Immune System and Cardiac Functions of Progeny Chicks from Dams Fed Diets Differing in Zinc and Manganese Level and Source[1,2]

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ABSTRACT This research was conducted to evaluate immunity (experiments 1 to 3), cardiac function, and ascities resistance (experiment 4) of progeny chicks from broiler breeders fed diets differing in trace metal level and source. Broiler breeders received a control diet (75 mg of Zn and 83 mg of Mn added/kg of diet), the control diet supplemented with inorganic Zn (75 mg/kg of diet) and Mn (80 mg/kg of diet), the control diet supplemented with organic Zn (75 mg/kg of diet) and inorganic Mn (80 mg/kg of diet), or the control diet supplemented with organic Zn (75 mg/kg of diet) and Mn (80 mg/kg of diet) in experiments 1, 2, and 3. In experiment 4, the control diet and diet supplemented with organic sources of Zn and Mn were fed to broiler breeders. Immune organ weights, circulating granulocytes vs. agranulocytes, CD4 and CD8 positive T cells, cutaneous basophil hypersensitivity, and antibody titers to SRBC and breeder vaccinations were measured in progeny. Some supplemental mineral treatments increased ($P \leq 0.05$) cutaneous basophil hypersensitivity and relative bursa weight. All supplemental mineral treatments increased ($P \leq 0.05$) relative thymus weight. In experiment 4, electrocardiograph, pulse oximetry, heart rate, hematocrits, ventricle weights, and ascites incidence were measured in progeny reared in a cold-stress environment. The supplemental organic minerals increased ($P \leq 0.05$) left ventricle plus septum and total ventricular weights. Although progeny ascites incidence did not differ between breeder mineral treatments, breeders fed supplemental Zn and Mn sired progeny with improved cardiac functional capacity and some improvements in immunity.

(*Key words*: broiler breeder, immunity, manganese, progeny, zinc)

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INTRODUCTION

Zinc is critical for proper immune function in animals. Its deficiency has been shown to decrease cellular immunity (Fletcher et al., 1988), thymus (Fraker et al., 1977) and spleen (Leucke et al., 1978) development, and interleukin production (Dowd et al., 1986). Abnormal T-lymphocyte development is thought to be the primary consequence of Zn deficiency (Dardenne and Bach, 1993). There is a paucity, however, of literature concerning the role of Mn and immune function in comparison to that of Zn. Manganese has been shown to be important in antibody production of many species (Fletcher et al., 1988). Both Mn and Zn contribute to superoxide dismutase function (Bannister et al., 1971; Fridovich, 1975), which is vital for macrophage and heterophil integrity (Cook-Mills and Fraker, 1993).

Zinc is important for proper disease resistance, and its deficiency has resulted in bacterimia (Flinchum et al., 1989; Kidd et al., 1992b, 1994), parasitic infections (Fraker et al., 1982), sickle cell anemia (Prasad, 1979), and alterations in high-density lipoprotein cholesterol (Hooper et al., 1980; Freeland-Graves et al., 1982). Also, Mn deficiency has been shown to increase skeletal abnormalities (Hurley, 1981). These former reports have resulted in poultry scientists investigating levels and sources of Zn and Mn to improve poultry immune function and disease resistance. For example, Zn impacts immunity in poultry (Stahl et al., 1989; Pimentel et al., 1991; Kidd et al., 1992a,b, 1994, 2000; Abou-Zeid, 1999). Also, Zn supplementation in breeder diets has been shown to enhance immunity of their progeny (Stahl

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Abbreviation Key: CBH = cutaneous basophil hypersensitivity; ECG = electrocardiograph; HCT = hematocrit; LV+S = left ventricle plus septum weight; TV = total ventricular weight; ZnaaMnaa = control diet plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn; ZnaaMni = control plus 75 mg Zn/kg from Zn amino acid complex Availa Zn and 80 mg Mn/kg from Mn sulfate.

et al., 1989; Kidd et al., 1992a,b, 2000). Much of the former research conducted by Kidd and coworkers utilized different Zn sources (i.e., inorganic vs. organic-amino acid complexed Zn). Zinc and Mn from amino acid complexes have been shown to be more bioavailable than Zn (Wedekind et al., 1992) and Mn (Henry et al., 1989; Smith et al., 1995) from inorganic sources, respectively. The main focus of the research presented herein was to determine the impact of Zn and Mn level and source on progeny immunity.

