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# Bioactivity of selenium-enriched exopolysaccharides produced by *Enterobacter cloacae* Z0206 in broilers

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ABSTRACT

To investigate effects of Se-enriched exopolysaccharides (Se-ECZ-EPS) produced by *Enterobacter cloacae* Z0206 on growth performance, antioxidant status and immune function, 240 broilers were randomly assigned into five groups: the control group was fed a basal diet supplemented with 0.15 mg/kg Se ($Na_2SeO_3$), and the other four groups were fed with diets supplemented with Se-ECZ-EPS at 280, 560, 840 and 1120 mg/kg, respectively. Administration of Se-ECZ-EPS (840 and 1120 mg/kg) significantly increased average daily gain, decreased feed/gain ratio and enhanced antioxidant enzyme activities. Serum cytokine concentrations showed positive responses in birds treated with 560 and 1120 mg/kg Se-ECZ-EPS. Serum antibody titers against Newcastle disease virus in birds treated with 840 mg/kg Se-ECZ-EPS were significantly increased. These results suggested that Se-ECZ-EPS could enhance antioxidant status and immune function, and could be developed to a potentiator of the immune response in broilers, with the 840 mg/kg addition level being the most effective.



## 1. Introduction

Certain polysaccharides are modifiers of biological responses, and are used in healthcare products and medicines (Sun, 2011). Many polysaccharides isolated from mushrooms, fungi, yeasts, algae, lichens and herbs have been reported to exhibit biological activities, including immunomodulatory (Na, Lim, Yun, Kweon, & Lee, 2010), anti-tumor (Ni et al., 2010; Zhang, Ren, & Chen, 2002), anti-adhesive (Lee, Shim, Chung, Lim, & Kim, 2009), antioxidation (Luo & Fang, 2008), antiviral (Wang, Ooi, & Ang, 2007) and anti-radiation (Kim et al., 2007; Park, Hwang, Song, & Jee, 2011) activities. Microorganisms are considered to be efficient producers of biologically active compounds (Jensen & Fenical, 1994). Exopolysaccharides produced by bacteria have been claimed to have a wide range of biological activities (Arena et al., 2006). Exopolysaccharides can be purified easily, which provides high yields in industrial production (Looijesteijn, Boels, Kleerebezem, & Hugenholtz, 1999).

Selenium (Se), an essential trace element for nutrition, is a cofactor of large numbers of Se-dependent enzymes such as glutathione peroxidase (GPx), which is involved in cellular protection from severe oxidation caused by free radicals (Martinez & Charlet, 2009; Ursini et al., 1995). Se also plays a preventive role in some degenerative conditions including cancer, inflammatory diseases and infections via a variety of selenoproteins (Kaur & Sandhu, 2008). The chemical form of Se is important for its bioavailability. It is generally accepted that organic selenium compounds have higher biological activities, lower toxicity compared with inorganic selenium as a dietary supplement, thus varieties of selenium-enriched biological products have been commercialized. Microorganism fermentation with selenium technique provides a feasible and economic approach for production of organic selenium compounds (Zhang, Benedict, & William, 2008).

The bacterial strain *Enterobacter cloacae* Z0206 (*E. cloacae* Z0206) can produce large amounts of viscous exopolysaccharides. Our previous studies (Xu, Wang, Jin, & Yang, 2009) demonstrated that *E. cloacae* Z0206 can accumulate selenium (Se) in the form of Se-enriched exopolysaccharides (Se-ECZ-EPS) during cultivation with Se. Structural analyses showed that the major component of Se-ECZ-EPS (with the average molecular weight of 29,300 Da) was glucose, galactose and mannose at a molar ratio of 8.530:0.061:0.706. Se-ECZ-EPS has been shown to improve the innate, cellular and humoral immune responses in immunosuppressed mice induced by cyclophosphamide (Xu et al., 2009; Yang, Jin, Xu, Shan, & Wang, 2009). Further studies suggested that Se-ECZ-EPS was an efficient immunostimulant and could protect crayfish

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against the white spot syndrome virus (Wang et al., 2010), and that it had significant anti-diabetic activities in alloxan-induced diabetic mice (Jin, Lu, Huang, & Wang, 2012).

