Effects of Different Levels of Zinc on the Performance and Immunocompetence of Broilers Under Heat Stress

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ABSTRACT Male broilers were used to evaluate the effect of zinc on performance and immune competence during heat stress (HS). Broilers raised in either a thermoneutral (TN, 23.9°C constant) or HS (23.9 to 35°C cycling) environment were fed a low zinc diet (LZ; 34 mg/kg), an adequate zinc diet (AZ; 68 mg/kg), or a supplemental zinc diet (HZ; 181 mg/kg). Humoral immunity was assessed by intravenous injection of 7% SRBC followed by evaluation of serum for antibody titers in primary and secondary responses. Cell-mediated immunity was assessed using a Sephadex stimulation method to recruit abdominal exudate cells (AEC) to evaluate macrophage phagocytic ability.

The HS birds consumed 12.5% less feed, gained 24.6% less weight, and had lower feed efficiency when compared to TN birds. Dietary zinc levels did not impact growth performance or plasma zinc concentration. Numbers of AEC, macrophages in AEC, phagocytic macrophages, and internalized opsonized and unopsonized SRBC were increased by HZ. Total, IgM, and IgG antibody titers for primary and secondary responses were significantly increased in birds receiving HZ under TN conditions. Tibia zinc concentration increased with increasing zinc levels but did not change with temperature. Lymphoid organ weights, primary and secondary antibody responses, incidences of macrophages in AEC, phagocytic ability of macrophages, and plasma zinc concentration were all significantly reduced by HS. These results indicate that the immune response of broilers can be influenced by the level of zinc in the diet and by environmental conditions.

(*Key words*: broiler, growth performance, heat stress, immune response, zinc)

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INTRODUCTION

Chronic heat stress (HS) is of great concern in all types of poultry production. Feed consumption, growth rate, hatchability, mortality, and other important traits governing the prosperity of the industry are adversely affected by severe HS. Heat loss in broilers is limited by feathering and the absence of sweat glands. When the temperature and relative humidity exceed the comfort level of a bird, it loses the ability to efficiently dissipate heat. This leads to physiological changes including a reduction in feed intake in order to reduce metabolic heat production (Teeter et al., 1985) and lower growth rate as well as a reduction in feed efficiency (Geraert et al., 1996). These authors along with many others have experimented with various temperature levels above what is considered thermoneutral (TN; approximately 23.9°C), and their reports have shown consistent effects of these temperatures on bird performance. Broilers exposed to an environmental temperature of 32°C showed a 14% decrease in feed intake by 4 wk of age and a 24% reduction by 6 wk of age (Geraert et al., 1996).

Other consequences of high environmental temperature have been reported. It has been established that high temperatures affect the development of a specific immune response in the chicken (Thaxton et al., 1968; Subba Rao and Glick, 1970; Thaxton and Siegel, 1972). When chicks were exposed to temperatures ranging from 32.2 to 43°C for short intermittent periods of constant high temperatures or cycling high temperature conditions, the resulting antibody response to SRBC was reduced significantly. Although limited information is available on the effect of high temperature on cell-mediated immune response, Miller and Qureshi (1991) reported a depression in the phagocytic potential of chicken macrophages during in vitro HS conditions.

The concentration of nutrients required to maintain health and productivity of the chicken is challenged due to the reduction in feed intake under HS conditions. Studies have shown a redirection of nutrient flow to meet the metabolic requirements of an immune or inflammatory

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Abbreviation Key: AEC = abdominal exudate cells; AZ = adequate zinc; HS = heat stress; HZ = high zinc; LZ = low zinc; ME = mercaptoethanol; TN = thermoneutral.

response (Bauman and Currie, 1980). There is evidence suggesting a redistribution of zinc during immunological stress. For example, plasma zinc was greatly reduced and hepatic zinc was found to be more than four times the amount lost from plasma (Klasing, 1984). It is therefore possible that the requirement for zinc is increased during exposure to HS conditions. It is believed that zinc is essential in all aspects of immunity (Chandra and Dayton, 1982; Sherman, 1992) and functions through its association with the enzymes critical for the integrity of the cells involved in the immune response (Dardenne et al., 1985). 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., 1992), whereas others have reported no effect (Stahl et al., 1989a; Pimentel et al., 1991b).