Very little research has been done to investigate the effects of trace metal status on ascites in poultry. Research with other species indicates that Zn can act as a protective agent in hearts with deficient blood supply (Powell et al., 1990, 1995; Aiuto et al., 1995). Zinc may act to protect heart tissue by interfering with the production of reactive oxygen intermediates (Lovering and Dean, 1992; Powell et al., 1995). High concentrations of Mn have been shown to decrease the force of ventricular contraction in dogs and rabbits (Conrad et al., 1966; Horner and Kligfield, 1982). Furthermore, intravenously administered Mn has been shown to cause tissue-specific alterations in regional blood flow in rats (Gerdin et al., 1985). Although the majority of research evaluating the impact of Zn and Mn on heart function has been conducted in other species, Rapp et al. (2001) evaluated the effect of Zn and Mn from amino acid complexes in broilers reared at high altitudes. It was demonstrated that supplemental organic Zn and Mn decreased mortality and ascites mortality (Rapp et al., 2001).

Three experiments were conducted to evaluate immune responses in progeny of broiler breeders receiving diets fortified with organic and inorganic sources and levels of Zn and Mn. In the final experiment, ascites resistance and cardiac function of progeny from hens fed supplemental organic Zn and Mn was evaluated.

MATERIALS AND METHODS

Broiler Breeder Husbandry

Cobb 500 broiler breeders received dietary treatments from 21 to 43 wk of age, and 4 hatches were used to evaluate progeny in 4 separate experiments. Treatments consisted of control diet (Table 1) containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix, control plus 75 mg Zn/kg from Zn sulfate and 80 mg Mn/kg from Mn sulfate (ZniMni), control plus 75 mg Zn/kg from Zn amino acid complex Availa Zn[4] and 80 mg Mn/kg from Mn sulfate (ZnaaMni), and control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn (ZnaaMnaa).[4] Breeders were reared in a floor pen facility with 24 pens (6 replications/treatment). Each pen was equipped with one nest site that contained 10 nest boxes, 1 bell drinker, 1 male tube feeder on the pen floor, and 1 female trough feeder on the slats. Male and female breeders were fed separately to control body weight. Each pen contained 15 females and 2 males. A total of 1,200 eggs (300 eggs per treatment) were obtained and set for each experiment. Breeder flocks when eggs were set were 29 wk of age for experiment 1, 33 wk of age for experiment 2, 43 wk of age for experiment 3, and 41 wk of age for experiment 4. Eggs set for experiment 4 were only from the control and ZnaaMnaa treatments due to design limitations.

Progeny Husbandry and Design

Parameters for experiment 3 were evaluated on the day of hatch; thus, no rearing was necessary. For experiments 1, 2, and 4, chicks were separated, weighed, and wing-banded by order of breeder pen on the day of hatch. Chicks in experiments 1 and 2 were placed into 24 pens of a battery facility.[5] The battery units were heated and thermostatically controlled. Each pen in the battery unit measured 33 by 99 cm, contained stainless steel feeders and waterers and raised-wire floors. Chicks were given 24 h of light throughout experiments 1 and 2. The use of stainless steel feeders and waterers was necessary to minimize Zn contamination from galvanized metals.

