Effects of *Salmonella typhymurium* Lipopolysaccharide Challenge on the Performance, Immune Responses and Zinc Metabolism of Laying Hens Supplemented with Two Zinc Sources*

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ABSTRACT : The study was conducted to determine the effect of *Salmonella typhymurium* lipopolysaccharide (LPS) challenge on egg-laying performance, inflammatory response, zinc metabolism in layer fed diets supplemented with organic or inorganic zinc since 3-wk-old. The three dietary treatments were corn-soybean meal basal diet without supplemental zinc or with supplemental zinc at 60 mg/kg zinc from $ZnSO_4$ or zinc amino acid complex (ZnAA). At the age of 58 wk-old, twelve hens from each dietary treatment were allotted into two sub-groups. On day 1, 3, 5, 7 of the 58th week of age, six birds of one sub-group were injected intraperitoneally (i.p.) with 2 ml LPS (1.0 μg/ml) or sterile saline. Neither zinc source × immune challenge interaction nor zinc source effect on egg production performance was observed ($p>0.05$), LPS-challenge decreased egg production ($p<0.04$) and increased percentage of cracked eggs ($p<0.01$). With LPS challenged, the fever response of hens fed ZnAA peaked and subsided earlier than in hens fed $ZnSO_4$ or basal diet. Serum IL-1β at 3 h was higher ($p<0.01$), but lower ($p<0.001$) at 12 h post-challenge with LPS in hens fed ZnAA than $ZnSO_4$. In saline-treated groups, serum IL-1β was higher in hens fed ZnAA than the basal diet at 3 h post-injection ($p<0.01$). LPS-challenged birds had lower serum zinc and higher zinc sequestered in liver and spleen ($p<0.001$). In saline-treated birds, there was no difference in zinc concentration of serum, liver and spleen among different dietary treatments ($p>0.05$). Supplementation of 60 mg/kg zinc from either ZnAA or $ZnSO_4$ significantly ($p<0.05$) elevated metallothionein (MT) concentration in liver and spleen. MT concentration in liver of birds fed ZnAA diet was higher than in those fed $ZnSO_4$ diet ($p<0.05$). The magnitude of increase of hepatic and splenic MT due to LPS challenge was higher by supplementation of ZnAA than $ZnSO_4$. The results suggest that zinc amino acid complex enhanceed MT synthesis and zinc sequestered in liver and spleen and increased the sensitivity to immune response due to LPS challenge. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 12 : 1717-1724)***

Key Words : Zinc Amino Acid Complex, Egg Production, Inflammatory Response, Metallothionein, Lipopolysaccharide, Laying Hens

INTRODUCTION

In the early 1960s, zinc was first identified as an essential element, and then as an integral part of more than 300 enzyme systems that are involved in major metabolic pathways. Its extreme important role in normal development, maintenance and function of immune systems is now widely accepted (Fraker et al., 1986; Dardenne and Bach, 1993). Zinc deficiency is manifested by lymphoid atrophy, lymphopenia and alterations in the proportions and functional activities of the various subsets of lymphocytes and mononuclear phagocytes. On the other hand, zinc repletion may correct the majority of observed change in immune function due to Zn deficiency (Wellinghausen et al., 1997).

Organic complexes of zinc have been proposed to be a more available source of zinc in chicks (Wedekinde et al., 1992), and may be metabolized differently compared with inorganic forms (Spears, 1989; Kidd et al., 1996). Potential benefits of organic zinc complexes on the immune function of poultry have also been proposed (Ferket and Qureshi, 1992; Kidd et al., 1994a,b; 2000). However, the exact mechanism of organic zinc affecting on immunity differing from inorganic forms has not been clarified yet.

Zinc concentration in serum or plasma have been demonstrated to be decreased following an experimental infection (Klasing, 1984; Tufft et al., 1988; Hill, 1989) or intravenous injection with endotoxin (Butler and Curtis, 1973) in poultry, and the zinc lost from the blood as a result of infection is sequestered in the liver and possibly other organs. Changes of zinc dynamic in lymphatic organ, and liver as well were also observed during both humoral and cellular type of immune reaction in mice (Verbanac et al., 1998). Singh and Singha (2002) also reported that glucocorticoid-induced stress caused a redistribution of zinc in body. It is well known that metallothionein (MT) can be induced tissue specifically by dietary zinc (Blalock et al., 1988; Fleet et al., 1988), and be able to regulate the plasma Zn concentration and redistribution of zinc to other organs (Cousins and Hempe, 1990). MT concentration and MT mRNA abundance were shown different in tissues of animals fed organic zinc from inorganic zinc sources (Cao et al., 2000,2002,2003). There is still no research reported so far on the MT induction by organic zinc and subsequent

