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Investigation of the zinc accumulation in the tissue and performance in broilers raised with high levels of zinc sulfate feed under heat stress

Thawinan WAEOTHONG, Jai Wei LEE, Liang Chou HSIA*
Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, Taiwan, ROC

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Abstract: The objectives of this study were to evaluate the effects of feeding zinc sulfate on the growth performance, Zn accumulation in the tissue, and the tibia quality. First day old of 100 chicks were randomly assigned to 10 experimental groups in a 5 (0 mg/kg Zn, 40 mg/kg ZnO, 40 mg/kg $ZnSO_4.H_2O$, 500 mg/kg $ZnSO_4$, and 1000 mg/kg $ZnSO_4$) × 2 (high and low temperature) factorial arrangement under randomized complete block design (RCBD). Each experimental group was replicated with 10 birds. Birds were kept in individual cage until 42 days. The results showed that broilers receiving the zinc sulfate supplementation throughout the experiment period (weeks 1 - 6) could improve their average daily feed intake (ADFI) and average daily gain (ADG); however, there were no significant differences in the feed conversion ratio (FCR). Under the high ambient temperature, both zinc oxide (40 mg/kg) and zinc sulfate (40 mg/kg) supplementation improves the FCR throughout the experiment period. Furthermore, during weeks 5-6, supplementation of zinc sulfate with the zinc dose of 1000 mg/kg increased the ADG. Comparing the two levels of temperature showed that broilers kept under a low environmental temperature with a zinc supplementation of 1000 mg/kg experienced more adverse effects than broilers kept under a high environmental temperature. In addition, there were no interactions between the diet and temperature on the zinc accumulation in the meat (breast and thigh), feathers, and organs (liver), as well as tibia length and breaking strength. In conclusion, the zinc supplementation of 40 mg/kg in the diet was a suitable dose for broiler, which were reared under high ambient temperatures for long period.

Key words: Zinc, temperatures, growth performance, zinc tissue, tibia quality, broilers

1. Introduction

Like many other aspects of agriculture, animal husbandry also has various issues to resolve one of which is heat stress due to the increase in the environmental temperature. Broilers are sensitive to heat stress as they do not have sweat glands to lower their body temperature. Although they have feathers covering their body for insulation, the feathers also inhibit heat loss during high temperature periods. Moreover, panting is one mechanism that the birds use to cool themselves, but it is not effective. In a situation where the external environmental temperature is higher than that of the broilers' internal body, this induces the extreme evaporation of water from their respiratory organs. If this is not resolved, it could become critical for the broilers. Hence, when they could not control the heat loss, this would result in the reduction of egg production, egg weight, feed intake, poor body weight, and potential mortality [1-3]. Another consequence of the adverse impact from of heat stress on chickens is having to lower the humoral immune response levels and reducing the immune organs' weight such as in the thymus, bursa, and spleen. Additionally, when broilers experience this critical condition, numerous minerals are excreted, which may result in a deficiency of minerals. Subsequently, broilers require optimal amounts of minerals to maintain their health and to recover from the consequences from of heat stress.

Zinc (Zn) is not only involved in various important bodily functions, such as protein synthesis, hormone mediation, and immune development, but it also has a role as an antioxidant and participates as an antioxidant defense system [4,5]. Resistance to heat stress has also been found in chickens and quails [6]. Furthermore, there are two types of Zn: Zn-organic and Zn-inorganic. More bioavailable properties have been found in Zn-organic sources [7]; nevertheless, the organic trace mineral is based in the inorganic source. However, it is still difficult to distinguish the effect of the mineral supplements from

* Correspondence: lchsia@mail.npust.edu.tw

the natural minerals in the diet; thus, Zn-inorganic, which is classified into two types: Zn-oxide and Zn-sulfate, has been commonly used in farm animals up to the present time. A very high level of pharmacological level of Zn-oxide has been used on pig farms due to its efficiency as a growth promoter. Zn-oxide not only increases the daily gain and feed intake but it is also used for the prevention of bacteria, such as E. coli, resulting in a reduced mortality rate in young pigs [8]. Compared to the utilization and mineral absorption in the bones, Zn-sulfate also provides more bioavailability than Zn-Oxide [9].