Each pen contained 10 straight-run chicks for experiments 1 and 2. In experiment 4, progeny were reared in 4 hypobaric chambers (2 pens/chamber). Each chamber was thermostatically controlled, contained 1 tube feeder, 1 bell drinker, and built-up pine shavings. Each pen contained 40 straight-run chicks for experiment 4. Progeny for experiment 4 received 24 h of light from d 1 to 5 and 23 h light from d 6 to 43. Chicks in experiment 4 were brooded at 32°C from d 1 to 5, 29°C from d 6 to 10, and 27°C from d 11 to 17. Temperature was decreased to 16°C from d 18 to 43 to provide cold stress to the progeny, thereby encouraging the development of ascites. Humidity was not controlled and airflow was maintained at 80 ft^3/min per chamber throughout experiment 4. In experiments 1, 2, and 4, chicks had ad libitum access to feed and water. Chicks for experiments 1 and 2 were fed a common starter diet (Table 1) that was based on corn, soybean meal, and poultry fat. Progeny for experiment 4 were fed common starter and grower diets. Progeny treatments were the dietary treatments of the broiler breeder flock.

Measurements

In experiment 1, 3 birds per breeder pen were used to measure immune organ weights on the day of hatch and on d 15. Birds were weighed and killed, and bursa, thymus, and spleen organs were harvested from each bird and weighed individually. Organ weights were expressed as a percentage of BW. Peripheral blood was obtained from 3 chicks per breeder pen. White blood cell populations were counted on blood smears treated with Wright's stain.[6] The first 100 cells observed in a random field per slide were

[4]Zinpro Corporation, Eden Prairie, MN.
[5]Petersime Brood Unit, Petersime Incubator Co., Gettysburg, OH.
[6]Fisher Diagnostics, Swedesboro, NJ.
[7]Becton Dickinson, San Jose, CA.
[8]Biopac Systems, Inc., Goleta, CA.

TABLE 1. Composition of broiler breeder and broiler diets

Ingredient	Breeder diet[1] (%)	Broiler diet (%)
Corn	67.56	56.78
Soybean meal	19.46	34.36
Poultry fat	1.35	4.55
Wheat middlings	2.00	
Dicalcium phosphate	1.71	1.78
Limestone	6.72	1.32
NaCl	0.50	0.46
Vitamin-mineral premix[2,3]	0.38	0.25
DL-Met	0.08	0.31
L-Lys HCl	0.04	0.11
L-Threonine		0.07
Filler[4]	0.19	
Calculated analysis		
Crude protein (%)	15.50	21.49
ME, kcal/kg	2,900.00	3,149.99
Lys, %	0.80	1.25
TSAA, %	0.60	0.99
Calcium, %	3.00	1.00
Available phosphorus, %	0.42	0.45
Thr, %		0.88

[1]Average analyzed Zn and Mn in the breeder diet were 95 mg Zn/kg and 83 mg Mn/kg.

[2]Breeder vitamin and mineral premix contained the following per kilogram of diet: vitamin A (vitamin A acetate) 11,731 IU; cholecalciferol 3,344 IU; vitamin E (source unspecified) 15 IU; menadione, 1.4 mg; B_{12}, 16.7 μg; choline, 576 mg; riboflavin, 7.6 mg; niacin, 50 mg; D-biotin, 0.09 mg; pyridoxine, 1.4 mg; ethoxyquin, 42.6 mg; Mn, 83 mg; Zn, 76 mg; iron, 42.6 mg; copper, 10.6 mg; iodine, 1.5 mg; selenium, 0.3 mg.

[3]Broiler vitamin and mineral premix contained the following per kilogram of diet: vitamin A (vitamin A acetate) 7,718 IU; cholecalciferol 2,200 IU; vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B_{12}, 11 μg; choline, 379 mg; riboflavin, 5.0 mg; niacin, 33 mg; D-biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; Mn, 55 mg; Zn, 50 mg; iron, 28 mg; copper, 7 mg; iodine, 1 mg; selenium, 0.2 mg.

[4]Breeder dietary treatments were added at the expense of an inert filler.

differentiated and scored. White blood cell differentiation was determined using the methods described by Burton and Guion (1968). However, because white blood cell populations were measured on the day of hatch, chicks were too young to successfully differentiate among granulocytes (heterophils, eosinophils, and basophils) and agranulocytes (lymphocytes and monocytes). It was possible, however, to accurately differentiate and quantify between granulocytes and agranulocytes. White blood cell populations (percentage granulocytes and percentage of agranulocytes per 100 cells counted) were also measured on the day of hatch.

