INTRODUCTION

Salmonella pullorum, a gram-negative bacteria pathogen, can cause pullorum disease (PD). Pullorum disease is an acute systemic disease more common in young birds and is associated with outbreaks characterized by high mortality and with reductions in productivity. Although PD is rare in a modern poultry industry of the developed countries due to extensive test and control of breeder birds, the disease gained a large number of incidents in recent years in many parts of the world, such as in South America, some countries of Europe, Africa, and especially Asia (Pan et al., 2009; Barrow and Freitas Neto, 2011). In China, more than 18,000 outbreaks of PD were reported from 2008 to June 2011 (OIE, 2012). Furthermore, infection with Salmonella leads to diarrhea and intestinal lesions and an influx of heterophils into the gut accompanied by inflammation and damage to villi (Barrow et al., 1987). Moreover, the leading cause of human foodborne infections in the world, associated with consumption of poultry products, however is Salmonella (Gomez et al., 1997). Besides slaughter and vaccines, antimicrobial agent treatment is still being used as a measure to control PD (Pan et al., 2009; Barrow and Freitas Neto, 2011). The widespread use of antibiotics has also raised concerns about increased antibiotic resistance of microorganisms for both animals and humans (Sarmah et al., 2006). In a study evaluating the antimicrobial resistance patterns of S. pullorum strains isolated between 1962 and 2007 from PD chickens in China, high levels of resistance were found to some antimicrobial agents and there was an increasing trend in the resistance among 2000 to 2007 strains (Pan et al., 2009). Therefore, attention has been drawn for possible alternatives to antibiotics (Joerger, 2003), with the perception that antibiotics should no longer be used in feed, and it is imperative that safe antibacterial agents or such feed additives must be found to replace the use of antibiotics for controlling Salmonella in poultry production (Van Immerseel et al., 2002). In recent years, many additives were found to act as antibacterial agents and protect chickens from Salmonella infection, such as probiotics (Carina Audisio et al., 2000; Al-Zenki et al., 2009), prebiotics (Spring et al., 2000; Haldar et al., 2011), and
The inorganic metal-bearing minerals, such as copper-bearing montmorillonite, were also found to be effective as antibacterial agents in animal production (Xia et al., 2004a, b, 2005). Zeolites are hydrated natural or synthetic microporous crystals with well-defined structures containing AlO₄ and SiO₄ tetrahedra linked through the common oxygen atoms (Pavelić et al., 2001). Clinoptilolite is one of the many varieties of natural zeolites and has a total pore volume in the range of 35%, with strong adsorptive and ion exchange capacity as well as clay mineral (Leung et al., 2007). Natural zeolites can be used as feed additives in the prevention and the treatment of certain farm animal diseases (Papaioannou et al., 2005; Shariatmadari, 2008) and improving performance of broilers (Suchy et al., 2006; Karamanlis et al., 2008). Antibacterial effect of modified clinoptilolite through silver, zinc, and copper ion exchange was reported in a previous in vitro study (Top and Uku, 2007). Antibacterial effect of modified clinoptilolite against S. pullorum and 0.8% zinc-bearing clinoptilolite (ZnCP) in saline solution almost completely inhibited Salmonella and 0.8% zinc-bearing clinoptilolite (ZnCP) in saline solution almost completely inhibited Salmonella growth in culture (T. T. Zhang, unpublished data). Therefore, this experiment was carried out to evaluate the effect of ZnCP addition in feed on protecting broilers from S. pullorum challenge.

