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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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High Levels of Zinc Stimulate Different Aspects of Immune System in Broiler Chicks

S. Sajadifar[1] and H. Miranzadeh[2]
[1]Faculty of Veterinary, Department of Epidemiology and Parasitology,
Armenian National Agrarian University, Yerevan, Armenia
[2]Department of Veterinary, University of Applied Science and Technology,
Institute of Science Applied Higher Education of Jahad-e-Agriculture, Isfahan, Iran

Abstract: In this study experimental treatments were as follows: broilers fed with 40 mg/kg Zn (A), broilers fed with 40 mg/kg Zn and vaccinated against coccidiosis (B), broilers fed with 120 mg/kg Zn (C),broilers fed with 120 mg/kg Zn and vaccinated against coccidiosis (D), broilers fed with 200 mg/kg Zn (E), broilers fed with 200 mg/kg Zn and vaccinated against coccidiosis (F). Average body weight gain, average daily feed intake and feed conversion ratio were obtained on d 21 and 42. 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 total protein, albumin, globulin (by an automated analyzer), weights of spleen and bursa of fabricius (on a relative live weight basis) and total leukocytes (using a hemocytometer) were measured. No dietary treatment significantly altered average daily feed intake, body weight gain and feed conversion ratio at days 21 and 42. The highest weights of spleen and bursa of fabrecius were observed at the combination of highest level of zinc and coccidiosis vaccine. At 32 d, treatment D and at 42 d, treatments D, E and F had the highest antibody titer against Newcastle disease vaccination. It is concluded that the treatments B, C, D, E and F showed significant ($p<0.05$) increase in white blood cell count as compare to control group (treatment A). Treatment F significantly improved total protein and albumin as compare to treatment A ($p<0.05$). Additional levels of zinc could be considered as natural promoter to increase different aspects of immune system without any harmful effect on performance traits.

Key words: Antibody titer, coccidiosis, immune organs, Newcastle disease, total white blood cells, zinc

INTRODUCTION

Studies on the relationships between animal nutrition and immunity have sought reliable methodologies to measure responses. It is clear that zinc affects multiple aspects of immune system, from the barrier of the skin to gene regulation within lymphocytes. Zinc is crucial for normal development and function of cells mediating nonspecific immunity such as neutrophils and natural killer cells (Shankar and Prasad, 1998). There are conflicting results regarding the level of zinc required to affect an immune response. 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) whereas the others have reported no effect (Pimentel *et al*., 1991b). Coccidiosis is regarded as the parasitic disease that has the greatest economic impact on the poultry industry, with total losses estimated at a devastating $3 billion annually worldwide (Dalloul and Lillehoj, 2006). Because of the resistance against anticoccidials that often develops, vaccination is the most appropriate method for disease control (Augustine *et al*., 2001). Present investigation was undertaken in broiler chicks given high levels of zinc and submitted with coccidiosis vaccine by measuring some aspects of immunity like produced antibodies against Newcastle disease vaccination, weight of immune organs and white blood cell counts.

MATERIALS AND METHODS

Animals and treatments: The study was carried out according to a completely randomized design, with six treatments and four replicates of 12 birds. Experimental treatments were as follows: broilers fed with 40 mg/kg Zn (A), broilers fed with 40 mg/kg Zn and vaccinated against coccidiosis (B), broilers fed with 120 mg/kg Zn (C), broilers fed with 120 mg/kg Zn and vaccinated against coccidiosis (D), broilers fed with 200 mg/kg Zn (E), broilers fed with 200 mg/kg Zn and vaccinated against coccidiosis (F). Zinc supplementation was provided by $ZnSO_4$ (MERCK ART, number 1.088833.1000) and was added to the basal diet (Table 1) to manufacture the treatments. Vaccine of coccidiosis (LIVACOX Q®, MERIAL) was used at d 7, in groups mentioned (B, D and F) at a dose of 1.5 mL/300mL of water distributed in 2mL for each broiler

Corresponding Author: S. Sajadifar, Faculty of Veterinary, Department of Epidemiology and Parasitology, Agrarian State University, Yerevan, Armenia

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

Ingredients	Starter (%)	Finisher (%)
Corn	53.55	59.57
Soybean meal 44%CP	38.93	33.34
Monodibasic Phosphate	1.43	1.21
Limestone	1.35	1.38
Vegetable oil	3.84	3.51
Salt	0.41	0.43
DL-methionine	0.207	0.214
L-Lysine HCl	0.129	0.197
Choline HCl 60%	0.06	0.05
Mineral-vitamin premix[1]	0.1	0.1
Total	100	100
Calculated nutrients		
Crude protein %	22	20
ME, kcal/kg	3,050	3,100
Calcium, %	0.9	0.85
Available phosphorus, %	0.4	0.35
Sodium, %	0.2	0.21
Chloride, %	0.27	0.29
Digestible Lys, %	1.15	1.07
Digestible Met,, %	0.49	0.48
Digestible Met+Cys %	0.81	0.77
Digestible Thr, %	0.78	0.71
Choline, mg/kg	1,420	1,300

[1]Composition (per kg): manganese, 75,000 mg; iron, 50,000 mg; copper, 8,000 mg; iodine, 750 mg; vitamin A, 8,000 kIU; vitamin D3, 2,000 kIU; vitamin K3, 1,800 mg; vitamin B1; 1,800 mg; vitamin B2, 6,000 mg; vitamin B6, 2,800 mg; vitamin B12, 12,000 μg; pantothenic acid, 10,000 mg; niacin, 40,000 mg; folic acid, 1,000 mg; biotin. 60,000 μg; selenium, 0.3 mg/kg. Basal diets Zn measured by atomic absorption spectrometer and Zinc contents were 74 and 72 mg/kg in starting and finishing basal diets

via oral administration. 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.