In terms of Zn utilization in broilers, many factors are causing antagonistic interference between the minerals, or the antagonistic mineral with phytate or phytic acid, fiber, and other chelating agents contained in basal corn and soybean meal. These reasons have led to inadequate mineral requirements for broiler growth and resistance to pathogenesis when reared under ambient temperature. Therefore, chicks may require a lot of minerals to dissipate their heat and better strengthen their body, especially those that are bred under new modern methods that have a rapid growth rate and induce the severity of various parts of the skeletal structure, such as leg weakness, fragile bones, and tibia dyschondroplasia. Therefore, to resolve these issues in broiler breeding, the objective of this experiment was to examine the effects of high dosages of Zn sulfate supplementation under different environmental conditions on broilers' performance, the Zn accumulation in different tissues, and bone quality.

2. Materials and methods

2.1. Chicks, treatment, and temperatures

This study was performed in collaboration with the Department of Tropical Agriculture and International Cooperation (DTAIC), National Pingtung University of Science and Technology, Taiwan. One hundred female day-old broiler chickens were divided into two groups of high and low environmental temperatures. Each group was randomly assigned to five dietary treatments with 10 replications. The chicks were maintained on a constant 24-h lighting regimen and allowed an ad libitum diet of feed and water. Individual chicks were kept in pens under the controlled temperature chamber for up to 42 days. In the first week, all chicks were maintained under 30 °C. In the second and third weeks, the chicks kept in low temperature groups had their ambient temperature reduced by two degrees. For the fourth to sixth weeks, the temperature was kept constant at 24 °C. The temperature differential with the high's temperature group was maintained at 30 °C until the end of the experiment. The data were individually recorded daily on the feed intake and weekly for the bodyweight gain.

2.2. Composition of the diets

The corn-soybean basal diets (Table 1) were formulated to meet or exceed the requirements for the initial and growth stages (*National Research Council*, 1994) except for the difference of Zn sulfate. The levels of Zn added in the diet were 0 (Diet 1), 40 mg/kg Zn oxide (ZnO) (Diet 2), 40 mg/kg Zn sulfate ($ZnSO_4.H_2O$ feed grade) (Diet 3), 500 mg/kg $ZnSO_4$ (Diet 4), and 1000 mg/kg $ZnSO_4$ feed (Diet 5).

2.3. Tibia length and breaking strength

The intact right tibia from each broiler was extracted and cleaned manually to remove the residual muscles. The tibia length was determined using a digital caliper. Following determining the tibia breaking strength, testing was conducted by using a Gotech, KT-7010-A2 machine, at a speed of 50 mm/min. The tibia breaking strength was defined as the amount of force (kg) applied to the center of the tibia (diaphysis) that was required to break the bone. The ultimate breaking point (kg/cm^2) was recorded, and the broken tibias were then stored at a temperature of -20 °C to determine the dry matter ash and mineral concentration.

2.4. Mineral concentration analysis in the tibia, feathers, and soft tissue

Post slaughter feathers, bones, dumpstick, breast meat, spleen, and liver were washed with deionized water. The fat content of the breast meat, dumpstick meat, and liver was removed, and the samples were cleaned again with deionized water. The feathers were cleaned with deionized water, incubated at 60 °C for four days followed by spinning. The tibias were boiled in deionized water for five minutes, and any residual flesh was removed retaining only the epiphyseal cartilages. Then, the tibias were extracted in petroleum ether for 72 h, incubated for 12 h at 105 °C, and finally spun into small pieces.

All the samples were determined as dry matter and ash by being placed into 60 °C for 2 h, 105 °C for 2 h, and 560 °C for 6 h, respectively. The weight of the ash samples was recorded and used for the calculation of the total mineral content. The ash was then digested with HNO_3 [10] until about 5 mL of solution was left inside a glass. Then, it was allowed to cool down, filtered, and increased to 100 mL with deionized water. Finally, all the samples were analyzed for the Zn accumulation in different tissues by using a polarized Zeeman atomic absorption spectrophotometer (Z-5000, Hitachi Instruments, Inc., USA).

2.5. Statistical analysis

The data of the growth performance, zinc in the tissues, and tibia quality were analyzed by using the general linear model (GLM) procedure of SAS 14 version 6.12 software [1] with the main effects being the environment, zinc source, and zinc level. Post hoc Duncan's new multiple range test was used with a level of significance set at $p < 0.05$ and $p < 001$, respectively.