Se-ECZ-EPS produced by *E. cloacae* Z0206 has been recognized to be an effective proponent of immunomodulatory actions in crayfish and mice, but there is little information about the potential role of Se-ECZ-EPS in broilers. The present study was designed to investigate the possible immunomodulatory and antioxidant roles of Se-ECZ-EPS in broilers, which would facilitate its use as a potential immunostimulant in broiler husbandry.

## 2. Materials and methods

### 2.1. E. cloacae Z0206 and reagents

The Se-ECZ-EPS-producing bacterial strain *E. cloacae* Z0206 was identified by conventional physiological and biochemical methods together with 16SrDNA sequence homology and phylogenetic analyses. It was collected by the China General Microbiological Culture Collection Center (CGMCC, Beijing, China) (Xu et al., 2009). Newcastle disease virus (NDV) vaccine (La Sota strain, No. 150132007) was purchased from Qingdao Yebio Bioengineering Co., Ltd. (Qingdao, China). All other reagents were of analytical grade and available commercially.

### 2.2. Preparation of Se-ECZ-EPS

Preparation and purification of Se-ECZ-EPS were carried out according to the previous report from our research team (Jin et al., 2012; Xu et al., 2009). Briefly, cultivation medium (sucrose, 2.5%; peptone, 0.5%; yeast extract, 0.5%, $K_2HPO_4$, 0.2%; $KH_2PO_4$, 0.1% and $MgSO_4{\cdot}7H_2O$, 0.05%) was prepared. Se-ECZ-EPS production was undertaken in a 1000 $dm^3$ bioreactor (Zhejiang CIFU Pharmaceutics, Inc., Shaoxing, China) with 700 $dm^3$ cultivation medium with stirring at 200 rpm at 30 °C for 2 days. The initial pH was 7.5 and the inoculation volume was 5.0% (v/v). The time of addition of Se (20 μg/mL) into the culture was the sixth hour. Aeration rate, growth temperature, foam level, dissolved oxygen tension and pH were measured and/or controlled by the bioreactor control unit. After cultivation, the broth was centrifuged to remove mycelia. The supernatant was precipitated upon addition of 3 vol. of cold 95% EtOH. The precipitate was collected by centrifugation and freeze-dried to yield a yellow powder, which was subjected to subsequent analyses. The content of Se in Se-ECZ-EPS was determined by a Hitachi 850 Fluorimeter (Hitachi, Tokyo, Japan) according to the method of Kessi, Ramuz, Wehrli, Spycher, and Bachefen (1999) and determined to be 536 mg/kg.

### 2.3. Animals and treatments

A total of 240, 1-day-old Avian × Avian broilers (average weight, 45.6 ± 0.8 g) were purchased from a commercial hatchery and randomly assigned into five groups with four replicates in each group and 12 chickens in each replicate. The control group was fed a basal diet supplemented with 0.15 mg/kg Se ($Na_2SeO_3$), and the other four groups were fed with diets supplemented with Se-ECZ-EPS at 280 mg/kg (0.15 mg/kg Se), 560 mg/kg (0.30 mg/kg Se), 840 mg/kg (0.45 mg/kg Se) and 1120 mg/kg (0.60 mg/kg Se), respectively. Broilers were fed a starter ration from 1 day to 21 days of age, and a finisher ration from 22 days to 42 days of age (Table 1). All rations were formulated in accordance with the requirements recommended by the NRC (NRC, 1994).

Broilers were housed in wire cages in a temperature-controlled room. The temperature of the animal facility on the first day of life was maintained at 33–35 °C and lowered by 2–3 °C every week until it was 22–23 °C in the final week. A 24-h light regimen was conducted throughout the 42-day trial. All birds had free access to food and water for the entire period. Average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G) were determined at 21 days and 42 days of age.

