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Immunomodulatory Effects of Graded Copper and Zinc on SRBC Titer and Lymphoid Organs in Broiler Chicks

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Abstract: A total of 400 pieces of 1 day old male broiler chicks were distributed into 9 treatments and 1 control diet with 4 replicates using a CRD design (3×3 factorial). Chicks for 42 days received graded levels of Cu (35, 70, 105 mg kg^{-1} diet) and Zn (40, 80, 120 mg kg^{-1} diet) in their diets (free of Cu and Zn mineral premix) to determine the antibody titer of SRBC and the weights of lymphoid organs (%BW) including: Thymus (TW), Bursa (BW), Spleen (SW) and Liver (LW). On days 28 and 35, two birds from each pen received SRBC (1 mL, 10%) injections and were bled 7 days after each inoculation to evaluate the primary and secondary responses for Total Immunoglobulin (TIg), Immunoglobulin M (IgM) and Immunoglobulin Y (IgY). On day 42, two birds from each pen were slaughtered to weigh the lymphoid organs. Since analysis of data showed no significant interaction between graded Cu and zinc, only the main effect of means were considered. The graded Cu increased titers of TIg and IgY linearly but reduced IgM titer quadratically in primary response. In secondary response, the titers of TIg and IgM decreased but IgY increased quadratically. The graded Zn increased TIg and decreased IgM titers quadratically but the titer of IgY increased linearly in primary response. In secondary response, the graded Zn increased IgM titer but decreased TIg and IgY titers quadratically. The graded Cu enhanced TW and SW linearly but BW and LW ($p<0.05$) quadratically. The graded Zn increased TW and decreased BW linearly but decreased SW and LW quadratically. Overall results indicated that adding Cu at 70-105 ppm and Zn at 80-120 ppm improved SRBC titers and weights of lymphoid organs. In conclusion, adding Cu and Zn at recommended levels may be useful as immunomodulators in broiler chicks.

Key words: SRBC titer, lymphoid organs, copper and zinc, broiler chicks, immunomodulators, Iran

INTRODUCTION

The poultry industry relies on the application of antibiotics or related medications to improve disease resistance within poultry flocks. However, this practice has accompanied with the prevalence and establishment of antibiotic-resistant species within the human population (Ratcliff, 2000). It is well known that trace elements in animal diets play a critical role on the modulation of immune responses (Klasing, 1998; Kidd, 2004). Copper and Zn are vital minerals and essential nutrient requirements for growth and development (Schumann *et al*., 2000; McCall *et al*., 2000).

Copper plays an important role in development and maintenance of the immune system (Percival, 1998). In experimental animals fed Cu-deficient diets, the antibodies forming cell response, decreased (Prohaska and Lukasewycz, 1981; Vyas and Chandra, 1983). Suttle and Jones (1986) found that Cu-deficient animals showed a decrease in antibody cell response along with increased susceptibility to infections. Zinc is a critical element for proper immune functions in animals and its deficiency decreased cellular immunity (Fletcher *et al*., 1988) of thymus (Fraker *et al*., 1977) and spleen (Leucke *et al*., 1978). A higher concentration of Zn is commonly added to poultry diets for its deficiency under commercial conditions due to poor availability in plants (Ellis *et al*., 1982).

Some studies observed that small increase in Zn consumption (NRC, 1994) depressed Cu status in experimental animals (L'Abbe and Fischer, 1984) and human (Fischer *et al*., 1984; Yadrick *et al*., 1989). Most nutrient requirements in broilers particularly vitamins and trace minerals are used based on researches conducted 40 years ago for old sues (Waldroup, 2004). Therefore, the aim of this study was to determine the effects of graded Cu and Zn on SRBC titer and lymphoid organs in new sues of broiler chicks.

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MATERIALS AND METHODS

Birds and treatments: This study was conducted at experimental animal house, Ferdowsi University of Mashhad (FUM), Department of Animal Sciences. About 400 pieces of 1 day old (Ross 308) male broiler chicks randomly assigned to 40 pens as 9 treatments and one control diet with 4 replicates using a CRD design (3×3 factorial). The treatments including Cu (35, 70, 105 mg kg^{-1} diet) and Zn (40, 80, 120 mg kg^{-1} diet) were added to basal ration (control diet; NRC, 1994) with minimum amounts of these minerals (3.9 and 3.1 mg kg^{-1} Cu and 19.09 and 17.31 mg kg^{-1} Zn) during starter and grower periods, respectively (Table 1). Chicks were maintained in a thermostatically-controlled room and had access to free water and feed during 42 days.

