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Immunological Responses of Broiler Chicks Can Be Modulated by Dietary Supplementation of Zinc-methionine in Place of Inorganic Zinc Sources

Hasan Nassiri Moghaddam* and Rahman Jahanian[1]

Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad 91775-1163, Iran

ABSTRACT : Male broiler chicks were fed graded levels of organic zinc (zinc-methionine) supplementation to investigate the effects of partial or complete substitution of the organic zinc source for inorganic ones on the development of lymphoid organs and immunological responses. A total of 450 day-old male broilers were distributed into groups of 10 chicks and randomly assigned to nine experimental diets during a 42-day feeding trial. Dietary treatments consisted of two basal diets supplemented with 40 mg/kg added zinc as feed-grade Zn sulfate or Zn oxide in which, Zn was replaced with that provided from zinc-methionine (ZnMet) complex at the levels of 25, 50, 75 or 100%. Two randomly-selected birds from each pen replicate were bled and then slaughtered by cervical cutting on the final day of the trial to measure leukocyte subpopulations and relative weights of lymphoid organs. Among lymphoid organs, only thymus weight was affected ($p<0.05$) by dietary treatments. The sulfate-supplemented birds were heavier ($p<0.01$) in relative weight of thymus than oxide-supplemented birds. The 10 days of age-assessed cutaneous hypersensivity reaction was stronger in chicks fed ZnMet-containing diets. Dietary ZnMet supplementation caused ($p<0.05$) an increase in proportion of lymphocytes and consequently a decrease in heterophil to lymphocyte ratio. Diet fortification by zinc-methionine complex increased ($p<0.01$) Newcastle antibody titer at 19 days of age. Also, a similar response was observed in antibody titers at 6 and 12 d after infectious bronchitis vaccine administration. There was no significant effect of replacement of dietary zinc on antibody titer against infectious bursal disease virus (IBDV) at the 6th d post-vaccine inoculation; however, at d 12 after vaccination, ZnMet-fortified diets improved antibody titer against IBDV. Although dietary inclusion of ZnMet had no marked effect on primary antibody titer against sheep erythrocytes, effective responses were observed during secondary reaction from the viewpoint of both total antibody and immunoglobulin Y (IgY) titers. From the present findings, it can be concluded that dietary supplementation with organic zinc improves both cellular and humoral immune responses. It is necessary to replace 75% of supplemental inorganic zinc with organic ZnMet complex to achieve the optimum immunological responses in broiler chicks. (**Key Words :** Broiler Chicks, Zinc-methionine, Lymphoid Organs, Primary and Secondary Immune Responses, Antibody Titer)

INTRODUCTION

Poultry possess limited natural resistance against many infectious diseases; hence, the poultry industry relies on the application of antibiotics or related medications to increase disease resistance within poultry flocks. However, this practice has accompanied the prevalence and establishment of antibiotic-resistant species within the human populations (Ratcliff, 2000; Phillips et al., 2004). Consequently, the use of antibiotics in livestock production is being faced with extensive limitations, and the poultry industry is compelled to find appropriate alternatives for antibiotics to increase disease resistance in poultry flocks as well as to maintain accessible markets for poultry products (Abdukalykova and Ruiz-Feria, 2006).

It is known that nutrition plays a critical role in the modulation of immune responses (Klasing, 1998; Kidd, 2004). Zinc has been shown to directly influence the immune system (Kirchgessner et al., 1976). Zinc has an important role in numerous biological processes and is an essential component of many enzymes (Vallee and Auld, 1990) with both structural and catalytic functions in metalloenzymes (O'Dell, 1992). This element is required for normal immune function (Dardenne and Bach, 1993; Kidd et al., 1996). When there are zinc deficiencies, thymic atrophy (Chandra and Au, 1980) and skeletal malformations (Blamberg et al., 1960) may occur in mice and laying hens, respectively.