### MATERIALS AND METHODS

#### Experimental Design and Dietary Treatments

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

A total of 240 straight-run 1-d-old Arbor Acres chickens was obtained from a commercial hatchery (Hewei, Anhui province, P. R. China) and randomly divided into 4 treatment groups with 6 replicates of 10 birds per treatment for a 21-d feeding trial. The average initial BW did not differ among the 4 groups. The 4 treatments were as follows: 1) nonchallenge control (CON) fed a basal diet; 2) S. pullorum challenge control (SCC) fed the basal diet; 3) S. pullorum challenge fed the basal diet plus antibiotic (50 mg of chlortetracycline/kg of diet; ANT); 4) S. pullorum challenge fed the basal diet plus 0.2% ZnCP (ZnCP). The basal diet was formulated based on the NRC (1994) to meet the nutrient requirements of the broilers and was devoid of antibiotics. The formulation and nutrient level of basal diet were shown in Table 1.

#### Preparation of Zinc-Bearing Clinoptilolite

The ZnCP was prepared using the ion exchange method. Clinoptilolite was first calcined at 400°C for 2 h in a muffle oven. After cooling down, clinoptilolite was added into a zinc chloride solution (1:10, wt/vol) of 1 mol/L. The mixture was blended at 60°C, at pH 4.0, and 90 rpm within a constant-temperature oscillated instrument for 3 h. The suspension was then further separated by centrifugation at 4,450 × g for 15 min. The lower sediments were repeatedly washed by deionized water until there was no white deposition in the washed solution when added with silver nitrate. 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 7.6 mg/g, as determined by microwave dissolution with inductively coupled plasma mass spectrometry. Clinoptilolite was provided by Zhenjiang Dantu Maoshan Zeolite Co. Ltd. (Zhenjiang, P. R. China) and was sieved through a 200-mesh sieve. The content of clinoptilolite in the natural zeolite was above 70% as determined by X-ray diffraction.

### Table 1. The formulation and calculated nutrient levels of broiler basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Content (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>578</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>325</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>30</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>27</td>
</tr>
<tr>
<td>Limestone</td>
<td>9.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>17.5</td>
</tr>
<tr>
<td>Salt</td>
<td>3</td>
</tr>
<tr>
<td>Premix¹</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
</tr>
</tbody>
</table>

Calculated nutrient levels²

- ME (kcal/kg): 2,990
- CP (g/kg): 211.5
- Calcium (g/kg): 9.7
- Available phosphorus (g/kg): 4.2
- Lysine (g/kg): 10.8
- Methionine (g/kg): 4.8
- Methionine + cysteine (g/kg): 8.1

¹Premix provided per kilogram of diet: limestone, 3.3 g; l-Lysine-HCl, 1.5 g; dl-Methionine, 1.3 g; vitamin A (transretinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (all-rac-α-tocopherol acetate), 50 IU; menadione, 1.3 mg; thiamine, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 600 mg; calcium pantothenate, 10 mg; pyridoxine-HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B₁₂ (cobalamine), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc oxide), 65 mg; iodine (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.

²The nutrient levels were on an as-fed basis.

All birds were placed in wired 3-level battery cages and housed in an environmentally controlled room maintained at 32 to 34°C for the first week and then reduced by 2 to 3°C per week. Birds were allowed ad libitum access to feed and water. The BW, feed intake, and mortality of chicks were recorded.
Salmonella Challenge

Birds in SCC, ANT, and ZnCP treatment groups were inoculated orally with \textit{S. pullorum} of $4 \times 10^4$ cfu bacteria per bird on d 3 posthatch, and the chicks of CON were inoculated with the same volume of sterilized saline. The strain of \textit{S. pullorum} (CVCC 533) was supplied by the China Veterinary Culture Collection Center (Beijing, P. R. China).

Sample Collection and Procedures

At 7 and 21 d, one male bird per replicate was randomly selected and weighed after feed deprivation for 12 h. Individual blood samples were taken and serum were separated by centrifugation at 3,900 $\times$ g for 15 min at 4°C. Serum samples were frozen at $-20°C$ for further analysis. After collection of blood samples, chickens were euthanized and necropsied immediately. The cecum tissues were quickly removed aseptically, and the \textit{Salmonella} and \textit{Lactobacillus} colonies in cecal contents were determined. The spleen, thymus, and bursa of Fabricius were calculated as weight of organ (g)/BW (kg).