Lack of sufficient evidence showing how HS broilers would respond immunologically if their diets were supplemented with varying levels of zinc has necessitated further study. An experiment was conducted to evaluate the effect of temperature and zinc levels on performance characteristics and immunocompetence of broilers raised under HS or TN conditions.

TABLE 1. Composition of low zinc diet

Ingredient	%
Yellow corn	51.26
Soybean meal (48% CP)	38.44
Corn oil	5.49
Dicalcium phosphate	1.43
Limestone	1.37
Salt	0.40
DL-Methionine	0.15
Vitamin-trace mineral mix[1]	1.00
Coban[2]	0.10
Sand[3]	0.36
Nutrient composition	
CP, %	22.77
ME, kcal/kg[4]	3189.70
Ca, %	1.21
P, %	0.67
Cu, mg/kg	13.00
Zn, mg/kg	34.00

[1]Supplied per kilogram of diets 2 and 3: copper, 8 mg; iodine, 0.4 mg; iron, 100 mg; selenium, 0.3 mg; vitamin A (retinyl acetate), 4,540 IU; vitamin D_3, 1,543 IU; vitamin E, 15.4 IU; choline, 284 mg; niacin, 34 mg; D-pantothenic acid, 5.7 mg; riboflavin, 3.4 mg; menadione, 0.85 mg; vitamin B_{12}, 0.01 mg; biotin, 0.1 mg; folic acid, 0.5 mg; thiamine, 0.6 mg.

[2]Coccidiostat, Elanco Products Co., Indianapolis, IN.

[3]Appropriate amount of zinc was added at the expense of an equivalent amount of sand in diets with adequate zinc (AZ) and high zinc (HZ).

[4]Metabolizable energy is calculated, whereas all other values are analyzed.

MATERIALS AND METHODS

Chickens and Diets

One hundred forty-four, 1-d-old Arbor Acres broiler males were used in this experiment. Chicks were randomly assigned to 12 experimental units in a Petersime brooder battery with 12 chicks per pen for the first 3 wk. They were fed one of three dietary treatments (Table 1) randomly assigned to each pen with four replications of each treatment. Water and feed were provided ad libitum, and feed intake and BW were recorded weekly. Experimental diets were as follows: low in zinc (LZ; 34 mg/kg), accomplished by adding a zinc-free vitamin-trace mineral mix to the ration; adequate in zinc (AZ; 68 mg/kg), served as control with no supplemental zinc; and high in zinc (HZ; 181 mg/kg). Zinc supplementation was provided by a zinc polyamino acid complex.[2]

Temperature Treatments

After 3 wk at recommended brooding temperatures, seven chicks per replicate from each dietary treatment (a total of 28 birds per treatment) were randomly selected and weighed. They were divided into two groups and transferred to individual cages in two environmental chambers. In one chamber, the ambient temperature was adjusted so that daily fluctuations were between 23.9 and 37°C, simulating diurnal temperature during HS. They were exposed to 12 h of 23.9°C, 3 h of 23.9 to 37°C, 6 h of 37°C, and 3 h of 37 to 23.9°C. In the other chamber, temperature was maintained at a constant 23.9°C. Relative humidity was allowed to fluctuate but not fall below 45%. For the next 4 wk birds were given the same dietary treatments as they received in the initial 3 wk of the study, and feed intake and BW were monitored weekly.

