

Effects of Dietary Supplementation with Different Levels of Pectin Oligosaccharide Chelated Zinc on Growth Performance, Immune Function, and Serum Antioxidant Function in Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of zinc-pectic oligosaccharide chelate (Zn-POS) on growth performance, immune function, and serum antioxidant capacity in Arbor Acres (AA) broiler chickens. A total of 480 healthy 1-day-old AA broiler chickens with relatively uniform body condition (half male and half female) were randomly divided into 5 groups with 6 replicates per group and 16 chickens per replicate based on the principle of uniform body weight. Group 1 was the basal diet group; Group 2 was the antibiotic group, with 62.5 mg/kg flavomycin added to the basal diet; Groups 3, 4, and 5 were Zn-POS groups, with 300, 600, and 900 mg/kg Zn-POS added to the basal diet, respectively. The experimental period lasted 42 days. The results showed: 1) At 1-21 days of age, compared with Group 1, the average daily gain of Groups 3 and 4 was significantly increased ($P < 0.05$); compared with Group 1, the body weight of Groups 3 and 4 was significantly increased ($P < 0.05$). At 22-42 days of age, compared with Group 1, the average daily gain of Groups 3 and 4 was significantly increased ($P < 0.05$), and the average daily feed intake of Group 3 was significantly increased ($P < 0.05$). At 1-42 days of age, compared with Group 1, the average daily gain of Group 3 was extremely significantly increased ($P < 0.01$), the average daily gain of Groups 3 and 4 was significantly increased ($P < 0.05$), and the average daily feed intake of Group 3 was significantly increased ($P < 0.05$); the average daily gain of Group 3 was extremely significantly higher than that of Group 1 ($P < 0.01$). 2) The bursa of Fabricius index of Group 3 was significantly higher than that of Group 1 ($P < 0.05$), but the serum immunoglobulin G level of Group 3 was significantly lower than that of Group 1 ($P < 0.05$). 3) The serum total superoxide dismutase activity

of Group was extremely significantly higher than that of Group ($P<0.01$), and the serum catalase activity of Groups and was significantly higher than that of Group ($P<0.05$); the serum malondialdehyde content of Groups and was significantly lower than that of Group ($P<0.05$). In conclusion, dietary supplementation with Zn-POS can improve the growth performance, immune function, and serum antioxidant function of AA broiler chickens aged 1-42 days, with the addition level of 600 mg/kg being more appropriate.

Full Text

Effects of Different Dietary Zinc-Pectic Oligosaccharide Chelate Supplemental Levels on Growth Performance, Immune Function and Serum Antioxidant Capacity of Broilers

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Abstract: This experiment was conducted to investigate the effects of different dietary zinc-pectic oligosaccharide chelate (Zn-POS) supplemental levels on growth performance, immune function and serum antioxidant capacity of Arbor Acre (AA) broilers. A total of 480 one-day-old healthy AA broilers with uniform body condition (half male and half female) were randomly allocated to 5 groups according to the principle of consistent body weight, with 6 replicates per group and 16 chicks per replicate. Group was fed a basal diet; group was fed the basal diet supplemented with 62.5 mg/kg flavomycin; and groups , and were fed the basal diet supplemented with 300, 600 and 900 mg/kg Zn-POS, respectively. The experiment lasted for 42 days. The results showed: 1) From 1 to 21 days of age, compared with group , the average daily gain (ADG) of groups and was significantly increased ($P<0.05$); compared with group , the body weight (BW) of groups and was significantly increased ($P<0.05$). From 22 to 42 days of age, compared with group , the ADG of groups and was significantly increased ($P<0.05$), and the average daily feed intake (ADFI) of group was significantly increased ($P<0.05$). From 1 to 42 days of age, compared with group , the ADG of group was extremely significantly increased ($P<0.01$), the ADG of groups and was significantly increased ($P<0.05$), and the ADFI of group was significantly increased ($P<0.05$); the ADG of group was extremely significantly higher than that of group ($P<0.01$). 2) The bursa of Fabricius index in group was significantly higher than that in group ($P<0.05$), but the serum immunoglobulin G level in group was significantly