[1] SAS Institute. SAS/STAT user's guide. Version 6.12. Cary, North Carolina, USA: SAS Institute Inc.; 1996.

Table 1. Composition of the dietary treatments.

Ingredient Percent (%)	Diet1	Diet 2, 3	Diet 4	Diet 5
Corn	60.4	60.39	60.26	60.11
Soybean meal	32.7	32.7	32.7	32.7
Soybean oil	3	3	3	3
Dicalcium phosphate	1.5	1.5	1.5	1.5
Limestone	1.2	1.2	1.2	1.2
Methionine	0.4	0.4	0.4	0.4
Common salt	0.3	0.3	0.3	0.3
Vitamin premix[1]	0.15	0.15	0.15	0.15
Trace mineral mix[2]	0.35	0.36	0.49	0.64
Nutrient composition				
CP (%)	20.2	20.2	20.2	20.2
Ca (%)	0.92	0.92	0.92	0.92
P (%)	0.35	0.35	0.35	0.35
Zn(mg/kg)	0	40	500	1,000

[1]Ingredients supplied per kilogram of the diet: vitamin A: 10,000,000 IU; vitamin D_3: 1,000,000 IU; vitamin E: 20,000 IU; vitamin B_1: 2,000 mg, vitamin B_2: 4,000 mg; B_6: 3,000 mg; vitamin B_{12}: 15 mg; niacinamide: 35,000 mg; vitamin K_3: 15,000 mg; D-calcium pantothenate: 15,000 mg; biotin: 50 mg; folacin: 1,000 mg; Cu ($CuSO_4.5H_2O$): 8 mg; Mg ($MgSO_4$): 600 mg; Na (Na_2SeO_3): 0.l5 mg; Mn ($MnSO_4.H_2O$): 60 mg; Fe ($FeSO_4.H_2O$): 80 mg; KI: 0.35.

3. Results

3.1. Growth performance

Table 2 presents the effects of the high and low levels of the Zn supplements of the basal diet on growth performance in broilers. The data show that during weeks 1–2 and 3–4, all supplementations of the Zn levels indicated a significant difference in the average daily feed intake (ADFI) ($p < 0.001$; $p < 0.05$). In the final period (weeks 5–6), 40 mg/kg Zn-sulfate, the broilers consumed more feed than other treatments; however, there were no significant differences ($p > 0.05$). Also, we observed that broilers consumed less feed when given 1000 mg/kg Zn-sulfate compared to other treatments. Throughout the experiment period, broilers fed with 40 mg/kg Zn sulfate had the greatest feed intake, which was followed by birds fed with 40 mg/kg Zn oxide and 500 mg/kg Zn oxide, respectively ($p < 0.01$). In addition, the ones in 1000 mg/kg Zn diet had ADFI better than nonsupplement group (Diet 1).

During the weeks 1-2 and 3-4, the average daily gain (ADG) for all supplement groups had a significantly higher than the nonsupplement group ($p < 0.001$, $p < 0.05$). A decrease in the ADG was observed during weeks 5-6 when the feed was supplemented with high Zn sulfate (1000 mg/kg). Through all the experiment period (week 1-6), a diet with Zn sulfate at the NRC levels (40 mg/kg) had a significantly higher ADG ($p < 0.05$). The feed conversion ratio (FCR) during weeks 1-4 showed no significant difference ($p > 0.05$). On the other hand, in weeks 5-6, an improvement of the FCR was found in broilers fed with 40 mg/kg Zn sulfate, 40 mg/kg Zn-oxide and 500 mg/kg, respectively ($p < 0.05$). Week 1-6, all dietary treatments were not found to have any significant differences ($p > 0.05$).

The effects of the temperature on broiler growth performance are shown in Table 3. The data showed that a high ambient temperature caused a significant decrease in the feed intake during weeks 1-2 and 5-6, as well as throughout the entire period of the experiment ($p < 0.001$). There were also effects of body gain during the weeks 1-2, 3-4, and 5-6 as well as during the period of the experiment ($p < 0.01$, $p < 0.05$, $p < 0.001$ and $P < 0.001$, respectively). The FCR was also significantly improved in weeks 1-2, 3-4, and 5-6 ($p < 0.001$, $p < 0.05$, and $p < 0.05$, respectively). However, throughout the entire period of the experiment, broilers reared under high ambient temperatures showed no improvement in the FCR ($p < 0.05$). Nonetheless, significant interactions between the dietary treatment and temperatures (diet × temp) were found in the ADG in weeks 5-6 ($p < 0.05$), FCR in weeks 5-6 ($p < 0.05$), and FCR in weeks 1-6 ($p < 0.05$) (Figure 1).