Immunocompetence Evaluations

Cell-Mediated Immunity. Avians lack a resident population of harvestable cells (macrophages and other inflammatory cells) in the abdominal cavity (Sabet et al., 1977; Trembicki et al., 1984). Therefore, the cell-mediated response of the birds was evaluated using a Sephadex stimulation method previously described (Qureshi et al., 1986). Briefly, on d 32 (12 d after transfer) three birds per treatment from each environmental chamber were randomly selected and weighed. The abdominal area was cleaned with alcohol and a 3% suspension of preswollen Sephadex G-50,[3] prepared in 0.85% sterile saline, was injected into the abdominal cavity at 1 mL/100 g BW.

Harvesting Abdominal Exudate Cells. Approximately 42 h after injection, birds were slaughtered by cervical dislocation. A small incision (approximately 1 cm) was made in an area of the abdomen that contained a minimal number of blood vessels. The cavity was then flushed with sterile heparinized saline solution (0.5 U/mL) and approximately 30 mL of exudates collected from each bird into conical siliconized glass tubes[3] on ice, using a fabricated harvester (created by M. A. Qureshi, 1998,

[2]Zinpro Corporation, Eden Prairie, MN.
[3]Sigma Chemical Co., St. Louis, MO.

North Carolina State University, personal communication). Tubes were allowed to sit for about 20 min to allow debris to settle. The exudate was pipetted into clean tubes and centrifuged at 500 × *g* at 8°C for 15 min. Cell pellets were resuspended in 5 mL of RPMI-1640 growth medium[4] supplemented with 5% fetal bovine serum and 1% antibiotics (penicillin-streptomycin).

Macrophage Culture. Total number of abdominal exudate cells (AEC) in suspension was determined by counting all nonerythroid cells from each chick on a hemacytometer utilizing the trypan blue exclusion technique (Phillips, 1977). Briefly, 10 μL of 0.4% trypan blue and 10 μL of sample were mixed and then 10 μL of that mixture was counted at 40× magnification. After determining total number of cells in the 5-mL suspension, 1 mL was placed in sterile 35-mm petri dishes,[5] (one for each chick) containing four sterile 13-mm coverslips. Dishes were incubated at 41°C at 5% CO_2 for 1 h. Coverslips were removed and washed with saline. The concentration of the rest of the cells in suspension was adjusted to 1×10^6 cells/mL for phagocytosis assay.

Preparation of Quail Anti-SRBC Serum. Five Japanese quail provided by the University of Tennessee poultry farm were injected by way of the jugular vein with 1 mL of 7% SRBC. After 7 d, 3 mL of blood was collected from each quail by jugular venipuncture, and serum was generated and evaluated for antibody production. Three days later the quail were given a booster injection of the 7% SRBC for secondary antibody production. This procedure was followed because the level of antibody produced during a secondary response is higher than during the primary response. The serum was evaluated for IgG and IgM titers. Serum from the quail with the highest titer was then used for opsonizing SRBC.

Preparation of Unopsonized and Opsonized SRBC. A 1% concentration of unopsonized SRBC was prepared by adding 250 μL/mL of cells in 25 mL of RPMI-1640 growth medium. For opsonized SRBC, cells were prepared as before except that 2 μL/mL of quail anti-SRBC serum was added. This mixture was incubated in a 37°C waterbath for 30 min. Tubes were removed, gently shaken to coat cells, and then centrifuged at 300 × *g* at 10°C for 10 min. Cells were washed and resuspended in 25 mL of RPMI-1640 medium.

In Vitro Phagocytosis Assay. The phagocytic ability of the macrophages was determined using an in vitro SRBC phagocytic assay as previously described (Qureshi et al., 1986). Percentage of phagocytic macrophages for opsonized and unopsonized SRBC, and the number of internalized SRBC per phagocytic macrophage were quantified. One milliliter of the 1×10^6 AEC suspension was placed in each of two sets of petri dishes per bird (1 unopsonized and 1 opsonized) containing four coverslips each and then incubated at 41°C at 5% CO_2 for 1 h. Dishes were removed and 1 mL each of opsonized and unopsonized SRBC was placed in a petri dish. They were again incubated for 1 h after which coverslips were removed and washed with 0.85% sterile saline to remove any free SRBC. Coverslips containing fixed cells were then stained using CMS Protocol HEMA 3 Staining Kit.[5] Coverslips were then mounted onto slides using Cytoseal 60 mounting medium.[5] After drying, 100 cells each were scored for total macrophages, phagocytic macrophages, and number of internalized opsonized and unopsonized SRBC for each coverslip per bird at 100× magnification.