lower than that in group (P<0.05). 3) The serum total superoxide dismutase activity in group was extremely significantly higher than that in group (P<0.01), and the serum catalase activity in groups and was significantly higher than that in group (P<0.05); the serum malondialdehyde content in groups and was significantly lower than that in group (P<0.05). In conclusion, dietary Zn-POS supplementation can improve the growth performance, immune function and serum antioxidant capacity of AA broilers aged 1 to 42 days, and the appropriate supplemental level is 600 mg/kg.

Keywords: Zn-POS; growth performance; immune function; serum antioxidant capacity; AA broilers

With increasing health awareness, research on prebiotics such as pectic oligosaccharides has become more extensive and in-depth. Pectic oligosaccharides, also known as oligogalacturonic acids or pectin oligosaccharides, are functional oligosaccharides discovered in plants in 1981 consisting of 2 to 20 galacturonic acid units linked by α -1,4 bonds, with some galacturonic acids existing in a methyl-esterified form. They appear as white solid powder, are easily soluble in water and slightly soluble in ethanol. Garthoff et al.'s Ames test results demonstrated that pectic oligosaccharides have no genotoxicity and exhibit good safety for food applications. Fanaro et al. evaluated the safety of pectic oligosaccharides as food additives by adding them to milk powder. Olano-Martin et al. found that pectic oligosaccharides can be well utilized by bifidobacteria in vitro and can protect HT29 colon cells from invasion by verotoxin (VT) secreted by *Escherichia coli* O157:H7. Rhoades et al. also reported that oligogalacturonic acid at pH 7.2 and concentration of 2.5 mg/mL can significantly inhibit the adhesion of enterohemorrhagic and enteropathogenic *E. coli*. Thomassen et al.'s test tube cultivation results indicated that pectic oligosaccharides have better bifidogenic effects than fructooligosaccharides. Nakanishi et al. found that adding pectic oligosaccharides to mouse diets could improve mineral element utilization by approximately 3-fold. Sun Lina's research showed that adding copper-oligogalacturonic acid chelate promoted mouse growth 4 times more effectively than adding Cu^{2+} alone, and significantly reduced Cu^{2+} content in mouse liver, blood and feces. Gullón et al. reported that pectic oligosaccharides can promote the growth of beneficial bacteria and production of short-chain fatty acids, which can lower intestinal pH and improve mineral solubility, thereby promoting intestinal mineral absorption.

Zinc is one of the most important trace elements in livestock and poultry, widely involved in animal digestion and metabolism with multiple physiological functions. Feng et al. demonstrated that dietary zinc glycine chelate can increase feed intake and growth performance in broilers while elevating serum immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG) levels and immune organ indices. Jiang Zongyong et al. found that dietary total zinc content significantly affected immune organ indices in yellow-feathered broilers, with maximum bursa, thymus and spleen indices achieved at 65 mg/kg

dietary zinc. Tian Lina et al. and Liu Xiping et al. reported that dietary nano-zinc oxide significantly improved serum total antioxidant capacity and reduced nitric oxide production in broilers. These findings indicate that dietary zinc supplementation can reduce free radical production to some extent, improve growth and immune performance, enhance antioxidant enzyme activity, and increase serum antioxidant capacity in broilers.

Zinc-pectic oligosaccharide chelate (Zn-POS) is a chelate of pectic oligosaccharides and inorganic zinc. Currently, no studies have reported the effects of Zn-POS on broilers. Research has shown that oligosaccharide chelated zinc can exert both the antibacterial effects of oligosaccharides and improve zinc absorption and utilization, including chitosan-chelated zinc and mannan oligosaccharide-chelated zinc. Therefore, this experiment aimed to investigate the effects of Zn-POS supplemental levels on growth performance, immune function and serum antioxidant capacity in Arbor Acre (AA) broilers, providing a reference for Zn-POS application in broiler production.