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Table 1. Composition and nutrients level of basal diets

Ingredients (%)	3-8 wk	9-18 wk	19-45 wk	46-65 wk
Corn	67.130	68.400	62.680	62.490
Soybean meal	29.430	20.480	27.000	26.000
Wheat bran	-	8.000	-	-
Dicalcium phosphate	1.630	1.290	1.400	1.200
Limestone	1.050	1.070	8.190	9.550
NaCl	0.350	0.350	0.300	0.350
Methionine	0.011	0.010	0.110	0.090
Choline (50%)	0.120	0.070	0.050	0.050
Multivitamin premix[1]	0.020	0.020	0.020	0.020
Chloroteracycline (15%)	0.010	0.060	-	-
Trace mineral premix[2]	0.250	0.250	0.250	0.250
Nutrients level				
Metabolizable energy (MJ/kg)	12.20	12.04	11.51	11.38
Crude protein (%)	18.02	15.50	16.50	16.00
Methionine (%)	0.30	0.26	0.45	0.35
Lysine (%)	0.80	0.70	0.80	0.80
Calcium (%)	0.90	0.80	3.50	3.80
Available phosphorus (%)	0.40	0.35	0.30	0.30
Zinc (mg/kg)[3]	35.00	31.00	29.00	31.00

[1] Supplied per kilogram of diet: vitamin A: 10,800 IU, vitamin D_3: 2,160 IU, vitamin E: 15 IU, vitamin K_3: 1.0 mg, vitamin B_1: 4 mg, riboflavin: 5 mg, vitamin B_6: 8 mg, vitamin B_{12}: 0.08 mg, pantothenic acid: 10 mg, niacin: 25 mg, folic acid: 0.4 mg, biotin: 0.15 mg.

[2] Supplied per kilogram of diet: manganese: 60 mg, copper: 10 mg, iron: 60 mg, selenium: 0.15 mg, iodine: 0.35 mg.

[3] Zinc concentrations in basal diet were analyzed by atomic absorption spectrophotometry.

redistribution of zinc in various tissues during endotoxin-induced immune stress.

The main objective of the present work was to investigate the laying performance and immune responses, and distributions of zinc and MT in both lymphoid and non-lymphoid organs of laying hens during immune stress induced by *Salmonella typhymurium* LPS challenge.

MATERIALS AND METHODS

Animals and experimental design

The experimental Hyline brown hens were fed with three different diets respectively from 3 wk old. The dietary treatments were as follows: A) basal diet (contain 29-35 mg/kg zinc by analysis) without additional zinc supplementation; B) basal diet supplemented with 60 mg/kg zinc from $ZnSO_4$; C) basal diet supplemented with 60 mg/kg zinc from Availa-Zn (provided by Zinpro Co., USA). The corn-soybean meal basal diet was formulated to meet National Research Council (1994) recommendation for brown-shell laying hens except zinc (Table 1). When the hens were at the age of 58 wk-old, twelve hens were randomly selected from each dietary treatment (72 hens of each dietary treatment) and allotted into two sub-group and then raised in individual cages, six birds of one sub-group were injected i.p. with 2 ml *Salmonella typhymurium* LPS (Sigma Chemical, MO, USA) or an equivalent amount of sterile saline. The LPS was reconstituted in saline (9 g/100 ml) at 1.0 μg/ml and sterilized by passing through a 0.45 μm filter. On the 1st 3rd, 5th and 7th day of the experiment, hens were injected with LPS consecutively. The same dose of saline was injected as control for the other half of hens.

Data collection and analytical determination

After the first injection of LPS or saline, rectal body temperature was measured using a 4.600 precision thermometer at 0, 1, 3, 6, 12, 24 h post-challenge. On the 5th day, 2 ml blood sample were collected at 3 h and 12 h after LPS or saline administration by wing-vein puncture. Serum concentrations of IL-1β were determined using ELISA method (ELISA kit offered by TPI Inc. Washington, USA), and serum zinc concentration was analyzed by atomic absorption spectrophotometry (3510, Agilent Technologies).

At 3 h post-injection with LPS or saline on the 7th d, all birds were killed by cervical dislocation. The livers and spleens of hens were then excised and frozen in liquid nitrogen for subsequent Zn and MT analysis. To determine tissue zinc concentration, 500 mg tissue samples were wet-ashed in concentrated nitric acid in calibrated centrifuge tube and placed in a 50°C water bath for 12 h. Subsequently, the samples were diluted to 25 ml with distilled water, and zinc concentration were analyzed by flame atomic absorption spectrophotometry (3510, Agilent Technologies). Tissue MT was measured by the modified Cd hemoglobin affinity assay of Eaton and Toal (1982). The concentration of Cd was measured with a graphite furnace atomic absorption spectrophotometer (3511G, Agilent Technologies), and the MT concentration was converted to nmol/g fresh tissue by using the Cd binding stoichiometry of 6:1. The formula was shown here as:

