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Short communication

Effect of copper nanoparticles on metabolic rate and development of chicken embryos

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ABSTRACT

The objective of the study was to investigate the effects of an *in ovo* injection of CuNano and the timing of injection on metabolic rate (O_2 consumption and heat production, HP) and development of layer hatchlings. On day 1 of incubation, 192 fertile eggs from 29-week-old Lohmann breeder strain chickens were distributed into four groups that were administered colloidal CuNano on: day 1 and/or 10. Gaseous exchange was measured in an open-air-circuit respiration unit, and HP was calculated for 16- and 19-day-old embryos. Yolk free body weight (YFBW) at 24 h after hatching and the relative organ weights were used as a measure of hatchling development. *In ovo* injection of CuNano on different days during incubation significantly decreased O_2 consumption and HP compared with the control group. The residual yolk sac weight in the treated groups was significantly higher than in the control group ($P<0.0001$), indicating that CuNano injection reduced lipid oxidation, which could be associated with the lower O_2 consumption ($P=0.001$). Accordingly, the organ weights (intestine, heart, liver) relative to YFBW were also lower in embryos injected with CuNano (all; $P<0.05$). Interestingly, the difference in metabolic rate and organ weights between treatments was not reflected in YFBW ($P>0.05$). Furthermore, the plasma concentrations of IgM and IgG and the mRNA expression of NF-kB and TNF-α were not affected (both; $P>0.05$), indicating the absence of inflammatory modulation by CuNano. These preliminary results demonstrated that CuNano, regardless of the day of injection, altered the metabolic rate of embryos and depressed the development of organs; however, it did not affect YFBW, immunoglobulin concentrations and the expression of immuno-related genes.

1. Introduction

Copper is an essential trace mineral that is involved in various physiological and biochemical processes, *i.e.*, foetal and early postnatal development, proper nerve function, connective tissue and bone development, and inflammatory processes, as well as being a constituent of several enzyme systems (ECDC, 2003). It has been shown that Cu supplementation, *ca.* 125 mg/kg, improves the performance of broilers and layers (Leeson, 2009; Karimi et al., 2011). However, only a small

Abbreviations: CO_2, carbon dioxide; CuNano, copper nanoparticles; HP, heat production; IgG, immunoglobulin G; IgM, immunoglobulin M; NF-kB, nuclear factor kappa-light-chain enhancer of activated B cells; O_2, oxygen; TNF-α, tumour necrosis factor alpha; YFBW, yolk-free body weight; YS, yolk-sac.

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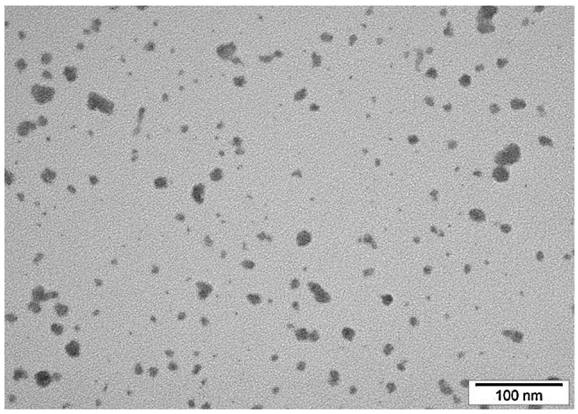

Fig. 1. Transmission electron microscopy image of copper nanoparticle.

fraction of dietary Cu is absorbed in the small intestine, regardless of its inclusion level, the major part of Cu (70–90%) is excreted in faeces and consequently, its effect on soil microorganisms, plants and aquatic species is currently one of the crucial environmental concerns (Zhao et al., 2010).

At present, there are no effective alternatives to Cu in promoting health and growth in chickens. Hence, the withdrawal of Cu from animal diets will cause severe health, performance and economical drawbacks in intensive poultry production.

Dietary Cu is traditionally supplied as inorganic copper sulphate (Leeson, 2009). However, in the recent past, the use of organic sources of Cu in the form of chelates, complexes or proteinates has been considered as an alternative to inorganic Cu due to better bioavailability and digestibility that could reduce Cu doses and decrease excretion to the environment (Zhao et al., 2010). However, comparative results of organic vs. inorganic Cu sources are not consistent (Chowdhury et al., 2004; Leeson, 2009).

Another solution that can be considered is the use of Cu nanoparticles in place of bulk Cu. Recent advancement in nanotechnology has enabled Cu to be engineered at the nano–size scale (1–100 nm), exhibiting ultrahigh physical activity and chemical neutrality. The activity of nanoparticles results from a large surface area, exposing their atoms to direct contact with target cells. Since CuNano has the same effect on animal health and performance as bulk Cu source and because of their high physical activity, the quantity of Cu added to animal diets and its consequent contamination of the environment can be significantly reduced. The results regarding application of CuNano as an alternative growth promoter are almost non-existent, except for the work of Gonzales-Eguia et al. (2009), who demonstrated a better performance of piglets supplied with CuNano.

We hypothesised that CuNano may affect prenatal development and postnatal performance of poultry. Thus, as the first step, potential effects of CuNano on the development of chicken embryos were investigated. The objective was to evaluate whether CuNano affected metabolic rate, body and organ weights, and several immunological indices in chicken embryos.

2. Material and methods

2.1. Nanosolution

CuNano solution with a concentration of 50 mg/kg and a particle size of 2–15 nm (Fig. 1) was obtained from Nano-Tech, Warsaw, Poland. The hydrocolloid solution was produced by a patented non-explosive high voltage method (Polish Patent 3883399) from high purity metals (99.9999%) and high purity demineralised water.

2.2. Experimental design

Fertilised chicken eggs ($n = 192$) from the Lohmann (29-weeks old) breeder strain were obtained from a commercial hatchery, randomly grouped into two batches and stored in a refrigerator (10 °C) for 1–3 days before being placed in the incubator. On day 1 of incubation, eggs from batch 1 were distributed into four groups that were administered with colloidal CuNano on: day 1 – $CuNano_{D1}$; day 10 – $CuNano_{D10}$; days 1 and 10 – $CuNano_{D1+D10}$; or not at all – Control. The eggs were injected into the air sac with 0.3 ml of CuNano using a sterile 27 gauge, 20 mm needle. Before and immediately after injection, the hole was sterilised with alcohol swabs and then sealed with hypoallergenic tape. The eggs were placed in an incubator for 21 days under standard conditions (37.8 °C, 55% humidity, turned once per hour during the first 18 days, and at 37 °C and 60% humidity from day 19 until hatching). The same procedure was repeated for the eggs in batch 2 on the next day. The experimental procedures followed Danish National Legislation.