**Table 1**
Composition and nutrients levels of basal diets (%).

| Ingredient (%) | Days 1–21 | Days 22–42 |
|---|---|---|
| Corn | 56.00 | 58.50 |
| Soybean meal | 31.48 | 28.00 |
| Corn gluten meal | 3.50 | 5.50 |
| Soybean oil | 3.00 | 4.20 |
| Fish meal | 2.30 | 0.00 |
| Limestone | 1.30 | 1.20 |
| Monocalcium phosphate | 1.50 | 1.60 |
| Salt | 0.30 | 0.30 |
| Vitamin premix[a] | 0.02 | 0.02 |
| Mineral premix[b] | 0.20 | 0.20 |
| DL-Methionine | 0.15 | 0.10 |
| L-Lysine | 0.06 | 0.14 |
| Allicin | 0.02 | 0.02 |
| Salinomycin | 0.05 | 0.05 |
| Antioxidant agent | 0.02 | 0.02 |
| Choline chloride | 0.10 | 0.15 |
| Total | 100 | 100 |
| Nutrient composition[c] | | |
| ME (MJ/kg) | 12.54 | 12.96 |
| Crude protein (%) | 21.52 | 20.01 |
| Ca (%) | 1.02 | 0.91 |
| Available phosphorus (%) | 0.45 | 0.40 |
| Lysine (%) | 1.17 | 1.05 |
| Tryptophan (%) | 0.24 | 0.21 |
| Methionine + cystine (%) | 0.83 | 0.73 |

[a] Mix supplied the following per kilogram of diet: vitamin A, 1,2500 IU; vitamin D, 2500 IU; vitamin E, 18.75 IU; vitamin $K_3$, 2.65 mg; vitamin $B_1$, 2 mg; vitamin $B_2$, 6 mg; vitamin $B_{12}$, 0.025 mg; D-biotin, 0.325 mg; folic acid, 1.25 mg; D-pantothenic acid, 12 mg; nicotinic acid, 50 mg.
[b] Mix supplied the following per kilogram of diet: copper, 8 mg; zinc, 75 mg; iron, 80 mg; manganese, 100 mg; iodine, 0.35 mg.
[c] Component based on calculated values.

### 2.4. Assay to ascertain antioxidant status in serum

Blood was collected on day 42 from the wing vein of 8 birds per treatment (2 birds/replicate) and serum was collected by centrifugation (2500 × *g* for 10 min at 4 °C). Serum was harvested and kept frozen until use. The activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) as well as the malondialdehyde (MDA) level in serum were determined by spectrophotometric methods with commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's manuals.

### 2.5. Cytokine determination in serum

The concentrations of tumor necrosis factor-α (TNF-α), interleukin (IL)-2 and IL-6 in serum were ascertained using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's manuals.

### 2.6. Antibody titer against NDV

Broilers were vaccinated with NDV "La Sota" vaccine using eye drops on day 7 and day 21 of the experiment. Blood samples were collected at 21, 35 and 42 days of age from the wing vein of 8 birds per treatment (2 birds/replicate). The humoral immune response against NDV was determined by the hemagglutination inhibition