1.1 Experimental Animals and Design

This experiment was conducted at the Nankou Pilot Base of the Institute of Feed Research, Chinese Academy of Agricultural Sciences. A single-factor completely randomized design was adopted. A total of 480 one-day-old healthy AA broilers with uniform body condition (half male and half female) were randomly allocated to 5 groups according to the principle of consistent body weight, with 6 replicates per group and 16 chicks per replicate. The Zn-POS used in the experiment was provided by the Institute of Feed Research, Chinese Academy of Agricultural Sciences, with purity 95%, zinc content of 7%, and chelation degree 96%. The experiment lasted for 42 days. Broilers were raised on high-bed floors with free access to feed and water, following conventional immunization procedures.

1.2 Basal Diet Composition and Experimental Design

The basal diet was formulated using corn, soybean meal, soybean oil and fish meal according to the nutritional requirements of AA broilers. The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of basal diets (air-dry basis) %

Note: The premix provided the following per kg of diet: VA 10,000 IU, VD₃ 2,500 IU, VE 15 mg, VK₃ 3 mg, VB₁ 0.01 mg, nicotinic acid 34 mg, calcium pantothenate 12 mg, folic acid 0.5 mg, biotin 0.2 mg, choline chloride 1,200 mg, Fe (as ferrous sulfate) 80 mg, Cu (as copper sulfate) 8 mg, Zn 80 mg, Mn (as manganese sulfate) 100 mg, I (as potassium iodide) 0.7 mg, Se (as sodium selenite) 0.3 mg. Nutrient levels were calculated values.

Experimental design: Group served as the control group fed a basal diet without antibiotics; group was the antibiotic group fed the basal diet supple-

mented with 62.5 mg/kg flavomycin; and groups , and were fed the basal diet supplemented with 300, 600 and 900 mg/kg Zn-POS, respectively.

1.3 Sample Collection and Processing

On day 42 of the experiment, 2 healthy broilers close to the average body weight were selected from each replicate (12 per group, 60 total). After weighing, blood samples were collected from the jugular vein, allowed to clot, then centrifuged at 3,000 r/min for 15 min to prepare serum, which was aliquoted into EP tubes and stored at -20 °C for subsequent serum biochemical analysis. After blood collection, broilers were completely exsanguinated and slaughtered, and immune organs (spleen, thymus, bursa of Fabricius) were excised, trimmed of connective tissue and fat, and weighed.

1.4 Measured Indices

1.4.1 Growth Performance Indices

On days 21 and 42 of the experiment, broilers were weighed by replicate after fasting, and residual feed was recovered to calculate body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F/G) at 21 and 42 days of age.

1.4.2 Immune Organ Index

Immune organ index (g/kg) = weight of immune organ / carcass weight.

1.4.3 Serum Biochemical Indices

Serum IgA, IgM, IgG and complement 3 (C3) levels were determined by enzyme-linked immunosorbent assay using kits (Zhongsheng Beikong Biotechnology Co., Ltd., Beijing) on a Selectra-E automatic biochemical analyzer (Selectra-E model, VITA LAB Instruments, Netherlands). Serum antioxidant indices [total superoxide dismutase (T-SOD), malondialdehyde (MDA), catalase (CAT)] were measured using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing) on a UV-visible spectrophotometer (SPECORD-50, Analytik Jena, Germany).

1.5 Statistical Analysis

Basic data processing was performed using Excel 2010. Experimental results were analyzed by one-way ANOVA using SPSS 17.0, with Duncan' s multiple comparison test used for post-hoc comparisons. Results are expressed as mean \pm standard error.

2.1 Effects of Zn-POS on Growth Performance of Broilers

As shown in Table 2 , from 1 to 21 days of age, compared with group , ADG was significantly increased in groups and (P<0.05), while BW and ADFI in groups , and showed an increasing trend and F/G showed a decreasing trend,

though not significantly ($P>0.05$). Compared with group , BW was significantly increased in groups and ($P<0.05$), while F/G showed a decreasing trend in groups , and , though not significantly ($P>0.05$). The ADG and ADFI in group were significantly higher than those in group ($P<0.05$).