SRBC test: humoral antibody response: The SRBC (sheep red blood cell) test was performed to quantify the specific antibody titer. On days 28 and 35, two birds from each pen were immunized intraperitoneally using 1 mL of 10% SRBC suspension. Seven days after SRBC inoculation, the birds were bled by brachial venipuncture and 3 mL of blood was collected to evaluate the primary and secondary antibody responses. The sera were inactivated by heat at 56°C for 30 min. Then, they were assayed for total anti-SRBC (Tig), 2-Mercaptoethanol-Sensitive antibody (2ME-S, IgM) and 2-Mercaptoethanol-resistant antibody (2ME-R, IgG) using a method described by Qureshi and Havenstein (1994). Briefly, 50 μL of serum was added to 50 μL PBS of the first column in a 96-well v-shaped bottom plate and then the solution was incubated for 30 min at 37°C. A serial of dilution was prepared as 1:2 and then 50 μL of 2% SRBC suspension was added to each well. The plates were read on a microplate reader at 650 nm (Molecular Devices, Sunnyvale, CA 94089) to evaluate the TIg titer against SRBC after 30 min of incubation at 37°C. Immediately, the well with a distinct SRBC bottom was considered as the endpoint of titer for agglutination. To evaluate 2ME-S (IgM) response, first 50 μL of 0.01 mol. of 2 ME in PBS was used and then followed the previous procedure. The difference between TIg and IgG responses was considered as IgM titer.

Lymphoid organs: On the last day of trial, two randomly selected birds from each replicate were weighted and slaughtered by cervical cutting. Thymus, spleen, bursa of fabricius and liver were precisely removed and weighted by a sensitive digital scale then calculated as percentage of body weight.

Statistical analysis: Collected data from the experiment was carried out in a CRD design (3×3 factorial) (Steel and

Table 1: Ingredients and composition (%, DM basis) of experimental diets in broilers during starter and grower periods

Ingredients	Starter (1-21 days)	Gower (21-42 days)
Corn	54.40	64.63
Soybean meal	39.01	31.00
Oil	2.33	1.00
Limestone	1.29	1.25
Dicalcium phosphate	1.85	1.20
Common salt	0.46	0.35
Mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.25	0.25
DL-methionine	0.16	0.07
Nutrient composition		
ME (Kcal kg^{-1})	3018.00	3050.00
Crude protein (%)	21.65	19.06
Ether extract (%)	4.71	3.70
Crude fiber (%)	2.72	2.63
Ca (%)	1.00	0.83
P (%)	0.48	0.35
Na (%)	0.20	0.15
Arginine (%)	1.56	1.32
Lysine (%)	1.37	1.10
Met + cys (%)	0.90	0.72
Methionine	0.50	0.38
Zinc (mg kg^{-1})	19.09	17.31
Copper (mg kg^{-1})	3.90	3.10

[1]Copper and Zinc-free mineral permix provided per kilogram of diet: Mn (from Mno), 55 mg; Fe (from $FeSO_4.7H_2O$), 96 mg; I (from Ca (IO_3) 2.H20) 1.4 mg. Se 0.4 mg^2; provided per kilogram of diet: vitamin A (vitamin A acetate) 8,700 IU; cholecalciferol 2,300 IU; vitamin E (from DL-α-tocopheryl acetate), 16 IU; menadione (from menadione dimethyl pyrimidinol), 1.50 mg; B12, 0.31 mg; riboflavin, 6.6 mg; niacin, 28 mg; folic acid 0.80 mg; thiamin 3 mg; pyridoxine 2.50 mg; biotin 30 mg; ethoxyquin 125 mg; calcium pentothenate 35 mg

Table 2: Main effect means of anti-SRBC antibody responses (primary and secondary) of broilers fed different levels of copper and zinc[1]

	Primary			Secondary		
Feed	Total Ig[2]	IgM[2]	IgY[2]	Total Ig[2]	IgM[2]	IgY[2]
Cu						
35	3.95	1.33	2.62	5.87	1.95	3.91
70	4.09	1.36	2.72	5.88	2.12	3.75
105	4.38	1.22	3.16	5.36	1.27	4.09
SE	0.53	0.27	0.43	0.31	0.33	0.36
40	3.40	1.27	2.13	5.63	1.79	3.95
80	4.54	1.63	2.90	5.87	1.70	4.16
Zn						
120	4.45	1.00	3.45	5.62	2.00	3.62
SE	0.53	0.27	0.43	0.31	0.33	0.36
Statistical effects (P)						
Cu	0.68	0.37	0.44	0.48	0.31	0.88
Zn	0.38	0.62	0.22	0.82	0.82	0.57
Cu×Zn	0.99	0.83	0.82	0.16	0.53	0.23
Zn (lin)[3]	0.28	0.38	0.09	0.92	0.63	0.62
Control vs. all[4]	0.04	0.99	0.04	0.23	0.23	0.16

[1]Values are least square treatment means. Data are from 40 birds per treatment; [2]Data represent means of log_2 of the reciprocal of the last dilution exhibiting agglutination; [3]Linear (Lin) responses estimated using orthogonal polynomial contrasts; [4]Estimated by using orthogonal contrasts