**Table 2**
Effects of Se-ECZ-EPS on growth performance in broilers.

| Time | Indexes | CON | Se-ECZ-EPS (mg/kg) | | | |
|---|---|---|---|---|---|---|
| | | | 280 | 560 | 840 | 1120 |
| Days 1–21 | ADFI[d] (g) | 47.69 ± 0.77[a] | 46.64 ± 0.75[a] | 47.02 ± 0.36[a] | 46.79 ± 0.81[a] | 47.75 ± 0.85[a] |
| | ADG[e] (g) | 28.19 ± 0.39[a] | 29.05 ± 0.27[a,b] | 30.28 ± 0.26[b,c] | 30.55 ± 0.56[c] | 30.51 ± 0.69[c] |
| | F/G[f] | 1.69 ± 0.03[a] | 1.61 ± 0.04[b] | 1.55 ± 0.02[b] | 1.53 ± 0.02[b] | 1.57 ± 0.01[b] |
| Days 22–42 | ADFI[d] (g) | 135.65 ± 2.66[a] | 128.22 ± 1.35[b,c] | 125.71 ± 2.29[c] | 133.92 ± 2.11[a,b] | 131.78 ± 2.68[a,b,c] |
| | ADG[e] (g) | 67.76 ± 1.02[a] | 68.09 ± 0.87[a] | 67.37 ± 0.76[a] | 72.68 ± 0.41[b] | 69.56 ± 0.83[a] |
| | F/G[f] | 2.00 ± 0.04[a] | 1.88 ± 0.01[b] | 1.87 ± 0.03[b] | 1.84 ± 0.03[b] | 1.89 ± 0.03[b] |
| Days 1–42 | ADFI[d] (g) | 91.67 ± 1.46[a] | 87.43 ± 1.00[b] | 86.37 ± 1.01[b] | 90.36 ± 1.45[a,b] | 89.77 ± 1.75[a,b] |
| | ADG[e] (g) | 47.98 ± 0.41[a] | 48.57 ± 0.35[a] | 48.83 ± 0.33[a,b] | 51.62 ± 0.26[c] | 50.03 ± 0.65[b] |
| | F/G[f] | 1.91 ± 0.03[a] | 1.80 ± 0.01[b] | 1.77 ± 0.02[b] | 1.75 ± 0.02[b] | 1.79 ± 0.02[b] |

[a] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 4$.
[b] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 4$.
[c] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 4$.
[d] Average daily feed intake.
[e] Average daily gain.
[f] Feed/gain ratio.

(HI) test (Fan et al., 2012). Briefly, twofold serial dilutions of 50-μL serum (after inactivation at 56 °C for 30 min) were added to a 96-well, V-shaped-bottom microtiter plate containing 50 μL of calcium- and magnesium-free phosphate buffered saline (CMF-PBS) in each well. Then, 50 μL of NDV antigen (4 hemagglutination (HA) units) was added into all wells (except for the last row because it was a control). Serum dilutions ranged from 1:2 to 1:2048. The antigen–serum mixture was incubated for 10 min at 37 °C, then 50 μL of 1% rooster erythrocyte suspension added into each well and the mixture allowed to incubate for 30 min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest dilution of serum that caused complete inhibition was considered to be the endpoint. The geometric mean titer was expressed as reciprocal $\log_2$ values of the highest dilution that displayed HI.

### 2.7. Statistical analyses

Data were analyzed using one-way analysis of variance (ANOVA) of software SPSS 16.0, followed by Fisher's least significant difference test. Results were presented as means ± standard error ($X \pm$ S.E.). Differences between groups were considered statistically significant at the 5% ($P < 0.05$) level.

## 3. Results

### 3.1. Growth performance

During days 1–21 of the experiment, as compared with the control group, supplemental Se-ECZ-EPS at 560, 840 and 1120 mg/kg significantly increased ADG ($P < 0.05$; Table 2) and decreased F/G ($P < 0.05$). However, Se-ECZ-EPS treatments did not affect ADFI ($P > 0.05$). During days 22–42 of the experiment, supplementation with Se-ECZ-EPS (840 mg/kg) significantly increased ADG by 7.26% ($P < 0.05$) and decreased F/G by 8.00% ($P < 0.05$). In the entire experimental period, supplementation with Se-ECZ-EPS at 840 and 1120 mg/kg significantly increased ADG and decreased F/G ($P < 0.05$), with the 840 mg/kg addition level being the most effective.

### 3.2. Activities of GPx and SOD as well as MDA concentration in serum

The effects of different levels of Se-ECZ-EPS on the activities of GPx and SOD as well as the MDA concentration in serum are shown in Table 3. Compared with the control, administration of Se-ECZ-EPS at 840 and 1120 mg/kg significantly increased the activities of GPx and SOD, and decreased MDA production, in serum ($P < 0.05$).