Table 2. Laying performance of hens administrated with LPS or saline on 1st, 3rd, 5th, 7th d at age of 58 wk[1]

Dietary treatments	Percentage of egg laying (%)[2]		Average egg weight (g)		Percentage of cracked egg (%)[2]	
	LPS	Saline	LPS	Saline	LPS	Saline
Basal diet	77.73[a]	77.60	62.20	62.37	3.07	2.63
$ZnSO_4$	76.77[ab]	78.17	62.80	62.30	3.37	2.00
ZnAA	75.63[b]	77.27	62.53	62.47	4.10	2.40
SEM	0.41	0.31	0.12	0.12	0.31	0.16
Main effects			P value			
Zinc source[3]	0.133		0.827		0.217	
Immunogen[4]	0.037		0.237		0.007	
Zinc source×immunogen	0.852		0.356		0.704	

[1] Mean value within columns with no common superscripts differs significantly ($p<0.05$).
[2] The data were arcsin-transformed before ANOVA.
[3] Zinc source effect refers to supplementation of ZnAA or $ZnSO_4$ at level of 60 mg/kg.
[4] Immunogen effect refers to LPS challenge or saline injection. Birds were intraperitoneally injected with LPS at 2 μg/bird or equivalent amount of sterile saline.

Table 3. The rectal temperature of hens after the first administered with LPS or saline[1]

Dietary treatments	Immune challenge	Rectal temperature (°C) at time post LPS or saline administration						SEM
		0 h	1 h	3 h	6 h	12 h	24 h	
Basal	LPS	$41.33_{(D)}$	$41.17^{b}_{(D)}$	$41.57^{b}_{(C)}$	$41.73^{b}_{(BC)}$	$42.10^{a}_{(A)}$	$41.80^{a}_{(B)}$	0.07
	Saline	41.23	41.10^{c}	41.27^{bc}	41.23^{c}	41.17^{d}	41.27^{b}	0.04
$ZnSO_4$	LPS	$40.86_{(C)}$	$41.56^{ab}_{(AB)}$	$42.00^{a}_{(A)}$	$42.13^{a}_{(A)}$	$41.77^{b}_{(A)}$	$41.07^{b}_{(BC)}$	0.13
	Saline	41.33	41.10^{c}	41.30^{bc}	41.27^{c}	41.30^{cd}	41.13^{b}	0.05
ZnAA	LPS	$41.33_{(C)}$	$41.76^{a}_{(B)}$	$42.27^{a}_{(A)}$	$42.07^{a}_{(A)}$	$41.37^{c}_{(C)}$	$41.23^{b}_{(C)}$	0.10
	Saline	41.33	41.27^{b}	41.43^{bc}	41.23^{c}	41.30^{cd}	41.07^{b}	0.05
SEM		0.08	0.08	0.10	0.10	0.07	0.07	
Parameter[2]						P value		
Zinc source[3]						0.234		
Immunogen[4]						<0.001		
Time post-injection[5]						<0.001		
Zinc source×immunogen						0.534		
Zinc source×time post-injection						0.224		
Immunogen×time post-injection						<0.001		
Zinc source×immunogen×time post-injection						0.396		

[1] Mean values within columns with no common superscripts (a, b, c, d) mean the significant ($p<0.05$) difference of the rectal temperatures at the same time post LPS or saline administration of hen between dietary groups; and mean values within rows with no common subscripts (A, B, C, D) mean the significant ($p<0.05$) difference of the rectal temperatures of hen in the same dietary group at different time post LPS or saline administration.
[2] The date were analyzed by three-way ANOVA, the main effects include zince source, immunogen and time post-injection and their interaction.
[3] Zinc source effect refers to supplementation of ZnAA or $ZnSO_4$ at level of 60 mg/kg.
[4] Immunogen effect refers to LPS challenge or saline injection. Birds were intraperitoneally injected with LPS at 2 μg/bird or equivalent amount of sterile saline.
[5] Time post-injection refers to 0, 1, 3, 6, 12, 24 h post-injected with LPS or saline.

$$MT\ (nmol/g) = Cd\ (\mu g/g)/112.4/6\times 1.000$$

During the 7 d of LPS administration, the egg production, egg weight, and cracked egg were recorded.

Statistical analyses

Data (excluding basal) were analyzed by two-way ANOVA with the GLM procedure of SPSS 10.0 and the main effects of Zn source, immunogen as main effects, and their interactions were analyzed. For an exception, the rectal temperatures were analyzed by three-way ANOVA with the GLM procedure of SPSS 10.0. Individual hen was used as experimental unit for all parameters. The data from all diets including basal were also subjected to one-way ANOVA and the differences between means were determined by the method of LSD using SPSS 10.0 software. The main effect or the difference between means was considered significant when $p<0.05$.

