



## Effects of Varying Dietary Zinc Levels and Environmental Temperatures on the Growth Performance, Feathering Score and Feather Mineral Concentrations of Broiler Chicks

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**ABSTRACT** : This study aimed to investigate the effects of dietary zinc (Zn), environmental temperatures and Zn×temperature interaction on growth, feathering score and mineral composition of broilers. A total of 256 d-old Avian male broiler chicks were randomly allocated to a 4×2 factorial arrangement with four corn-soybean meal basal diets (containing 44 mg Zn/kg) supplemented with 0, 40, 60 mg/kg Zn (Diets 1, 2 and 3, respectively; 0.8% Ca for these three diets) and non-Zn supplementation, 1.6% Ca (Diet 4) and two temperature conditions (low: 26, 24, 22°C vs. high: 30, 28, 26°C). All birds were given feathering coverage scores for back, breast, wing, under-wing and tail. The wing and tail were further evaluated for the occurrence and severity of defect feathers. Feathers were then pooled for mineral composition analysis. The results showed that in high temperature conditions, broilers fed Zn-unsupplemented, 0.8% Ca ration (Diet 1) had significantly ( $p<0.05$ ) lower ADFI and ADG (wk 1-6) than birds under low temperature conditions. However, when the birds were fed 40 and 60 mg/kg Zn supplementation (Diets 2 and 3), the ADFI and ADG in both temperature conditions were not significantly different. In low temperature conditions, the ADFI, ADG ( $p<0.05$ ), all feather coverage ( $p<0.01$ ) and tail defect scores ( $p<0.001$ ) of birds fed Diet 4 (excess Ca) were significantly poorer than those fed Diet 1. More Ca ( $p<0.05$ ) was retained in the feathers of broilers fed Diet 4 under high temperature conditions. Broilers fed the Zn-unsupplemented ration (Diet 1) had significantly higher feather phosphorus ( $p<0.01$ ) and potassium ( $p<0.05$ ) concentrations than those fed the 60 mg/kg Zn-supplemented ration (Diet 3). A reduction of feather phosphorus ( $p<0.01$ ) and potassium ( $p<0.05$ ) and higher manganese ( $p<0.05$ ) concentrations were observed in Diet 4 broilers as compared to those fed Diet 1. Under high temperature conditions, broilers had lower iron ( $p<0.05$ ) and higher manganese ( $p<0.05$ ) concentrations in feathers. Broilers kept in high temperature conditions had a higher Zn requirement and 40 mg/kg Zn supplementation was sufficient for the birds to achieve optimum growth. Supplemental Zn ameliorated the adverse effect of high temperature on growth and occurrence of tail feather defects. Excess Ca disrupted Zn metabolism to exert a detrimental effect on growth performance and normal feathering and this was elucidated in the birds kept in low temperature conditions. (**Key Words** : Zinc, Environmental Temperature, Growth, Feathering Score, Minerals, Broiler)

### INTRODUCTION

Zinc (Zn) is an essential trace element for growth, enzyme

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structure and function, appetite, normal immune function, proper skeletal development and maintenance. Zinc is commonly supplemented in commercial poultry diets because practical corn-soybean meal diets provide only marginal levels of Zn relative to requirements. The presence of fiber, phytate, concentrations of other chelating agents and divalent metals such as calcium (Ca) further limit Zn utilization by the animal. Calcium tends to form insoluble complexes with phytate and Zn, and consequently inhibiting Zn absorption. For broilers from 0 to 8 wk-old, the National Research Council (NRC, 1994) recommended a level of 40 mg Zn/kg, which depended largely on historical data about 50 years ago. The rapid growth rate of modern commercial broilers with different productivity

potentials and the aforementioned dietary factors have brought the NRC recommendation into question for current use.

In chicks, signs of Zn deprivation include dermatitis, especially of the feet, and poor feathering (Sunde, 1972). Hair analysis may be a useful biopsy tool to predict body stores of minerals including Zn. Growing hair is metabolically active and can be easily collected and stored. Combs et al. (1982) have reported that hair may reflect concentrations of minerals that are incorporated in the follicle during hair synthesis. Although growth and mineral retention in plasma and tissues are reliable response criteria of bioavailability of minerals in diets, feathering score may be a rapid visual field indicator reflecting the Zn status in poultry.

The deleterious effects of high environmental temperature on the performance of broilers are well documented (Charles 1986; Geraert et al., 1996). According to Ojano-Dirain and Waldroup (2002), as temperature increases above the upper critical limit of the bird's zone of comfort, heat stress is initiated. In avian species, sweat glands are lacking and feathering limits heat loss. In order to reduce metabolic heat production, feed intake is reduced (Geraert et al., 1996) and consequently growth rate decreases due to impaired nutrient digestion and absorption. Hence, nutrient requirements including Zn may differ for birds under heat stress as the published nutrient requirements, such as those by NRC (1994), are estimates for the average gross demand of the population under common environmental conditions. Dietary manipulation is a practical way to overcome the negative effects of environmental stress on broiler performance. Antioxidant mineral such as Zn has been reported to alleviate the adverse effects of heat stress on laying quails (Sahin and Kucuk, 2003). Although the mechanism is not well defined, Zn has been reported to be involved in the antioxidant defense system (Powell, 2000).

