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IMMUNE RESPONSES OF BROILER CHICKS SUPPLEMENTED WITH HIGH LEVELS OF ZINC

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ABSTRACT: *The objective of this study was to evaluate if the high levels of zinc can improve different aspects of broilers' immune system. One hundred and forty-four 1-d old broiler chicks were used in the current study with three dietary zinc (40, 120 and 200 mg/kg). At 2, 22, 32, 42 days of age, the blood serums were tested for antibody titer against Newcastle disease vaccination, using the standard Haemagglutination Inhibition test. On day 42 the sum of nitrite and nitrate (based on the reduction of nitrate to nitrite by cadmium) were measured and the weights of spleen and bursa of fabricius were recorded on a relative live weight basis. At 42 d, antibody response of level 200 mg/kg diet was significantly higher than control. Adding levels of 120 and 200 mg Zn/kg diet significantly increased the weight of bursa and spleen respectively (P< 0.05) compared with the control. Also level of 200 mg Zn/kg diet showed the highest amount of nitrite and nitrate in compare with other levels. The use of 200 mg Zn/kg diet in broilers diet could be considered as a natural promoter of cell-mediated immunity.*

ORIGINAL ARTICLE

Key words: *Antibody titer, Broiler, Immune organs, Newcastle disease, Nitrite and nitrate, Zinc*

INTRODUCTION

Nowadays the use of specific dietary supplements to boost the intrinsic potential of poultry to perform better immunologically is so important. Trace elements are involved in the metabolic activities via metalloenzymes which are essential for the antioxidant protection of cells in poultry (Petrovic et al., 2010).

Zinc is essential for highly proliferating cells, especially in the immune system and is an essential cofactor for thymulin which modulates cytokine release and induces proliferation (Maggini et al., 2007). Some studies indicate that supplementing the diet of broilers above 40 ppm recommended by the National Research Council (1994) enhances antibody production (Kidd et al., 2004).

Bartlett and Smith (2003) reported the broilers receiving 68 and 181 mg Zn had a higher response for total, IgM, and IgG antibodies.

The objective of this study was to evaluate if the high levels of zinc can improve different aspects of broilers' immune system like macrophages activity and immune organs.

MATERIALS AND METHODS

One hundred and forty-four 1-d old Ross 308 broiler chicks were used in this experiment. The study was carried out according to a completely randomized design, with three dietary Zn-So_4 (Merck Art, number 10888331000) levels and four replicates of 12 birds. The experimental diets were manufactured from a basal diet (Table 1), which was formulated to meet the nutrient requirements of broiler chickens (NRC, 1994). Three zinc levels (40, 120 and 200mg/kg) were added to the basal diet to establish the treatments. Zinc contents in starting, finishing basal diets and potable water were 72, 70 and 5 mg/kg respectively, as measured by atomic absorption analysis. Birds were kept in floor pens, and diets and fresh water were provided *ad libitum* from day one.

The lighting program used was 24 hours of artificial light during the entire experimental period, which Lasted 42 days. At 42 day of age, eight birds from each treatment were chosen at random, weighed and then slaughtered. The weights of carcass, spleen and bursa of fabricius were recorded. Organ weights were expressed on a relative

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carcass weight basis. Birds of all groups were intramuscularly injected with 0.1 ml of killed Newcastle disease (ND) vaccine (Cevac®Broiler NDK) at eight days of age. Blood samples from each replicate were collected at 2, 22, 32, 42 days of age. All the blood samples obtained from wing vein and serums were separated by 3000 rpm centrifuging for 15 min. The serums were tested for antibody against NDV, using the standard Haemagglutination Inhibition (HI) test (Allan and Gough, 1974) and the results were expressed as the logarithm base 2. Also at 42 d serum samples were prepared from eight chicks per each treatment and sum nitrite and nitrate was measured based on the reduction of nitrate to nitrite by cadmium. The nitrite produced was determined by Griess reaction. The serum sample was deproteinized by adding ZnSO (75 mmol/l) and NaOH (55 mmol/l) solutions. After centrifuging, the supernatant was recovered and diluted in glycine buffer (45 g/l, pH 9.7). Cadmium granules (2 - 2.5 g) were rinsed three times with deionized distilled water and swirled in a CuSO4 solution (5 mmol/l) in glycine-NaOH buffer (15 g/l, pH 9.7) for five min to become activated. Freshly activated cadmium granules were added to pretreated deproteinized serum. After continuous stirring for 10 min, the samples were transferred to appropriately labeled tube for nitrite determination by Griess reaction. Griess reagent 1 (1% sulfanilamide in 5% phosphoric acid) was added to the sample tubes and then incubated for 10 minutes at room temperature, protected from light. Griess reagent 2 was added (0.1% N-napthylethylenediamine dihydrochloride in water) to all samples and absorbance was measured within 10 minutes in a spectrophotometer at a wavelength of 540 nm (Pirali-Kheirabadi et al., 2011). At the final stage, the sum of the nitrite and nitrate was measured.