RESULTS

Egg production performance

The effects of dietary zinc sources and immune challenge on egg production, egg weight, and percentage of cracked egg were shown in Table 2. There was neither zinc source nor zinc source×immune challenge interaction effect on egg production, egg weight, and percentage of cracked eggs ($p>0.05$). However, consecutive LPS challenge decreased egg production ($p<0.05$) and increased

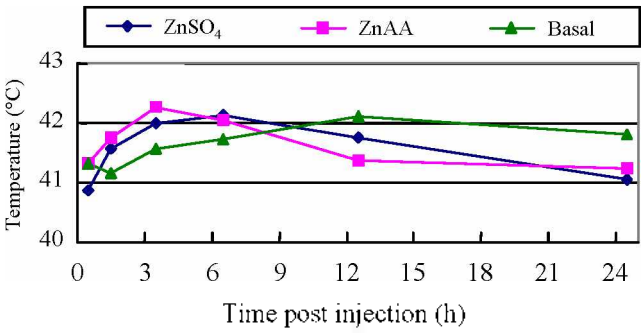

Figure 1. Rectal temperature of hens fed different dietary zinc after challenged with LPS.

percentage of cracked eggs ($p<0.01$). There were no differences in egg production performance of saline-administrated birds fed basal diet and supplemented with either $ZnSO_4$ or ZnAA. When challenged with LPS, hens received ZnAA had a decreased egg production compared to that fed with basal diet ($p<0.05$), but no significant difference was shown between birds fed basal diet with or without $ZnSO_4$ supplementation.

Rectal temperature

Body temperature was significantly ($p<0.001$) affected by immunogen, time post-injection and the interaction between immunogen and time (Table 3). Hens fed either $ZnSO_4$ or ZnAA demonstrated an increase in internal body temperature after LPS injection, however the temperature profiles was different in hens fed different source of zinc (Figure 1). When challenged with LPS, the fever response of hens given ZnAA was peaked earlier as 3 h post-injection, whereas in hens fed $ZnSO_4$ and basal, the highest fever was occurred later at 6 h and 12 h after LPS administration respectively. In addition, the fever of birds fed ZnAA subsidized faster than those fed the basal diet with or without $ZnSO_4$.

Serum IL-1β concentration

The effects of dietary zinc source and immune challenge on serum IL-1β concentration of hens are shown in Table 4. Compared to hens fed basal diet, hens supplemented with ZnAA was shown a higher serum IL-1β concentration at 3 h post either LPS or saline administration ($p<0.05$). Whereas the concentration of serum IL-1βin hens fed either ZnAA or ZnSO4 was lower than that of hens fed basal diet at 12 h post challenged with LPS ($p<0.05$). Among LPS-challenged birds, serum IL-1β was higher in hens fed ZnAA diet than hens fed $ZnSO_4$ diet at 3 h post challenge ($p<0.01$). However, birds fed ZnAA had the lower serum IL-1β concentration than those fed $ZnSO_4$ at 12 h post-challenge with LPS ($p<0.001$).

Table 4. Serum IL-1β concentration (pg/100 μl) of hens after the 5th d administered with LPS or saline[1]

Dietary treatments	3 h post injection		12 h post injection	
	LPS	Saline	LPS	Saline
Basal diet	70.23^{b}	40.27^{b}	81.60^{a}	39.43
$ZnSO_4$	72.60^{b}	43.33^{ab}	64.03^{b}	41.87
ZnAA	81.07^{a}	43.83^{a}	50.83^{c}	40.83
SEM	1.77	0.73	4.49	0.52
Main effects	P value			
Zinc source[2]	0.005		<0.001	
Immunogen[3]	<0.001		<0.001	
Zinc source×immunogen	0.008		<0.001	

[1] Mean values within columns with no common superscripts differ significantly ($p<0.05$).
[2] Immunogen effect refers to LPS challenge or saline injection. Birds were intraperitoneally injected with LPS at 2 μg/bird or equivalent amount of sterile saline.
[3] Zinc source effect refers to supplementation of ZnAA or $ZnSO_4$ at level of 60 mg/kg.