The interaction effect of Zn and Ca is well known and extensively documented in the literature. However, there is very little published information on Zn×temperature interaction and the Zn-Ca antagonism in broilers under different environmental temperatures. The objectives of the present study were i) to validate NRC (1994) recommendations on Zn in modern commercial broilers under high environmental temperature; ii) to examine the viability of using feathering score in conjunction with its mineral composition as an index to reflect the Zn status in commercial broilers, and iii) to determine the occurrence of dietary Zn×temperature interactions.

## MATERIALS AND METHODS

### Birds, experimental diets and management

The study was conducted in the Innovation and

Practical Training Centre, National Pingtung University of Science and Technology, Taiwan, Republic of China. A total of two hundred and fifty six d-old Avian male broilers were randomly assigned to a 4 (corn-soybean meal diets)×2 (temperature conditions) factorial arrangement. The chicks were kept in 32 experimental units in two environmental-controlled chambers for 42 d. Each treatment with eight chicks per pen (1.8×0.9 m) was replicated four times. The experimental animals received humane care as outlined in the Guide for the Care and Use of Experimental Animals (Research Policy, University Putra Malaysia).

Four experimental diets, which contained 44 mg/kg basal Zn (Table 1) were formulated to meet NRC (1994) recommendations, except for different Zn supplementations using Zn oxide, i.e., 0 (Diet 1), 40 (Diet 2), 60 (Diet 3) mg Zn supplementation per kg feed (all the three diets were with 0.8% Ca), and 0 supplemental Zn with 1.6% Ca (Diet 4). The chicks were maintained on a 24-h constant lighting regimen. Drinking water containing non-detectable Zn (Table 2) and feed were given *ad libitum*. Feed trough and drinker were made of plastic materials to prevent Zn contamination. The daily feed consumption and weekly body weight were recorded for ADFI, ADG and feed conversion ratio (FCR) calculations.

### Temperature treatments

The two environmental controlled chambers were

**Table 1.** Composition of dietary treatments

Ingredient (%)	Diets 1-3	Diet 4
Corn	49.2	47.2
Soybean meal (44% CP)	43.2	43
Soybean oil	4.0	4.0
Dicalcium phosphate	1.9	1.9
Limestone	0.5	2.7
Methionine	0.5	0.5
Common salt	0.3	0.3
Vitamin premix <sup>1</sup>	0.1	0.1
Trace mineral mix <sup>2</sup>	0.3	0.3
Nutrient composition		
ME (kcal/kg) <sup>3</sup>	2,990	2,917
CP (%)	23.30	23.39
Ca (%)	0.80	1.62
P (%)	0.87	0.90
Zn (mg/kg) <sup>4</sup>	44.30	44.74

<sup>1</sup> Vitamin premix supplied per kilogram diet: vitamin A, 10,000,000 IU; vitamin D<sub>3</sub>, 1,000,000 IU; vitamin E, 20,000 IU; vitamin B<sub>1</sub>, 2,000 mg; vitamin B<sub>2</sub>, 4,000 mg; vitamin B<sub>6</sub>, 3,000 mg; vitamin B<sub>12</sub>, 15 mg; niacinamide, 35,000 mg; vitamin K<sub>3</sub>, 1,500 mg; D-calcium pantothenate, 15,000 mg; biotin, 50 mg; folacin, 1,000 mg.

<sup>2</sup> Supplied individually per kilogram of diet: Cu, 8 mg; I, 0.35 mg; Fe, 80 mg; Mg, 60 mg; Se, 0.15 mg; Mg, 600 mg.

<sup>3</sup> ME is calculated, whereas all other values are analyzed.

<sup>4</sup> Total Zn content analyzed per kilogram of diet: Diet 1, 44.13 mg; Diet 2, 82.77 mg; Diet 3, 101.94 mg; Diet 4, 44.74 mg.

**Table 2.** Ionic composition of drinking water

Ionic composition	Concentration (mg/kg)
Zn	Non-detectable
Cu	0.00002
Fe	0.00018
Mn	Non-detectable
Mg	0.00031
Ca	0.00065
K	0.00027

adjacent to each other and of similar dimensions and set up. On d 1 to 7 post-hatching, all the chicks were kept at recommended brooding temperature of 30-32°C and the temperature was adjusted from the second week onwards. Throughout the duration of the experiment, the temperature difference between the two chambers was maintained at 4°C commencing from 2 wk-old. In the lower temperature chamber, the ambient temperatures were 26°C (wk 2-3), 24°C (wk 4-5) and 22°C (wk 6), whereas in the higher temperature chamber, ambient temperatures were 30°C (wk 2-3), 28°C (wk 4-5) and 26°C (wk 6). The temperatures in

