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Effects of Calcium, Phosphorus and Zinc in Wheat-Based Diets on Broiler Chickens' Performance, Immunity and Bone Parameters

Research Article

M. Askari[1], A. Khatibjoo[1*], K. Taherpoor[1], F. Fattahnia[1] and H. Souri[1]

[1] Department of Animal Science, University of Ilam, Ilam, Iran

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*Correspondence E-mail: a.khatibjoo@mail.ilam.ac.ir

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ABSTRACT

An experiment was conducted to investigate the effect of zinc (Zn) supplementation and different concentrations of calcium (Ca) and phosphorus (P) in wheat-based diets on the performance, immune responses and bone parameters of broiler chickens. A randomized complete block design with factorial arrangement was used (three concentrations of Zn supplementation×two concentrations of dietary Ca-P), 300 day-old broilers were assigned to six dietary treatments with five replicates of ten birds. Dietary treatments were the basal diet (control; TRT1), control plus 50 ppm Zn (TRT2), control plus 70 ppm Zn (TRT3), low Ca-P diet (0.60 to 0.30%; TRT4), low Ca-P diet plus 50 ppm Zn (TRT5) and low Ca-P diet plus 50 ppm Zn (TRT6). Ca and P in the control diet were 0.90 and 0.45% in the grower phase and 0.85 and 0.42% in the finisher phase. Changes in dietary Ca-P had no effect on body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) or serum Ca and P concentrations ($P>0.05$) whereas Zn supplementation increased FI ($P<0.05$). The addition of 50 ppm Zn increased serum P concentration ($P<0.05$) and dietary treatments had no effect on antibody titers against sheep red blood cells (SRBC) ($P>0.05$). The lowest blood heterophil (H) and the highest lymphocyte (L) percentages and lowest H:L ratio were observed in birds fed with the diet containing a standard Ca-P with 70 ppm Zn supplementation ($P<0.05$). Dietary treatments had no effect on bone length, thickness and breaking strength ($P>0.05$). Tibia and fibula ash decreased by feeding lower Ca-P than the standard diet ($P<0.05$). It is concluded that low Ca-P diets did not have a detrimental effect on performance or blood and bone parameters and that Zn supplementation did not improve those parameters when feed was low in Ca-P.

KEY WORDS bone, broiler, calcium, immunity, performance, phosphorus, zinc.

INTRODUCTION

Environmental pollution due to the excretion of unutilized mineral compounds such as phytate phosphorus (PP) from large-scale poultry farming has compelled nutritionists to redefine the optimum concentrations of phosphorus (P) and calcium (Ca) for broiler chickens. Also, genetic selection for higher growth rate has increased broilers' body weight to about 2.5 kg at age 45 days. Since corn and soybean meal make up a substantial portion of broiler diets, much of the phosphorus of these ingredients is unavailable for absorption because of its binding to phytic acid (Harland and Oberleas, 1999; Ravindran *et al.* 1999). Therefore, inorganic P must be added to corn-soybean based broiler diets. Phytates, the salts of phytic acid, are the main storage form of P in plants (Pallauf and Rimbach, 1997; Ravindran *et al.* 1995) and render the P relatively unavailable to monogastric animals. Phytates are hydrolyzed by phytase to inositol and phosphoric acid, making the P available to the animals (Liu *et al.* 1998). Besides Ca and P, zinc (Zn) is important in biological processes such as immunity and bone formation in birds and mammals. For example, Zn is an essential

component of many enzymes and it has both structural and catalytic functions in metalloenzymes (O'Dell, 1992). Furthermore, dietary Zn is required for normal immune function (Kidd *et al.* 1996) as well as proper skeletal development and maintenance (Brandão-Neto *et al.* 1995). Dietary zinc methionine complex (Zn-Met) supplementation (80 mg/kg for old broilers and 40 mg/kg for young broilers) in the diet improves immunity in the progeny of old (Kidd *et al.* 1992) and young broiler breeders (Kidd *et al.* 1993). Supplemental Zn-Met in either a corn soybean or a milo and corn-soybean meal diet fed to broiler breeders at age 20 weeks increased cutaneous basal hypersensitivity to phytohemagglutinin-P in their progeny and supplemental Zn oxide (Zn-O) increased antibody titers to SRBC in the progeny of broiler breeders (Kidd *et al.* 1993).