Humoral Immunity. Sheep red blood cells were used as a test antigen to quantify specific antibody responses. On d 32, three birds per treatment per environmental chamber were bled by cervical venipuncture, and 3 mL of blood was collected from each bird for prechallenge antibody titer analysis. This procedure was followed to check for the presence of antibodies prior to challenge with SRBC. The same birds were then immunized intravenously via the wing with 1 mL of 7% SRBC suspension. Seven days post-injection, all birds were bled by brachial venipuncture, and 3 mL of blood was collected for primary antibody response. The blood was left at room temperature for 2 h to clot, then wrung with a wooden applicator stick, and placed in a 4°C refrigerator overnight for maximum sera yield. The antigenic challenge was repeated 14 d after the first challenge, and blood samples were collected 3 d after injection to determine secondary antibody response.

Antibody Assay. Serum samples were tested for total antibody response, then specifically for IgM and IgG using the 2-mercaptoethanol (ME) technique as previously described (Qureshi and Havenstein, 1994; Lepage et al., 1996). Briefly, serum was pipetted into microcentrifuge tubes and inactivated by heat in a 56°C waterbath for 30 min. To assess total antibodies, 50 μL of PBS was placed in the first row of wells in a 96-well V-bottom microtitration plate.[5] To the same wells, 50 μL of serum was added, and plates were sealed and incubated at 37°C for 30 min. Plates were removed from incubator, and 50 μL PBS was added to the 11 remaining wells in each row. A twofold serial dilution of the samples was made on successive rows, 50 μL of a 2.5% SRBC suspension was added to each well, and plates were again sealed and incubated for 30 min. The IgM ME-sensitive and IgG (ME-resistant) antibody titers were assessed using the same procedure as for total titers except that 50 μL of 2-ME was added to the first row of wells. Titers were read by holding plates over a lighted mirror to observe wells showing agglutination. All antibody titers were reported as $\log_2$ of the reciprocal of the last dilution in which agglutination was observed.

Plasma and Tibia Zinc Concentrations. Three birds per treatment per environmental chamber were bled by brachial venipuncture on d 48 by using syringes coated with heparin saline (100 U/mL), and 6 mL of blood was collected into NH_4-heparin tubes. The blood was centrifuged at 1,500 × *g* for 20 min, and plasma was pipetted into polypropylene culture tubes. Analysis was done using a Thermo Jarrel Ash Solaar 969 Atomic Absorption Spectro-

[4]Gibco Laboratories, Grand Island, NY.
[5]Fisher Scientific Co., LLC, Suqanee, GA.

TABLE 2. Gain, feed conversion and intake of 7-wk-old broilers fed different levels of zinc under thermoneutral (TN) and heat stress (HS) conditions[1]

Diet[2]	Temperature	Gain (g)	Intake (g)	Feed conversion (feed/gain)
LZ	TN	1,690.17	3,549.71	2.10
	HS	1,462.01	3,322.33	2.27
AZ	TN	1,793.64	3,692.64	2.06
	HS	1,363.13	3,191.10	2.34
HZ	TN	1,702.67	3,689.40	2.17
	HS	1,336.46	3,207.56	2.40
Pooled SEM		56.67	111.36	0.05
Source of variation			Probability	
Diet		0.29	0.99	0.14
Temperature		0.0001	0.0001	0.0001
Diet × temperature		0.07	0.16	0.26
Main effect means				
Diet				
LZ		1,576.09	3,436.02	2.18
AZ		1,578.39	3,441.87	2.18
HZ		1,519.57	3,448.48	2.27
Temperature				
TN		1,728.83[a]	3,643.92[a]	2.11[b]
HS		1,387.20[b]	3,240.33[b]	2.34[a]

[a,b]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Values are least square treatment means. Data from 72 birds per treatment.