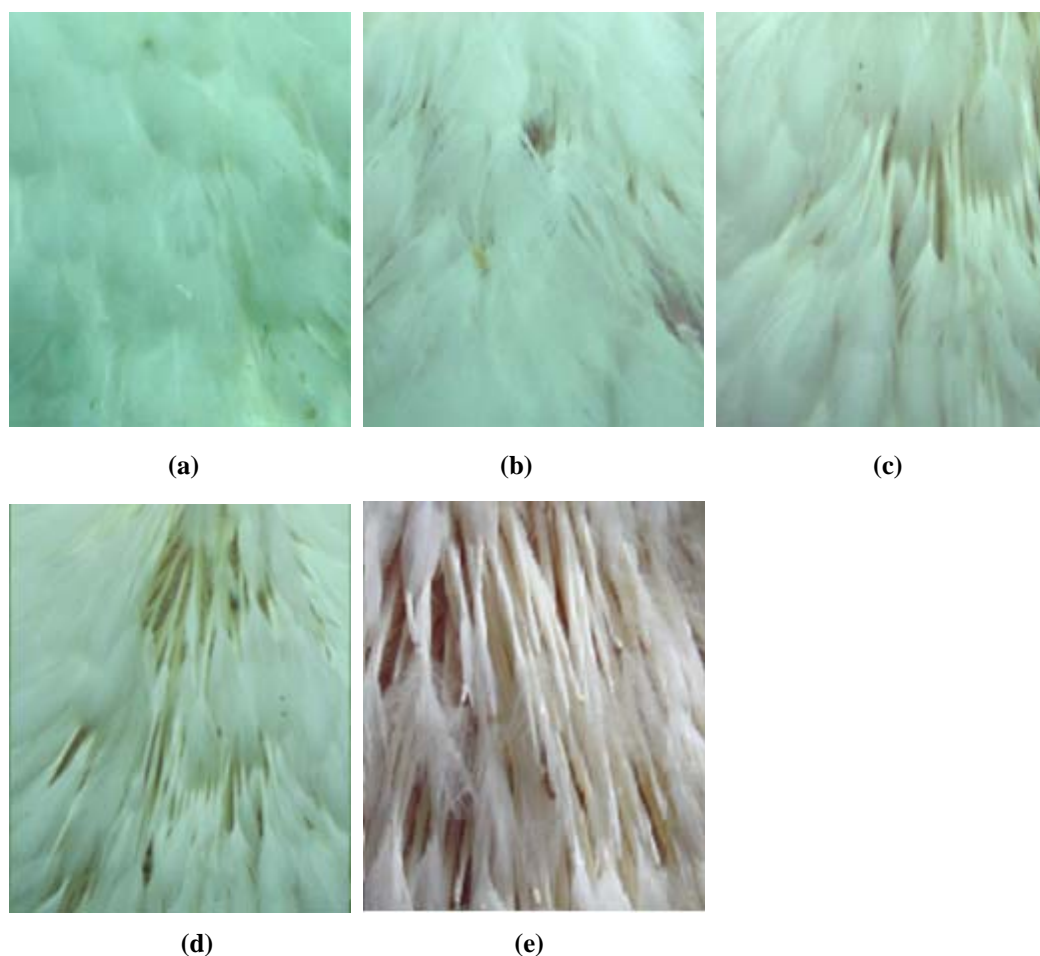
the chambers were monitored with thermometers and recorded four times daily.

### Feathering score

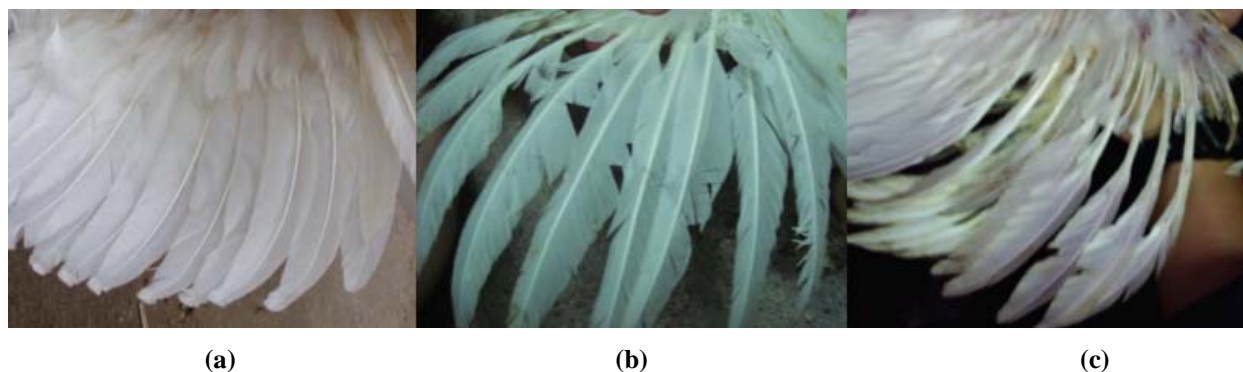
At the end of the experiment, all the birds were given feathering scores for back, breast, wing, under-wing and tail using scores of 1 to 5 for feather coverage with 1 representing minimal coverage, i.e. <25% coverage; 2 for 25-50% coverage; 3 for 50-75% coverage; 4 for >75% coverage and 5 for complete coverage (Figure 1a-e). The wing and tail were further evaluated on a scale of 0 to 2 for the occurrence and severity of poor feathering with 0 indicating no defect; 1 for lesion and torn feathers, and 2 indicating blisters had developed on the shaft, near failure of feather to emerge from the follicle, broken feathers and retarded feathering (Figure 2a-c).

### Feather mineral concentrations

Six birds per treatment were randomly selected and euthanized by stunning and then severing the jugular vein. Samples of feathers were collected, washed with deionized water and dry-ashed in porcelain crucibles at 560°C for 6 h.



**Figures 1.** Feathering score of broilers, (a) Score 5, complete coverage; (b) Score 4, >75% coverage; (c) Score 3, 50-75% coverage; (d) Score 2, 25-50% coverage and (e) Score 1, <25%, minimal coverage.



**Figures 2.** Feathering defect score, (a) Score 0, no feather defects; (b) Score 1, lesion and torn feathers and (c) Score 2, blisters had developed on the shaft, near failure of feather to emerge from the follicle, broken feathers and retarded feathering.