On the day of hatch in experiment 2, 3 birds per pen were selected randomly and bled at d 1. T cells were separated and analyzed by flow cytometry for CD4 and CD8 receptor sites on T lymphocytes. Approximately 1 mL of peripheral blood was taken from each chick. Samples were then pooled by breeder pen. Leukocytes were isolated from blood by Ficoll-Paque technique and incubated for 5 min using the appropriate conjugates as described previously (Willeford et al., 2000). The CD4 and CD8 subsets were then determined using a FACSCalibur flow cytometer.[7] On d 9 and 10, a cutaneous basophil hypersensitivity (CBH) test was administered to 3 birds per pen. Each chick was injected in the right toe web with 100 μg of phytohemagglutinin-P suspended in 0.1 mL of PBS. Toe web thickness was measured before and 24 h after injection with a pressure-sensitive caliper. Antibody responses to SRBC were measured on d 14. On d 7, 5 birds per pen were injected peritoneally with a 10% solution of SRBC suspended in 0.85% saline. Serum was obtained after bleeding birds for primary antibody titer measurement on d 14. Serum was frozen until analysis. Total primary antibody titers were measured by a microhemagglutination assay in 96-well plates using methods previously described (Becker and Rudback, 1979; Tsiagbe et al., 1987). Primary antibody titers were expressed as the $\log_2$ of the highest serum dilution that agglutinated 0.05 mL of a 2% suspension of SRBC in sterile saline.

For experiment 3, birds were bled at hatch (3 chicks/breeder pen) to measure antibodies acquired from the hen (infectious bursal disease, infectious bronchitis, Newcastle disease, and reovirus) Serum samples were allowed to thaw at room temperature and diluted 1:500 in standard diluent. Diluted serum samples were added to 96-well plates coated with infectious bursal disease, infectious bronchitis, Newcastle disease, or reovirus antigen. Plates were then covered and incubated at room temperature for 30 min. One hundred microliters of substrate was then added to the plates.

TABLE 2. Organ weights[1] of 1-d-old progeny from broiler breeders fed diets differing in Zn and Mn level and sources

Treatment[2]	Bursa (%)	Thymus (%)	Spleen (%)
Control	0.10	0.22	0.04
ZniMni	0.12	0.26	0.03
ZnaaMni	0.10	0.21	0.03
ZnaaMnaa	0.12	0.31	0.04
SEM	0.01	0.05	0.004
$P > F$	0.24	0.45	0.38

[1]Bursa, thymus, and spleen are expressed relative to BW.

[2]Treatments represent dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZniMni = control plus 75 mg Zn/kg from Zn sulfate and 80 mg Mn/kg from Mn sulfate; ZnaaMni = control plus 75 mg Zn/kg from Zn amino acid complex Availa Zn and 80 mg Mn/kg from Mn sulfate; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

TABLE 3. Organ weights[1] of 15-d-old progeny from broiler breeders fed diets differing in Zn and Mn level and sources

Treatment[2]	Bursa (%)	Thymus (%)	Spleen (%)
Control	0.13[ab]	0.12[b]	0.11
ZniMni	0.15[a]	0.18[a]	0.10
ZnaaMni	0.12[b]	0.16[a]	0.10
ZnaaMnaa	0.13[b]	0.17[a]	0.10
SEM	0.01	0.01	0.01
$P > F$	0.005	0.025	0.941

[a,b]Means within a column with no common superscripts differ ($P < 0.05$).

[1]Bursa, thymus, and spleen are expressed relative to BW.