Because of the roles of Ca, P and Zn in immunity and bone formation, we hypothesized that feeding diets including different concentrations of Ca and P with constant Ca:P ratio and Zn supplementation in wheat-based diets may improve broilers' performance, immunity and bone parameters.

MATERIALS AND METHODS

Birds and housing

300 day-old Ross 308 broiler chicks were randomly allocated to a four-floor battery cage. A three-phase feeding program was used: starter (1-12 days), grower (13-24 days), and finisher (25-42 days). Birds were fed wheat-soybean-based diets formulated according to standardized ileal digestible (SID) amino acid (Table 1) (Hoehler *et al.* 2005). The experimental diets were isonitrogenous and isocaloric and fed from 15-42 days of age. At the age of 14 days, birds were weighed and divided into groups of six of similar body weight (465±10 g).

A randomized complete block design with factorial arrangement was used (three concentrations of Zn supplementation × two concentrations of dietary Ca-P), 300 day-old broilers were assigned to six dietary treatments with five replicates of ten birds. Dietary treatments were the basal diet (control; TRT1), control plus 50 ppm Zn (TRT2), control plus 70 ppm Zn (TRT3), low Ca-P diet (0.60 to 0.30%; TRT4), low Ca-P diet plus 50 ppm Zn (TRT5) and low Ca-P diet plus 50 ppm Zn (TRT6). Ca and P in the control diet were 0.90 and 0.45% in the grower phase and 0.85 and 0.42% in the finisher phase. Ca, P and Zn in the ingredients and experimental diets were analyzed by Atomic Absorption Spectrometry showing that the basal diet had 100 mg/kg Zn. Zn was added to diets in the form of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$). Birds were kept under conventional conditions for vaccination, temperature, ventilation, and lighting based on Ross catalogue recommendations and other requirements were provided following Ross production manual recommendations. Water was provided *ad libitum*. BWG and FI were recorded every two weeks during the whole experimental period and FCR was calculated.

Antibody response to SRBC

In order to investigate humoral immunity, sheep red blood cells (SRBC) were used as T-dependent antigen. Two birds from each replicate within the average body weight of each pen were injected intramuscularly with SRBC (2.5% suspension in PBS, 1 mL/bird) twice, at 23 and 31 days of age. Blood samples were collected seven days after the first and second injections. The serum of each sample was separated, heat inactivated at 56 °C for 30 min and then analyzed for total, mercaptoethanol-sensitive (MES) IgM and mercaptoethanol-resistant IgG anti-SRBC antibodies (Delhanty and Solomon, 1966; Qureshi and Havenstein, 1994). Briefly, 50 µL of serum was added to an equal volume of PBS in the first column of a 96-well V-bottomed plate, and incubated for 30 min at 37 °C. A 1:2 serial dilution was then made and 50 µL of 2% SRBC suspension was added to each well. Total antibody titers were read after incubation. The well immediately preceding a well with a distinct SRBC button (agglutinated RBC) was considered as the endpoint titer for agglutination. To measure MES (IgM), 50 µL of 0.01 *M* mercaptoethanol in PBS was used in this procedure instead of PBS alone. The difference between the total antibody concentration and the IgG concentration was considered to be equal to the IgM concentration (Cheema *et al.* 2003).

At 42 days of age, blood samples were collected and analyzed for H, L and H:L ratio. Serum Ca and P were determined using the Calcium Colorimetric Assay Kit (K380-250) and Phosphate Colorimetric Assay Kit (K410-500). At the end of the experiment one broiler from each replicate was killed by cervical dislocation and its bursa, spleen and thymus were weighed.