The ashes were then dissolved in hot dilute nitric acid and hydrochloric acid, filtered and brought to 100 ml with deionized water. Zinc, iron (Fe), copper (Cu), manganese (Mn), magnesium (Mg), potassium (K) and Ca were determined by Polarized Zeeman Atomic Absorption Spectrophotometer (Z-5000, Hitachi Instruments, Inc. USA). Phosphorus (P) was quantified by the calorimetrically vanamolybdate procedure (AOAC, 1975) using a spectrophotometer (U-2001, Hitachi Instruments, Inc. USA).

#### Statistical analysis

The experimental data were analyzed using the general linear model procedure of SAS software (SAS, 1996). Differences among treatments means were determined using Duncan's New Multiple Range Test at  $p < 0.05$  significant level.

## RESULTS AND DISCUSSION

#### Growth performance

The dietary treatments and ambient temperature significantly influenced broiler growth performance during the entire 6-wk study (Table 3). During the entire experimental period (wk 1-6), ADFI and ADG were affected significantly ( $p < 0.05$ ) by Zn (diet)  $\times$  temperature effect. Under high temperature condition, broilers fed non-Zn supplementation, 0.8% Ca (Diet 1) had less ADFI (98 vs. 112 g/bird) and ADG (56 vs. 64 g/bird) than birds in the low temperature condition. When the birds were supplemented with Zn (Diets 2 and 3), there were no significant differences in ADFI and ADG of birds kept under both temperature conditions. These observations indicated that Zn supplementation ameliorated the adverse effect of high ambient temperature on the growth of the birds and higher Zn requirement was necessary for broilers kept under higher temperature condition to compensate for the loss of production performance.

The dietary Zn level (44 mg/kg) in the basal diet (Table

1) of the present study was in accordance with the NRC (1994) recommendation for the entire growing phase of broilers. Under low temperature condition, the ADFI of birds fed Zn-unsupplemented basal diet (Diet 1) was significantly higher than 40 mg/kg Zn-supplemented birds (Diet 2), but was not significantly different from 60 mg/kg Zn-supplemented birds (Diet 3). However, when the birds were kept in low temperature condition, there were no significant differences in the ADG of the birds fed Diets 1, 2 and 3, indicating that NRC (1994) recommendation of 40 mg/kg Zn was sufficient for the birds to obtain optimum ADG and dietary Zn concentration exceeding NRC (1994) recommendation did not further improve the ADG of the birds.

For broilers fed Diet 4 (non-Zn supplementation, 1.6% Ca), low temperature condition ameliorated the detrimental effect of high Ca on ADFI (99 vs. 92 g/bird) but not on ADG. Under low temperature condition and non-Zn supplementation, broilers fed Diet 4 (high Ca level, 1.6%) had reduced ADFI and ADG when compared to those fed Diet 1 (low Ca level, 0.8%). However, under high temperature condition, there were no significant differences in ADFI and ADG between broilers fed Diet 1 and Diet 4. This suggested that the detrimental effect of excess Ca on growth was not prominent and was masked in birds kept in high temperature condition.

Diet  $\times$  temperature effect was not significant ( $p > 0.05$ ) on the FCR of the birds in the current study. Broilers fed 40 mg/kg Zn-supplemented ration (Diet 2) had significantly better ( $p < 0.001$ ) FCR during the entire experimental period (Table 3, Diet main effect). The findings in this study are contrary to that of Bartlett and Smith (2003) who found no significant differences in the FCR, intake and weight gain of 7 wk-old broilers fed 33, 68 and 181 mg/kg Zn diets under thermoneutral and heat-stressed conditions.

Excess dietary Ca aggravates Zn deficiency (O'Dell et al., 1964) and the Ca effect is dependant on the phytate level present in the diet (Bafundo et al., 1984a). When Ca level is in excess, practical corn-soybean meal ration

**Table 3.** Effects of dietary Zn levels and environmental temperatures on the growth performance of broilers<sup>1</sup>