Growth Performance and Relative Weights of Immune Organs

Body weights were recorded for each replicate at 1 and 21 d of age. Feed was withdrawn for 12 h and water was provided for ad libitum drinking before weighing at 21 d. Feed intake was recorded during the 21-d trial.

Table 2. Effects of zinc-bearing clinoptilolite on growth performance of 1- to 21-d broilers challenged with \textit{Salmonella pullorum}1,2

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>SCC</th>
<th>ANT</th>
<th>ZnCP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG (g/d)</td>
<td>24.94a</td>
<td>21.67b</td>
<td>26.55a</td>
<td>25.39a</td>
<td>0.58</td>
<td>0.004</td>
</tr>
<tr>
<td>ADFI (g/d)</td>
<td>40.12</td>
<td>38.45</td>
<td>42.62</td>
<td>42.10</td>
<td>1.03</td>
<td>0.504</td>
</tr>
<tr>
<td>F/G ratio (g/g)</td>
<td>1.61</td>
<td>1.77</td>
<td>1.60</td>
<td>1.66</td>
<td>0.03</td>
<td>0.146</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>1.67</td>
<td>5.00</td>
<td>1.67</td>
<td>3.33</td>
<td>1.05</td>
<td>0.674</td>
</tr>
</tbody>
</table>

a,bValues within a row not sharing the same superscript are different at $P < 0.05$; n = 6.

1CON = nonchallenge control, SCC = \textit{Salmonella}-challenged control, ANT = antibiotic, ZnCP = zinc-bearing clinoptilolite.

2F/G ratio = feed/gain ratio.

Salmonella and Lactobacillus Colonies Assay

The cecum were aseptically removed, 0.2 g of cecal contents were diluted in 2 mL of sterilized saline, and three 10-fold serial dilutions ($10^{-3}$, $10^{-4}$, and 10$^{-5}$) were made from diluted cecal contents. A 100-μL portion of the last 3 dilutions was spread evenly onto plates. Lactobacillus were enumerated on MRS agar medium prepared according to De Man et al. (1960) at 37°C for 48 h, whereas \textit{Salmonella} colonies were determined on Bismuth sulfite agar incubated for 24 h at 37°C. The bismuth sulfite agar was purchased from Qingdao Hope Bio-Technology Co. Ltd. (Qingdao, P. R. China). The bacterial population from plates with countable colonies were enumerated and averaged to express log$_{10}$ cfu/g of cecal contents.

Determination of Serum Diamine Oxidase and Parameters of Intestinal Mucosa

The activity of serum diamine oxidase (DAO) was determined according to a test kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, P. R. China). Approximately 0.3 g of jejunal and ileal mucosa was used to prepare the mucosa homogenate. The mucosa was diluted 1:9 (wt/vol) with PBS solution and homogenized using an Ultra-Turrax homogenizer (Tek-
The protein concentrations, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content of the mucosa homogenate were determined using a corresponding diagnostic kit (Nanjing Jiancheng Bioengineering Institute) according to the instructions of the manufacturer. Briefly, the MDA was measured by barbiturate thiosulfate assay, and SOD enzymes were measured by xanthine oxidase method. The MDA concentrations were expressed as nmol per mg of protein of mucosa tissue, and the SOD activity was expressed as unit per mg of protein of mucosa.

**Statistical Analysis**

Data were analyzed by one-way ANOVA with the post hoc Duncan multiple comparison tests using SPSS statistical software (ver.16.0 for Windows, SPSS Inc., Chicago, IL). The means and total standard errors (SEM) are presented. Significance (P-value) was evaluated at the 0.05 level.