[2]Treatments represent dietary treatments of the broiler breeder flock. Treatments were: Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZniMni = control plus 75 mg Zn/kg from Zn sulfate and 80 mg Mn/kg from Mn sulfate; ZnaaMni = control plus 75 mg Zn/kg from Zn amino acid complex Availa Zn and 80 mg Mn/kg from Mn sulfate; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

TABLE 4. White blood cell differentials[1] from progeny of broiler breeders fed diets differing in Zn and Mn level and sources

Treatment[2]	Mononuclear cells (%)	Granulocytes (%)
Control	23.25	76.75
ZniMni	23.47	76.53
ZnaaMni	26.80	73.20
ZnaaMnaa	24.17	75.83
SEM	1.74	1.74
$P > F$	0.48	0.48

[1]Granulocytes = average number of granulocytes (heterophils, basophils, and eosinophils) per 100 white blood cells; mononuclear cells = average number of mononuclear cells (lymphocytes and monocytes) per 100 white blood cells.

[2]Treatments represent dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZniMni = control plus 75 mg Zn/kg from Zn sulfate and 80 mg Mn/kg from Mn sulfate; ZnaaMni = control plus 75 mg Zn/kg from Zn amino acid complex Availa Zn and 80 mg Mn/kg from Mn sulfate; ZnaaMnaa = control plus 75 mg Zn/kg from AvailaZn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

TABLE 6. Primary antibody and CBH response from progeny of broiler breeders fed diets differing in Zn and Mn level and sources

Treatment[2]	SRBC[3] (log_2)	CBH (mm)	CBH (%)
Control	2.75	0.90[c]	90.32[c]
ZniMni	2.00	1.12[ab]	112.08[ab]
ZnaaMni	1.95	0.94[bc]	94.16[bc]
ZnaaMnaa	1.92	1.19[a]	118.55[a]
SEM	0.50	0.08	7.78
$P > F$	0.59	0.03	0.03

[a–c]Means within a column with no common superscripts differ ($P < 0.05$).

[1]CBH = cutaneous basophil hypersensitivity response to phytohemagglutinin-P.

[1]Treatments represent dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZniMni = control plus 75 mg Zn/kg from Zn sulfate and 80 mg Mn/kg from Mn sulfate; ZnaaMni = control plus 75 mg Zn/kg from Zn amino acid complex Availa Zn and 80 mg Mn/kg from Mn sulfate; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

[3]SRBC = antibody titer response to sheep red blood cells.

Plates were allowed to incubate at room temperature for 15 min to allow for a color change reaction. A standard stop solution was then added to the plates to halt the enzyme-substrate reaction. Plates were then read using a microplate reader to measure antibody titers to infectious bursal disease, infectious bronchitis, Newcastle disease, and reovirus.

For experiment 4, electrocardiograph (ECG) measurements were obtained from 7 birds per pen on d 34 using methods previously described (Wideman and Kirby, 1996). Briefly, birds were restrained in dorsal recumbency with wings and legs extended. Needle electrodes were inserted subcutaneously at the base of wings and at the base of the left leg. The ECG values were recorded using a Biopac MP100 data acquisition system, ECG100A amplifiers, and Acknowledge software.[8] Lead II R, S, and RS waves were recorded for analysis. Percentage oxygen saturation of hemoglobin was measured in 7 birds per pen with a pulse oximiter on d 35 using methods previously described (Wideman and Kirby, 1995a,b). On d 36, blood was removed via puncture of wing web in duplicate from the same birds examined on d 35, and hematocrit (HCT) percentages were determined with methods previously described (Wideman et al., 1998). On d 42, all birds suspected of having ascites were killed, and necropsies were performed. Hearts were removed from each bird and dissected. Right ventricle weight (RV), left ventricle plus septum weight (LV+S), and total ventricular weight (TV) were measured in each bird using methods previously described (Wideman et al., 1998). On d 43, all birds used for experimental parameters on d 35 and 36 were killed and necropsied. Heart measurements (RV, LV+S, and TV) were then obtained from each bird, and overall incidence of ascites was calculated.