Diet <sup>2</sup>	Temperature	ADFI (g/bird) <sup>3</sup>			ADG (g/bird) <sup>3</sup>			FCR <sup>3</sup>		
		Wk 1-3	Wk 4-6	Wk 1-6	Wk 1-3	Wk 4-6	Wk 1-6	Wk 1-3	Wk 4-6	Wk 1-6
1	High	59	137	98 <sup>de</sup>	39	73	56 <sup>c</sup>	1.48	1.86	1.67
	Low	62	162	112 <sup>a</sup>	41	87	64 <sup>a</sup>	1.52	1.85	1.68
2	High	60	140	100 <sup>cd</sup>	40	82	61 <sup>ab</sup>	1.48	1.71	1.59
	Low	59	149	104 <sup>bc</sup>	38	88	63 <sup>a</sup>	1.54	1.68	1.61
3	High	64	148	106 <sup>bc</sup>	43	80	61 <sup>ab</sup>	1.5	1.84	1.67
	Low	64	152	108 <sup>ab</sup>	41	83	62 <sup>a</sup>	1.56	1.84	1.7
4	High	53	132	92 <sup>e</sup>	29	80	55 <sup>c</sup>	1.79	1.63	1.71
	Low	57	142	99 <sup>cd</sup>	32	83	58 <sup>bc</sup>	1.76	1.69	1.73
SE		1.05	3.53	2.02	1.11	2.04	1.27	0.04	0.02	0.02
Diet										
1		60 <sup>b</sup>	149	105 <sup>ab</sup>	40 <sup>ab</sup>	80	60 <sup>a</sup>	1.50 <sup>b</sup>	1.86 <sup>a</sup>	1.68 <sup>a</sup>
2		59 <sup>b</sup>	144	102 <sup>b</sup>	39 <sup>b</sup>	85	62 <sup>a</sup>	1.51 <sup>b</sup>	1.69 <sup>b</sup>	1.60 <sup>b</sup>
3		64 <sup>a</sup>	150	107 <sup>a</sup>	42 <sup>a</sup>	81	62 <sup>a</sup>	1.53 <sup>b</sup>	1.84 <sup>a</sup>	1.68 <sup>a</sup>
4		55 <sup>c</sup>	137	96 <sup>c</sup>	30 <sup>c</sup>	82	56 <sup>b</sup>	1.77 <sup>a</sup>	1.66 <sup>b</sup>	1.72 <sup>a</sup>
SE		0.74	2.49	1.42	0.79	1.44	0.9	0.03	0.01	0.02
Temperature										
High		59 <sup>b</sup>	139 <sup>b</sup>	99 <sup>b</sup>	38	79 <sup>b</sup>	58 <sup>b</sup>	1.56	1.76	1.66
Low		60 <sup>a</sup>	151 <sup>a</sup>	106 <sup>a</sup>	38	85 <sup>a</sup>	62 <sup>a</sup>	1.6	1.76	1.68
SE		0.52	1.76	1.01	0.56	1.02	0.64	0.02	0.01	0.01
Source of variation										
		----- Probability -----								
Diet		p<0.001	ns	p<0.001	p<0.001	ns	p<0.001	p<0.001	p<0.001	p<0.001
Temperature		p<0.05	p<0.001	p<0.001	ns	p<0.001	p<0.001	ns	ns	ns
Diet×temperature		ns	ns	p<0.05	ns	ns	p<0.05	ns	ns	ns

<sup>a-e</sup> Means with different superscripts in the same column differ significantly, p<0.05; ns: not significant.

<sup>1</sup> Each value represents the mean of 4 pens with 8 chicks per pen.

<sup>2</sup> Diet 1: no Zn supplementation, Diet 2: 40 mg/kg Zn supplementation, Diet 3: 60 mg/kg Zn supplementation (Ca supplementation of 0.8% for Diets 1-3), Diet 4: no Zn supplementation but high Ca supplementation (1.6%).

<sup>3</sup> ADFI = Average daily feed intake; ADG = Average daily gain; FCR = Feed conversion ratio.

contains sufficient amount of natural phytate to hinder Zn utilization and, consequently, impairs growth performance (Bafundo et al., 1984a). In the present study, comparing the growth of broilers fed Diet 1 (0.8% Ca) and Diet 4 (1.6% Ca), the impact of high Ca level was significant (p<0.001) at the starter stage, where ADFI (60 vs. 55 g/bird), ADG (40 vs. 30 g/bird) and FCR (1.50 vs. 1.77) were impaired (p<0.001).

When the birds were exposed to higher temperature in this study, there was a significant reduction in ADFI (1.7% and 7.9% during starter and finishing stage, respectively) when compared to birds in lower temperature condition (Table 3, temperature main effect). It was also noted that the ADG during the starter period was not affected by temperature treatments but at the finisher stage, broilers kept under high temperature showed 7.1% less (p<0.001) ADG than those under low temperature. During the first few weeks post-hatching, it is essential to provide supplemental heat for the chicks to maintain constant body temperature for normal growth and development (Ojano-Dirain and Waldroup, 2002). In contrast, as the birds grow

or as the weight increases, they are more susceptible to heat stress (Reece et al., 1972) owing to the development of insulating feathers and higher metabolic heat production. Although the birds in the present study were not exposed to chronic heat stress as demonstrated in other studies (Geraert et al., 1996; Sahin and Kucuk, 2003), the results obtained are consistent with the general trend observed in heat-stressed broilers. In certain conditions, although ambient temperature may be slightly higher than the comfort zone without compromising the birds' welfare and survival, impaired production performance is still observable. The 4°C increment in temperature, which was sufficient to significantly impair the growth rate, however, did not significantly affect (p>0.05) the FCR at the finisher stage.

#### Feathering score

Clinical manifestations of Zn deficiency were noted even when dietary Zn appeared to be sufficient or at levels higher than the recommendations as observed by Sunde (1972), and in some cases might be due to dietary antagonists and interaction with other minerals (Sahin et al.,

2005). Goswami et al. (2005) reported that long before the body Zn storage is depleted, pathological expression of deficiency usually occurs. The signs of mild Zn deficiency are non-specific and often equivocal. Sunde (1972) reported that feather abnormalities and frayed feather symptoms occurred in the primaries and secondaries of Zn-deficient birds. Hence, in the present study, feathering score was introduced to be used as a potential visual aid for the rapid detection of Zn deficiency. Based on our observations, the feathering growth on the back and wing (Figures 1a-e and 2a-c) are feasible for the detection of Zn deficiency.