From 22 to 42 days of age, compared with group , ADG was significantly increased in groups and ($P<0.05$), and ADFI was significantly increased in group ($P<0.05$). Compared with group , no significant differences were observed in ADG and ADFI among groups , and ($P>0.05$). The ADG and ADFI in group were higher than those in group , but not significantly ($P>0.05$). Group had the highest BW, but no significant differences were observed among groups ($P>0.05$). No significant differences were found in F/G among all groups ($P>0.05$).

From 1 to 42 days of age, compared with group , ADG was extremely significantly increased in group ($P<0.01$), significantly increased in groups and ($P<0.05$), and ADFI was significantly increased in group ($P<0.05$). No significant differences were observed in ADG and ADFI among groups , and compared with group ($P>0.05$). The ADG in group was extremely significantly higher than that in group ($P<0.01$), though ADFI showed no significant difference ($P>0.05$). No significant differences were observed in F/G or mortality among all groups ($P>0.05$).

Table 2 Effects of different dietary Zn-POS supplemental levels on growth performance of broilers

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($P>0.05$), while different small letter superscripts mean significant difference ($P<0.05$), and different capital letter superscripts mean extremely significant difference ($P<0.01$). The same as below.

2.2 Effects of Zn-POS on Immune Function of Broilers

As shown in Table 3 , serum IgG level in group was significantly higher than that in group ($P<0.05$). Compared with group , serum IgG levels in groups , and were increased by 18.75%, 12.50% and 18.75% respectively ($P>0.05$). Serum IgA levels in groups and were higher than those in groups , and , but did not reach significant levels ($P>0.05$). Dietary Zn-POS supplementation showed a trend toward increasing serum IgM and C3 levels in 1- to 42-day-old AA broilers, but no significant differences were observed ($P>0.05$).

Table 3 Effects of different dietary Zn-POS supplemental levels on serum immune indices of broilers (g/L)

As shown in Table 4 , the bursa of Fabricius index in group was significantly higher than that in group ($P<0.05$). Dietary Zn-POS supplementation showed a trend toward increasing immune organ indices, but no significant differences were observed compared with groups and ($P>0.05$).

Table 4 Effects of different dietary Zn-POS supplemental levels on immune organ indices of broilers

2.3 Effects of Zn-POS on Serum Antioxidant Function of Broilers

As shown in Table 5, compared with group , serum T-SOD activity in group was extremely significantly increased ($P < 0.01$), serum MDA content was significantly decreased ($P < 0.05$), and serum CAT activity was significantly increased ($P < 0.05$); serum CAT activity in group was also significantly increased ($P < 0.05$). Serum T-SOD activity in group was significantly higher than that in group ($P < 0.05$). Serum MDA content in groups and was significantly lower than that in group ($P < 0.05$). No significant differences were observed in serum antioxidant indices between any Zn-POS group and group ($P > 0.05$).

Table 5 Effects of different dietary Zn-POS supplemental levels on serum antioxidant indices of broilers

The mechanism of action of Zn-POS in broilers may involve two aspects: first, the pectic oligosaccharides in Zn-POS can be selectively fermented by beneficial intestinal bacteria such as bifidobacteria to produce short-chain fatty acids, thereby reducing intestinal pH, inhibiting the adhesion and proliferation of harmful bacteria, and promoting nutrient digestion and absorption; second, the pectic oligosaccharides in Zn-POS can form complexes with trace metal elements through ionic and covalent bonds, promoting their absorption. The zinc element in Zn-POS is an essential component of many digestive enzymes in livestock and poultry, playing important physiological roles in promoting growth and enhancing immunity.

