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Effects of zinc-bearing clinoptilolite on growth performance, cecal microflora and intestinal mucosal function of broiler chickens

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ABSTRACT

This study was conducted to evaluate the effect of zinc-bearing clinoptilolite (ZnCP) addition on growth performance, cecal microflora, intestinal antioxidant function and status, immune function of broiler chickens. A total of 300 one-day-old Arbor Acres chickens were randomly divided into 5 groups with 6 replicates of 10 birds each for a 42-day feeding trial. The dietary treatments were as follows: (1) control (CON) fed a basal diet; (2) the basal diet plus antibiotic (40 mg chlortetracycline per kg of diet, ANT); (3) the basal diet plus 0.1% ZnCP; (4) the basal diet plus 0.2% ZnCP; (5) the basal diet plus 0.4% ZnCP. ZnCP treatments had positive effect on average daily gain (ADG) (linear, $P = 0.005$; quadratic, $P = 0.093$) of broilers during 1–21 days. The population of *Escherichia coli* in cecal content decreased linearly ($P = 0.048$) and quadratically ($P = 0.045$) on day 21 and day 42 (linear, $P = 0.032$; quadratic, $P = 0.081$) with increasing ZnCP levels. The activities of total superoxide dismutase (T-SOD) (linear, $P = 0.001$) and Cu–Zn superoxide dismutase (Cu–Zn SOD) (linear, $P < 0.001$) in jejunal mucosa were increased by supplementary ZnCP at 21 day. Malondialdehyde (MDA) contents in jejunum (linear, $P = 0.001$; quadratic, $P = 0.008$) and ileum (linear, $P = 0.018$; quadratic, $P = 0.012$) were reduced by ZnCP treatments at 21 day. GSH content in jejunum was significantly increased at 21 day (linear, $P < 0.001$; quadratic, $P = 0.009$) by ZnCP treatments. Compared to CON and ANT, 0.2% and 0.4%ZnCP treatments significantly increased immunoglobulin G (IgG) in jejunal mucosa at 21 day ($P < 0.05$), and 0.1% ZnCP supplementation improved the content of secretory immunoglobulin A (sIgA) in jejunum at 42 day ($P < 0.05$). The results suggested that supplementation with 0.1% or 0.2% ZnCP modulated microbial populations and improved immune function and antioxidant status in the gastrointestinal (GI) tract, which may exert protective effects on the integrity of the mucosal barrier function of broilers, was as efficacious as 40 mg chlortetracycline per kg in enhancing growth performance. The results indicated that ZnCP may be used as a new growth promoter instead of antibiotics in poultry feed.

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; ANT, antibiotic; CFU, colony-forming units; CON, control; Cu–Zn SOD, Cu-Zn superoxide dismutase; F/G, feed/gain ratio; GI, gastrointestinal; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; IgG, immunoglobulin G; MDA, malondialdehyde; IAA, inorganic antibacterial agents; S.E.M., total standard error of means; sIgA, secretory immunoglobulin A; T-SOD, total superoxide dismutase; ZnCP, zinc-bearing clinoptilolite.

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1. Introduction

Overgrowth of pathogens in the intestinal tract causes induced tissue oxidative stress and immune response (Spurlock, 1997). This frequently results in increased animal mortality, decreased productivity, and contaminated poultry products (Barrow et al., 1987; Gomez et al., 1997). Sub-therapeutic antibiotics have long been used in broiler diets for growth improvement and control of intestinal pathogens. However, issues regarding the development of antibiotic-resistant bacteria and food safety have led to public demand to limit the use of antibiotics in animal feed (Smith et al., 2003). Inorganic materials carrying anti-bacterial ions, termed as inorganic antibacterial agents (MIAA), have recently emerged as a novel alternative to chemical antibiotics with their long-lasting, free of resistance induction in use in animals. (Matsumura et al., 2003; Xia et al., 2005; Hu and Xia, 2006; Hrenovic et al., 2012). Malachova et al. (2011) demonstrated that silver, zinc or copper montmorillionites exerted significant antibacterial and antifungal activities *in vitro* and suggested that the bactericidal activity was partly due to the delivery of metal ions into the gut environment via IAA, which can damage cell walls and inhibit some enzymes, e.g., copper and zinc ions, or can inactivate proteins with SH groups and prevent the ability of DNA to replicate, e.g., silver ion.