Effects of dietary Zn and environmental temperature on the feathering scores of broilers are shown in Table 4. The results showed that the effect of Zn (diet)×temperature was significant for all parameters measured for feather coverage score ( $p<0.01$ ) and defect feather score in tail ( $p<0.001$ ). In high temperature, higher feather coverage scores for back and wing were noted from 60 mg/kg Zn-supplemented broilers (Diet 3) as compared to other dietary treatment birds. Except for under-wing, feather coverage scores of the birds fed Diet 1 and Diet 2 kept in high temperature did not

differ significantly. Under high ambient temperature, birds fed 40 and 60 mg/kg supplemental Zn (Diets 2 and 3) had lower occurrence and severity of tail defect feathers as compared to Zn-unsupplemented (Diet 1) birds at 0.8% dietary Ca. This indicated that supplemental Zn ameliorated the deleterious effect of high temperature on the tail defect feathering in broilers.

Under low ambient temperature, no significant differences were found in feather coverage score for the back, breast, wing, under-wing and tail defect score of birds in Diets 1 and 3. However, under similar environmental condition, birds in the above two diets had significantly higher feather coverage score determined for back, breast and wing than those in Diet 2. The above results seem to suggest that the basal Zn content (44 mg/kg) in Diet 1 is sufficient for proper feathering for birds under low temperature condition. No explanation could be provided at this moment to explain for the poorer coverage for birds in Diet 2 (supplemented with 40 mg/kg Zn) as compared to their counterparts in Diet 1 (with no Zn supplementation).

When the birds were kept in low temperature and fed

**Table 4.** Effects of dietary Zn levels and environmental temperatures on feathering score of broilers<sup>1</sup>

Diet <sup>2</sup>	Temperature	Coverage score					Defect score	
		Back	Breast	Wing	Under-wing	Tail	Wing	Tail
1	High	3.07 <sup>e</sup>	3.61 <sup>bcd</sup>	3.26 <sup>ef</sup>	3.80 <sup>d</sup>	2.93 <sup>de</sup>	1.19	0.87 <sup>a</sup>
	Low	4.43 <sup>a</sup>	4.39 <sup>a</sup>	4.20 <sup>a</sup>	4.71 <sup>a</sup>	4.25 <sup>a</sup>	1.18	0.45 <sup>d</sup>
2	High	3.31 <sup>de</sup>	3.46 <sup>cd</sup>	3.46 <sup>de</sup>	4.19 <sup>c</sup>	3.19 <sup>cd</sup>	1.06	0.63 <sup>bcd</sup>
	Low	3.89 <sup>b</sup>	3.64 <sup>bc</sup>	3.70 <sup>dc</sup>	4.50 <sup>ab</sup>	3.95 <sup>b</sup>	1.05	0.50 <sup>d</sup>
3	High	3.61 <sup>c</sup>	3.85 <sup>b</sup>	3.81 <sup>bc</sup>	4.22 <sup>bc</sup>	3.44 <sup>c</sup>	1.00	0.50 <sup>d</sup>
	Low	4.44 <sup>a</sup>	4.13 <sup>a</sup>	4.00 <sup>ab</sup>	4.65 <sup>a</sup>	3.78 <sup>b</sup>	0.89	0.54 <sup>cd</sup>
4	High	3.17 <sup>e</sup>	2.76 <sup>e</sup>	3.00 <sup>f</sup>	2.93 <sup>f</sup>	2.57 <sup>f</sup>	0.98	0.72 <sup>abc</sup>
	Low	3.54 <sup>cd</sup>	3.34 <sup>d</sup>	3.20 <sup>f</sup>	3.27 <sup>e</sup>	2.79 <sup>ef</sup>	0.96	0.79 <sup>ab</sup>
SE		0.10	0.10	0.09	0.10	0.09	0.09	0.07
Diet								
1		3.76 <sup>b</sup>	4.01 <sup>a</sup>	3.74 <sup>ab</sup>	4.26 <sup>a</sup>	3.60 <sup>a</sup>	1.18 <sup>a</sup>	0.65 <sup>ab</sup>
2		3.61 <sup>b</sup>	3.55 <sup>b</sup>	3.58 <sup>b</sup>	4.35 <sup>a</sup>	3.57 <sup>a</sup>	1.05 <sup>ab</sup>	0.56 <sup>b</sup>
3		4.03 <sup>a</sup>	3.99 <sup>a</sup>	3.91 <sup>a</sup>	4.44 <sup>a</sup>	3.61 <sup>a</sup>	0.94 <sup>b</sup>	0.52 <sup>b</sup>
4		3.35 <sup>c</sup>	3.05 <sup>c</sup>	3.10 <sup>c</sup>	3.10 <sup>b</sup>	2.68 <sup>b</sup>	0.97 <sup>b</sup>	0.75 <sup>a</sup>
SE		0.07	0.07	0.06	0.07	0.07	0.06	0.05
Temperature								
High		3.29 <sup>b</sup>	3.42 <sup>b</sup>	3.38 <sup>b</sup>	3.78 <sup>b</sup>	3.03 <sup>b</sup>	1.06	0.68 <sup>a</sup>
Low		4.07 <sup>a</sup>	3.87 <sup>a</sup>	3.77 <sup>a</sup>	4.28 <sup>a</sup>	3.69 <sup>a</sup>	1.02	0.57 <sup>b</sup>
SE		0.05	0.05	0.05	0.05	0.05	0.04	0.04
Source of variation		Probability						
Diet		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.05	p<0.01
Temperature		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	ns	p<0.05
Diet×temperature		p<0.001	p<0.01	p<0.001	p<0.01	p<0.001	ns	p<0.001

<sup>a-f</sup> Means with different superscripts in the same column differ significantly,  $p<0.05$ ; ns: not significant.