Table 3: Main effect means of lymphoid organ and liver weights of 42 days old broilers fed different levels of copper and zinc (BW %)

Feed	Thymus	Bursa	Spleen	Liver
Cu (ppm)				
35	0.322	0.091	0.105	1.741[b]
70	0.323	0.088	0.108	2.557[a]
105	0.378	0.100	0.126	2.06[ab]
SE	0.045	0.015	0.007	0.190
Zn (ppm)				
40	0.314	0.111	0.112	1.857
80	0.318	0.089	0.118	2.379
120	0.386	0.079	0.107	2.105
SE	0.045	0.015	0.007	0.190
Statistical effects (P)				
Cu	0.508	0.688	0.097	0.066
Zn	0.537	0.244	0.555	0.604
Cu×Zn	0.569	0.230	0.201	0.350
Cu (lin)[1]	0.311	0.500	0.040	0.735
Cu (quad)[1]	0.572	0.580	0.390	0.020
Control vs. all[2]	0.380	0.850	0.080	0.740

[a,b]Means within columns with different superscripts differ significantly ($p<0.05$); [1]Linear (Lin) or Quadratic (Quad) response estimated using orthogonal polynomial contrasts; [2]Estimated by using orthogonal contrasts

Torrie, 1980). The data were subjected to ANOVA according to GLM procedure of SAS software. The main effect of means was compared at significant level of $p<0.05$ using a Duncan's multiple range test (Duncan, 1955). Since we had no significant interaction between graded Cu and Zn, only the main effect means were considered (Table 2 and 3). All data were checked for normality using Shapiri-Wilk test.

RESULTS AND DISCUSSION

Humoral antibody response (SRBC): Data analysis showed no significant interaction between graded Cu and zinc. The main effect means of antibody titers by graded Cu and Zn on primary and secondary responses of SRBC are shown in Table 2. The primary and secondary responses of anti-SRBC titer in broilers fed different levels of Cu and Zn showed no significant effects. Contrast comparison between control and treatment diets showed improvement of TIg and IgY in primary response ($p<0.05$). The graded Cu increased titers of TIg and IgY linearly but IgM reduced quadratically in the primary response. In the secondary response, the titers of TIg and IgM were reduced but IgY was induced quadratically.

The graded Zn increased TIg and decreased IgM titers quadratically but the titer of IgY increased linearly in the primary response. In the secondary response, the graded Zn increased IgM titer but decreased titers of TIg and IgY quadratically. The graded Zn increased IgY titer in primary response linearly ($p = 0.09$). Similarly, Pimentel *et al*. (1991) reported that excess levels of Zn enhanced IgG titer on day 9 after SRBC inoculation. Virden *et al*. (2004) observed that primary antibody response to SRBC didn't differ between treatments in progeny of broiler breeders fed diets with different levels and sources of zinc. While, Bartlett and Smith (2003) reported that high organic Zn diet increased TIg, IgM and IgG titers in birds in comparison with adequate or low organic Zn diets during primary response ($p<0.05$).

Relative lymphoid organ weights: Since there was no significant interaction between Cu and Zn at 42 days of age on organ weights in broilers, only the main effect means of lymphoid organ weights were considered in Table 3. The LW was affected by dietary Cu with the highest for 70 ppm ($p<0.05$). The response of TW, BW and SW to Cu levels followed a linear trend with a significant increase for SW up to 105 ppm ($p<0.05$). In addition, the trend of LW responded quadratically ($p<0.02$). In contrast, Arias and Koutsos (2006) reported no significant differences between treatments containing Cu sulfate and control group in broilers.

Previous reports showed that a diet deficient in Zn led to atrophy of thymus (Prasad and Oberleas, 1971) and reduction of SW in rats (Mengheri *et al*., 1988). In contrast, the results showed the graded Zn increased TW and LW linearly and quadratically, respectively. Several studies reported that Zn deficiency caused thymus (50% of normal) (Dowd *et al*., 1986) and spleen involution (61% of normal) (Leucke *et al*., 1978) and depression of immune functions in mice and rats (Beach *et al*., 1980).

On the other hands, the birds that received high, low or adequate Zn showed no effects on TW, SW and BW (Bartlett and Smith, 2003). Earlier study showed that Cu has an important role on development of immune system (Mulhern and Koller, 1988). It seems that low levels of Zn in diet can display Cu roles on improvement of immune system. Fischer *et al*. (1984) reported that Cu absorption decreased as the dietary Zn increased.

CONCLUSION

Considering the results, the graded Cu at 70-105 ppm and Zn at 40-80 ppm improved SRBC titer and weights of some lymphoid organs in male broiler chicks. In conclusion, adding Cu and Zn at recommended levels is useful as immunomodulators. Apart from present study, further researches should be done to determine the exact levels of Cu and Zn with different sources on immune system in broiler chicks.

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