Bone breaking strength, diameters, length and ash content

After killing, from one broiler with average body weight of each replicate, the right femur, tibia and phalanges were excised. Soft tissues were removed manually and the bone cleaned with gauze. Bone samples were stored in plastic bags and frozen at -20 °C until analysis for breaking strength.

Bones were brought to room temperature then bone-breaking strength was measured in the center of each bone using a Zwic/roell tensile testing machine (model BT1-FR0.5TH.D14) with automated materials test system software. Fulcrum width was altered to accommodate bone length. A round-based probe was attached to a 50-kg load cell with a crosshead speed of 1 mm/s.

Table 1 Ingredients and nutrient composition of experimental diets

Item	Grower		Finisher	
	Standard	Low Ca-P	Standard	Low Ca-P
Ingredient (g/kg)				
Corn	200.0	200.0	200.0	200.0
Wheat	468.4	481.2	479.2	504.0
Soybean meal	209.0	236.0	208.8	188.0
Corn gluten	37.4	11.0	26.4	41.6
Vegetable oil	20.0	20.0	20.0	20.0
Fish meal	30.0	30.0	30.0	30.0
Methionin	1.2	1.4	1.2	1.0
Lysin	1.7	1.1	1.3	1.8
Threonin	0.00	0.00	0.00	0.00
Di Calcium Phosphate	13.9	5.4	10.0	6.9
Oyster shell	10.4	7.2	12.2	7.7
Salt	2.2	2.4	2.3	2.23
$NaHCO_3$	4.7	0.15	0.9	2.25
Vitamin premix[1]	2.5	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5	2.5
Nutrient composition				
AME (kcal/kg)	3000	3000	3030	3030
Crude protein %	20.6	20.4	19.7	19.47
Lys (SID) %	0.97	0.97	0.90	0.90
Met (SID) %	0.43	0.43	0.40	0.41
Cys (SID) %	0.30	0.30	0.30	0.297
Met + Syc (SID) %	0.73	0.73	0.70	0.70
Thr (SID) %	0.61	0.61	0.58	0.58
Trp (SID) %	0.21	0.22	0.196	0.20
Arg (SID) %	1.08	1.13	1.03	1.05
Ca %	0.90	0.60	0.85	0.60
Available P %	0.45	0.30	0.42	0.30
Ca/Ave. P	2	2	2	2
Na %	0.20	0.20	0.20	0.20
Cl %	0.23	0.23	0.23	0.23
DCAB meq/kg	186	198	195	201
Linoleic acid %	1.50	1.50	1.50	1.50
Fiber %	4.33	4.35	4.25	4.25

1: provides per kg of diet: vitamin A (from vitamin A acetate): 7714 IU; Cholecalciferol: 2204 IU; vitamin E (from DL-tocopheryl acetate): 16.53 IU; vitamin B_{12}: 0.013 mg; Riboflavin: 6.6 mg; Niacin: 39 mg; Pantothenic acid: 10 mg; Choline: 465 mg; Menadione (from menadione dimethylpyrimidinol): 1.5 mg; Folic acid: 0.9 mg; Thiamin (from thiamine mononitrate): 1.54 mg; Pyridoxine (from pyridoxine hydrochloride): 2.76 mg; D-biotin: 0.066 mg; Ethoxyquin: 125 mg and Se: 0.1 mg.
2: provided per kg of diet: Mn (from $MnSO_4.H_2O$): 100 mg; Zn (from $ZnSO_4.7H_2O$): 100 mg; Fe (from $FeSO_4.7H_2O$): 50 mg; Cu (from $CuSO_4.5H_2O$): 10 mg and I (from $Ca(IO_3)_2.H_2O$): 1 mg.
SID: standardized ileal digestibility.
DCAB: dietary anion-cation balance.