<sup>1</sup> Each value represents the mean of 4 pens with 8 chicks per pen.

<sup>2</sup> Diet 1: no Zn supplementation, Diet 2: 40 mg/kg Zn supplementation, Diet 3: 60 mg/kg Zn supplementation (Ca supplementation of 0.8% for Diets 1-3), Diet 4: no Zn supplementation but high Ca supplementation (1.6%).

Diet 4 (non-supplemental Zn, high Ca inclusion), they had lower feather coverage and higher tail defect score as compared to the other dietary treatment birds. A similar trend was observed in the feather coverage score on the breast, under-wing and tail of broilers reared under high temperature environment. Feathers are mainly composed of keratin (Richards et al., 2007) and this fibrous protein requires Zn for synthesis (Underwood and Suttle, 1999). In the present study, the antagonistic relationship of Zn×Ca which could lead to poor feathering was striking, particularly for birds in Diet 4 in the low temperature environment. Excess Ca inhibits Zn utilization and absorption which might reduce keratin synthesis rates resulting impaired feathering and occurrence of defect feathers.

The insulating properties of feathers might be detrimental to the heat-stressed birds (Geraert et al., 1996). In almost all cases, broilers kept at higher temperature had lower feather coverage score than those at lower temperature, regardless of dietary treatments. Besides the low dietary Zn concentrations or reduced nutrient consumption, which might lead to poor feathering, less feather coverage birds are more capable to withstand high temperature environment owing to their ability to dissipate excess body heat via sensible heat loss.

### Mineral compositions in feathers

Antagonism can occur between one mineral and another (Richards et al., 2007) and may be one- or two-sided (Georgievskii, 1982). The latter author stated that deviations on any biochemical functions due to metabolic processes are only noted if the mutual proportions between the individual elements have been disturbed to a major extent. In the current study, Zn (diet)×temperature interaction was significant ( $p<0.05$ ) for the Ca content in feathers (Table 5). It was expected that Ca retention in feathers of birds under high temperature condition would be lower than those kept in low temperature because of the reduced ADFI observed in Table 3. However, the results showed that birds fed excess dietary Ca (Diet 4) under high temperature condition had significantly higher concentration of Ca in the feathers than those under low temperature condition. This might be due to the vasodilation of blood vessels which increases the flow of nutrients to the follicles (Montagna and Ellis, 1958) or hormonal changes that occur (Wysocki and Klett, 1971) during high temperature conditions that cause increased feather Ca content.

Feather P ( $p<0.01$ ), K and Mn ( $p<0.05$ ) concentrations were affected by the dietary treatments (Table 5). Broilers fed non-supplemented Zn ration (Diet 1) had significantly higher feather P and K concentrations (by 21.1% and 28%,

**Table 5.** Effects of dietary Zn levels and environmental temperatures on feather mineral concentrations of broilers<sup>1</sup>

Diet <sup>2</sup>	Temperature	P (%)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	Mn (mg/kg)
1	High	0.25	176.14	16.45	58.83	329.84 <sup>c</sup>	265.87	1056.92	4.99
	Low	0.22	169.59	14.80	72.34	295.29 <sup>c</sup>	226.11	1147.21	3.99
2	High	0.22	184.48	14.96	53.02	290.80 <sup>c</sup>	245.98	967.35	4.99
	Low	0.21	172.87	14.80	71.14	295.26 <sup>c</sup>	229.38	877.38	4.33
3	High	0.20	179.69	13.14	61.57	326.18 <sup>c</sup>	232.90	896.88	4.66
	Low	0.19	172.77	14.12	88.54	346.30 <sup>c</sup>	259.20	825.62	4.82
4	High	0.20	174.68	15.31	86.33	708.68 <sup>a</sup>	276.15	861.75	6.82
	Low	0.20	182.80	14.62	83.43	566.71 <sup>b</sup>	252.59	772.78	4.99
SE		0.01	6.10	0.95	9.36	26.80	14.23	103.11	0.47
Diet									
1		0.23 <sup>a</sup>	172.87	15.62	65.59	312.57 <sup>b</sup>	245.99	1102.1 <sup>a</sup>	4.49 <sup>b</sup>
2		0.22 <sup>ab</sup>	178.67	14.88	62.08	293.03 <sup>b</sup>	237.68	922.4 <sup>ab</sup>	4.66 <sup>b</sup>
3		0.19 <sup>b</sup>	176.23	13.63	75.06	336.24 <sup>b</sup>	246.05	861.2 <sup>b</sup>	4.74 <sup>b</sup>
4		0.20 <sup>b</sup>	178.74	14.96	84.88	637.69 <sup>a</sup>	264.37	817.3 <sup>b</sup>	5.90 <sup>a</sup>
SE		0.01	4.31	0.67	6.62	18.95	10.07	72.91	0.33
Temperature									
High		0.22	178.75	14.96	64.94 <sup>b</sup>	413.87	255.23	945.73	5.36 <sup>a</sup>
Low		0.20	174.51	14.58	78.86 <sup>a</sup>	375.89	241.82	905.75	4.53 <sup>b</sup>
SE		0.01	3.05	0.47	4.68	13.40	7.12	51.56	0.23
Source of variation						Probability			
Diet		$p<0.01$	ns	ns	ns	$p<0.001$	ns	$p<0.05$	$p<0.05$
Temperature		ns	ns	ns	$p<0.05$	ns	ns	ns	$p<0.05$
Diet×temperature		ns	ns	ns	ns	$p<0.05$	ns	ns	ns

<sup>a-c</sup> Means with different superscripts in the same column differ significantly,  $p<0.05$ ; ns: not significant.