All experiments were randomized complete block designs. Breeder pen was the experimental unit. All data were analyzed by the GLM procedure of SAS software (1996). Percentage data were transformed using arcsin. When differences among means were found, differences were separated using the least squares means option of SAS software (1996).

TABLE 5. CD4 and CD8[1] receptor ratios from progeny of broiler breeders fed diets differing in Zn and Mn level and sources

Treatment[2]	CD4 (%)	CD8 (%)
Control	32.16	6.49
ZniMni	18.46	5.33
ZnaaMni	25.81	7.69
ZnaaMnaa	23.83	10.87
SEM	5.24	2.42
$P > F$	0.35	0.46

[1]CD4 = number of CD4 receptors on T lymphocytes; CD8 = number of CD8 receptors on T-lymphocytes.

[B]Treatments represent dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZniMni = control plus 75 mg Zn/kg from Zn sulfate and 80 mg Mn/kg from Mn sulfate; ZnaaMni = control plus 75 mg Zn/kg from Zn amino acid complex Availa Zn and 80 mg Mn/kg from Mn sulfate; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

RESULTS

Experiment 1

There were no differences in lymphoid organ weights at hatch (Table 2). On d 15 (Table 3), chicks from breeders

TABLE 7. Day 34 electrocardiographs for broilers

Treatment[1]	R wave (mV)	S wave (mV)	RS wave (mV)
Control	0.063	0.088	0.151
ZnaaMnaa	0.063	0.094	0.153
SEM	0.007	0.012	0.012
$P > F$	0.979	0.735	0.902

[1]1Treatments represents the dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

TABLE 8. Day 35 and 36 pulse oximetries, hematocrits, and heart rates of broilers[1]

Treatment[2]	Pulse O_2 (%)	Hematocrit readings (%)		HR (beats per min)
		1	2	
Control	82.21	36.46	36.36	362.36
ZnaaMnaa	82.64	37.44	37.96	376.07
SEM	1.79	0.84	0.91	7.39
$P > F$	0.866	0.415	0.217	0.195

[1]Pulse O_2 = percentage O_2 saturation of hemoglobin; hematocrit readings 1 and 2 = duplicate percentage hematocrit; HR = heart rate in beats per min. Seven birds per pen were selected at random, measured, identified, and returned to pens.

[2]Treatments represents the dietary treatments of the broiler breeder lock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

fed supplemental Zn and Mn from inorganic sources had higher ($P < 0.01$) percentage bursa weight than chicks from breeders fed ZnaaMni and ZnaaMnaa (Table 3). Also, chicks from breeders fed supplemental Zn and Mn from all sources had higher ($P < 0.03$) percentage thymus weights than chicks from breeders receiving the control diet on d 15 (Table 3). No differences were found among treatments for white blood cell populations (Table 4).

Experiments 2 and 3

Ratios of CD4 and CD8 receptor sites on T lymphocytes did not differ among treatments (Table 5). No beneficial humoral immune responses were observed in this research. Primary antibody responses to SRBC did not differ among treatments in experiment 2 (Table 6). Furthermore, antibody titer levels in the progeny from vaccinations in the parent flock did not differ among treatments (data not presented). Chicks from parents fed diets with supplemental Zn and Mn from amino acid complexes had increased ($P < 0.03$) CBH to phytohemagglutinin-P compared with chicks from parents fed the control diet (Table 6).

Experiment 4

The ECG R, S, and RS wave values (Table 7) did not differ among treatments. No effect was observed on hemoglobin as neither percentage hemoglobin oxygen saturation nor percentage HCT (Table 8) differed among treatments. There was no difference in heart rate among treatments (Table 8). On d 42, birds suspected of having ascites did not have different absolute or relative RV, LV+S, or TV weights between treatments (Table 9). On d 43, birds used for experimental parameters on d 35 and 36 displayed no differences in RV weight between treatments (Table 10). However, absolute LV+S and TV weights were higher ($P < 0.03$ and $P < 0.02$, respectively) for progeny of broiler breeders receiving a diet containing supplemental Zn and Mn from amino acid complexes than progeny of breeders receiving control diet containing no supplemental Zn and Mn (Table 10). The overall ascites incidence did not differ among treatments (Table 11).