[2]Low zinc (LZ; 34 mg/kg zinc) and adequate zinc (AZ; 68 mg/kg zinc) had no supplemental zinc, and high zinc (HZ; 181 mg/kg zinc) was supplemented with zinc.

photometer, utilizing the method of Gorush (1973). Tibias were collected on day of slaughter for tibia zinc concentration.

Organ Collection. Birds were slaughtered on d 50, and thymus, spleen, liver, and bursa of Fabricius were collected and weighed.

Experimental Design and Statistical Analysis

The experiment outline above was repeated four times. The design used was a randomized block design blocking on time. The experimental data were analyzed using the mixed models procedure of SAS software (SAS, 1995). Differences among treatment means were determined using least significance difference test. Log_2 transformations were done on antibody titers prior to statistical analysis.

RESULTS AND DISCUSSION

Growth Performance

The level of zinc in the diet did not significantly influence broiler growth performance during the entire 7 wk of the study (Table 2). These results were not surprising based on the inconsistencies reported on zinc status and growth rate in broilers. Pimental et al. (1991b) in one experiment found no differences in feed intake and growth of broilers fed up to 88 μg zinc/g diet, whereas Kidd et al. (1992, 1994) observed no differences in BW and feed conversion of broilers supplemented with 140 or 164 μg zinc/g diet. Reports by Miller et al. (1968) and Swinkel et al. (1994) show that diets low in zinc lead to depressed appetite resulting in lowered feed intake and reduced weight gain. However, in this study zinc levels were apparently not low enough to affect feed intake or weight gain. There was a significant reduction in BW, feed conversion, and intake when the birds were exposed to HS (Table 2). This result was consistent with the general trend observed in HS broilers (Austic, 1985; Geraert et al., 1996). It is believed that for every 10°C increase in ambient temperature above 20°C, there is a 17% reduction in feed intake (Austic, 1985). Geraert et al. (1996) saw a 14% reduction in BW at 2 to 4 wk of age and a 24% reduction at 4 to 6 wk of age when birds were exposed to 32°C and suggested that the reduced efficiency could be due to changes in metabolic utilization of nutrients.

Lymphoid Organ Weights

Organ weights were measured as a percentage of BW (Table 3). None of these organs (thymus, bursa, spleen, or liver) were significantly affected by the level of zinc in the diet. This finding was inconsistent with previous reports that a diet deficient in zinc leads to atrophy of the thymus (Prasad and Oberleas, 1971) and a reduction in spleen weight of rats (Mengheri et al., 1988). Although not significant, birds on the HZ diet showed a slight increase in thymus weight as a percentage of BW (0.34% compared to 0.30%) each for LZ and AZ. All organ weights were significantly reduced by HS. This could have been a result of the reduction in feed intake, thereby providing less nutrients for the proper development of these organs. Supplementing the zinc in the diets did not seem to improve the weight of these organs under HS conditions.

TABLE 3. Lymphoid organ and liver weights of 7-wk-old broilers fed different levels of zinc under thermoneutral (TN) and heat stress (HS) conditions[1]

Diet[2]	Temperature	Thymus[3] (% BW)	Bursa (% BW)	Spleen (% BW)	Liver (% BW)
LZ	TN	0.36	0.18	0.12	1.63
	HS	0.24	0.11	0.10	1.57
AZ	TN	0.37	0.18	0.15	1.55
	HS	0.24	0.10	0.09	1.45
HZ	TN	0.43	0.19	0.14	1.69
	HS	0.24	0.10	0.08	1.48
Pooled SEM		0.02	0.11	0.02	0.70
Source of variation			Probability		
Diet		0.52	0.872	0.84	0.06
Temperature		0.0001	0.0001	0.0001	0.01
Diet × temperature		0.62	0.88	0.07	0.25
Main effect means					
Diet					
LZ		0.30	0.15	0.11	1.60
AZ		0.30	0.14	0.12	1.50
HZ		0.34	0.14	0.11	1.59
Temperature					
TN		0.39[a]	0.18[a]	0.14[a]	1.62[a]
HS		0.24[b]	0.11[b]	0.09[b]	1.50[b]

[a,b]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Values are least square treatment means. Data from 24 birds per treatment.