Tissue zinc concentration

As shown in Table 5, although there were no differences in zinc concentration of serum, liver and spleen of saline-treated birds among different dietary treatments ($p>0.05$), the concentration of zinc in liver and spleen was higher in LPS challenged birds fed ZnAA than fed basal diet ($p<0.05$). LPS challenged birds had decreased serum zinc and increased Zn accumulation in liver and spleen compared to

Table 5. Zinc concentration of serum, liver and spleen of hens at 3 h after the 7th d administration with saline or LPS challenge[1]

Dietary treatments	Serum zinc (μg/ml)		Hepatic zinc (mg/kg)		Splenic zinc (mg/kg)	
	LPS	Saline	LPS	Saline	LPS	Saline
Basal diet	1.19	1.70	40.87^{b}	30.47	16.27^{b}	15.47
$ZnSO_4$	1.02	1.75	44.13^{ab}	32.00	17.43^{ab}	15.23
ZnAA	1.03	1.78	45.47^{a}	31.97	18.57^{a}	15.17
SEM	0.04	0.02	1.25	0.86	0.48	0.33
Main effects	P value					
Zinc source[2]	0.670		0.081		0.088	
Immunogen[3]	<0.001		<0.001		0.001	
Zinc source×immunogen	0.818		0.077		0.073	

[1] Mean values within columns with no common superscripts differ significantly ($p<0.05$).
[2] Zinc source effect refers to supplementation of ZnAA or $ZnSO_4$ at level of 60 mg/kg.
[3] Immunogen effect refers to LPS challenge or saline injection. Birds were intraperitoneally injected with LPS at 2 μg/bird or equivalent amount of sterile saline.

Table 6. MT concentrations in liver and spleen of hens at 3 h after the 7th d administration with saline or LPS challenge[1]

Dietary treatments	Hepatic MT (nmol/g)		Splenic MT (nmol/g)	
	LPS	Saline	LPS	Saline
Basal diet	52.80[c]	35.61[c]	17.19[c]	14.01[b]
$ZnSO_4$	83.52[b]	48.10[b]	26.03[b]	17.82[a]
ZnAA	140.65[a]	56.10[a]	38.66[a]	18.38[a]
SEM	12.95	9.17	3.18	2.09
Main effects	P value			
Zinc source[1]	<0.001		<0.001	
Immunogen[2]	<0.001		<0.001	
Zinc source×immunogen	<0.001		0.001	

[1] Mean values within columns with no common superscripts differ significantly ($p<0.05$).

[2] Zinc source effect refers to supplementation of ZnAA or $ZnSO_4$ at level of 60 mg/kg.

[3] Immunogen effect refers to LPS challenge or saline injection. Birds were intraperitoneally injected with LPS at 2 μg/bird or equivalent amount of sterile saline.

saline-treated hens ($p<0.001$). The concentration of zinc in liver and spleen tended to be higher in LPS challenged birds fed ZnAA than fed $ZnSO_4$ ($p<0.09$).

Tissue metallothionein

As shown in Table 6, the MT concentration in both liver and spleen were affected by zinc source, immune challenge and their interaction as well ($p<0.001$). In both LPS and saline-administrated birds, supplementation of ZnAA or $ZnSO_4$ significantly ($p<0.05$) elevated MT concentration in liver and spleen compared to basal diet. In saline-treated birds, although there was no difference in MT concentration in splenic tissue fed ZnAA or $ZnSO_4$, MT concentration in liver of birds fed ZnAA was higher than those fed $ZnSO_4$ ($p<0.05$), the MT concentration was increased 158% and 135% by supplementation of ZnAA and $ZnSO_4$ respectively. When challenged with LPS, birds fed ZnAA diet have a higher concentration of MT in both liver and spleen than those fed $ZnSO_4$ ($p<0.05$) Supplementation of ZnAA increased MT concentration by 266% and 225% in liver and spleen respectively, whereas supplementation of $ZnSO_4$ increased MT concentration only by 158% and 151% in liver and spleen respectively.

DISCUSSION

Lipopolysaccharide (LPS) isolated from the cell wall of gram-negative bacteria is often used to induce acute phase response and the subsequent systemic inflammation. The present work showed that a *Salmonella typhimurium* LPS challenge reduced eggs production and increased percentage of cracked egg, which was in agreement with previous research reports. Klasing and Austic (1984) reported that activation of the immune response led to depressed growth in chicks. In a turkey study, *B. avium* infection decreased the body weight at age of 21 day (Kidd et al., 2000). Klasing and Austic (1984) also reported that a live *Escherichia coli* challenge caused a decrease in skeletal muscle protein synthesis and an increase in the rate of protein degradation. It was suggested that immunologic stress occur from infectious challenges that activate the immune system was accompanied with metabolic changes (Klasing et al., 1987). Therefore, when hens were imposed with immunological challenged (such as LPS), more nutrients may be mobilized to maintain the normal function of immunity and then less available for egg production, which might result in a poor laying performance of hens. In the present work, birds fed ZnAA diet had a decreased egg production when challenged with LPS compared to those fed basal diet. The course may be due to their more intense immune response to LPS-challenge.