DISCUSSION

No differences were observed in lymphoid organ weights on the day of hatch. However, chicks from parents fed all diets containing supplemental Zn and Mn had heavier ($P < 0.03$) thymus weights than progeny of dams receiving the control diet on d 15. Furthermore, chicks from parents receiving diets supplemented with inorganic Zn and Mn sources had heavier ($P < 0.01$) bursa weight than chicks from parents fed diets containing supplemental Zn and Mn from organic sources on d 15. Previous reports of similar results involving broiler breeder progeny could not be found. These results are contradictory to results obtained by Kidd et al. (2000) in a study involving the progeny of turkeys. Poults from parents given diets supplemented with Zn from an amino acid complex had higher ($P < 0.04$) bursa weights than poults from parents receiving a diet containing equal additions

TABLE 9. Day 42 heart characteristics for broilers suspected of having ascites[1]

Treatment[2]	BW (g) *RV (g)*	*LV + S (g)*	*TV (g)*	*RV (%)*	*LVS (%)*	*TV (%)*	*RV/TV (g/g)*	
Control	*1,970.00*	*3.17*	*6.02*	*9.19*	*0.17*	*0.31*	*0.48*	*0.27*
Control + ZnaaMnaa	*1,941.82*	*2.90*	*6.56*	*9.45*	*0.15*	*0.34*	*0.49*	*0.27*
SEM	*105.06*	*0.30*	*0.54*	*0.67*	*0.02*	*0.02*	*0.03*	*0.01*
P > F	*0.852*	*0.536*	*0.497*	*0.784*	*0.515*	*0.413*	*0.798*	*0.956*

[1]RV = right ventricle weight; LV + S = left ventricle + septum; TV = total ventricular weight; RV/TV = ratio of right ventricular weight to total ventricular weight.

[2]Treatments represent dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

TABLE 10. Day 43 heart characteristics for broilers[1]

Treatment[2]	BW (g)	RV (g)	LV + S (g)	TV (g)	RV (%)	LVS (%)	TV (%)	RV/TV (g/g)
Control	2,649.2	2.98	8.01	10.99	0.11	0.30	0.42	0.27
Control + ZnaaMnaa	2,680.0	3.26	8.65	11.91	0.12	0.32	0.45	0.27
SEM	42.9	0.18	0.19	0.27	0.01	0.01	0.01	0.01
$P > F$	0.614	0.268	0.026	0.021	0.355	0.055	0.064	0.956

[1]RV = right ventricle weight; LV + S = left ventricle + septum; TV = total ventricular weight; RV/TV = ratio of right ventricular weight to total ventricular weight. These measurements were obtained from the same 7 birds per pen that were evaluated on d 35 and 36.

[2]Treatments represent dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

of Zn from Zn sulfate (Kidd et al., 2000). However, parent birds in the work of Kidd et al. (2000) received Zn from a different amino acid complex than parent birds in this research.

In this research, white blood cell populations did not differ among treatments. Also, the ratios of CD4 and CD8 antigen expression on T lymphocytes in the progeny were not affected by parental dietary treatments. Past research has shown that progeny of turkeys that received Zn from amino acid complexes had more macrophages recruited in the peritoneal cavity (Kidd et al., 2000). Although macrophages were not counted in this research, agranulocytes, which include the subpopulation monocytes (immature macrophages) were, and no differences among treatments were found. Neutrophils, the equivalent of avian heterophils, have been shown to decrease in number when Zn is deficient in other species (Vruwink et al., 1993). In this research, heterophils could not be quantified due to the age of the chicks. However, no differences in circulating granulocytes were observed.