Zinc must be supplemented to most poultry and pig diets to meet the nutritional requirements and to prevent the problems associated with zinc deficiency. National

* Corresponding Author: Hasan Nassiri Moghaddam. Tel: +98-915-511-1197, Fax: +98-511-8787430, E-mail: hnassirim@gmail.com
[1] Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran.
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Table 1. Chemical composition of basal diets in starter and grower stages

Ingredients (%)	Starter (1-21 d)	Grower (21-42 d)
Corn, yellow	54.31	62.01
Soybean meal	38.41	31.31
Sunflower oil	3.00	0.75
Poultry fat	-	2.25
Dicalcium phosphate	1.85	1.35
Limestone	1.22	1.32
Common salt	0.40	0.30
Mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.25	0.25
DL-methionine	0.16	0.06
Variable[3]	0.15	0.15
Nutrient composition		
ME (kcal/kg)	3,000	3,030
Crude protein (%)	21.57	18.94
Ether extract (%)	5.99	6.26
Methionine (%)	0.49	0.36
TSAA[4] (%)	0.84	0.68
Lysine (%)	1.18	1.01
Threonine (%)	0.84	0.74
Tryptophan (%)	0.32	0.27
Arginine (%)	1.40	1.21
Calcium (%)	0.94	0.85
Non-phytate P (%)	0.43	0.34
Sodium (%)	0.17	0.14
Zinc (mg/kg)	25.9	24.2

[1] Zinc-free mineral premix. Provided per kilogram of diet: Mn (from $MnSO_4 \cdot H_2O$), 60 mg; Fe (from $FeSO_4 \cdot 7H_2O$), 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 6 mg; I (from $Ca(IO_3)_2 \cdot H_2O$), 1 mg; Se, 0.20 mg.

[2] Provided per kilogram of diet: vitamin A (from vitamin A acetate), 8,700 IU; cholecalciferol, 2,300 IU; vitamin E (from DL-α-tocopheryl acetate), 16 IU; vitamin B_{12}, 0.31 mg; riboflavin, 6.6 mg; niacin, 28 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidinol), 1.50 mg; folic acid, 0.80 mg; thiamine, 3 mg; pyridoxine, 2.50 mg; biotin, 30 mg; ethoxyquin, 125 mg.

[3] Variable amounts of zinc sources and inert filler (washed builders sand). Zinc sources added in place of an equivalent weight of sand.

[4] TSAA = Total sulfur amino acids.

Research Council (1994) recommends that broiler diets should be supplemented with 40 mg/kg zinc to provide sufficient mineral for optimal growth performance; however, broiler diets are often formulated to contain dietary zinc concentrations greater than 80 mg/kg. A higher concentration of dietary zinc is commonly added to reduce the possibility of zinc deficiency under commercial conditions because of the poor availability of zinc in plant feed ingredients (Ellis et al., 1982; Fordyce et al., 1987). However, diets containing high zinc potentially produce faecal waste with higher zinc content and cause environmental contamination with this mineral.

Recent research has shown that the problems associated with poor bioavailability of zinc could be overcame by dietary organic zinc supplementation (Wedekind et al., 1992; Jahanian et al., 2008a,b). Zinc-methionine (ZnMet) is a specific organo-amino acid zinc complex which differs from inorganic zinc, zinc proteinates, and zinc polysaccharide complexes (American Association of Feed Control Officials, 1990). The bioavailability of ZnMet for chicks has been shown to be higher than that of Zn sulfate (Wedekind et al., 1992; Jahanian et al., 2008b) but equal to zinc oxide (Pimentel et al., 1991). Furthermore, the zinc-methionine supplementation of diets fed to broiler breeders has been shown to promote cellular immunity in the progeny (Kidd et al., 1992, 1993). In addition, Flinchum et al. (1989) showed that zinc-methionine supplementation of the diet fed to aged hens improved progeny survival after an *Escherichia coli* challenge. Thus, zinc-methionine, when added to the diet of poultry, may improve the immune system function and augment disease resistance (Kidd et al., 1996). However, some studies with pigs (Hill et al., 1986; Swinkels et al., 1991; Wedekind et al., 1994) have failed to show differences in Zn bioavailability between organic (complexes or chelates) and inorganic zinc sources. There are few and conflicting data regarding the relative efficacy of different organic versus inorganic Zn sources in promoting immunological responses of broilers. The present study, therefore, aimed to evaluate the influences of supplemental ZnMet individually or in combination with inorganic zinc sources on immune functions of male broiler chicks.