**RESULTS**

**Growth Performance, Microflora Population, and Serum Diamine Oxidase**

Growth performance of chickens was shown in Table 2. *Salmonella* challenge (SCC) caused adverse effects on ADG (P < 0.05) and a nonsignificant negative effect on ADFI and F/G ratio of broilers during the 21-d experiment as compared with CON. However, ADG, ADFI, and F/G ratio of birds fed ZnCP, similar to ANT, were ameliorated compared with SCC, and the ADG was improved significantly (P < 0.05). There was no significant difference on mortality of broilers among groups.

Compared with CON, *Salmonella* colonies of cecal contents of *Salmonella*-challenged broilers were significantly increased on 7 d (P < 0.05) and numerically increased on 21 d (Table 3). The addition of ZnCP in feed significantly decreased the *Salmonella* population in cecal contents (P < 0.05) both at 7 and 21 d, compared with SCC. Adversely, *Salmonella* challenge caused significant reduction in *Lactobacillus* colonies on 7 d (P < 0.05). No significant effect of ZnCP supplementation was observed on viable counts of *Lactobacillus* in the cecal contents of chicks during the experiment compared with SCC. However, ANT treatment decreased *Salmonella* population (P < 0.05) and tended to increase *Lactobacillus* colonies in cecal contents at 7 d as compared with the SCC treatment but significantly increased both the *Salmonella* viable counts and *Lactobacillus* colonies at 21 d (P < 0.05), compared with SCC.

*Salmonella*-challenged chickens had numerically higher serum DAO activity, compared with CON. However, the ZnCP treatment significantly decreased the serum DAO activity (P < 0.05) as compared with SCC (Table 4).

**Relative Weights of Immune Organs**

Relative weights of spleen, thymus, and bursa of Fabricius of 7-d-old chicks and those of thymus and bursa of Fabricius of 21-d-old broilers were not affected by treatments (Table 5). However, the relative weight of spleen of 21-d birds was significantly increased by *Salmonella*-challenged treatment (P < 0.05), and that

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>SCC</th>
<th>ANT</th>
<th>ZnCP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 d (g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.71</td>
<td>0.91</td>
<td>0.76</td>
<td>0.89</td>
<td>0.04</td>
<td>0.223</td>
</tr>
<tr>
<td>Thymus</td>
<td>2.84</td>
<td>3.07</td>
<td>2.95</td>
<td>3.01</td>
<td>0.12</td>
<td>0.923</td>
</tr>
<tr>
<td>Bursa F</td>
<td>1.78</td>
<td>1.80</td>
<td>1.44</td>
<td>1.84</td>
<td>0.08</td>
<td>0.326</td>
</tr>
<tr>
<td>21 d (g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.90b</td>
<td>2.06a</td>
<td>1.11b</td>
<td>1.06b</td>
<td>0.17</td>
<td>0.057</td>
</tr>
<tr>
<td>Thymus</td>
<td>1.98</td>
<td>1.93</td>
<td>2.02</td>
<td>2.28</td>
<td>0.12</td>
<td>0.758</td>
</tr>
<tr>
<td>Bursa F</td>
<td>2.31</td>
<td>2.20</td>
<td>2.21</td>
<td>2.21</td>
<td>0.14</td>
<td>0.993</td>
</tr>
</tbody>
</table>

* a,bValues within a row not sharing the same superscript are different at P < 0.05; n = 6.

1 CON = nonchallenge control, SCC = *Salmonella*-challenged control, ANT = antibiotic, ZnCP = zinc-bearing clinoptilolite.
of ANT or ZnCP was decreased significantly compared with SCC \((P < 0.05)\).

**Antioxidant Parameters of Intestinal Mucosa**

As observed from Table 6, MDA content in jejunal and ileal mucosa of broilers in SCC was improved on 21 d \((P < 0.05)\), compared with CON. Moreover, supplementation with ZnCP and ANT reduced MDA content in intestinal mucosa significantly on 21 d \((P < 0.05)\) compared with SCC.