Single Comb White leghorn hens fed a corn-soybean meal diet containing supplemental Zn had progeny with increased antibody titers to SRBC (Stahl et al., 1989). Progeny of broiler breeders receiving a diet supplemented with Zn from Zn oxide displayed higher ($P < 0.04$) antibody titers to SRBC over progeny of breeders receiving a control diet (Kidd et al., 1992b). This response was not observed in other experiments involving broiler breeders (Kidd et al., 1992 a). However, supplemental Zn from amino acid complexes in diets of the parents and chicks increased chick antibody responses to SRBC and *Salmonella pullorum* (Kidd et al., 1992a). No beneficial humoral immune responses were observed in this research. Neither primary antibody titers to SRBC nor progeny antibody titer levels from breeder flock vaccinations (data not presented) differed among treatments.

In this research, progeny of breeders fed diets containing supplemental Zn and Mn from amino acid complexes had higher ($P < 0.03$) CBH response to phytohemagglutinin-P than chicks from parents fed the control diet (Table 6). Broiler breeders receiving diets supplemented with Zn from amino acid complexes have been shown to have progeny with enhanced CBH responses (Kidd et al., 1992a,b). Similar results have also been observed in the progeny of turkeys fed a diet supplemented with Zn from amino acid complexes (Kidd et al., 2000).

In the cardiovascular research portion of this research, there were no differences between treatments observed on ECG measurements, percentage hemoglobin oxygen saturation, percentage HCT, or heart rate. On d 42, neither absolute nor relative RV, LV+S, or TV weights differed among treatments in birds suspected of having ascites. On d 43, broilers used for experimental measurements on d 35 and 36 had no observed differences among treatments for either absolute or relative RV weight. However, on d 43, absolute LV+S and TV weight were higher ($P < 0.03$ and $P < 0.02$, respectively) for progeny of breeders fed diets with supplemental Zn and Mn from amino acid complexes than progeny from parents receiving a control diet (Table 10). Heart Zn level has been correlated to the ejection fraction of the left ventricle (amount of blood output from the left ventricle relative to total cardiac output; Oster et al., 1989). The left ventricle in preascitic broilers must pump a higher volume of blood at a lower than normal pressure to provide enough systemic oxygen for proper metabolism (Wideman, 2000). Perhaps the observed increase in LV+S and TV weight could allow preascitic broilers to meet systemic blood flow demands more easily. Research by Rapp and coworkers (2001) demonstrated that broilers reared at high altitudes had lower mortality due to ascites when fed supplemental Zn and Mn from amino acid complexes. Perhaps no differences were observed in the current study because mineral treatments were only provided in the parent diets. It has been shown that turkey hen Zn source did not affect the heart

TABLE 11. Total mortality and mortality attributable to ascites for all broilers from Days 1 to 43[1]

Treatment[2]	Mortality (%)	Ascites mortality (%)
Control	13.13	88.25
Control + ZnaaMnaa	14.38	90.75
SEM	4.07	8.56
$P > F$	0.835	0.843

[1]Mortality = percentage of total mortality; ascites mortality = percentage mortality attributable to ascites.

[2]Treatments represents the dietary treatments of the broiler breeder flock. Control = control diet containing 75 mg Zn/kg and 83 mg Mn/kg from the mineral premix; ZnaaMnaa = control plus 75 mg Zn/kg from Availa Zn and 80 mg Mn/kg from Mn amino acid complex Availa Mn.

Zn level or heart weight of their progeny (Kidd et al., 2000). However, Kidd et al. (2000) provided no supplemental Mn in the turkey breeder diet. Furthermore, in the research by Rapp et al. (2001), ascites was induced by altitude, whereas cool-temperature challenge was used in the current study. Perhaps the method of ascites induction could be another contributing factor to the presence or absence of treatment effects.

This study indicates that dietary supplements of Zn and Mn in broiler breeder diets may improve cardiac output and some immune system endpoints of their progeny. Future research should address the ability of these trace metals in hen diets to allow progeny chicks to overcome infectious challenges. Hence, broiler breeders receiving diets with supplemental Zn and Mn have been shown to reduce mortality when reared in floor pen environments (Virden et al., 2003).

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