Bone diameter and length were measured using a Lutron Digital Caliper (model DC-515, Lutron Electronic Enterprise Co., Ltd, Taiwan) at the narrowest and widest points. The mean of each pair of measurements was calculated. Then bones of each bird were collected for ash determination on a fat-free dry weight basis, according to AOAC (1990).

Statistical analysis

Results were analyzed by two-way ANOVA using the GLM procedure of SAS institute (SAS, 2001) with Ca-P and Zn supplementation as main effects. Tukey's multiple range tests was used to compare means taking $P < 0.05$ to indicate statistical significance.

RESULTS AND DISCUSSION

Performance and blood calcium and phosphorus

Effects of dietary treatments on birds' performance (BWG, FI and FCR) and blood Ca and P concentration are illustrated in Tables 2 and 3 respectively. Results showed that dietary treatment did not significantly influence BWG, FI and FCR of birds from 15-42d of age ($P>0.05$) whereas among the main effects of each factor, Zn supplementation more than 50 ppm significantly increased FI from 15-42 d of age ($P<0.05$) while having no effect on BWG and FCR ($P>0.05$). The results in Table 3 demonstrated that altering Ca and P concentration of diets had no significant effects on blood Ca and P concentration ($P>0.05$).

White blood cell count

Effects of dietary treatments on white blood cell (WBC) count are shown in Table 4. Using low Ca-P diets decreased blood lymphocyte percentage and supplemental Zn had no consistent effect on blood lymphocytes ($P<0.05$). Dietary treatments significantly affected blood heterophil and H:L ratio but there were no clear trends because for each of the Ca-P diets, the numerical response to Zn was not the same ($P<0.05$). Addition of 70 ppm Zn had the opposite effect on heterophil percentage and H:L ratio when compared with 50 ppm Zn and control groups ($P>0.05$).

Antibody titer against SRBC

The antibody titers against SRBC inoculation, a measure of humoral immune response, varied significantly ($P<0.05$) with the concentration of supplemental Zn (Table 5). There was no significant effect of dietary treatments on first and second IgG and IgM response against SRBC. However, the main effect of supplemental Zn on the first IgM response was significant with the IgM response increasing with the Zn concentration in the diets ($P<0.05$).

Bone parameters

The effects of dietary treatments on bone parameters ash percentage, diameters, lengths and breaking strength are shown in Tables 6, 7, 8 and 9 respectively. There were few significant differences among dietary treatments for bone morphology measurements ($P>0.05$). There were significant effects of Ca-P on tibia and fibula ash percentage and fibula diameter. Zn supplementation had significant effects on the percentage of ash and the mean breaking strength of the fibula. Low Ca-P diets significantly decreased the fibula and tibia ash content ($P<0.05$) whereas they did not influence femur ash percentage ($P>0.05$). At the other hand, the

70 ppm Zn supplementation significantly decreased fibula ash percentage when compared with those of broilers that received 0 and 50 ppm Zn in their diets (P<0.05).

Growth performance

Determining the optimal Ca:P ratio as well as the Ca-P concentrations is very important in broiler nutrition. Our study was similar to that of other authors who used lower concentrations of Ca and P (Ca at 0.6% and non-phytate phosphorus (NPP) at 0.225 and 0.325%) to investigate the requirements of broilers for Ca and P (Sebastian *et al.* 1996; Sohail and Roland, 1999).