Table 1 - Ingredients and calculated composition of the starter and finisher diets

Ingredients	Starter (g/kg)	Finisher (g/kg)
Corn	535.5	595.7
Soybean meal 44%CP	389.3	333.4
Monodibasic Phosphate	14.3	12.1
Limestone	13.5	13.8
Vegetable oil	38.4	35.1
Salt	4.1	4.3
DL-methionine	2.07	2.14
L-Lysine HCl	1.29	1.97
Choline HCl 60%	0.6	0.5
Mineral-vitamin premix[1]	1	1
Total	1,000	1,000
Calculated Nutrients		
Crude protein	220	200
ME, kcal/kg	3,050	3,100
Calcium	9	8.5
Available phosphorus	4	3.5
Digestible Lys	11.5	10.7
Digestible Met	4.9	4.8
Digestible Met+Cys	8.1	7.7

[1]Composition (per kg): Mn (from MnSO4·H2O), 40,000 mg; Fe (from FeSO4·7H2O), 20,000 mg; Cu (from CuSO4·5H2O), 40,000 mg; I (from Ca (IO3) 2·H2O), 400 mg; vitamin A (from vitamin A acetate), 3,600,000 IU; cholecalciferol, 800,000 IU; vitamin E (from DL-α-tocopheryl acetate), 7,200 IU; menadione, 800 mg; thiamine; 720 mg; riboflavin, 2,640 mg; niacin, 4,000 mg; calcium pantothenate, 12,000 mg; pyridoxine, 1,200 mg; folic acid, 400 mg; cyanocobalamin, 6 mg; biotin, 40 mg; choline, 100,000 mg.

Statistical analysis

Statistical analyses were conducted using the ANOVA general linear models procedure of SAS software (SAS Institute, 1997). When ANOVA revealed significant effects, means were separated by Duncan's multiple range tests. The values were considered significant at $P< 0.05$.

RESULTS

Immune organs (spleen and bursa of fabrecius) weight were measured on a relative carcass weight basis (Table 2). Adding levels of 120 and 200 mg Zn/kg diet significantly increased the weight of bursa and spleen respectively ($P< 0.05$) compared with the control.

Table 2 - The Immune organ weights of broilers fed different levels of zinc

Zn level (mg/kg)	Bursa (%)	Spleen (%)
40	0.066b±0.008[b]	0.12±0.011[b]
120	0.084±0.006[a]	0.139±0.002[ab]
200	0.078±0.009[ab]	0.165±0.026[a]
SEM	0.008	0.016

[a,b] Means within a column with no common superscript are significantly different ($p<0.05$)

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Fig 1 shows the effects of graded levels of Zn on antibody titer against NDV of broiler chicks. Although at 2, 22, 32 d there was no significant difference in antibody titer, but at 42 d, antibody response of level 200 mg/kg diet was significantly (P< 0.05) higher than control, however the difference between 120 and 200 mg/kg diet was not significant.

Fig 2 shows the sum of nitrite and nitrate in serum after using different treatments. Results showed that adding additional Zn significantly (P< 0.05) increased the sum of nitrite and nitrate in the serum of broilers. Also level of 200 mg Zn/kg diet showed the higher amount of nitrite and nitrate in compare with the level of 120 mg Zn/kg diet; however this difference was not significant.

