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Effects of nanosize zinc oxide on zinc retention, eggshell quality, immune response and serum parameters of aged laying hens

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ABSTRACT

The aim of this study was to investigate how dietary supplementation of nanosize zinc oxide affect zinc retention, egg production and eggshell quality, immune response and serum parameters of aged layers. In trial 1, twenty white Leghorn laying hens (68 weeks-old) were assigned to the control, ZnO, organic-Zn (Zn-methionine) and nano-Zn (nanosize ZnO) groups. The Zinc was maintained at a 60 mg/kg level in the treatment groups' diet, while the control group's diet contained 40 mg/kg Zn to evaluate the nutrient retention and zinc bioavailability. In trial 2, eighty old white Leghorn laying hens (68 weeks-old) were randomly allotted to four dietary treatments (as trial 1) to evaluate the egg production and eggshell quality, immune response and serum parameters. The results of trial 1 indicated that there were no differences in nutrient retention among the groups, but zinc retention was significantly higher in the nano-Zn and organic-Zn groups than that in the control and ZnO groups ($P < 0.05$). Trial 2 results indicated that eggshell thickness was increased in the nano-Zn and organic-Zn groups compared to the control group ($P < 0.05$); immune responses parameters, including: PHA (phytohemagglutinin) skin challenge test result, GRBC (goat red blood cells) antibody titer and IgG levels exhibited no differences among the groups; serum growth hormone concentration and carbonic anhydrase activity was significantly higher in the nano-Zn and organic-Zn groups compare to control group ($P < 0.05$); serum albumin concentration in organic-Zn group was lower than that of the control group ($P < 0.05$). In conclusion, nanosize zinc for dietary supplementation can increase zinc retention, as well as enhance eggshell thickness, serum carbonic anhydrase activity and growth hormone level of layers. We therefore concluded that nanosized zinc oxide can enhance zinc absorption in the intestine of layers compare to regular zinc oxide.

1. Introduction

Zinc bioavailability in monogastric animals is low (Brody, 1994). Notably, cereal grain contains phytic acid which impairs zinc absorption, calcium, also antagonist with zinc absorption (McDowell, 2003). Although absorption can be increased by using organic zinc, improvement is still needed (Schell and Kornegay, 1996; Cao et al., 2002).

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Zinc is an essential nutrient with with widely varying functions in many important enzymatic processes of glucose, protein and lipid metabolism, and in hormone production and secretion (*e.g.,* growth hormone, insulin and sex hormone); thus, it can influence animals production and reproduction performance. Since zinc is a conjugate with DNA-binding protein, it regulates gene expression and participates in nucleic acid and protein synthesis, which affects. Moreover, zinc keeps the skin healthy (Brody, 1994; McDowell, 2003).

Zinc influences immune system functioning and can affect the thymus secretion of thymulin, which stimulates T-cell production. Thus, a zinc deficiency can cause thymus withering, which causes immune function problems (Brody, 1994; Mocchegiani et al., 1998b; Saha et al., 1995; Wellinghausen et al., 1997), so that bacterial infection can easily occur (Mugenio et al., 2000). Zinc is also involved in the production of cytokines such as IL-1, IL-6 and TNF-α (Wellinghausen et al., 1997). Zinc also plays a role in regard to comitogenic action: it can enhance the immune response of PHA (phytohemagglutinin) and LPS (lipopolysaccharide) injection (Mocchegiani et al., 1998a; Wellinghausen et al., 1997).

A common problem in old layers is thin eggshells, which result in easily broken eggs. Carbonic anhydrase is plays a role in the reaction of $CO_2 + H_2O$ becoming HCO_3^- which is the key component of eggshells (HCO_3^- +Ca incorporation to $CaCO_3$). Thus, zinc is important in eggshell quality of layers.

In practice, chickens generally have high levels of zinc (200–2000 ppm) added to their diet to minimize the problem of watery excreta, though the recommended NRC is only 35 ppm (NRC, 1994). This has resulted in a high residue of zinc in the excreta of chicken, as well as an environment pollution problem. Therefore, enhancement of zinc bioavailability can help to solve this problem.

Recent developments in nanotechnology has brought new trends to many fields. As nutrients are digested, large molecules are degraded into small ones As nutrients are digested, large molecules are degraded into small ones so that they can pass easily through the intestinal mucosa for absorption. The surface area of nanosize minerals is 1250 times of that of macrosize minerals (Rajendran, 2013). Thus, reducing the material size to nanoscale may increase their absorption and utilization. Some reports indicate that nanosize drugs and minerals have increased absorption (Florence et al., 1995; Desai et al., 1997; Davda and Labhasetwar, 2002; Win and Feng, 2005). Lien et al. (2009) reported that chromium nanoparticles significantly elevated the chromium availability and serum chromium level in rats. Nanosize copper has also been demonstrated to increase its availability (Gonzales-Eguiaet al., 2009).

This study hypothesized that the nanoparticles zinc supplementation in aged layers have positive effects on zinc retention, eggshell quality and immunity. Therefore, this study examined the effect of nanosize zinc on zinc retention, egg production and egg shell quality, immune response and serum parameters of old layers.

2. Materials and methods

In this study, two trials were conducted. Trial 1 was designed to examine the effects of nanosize zinc supplementation on nutrient retention. Trial 2 was conducted to determine the effects of nanosize zinc supplementation on egg production, eggshell quality, immune response and the serum parameters of layers. Animals used in this study were cared for according to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.*

2.1. Zinc nanoparticle preparation

Zinc oxide nanoparticles (nano-Zn) were prepared using the wet polish method with a ball grinding machine (Just Nanotech Co., JBM-B035, Taiwan). A mixture of dry ingredients composed of 10 g of zinc oxide (ZnO, 0.045 ± 0.015 mm) and 2.5 g of dispersed reagent-silica was added to 240 mL of 95% ethanol to form a slurry. The slurry was pre-mixed for 1.5 h and then placed in a grind chamber with 500 g of 0.2 mm zirconium particles. The mixture then was ground for 1.5 h at 2388 rpm. After grinding, the slurry passed through a 200 mesh sieve to remove large particles. The slurry was then oven-dried at 50 °C overnight. The zinc nanoparticles powder was then again passed through a 200 mesh sieve.

The zinc content of nano-Zn was determined by atomic absorption spectrometry (PerkinElmer, Atomic Analyst 100, USA).

2.2. Nanoparticle size determination

A small amount of nano-Zn powder was added to 5.0 mL methanol, and then shaken in a water bath for 5 min. A small drop of sample was added onto a 3 mm diameter TEM sample support film mesh grid (TED PELLA, Inc, 200-Cu), and then vaporized for TEM measuring. Particle size was determined using a transmission electronic microscope (JEM 2100, JOEL, Japan).

2.3. Trial 1: nutrients retention of layers

Twenty layers (Hendrix,68 weeks-old) were housed in individual metabolism crates. Treatments included the control (basal diet, Table 1) (NRC, 1994), the ZnO (Shimakyu's Co., Japan), the organic-Zn (Zn-methionine, American Elements Inc., USA) and the nano-Zn groups. The dietary zinc supplements were 60 mg/kg in the experimental group and 40 mg/kg in the control group. The layers were kept in individual cages (35 cm × 20 cm), and plastic plates were used for collecting total excreta. Feed was restricted to about 100 g/day, and water was given *ad libitum*. After 7 day of adaptation to the metabolic