Table 2 Effect of dietary treatments on broiler performance from 15-42 days of age

Treatment	FI (kg)	BWG (kg)	FCR
Ca-P			
Standard	3.51	2.01	1.74
Low	3.47	1.96	1.77
P-value	0.72	0.32	0.61
SEM	0.07	0.04	0.04
Supplementation Zinc			
0	3.26^{b}	1.91	1.71
50 ppm	3.59^{a}	2.07	1.74
70 ppm	3.61^{a}	1.97	1.83
P-value	0.02	0.08	0.19
SEM	0.09	0.50	0.05
Ca-P × supplementation Zn			
Low CaP 0 Zn	3.16	1.87	1.69
Low CaP 50 Zn	3.72	2.09	1.78
Low CaP 70 Zn	3.52	1.91	1.84
Std CaP 0 Zn	3.35	1.94	1.73
Std CaP 50 Zn	3.47	2.06	1.68
Std CaP 70 Zn	3.70	2.04	1.81
P-value	0.18	0.57	0.58
SEM	0.13	0.07	0.07

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Table 3 Effect of dietary treatments on blood Ca and P of broilers

Treatment	Ca (mg/dl)	P (mg/dl)
Ca-P		
Standard	13.24	8.38
Low	11.91	7.84
P-value	0.77	0.23
SEM	2.62	0.31
Supplementation Zinc		
0	13.22	8.33
50 ppm	12.49	8.29
70 ppm	12.01	7.72
P-value	0.96	0.43
SEM	3.18	0.38
Ca-P × Supplementation Zn		
Low CaP 0 Zn	12.82	7.34
Low CaP 50 Zn	11.05	7.93
Low CaP 70 Zn	11.86	8.05
Std CaP 0 Zn	13.61	8.47
Std CaP 50 Zn	13.94	8.93
Std CaP 70 Zn	12.16	8.12
P-value	0.95	0.33
SEM	1.54	0.53

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Table 4 Effect of dietary treatments on blood immune cells of broilers

Treatments	L%	H%	H:L Ratio
Ca-P			
Standard	69.17^{a}	17.83	0.27
Low	65.92^{b}	19.00	0.29
P-value	0.001	0.08	0.15
SEM	0.61	0.45	0.01
Sup. Zinc			
0	67.62^{b}	18.50^{b}	0.28^{b}
50 ppm	74.75^{a}	14.25^{c}	0.19^{c}
70 ppm	60.25^{c}	22.50^{a}	0.38^{a}
P-value	0.01	0.01	0.01
SEM	0.75	0.55	0.01
Ca-P × Sup. Zn			
Low CaP 0 Zn	63.00^{c}	19.50^{bc}	0.31^{b}
Low CaP 50 Zn	71.25^{d}	16.25^{d}	0.23^{c}
Low CaP 70 Zn	63.50^{c}	21.25^{b}	0.34^{b}
Std CaP 0 Zn	72.25^{b}	17.75^{cd}	0.24^{c}
Std CaP 50 Zn	78.25^{a}	12.25^{e}	0.16^{d}
Std CaP 70 Zn	75.00^{b}	23.75^{a}	0.42^{a}
P-value	0.01	0.01	0.01
SEM	1.06	0.77	0.01

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
L: lymphocyte and H: heterophil.
SEM: standard error of mean.

Table 5 Effect of dietary treatments on humoral immunity of broilers against SRBC[1]

Treatment	First-IgG	Second-IgG	First-IgM	Second-IgM
Ca-P				
Standard	2.83	2.46	2.16	2.08
Low	2.66	2.42	2.04	2.58
P-value	0.41	0.22	0.78	0.001
SEM	0.20	0.19	0.20	0.16
Supplementation Zinc				
0	1.69^{b}	2.44	1.93	2.25
50 ppm	2.52^{ab}	2.73	2.12	2.27
70 ppm	2.62^{a}	2.90	2.25	2.78
P-value	0.41	0.05	0.78	0.01
SEM	0.25	0.23	0.25	0.19
Ca-P × Supplementation Zn				
Low CaP 0 Zn	2.62	2.62	2.00	2.50
Low CaP 50 Zn	2.62	2.37	2.00	3.00
Low CaP 70 Zn	2.75	2.37	2.12	2.25
Std CaP 0 Zn	2.75	2.25	1.87	2.00
Std CaP 50 Zn	3.25	2.37	2.25	2.75
Std CaP 70 Zn	2.50	2.62	2.37	1.50
P-value	0.21	0.22	0.23	0.50
SEM	0.35	0.33	0.35	0.28

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
1: the data represent mean ± standard errors of log2 of the reciprocal of the last dilution exhibiting agglutination.
SEM: standard error of mean.