MATERIALS AND METHODS

Experimental diets and general protocol

This study was performed in the Research Farm of Ferdowsi University of Mashhad (Mashhad, Iran). Four hundred and fifty day-old, male Ross×Ross broiler chicks were weighed and randomly assigned to five pen replicates for each of nine experimental diets in a completely randomized design. Dietary treatments included two basal corn-soybean meal diets containing 40 mg/kg supplemental Zn as feed-grade Zn sulfate or Zn oxide in which, Zn was substituted by zinc-methionine complex (Zinpro Corporation, Edina, MN) by 25, 50, 75 or 100%. The basal diets (Table 1) during both starter and grower periods were prepared using Zn-free mineral premix to contain a minimum amount of zinc (25.9 and 24.2 mg/kg during starter and grower stages, respectively). Treatment additions were made at the expense of inert filler (washed builders sand) so that all of the experimental diets contained equal concentrations of all nutrients according to NRC (1994) recommendations, except zinc. The chicks had free access to the diets and to tap water containing no detectable Zn throughout the 42 d feeding trial. Feed and water were provided using plastic equipments to minimize environmental Zn contamination. Chicks were maintained on a 23 L:1 D lighting schedule in the floor pens made of painted partitions, which were placed in a thermostatically-controlled room. Production

temperature was 33°C during the first week of age and then reduced by 3°C/wk until the birds were 4 wk old. At the final day of the trial (42 d), two randomly-selected birds from each pen replicate were weighed and bled individually, then slaughtered by cervical cutting. Thymus, bursa of Fabricius and spleen were then precisely removed and weighed separately on a sensitive digital scale. To study the effect of different dietary treatments on blood leukocyte subpopulations, EDTA-containing blood samples were counted for different leukocyte subsets after processing of blood samples as previously described by Lucas and Jamroz (1961).

Immunological assays

Cell-mediated immune response : Cellular immunity was assessed by a cutaneous basophil hypersensivity test *in vivo* using phytohemagglutinin P (PHA-P). At Day 10, the toe web thickness of the right foot (two birds per pen) was measured in millimeters with a sensitive caliper and then 100 μg of PHA-P (suspended in 0.10 ml sterile saline) was injected into the toe web. The toe web swelling was measured 12 and 24 h after injection. The response was quantified by subtracting the skin thickness prior to and after PHA-P injection into the toe web (Corrier and DeLoach, 1990).

Hemagglutination inhibition antibody titer against Newcastle disease virus : At Day 13, two chicks per cage (wing-banded) were inoculated by eyedrop with a 10^{-3} dilution of stock solution of B1 strain of Newcastle disease virus. The chicks were bled from a wing vein, 6 days after inoculation and sera were collected individually in separate sterile vials. The hemagglutination inhibition test (Marduardt et al., 1984) was set up to determine the antibody production titer as $\log_2$ of the reciprocal of the last dilution.

ELISA antibody titers against infectious bronchitis and infectious bursal disease viruses : Vaccinations to infectious bronchitis and infectious bursal disease viruses were conducted at Days 18 and 23, respectively, via eye dropping of two wing-banded chicks per pen for each disease virus. At days 6 and 12 after each vaccine inoculation, the birds (subjected to each vaccination administration) were bled via wing vein, and sera were frozen until antibody titer assays could be performed. Briefly, serum samples were thawed at room temperature and diluted 500-fold (1:500) in diluent. Diluted serums (100 μl) were added to 96-well microplates coated with either infectious bronchitis or infectious bursal antigens (IDEXX Inc., Westbrook, ME 04092). Plates were covered and allowed to incubate at room temperature. After 30 min incubation, microplates were aspirated and washed with 350 μl of sterile distilled water. Thereafter, 100 μl of the conjugate was dispensed into the wells and plates were allowed to incubate at room temperature again for 30 min. Substrate was then dispensed (100 μl) into the wells to facilitate a color reaction and plates were allowed to incubate at room temperature for an additional 15 min. A stop solution was eventually added to end the enzymatic process. Plates were read on a microplate reader (Molecular Devices, Sunnyvale, CA 94089) at 650 nm to measure the antibody titers against infectious bronchitis or infectious bursal disease virus (Kidd et al., 2001).