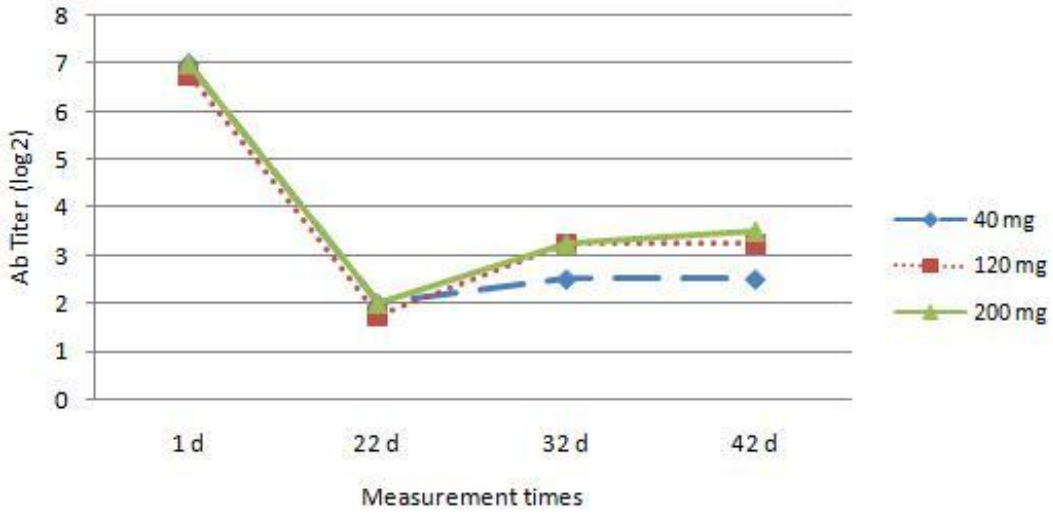

Figure 1 - Antibody responses of broilers fed different levels of zinc

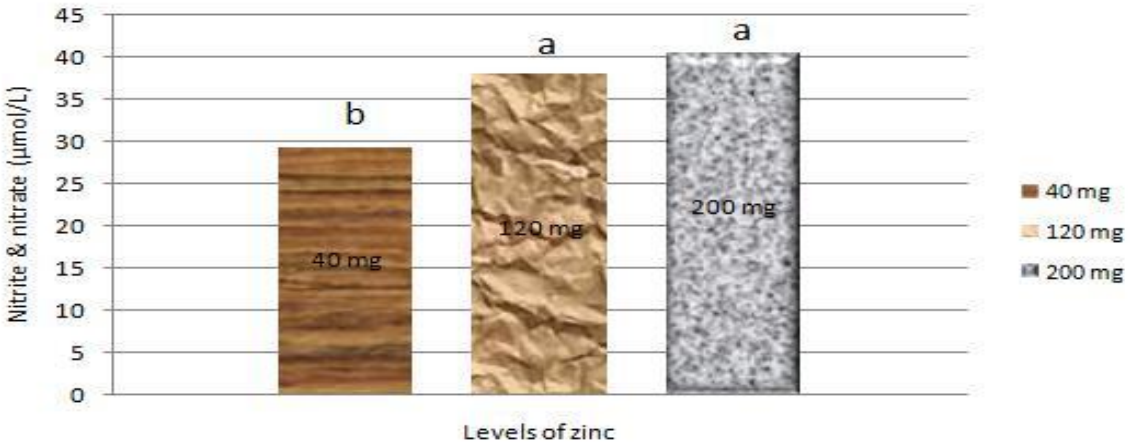

Figure 2 - Effects of Zn supplementation on sum of nitrite & nitrate in the serum of broilers.
[a,b] Columns that do not share the same letters differ significantly (P< 0.05).