3.2. Accumulation of Zn in the bone, feathers, and tissues

Zn accumulation is shown in Table 4. It can be seen that the Zn uptake by the bones influenced with-on all supplementary Zn levels ($p < 0.001$). There was a

Table 2. The effect of zinc addition as oxide or sulfate on growth performance of broilers.

Performance	Period (week)	Diet (mg/kg)					SE	Sig.
		0	40-ZnO	40-$ZnSO_4$	500-$ZnSO_4$	1000-$ZnSO_4$		
ADFI (g/bird)	1-2	29.23^{b}	35.35^{a}	35.66^{a}	34.34^{a}	35.84^{a}	2.71	***
	3-4	79.57^{b}	86.92^{a}	89.91^{a}	87.09^{a}	87.75^{a}	3.91	*
	5-6	117.32	121.04	125.34	117.38	113.81	4.49	NS
	1-6	76.37^{c}	81.11^{ab}	83.67^{a}	79.60^{abc}	78.97^{bc}	2.76	**
ADG (g/bird)	1-2	20.78^{b}	24.85^{a}	25.90^{a}	25.18^{a}	25.68^{a}	2.11	**
	3-4	51.62^{b}	58.84^{a}	59.13^{a}	56.78^{ab}	58.10^{a}	3.09	*
	5-6	57.54^{bc}	61.50^{ab}	65.18^{a}	59.54^{ab}	52.55^{c}	4.78	**
	1-6	44.26^{b}	48.40^{ab}	50.07^{a}	47.16^{ab}	45.44^{b}	2.36	**
FCR	1-2	1.41	1.42	1.38	1.32	1.37	0.03	NS
	3-4	1.66	1.48	1.54	1.57	1.52	0.07	NS
	5-6	2.11^{ab}	1.99^{b}	1.96^{b}	2.01^{b}	2.26^{a}	0.13	*
	1-6	1.68	1.63	1.62	1.65	1.72	0.04	NS

Sig.: significant; ^{a,b} Means within the row without a common superscript difference ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
NS: not significant ($p > 0.05$), ADFI: average daily feed intake (g/bird), ADG: average daily gain (g/bird), FCR: feed conversion ratio.

Table 3. The effect of the ambient temperature on growth performance.

Performance	Period (week)	Temperature		SE	Sig.	Interaction	SE
		Low	High			D×T	
ADFI	1-2	36.06^{a}	32.31^{b}	2.93	***	NS	0.25
	3-4	86.98	85.63	1.07	NS	NS	1.90
	5-6	125.71^{a}	112.04^{b}	9.66	***	NS	6.05
	1-6	86.92^{a}	76.98^{b}	4.19	***	NS	2.01
ADG	1-2	25.19^{a}	23.76^{b}	1.01	**	NS	0.56
	3-4	58.94^{a}	54.91^{b}	2.85	*	NS	2.26
	5-6	65.18^{a}	53.17^{b}	8.49	***	*	6.05
	1-6	49.77^{a}	44.37^{b}	3.82	***	NS	2.20
FCR	1-2	1.34^{b}	1.44^{a}	0.07	***	NS	0.03
	3-4	1.49^{b}	1.61^{a}	0.07	*	NS	0.11
	5-6	1.99^{b}	2.14^{a}	0.10	*	*	0.16
	1-6	1.65	1.68	0.02	NS	*	0.06

Sig.: significant; ^{a,b} Means within the row without common superscript difference ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
NS: not significant ($p > 0.05$), ADFI: Average daily feed intake (g/bird), ADG: average daily gain (g/bird), FCR: feed conversion ratio, D: diet, and T: temperature.

significant difference between the low and high dietary Zn. High accumulation was found in broilers fed with high Zn sulfate (1000 mg/kg). In comparing the two Zn sources at the same level, Zn oxide had lesser bone Zn concentration than Zn sulfate. Also, broilers fed with nonsupplementary Zn had poor retention in the bones. Similarly, with Zn in the feathers, nonsupplementation was found to have the lowest Zn accumulation ($p < 0.001$). There was an adverse