Humoral antibody response : Sheep red blood cells (SRBC) were used as a test antigen to quantify specific antibody response. The birds (two per pen replicate) were immunized intraperitoneally with 0.5 ml of 10% SRBC suspension at Day 25. The same birds (wing-banded) were immunized against SRBC again seven days post primary injection. Seven days after each injection, all SRBC-inoculated birds were bled by brachial venipuncture, and 2-3 ml of blood was collected for evaluating primary and secondary antibody responses using the 2-mercaptoethanol (ME) technique as previously described (Qureshi and Havenstein, 1994; Lepage et al., 1996). All SRBC antibody titers were expressed as $\log_2$ of the reciprocal of the highest serum dilution causing agglutination of SRBC.

Statistical analysis

Data obtained were analyzed by the General Linear Model (GLM) procedures of SAS statistical software (SAS Institute, 1999). Pen was the experimental unit for all measurements. Percentage data were first arcsin transformed. Whereas the statistical differences were determined from the transformed data, only non-transformed data are presented for relevance. Treatment means were compared for significant ($p<0.05$) differences using Duncan's multiple range tests (Duncan, 1955). Contrast comparisons were made among treatment means to compare sulfate- and oxide-supplemented diets, replacement levels, and level of 100% for all three Zn sources as well.

RESULTS

The influences of dietary organic zinc supplementation on relative lymphoid organ weights are presented in Table 2. Among lymphoid organs, only relative weight of thymus was affected ($p<0.05$) by dietary treatments. Dietary supplementation with organic zinc increased thymus weight as a proportion of live body weight. Interestingly, the relative weight of bursa of Fabricius was greater ($p<0.05$) in birds fed on Zn sulfate-containing diets than those fed diets containing Zn oxide.

Dietary treatments significantly ($p<0.01$) influenced cutaneous hypersensivity reaction in the 10 d-aged chicks (Table 3). In addition, dietary inclusion of ZnMet partially or completely in replace of conventional inorganic zinc

Table 2. Influence of partial or complete substitution (% of Zn supplied) of zinc-methionine (ZnMet) for dietary inorganic zinc sources on lymphoid organ weights (% of live body weight) of broiler chicks at 42 d of age

Source and level of inorganic Zn		ZnMet	Thymus	Bursa of Fabricius	Spleen
Zn sulfate	100	-	0.217	0.167	0.127
	75	25	0.211	0.175	0.120
	50	50	0.204	0.180	0.124
	25	75	0.225	0.159	0.128
Zn oxide	100	-	0.185	0.163	0.133
	75	25	0.198	0.125	0.123
	50	50	0.189	0.145	0.130
	25	75	0.213	0.136	0.115
	-	100	0.235	0.168	0.125
			---------- p values ----------		
Treatment			0.0127	0.4536	0.8299
Contrasts					
Sulfate vs. oxide			0.0033	0.0120	0.9206
Replacement level			0.0207	0.8049	0.7290
100% of each source			0.0593	0.9841	0.7027
Pooled SEM			0.0093	0.0184	0.0075

sources (Zn sulfate or oxide) caused a significant ($p<0.001$) increase in skin thickness 12 or 24 h following PHA-P injection.

The influences of dietary treatments on the proportions of peripheral blood leukocyte subpopulations are shown in Table 3. As noted, dietary treatment overall had no significant effect on proportions of heterophils, lymphocytes or heterophil to lymphocyte (H/L) ratio. However, dietary introduction (replacement level) of ZnMet into the experimental diets tended ($p = 0.0543$) to reduce heterophil number in a curvilinear manner. Furthermore, dietary substitution of ZnMet partially or completely in replacement of inorganic zinc sources led an increased ($p<0.01$) lymphocyte proportion, and consequently a decreased H/L ratio.