In the current experiment, different Ca and P in the diet made no difference to performance. In other studies depression in weight gain and FI were observed at higher dietary concentrations of Ca (8 and 9 g kg^{-1}) with lower

Table 6 Effect of dietary treatments on bone ash percentage

Treatment	Ash (%)		
	Femur	Tibia	Fibula
Ca-P			
Standard	30.93	35.83[a]	33.57[a]
Low	31.29	33.57[b]	31.54[b]
P-value	0.73	0.03	0.02
SEM	0.72	0.71	0.58
Supplementation Zinc			
0	31.06	34.51	32.16[ab]
50 ppm	30.07	34.98	34.07[a]
70 ppm	31.88	34.53	31.45[b]
P-value	0.35	0.91	0.04
SEM	0.88	0.78	0.71
Ca-P × Supplementation Zn			
Low CaP 0 Zn	30.44	34.01	32.67
Low CaP 50 Zn	31.35	33.33	29.87
Low CaP 70 Zn	30.66	35.81	32.22
Std CaP 0 Zn	32.05	33.21	32.09
Std CaP 50 Zn	29.70	35.94	35.46
Std CaP 70 Zn	32.42	35.73	33.03
P-value	0.60	0.96	0.28
SEM	1.24	1.22	1.00

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Table 7 Effect of dietary treatments on bone thickness (mm)

Treatment	Bone type		
	Fibula	Tibia	Femur
Ca-P			
Standard	3.05[a]	7.58	9.02
Low	2.50[b]	7.56	9.03
P-value	0.04	0.96	0.96
SEM	0.17	0.21	0.25
Supplementation Zinc			
0	2.92	7.42	8.72
50 ppm	2.42	7.68	9.39
70 ppm	2.92	7.61	8.97
P-value	0.28	0.77	0.33
SEM	0.21	0.26	0.31
Ca-P × Supplementation Zn			
Low CaP 0 Zn	2.47	7.54	8.67
Low CaP 50 Zn	3.39	7.49	9.52
Low CaP 70 Zn	2.63	7.80	8.90
Std CaP 0 Zn	3.38	7.40	8.76
Std CaP 50 Zn	2.57	7.73	9.26
Std CaP 70 Zn	3.20	7.56	9.03
P-value	0.51	0.81	0.88
SEM	0.30	0.36	0.44

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

concentrations of NPP (3 and 3.5 g×kg^{-1}) (Rama Rao *et al.* 2006). When broilers' diets contained 110 mg Zn kg^{-1}, performance improved (Burrell *et al.* 2004); however the NRC requirement of Zn for broilers is only 40 mg/kg (NRC, 1994).

Table 8 Effect of dietary treatments on bone length (mm)

Treatment	Bone type		
	Fibula	Tibia	Femur
Ca-P			
Standard	77.47	100.55	71.00
Low	75.13	97.20	70.70
P-value	0.41	0.19	0.86
SEM	1.96	1.76	1.23
Supplementation Zinc			
0	74.48	98.71	71.58
50 ppm	75.42	99.83	69.34
70 ppm	79.02	98.08	71.62
P-value	0.38	0.84	0.48
SEM	2.39	2.15	1.50
Ca-P × Supplementation Zn			
Low CaP 0 Zn	78.13	100.58	70.82
Low CaP 50 Zn	72.02	97.36	70.22
Low CaP 70 Zn	75.24	97.26	71.05
Std CaP 0 Zn	70.83	86.84	72.34
Std CaP 50 Zn	78.81	102.41	68.46
Std CaP 70 Zn	82.80	102.41	72.19
P-value	0.07	0.14	0.70
SEM	3.39	3.04	2.13