3.1 Effects of Zn-POS on Growth Performance of Broilers

Studies have shown that pectic oligosaccharides can be utilized and fermented by microorganisms in the posterior digestive tract such as bifidobacteria and lactobacilli to produce short-chain fatty acids, reduce intestinal pH, directly inhibit pathogenic microorganisms, and promote nutrient digestion and absorption. Additionally, they can form stable complexes with trace metal elements through covalent and ionic bonds, promoting their absorption and utilization. Jin Yue et al. found that adding chitosan oligosaccharide-chelated zinc containing 30 mg/kg zinc significantly improved ADFI and ADG in mice and significantly reduced F/G. Sun Lina et al. reported that adding copper-pectic oligosaccharide chelate to mouse diets did not significantly change feed intake but showed better growth-promoting effects than CuSO_4 and a mixture of pectic oligosaccharides with CuSO_4 . Yi Zhonghua et al. demonstrated that 0.25% and 0.50% fructooligosaccharides significantly improved ADG and ADFI in broilers, with better effects during weeks 1-3 than weeks 3-6, where 0.50% fructooligosaccharides increased ADG by up to 14.64% during weeks 1-3. Yu Guiyang et al. found that dietary supplementation with 1.0 and 1.5 g/kg mannan oligosaccharides significantly improved ADG by 8.89% and 6.67% respectively, with the 1.0 g/kg

group significantly reducing F/G by 5.75%. Guo Yungui et al. reported that adding 4 g/kg mannan oligosaccharides to basal diets of three-yellow chickens significantly improved ADG and showed a trend toward reducing F/G. The present results indicate that dietary supplementation with 600 mg/kg Zn-POS significantly improved ADG and ADFI in AA broilers, which is consistent with the aforementioned findings.

3.2 Effects of Zn-POS on Immune Function of Broilers

Studies have shown that oligosaccharides can bind to the surfaces of certain toxins, viruses and eukaryotic bacteria, acting as adjuvants for these foreign antigens to enhance both B lymphocyte-mediated humoral immunity and T lymphocyte-mediated cellular immunity. Oligosaccharides can promote the proliferation of beneficial bacteria such as bifidobacteria to improve animal immunity and can activate the immune system by acting on specific and non-specific immune systems. Wang Jitan et al. found that compared with antibiotics, galactomannan oligosaccharides showed no significant differences in relative weights of thymus, spleen and bursa of Fabricius, but exhibited a trend toward increasing relative weights of spleen and bursa. Jin Yue et al. reported that adding 40 mg/kg chitosan oligosaccharide-chelated zinc (as zinc) significantly improved immune organ indices including thymus, spleen and bursa of Fabricius in laying chicks. Chen Shaohong et al. found that dietary supplementation with 0.2% and 0.3% oligosaccharides increased thymus index, bursa of Fabricius index and spleen index by 16.41%, 2.62%, 25.53% and 2.65%, 11.07%, 1.65% respectively, showed a trend toward increasing leukocyte phagocytic index and serum IgG level, and significantly improved Newcastle disease antibody titer in chickens. Gao Lin reported that different supplemental levels of mannan oligosaccharides had certain effects on immune function and immune organ indices in broilers, promoting increases in serum IgA, IgG, IgM, C3, C4 and interleukin-2 levels. The present results showed that Zn-POS supplemental levels had no significant effects on serum IgA, IgG, IgM and C3 levels in broilers but showed a trend toward increasing IgG level, and exhibited a trend toward improving immune organ indices in AA broilers without significant differences compared with the control group. These differences from previous studies may be related to factors such as oligosaccharide type, supplemental level and broiler breed.

3.3 Effects of Zn-POS on Serum Antioxidant Function of Broilers

Studies have demonstrated that oligosaccharides possess strong antioxidant capacity and can effectively scavenge free radicals and toxins in vivo. Ngo et al. proved that chitin oligosaccharides can inhibit protein oxidation. Li et al. and Du Lijuan et al. reported that haw pectic oligosaccharides can significantly inhibit MDA content, improve superoxide dismutase activity in hyperlipidemic mice, and effectively scavenge superoxide anion and hydroxyl radicals in vitro. Chen et al. reported that oral administration of soybean oligosaccharides at 450 mg/kg daily significantly improved CAT, glutathione peroxidase and superox-

ide dismutase activities in rat serum, reduced thiobarbituric acid reactive substances, and prevented oxidative stress in tissues and cells. The present results showed that dietary Zn-POS supplementation significantly improved serum T-SOD and CAT activities and significantly reduced serum MDA content, which is consistent with the aforementioned findings.