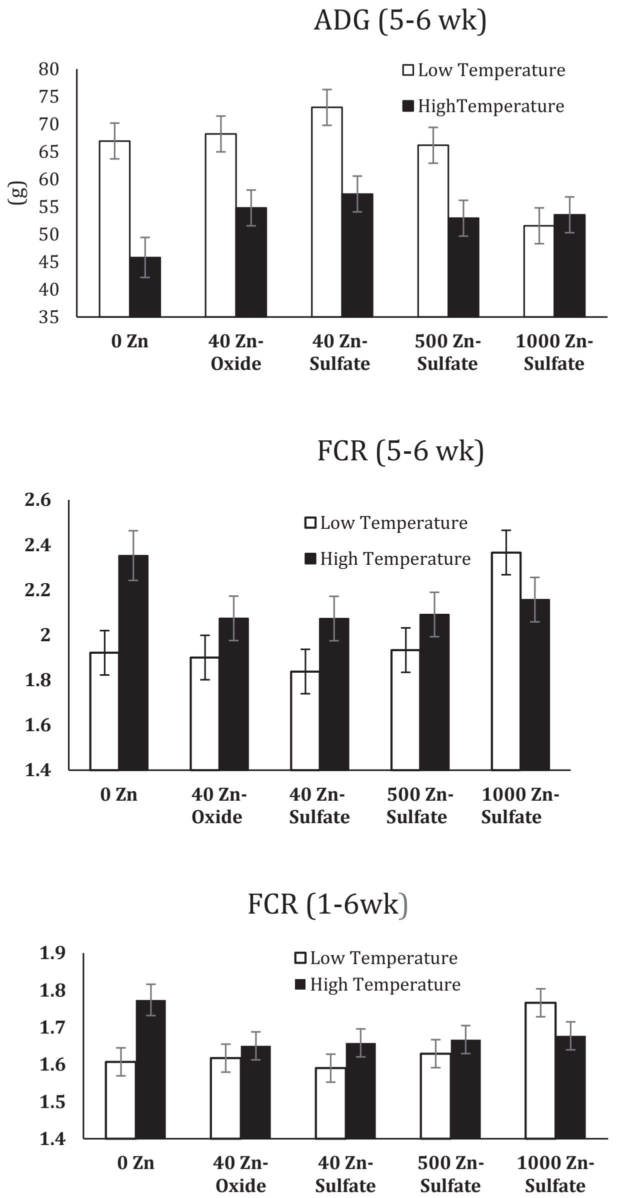

Figure 1. Interaction between the diet and ambient temperature on the average daily gain and feed conversion ratio.

symptom from the deficiency (Figure 2). Broken feather wings and undeveloped tails were found with chicks fed with nonsupplementary Zn (Figure 2A, 2B, 2C, and 2D, respectively). Serious symptoms of feathers were also found in broilers fed with nonsupplementary Zn reared under high ambient temperatures (Figures 2A and 2B) together with the result of Zn in the feathers, which had the lowest Zn accumulation (Table 5). Figures 2C and 2D display the symptoms from broilers fed with nonsupplementary Zn reared under low ambient temperatures. Additionally, Zn in the liver did not show any significant difference ($p > 0.05$). Zn in the thighs had a high accumulation in all supplemented Zn levels ($p < 0.01$). The highest Zn supplemental level (1000 mg/kg) showed a high Zn accumulation in the breast meat ($p < 0.001$). The effects between the high and low ambient temperatures did not show any significant differences in the bones, feathers, liver, thigh, and breast ($p > 0.05$).

3.3. Tibia length, tibia breaking strength, and percentage of the tibia weight

The tibia length was affected by all supplementations of Zn ($p < 0.01$) (Table 4). However, the tibia breaking strength was not found to have any significant difference ($p > 0.05$). The percentage of the tibia weight was significant in the dietary supplementation with all dosages of Zn sulfate ($p < 0.05$). Under the different ambient temperatures, there were no significant differences in the tibia length, tibia breaking strength, and percentage of the tibia weight ($p > 0.05$) (Table 5), as well as the interaction between the diet and temperature.