It was shown in present experiment that LPS-challenge has induced marked fever response in hens. Though there was no difference in fever response by different dietary zinc source, among LPS-challenged birds, the peak of fever appeared earlier in hens supplemented with ZnAA than those fed $ZnSO_4$. The difference in the fever response profiles implicated different sensitivities of birds to LPS. Moderate fever is considered to be a beneficial response to infection because it enhances the activity of immune cells and increases heat shock protein (HSP) synthesis, which provides protection against the detrimental effects of various stress and immune stimuli and amplify the local immune responses (de Boer and Breimer, 1998). Suppressed febrile response contributes to increased mortality and prolonged recovery from infection (Fraifeld and Kaplanski, 1998). Therefore, the ability to initiate earlier febrile response and quicker fading away from fever in birds fed ZnAA diet may contribute to their stronger resistance to pathogenic infection and recovery from immunological stress.

LPS can induce the release of proflammatory cytokine such as IL-1β, IL-6 and TNF-α, in which these cytokines activate the hypothalamic-pituitary-adrenal (HPA) axis and mediate systemic components of the inflammatory response such as fever (Luheshi, 1999). Researches had also suggested that zinc stimulated peripheral blood mononuclear cell (PBMC) in dose-dependent manner to release IL-1, IL-6, tumor necrosis factor (TNF)-α and IFN-γ (Driessen et al., 1994), and acted synergistically with LPS with respect to cytokine induction in leukocytes (Driessen et al., 1995a,b). In the present experiment, among LPS-challenged birds, serum IL-1β was higher in hens fed ZnAA diet than those fed the basal or $ZnSO_4$ diet at 3 h after challenge. However, birds fed ZnAA diet had the lowest serum IL-1β concentration among dietary treatments at 12 h post-challenge with LPS. Therefore, compared to the basal

or $ZnSO_4$ diet, birds fed ZnAA might induce earlier release and quicker fading away of proflammatory cytokine, which was quite in agreement with the time frame of fever response. It is also possible that the enhanced sensitivity of birds fed ZnAA to LPS may be due to its more bioavailabity than $ZnSO_4$. More bioavailable Zn may augment the proliferation as well as cytokine secretion in monocytes and macrophage and amplify the immuno-stimulative effects of LPS. But the exact underlying mechanism is now not clear. Interestingly, among saline-treated birds, serum IL-1β concentration was higher in hens fed ZnAA diet than those fed basal diet at 3 h after saline administration. This may imply zinc-induced cytokine production might be caused by a direct interaction of Zn with monocytes and macrophage independent of immune challenge.

The present experiment demonstrated that LPS-challenge decreased the serum zinc concentration and increased zinc concentration in liver and spleen, and that was in consistent with previous reports with experimental infection (Klasing, 1984; Tufft et al., 1988; Hill, 1989) and/or intravenous injection with endotoxin (Butler and Curtis, 1973). This dramatic decrease of serum zinc and internal redistribution of zinc accompanied with the acute phase response was thought to withhold important minerals such as zinc necessary for bacterial growth in blood stream and to reprioritize hepatic protein synthesis in a fashion beneficial to the host in times of stress or infection (Cousins, 1985; Sugarman, 1983). Among LPS-challenged birds, feeding ZnAA diet resulted in higher hepatic and splenic zinc concentration compared to $ZnSO_4$ diet, which implied that dietary supplementation of ZnAA may supply a more bioavailable zinc for liver and spleen and thereby contributed to better immune defense than $ZnSO_4$. It has been reported that intracellular functions that require zinc are dependent upon the availability of zinc for proper functioning (Dardenne and Bach, 1993), and that the organic zinc such as zinc methionine is 206% more available than $ZnSO_4$ (Wedekind et al., 1992).