Conclusions:

- 1) Dietary supplementation with Zn-POS in 1- to 42-day-old AA broiler diets can improve growth performance, immune function and serum antioxidant capacity. The growth-promoting effect of adding 600 mg/kg Zn-POS is comparable to that of adding 62.5 mg/kg flavomycin.
- 2) The appropriate supplemental level of Zn-POS in 1- to 42-day-old AA broiler diets is 600 mg/kg.

References:

- [1] DONG Xiangyan, PENG Qing, EROMOSELE O, et al. Research progress on biological activities of oligogalacturonic acid[J]. *Journal of Nuclear Agricultural Sciences*, 2014, 28(6): 1076-1082.
- [2] WANG Jianglang. Study on enzymatic preparation, separation and antibacterial activity of apple pectin oligosaccharides[D]. Master' s thesis. Yangling: Northwest A&F University, 2009.
- [3] BISHOP P D, MAKUS D J, PEARCE G, et al. Proteinase inhibitor-inducing factor activity in tomato leaves resides in oligosaccharides enzymically released from cell walls[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1981, 78(6): 3536-3540.
- [4] WANG Danbo. Study on enzymatic preparation and application of pectin oligosaccharides[D]. Master' s thesis. Hangzhou: Zhejiang University of Technology, 2006.
- [5] GARTHOFF J A, HEEMSKERK S, HEMPENIUS R A, et al. Safety evaluation of pectin-derived acidic oligosaccharides (pAOS): genotoxicity and sub-chronic studies[J]. *Regulatory Toxicology and Pharmacology*, 2010, 57(1): 31-42.
- [6] FANARO S, MARTEN B, BAGNA R, et al. Galacto-oligosaccharides are bifidogenic and safe at weaning: a double-blind randomized multicenter study[J]. *Journal of Pediatric Gastroenterology and Nutrition*, 2009, 48(1): 82-88.
- [7] OLANO-MARTIN E, GIBSON G R, RASTALL R A. Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides[J]. *Journal of Applied Microbiology*, 2002, 93(3): 505-511.
- [8] RHOADES J, MANDERSON K, WELLS A, et al. Oligosaccharide-mediated inhibition of the adhesion of pathogenic *Escherichia coli* strains to human gut epithelial cells in vitro[J]. *Journal of Food Protection*, 2008, 71(11): 2272-2277.