4. Discussion

4.1 Growth performance

In the present study, it is noteworthy to state that Zn is an essential mineral for broilers' growth, and they cannot lack this mineral from the time they are born. This is because Zn stimulates bone growth by increasing the bone mass, has a relationship with the growth hormone (GH), and is involved with the insulin growth factor - I (IGF - I) [11]. In the gene mechanism, Zn is found in the chromatin structures, DNA replication, and RNA transcription that is beneficial for protein synthesis and cell division in conjunction with increasing the body mass [11]. The trend of increasing the feed intake and body weight was shown in this result, which was found in the broiler diet with high and low Zn levels. This was unlike broilers fed with no Zn supplementation in their diet, which had the lowest performance and showed an adverse symptom in the wing feathers by observation (Figure 2). Lai et al. [12] also reported that Zn deficiency symptoms could be found on the feathers (wing, under the wing, breast, tail, and back). The appearance of a Zn deficiency in the feathers was observed from broken, retarded, or undeveloped feathers and near failure of the feathers to emerge from the follicle. Furthermore, leg abnormalities symptoms, such as shortening and thickening of leg bones [13], enlargement of the hock joint, and scaling of the skin on the feet were observed.

In addition, various previous studies had found the Zn supplementation being over the level of the NRC recommendations, which was a result of a several reasons. First, Zn interfered with other minerals, such as Co, Pb, Cr, Hg, Se, Ca, Cu, P, Fe, Mn, Ni, and Cd. On the other hand, Zn could also result in other mineral deficiencies or an antagonism of the metabolic functions by the excessive

Table 4. The effect of the diet on Zn accumulation in different tissues, tibia length, and breaking strength.

Trials	Diet (mg/kg)					SE	Sig.
	0	40-ZnO	40-$ZnSO_4$	500-$ZnSO_4$	1000-$ZnSO_4$		
Zn accumulation (ppm)							
Feather	67.17^{d}	76.211^{c}	86.17^{b}	95.55^{a}	99.81^{a}	13.48	***
Bone	50.27^{d}	98.44^{c}	117.55^{cb}	129.04^{b}	158.69^{a}	40.26	***
Liver	45.58	70.96	88.24	73.79	70.48	15.37	NS
Thigh	33.87^{b}	40.98^{a}	39.40^{ab}	45.90^{a}	44.18^{a}	4.68	**
Breast	13.30^{b}	13.98^{b}	15.86^{b}	17.25^{b}	23.37^{a}	4.01	***
Tibia length (cm)	10.84^{b}	11.57^{a}	11.67^{a}	11.75^{a}	11.79^{a}	0.38	**
Breaking strength (kg/cm^2)	28.43	24.81	26.82	24.76	22.12	2.38	NS
Tibia weight (%)	2.38^{ab}	2.09^{b}	2.03	2.26^{ab}	2.49^{a}	13.48	NS

Sig.: significant; ^{a,b} Means within a row without common superscript difference ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
NS: not significant ($p > 0.05$).

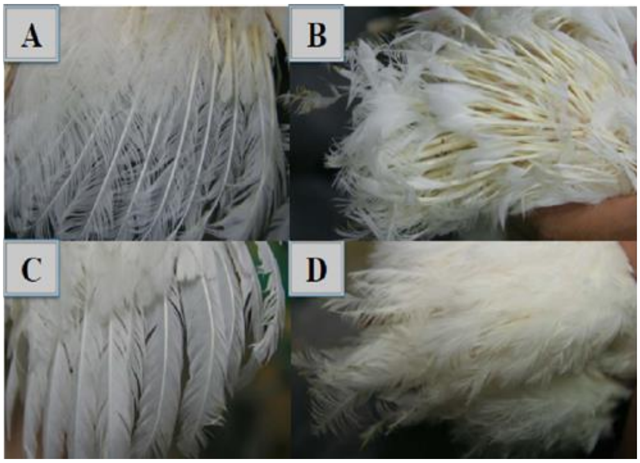

Figure 2. The observation of Zn-deficiency on the tail and wing: A and B: Zn deficiency was observed in broilers fed with nonsupplemented Zn reared under a high ambient temperature. C and D: Zn-deficiency was observed in broilers fed with nonsupplemented Zn reared under a low ambient temperature.