[2]Low zinc (LZ; 34 mg/kg zinc) and adequate zinc (AZ; 68 mg/kg zinc) had no supplemental zinc, and high zinc (HZ; 181 mg/kg zinc) was supplemented with zinc.

[3]All thymic lobes from both sides of the neck were weighed for each chick.

Primary and Secondary Antibody Responses

Prior to antigenic challenge, sera from birds were analyzed for prechallenge antibody titers that could influence the experiment and were found to be negative. Table 4 shows the main effect means of diets and temperature and their effects on primary and secondary antibody responses. The data represent the means of $\log_2$ of the reciprocal of the last dilution exhibiting agglutination. Birds receiving the HZ diet had significantly higher titers of total (5.80), IgM (3.72), and IgG (2.08) antibodies than those receiving the other diets for primary response. Those receiving AZ and HZ were similar with a higher response (6.50 and 6.61, 1.70 and 1.86, 4.80 and 4.75, respectively) for total, IgM, and IgG antibodies during the secondary challenge. These results are consistent with previous reports (Beach et al., 1980; Burns, 1983) that diets supplemented with zinc tend to improve the ability of the birds to produce antibodies. Birds reared under HS conditions showed a significant reduction in all three parameters during primary and secondary responses. This response was similar to observations by Thaxton and Siegel (1970) and Donker et al. (1990) when birds were exposed to temperatures of up to 42°C. However, this is not always the case. Along with the immununosuppression observed in these studies, there has been evidence of immunostimulation (Kelley, 1985; Siegel, 1987), as well as no response (Regnier et al., 1980). Due to the considerable variation found in the above-mentioned re-

TABLE 4. Main effect means of anti-SRBC antibody response (primary and secondary) of broilers fed different levels of zinc under thermoneutral (TN) and heat stress (HS) conditions[1]

	Primary			Secondary		
Treatment	Total Antibody[2]	IgM[2]	IgG[2]	Total Antibody[2]	IgM[2]	IgG[2]
Diet[3]						
LZ	3.76[c]	2.45[b]	1.31[b]	4.64[b]	1.44[b]	3.20[b]
AZ	4.77[b]	2.92[b]	1.85[a]	6.50[a]	1.70[a]	4.80[a]
HZ	5.80[a]	3.72[a]	2.08[a]	6.61[a]	1.86[a]	4.75[a]
Pooled SEM	0.16	0.17	0.13	0.24	0.12	0.21
Temperature						
TN	5.53[a]	3.57[a]	1.96[a]	6.96[a]	1.92[a]	5.04[a]
HS	4.03[b]	2.49[b]	1.54[b]	4.88[b]	1.43[b]	3.45[b]

[a–c]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Values are least square treatment means. Data are from 18 birds per treatment.

[2]Data represent means of $\log_2$ of the reciprocal of the last dilution exhibiting agglutination.

[3]Low zinc (LZ; 34 mg/kg zinc) and adequate zinc (AZ; 68 mg/kg zinc) had no supplemental zinc, and high zinc (HZ; 181 mg/kg zinc) was supplemented with zinc.