It is widely reported that metallothionein, a zinc binding protein, is a major factor causing serum hypozincemia and zinc sequestered in the liver and other organs after LPS administration (Cousins, 1985; Gaetke et al., 1997). Reduction in plasma Zn concentration is usually associated with enhanced hepatic MT gene expression and increased synthesis of MT (Dunnm and Cousins, 1989). Furthermore, an increase in Zn status of an animal resulted in increased synthesis of MT in many different tissues (Richards, 1989). Our results showed that MT concentration of liver and spleen was significantly elevated by LPS-challenge. The positive correlation of tissue MT and zinc sequestered in these tissues confirms the pivotal role of MT in zinc dynamics during immunological stress. In LPS-challenged birds, feeding ZnAA tended to increase MT level in liver and spleen more than that of feeding $ZnSO_4$ (266% vs. 158%, 225% vs. 135%, respectively), which indicated that organic zinc source might be a better source for zinc accumulation and MT synthesis to alleviate immune challenge. The effect on MT concentration by interaction between immune-challenge and Zn supplementation was in agreement with the report of Hernandez et al. (1996), in which they found that administration of zinc followed by endotoxin treatment increased MT levels in rat liver by 40 folds, whereas simple endotoxin challenge or zinc supplementation increased MT levels only 8-12 times. The synergism between zinc and endotoxin in tissue MT induction *in vivo* is hypothetically attributed to zinc levels in body and the mobilization capacity of zinc, because the effect was abolished in an *in vitro* experiment (Hernandez et al., 1996). Transcription regulation of MT gene by zinc is conferred by metal response elements (MRE) in the MT promoter. Only metal occupancy of a transcription factor that binds specifically to the MRE sequence of DNA can initiate transcription (Davis and Cousins, 2000). Dietary ZnAA supplementation may result in a larger intracellular zinc pool, thereby increase the opportunity for occupancy of one specific zinc finger. In addition, zinc amino acid complex may be absorbed intact and circulated to target tissues very efficiently (Power and Horgan, 2000). It is also possible that the intact zinc amino acid complex may bind with a higher affinity with zinc finger protein of transcription factor, therefore enhance gene transcription and protein synthesis of MT.

CONCLUSION

Compared with $ZnSO_4$, supplementation of Zn amino acid complex seemed to elevate the sensitivity to LPS challenge and shorten acute phase response. Dietary supplementation of Zn amino acid complex increased zinc retention and metallothionein synthesis in liver and spleen, which might result in a more effective immunological response to limit the serum Zn available for pathogens and improve utilization of zinc for the tissues requiring zinc.

REFERENCES

Blalock, T. L., M. A. Dunn and R. J. Cousins. 1988. Metallothionein gene expression in rats: Tissue-specific regulation by dietary copper and zinc. J. Nutr. 118:222-228.

Butler, E. J. and M. J. Curtis. 1973. The effect of *Escherichia coli* endotoxin and ACTH on the plasma zinc concentration in the domestic fowl. Res. Vet. Sci. 15:363-367.

Cao, J., P. R. Henry, S. R. Davis, R. J. Cousin, R. D. Miles, R. C. Littell and C. B. Ammerman. 2002. Relative bioavailability of organic zinc sources based on tissue zinc and metallothionein

in chicks fed conventional dietary zinc concentration. Anim. Feed Sci. and Technol. 101:161-170.

Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, S. R. Miles and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-2054.

Cao Jia-yin, Luo Xu-gang, S. R. Davis, P. R. Henry, R. J. Cousin, R. D. Miles and C. B. Ammerman. 2003. Tissue zinc and metallothionein concentrations and Metallothionein gene expression as criteria for relative bioavailability assays of zinc sources in chicks. Chinese J. Anim. Vet. Sci. 34 (3):227-231.

Cousin, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65:238-309.

Cousins, R. J. and J. M. Hempe. 1990. Zinc, in Present Knowledge in Nutrition, (Ed. M. L. Brown), International Life Science Institute, Nutrition Foundation, Washington, DC. pp. 251-261.

Dardenne, M. and J. M. Bach. 1993. Rational for the mechanism of zinc interaction in the immune system. In: Nutrient Modulation of the Immune Response (Ed. S. Cunning ham-Rundles), Marcel Dekker Inc. New York. pp. 501-509.

Davis, S. R. and R. J. Cousins. 2000. Metallothionein expression in animals: A physiological perspective on function. J. Nutr. 130:1085-1088.

de Boer, A. G. and D. D. Breimer. 1998. Cytokines and blood-brain barrier permeability. Prog. Brain Res. 115:425-451.

Driessen, C., K. Hirv, L. Rink and H. Kirchner. 1994. Induction of cytokine by zinc ions in human peripheral blood mononuclear cell and separated monocytes. Lymphokine Cytokine Res. 13:15-20.

Driessen, C., K. Hirv, H. Kirchner and L. Rink. 1995a. Divergent effects of zinc or different bacterial pathogenic agents. J. Infect. Dis. 171:486-489.

Driessen, C., K. Hirv, H. Kirchner and L. Rink. 1995b. Zinc regulates cytokine induction by superantigens and lipopolysaccharide. Immunology. 84:272-277.

Dumm, M. A. and R. J. Cousins. 1989. Kinetics of zinc metabolism in the rate: effect of dibutyryl cyclic AMP. Am. J. Physiol. 256, E420-E430.

Eaton, D. L. and B. F. Toal. 1982. Evaluation of the Cd/hemoglobin affinity assay for rapid determination of metallothionein in biological tissues. Toxicol. Appl. Pharmacol. 66:134-142.

Ferket, P. R. and M. A. Qureshi. 1992. Effect of level of inorganic and organic zinc and manganese on the immune function of turkey toms. Poult. Sci. 71 (Suppl.):60 (Abstr.).