intake of Zn, such as Pb, Cr, Hg, Cu, Fe, Mn, Ni, and Cd [14]. Second, Zn could antagonize vitamins. For example, giving a high dosage of vitamin D could increase the Ca absorption affecting the Zn deficiency. Furthermore, a high intake of inositol and vitamins B1, B10, B12, D and E could interfere with the Zn levels. However, an excessive Zn intake could also harm those vitamin levels [14]. Third, anti-inflammatory drugs, such as corticosteroids, which could reduce pain by suppressing inflammatory responses to damaged tissue, may therefore adversely affect the Zn plasma levels [15]. Fourth, binding divalent cations of inositol hexaphosphate (phytate), which is contained in corn and soybean meal, depress Zn absorption and would reduce the utilization in the intestinal tract of chickens. Yu et al. [16] found that Zn was more likely to be absorbed in the ileum of the broiler's intestinal tract. Fifth is about the genetic selection. At present, some chick breeding has rapid growth resulting in leg weakness; thus, it may require more Zn than the formulated NRC level to maintain their need for growth. Sixth, element minerals including Zn may disappear while mixing feed. The last reason is because of the high ambient environment temperatures. This condition could lead Zn and other minerals (Na, Cu, Fe, Mg, Se, and Cr) to be excreted by urination and defecation because of the high rate of consuming water, which is a method for the disposal of heat for broilers (1). El Husseiny and Creger [17] mentioned that a high ambient temperature also decreased the retention rate of Zn, Fe, Na, K and Ca in the broiler's body. Those situations could be sensitive to modern broilers. Because of their rapid body growth and the consequence of more heat generated in the growth and greater metabolic activity, they would need more vitamins and Zn to recover from the adverse symptoms [6]. Less or nonoptimal Zn consumption during this temperature over a long period may not be sufficient enough to recover from any adverse symptoms; consequently, marginal minerals or mineral deficiency would occur. There was clear evidence from this experiment of the dramatic reduction of the bodyweight of broilers reared in a 30 °C controlled chamber in nonsuppemental Zn diet. Besides, our studies revealed that the features of the feathers had serious side effects showing abnormal or retarded growth. This could be explained by the fact that the broilers being consumed less than usual results in low diet proteins inducing amino acids and other nutrient deficiencies. Reduced immune organ weight, such as, bursa, spleen, liver and thymus, as well as humoral, and cell-mediated immune responses were also noted [18]. These findings found that supplements of 40 mg/kg Zn sulfate into the diet could improve the growth rate of broilers reared in high ambient temperature. This concurred with Lai et al. [12] where broilers fed with 40 mg/kg Zn had positive results of the FCR. Wang et al. [19] mentioned that the basal feeding diet supplemented with 30 mg/kg or 45 mg/kg Zn sulfate improved the productive performance and increased the total superoxide dismutase and glutathione peroxidase in ducks reared in high ambient temperatures. Sahin et al. [2] compared supplements of 30 mg/kg and 60 mg/kg Zn sulfate fed to quails reared under heat stress conditions (34 °C/h/day). The highest final body weight was found at 60 mg/kg Zn supplementation. Salabei et al. [20] reported rearing broilers under heat stress conditions by changing the temperatures daily by exposing them to 12 h at 23.9 °C, 3 h at 23.9–37 °C, 6 h at 37 °C and 3 h at 37-23.9 °C until day 42. The 90 mg/kg Zn supplementation resulted in a high feed intake and body weight gain, but there was reduced weight gain when the birds were given 135 mg/kg Zn. Salabei et al. [20] made an experiment under the same temperature conditions with a supplement of 181 mg/kg Zn that reduced the growth performance at week 7 compared to 68 mg/kg Zn [21]. Lai et al. [12] found that body weight gain were higher when a supplement of 60 mg/kg Zn sulfate was provided at temperatures lower than 30 °C during weeks 1-3. Interestingly, these findings, the excessive supplement of 1000 mg/kg increased bodyweight and FCR during week 5–6 in broilers reared under high ambient temperature (1–42 days). This happening might be due to broilers pulling Zn to recover negative effect, and remaining Zn might go through defecation and urination. Meanwhile, it also accumulates in tissues of the liver and meat, for example. Moreover, an excessive supplement of 500 mg/kg and 1000 mg/kg Zn did not perform well when compared to a supplement of 40 mg/kg Zn-sulfate.