Specific antibody productions against Newcastle, infectious bronchitis and infectious bursal disease viruses were measured (see Table 4). None of the antibody titers were significantly affected by dietary Zn sulfate versus Zn oxide supplementation (regardless of whether diets contained ZnMet complex). Dietary supplementation with ZnMet caused a significant ($p<0.01$) increase in antibody production titers against Newcastle and infectious bronchitis (both 6 and 12 days post inoculation) disease viruses. For infectious bursal disease virus, organic zinc supplementation was observed to increase antibody titer only at 12th day post-vaccine administration. In addition, the birds fed 100% of each Zn source did not differ in antibody titer against infectious bursal disease virus at d 6 or 12 post vaccine inoculation.

Although dietary supplementation with ZnMet complex partially or completely in place of inorganic zinc sources had not as much effect on primary antibody titers to SRBC, secondary responses were observed to be stronger in birds fed on ZnMet-containing diets (Table 5). Single degree of freedom contrast comparisons showed that Zn sulfate versus Zn oxide supplementation had no significant effect on SRBC antibody titers, neither in primary nor in secondary response.

DISCUSSION

Dietary supplementation with a more bioavailable zinc source (i.e. zinc-methionine complex) caused a significant increase in relative weight of thymus. In addition, thymus

Table 3. Influence of partial or complete substitution (% of Zn supplied) of zinc-methionine (ZnMet) for dietary inorganic zinc sources on skin reaction to phytohemagglutinin P and peripheral blood leukocyte subpopulations

Source and level of inorganic Zn		ZnMet	CBH[1] (mm)	Heterophil (%)	Lymphocyte (%)	H/L[2]
Zn sulfate	100	-	0.482	24.13	69.13	0.349
	75	25	0.502	22.88	68.75	0.333
	50	50	0.543	23.63	69.38	0.341
	25	75	0.525	23.13	70.88	0.326
Zn oxide	100	-	0.478	24.75	69.50	0.356
	75	25	0.465	23.88	68.13	0.351
	50	50	0.524	22.88	68.50	0.334
	25	75	0.539	23.75	71.13	0.334
	-	100	0.563	21.63	70.38	0.308
			---------- p values ----------			
Treatment			0.0039	0.2044	0.1247	0.0670
Contrasts						
Sulfate vs. oxide			0.4502	0.4586	0.7229	0.3471
Replacement level			0.0004	0.0543	0.0153	0.0113
100% of each source			0.0003	0.0386	0.6426	0.0276
Pooled SEM			0.0171	0.7379	0.7898	0.0100

[1] Cutaneous basophil hypersensivity reaction. [2] Heterophil to lymphocyte ratio.

Table 4. Influence of partial or complete substitution (% of Zn supplied) of zinc-methionine (ZnMet) for dietary inorganic zinc sources on antibody titers against Newcastle (log_2), infectious bronchitis and infectious bursal (log_{10}) disease viruses

Source and level of inorganic Zn		ZnMet	Newcastle	Infectious bronchitis		Infectious bursal disease	
				6 dpi[1]	12 dpi	6 dpi	12 dpi
Zn sulfate	100	-	3.86	3.36	3.15	3.51	3.22
	75	25	3.94	3.44	3.23	3.44	3.16
	50	50	4.21	3.33	3.31	3.55	3.23
	25	75	4.12	3.58	3.49	3.51	3.39
Zn oxide	100	-	4.05	3.41	3.11	3.41	3.21
	75	25	4.01	3.39	3.16	3.45	3.18
	50	50	4.31	3.56	3.42	3.45	3.20
	25	75	4.45	3.79	3.60	3.52	3.43
	-	100	4.64	3.73	3.62	3.47	3.38
			---------- p values ----------				
Treatment			0.0251	0.0232	0.0002	0.9478	0.1416
Contrasts							
Sulfate vs. oxide			0.1558	0.1831	0.7105	0.3919	0.9357
Replacement level			0.0046	0.0091	0.0001	0.8881	0.0122
100% of each source			0.0001	0.0185	0.0097	0.6650	0.2720
Pooled SEM			0.1538	0.0996	0.0813	0.0797	0.0787