Newcastle Disease Vaccine (NDV): Birds of all groups were intramuscularly injected with 0.1 ml of killed NDV (Cevac®Broiler NDK) at eight days of age.

Performance traits: Birds were weighed on d 1, 21 and 42 as a group. Average Body Weight Gain (BWG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) were obtained by pen on d 21 and 42.

Immune organs: At 42 day of age, eight birds from each treatment were chosen at random, weighted and then slaughtered. The weights of spleen and bursa of fabricius were recorded. Organs weights were expressed on a relative live weight basis.

Antibody titer against NDV: Blood samples from each replicate were collected at 2, 22, 32, 42 days of age. 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 $\log_2$.

Total White Blood Cells (WBC) counts: On 42d, blood samples were collected from wing vein using sterile lancet. Briefly, 490μ / of brilliant cresly blue dye was mixed with 10μ / whole blood sample and total leukocytes were counted using a hemocytometer.

Total protein, albumin and globulin: At the end of the experimental period (42 d of age), blood samples were collected from wing vein using sterile lancet and centrifuge tubes containing EDTA and then centrifuged at 3000 rpm for 15 min. Plasma was separated and used for measurement of the total protein and albumin. Globulin concentration was computed by subtracting albumin from total protein and consequently albumin to globulin ratio was calculated. The experiments were performed by an automated analyzer (RA 1000).

Statistical analysis: Statistical analyses were conducted using the ANOVA general linear models procedure of SAS software (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

The influence of dietary treatments on performance traits are listed in Table 2. No dietary treatment significantly altered Average Daily Feed Intake (ADFI), Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) at days 21 and 42. Immune organs weight (spleen and bursa of fabrecius) were measured on a relative carcass weight basis (Table 3). The highest weight of spleen and bursa of fabrecius were observed at the combination of highest level of zinc and coccidiosis vaccine (treatment F). Table 4 shows the effects of different treatments on antibody titer against NDV. At 32 d, treatment D and at 42 d, treatments D, E and F had the highest antibody titer against NDV. Results show that coccidiosis vaccine did not significantly affect on antibody response against NDV of broilers in different ages. Table 5 displays the effects of Zn supplementation on White Blood Cell (WBC) count. Results show that treatment A had the lowest WBC count in compare with the other treatments ($p<0.05$). In other hand, however treatment F showed the highest WBC count but its difference with treatments D was not significant. Also WBC count was significantly ($p<0.05$) increased in all three levels of 40, 120 and 200 mg Zn/kg diet after vaccination against coccidiosis. The effects of different levels of zinc on total protein, albumin, globulin and albumin/globulin (A/G) ratio in the

Table 2: Average Daily Feed Intake (ADFI), Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) of broilers fed different levels of zinc

	0-21			0-42		
Treatment	ADFI (g/bird)	BWG (g/bird)	FCR (g/g)	ADFI (g/bird)	BWG (g/bird)	FCR (g/g)
A	30.33±0.82	18.18±0.73	1.67±0.03	67.15±4.08	36.13±1.87	1.86±0.11
B	30.8±0.86	18.83±0.77	1.64±0.06	69.65±0.29	36.53±0.62	1.91±0.02
C	29.78±0.26	18.56±0.41	1.6±0.05	63.95±1.16	35.9±0.75	1.87±0.04
D	30.45±0.93	19.6±0.7	1.53±0.05	69.33±0.82	36.35±0.33	1.91±0.03
E	31.35±0.84	19.48±0.87	1.68±0.07	70.4±1.16	36.3±1.33	1.94±0.03
F	30.35±0.29	19.12±0.72	1.59±0.05	67.85±.82	35.7±0.99	1.9±0.03
SEM	1.33	1.097	0.074	5.22	2.58	0.01

Table 3: Effects of different treatments on immune organs weights of broiler chicks

Treatment	Spleen (% live body weight)	Bursa (% live body weight)
A	0.066±0.008 ^{d}	0.112±0.011^{d}
B	0.081±0.01^{dc}	0.156±0.021^{bc}
C	0.085±0.006^{bc}	0.139±0.002^{dc}
D	0.097±0.006^{b}	0.177±0.008^{bc}
E	0.077±0.009^{dc}	0.165±0.026^{bc}
F	0.126±0.018^{a}	0.243±0.027^{a}
SEM	0.01	0.02