TABLE 5. Incidence of macrophages in abdominal exudates cells of broilers fed different levels of zinc under thermoneutral (TN) and heat stress (HS) conditions[1]

Diet[2]	Temperature	AEC[3] ($\times 10^6$)	Macrophages[4] (%)
LZ	TN	5.38	95.22
	HS	5.91	92.40
AZ	TN	7.77	93.75
	HS	7.10	94.29
HZ	TN	8.09	95.86
	HS	9.64	94.93
Pooled SEM		1.10	0.53
Source of variation		Probability	
Diet		0.01	0.01
Temperature		0.58	0.02
Diet × temperature		0.57	0.01
Main effect means			
Diet			
LZ		5.64[b]	93.81[b]
AZ		7.44[ab]	94.02[b]
HZ		8.87[a]	95.40[a]
Temperature			
TN		7.08	94.95[a]
HS		7.55	93.87[b]

[a,b]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Values are least square treatment means. Data are from 24 birds per treatment.

[2]Low zinc (LZ; 34 mg/kg zinc) and adequate zinc (AZ; 68 mg/kg zinc) had no supplemental zinc, and high zinc (HZ; 181 mg/kg zinc) was supplemented with zinc.

[3]Mean AEC counted on a hemacytometer after Sephadex stimulation.

[4]Represents percentage of macrophages scored out of approximately 300 adherent cells per coverslip.

sponses, these researchers believe there are apparent differences in susceptibility to HS based on genetic lines. These differences may have implications during the application of selection programs for immune responsiveness. Another explanation could be the decrease in feed intake caused by HS during this study leading to a reduction in the nutrients available to mount an effective immune response. Supplementing the diet with zinc did not significantly improve antibody response when the birds were under HS during this study. Because of the lack of sufficient evidence on the use of these zinc polyamino acid complexes and their effect on the immune system, we can only postulate that the level of zinc could be manipulated further before a definite recommendation is made.

Cellular Immune Response

Total mean AEC (Table 5) was highest in birds receiving HZ with 57% more than LZ. This finding supports those of other researchers that zinc is important in maintaining the integrity of the cells of the innate immune system, because avian macrophages are the first line of defense against microorganisms (Dietert et al., 1990). The number of AEC was not affected by HS. The incidence of macrophages in AEC were highest for birds fed the HZ diets with 95.4% compared to 94.02 and 93.81% for AZ and LZ, respectively. This finding compared favorably with the results by Kidd et al. (1994) who supplemented turkey poult diets with zinc, showing zinc to be important in maintaining the integrity of the cells of the innate immune system. Although HS did not significantly affect the total number of AEC, there was a reduction in the percentage of macrophages. Table 6 shows the phagocytic ability of macrophages for opsonized and unopsonized SRBC. Birds fed the HZ diet had more activated macrophages for opsonized and unopsonized SRBC than those fed the LZ diet. However, those macrophages for the opsonized SRBC had more activity. In both cases HS significantly reduced the number of activated macrophages. The trend was similar for the number of engulfed SRBC. Birds fed the HZ diet engulfed more SRBC then the other diets for opsonized and unopsonized SRBC with opsonized SRBC having greater phagocytic activity.

Similar results were obtained by Wirth et al. (1989) working with mice. To demonstrate that zinc was essential for the activity of these macrophages, these researchers (Wirth et al, 1989) supplemented the culture medium containing the macrophages from the zinc-deficient diet with zinc and the phagocytosis and killing functions of the macrophages were restored. With the limited availability of evidence on cellular response to HS, there is some uncertainty with regard to the type of response that would be generated under similar conditions.

Plasma and Tibia Zinc Concentration

The level of zinc in the diet did not affect the concentration of zinc in plasma, however, tibia zinc concentration was significantly lower in LZ birds, 400.08 μg/g, compared to 423.69 and 437.70 μg/g for AZ and HZ birds, respectively (Table 7). Moreover, plasma zinc was significantly reduced by HS, whereas tibia zinc was not affected. A similar observation was made by Sandoval et al. (1998), even though their basal ration was supplemented with between 1,000 and 1,500 mg/kg zinc. Also in their

TABLE 6. Phagocytosis of opsonized and unopsonized SRBC by abdominal macrophages of broilers fed different levels of zinc under thermoneutral (TN) and heat stress (HS) conditions[1]