Under thermal neutral zones or normal temperature, an excessive supplement of Zn (500 and 1000 mg/kg) showed a decline in growth performance. Wedekind et al. [22] found a reduction of feed intake when a dietary supplementation of 750 mg/kg Zn was given to broilers. A high diet of 200-1200 mg/kg Zn - organic also depressed the feed intake [23]. Sandoval et al. [24] also found a decrease in growth performance in broilers over the entire experimental period when supplemented with 500 ppm and 1500 ppm Zn in the diet. This situation could be explained by the fact that the broiler's body attempted to reduce the harmfulness of the toxicity of Zn by inducing an intestinal metallothionine (MT) synthesis as too much prevalence of Zn would bind to the MT resulting in reduced Zn absorption, and it would be excreted in the feces. As such, these factors may be a consequence of the decreased bodyweight together with the symptoms of toxicity of Zn occurring.

A study on the growth performance under high ambient temperature conditions compared Zn inorganic with Zn sulfate and Zn oxide. This experiment found that supplementation at 40 mg/kg Zn sulfate performed better than Zn oxide by enhancing the growth performance in the final stages of the bird's age before going to the market (weeks 5-6). As Zn sulfate had more bioavailability than Zn oxide, the outcome for broilers reared under these conditions for long periods could result in the resistance of the adverse effects. Moreover, Zn oxide supplementation, as well as Zn sulfate, improved the FCR throughout the experiment period. This could imply that Zn oxide was also enhanced under these conditions. Furthermore, high supplemental Zn oxide levels were nontoxic, like Zn sulfate, as this prevented nitrogen loss to the atmosphere without detrimental effects on growth performance [25]. In addition, a high Zn oxide supplement was used for hens molting without creating adverse effects on the broilers' performance [26]. In piglets, a high diet of Zn oxide could improve the pigs' body weight gain and health [8].

4.2. Accumulation of Zn in the tissue, bone, and feathers

Based on mineral retention, different tissues were investigated for Zn bioavailability and accumulation. This could determine the toxicity from the high deposition of Zn when fed at high levels. Many researchers used bones to determine Zn accumulation, as it was the most sensitive index for assessment in chicks [24]. The linear relationship of the bone Zn increased with the dietary levels (10-320 mg/kg) [18]. Moreover, the deposition of bone Zn could

Table 5. The effect of the ambient temperature on Zn accumulation in different tissues, tibia length, and breaking strength.

Trials	Temperatures		SE	Sig.	Interaction	SE
	Low	High			D×T	
Zn accumulation (ppm)						
Feather	84.72	85.24	0.37	NS	NS	1.42
Bone	117.46	104.11	5.86	NS	NS	15.76
Liver	76.21	63.41	9.05	NS	NS	12.85
Thigh	41.61	40.57	0.42	NS	NS	0.33
Breast	17.01	16.46	0.42	NS	NS	0.71
Tibia length (cm)	11.63	11.43	0.14	NS	NS	0.21
Breaking strength (kg/cm^2)	26.25	24.52	1.22	NS	NS	1.41
Tibia weight (%)	2.23	2.27	0.37	NS	NS	1.42

Sig.: significant; [a,b] Means within a row without common superscript difference ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
NS: not significant ($p > 0.05$), D: diet, and T: temperature.

decrease as the chick became older [24]. In this experiment, the authors observed the high bioavailability of Zn sulfate compared with Zn oxide in the bone.

The liver is an important organ, which plays a key role in controlling the detoxification of the body. In this experiment, the liver did not show that it was accumulating high Zn levels. This may be due to the Zn either passing through the metabolic function or endogenous excretion. In concurring with Wedekind et al. [22], excessive dietary Zn feed grade or reagent grade showed no differences in the accumulation of Zn in the liver and kidney. Feathers were the best indicators to show a Zn deficiency in broilers [12,13]. In this experiment, the authors observed the adverse symptoms of feathers in broilers fed with nonsupplemented Zn; however, the feather Zn concentration showed no difference in the statistics compared with broilers fed with Zn at low levels (40 mg/kg). Lai et al. [12] observed that supplementary Zn in a corn soybean basal diet at 0 mg/kg, 10 mg/kg, 40 mg/kg, and 60 mg/kg found no significant differences in layers' and broilers' feathers. The thigh and breast muscle Zn were less sensitive when supplemented at low levels; however, excessive Zn supplementation increased the Zn levels when administered at high levels; such as 500 mg/kg and 1000 mg/kg. