Effects of Dietary Zinc Bearing Palygorskite Supplementation on the Carcass Traits, Chemical Composition of Muscle, and Muscular Lead and Chromium Contents of Broilers

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The present study was conducted to investigate the effects of zinc (Zn) bearing palygorskite (ZnP) inclusion on the carcass traits, chemical composition of muscle, and muscular lead (Pb) and chromium (Cr) contents of broilers. A total of 240 1-day-old Arbor Acres broiler chicks were randomly divided into 5 dietary treatments with 6 replicates of 8 chicks each. Broilers in the 5 treatments were fed a basal diet supplemented with 0 (Control group), 20, 40, 60, and 80 mg/kg Zn in the form of ZnP for 42 days, respectively. There were no differences in the carcass yield, abdominal fat yield, subcutaneous fat thickness, and intramuscular fat width among treatments ($P > 0.05$). Compared with the control group, the eviscerated yield ($P = 0.010$) and thigh muscle yield ($P = 0.046$) were quadratically increased by the supplementation of ZnP ($P < 0.05$). Similarly, the breast muscle yield was linearly ($P = 0.024$) and quadratically ($P = 0.011$) increased by ZnP inclusion. The addition of ZnP to diets of broilers also linearly ($P = 0.002$) increased fat content in the thigh. Moreover, the supplementation of ZnP linearly and quadratically reduced the content of muscular Pb and the content of Cr in the thigh muscle ($P < 0.05$). It was concluded that ZnP inclusion could improve carcass traits, increase fat content in the thigh, and reduce the accumulations of Pb and Cr in the muscles, and this effect was more pronounced when extra Zn dosage in the form of ZnP was 40 mg/kg.

Key words: broilers, carcass traits, chemical composition, heavy metal, zinc bearing palygorskite

Introduction

Zinc (Zn) is an essential trace mineral and acts both structurally and catalytically in various metalloenzymes (Vallee and Falchuk, 1993; Gaither and Eide, 2001; Park et al., 2004; Salim et al., 2008). These enzymes play multiple roles in metabolism (Underwood and Suttle, 1999) and other functions, such as immune response (Kidd et al., 1996) and antioxidation status maintenance (Powell, 2000). However, lots of feedstuffs are marginally deficient in Zn. Therefore, Zn is commonly supplemented to poultry diet in the form of inorganic Zn sources as Zn sulfate or Zn oxide (Sandoval et al., 1997; Batal et al., 2001; Huang et al., 2009). Although the NRC (1994) recommends that broiler diets contain 40 mg/kg of Zn, these diets are often formulated to contain dietary Zn at about 120 mg/kg since a higher concentration of dietary Zn can reduce the possibility of Zn deficiency under commercial conditions (Burrell et al., 2004). In broilers, extra Zn supplementation, irrespective of sources, has been proved to improve carcass traits and chemical composition of muscles (Liu et al., 2011).

Palygorskite (Pal) is a natural hydrated magnesium aluminum silicate clay mineral with chain-layered crystal structure, and it has considerable micropores and channels that are comprised by silicon oxygen tetrahedron and aluminum-oxygen octahedral chains (Galan et al., 1994; Bergaya et al., 2006; Brigatti et al., 2006; Bergaya and Lagaly, 2013). The chemical and physical characteristics of Pal endow it with adsorption property, good adhesive ability and cation exchange capacity (Galan, 1996; Galan and Carretero, 1999; Murray, 2000; Carretero, 2002; Chen and Wang, 2007; Huang et al., 2007; Zhou, 2011). In animal nutrition, Pal has been widely used as pellet binder, feed ingredients or feed supplement to promote growth, maintain health, enhance immunity, and detoxify toxins (Schell et al., 1993; Zaid et al., 1995; Pappas et al., 2010; Ministry of Agriculture of China, 2013; Zhang et al., 2013). Due to their potential neurotoxic, teratogenic and lethal effect, the accumulations of heavy metals including lead (Pb), chromium (Cr) and cadmium (Cd) would pose a threat to all forms of life including human, animal, plant and aquatic life through the
food chain (Carpenter, 1987; Goyer, 1996; Ruff et al., 1996; Zhuang et al., 2009). Previous studies have shown that Pal, as an effective solution, could be used for the removal of heavy metals both in vitro (Alvarez-Ayuso and Garcia-Sanchez, 2003; Potgieter et al., 2006; Fan et al., 2009) and in vivo (Zhang et al., 2015; Cheng et al., 2016).

Zn bearing Pal (ZnPal) has been recently prepared using solid state ion exchange method (Yan et al., 2016a, 2016b). In an in vivo study, Yan et al. (2016a) have newly found that ZnPal as a potential Zn source displayed a higher bio-availability than Zn sulfate during a 42-day study in broilers. In a previous study (Yang et al., 2016b), it has been demonstrated that ZnPal supplementation could also improve meat quality and its antioxidant capacity whereas did not affect growth performance and muscular Zn content of broilers. However, little was known about extra ZnPal supplementation on carcass traits, chemical component of muscle, and the accumulations of muscular Pb and Cr in broilers. Therefore, the current study was conducted to investigate the effects of ZnPal inclusion on carcass traits, chemical component of muscle, and the accumulation of Pb and Cr in broilers.

### Materials and Methods

#### Preparation of ZnPal

The Pal was kindly provided by Jiangsu Sinitic Biotech Co., Ltd. (Xuyi, Jiangsu, P. R. China) and sieved by a 200-mesh sieve (diameter, 0.074 mm). The main chemical compositions of Pal determined by a Minipal 4X ray fluorescence spectrometer (PANalytical, Netherland) are listed in the following: SiO2, 59.11%; MgO, 12.75%; Al2O3, 10.31%; CaO, 7.42%; Fe2O3, 6.21%; Na2O, 1.27% and K2O, 1.19%.

ZnCl2 (≥ 98.0%; 4:1, wt/wt) purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, Jiangsu, P. R. China) in a stainless steel blade grinder. The mixture was subsequently calculated at around 300°C for 3 h in a muffle oven. After cooling down, Pal was mixed with ZnCl2 (ZnCl2 ≥ 98.0%; 4:1, wt/wt) purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, Jiangsu, P. R. China) in a stainless steel blade grinder. The mixture was subsequently calculated at around 300°C for 3 h in a muffle oven. After cooling down to room temperature, the mixture was washed repeatedly by deionized water until there was no white deposition generated in the washed solution when swigged with

### Table 1. Composition and nutrient level of basal diet (g/kg, as fed basis unless otherwise stated)

| Ingredients                  | 1–21 days | 22–42 days |
|------------------------------|-----------|------------|
| Corn                         | 576.1     | 622.7      |
| Soybean meal                 | 310       | 230        |
| Corn gluten meal             | 32.9      | 60         |
| Soybean oil                  | 31.1      | 40         |
| Limestone                    | 12        | 14         |
| Dicalcium phosphate          | 20        | 16         |
| L-Lysine                     | 3.4       | 3.5        |
| DL-Methionine                | 1.5       | 0.8        |
| Sodium chloride              | 3         | 3          |
| Premix a                     | 10        | 10         |
| Calculated nutrient levels   |           |            |
| Apparent metabolizable energy (MJ/kg) | 12.56  | 13.19      |
| Crude protein                | 211       | 196        |
| Calcium                      | 10.00     | 9.50       |
| Available phosphorus         | 4.60      | 3.90       |
| Lysine                       | 12.00     | 10.50      |
| Methionine                   | 5.00      | 4.20       |
| Methionine + cystine         | 8.50      | 7.60       |
| Analyzed compositionb        |           |            |
| Crude protein                | 208       | 192        |
| Ash                          | 57.2      | 56.5       |

*a Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (all-rac-tocopherol), 30 IU; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine-HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamin), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.

b Values based on analysis of triplicate samples of diets.
and defeathering. The head, feet, abdominal fat (fat surrounding the cloaca and the gizzard), and all of the viscera except the kidney were then further removed to determine eviscerated yield based on live weight. The left pectoralis major muscle and thigh muscle were excised without skin and immediately weighed to calculate the yield based on eviscerated weight. After that, the left pectoralis major muscle and thigh muscle samples were immediately frozen and stored at −20°C for further analysis.

Table 2. Analyzed mineral elements content in the diets (mg/kg)

| Items | 0 (Basal diet) | 20 | 40 | 60 | 80 |
|-------|----------------|----|----|----|----|
| 1–21 d |                |    |    |    |    |
| Zn    | 81 ± 2         | 102 ± 3 | 120 ± 2 | 144 ± 4 | 165 ± 3 |
| Fe    | 277 ± 4        | 280 ± 7 | 276 ± 5 | 283 ± 7 | 286 ± 6 |
| Cu    | 22.8 ± 0.3     | 21.9 ± 0.7 | 22.5 ± 0.5 | 22.9 ± 0.6 | 23.5 ± 0.4 |
| Mn    | 164 ± 6        | 163 ± 3 | 168 ± 4 | 170 ± 5 | 174 ± 6 |
| Mg    | 1825 ± 13      | 1821 ± 19 | 1848 ± 14 | 1831 ± 12 | 1817 ± 14 |
| Pb    | 2.15 ± 0.19    | 2.05 ± 0.09 | 2.17 ± 0.28 | 1.98 ± 0.08 | 2.13 ± 0.06 |
| Cr    | 1.26 ± 0.18    | 1.01 ± 0.21 | 0.96 ± 0.08 | 1.08 ± 0.13 | 1.20 ± 0.03 |
| 22–42 d |               |    |    |    |    |
| Zn    | 88 ± 4         | 106 ± 5 | 128 ± 3 | 146 ± 3 | 170 ± 2 |
| Fe    | 271 ± 6        | 264 ± 5 | 260 ± 4 | 274 ± 7 | 283 ± 4 |
| Cu    | 24.8 ± 1.0     | 23.5 ± 0.8 | 22.7 ± 0.6 | 23.9 ± 0.4 | 23.0 ± 0.5 |
| Mn    | 194 ± 6        | 197 ± 4 | 186 ± 4 | 188 ± 5 | 193 ± 7 |
| Mg    | 1819 ± 17      | 1835 ± 11 | 1825 ± 18 | 1856 ± 22 | 1885 ± 19 |
| Pb    | 1.93 ± 0.12    | 2.01 ± 0.06 | 1.83 ± 0.11 | 1.79 ± 0.08 | 1.91 ± 0.17 |
| Cr    | 1.21 ± 0.09    | 1.24 ± 0.19 | 1.17 ± 0.14 | 1.31 ± 0.11 | 1.19 ± 0.07 |

1 Values based on analysis of triplicate samples of diets.
2 Means and standard error were presented.

Experimental Design, Diets and Management

All procedures were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee. A total of 240 1-day-old Arbor Acres broiler chicks (initial weight, 36.71 ± 0.18 g) obtained from a commercial hatchery were randomly allocated into five dietary treatments with six replicates (cages) of 8 chicks each (4 males and 4 females/cage). Birds in the 5 treatments were given a basal diet supplemented with 0 (control group), 20, 40, 60, and 80 mg/kg Zn diet as ZnPalm for 42 days, respectively. The formulation and calculated nutrient level of basal diet are described by Zhang (2003). The measured mineral elements contents in the diets are shown in Table 1. The formulation and calculated nutrient level of basal diet are shown in Table 1. The measured mineral elements contents in the diets are shown in Table 2. Birds were allowed ad libitum access to mash feed and water. The water met the national drinking water standard of China (Ministry of Health of China, 2006), in which the concentrations of As <0.01 mg/L, Cd <0.005 mg/L, Cr (VI) <0.05 mg/L, Pb <0.01 mg/L, Hg <0.001 mg/L, Zn <1.0 mg/L. All birds were placed in wire cages and housed in an environmentally controlled room with continuing light. Temperature was maintained at 32 to 34°C for the first 3 days and then reduced by 2 to 3°C per week to a final temperature of 20°C.

Carass Traits

At 42 days of age, 1 male bird from each replicate (cage) was randomly selected and weighed after feed deprivation for 12 h. Chickens were euthanized by cervical dislocation. Subcutaneous fat thickness and intramuscular fat width were measured by a vernier caliper as previously described (Wu et al., 2012). The hot carcasses were weighted after bleeding and defeathering. The head, feet, abdominal fat (fat surrounding the cloaca and the gizzard), and all of the viscera except the kidney were then further removed to determine eviscerated yield based on live weight. The left pectoralis major muscle and thigh muscle were excised without skin and immediately weighed to calculate the yield based on eviscerated weight. After that, the left pectoralis major muscle and thigh muscle samples were immediately frozen and stored at −20°C for further analysis.

Chemical Composition of Muscles

The contents of moisture, crude protein and crude fat of muscle samples (pectoralis major muscle and thigh muscle) were determined according to the standardized procedures as described by Zhang (2003).

Determination of Muscular Mineral Elements

The contents of Zn, Pb, Cr, Cd, and mercury (Hg) in the muscle samples (left major muscle and thigh muscle) were determined according to the method described by Tang et al. (2015) and Yan et al. (2016a). Approximately 2.0 g of muscle sample was firstly weighed into a glass digestion tube, mixed with 10 mL of a mixture acid of nitric acid and perchloric acid (4:1, vol/vol) at room temperature for 12 h, and then digested on a heating block (LabTech DigiBlock Digester, EHD36, Labtech Co., Ltd., P. R. China) to acquire a clear digested solution. The procedure of digestion was in the following: 90°C for 30 min; 120°C for 30 min; 160°C for 120 min; 180°C for 180 min. After that, digested solutions were diluted with ultra-pure water to a final volume of 25 mL. The contents of Zn, Pb, Cr, Cd and Hg in the digested liquid were determined using ICP-MS (Optimal 2100DV, Perkin-Elmer-Sciex, Norwalk, NY, USA). The operating conditions are presented in the following: power, 1300 W; plasma gas flow rate, 12 L/min; auxiliary gas flow rate, 0.2 L/min; nebuliser gas flow rate, 0.55 L/min; sample flow rate,
1.5 mL/min; sample uptake rate, 1.0 mL/min. Validation of the minerals analysis was conducted using a certified bovine liver powder (GBW (E) 080193; National Institute of Standards and Technology, Beijing, P. R. China) as a standard reference material. The Cd and Hg were not detected in the muscles and diets, and therefore are not presented in the Tables.

**Statistical Analysis**

Data were analyzed by one-way ANOVA using SPSS (2008) statistical software (Ver. 16.0 for Windows, SPSS Inc., Chicago, IL, USA). Polynomial contrasts were used to determine the linear and quadratic effects of dietary ZnPal inclusion level. The differences among treatments were examined by Tukey’s test, which were considered to be significant at $P<0.05$. The means and total standard error of means (SEM) were presented.

**Results**

**Carcass Traits**

Compared with the control group (Table 3), the eviscerated yield ($P=0.010$) and thigh muscle yield ($P=0.046$) were quadratically increased by the inclusion of ZnPal. Likewise, the addition of ZnPal linearly ($P=0.024$) and quadratically ($P=0.011$) increased breast muscle yield.

However, no differences were observed in carcass yield, abdominal fat yield, subcutaneous fat thickness, and intramuscular fat width among groups ($P>0.05$).

**Chemical Composition of Muscle**

The supplementation of ZnPal linearly increased the fat content in thigh muscle (Table 4, $P=0.002$) whereas the similar effect was not observed for the fat content in the breast muscle ($P>0.05$). Additionally, neither muscular moisture nor crude protein was altered by ZnPal inclusion ($P>0.05$).

**Muscular Mineral Elements**

As indicated in the Table 5, the supplementation of ZnPal linearly and quadratically reduced the content of muscular Pb and the content of Cr in the thigh muscle ($P<0.05$). No difference was observed in the content of Cr in breast muscle ($P>0.05$).

**Discussion**

In broilers, Saenmahayak et al. (2010) found that complexed Zn supplementation did not influence carcass traits and component yields whereas increased deboned fillet and total breast yields. In the current study, the growth performance of broilers at 42 days of age was similar among groups, and the live weight of broilers at 42 days of age in the
control group and the four ZnPal groups (from 20 to 80 mg/kg Zn) was 2253, 2272, 2233, 2200, and 2309 g, respectively (Yang et al., 2016). However, dietary ZnPal supplementation increased eviscerated yield and muscle yield, indicating that the inclusion of ZnPal could improve the carcass composition of broilers. The increased eviscerated yield resulting from Zn supplementation may be in related with the simultaneously improvement of muscle yield. This result was in agreement with finding of Liu et al. (2011) who reported that extra Zn supplementation, irrespective of sources, increased the percentage of eviscerated yield in broilers. Similar results were also observed by Sahin et al. (2005) in Japanese quail, and Spears and Kegley (2002) in steers. The improved carcass traits induced by the supplementation of ZnPal may be associated with the biological function of Zn which is involved in endocrine function such as the synthesis and secretion of various hormones including osteocalcin, testosterone, thyroid hormones, insulin-like growth factor-1, and insulin, which play vital roles in the growth and development of bone and muscle (Giugliano and Millward, 1984; Ploysangam et al., 1997; Fukada et al., 2011). Huang et al. (2007) demonstrated that the optimal dietary Zn of chicks from hatch to 21 days of age was 84 mg/kg. Similarly, Sunder et al. (2008) reported that basal diet Zn (29 ppm) was adequate to support optimum performance of broilers, and 80 ppm of Zn was required up to 4 wk of age for better mineral retention, immune response, and alleviation of stress. These studies together showed that a higher intake of Zn that exceeded the NRC recommendation (40 mg/kg) was beneficial for the growth of broilers. In this study, the carcass characteristics of broilers were optimized by the extra Zn supplementation at the dosage of 40 mg/kg as ZnPal, which in turn suggested that a higher level of Zn intake was required for the optimal carcass composition.

Muscular fat is very important from the sensory aspect since it is a source of many aromatic substances affecting the meat taste (Suchý et al., 2002). In this study, dietary ZnPal supplementation linearly increased the fat content in thigh. Likewise, Liu et al. (2011) found that the addition of extra Zn to broiler diet increased the dry matter and intramuscular fat contents of the breast muscle. Additionally, Greene et al. (1988) observed that the addition of organic Zn (Zn-Met) increased USDA carcass quality grade, external fat, and kidney, pelvic, and heart fat of steers. Previous studies have shown that the supplementation of Zn bearing clay improved the intestinal morphology and digestibilities of nutrients (Hu et al., 2013; Tang et al., 2014). Thus, the improved muscle composition may be associated with the possibly enhanced nutrient availability resulting from ZnPal supplementation.

In *in vitro* studies, Pal used as an adsorbent can remove Pb and Cr in heavy metal contained water or soil (Alvarez-Ayuso and Garcia-Sanchez, 2003; Potgieter et al., 2006; Fan et al., 2009). In the current study, dietary ZnPal did not alter muscular Zn content (Yang et al., 2016), and it may due to that muscle, unlike pancreas and tibia, was not sensitive to reflect Zn retention (Huang et al., 2007, 2009). However, the supplementation of ZnPal in this study reduced the accumulations of Pb and Cr in the muscles, and it indicated that ZnPal supplementation could improve the safety of meat products. Similar results were also observed by Cheng et al. (2016) who reported that dietary Pal inclusion, at either 10 or 20 g/kg, significantly decreased Pb accumulation in the breast or thigh muscle. Additionally, Zhang et al. (2015) reported that 2% Pal supplementation reduced muscular Cd accumulation in blunt snout bream. Inclusion of diets with excess minerals could lead to antagonism. The antagonistic effects between Zn and heavy metals have been well demonstrated (Waalke and Poirier, 1984; Fosmire, 1990; Kargin and Çogun, 1999; McDonald et al., 2011). Thus, the reduced accumulation of heavy metals may also result from the antagonism induced by extra Zn supplementation in the form of ZnPal.