### 3.3. Concentrations of TNF-α, IL-2 and IL-6 in serum

The effects of Se-ECZ-EPS on cytokine levels in serum are shown in Table 4. Compared with the control, the serum concentrations of TNF-α and IL-2 showed positive responses in four groups supplied with Se-ECZ-EPS ($P < 0.05$). Significant increases in the level of IL-6 were observed only in the serum of birds treated with Se-ECZ-EPS at 560 and 1120 mg/kg as compared with the control group ($P < 0.05$).

### 3.4. Antibody titers against NDV

The effects of Se-ECZ-EPS on antibody titers against NDV are shown in Table 5. At 21 days and 35 days of age, the HI titers in groups supplied with Se-ECZ-EPS at 840 and 1120 mg/kg were significantly higher than that in the control ($P < 0.05$). At 42 days of age, a significant increase in HI titer was observed only in birds treated

**Table 3**
Effects of Se-ECZ-EPS on the activities of GPx and SOD as well as MDA concentration in serum.

| | CON | Se-ECZ-EPS (mg/kg) | | | |
|---|---|---|---|---|---|
| | | 280 | 560 | 840 | 1120 |
| GPx (unit/mL) | 1733.18 ± 43.73[a] | 2235.00 ± 43.63[b] | 2391.82 ± 47.70[c] | 2434.09 ± 63.11[c] | 2389.77 ± 38.82[c] |
| SOD (unit/mL) | 134.44 ± 4.10[a] | 131.92 ± 4.37[a] | 141.46 ± 3.54[a,b] | 150.39 ± 5.01[b] | 152.29 ± 4.56[b] |
| MDA (nmol/mL) | 5.07 ± 0.14[a] | 4.75 ± 0.10[a] | 4.24 ± 0.13[b] | 4.10 ± 0.15[b] | 4.34 ± 0.09[b] |

[a] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.
[b] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.
[c] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.

**Table 4**
Effects of Se-ECZ-EPS on the concentrations of TNF-α, IL-2 and IL-6 in serum.

| | CON | Se-ECZ-EPS (mg/kg) | | | |
|---|---|---|---|---|---|
| | | 280 | 560 | 840 | 1120 |
| TNF-α (pg/mL) | 275.15 ± 8.07[a] | 323.05 ± 10.81[b] | 348.80 ± 10.55[b] | 334.20 ± 7.82[b] | 338.08 ± 8.96[b] |
| IL-2 (pg/mL) | 720.25 ± 16.74[a] | 821.15 ± 14.94[b] | 848.58 ± 21.10[b,c] | 864.88 ± 20.75[b,c] | 911.62 ± 30.58[c] |
| IL-6 (pg/mL) | 341.58 ± 9.43[a] | 359.65 ± 11.17[a,b] | 381.50 ± 13.95[b] | 369.88 ± 8.15[a,b] | 390.45 ± 11.57[b] |

[a] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.
[b] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.
[c] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.

**Table 5**
Effects of Se-ECZ-EPS on antibody titers against NDV.

| HI titer ($log_2$) | CON | Se-ECZ-EPS (mg/kg) | | | |
|---|---|---|---|---|---|
| | | 280 | 560 | 840 | 1120 |
| 21 days | 6.67 ± 0.33[a] | 6.83 ± 0.17[a,b] | 6.83 ± 0.31[a,b] | 7.75 ± 0.25[b] | 7.60 ± 0.40[b] |
| 35 days | 5.17 ± 0.40[a] | 5.67 ± 0.21[a,b] | 5.50 ± 0.34[a,b] | 6.17 ± 0.31[b] | 6.33 ± 0.33[b] |
| 42 days | 3.80 ± 0.37[a] | 4.40 ± 0.24[a,b] | 4.20 ± 0.20[a,b] | 4.80 ± 0.37[b] | 4.40 ± 0.24[a,b] |

[a] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.
[b] Means within a row lacking a common superscript differ ($P < 0.05$), $n = 8$.

with Se-ECZ-EPS at 840 mg/kg as compared with the normal control ($P < 0.05$).

