions with concentrations of 50 and 100 mg/kg and a particle size 2–15 nm were purchased from Nano-Tech, Warsaw, Poland. The solutions were manufactured by a patented non-explosive high voltage method (Polish Patent 3883399) from high purity metals (99.9999%) and high purity demineralised water.

The $CuSO_4$ solution was dissolved in ultra-pure water purchased from Sigma-Aldrich, St Louis, MO, USA. The placebo group was injected with the high purity (99.9999%) demineralised water obtained from Nano-Tech, Warsaw, Poland.

1.1.3. Gaseous exchange measurement

The eggs were candled and weighed prior to measurements. Eggs without an embryo or with dead embryo were discarded and replaced with eggs of the same age from the same treatment kept in the incubator as reserves. The measurements were carried out on the 10th, 13th, 16th and 19th day of incubation. Oxygen consumption and carbon dioxide production (CO_2) were measured according to the procedure described by Chwalibog et al. (2007) in an open-air circuit respiration unit (Micro-Oxymax calorimeter from Columbus Instruments, Columbus, OH, USA), equipped with four respiration chambers, each with a capacity of 2000 cm^3. The temperature and relative humidity were maintained similar to the conditions in the incubator (37.8 °C,60% humidity). Six eggs from each treatment group were placed in each chamber and measured for 3 h in the morning from 8:00–11:00, followed by another six eggs from the same groups in the afternoon from 12:00–15:00. After each measurement, the eggs were put back into the incubator. All gas exchange results were standardised to a 50 g egg mass to account for weight differences. Energy expenditure was calculated from O_2 consumption and CO_2 production by the formula: $EE\ (J) = 16.18 \times O_2\ (ml) + 5.02 \times CO_2\ (ml)$ (Brouwer, 1965).

1.1.4. Blood parameters and organ weight

The hatched chickens were weighed and euthanized and blood samples (n = 10 per group) were taken directly from the neck of the one-day old broilers and collected in heparinized tubes. After centrifugation at 2000*g* for 10 min at 4 °C, blood plasma was obtained and kept at −20 °C for biochemical analysis. The chicks were then dissected and the yolk sac, heart, liver, breast and intestine were weighed in order to measure their development.