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RESEARCH ARTICLE

Effect of *in ovo* zinc injection on the embryonic development, tissue zinc contents, antioxidation, and related gene expressions of broiler breeder eggs

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Abstract

Two experiments were conducted to investigate the effect of *in ovo* zinc (Zn) injection on the embryonic development, tissue Zn contents, antioxidation and related gene expressions of fertilized eggs of Arbor Acres broiler breeders. Experiment 1 was conducted to determine an optimal embryonic age for early *in ovo* injection. A total of 720 fertilized eggs with similar weights were randomly allotted to 4 treatments with 6 replicates per treatment and 30 eggs per replicate in a completely randomized design. The eggs were injected with 0.1 mL sterilized water at 3, 6 and 9 embryonic days of incubation (E3, E6 and E9) or non-injection (the control), respectively. The results from experiment 1 showed that E3 and E6 injections increased (P<0.05) the embryonic mortalities, and decreased (P<0.05) hatchabilities compared to the non-injected control, but no differences (P>0.05) between E9 injection and the non-injected control were observed in either embryonic mortality or hatchability. The findings suggest that the E9 is the optimal embryonic age for early *in ovo* injection. In experiment 2, a total of 672 fertilized eggs with similar weights were randomly allocated to 7 treatments with 6 replicates per treatment and 16 eggs per replicate in a completely randomized design. The eggs were injected with 0 (the negative control), 50, 100, 150, 200, or 250 μg Zn/egg as reagent grade $ZnSO_4 \cdot 7H_2O$ in a 0.1-mL solution, or non-injection (the positive control), respectively at E9–10. The results from the experiment 2 demonstrated that no differences (P>0.05) among 50 and 100 μg Zn/egg groups and the negative control were observed in the embryonic mortality and hatchability, however, the injection of 200 μg Zn/egg increased (P<0.05) the embryonic mortality, and injections of 150 and 200 μg Zn/egg decreased (P<0.05) hatchabilities compared with the controls. The embryonic tibia Zn contents at E20 were increased (P<0.05) by injections of 150, 200 and 250 μg Zn/egg. Zinc injection did not affect (P>0.05) malonaldehyde (MDA) contents, copper- and Zn-containing superoxide dismutase (CuZnSOD) activities and mRNA expression levels in the liver and heart of chick embryos at E15 and E20. Compared with the negative control, injections of 50, 150 and 200 μg Zn/egg up-regulated (P<0.05) the

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metallothionein (MT) mRNA expression levels in the embryonic liver at E20. These results indicated that *in ovo* Zn injections increased Zn contents in the embryonic tibia and MT mRNA expression levels in the embryonic liver at E20, however, injections of 150–200 μg Zn/egg were harmful to the embryonic development.

Keywords: *in ovo* injection, zinc, embryonic development, gene expression, broiler breeder egg

1. Introduction

Because of the increased metabolic rate of the embryos of today, the embryonic nutrient reserves are insufficient and might be depleted in the prenatal period (Yair *et al*. 2013). Such nutritional insufficiencies may induce long-lasting adverse consequences on progeny performance (Petry and Hales 2000). Therefore, intervention strategies involving pre-hatch nutrient supplementation have been developed to reduce nutritional restrictions (Oliveira *et al*. 2015). *In ovo* injection technology provides a practical means to safely introduce external nutrients into developing embryos (Foye *et al*. 2007; Kadam *et al*. 2008; Bello *et al*. 2014). Feeding the embryo before hatch by *in ovo* administration of external feed components was reported to cause a positive effect on hatchability, development of the digestive tract, body weight and nutritional status of the hatchling (Uni and Ferket 2004).

Zinc (Zn) is an essential trace element for normal growth, bone development, feathering, enzyme structure and function, and appetite regulation for all avian species (Park *et al*. 2004). Zinc plays an essential role in a wide variety of biochemical processes (Vallee and Auld 1990), and it acts as an antioxidant supplement for protecting poultry against oxidative damage. Dietary Zn supplementation or intravenous Zn injection could enhance expression levels of metallothionein (MT) and copper- and Zn-containing superoxide dismutase (CuZnSOD) as free radical scavengers in tissues of broilers (Huang *et al*. 2007, 2009, 2013; Liu *et al*. 2011, 2013, 2015; Liao *et al*. 2013; Shen *et al*. 2013; Li *et al*. 2015; Suo *et al*. 2015). Zinc deficiency in maternal purified or semi-purified diets of laying breeder hens resulted in a decreased hatchability, abnormal embryonic development, and poor growth performance of offspring birds, whereas maternal dietary Zn supplementation could eliminate these negative effects (Kienholz 1961; Zhu 2016; Zhu *et al*. 2017). *In ovo* injection of a nano form of either Zn (20 μg/egg) or copper or selenium through the amniotic cavity at 18 d of incubation did not affect the embryonic development or hatchability (Joshua *et al*. 2016). Yair and Uni (2011) reported that injecting a mixture of Zn (600 μg/egg) and other minerals into the amniotic cavity at 17.5 d of incubation increased yolk Zn level and Zn consumption of the broiler embryos. *In ovo* injection of a solution containing Zn (600 μg/egg), vitamins and carbohydrates improved mechanical properties of bone of offspring broilers at 3 and 14 d of age posthatching (Yair *et al*. 2013). Bakyaraj *et al*. (2012) injected a mixture of Zn (80 μg/egg) and other trace elements into the amniotic cavity of the 18-d-old embryos, and found that the enrichment might modulate the cell-mediated immunity of offspring broilers. In another study, Oliveira *et al*. (2015) reported that the injection of organic Zn (81.6 μg/egg), manganese and copper into the amniotic cavity at 17 d of incubation had the potential to improve bone mineralization, but negatively affected the hatchability of fertilized eggs.

During incubation, the first 2 weeks are an important period for the formation of embryonic organs and tissues (Macalintal 2012). The yolk sac is mainly responsible for the transfer of nutrients needed for energy and tissue growth (Noble and Cocchi 1990). Yair and Uni (2011) reported that broiler embryo's consumption of Zn was medium until E 11, and then increased between E11 and E17, suggesting an increased need of Zn during the middle stage of incubation. However, in previous studies (Yair and Uni 2011; Bakyaraj *et al*. 2012; Yair *et al*. 2013; Oliveira *et al*. 2015; Joshua *et al*. 2016), *in ovo* injections of Zn (20 to 600 μg/egg) and other nutrients mainly focused on the later stage (E17–18) of incubation and their effects on embryos and offspring birds. No studies on the effect of *in ovo* Zn injection of chick embryos at the early stage of incubation on the embryonic development and antioxidation have been reported before, although maternal dietary Zn supplementation has been shown to play an important role in the embryonic development and antioxidation (Kienholz 1961; Zhu 2016; Zhu *et al*. 2017). We hypothesized that *in ovo* Zn injection at the early stage of incubation might be able to increase the embryonic Zn content and antioxidation during the prenatal period, which would have a positive effect on the embryonic development. Therefore, the objective of the current study was to investigate the effect of graded levels of *in ovo* Zn injection at the early stage of incubation on the embryonic development, tissue Zn contents, antioxidation, and related gene expressions of broiler breeder eggs.

2. Materials and methods

2.1. Experimental design and treatments

All experimental procedures were approved by the Animal