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Consistent with our results, some researchers have reported that dietary Zn supplementation increased feed intake, growth rate, and feed efficiency in broiler chicks (Sadoval *et al.* 1999) and quail (Sahin and Kucuk, 2003). Zn is required for the biological function of more than 300 enzymes. In particular, Zn is essential and directly involved in catalysis and cocatalysis by enzymes which control many cell processes including DNA synthesis, growth, brain development, behavioral responses, reproduction, fetal development, membrane stability, bone formation and wound healing (Dołęgowska *et al.* 2003; Mocchegiani *et al.* 2000). Trace mineral supplementations recommended by NRC (1994) were determined by maximal weight gain at that time, which was far below the current broiler weight gain (Bao *et al.* 2009). Thus, it is reasonable to assume that, in modern, rapidly growing broiler strains, higher levels of supplementation than those recommended by NRC will be required. However these trace minerals may not act as growth promoters and supplementing organic Zn close to NRC recommendations may support optimal growth of broilers due to the inherent high bioavailability of this form of Zn. In our study, supplemental Zn increased broiler feed intake from 15-42 days of age. Others have provided information regarding neuropeptide Y (NPY) and galanin concentrations during Zn deficiency (Kennedy *et al.* 1998; Selvais *et al.* 1997). These studies suggested that supplemental Zn increased expression of NPY and galanin and induced anorexia so that higher concentrations of NPY mRNA, but not of NPY peptide concentrations were ob-

served in the hypothalamus of Zn-deficient rats (Selvais *et al.* 1997).

Taken together, these reports suggested that an NPY "paradox" or "resistance" may exist during Zn deficiency. Possible explanations for this apparent resistance include impairments in the processing of pro-NPY into active NPY, reduced secretion of NPY from neurons and attenuation of NPY signal transduction (Selvais *et al.* 1997). Circulating leptin concentrations are reduced during Zn deficiency in the rat (Mangian *et al.* 1998). Reduced concentrations of leptin as a result of Zn deficiency may explain reports of increases in hypothalamic NPY (Lee *et al.* 1998; Selvais *et al.* 1997). Leptin secretion from adipose tissue is reduced by Zn deficiency (Ott and Shay, 2001) and insulin action is a major factor stimulating leptin synthesis and secretion (Barr *et al.* 1997). Thus the results of the present study suggest that minimum concentrations of Ca, P and Zn are optimal for commercial broilers up to 42 days of age.

Antibody response to SRBC and WBC count

Zn is required for the normal development of lymphocytes and Zn deficiency leads to thymocyte depletion in the thymus and reduction in peripheral T-cell numbers and T-cell helper functions. Zn plays an important role in immune modulation by increasing the counts of thymocytes and peripheral T-cells and by enhancing interferon production (Kidd *et al.* 2000).

(Sunder *et al.* 2008), using different concentrations of Zn in broiler diets reported that maximum immune response was observed at 80 ppm, which was lower than values reported earlier.

However, Zn supplementation at 40 ppm was adequate to support optimum development of lymphocytes, which alleviated stress, as observed here (Sunder *et al.* 2008). The results of the present study, suggest that the Ca, P and Zn concentrations used in broiler diets did not have detrimental effects on the immune response. It may be due to lower requirement of broilers for these minerals to show humoral immune response to SRBC antigen. Lower Zn concentration had a positive effect on immune response so it is suggested that supplementation with Zn was useful to reducing stress in young broilers.