[1] dpi: days post inoculation

weights in sulfate-supplemented groups were heavier than in oxide ones. Presumably, higher bioavailability of Zn sulfate than Zn oxide is responsible for this observation. Zinc deprivation has been shown to cause involution of the thymus (to 50% of normal) in zinc-deficient mice and rats (Fraker et al., 1977; Leucke et al., 1978; Dowd et al., 1986); involution of the spleen (to 61% of normal) in zinc-deficient mice (Leucke et al., 1978), and depression of immune function in both mice and rats with only a moderate depression in body weight (Leucke et al., 1978; Beach et al., 1980). Although the animals in the present study did not appear to be deficient in zinc (from the viewpoint of performance parameters; data not shown), it appears that more dietary zinc or a more bioavailable zinc source is needed to assure functionality of the immune system, particularly cell-mediated immune responses. The immune system is dependent on the functions of cellular metabolism. Zinc is ubiquitous in cellular metabolism and functions both structurally and catalytically in metalloenzymes (O'Dell, 1992). In contrast with our observations, Kidd et al. (2000) reported that bursa weight, expressed as a proportion of live body weight, was higher in poults from turkey hens supplemented with ZnMet than in Zn sulfate ones. However, the same authors observed no differences in the relative weights of spleen, liver and heart among progenies of ZnMet- and Zn sulfate-hens (Kidd et al.,

Table 5. Influence of partial or complete substitution (% of Zn supplied) of zinc-methionine (ZnMet) for dietary inorganic zinc sources on primary and secondary antibody responses to sheep erythrocytes (log_2)

Source and level of inorganic Zn		ZnMet	Primary antibody titer			Secondary antibody titer		
			Total Ig[1]	IgM[2]	IgY[3]	Total Ig	IgM	IgY
Zn sulfate	100	-	3.85	2.56	1.29	4.17	1.42	2.75
	75	25	4.04	2.86	1.18	4.96	1.54	3.42
	50	50	4.37	2.95	1.42	5.31	1.34	3.97
	25	75	4.25	2.71	1.54	5.25	1.28	3.97
Zn oxide	100	-	4.06	3.01	1.05	4.42	1.48	2.94
	75	25	4.39	2.87	1.52	5.09	1.37	3.72
	50	50	4.12	2.74	1.38	5.01	1.45	3.56
	25	75	4.63	3.14	1.49	5.36	1.29	4.07
	-	100	4.36	3.03	1.33	5.51	1.24	4.27
			---------- p values ----------					
Treatment			0.0220	0.9409	0.9503	0.0001	0.1191	0.0001
Contrasts								
Sulfate vs. oxide			0.1633	0.4332	0.9900	0.7671	0.9644	0.8118
Replacement level			0.0458	0.9701	0.7960	0.0001	0.0533	0.0001
100% of each source			0.1050	0.3942	0.5643	0.0001	0.1613	0.0002
Pooled SEM			0.1413	0.3097	0.2874	0.0991	0.0750	0.1383

[1] Immunoglobulin. [2] Immunoglobulin M. [3] Immunoglobulin Y.

2000).

Cellular immune response to PHA-P injection was enhanced when the diets were supplemented with ZnMet complex (apart from that the remaining part of dietary added zinc was Zn sulfate or Zn oxide). Other researchers have demonstrated that supplementation of broiler breeder hen diets with zinc-methionine rather than inorganic zinc sources promoted the cellular immune response of progeny to PHA (Kidd et al., 1992, 2000). In addition, hens provided diets with added zinc from amino acid complexes or chelates had increased thymus weights (Virden et al., 2002) and improved livability of progeny (Flinchum et al., 1989).