<sup>1</sup> Each value represents the mean of 6 chicks per treatment.

<sup>2</sup> Diet 1: no Zn supplementation, Diet 2: 40 mg/kg Zn supplementation, Diet 3: 60 mg/kg Zn supplementation (Ca supplementation of 0.8% for Diets 1-3), Diet 4: no Zn supplementation but high Ca supplementation (1.6%).

respectively) than those fed 60 mg/kg supplemental Zn (Diet 3). It is not surprising that Zn supplementation led to reductions of P concentration in feathers as it has been reported that Zn and P inhibit the absorption of each other (Georgievskii, 1982). Potassium inhibits the absorption of Zn (Georgievskii, 1982), but the reverse is not documented.

In the current study, broilers fed a high dietary Ca level in Diet 4 had 13% and 25.8% reduction in feather P and K concentrations, respectively, when compared to those fed low dietary Ca level in Diet 1. Similarly, a study using rats by Forbes (1964) showed that increasing dietary Ca decreased P absorption by 13%. The reason for the reduction in feather K in the high Ca treatment (Diet 4) as compare to low Ca treatment (Diet 1) is unknown. No metabolic interaction between Ca and K was found in the literature. It seems as though the reduced feather K content observed in the present study was a secondary effect resulted from the interaction of Ca with other dietary components or elements. Feather Mn concentration was not affected by increasing dietary Zn levels. However, when no Zn was supplemented but high dietary Ca (Diet 4) was given, feather Mn content increased ( $p < 0.05$ ) by 31.4% when compared to low dietary Ca supplementation (Diet 1). Ji et al. (2006) observed a stimulatory effect of high Ca on Mn uptake in the intestine of broilers. This might be the reason for the higher Mn retention in the feathers of birds fed high Ca ration (Diet 4).

Feather Zn concentration was not affected ( $p > 0.05$ ) by the dietary treatments. Inconsistent results on the Zn status and hair Zn content have been reported in the literature. According to McDowell (2003), hair Zn may or may not decrease during Zn deprivation. In agreement with the present study, Shan (1990) found no significant differences in feather Zn contents of layers when 0, 10 and 40 mg/kg Zn, 0.88% vs. 1.78% Ca, were supplemented to a corn-soybean meal basal diet which contained 29 mg/kg Zn. In contrast, a reduction of hair Zn content was noted in pigs fed corn-soybean meal diet without supplemental Zn when compared to the control group fed normal Zn diet (Hsia and Chang, 2006). In rats, Zn consumption determined the Zn concentration in hair (Reinhold et al., 1968). Hair Zn content depend not only on the Zn incorporated into the roots, but also on hair growing rate; Zn deficiency impair hair growth, and actually increased hair Zn content (Fairweather-Tait, 1988). Therefore, hair or feather Zn may reflect dietary Zn intake, but they do not necessarily reflect Zn nutrition status as measured by production performance and feather growth.

Feather Cu and Fe levels were not affected ( $p > 0.05$ ) by the dietary treatments in the present study. However, other workers found reduction in the availability of Cu (Ritchie et al., 1963) and Fe (Bafundo et al., 1984b) when excess Zn was added. The Zn levels in the diets in this study might not

be high enough to create an adverse impact on the concentrations of Cu and Fe in feathers. Wauben and Atkinson (1999) have reported no differences in Fe uptake from piglets fed high and normal Ca due to adaptation to high Ca feed. In the current study, the birds were fed experimental diets for 42 d, an adaptive response to the antagonism of Ca on Fe may present as the feather Fe concentration was not affected by dietary Ca levels.

Ambient temperature effect was significant ( $p < 0.05$ ) on the Fe and Mn contents of the feathers. Broilers kept at higher temperature had lower Fe and higher Mn concentrations in the feathers than those kept at lower temperature. It has been indicated that Fe retention decreased in heat-stressed condition (Sahin et al., 2003). O'Mary et al. (1969) conducted hair analysis of Hereford cattle in USA during March and August. They found higher Cu, Mn, Ca, Mg and K, and lower Fe concentration in August samples, but Zn and P contents did not change between seasons. One may assume that the mineral concentrations in feathers may be affected by feed intake regulated by environmental temperature. Combs et al. (1982) stated that factors such as season, breed, age, hair color, body location and contamination affect hair mineral concentrations. Conclusive information on the retention of minerals in feathers is lacking. Hence, factors affecting mineral deposition and the mechanisms of mineral incorporation into feathers should be identified when feathers are used to predict mineral status.

In conclusion, the results of the present study showed that higher Zn requirement was necessary for birds under high temperature condition and supplementation of 40 mg Zn per kg corn-soybean meal diet was sufficient for the optimum growth of the birds. Zinc supplementation ameliorated the adverse effect of high ambient temperature on the growth performance and tail defect feathering of the birds. It appeared that dietary Zn and Ca did alter the Zn metabolism sufficiently to exert a deleterious effect at the growth performance and feathering level. Feathering score could be a useful visual field indicator for Zn status in broilers, particularly in the presence of dietary antagonist when Zn absorption and utilization were inhibited. Feather mineral analysis may be useful when combined with other indicators to provide a more precise assessment of mineral status in poultry.

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