Diet[2]	Temperature	% Phag.mac[3] (ops)	# SRBC/phag.mac (ops)	% Phag.mac (unops)	# SRBC/phag.mac (unops)
LZ	TN	31.29	3.00	23.04	1.87
	HS	27.74	2.93	22.06	1.81
AZ	TN	37.30	4.92	27.94	2.82
	HS	31.99	3.59	24.11	2.34
HZ	TN	37.22	6.04	29.07	2.71
	HS	32.24	3.81	25.79	2.38
Pooled SEM		0.83	0.27	0.70	0.14
Source of variation			Probability		
Diet		0.0001	0.0001	0.0001	0.0001
Temperature		0.0001	0.0001	0.0001	0.02
Diet × Temperature		0.53	0.0001	0.04	0.35
Main effect mean					
Diet					
LZ		29.51[b]	2.97[c]	22.55[c]	1.84[b]
AZ		34.64[a]	4.26[b]	26.02[b]	2.58[a]
HZ		34.73[a]	4.93[a]	27.43[a]	2.54[a]
Temperature					
TN		35.27[a]	4.66[a]	26.68[a]	2.47[a]
HS		30.66[b]	3.44[b]	23.99[b]	2.18[b]

[a–c]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Values are least square treatment means. Data are from 18 birds per treatment.

[2]Low zinc (LZ; 34 mg/kg zinc) and adequate zinc (AZ; 68 mg/kg zinc) had no supplemental zinc, and high zinc (HZ; 181 mg/kg zinc) was supplemented with zinc.

[3]Macrophages with engulfed opsonized and unopsonized SRBC were scored out of 300 cells per coverslip.

study, dietary zinc supplementation resulted in increased tibia zinc concentration as was found in the present study. This was consistent with findings by Pimentel et al. (1991a) who observed a significant increase in bone zinc with increased dietary zinc. It is believed that zinc from bone can be utilized when zinc deficiency occurs; therefore, bone zinc constitutes a functional reserve that can be mobilized.

The observations in this study corroborate and extend previous studies on the role of zinc and the effect of zinc deprivation on the immunological capacity. Although the levels of zinc used in this study did not seem to impact the growth performance of the birds, no final conclusions can be drawn until these polyamino acid complexes are investigated further. The relationship between nutrition and the immune response has received

TABLE 7. Plasma and tibia zinc concentration of broilers fed different levels of zinc under thermoneutral (TN) and heat stress (HS) conditions[1]

		Zinc concentration	
Diet[2]	Temperature	Plasma (μg/dL)	Tibia (μg/g)
LZ	TN	198.84	400.57
	HS	177.33	399.59
AZ	TN	197.71	420.88
	HS	192.40	426.50
HZ	TN	220.49	432.95
	HS	183.43	442.46
Pooled SEM		12.48	14.20
Sources of variation		Probability	
Diet		0.33	0.0001
Temperature		0.01	0.45
Diet × temperature		0.22	0.79
Main effect mean			
Diet			
LZ		188.09	400.08[b]
AZ		195.05	423.69[a]
HZ		201.96	437.70[a]
Temperature			
TN		205.68[a]	418.13
HS		184.39[b]	422.85

[a,b]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Values are least square treatment means. Data are from 24 birds per treatment.

[2]Low zinc (LZ; 34 mg/kg zinc) and adequate zinc (AZ; 68 mg/kg zinc) had no supplemental zinc, and high zinc (HZ; 181 mg/kg zinc) was supplemented with zinc.

much attention, and the results of this study indicate that this relationship is a crucial one. In this study, both cellular and humoral immune responses were compromised in birds exposed to HS demonstrating the effects of high environmental temperature. Producers in hot climates should avail themselves of management and nutritional practices that would help to alleviate some of the consequences of HS. The impact of zinc on performance and immunity in broilers is still not completely understood, and future studies rest with continued understanding of the dynamics of this mineral on overall productivity and health.

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