The SOD activity in jejunal mucosa of broilers on 7 d or 21 d did not differ among groups (Table 7). Compared with CON, *Salmonella* challenge induced a negative effect on the SOD activity in ileal mucosa at 21 d \((P < 0.05)\). However, compared with SCC, there was a significant increase on the SOD activity in ileal mucosa of 7-d-old chicks in ZnCP and ANT groups \((P < 0.05)\). However, there was no statistical difference on SOD activity of jejunal mucosa among groups.

**DISCUSSION**

**Growth Performance, Microflora Population, and Serum Diamine Oxidase**

The adequate infection of *Salmonella* induced a significant deterioration or side effect on growth performance of poultry (Van Immerseel, et al., 2002; Vicente et al., 2007; Vandeplas et al., 2009; Marcq et al., 2011). In the present trial, *S. pullorum* challenge caused adverse effects on ADG \((P < 0.05)\), ADFI, and F/G ratio of broilers; however, mortality of birds was not affected significantly by treatments. Supplementation with ZnCP improved growth performance as similar to ANT compared with SCC. The mortality observed in this experiment was in agreement with that of a previous study (Bohez et al., 2008), in which chicks challenged with \(10^8\) cfu *Salmonella* Enteritidis did not have a statistically significant mortality and the mortality was below 0.7%. No death of broilers was caused after infection with \(10^6\) and \(10^9\) *Salmonella* Typhimurium at 21 d of age (Marcq et al., 2011). However, some previous reports on the effects of *Salmonella* on chicken mortality were inconsistent. Berchieri and Barrow (1996) reported that chickens inoculated orally with \(10^8\) cfu of *Salmonella* gallinarum caused a mortality of 31%. Similarly, chicks challenged by *S. pullorum* at 4 d showed a mortality of 50%, and even the treatment protected by probiotics had 25% mortality (Carina Audisio et al., 2000). Moreover, challenged with \(4.7 \times 10^4\) cfu/mL of *S. pullorum* on 3 d caused 58.3% mortality of chicks in the challenged control group, and the organic acids treatment had the lowest mortality of 8.3% (Al-Tarazi and Alshawabkeh, 2003). The difference in resultant performance responses may be affected by chick infection age (Gast and Beard, 1989), bacterial organism strains or serotypes (Totton et al., 2011), challenging bacterial dosage, and environmental condition (Soleiman et al., 2012).

*Salmonella* challenge caused higher *Salmonella* colonization in cecal or fecal contents (Carina Audisio et al., 2000; Al-Tarazi and Alshawabkeh, 2003; Bohez et al., 2008; Borsoi et al., 2011) and decreased *Lactobacillus* colonies (Carina Audisio et al., 2000). In agreement

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>SCC</th>
<th>ANT</th>
<th>ZnCP</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 d (nmol/mg prot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.20</td>
<td>0.28</td>
<td>0.15</td>
<td>0.20</td>
<td>0.02</td>
<td>0.217</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.06</td>
<td>0.10</td>
<td>0.06</td>
<td>0.04</td>
<td>0.01</td>
<td>0.358</td>
</tr>
<tr>
<td>21 d (nmol/mg prot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.39(a)</td>
<td>0.97(a)</td>
<td>0.50(b)</td>
<td>0.37(b)</td>
<td>0.08</td>
<td>0.011</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.31(b)</td>
<td>0.61(a)</td>
<td>0.30(b)</td>
<td>0.28(b)</td>
<td>0.05</td>
<td>0.054</td>
</tr>
</tbody>
</table>

\(a,b\)Values within a row not sharing the same superscript are different at \(P < 0.05\); \(n = 6\).