## 4. Discussion

The overall trend in livestock production has been to keep a greater number of animals on fewer farms (Johnston, 2001). Numerous additives (especially antibiotics) are administered intensively through feed or water for prophylaxis, therapy and growth promotion (Singer et al., 2003; Wegener, 2003). However, increasing public concern about antibiotic resistance and residues in animal products have resulted in the search for alternatives, such as prebiotics, probiotics and other feed additives (Guo et al., 2004). Certain plant polysaccharides are recognized as having a prebiotic effect (Cummings & Macfarlane, 2002). Polysaccharides derived from *Astragalus membranacea Radix*, *Tremella fuciformis* and *Aloe vera* have been widely used as feed additives for farm animals in China due to their biological activities, such as immunomodulatory (Shao et al., 2004), antioxidant (Yu, Jin, Xin, & He, 2009), and antiviral (Jiang, Wu, Gao, Song, & Li, 2010) activities as well as the regulation of intestinal microbiota (Guo et al., 2004). In recent years, interest in high-molecular-weight polysaccharides produced by microorganisms has increased because these biopolymers have advantages over the polysaccharides that are currently in use, and they can be produced economically from local sources of utilizable fermentable substrates (Sutherland, 1996). Here, we studied the bacterial exopolysaccharide Se-ECZ-EPS to explore its effects on growth performance, antioxidant status and immune function in broilers.

The current results indicated that Se-ECZ-EPS had a better role in promoting the growth of broilers in the early period. In the entire period, supplementation with Se-ECZ-EPS at 840 mg/kg was the most effective for growth promotion. These results were consistent with the results of the effects of *Astragalus* polysaccharides on chickens (Guo et al., 2004) and provided the basis for the application of Se-ECZ-EPS in broiler husbandry.

Reactive oxygen species (ROS) such as the superoxide anion, hydrogen peroxide ($H_2O_2$) and the hydroxyl radical can lead to membrane damage and the pathogenesis of various diseases (Pahlavani & Harris, 1998). The cellular defense system against antioxidants operates through enzymatic and non-enzymatic components. SOD is an important antioxidants enzyme that converts superoxide to $H_2O_2$ and maintains low superoxide concentrations (Jiang et al., 2008). GPx is an equally important antioxidant which can catalyze the reduction of $H_2O_2$ and other lipid peroxides, thereby protecting cell membranes from peroxidation (Reed & Beatty, 1980). Se-containing polysaccharide is considered to be a novel Se source in dietary supplements, with potent antioxidant properties (Wang, Zhao, Wang, Yao, & Zhang, 2012). According to the present study, administration of Se-ECZ-EPS at 560, 840 and 1120 mg/kg significantly enhanced the activities of SOD and GPx and reduced the concentration of MDA (an indicator of lipid peroxidation) in serum. These findings suggested that Se-ECZ-EPS has antioxidant activity in broilers, and the results were in line with previous studies on rats (Xu et al., 2009).

Several studies have shown that many types of polysaccharides derived from herbs can modulate the production of a range of cytokines (Schepetkin & Quinn, 2006). Cytokines such as TNF-α, IL-2 and IL-6 can induce a cell-mediated immune response and directly or indirectly regulate immune reactions (Mosmann & Coffman, 1989). TNF-α can induce the production of cytokines that could participate in the host response against infecting pathogens (Jablons et al., 1989). IL-2 is essential for T-lymphocyte proliferation, differentiation of B lymphocytes into plasma cells, and the cloning of antigen-specific T lymphocytes (Thiagarajan, Ram, & Bansal, 1999). IL-6 has pro-inflammatory and anti-inflammatory activities, and induces B-cell differentiation, monocyte proliferation, and neutrophil recruitment to the sites of inflammation (Sitaraman et al., 2001). Thus, TNF-α, IL-2 and IL-6 were measured to describe the profile of Se-ECZ-EPS in the induction of cytokines. Administration of Se-ECZ-EPS at 560, 840 and 1120 mg/kg significantly increased the concentrations of the cytokines described above compared with control. These findings suggested that Se-ECZ-EPS produced by bacteria had the same effect on the cellular immune response as the polysaccharides isolated from fungi and herbs (Fan et al., 2012; Yu et al., 2009).