Fleet, J. C., M. A. Qureshi, R. R. Dietert and C. C. McCormick. 1988. Tissue-specific accumulation of metallothionein in chickens as influenced by the route of zinc administration. J. Nutri. 118:176-182.

Fraifeld, V. and J. Kaplanski. 1998. Brain eicosanoids and LPS fever: species and age differences. Prog. Brain Res. 115:141-156.

Fraker, P. J., M. E. Gershwin, R. A. Good and A. Prasad. 1986. Interrelationships between zinc and immune function. Fed. Proc. 45:1474-1479.

Gaetke, L. M., C. J. McClain, R. T. Talwalkar and S. T. Shedlofsky. 1997. Effects of endotoxin on zinc metabolism in human volunteers. Am. J. Physiol. 272 (Endorcrinol. Metab. 35:E952-956.

Hernandez, J., M. Giralt, E. Belloso, D. V. Rebollo, B. Romero and J. Hidaigo. 1996. Interaction between metallothionein inducers in rat liver and primary culture of rat hepatocytes. Chem. Bio. Interact. 100:27-40.

Hill, C. H. 1989. Effect of *Salmonella gallinarum* infection on zinc metabolism in chicks. Poult. Sci. 68:297-305.

Kidd, M. T., M. A. Qureshi, P. R. Ferket and L. N. Thomas. 1994a. Blood clearance of *escherichia coli* and evaluation of mononuclear-phagocytic system as influenced by supplemental dietary zinc methionine in young turkeys. Poult. Sci. 73:1381-1389.

Kidd, M. T., M. A. Qureshi, P. R. Ferket and L. N. Thomas. 1994b. Dietary zinc methionine enhances mononuclear-phagocytic function in young turkeys. Bio. Trace Elem. Res. 42:217-229.

Kidd, M. T., P. R. Ferket and M. A. Qureshi. 1996. Zinc metabolism with special reference to its role in immunity. World's Poult. Sci. J. 52:309-324.

Kidd, M. T., M. A. Quershi, P. R. Ferket and L. N. Thomas. 2000. Turkey hen Zinc source affects progeny immunity and disease resistance. J. Appl. Poult. Res. 9:414-423.

Klasing, K. C. and R. E. Austic. 1984. Changes in protein degradation in chickens due to an inflammatory challenge. Proc. Soc. Exp. Biol. Med. 176:292-296.

Klasing, K. C. 1984. Effect of inflammatory agents and interleukine-1 on iron and zinc metabolism. Am. J. Physiol. 247:901-904.

Klasing, K. C., D. E. Laurin, R. K. Peng and D. M. Fry. 1987. Immunologically mediated growth depression in chicks: Influence of feed intake, corticosterone, and interleukine-1. J. Nutr. 117:1629-1637.

Luheshi, G. L. 1999. Cytokines and fever. An NY Acad. Sci. 856:83-89.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. Washington, DC. National Academy Press.

Power, R. and K. Horgan. 2000. Biological chemistry and absorption of inorganic and organic trace metals. In: Biotechnology in the Feed Industry. (Ed. T. P. Lyon and K. A. Jacques) Nottingham University Press, Nottingham, UK. pp. 277-291.

Richards, M. P. 1989. Characterization of the metal composition of metallothionein isoforms using reversed-phase high-performance liquid chromatography with atomic absorption spectrophotometric detection. J. Chromatogr. 482:87-97.

Singh, C. and S. P. S. Singha. 2002. Effect of dexamethasone stress on concentrations of zinc in blood plasma and in sub-cellular fractions of various tissues of neonatal buffalo calves. Asian-Aust. J. Anim. Sci. 15:1022-1025.

Spear, J. W. 1989. Zinc methionine for ruminant: relative bioavailability of zinc in lambs and effects on growth and performance of growing heifer. J. Anim. Sci. 67:835.

Sugarman, B. 1983. Zinc and infection. Rev. Infect. Dis. 5:137-147.

Tufft, L. S., C. F. Nockels and M. J. Fettman. 1988. Effects of *escherichia coli* on iron, copper, and zinc metabolism in chicks. Avian Diseases, 32:779-786.

Verbanac, D., C. Milin, B. Radosevic-Stasic, Z. Trobonjaca, R. Domitrovic, J. Giacometti, M. Petkovic, M. Cuk, Z. Ciganj, J. Rupcic, and D. Rukavina. 1998. Tissue zinc dynamics during the immune reaction in mice. Bio. Trace Elem. Res. 65:97-108.

Wedekind, K. J., A. E. Hortin and D. H. Baker. 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70:178-187.

Wellinghausen, N., H. Kirchner and L. Rink. 1997. The immunobiology of zinc. Immunol. Today 18:519-531.