**Table 6.** Effects of zinc-bearing clinoptilolite on malondialdehyde content of intestine mucosa of broilers challenged with *Salmonella pullorum*\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>SCC</th>
<th>ANT</th>
<th>ZnCP</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 d (unit/mg prot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>210.79</td>
<td>243.00</td>
<td>238.31</td>
<td>291.30</td>
<td>13.89</td>
<td>0.233</td>
</tr>
<tr>
<td>Ileum</td>
<td>81.61(b)</td>
<td>67.75(b)</td>
<td>130.43(a)</td>
<td>112.54(a)</td>
<td>7.49</td>
<td>0.001</td>
</tr>
<tr>
<td>21 d (unit/mg prot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>173.25</td>
<td>133.76</td>
<td>155.60</td>
<td>137.24</td>
<td>6.93</td>
<td>0.151</td>
</tr>
<tr>
<td>Ileum</td>
<td>116.29(a)</td>
<td>69.51(b)</td>
<td>96.66(ab)</td>
<td>92.01(ab)</td>
<td>7.14</td>
<td>0.133</td>
</tr>
</tbody>
</table>

\(a,b\)Values within a row not sharing the same superscript are different at \(P < 0.05\); \(n = 6\).

**Table 7.** Effects of zinc-bearing clinoptilolite on superoxide dismutase activity of intestine mucosa of broilers challenged with *Salmonella pullorum*\(^1\)
with the results of these studies, Salmonella colonies in cecal contents of Salmonella-challenged broilers were increased significantly on 7 d and numerically on 21 d, whereas Salmonella challenge caused a significant reduction in Lactobacillus population on 7 d and a slight decrease on 21 d. The different changes of cecal microbial population registered in 7 d and 21 d could be explained by the fact that chicks' susceptibility to Salmonella decreased naturally with age and they could obtain a mature microflora from the environment (Edens et al., 1997). The addition of ZnCP to the diet produced a decreasing effect on the Salmonella population ($P < 0.05$), suggesting that ZnCP can regulate the cecal microflora of broilers after Salmonella infection. Interesting, compared with SCC, ANT treatment significantly increased both Salmonella and Lactobacillus populations simultaneously at 21 d, which was different from previous reports and should be further studied.

The DAO is normally present in very small amounts in the circulation, and plasma DAO may serve as a marker of the injury and integrity of the intestinal mucosa (Wolvekamp and de Bruin, 1994). The intestinal mucosa has an important barrier function in health and disease (Turner, 2009). Salmonella can attach to and invade the intestinal mucosa and multiply in the host cells, and they can produce toxins and affect gut microflora, causing direct injury to the intestine (Stavric and Daoust, 1993). However, the present results showed that serum DAO of broilers challenged with Salmonella was numerically higher but not significantly different. It was in agreement with the results of mortality, and it could be explained that the lower dosage of Salmonella infection in the current trial did not cause serious damage to the intestine of chickens. The ZnCP addition into feed decreased the serum DAO activity significantly, meaning that ZnCP could protect gut integrity and gut health for broilers.

The protective effects of ZnCP on growth performance, microflora colonies, and serum DAO of broilers infected by Salmonella might attribute to the antibacterial activity and the adsorption of bacteria or toxins onto ZnCP. Therefore, ZnCP could act as an antibacterial agent against Salmonella, inhibiting Salmonella growth and improving the gut health. Our in vitro experiment showed that adequate ZnCP inhibited Salmonella growth, which agreed with the report of Top and Ulku (2004), who found that zinc exchanged clinoptilolite and resulted in antibacterial effects on Pseudomonas aeruginosa and Escherichia coli. The antibacterial mechanism of ZnCP can be explained with the antibacterial activity of the released zinc ions from clinoptilolite (Top and Ulku, 2004; Xia et al., 2007; Hrenovic, et al., 2012), and sequential adsorption of bacteria or toxins onto zeolite, thereby reducing the bacterial bioactivity in the gastrointestinal tract (Ramu et al., 1997; Hrenovic et al., 2008; Kubota et al., 2008; Narin, et al., 2010) and interactions of bacteria and metal-loaded minerals (Tong et al., 2005; Xia et al., 2007; Malachova et al., 2011). It was reported that copper-bearing montmorillonite improved growth performance as well as lowered viable counts of Escherichia coli in cecal contents (Xia et al., 2004a), while copper-bearing montmorillonite as similar to ZnCP was a metal-loaded mineral-type antibacterial agent (Hrenovic et al., 2012).