Dietary supplementation with ZnMet partially in place of inorganic zinc sources caused an increase in proportion of lymphocytes and a decreased H/L ratio. Similarly, Virden et al. (2004) observed that the relative proportion of mononuclear cells to total leukocytes increased in progeny of broiler breeders fed diets containing a zinc-amino acid complex. An inadequate intracellular level of zinc has been shown to impair lymphocyte proliferation (Cunningham-Rundles and Cunningham-Rundles, 1988), probably because DNA synthesis is dependent upon zinc. Deoxythymidine kinase activity, a zinc-dependent enzyme, is severely altered during states of only mild zinc deficiency (Prasad, 1993). DNA and RNA polymerases, both zinc metalloenzymes, are decreased in cells in cultures when zinc-chelating agents are added (Prasad, 1982). These observations suggest that inadequate zinc or supplementing diets with poorly available zinc sources results in impaired cellular functions, largely due to a decrease in DNA and/ or RNA synthesis.

As noted in Table 4, except for antibody titer against infectious bursal disease virus at 6^{th} day post-vaccine inoculation, antibody production titers to Newcastle (NDV) and infectious bronchitis disease viruses were improved by dietary supplementation of ZnMet partially (or completely) in place of inorganic sources. Hudson et al. (2004) reported that antibody titers to NDV were higher when supplemental zinc was provided by Zn-amino acid complex. Similarly, in their study with broiler breeders, Khajarern et al. (2002) found that NDV, infectious bursal disease and infectious bronchitis titers were increased when hen diets were supplemented with zinc-amino acid complex. Zinc is essential for thymulin, a thymic hormone that regulates T-lymphocyte maturation (Fraker et al., 1986). Birds provided diets supplemented with a more available zinc source (ZnMet) 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.

Zinc-methionine has been shown to be more bioavailable than Zn sulfate and Zn oxide when fed to chicks in either purified crystalline amino acid, semipurified (soy isolate) or complex corn-soybean meal diets (Wedekind et al., 1992; Jahanian et al., 2008a). However, greater bioavailability values of ZnMet in complex diets (Wedekind et al., 1992) suggests that phytic acid and fiber contained in mixed corn-soybean meal diets may have reduced the availability of zinc oxide and zinc sulfate more than that of zinc-methionine. An increased absorption of zinc from zinc-methionine complex may lead to a larger zinc pool, thereby increasing zinc metalloprotein activities, plasma levels of zinc and immune cell functions that require zinc (Kidd et al., 1996). Zinc-associated intracellular functions are dependent upon the availability of this nutritionally essential trace mineral for proper functioning (Dardenne and Bach, 1993).

This fact that dietary supplementation with organic zinc had a considerable effect on infectious bronchitis antibody titer, but had little or no influence on antibody production titer against infectious bursal disease virus, indicates that zinc affects cellular immunity to a greater extent than humoral immune responses. The protective role of humoral immunity is evident from the fact that chickens immunocompromised by infectious bursal disease virus were unable to clear the virus and suffered more severe disease symptoms compared to immunocompetent birds in response to infection with infectious bronchitis virus (Thompson et al., 1993). However, humoral immunity does not seem to be the only route of protection because bursectomized chickens, which did not produce antibody, nevertheless could successfully resist infectious bronchitis challenge (Cook et al., 1991).

Our results showed that dietary organic zinc improved SRBC antibody titers during both primary and secondary responses; however, this effect was stronger during secondary response, and IgY particularly was also affected during this stage. Consistent with our findings, Beach et al. (1980) reported that diets supplemented with zinc tended to improve the ability of birds to produce antibodies. Also, Bartlett and Smith (2003) showed that birds receiving a high zinc diet had significantly higher titers of total, IgM and IgG antibodies than those receiving adequate or low zinc diets during the primary response. Furthermore, those birds receiving adequate and high zinc diets were similar with higher responses for total, IgM, and IgG antibodies during the secondary challenge with SRBS than those in the low zinc group (Bartlett and Smith, 2003). Kidd et al. (1992) also reported that supplemental Zn from amino acid complexes in the diets of the parents and chicks increased antibody responses of chicks to SRBC and *Salmonella pullorum* challenges.

Overall, the zinc-methionine complex seems to provide more bioavailable zinc than inorganic zinc sources and to enhance some cell functions important for immunity and disease resistance when added to the diet. It appears that

dietary ZnMet supplementation by 75% in replacement of inorganic sources can improve both cellular and humoral functions of the broiler immune system with more influence on cell-mediated immune responses.

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