Zeolite clinoptilolite, one of the non-metallic mineral clays, has been incorporated in animal diets as an enhancer of nutrient digestibility and growth performance in animals (Mumpton and Fishman, 1977; Nakaue et al., 1981). *In vitro* studies showed that clinoptilolite could adsorb *Escherichia coli*, but lack of bacteriostatic or bactericidal effects (Ramu et al., 1997). However, the addition of silver ion to clinoptilolite via ions exchange exhibited antibacterial effects on *Pseudomonas aeruginosa* and *E. coli* (Top and Ulku, 2004). Zinc-loaded zeolite showed better antibacterial activity for *E. coli* than copper-loaded zeolite (Hrenovic et al., 2012). To our knowledge, no study about the use of zinc-loaded clinoptilolite in replacement of antibiotics in broiler diets has been reported. Our previous study showed that the addition of zinc-bearing clinoptilolite (ZnCP) in broiler diets exerted positive effects on growth performance of broilers against *S. pullorum* infection (Wang et al., 2012). We hypothesize that ZnCP may be a potential material to replace the antibiotic use in broiler diets by improving antioxidant status and immune function in the intestine. Therefore, this experiment was carried out to evaluate the effect of ZnCP addition on growth performance, cecal microflora, intestinal antioxidant function and status, immune function of broiler chickens in comparison to antibiotic treatment.

2. Materials and methods

2.1. Preparation of zinc-bearing clinoptilolite

Zeolite clinoptilolite was provided by Zhenjiang Dantu Maoshan Zeolite Co., Ltd. (Zhenjiang, PR China) and sieved through a 200-mesh sieve. The content of clinoptilolite in the natural zeolite was above 75% as determined by X-ray diffraction.

The ZnCP was prepared using ion exchange method according to Wang et al. (2012). Clinoptilolite was firstly calcinated at around 350 °C for 2 h in muffle oven. After cooling down, clinoptilolite was added into (1: 10 wt./vol) $ZnCl_2$ solution of 2.5 mol/L. The mixture was blended at 70 °C, pH 4.5 and 120 r/min within a constant temperature oscillated instrument for 4 h. The suspension was then further separated by centrifugation at 4,550 × g for 15 min. The sediments were repeatedly washed by deionized water until there was no white deposition in the washed solution when swinging in with 0.1 mol/L $AgNO_3$ solution. Finally, the washed materials were collected and dried at around 105 °C for 2 h in an air oven, and then ground through a 200-mesh sieve. The amount of zinc adsorption onto clinoptilolite was 15.5 mg/g, as determined by microwave dissolution with inductively coupled plasma mass spectrometry.

2.2. Experimental design, diets, and management

The experimental design and procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

A total of three hundred one-day-old Arbor Acres chickens obtained from a commercial hatchery (Hewei Agricultural Development Co., Ltd., Anhui province, PR China), were randomly divided into 5 groups with 6 replicates of 10 birds for a 42-day feeding trial. The average initial body weight did not differ among all groups. The treatments were as follows: (1) control (CON) fed a basal diet; (2) the basal diet plus antibiotic (40 mg chlortetracycline per kg of diet, ANT); (3) the basal diet plus 0.1% ZnCP; (4) the basal diet plus 0.2% ZnCP; (5) the basal diet plus 0.4% ZnCP. The basal diets were formulated based on the NRC (1994) to meet the nutrient requirements of broilers and were devoid of antibiotics. The formulation and calculated nutrient level of basal diet were shown in Table 1.

All birds were placed in wired cages and housed in an environmentally controlled room. Temperature was maintained at 32–34 °C for the first week and then reduced by 2,3 °C per week. Birds were allowed *ad libitum* access to mash feed and water. Chicks were weighed individually at 21 day and 42 day to determine weight gain. Feed consumed on cage basis was recorded, and feed/gain ratio (*F*/*G*, g feed/g gain) was calculated. The number of dead chickens was recorded on a per-cage basis as it occurred, and the mortality rate was calculated.