^{a,b,c}Columns that do not share the same letters differ significantly ($p<0.05$)

Table 4: Effects of different treatments on blood antibody titer against NDV at different ages in broilers

Treatment	1 d	22 d	32 d	42 d
A	7±0	2±0^{ab}	2.5±0.5^{b}	2.5±0.5^{b}
B	6.5±0.5	1.5±0.5^{b}	2.5±0.5^{b}	2.5±0.5^{b}
C	6.75±0.5	1.75±0.5^{b}	3.25±0.5^{ab}	3.25±0.5^{ab}
D	7±0	2±0^{ab}	3.5±0.5^{a}	3.5±0.5^{a}
E	7±0	2±0^{ab}	3.25±0.5^{ab}	3.5±0.5^{a}
F	7±0	2.5±0.5^{a}	3±0^{ab}	3.5±0.5^{a}
SEM	0.31	0.39	0.5	0.57

^{a,b}Columns that do not share the same letters differ significantly ($p<0.05$)

Table 5: Effects of different treatments on White Blood Cell (WBC) count of broiler chicks

Treatment	WBC (x10^{3}/µL)
A	21.13±0.28^{e}
B	21.83±0.71^{d}
C	24.51±0.55^{c}
D	25.66±0.5^{ab}
E	25.12±0.24^{bc}
F	26.08±0.14^{a}
SEM	0.45

^{a,b,c,d,e}Columns that do not share the same letters differ significantly ($p<0.05$)

blood of the broilers were shown in Table 6. Treatment F improved total protein and albumin whereas treatments E and F increased globulin as compareto the other treatments ($p<0.05$). Also using coccidiosis vaccine at level of 200 mg Zn/kg diet did not significantly change the amount of globulin.

DISCUSSION

Our findings about performance traits were in accordance with the results of Bartlett and Smith (2003) who found no difference in weight gain, feed intake and feed conversion of broilers fed at 34, 68 and 181 mg Zn/kg. In the current study, birds provided treatment F showed the highest weight of spleen and bursa of fabrecius. Feng *et al.* (2011) reported that thymus, spleen and bursa of fabricius indexes increased linearly with increasing dietary Zn. Tanaka *et al.* (1990) reported that zinc is required for lymphocyte proliferation. Increase of antibody titer against NDV in higher levels of zinc was similar to the findings of Bartlett and Smith (2003) who reported that broilers receiving 68 and 181 mg Zn had a higher response for total, IgM and IgG antibodies. Also Kidd *et al.* (2000) demonstrated that higher Zn levels produce better immune status in broiler chickens. Improvement of blood WBC count by additional zinc in the current study is due to antioxidant role of zinc that is known to modulate immune function by protecting cells from the damaging effects of oxygen radicals generated during immune activation (Bray and Bettger, 1990). Also increase of WBC count after vaccination against coccidiosis, in all three levels, could be due to effect of vaccination on improve of immune system functions and produce of more white blood cells. The highest amounts of total protein, albumin and globulin were observed in treatments E and F. Feng *et al.* (2011) suggested that adding additional Zn improved the levels of total protein in broiler chicks, because zinc is an essential component of a large number of enzymes participating in the synthesis of proteins and nucleic acids (Maggini *et al.*, 2007). Also after using coccidiosis vaccine at level of 200 mg Zn/kg diet we did not observe significant increase in amount of globulin that can demonstrate just adding 200 mg Zn/kg diet would be enough to increase globulin in serum. In the current research, supplementing the diet with 120 and 200 mg Zn with or without coccidiosis vaccine did not significantly affect on performance traits of broilers. Also the overall results of this study show the optimal level of zinc to obtain the best response of immune system in different aspects of immunity can be varied. Level of 120 mg Zn/kg diet along with coccidiosis vaccine was enough to achieve the maximum WBC count and antibody titer against NDV in blood, while level of 200 mg Zn/kg diet along with coccidiosis vaccine showed more efficiency to increase the spleen and bursa of fabrecius weights, total protein, albumin and globulin. Level of 120

Table 6: Effects of different treatments on total protein, albumin, globulin and A/G ratio in the blood of the broilers

Treatment	Total protein (g/dL)	ALB (g/dL)	GLO (g/dL)	A/G
A	4.23±0.05c	2.18±0.05dc	2.05±0.06b	1.06±0.05ab
B	4.33±0.05c	2.28±0.09c	2.05±0.06b	1.11±0.08a
C	4.23±0.05c	2.2±0.03dc	2.03±0.05b	1.09±0.02ab
D	4.08±0.15d	2.1±0.11d	1.98±0.05b	1.06±0.04ab
E	4.85±0.06b	2.4±0.08b	2.45±0.13a	0.98±0.08b
F	5±0.12a	2.53±0.09a	2.48±0.12a	1.02±0.08ab
SEM	0.089	0.08	0.08	0.06

[a,b,c,d]Columns that do not share the same letters differ significantly ($p<0.05$)

and 200 mg Zn/kg diet could be considered as a natural promoter to improve different aspects of immune system without any harmful effect on performance traits.

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