- [9] THOMASSEN L V, VIGSNÆS L K, LICHT T R, et al. Maximal release of highly bifidogenic soluble dietary fibers from industrial potato pulp by minimal enzymatic treatment[J]. *Applied Microbiology and Biotechnology*, 2011, 90(3): 873-884.
- [10] NAKANISHI N, KITADA Y. Mineral absorption promoting agent: US, 5952308[P]. 1999-09-14.
- [11] SUN Lina. Enzymatic preparation of oligogalacturonic acid and its effects of copper chelate on growth characteristics in mice[D]. Master's thesis. Beijing: Chinese Academy of Agricultural Sciences, 2007.
- [12] GULLÓN B, GÓMEZ B, MARTÍNEZ-SABAJANES M, et al. Pectic oligosaccharides: manufacture and functional properties[J]. *Trends in Food Science & Technology*, 2013, 30(2): 153-161.
- [13] YUAN Tao, ZHANG Weili, WU Hongzhong, et al. Research progress on oligosaccharides in animal nutrition[J]. *Cereal and Feed Industry*, 2003(5): 26-28.
- [14] SONG Yicheng, ZHAO Huxiang. Special effects of feed oligosaccharides on animals[J]. *Veterinary Pharmaceuticals and Feed Additives*, 2000, 5(2): 34.
- [15] FENG J, MA W Q, NIU H H, et al. Effects of zinc glycine chelate on growth, hematological, and immunological characteristics in broilers[J]. *Biological Trace Element Research*, 2010, 133(2): 203-211.
- [16] JIANG Zongyong, LIU Xiaoyan, JIANG Shouqun, et al. Zinc requirement of 1- to 21-day-old yellow-feathered broilers[J]. *Chinese Journal of Animal Nutrition*, 2010, 22(2): 301-309.
- [17] TIAN Lina, ZHU Fenghua, REN Huiying, et al. Effects of nano-zinc oxide on antioxidant capacity in broilers[J]. *Chinese Journal of Animal Nutrition*, 2009, 21(4): 534-539.
- [18] LIU Xiping, SUI Meixia, XU Zhongxiang, et al. Research progress on trace element zinc and different zinc sources in livestock and poultry nutrition[J]. *China Animal Husbandry and Veterinary Medicine*, 2014, 41(6): 94-98.
- [19] JIN Yue. Preparation of chitosan oligosaccharide-chelated zinc and comparative study on its nutritional functions[D]. Master's thesis. Dalian: Liaoning Normal University, 2011.
- [20] YI Zhonghua, XU Chuanlai, CHEN Xudong, et al. Effects of fructooligosaccharides supplementation in low-oligosaccharide diets on broiler performance[J]. *China Poultry*, 2004, 26(16): 15-17.
- [21] YU Guiyang, ZHENG Chunfang, QIN Kaiquan, et al. Effects of mannan oligosaccharides on growth performance of broilers[J]. *Poultry Science*, 2009(9): 36-37.

- [22] GUO Yungui, ZHU Huixia, TANG Yegang, et al. Effects of mannan oligosaccharides on growth performance and lipid metabolism in three-yellow chickens[J]. Jiangsu Agricultural Sciences, 2011, 39(4): 245-247.
- [23] LING Baoming, QU Mingren, LU Dexun. Immunological functions and mechanisms of functional oligosaccharides[J]. Guangdong Feed, 2005, 14(1): 29-31.
- [24] QIN Chunfu, SUN Peng, ZHANG Peihua, et al. Research progress on effects of functional oligosaccharides on animal immune function[J]. Acta Agriculturae Boreali-Sinica, 2012, 27(Supplement): 421-424.
- [25] WANG Jitan, LI Defa, GONG Limin, et al. Effects of galactomannan oligosaccharides on performance and immune function in broilers[J]. Chinese Journal of Animal Science, 2003, 39(2): 5-7.
- [26] CHEN Shaohong, WAN Jianghong, HUANG Yinji, et al. Effects of oligosaccharides on performance and immune function in three-yellow chickens[J]. Journal of Zhanjiang Ocean University, 2005, 25(3): 76-78.
- [27] GAO Lin. Effects of mannan oligosaccharides on immune performance in broilers[J]. Agricultural Science and Technology and Equipment, 2015(4): 56-58.
- [28] NGO D N, KIM M M, KIM S K. Chitin oligosaccharides inhibit oxidative stress in live cells[J]. Carbohydrate Polymers, 2008, 74(2): 228-234.
- [29] LI T P, LI S H, DU L J, et al. Effects of haw pectic oligosaccharide on lipid metabolism and oxidative stress in experimental hyperlipidemia mice induced by high-fat diet[J]. Food Chemistry, 2010, 121(4): 1010-1013.
- [30] DU Lijuan, LI Tuoping, WANG Na, et al. Study on antioxidant activity of haw pectin hydrolysates[J]. Food Research and Development, 2009, 30(6): 18-21.
- [31] CHEN H, LIU L J, ZHU J J, et al. Effect of soybean oligosaccharides on blood lipid, glucose levels and antioxidant enzymes activity in high fat rats[J]. Food Chemistry, 2010, 119(4): 1633-1636.

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