Bone diameter, length, thickness and ash percentage

Tibiotarsus width decreased linearly with increasing dietary Ca content but no dietary effects on cortical thickness or BW were found. Body weight tended to be highest in birds fed diets containing 0.7-0.9% Ca and 0.4-0.5% available P. Hence these authors reported that changing Ca and available P of diets did not influence bone ash percentage (Williams *et al.* 2000). The author's original hypothesis was that the consistently lower bone ash content observed in the low Ca-P diet, as compared with the control diet, might be due to a dietary deficiency in Ca or available P

Table 9 Effect of dietary treatments on bone breaking strength (N/m^2)

Treatment	Breaking strength			Breaking strength (Max)		
	Fibula	Femur	Tibia	Fibula	Femur	Tibia
Ca-P						
Standard	115.45	246.00	297.33	155.17	267.58	296.58
Low	92.57	221.82	262.67	131.99	240.25	280.00
P-value	0.20	0.09	0.23	0.08	0.08	0.56
SEM	12.30	16.82	20.86	8.49	10.34	19.87
Supplementation Zinc						
0	111.90[a]	242.75	258.25	139.55	246. 50	285.75
50 ppm	66.66[b]	241.88	283.00	158.21	270.88	293.75
70 ppm	133.44[a]	244.10	274.25	132.98	244.38	285.33
P-value	0.01	0.99	0.96	0.26	0.28	0.96
SEM	15.07	20.58	25.56	10.95	12.67	24.34
Ca-P × supplementation Zn						
Low CaP 0 Zn	90.55[ab]	209.25	242.50	127.35	212.00	258.75
Low CaP 50 Zn	60.28[b]	235.50	245.25	111.20	237.50	247.25
Low CaP 70 Zn	126.88[ab]	220.70	300.25	157.43	271.25	334.00
Std CaP 0 Zn	133.25[a]	276.25	308.00	151.75	281.00	312.75
Std CaP 50 Zn	73.05[ab]	248.25	320.75	154.75	251.25	323.50
Std CaP 70 Zn	140.00[a]	267.50	248.25	159.00	270.50	253.50
P-value	0.73	0.65	0.17	0.42	0.15	0.07
SEM	21.31	29.11	36.14	15.48	17.92	34.42

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

and they also reported that dietary mineral content of broiler diets did not significantly affect cortical bone thickness (Williams *et al.* 2000).

Although there was a tendency for lower ash values of fibula and phalanges to occur at the lowest Ca-P contents in the present study, there appeared to be no simple dietary effect on bone ash content. Hence we concluded that the administration of Zn in broiler diets could increase ash content and alleviate detrimental effects of low Ca-P diets on bone ash content, and that there was some evidence that the low bone ash content observed was due to a nutritional problem. Also the significant difference between Ca-P diets may be due to purely genetic factors between different strains, or due to the growth rate of these birds accelerating past the maximum bone mineralization rate.

Bone breaking strength

The values for bone breaking strength observed here were considerably lower than the values (160-730 N) previously reported (Moran and Todd, 1994). The lower values observed here could be due to wider gauge length (5 cm instead of 3-3.2 cm) and faster speed of load cell (5 cm min^{-1} instead of 2 cm min^{-1}), Applying four concentrations of Ca and available P and different ratios of Ca:P, Williams *et al.* (2000) reported no effects of dietary Ca or available P content on bone breaking strength or collagen content.

Bone strength depends in part on the relative amounts and properties of the mineral content and organic matrix (Boskey *et al.* 1999). It has also been suggested that variations in mineral composition, as demonstrated by different bone Ca:P ratios, might affect bone strength (Thorp and Waddington, 1997). The present study demonstrated no observable dietary mineral content effects on bone ash content, and bone breaking strength was unaffected by dietary Ca-P content at 6 weeks of age. Reducing the Ca:P of diets did not affect bone strength and bone mineral composition.

CONCLUSION

FI and WBC counts were significantly affected by dietary Ca-P concentration and Zn supplementation whereas BWG, FCR and humeral immune response against SRBC antigen were not affected by dietary treatments. It appears that currently recommended broiler commercial diets have a partial deficiency of Zn and addition of supplemented Zn may be important to maintain bone quality and good immune responses.

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