DISCUSSION

Increase of the weight of bursa and spleen in this study was similar with result of Bartlett and Smith (2003) that showed a slight increase in lymphoid organs weight. Also Feng et al. (2011) reported that thymus, spleen, and bursa of fabricius indexes increased linearly with increasing dietary Zn. These finding could be due to role of Zn in growth and function of lymphocytes. Our results showed that antibody response at the level 200 mg/kg diet was significantly higher than control. Bartlett and Smith (2003) reported the broilers receiving 68 and 181 mg Zn had a higher response for total, IgM, and IgG antibodies. Also Hosseini et al. (2010) demonstrated that the graded Zn increased IgM and IgY titer against SRBC. Zinc is essential for thymulin, a thymic hormone that regulates T-lymphocyte maturation. Birds provided diets supplemented with a more available zinc source might have induced thymulin activity, and therefore promoted immune responses through increased maturation of T-lymphocytes and activation of B-lymphocytes by T-helper cells (Nassiri-Moghadam and Jahanian, 2009). Increase of sum of nitrite and nitrate has been caused by increase in production of macrophages. Zinc has effect on some aspects of

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macrophage function. It was proposed that resistance to some diseases after supplementation with Zn, may be due to role it in nitric oxide–mediated microbicidal activity of macrophages (Shankar and Prasad, 1998). This result was in agreement with finding of Bartlett and Smith (2003) that birds fed the 181 mg Zn/kg diet had more activated macrophages for opsonized and unopsonized sheep red blood cell (SRBC) than those fed the lower Zn. There is much speculation regarding the role of zinc in the killing of pathogens by oxygen radicals produced by macrophages. Rapid therapeutic effects of zinc supplementation on diarrhea or the common cold may involve some aspects of macrophage function.

CONCLUSION

The overall results of this study showed that supplementation of diet of broilers above 40 mg Zn/kg increased different aspects of immunity. Level of 120 mg Zn/kg diet was enough to achieve the maximum weight of bursa of fabrecius, while level of 200 mg Zn/kg diet was more efficient to increase spleen weight, antibody titer against NDV and sum of nitrite and nitrate in serum. Then the use of 200 mg Zn/kg diet in broilers diet could be more considered as a natural promoter of immunity in broiler chicks.

REFERENCES

Allan WH and Gough RE (1974). A standard Haemagglutination Inhibition test for Newcastle disease. A comparison of macro and micro methods. Veterinary Record, 95: 120-123.

Bartlett JR and Smith MO (2003). Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poultry Science, 82: 1580-1588.

Feng J, Ma WQ, Niu HH, Wu XM, Wang Y and Feng J (2011). Effects of zinc glycine chelate on growth, hematological, and immunological characteristics in broilers. Biology Trace Element Research, 431-439.

Hosseini S, Arshami J and Torshizi ME (2010). Immunomodulatory effects of graded copper and zinc on SRBC titer and lymphoid organs in broiler chicks. Journal of Animal Veterinary Advance, 9(11): 1650-1653.

Kidd MT (2004) Nutritional modulation of immune function in broilers. Poultry Science, 83: 650–657.

Maggini S, Wintergerst ES, Beveridge S and Hornig DH (2007). Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. British Journal Nutrition, 98(1): 29–35.

Nassiri-Moghadam H and Jahanian R (2009). Immunological responses of broiler chicks can be modulated by dietary supplementation of zinc-methionine in place of inorganic zinc sources. Asian-Australian Journal of Animal Science, 22(3): 396–403.

National Research Council (1994). Nutrition Requirements of Poultry. 9th Rev. Edition National Academy Press, Washington, DC.

Petrovič V, Kushev J, Nollet L and Kováč G (2011). Effect of dietary supplementation of trace elements on blood chemistry and selected immunological indices depending on the age of broiler chickens. Acta Veterinary Brno, 80: 057–064.

Pirali-Kheirabadi KH, Hassanpour H, Nourani H, Farahmand E, Cheraghchi Bashi M and Hossainpour Jaghdani F (2011). Increasing of serumnitric oxidemetabolites in chicken Eimeria infection. International Journal of Veterinary Research, 5(2): 99-10.

SAS Institute (1997). SAS User's Guide. Statistics. SAS Institute, Inc., Cary, NC. Virden WS, Yeatman JB, Barber SJ, Willeford KO, Ward TL.

Shankar AH and Prasad AS (1998). Zinc and immune function: the biological basis of altered resistance to infection. American Journal of Clinical Nutrition, 68(suppl): 447S–463S.