Newcastle disease (ND) is one of the notifiable diseases in the Office International des Epizooties (1996). An outbreak of ND can cause severe morbidity and mortality in broilers. The maternal antibody protects the birds during only the first week of life, so vaccination against this disease is warranted (Haldar, Ghosh, Toshiwati, & Bedford, 2011). Humoral immunity mediated by B lymphocytes is one of the main factors to resist infectious diseases (Guo et al., 2012). The serum antibody titer is an indicator of humoral immunity. The present study suggested that the HI titers against NDV in all treatment groups were numerically higher than

that of the control group at nearly all time points, and that the titer in the group supplied with Se-ECZ-EPS at 840 mg/kg was significantly higher than that in the control at 21, 35 and 42 days of age. The improvement in humoral immunity against NDV due to dietary treatments might be the result of the combined effects of the glucans and the mannans in Se-ECZ-EPS (Xu et al., 2009) as has been reported in yeast (Haldar et al., 2011). It has been reported that the glucans and mannans could improve non-specific immunity by increasing the release of lysozyme from the phagocytic cells of broilers (Jensen, Patterson, & Yoon, 2008).

The combined effects of Se and exopolysaccharide are responsible for the bioactivity of Se-ECZ-EPS produced by *E. cloacae* Z0206 in broilers. Se is an essential trace element of GPx, Type I, II and III iodothyronine deiodinase, and thioredoxin reductase in human and animal bodies (Tamura & Stadtman, 1996; Ursini et al., 1995). Se also participates in the synthesis of enzymes and protects the structure and function of biomembrane from overoxidation and damage (Wang et al., 2011). Dietary Se supplementation, especially selenomethionine and Se-enriched yeast, has been shown to positively affect the growth performance, antioxidation and immune functions of birds (Skrivan, Marounek, Englmaierova, & Skrivanova, 2012; Wang & Xu, 2008). Bacterial exopolysaccharides have been widely used as biological response modifiers in recent years. It is currently unclear how exopolysaccharides affect the intracellular immune system. Monosaccharide component, molecular weight and branching, chain conformation, and water-solubility may affect their activities (Zjawiony, 2004). The exopolysaccharides contain types of essential sugars (e.g. glucose, mannose and xylose) that predominate in glycoproteins and glycoprotein receptors. The major component of Se-ECZ-EPS is composed of glucose, galactose and mannose with α-configuration, pyranoside and some branches (Xu et al., 2009), which may be related to its immunomodulatory activity in broilers. The biological activity of Se-ECZ-EPS was consistent with some selenium-containing polysaccharides isolated from fungi and plants (He et al., 2013; Malinowska et al., 2009; Wang et al., 2011).

## 5. Conclusions

The present study was the first to demonstrate the biological activities of Se-ECZ-EPS produced by *E. cloacae* Z0206 in broilers. Preliminary tests suggested that Se-ECZ-EPS could significantly enhance aspects of immunomodulatory and antioxidant activity, and that Se-ECZ-EPS could be a new, effective potentiator of the immune system. Dietary supplementation with Se-ECZ-EPS increased the ADG and decreased F/G of broilers, with 840 mg/kg of Se-ECZ-EPS being the most effective. However, the exact chemical structure of Se-ECZ-EPS is not clear, and little is known about the essential structure responsible for its biological activity. More studies are needed to examine the observed dosage effect and to determine the immunofunctional effects and mechanisms of Se-ECZ-EPS.

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