**Relative Weights of Immune Organs**

The bursa of Fabricius and the thymus serve as the 2 primary or central lymphoid organs of the immune system. These organs secrete B cells and T cells, respectively, which are commonly referred to as acting as humoral immunity and cellular immunity (Sharma, 1999). The spleen is the main peripheral lymphoid organ of systemic immunity in birds, and is important in disease resistance with the scarcity of avian lymph nodes (John, 1994). Some bacteria can elicit an immune response, which, as a side effect, causes reduction of appetite and catabolism of muscle protein (Bedford, 2000). In the present study, the relative weight of spleen of 21-d birds was significantly increased by Salmonella challenge treatment. It may be due to the immune response against Salmonella challenge, which is in agreement with Barrow and Lovell (1988), who reported that S. pullorum organisms were transported rapidly to the liver and spleen, which are the major sites of multiplication. Several recent studies also proved that Salmonella could transfer and multiply in the spleen cell or tissue (Barrow, 1991; Wigley et al., 2002; Salazar-Gonzalez et al., 2007). Results indicated that supplementation with ZnCP significantly decreased the relative weight of spleen compared with SCC in our experiment. It could be explained that ZnCP could adsorb Salmonella bacteria or toxins and inhibit its activity and decrease the transportation of Salmonella into spleen, thus reducing the immune reactions against Salmonella and spleen weight.

**Antioxidant Parameters of Intestinal Mucosa**

Oxidative stress is strongly implicated in several diseases and is emerging as one of the most important causative agents of mutagenesis, tumorigenesis, and aging. Bacteria such as Escherichia coli and S. typhimurium respond to oxidative stress by invoking 2 distinct stress responses, the peroxide stimulon and the superoxide stimulon, depending on whether the stress is mediated by peroxides or by the superoxide anion (Farr and Kogoma, 1991). On the other hand, the antioxidant system is important in maintaining intestinal barrier integrity against bacterial infection (Kelly et al., 2004; van Auping et al., 2009). Malondialdehyde is one of the most frequently used indicators of lipid peroxidation or biomarker for oxidative stress (Nielsen et al., 1997). The effective antioxidant defense system requires an increase in antioxidant enzyme activity, and SOD is one of the most important antioxidant enzymes (Zelko et al., 2002). An in vitro experiment indicat-
ed that a strong oxidative burst was essential for the elimination of intracellular *Salmonella* (Withanage et al., 2005). In the current results, *Salmonella* challenge caused side effects on MDA content in jejunal and ileal mucosa and SOD activity of ileal mucosa at 21 d ($P < 0.05$); however, there was no statistical difference at 7 d. This phenomenon may be explained by the fact that 4 d after infection (7 d) was too short a time period and the serious adverse effects on oxidative damage of intestinal mucosa did not emerge. Supplementation with ZnCP significantly decreased MDA contents in intestinal mucosa at 21 d and increased SOD activity, particularly in ileal mucosa. The improvement on antioxidant effects of ZnCP may be attributed to the antibacterial activity, thus reducing the oxidative stress by *Salmonella* infection and the protective effects of ZnCP on intestine health, plus that ZnCP may adsorb free radicals generated. The phenomenon that antioxidant parameters were affected more notably in ileum than jejunum may be explained by ZnCP mainly changing the microflora of the hind gut, thus the antioxidant defense function of hind gut was more likely influenced.

It was concluded that ZnCP made protective effects on broilers challenged with *S. pullorum* and might be used as a new antibacterial agent in poultry feed with further study.

**REFERENCES**


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