The newly total maximum Zn content recommended by EU are 150 mg/kg Zn complete feed for piglets, sows, rabbits, salmonids, cats and dogs; 120 mg/kg Zn complete feed for turkeys for fattening; 100 mg/kg Zn complete feed for all other species and categories, and it would result in an overall reduction of Zn emission from animal production of about 20% (EFSA, 2014). Under commercial conditions, diets for broiler chickens are always designed to contain Zn at approximately 120 mg/kg to acquire the optimal growth performance (Burrell et al., 2004). However, the major of

### Table 5. The contents of heavy toxic metals in breast and thigh muscles (mg/kg fresh sample)

| Items | Zn as ZnPal<sup>1</sup> (mg/kg) | SEM<sup>2</sup> | P-value |
|-------|-------------------------------|---------------|---------|
|       | Control | 20 | 40 | 60 | 80 | Control vs ZnPal | Linear | Quadratic |
| Breast | 1          | 0.124<sup>a</sup> | 0.118<sup>b</sup> | 0.113<sup>b</sup> | 0.112<sup>b</sup> | 0.120<sup>ab</sup> | 0.001 | 0.015 | 0.039 | 0.016 |
| Pb     | 0.286     | 0.253 | 0.254 | 0.290 | 0.313 | 0.010 | 0.357 | 0.406 | 0.070 |
| Cr     | 0.281<sup>ab</sup> | 0.300<sup>a</sup> | 0.266<sup>ab</sup> | 0.242<sup>bc</sup> | 0.218<sup>c</sup> | 0.007 | <0.001 | <0.001 | <0.001 |
| Thigh  | 1          | 0.120<sup>a</sup> | 0.128<sup>a</sup> | 0.123<sup>a</sup> | 0.083<sup>b</sup> | 0.083<sup>b</sup> | 0.004 | <0.001 | <0.001 | 0.003 |

<sup>a,b</sup> Means within a row with different superscripts are different at P<0.05.

<sup>1</sup> ZnPalm, zinc bearing palygorskite.

<sup>2</sup> SEM, total standard error of means.
ingested Zn (around 94%) in broilers is excreted actually, and it can in turn lead to Zn resource waste, and subsequently increase Zn load on the environment (Mohanna and Nys, 1997, 1999). To avoid these problems, Zn sources with a higher bioavailability would be a new prospect. Clays (as Pal and zeolite) with plentiful pores, high aspect ratio, high specific surface area, and good ion-exchange capacity can be used as carriers of active components (Murray, 2000; Tang et al., 2015; Yan et al., 2016a). In broilers, Yan et al. (2016b) have shown that Zn-Pal had a higher bioavailability than ZnSO₄ in broiler diets, and the optimal level of supplemented Zn for broilers was 60 mg/kg in the form of Zn-Pal. Previous studies have also indicated, zeolite bearing Zn exhibited a higher bioavailability than ZnSO₄ in broilers (Tang et al., 2015) and laying hens (Li et al., 2015). These studies together indicated that clay bearing Zn may be a prospect Zn source in the future.

In conclusion, the present study demonstrated that Zn supplementation as ZnPal (from 0 to 80 mg/kg) espically at the dosage of 40 mg/kg improved the eviscerated and muscle yields, increased the fat content in thigh muscle, and decreased the accumulation of Pb and Cr in breast and thigh muscles.

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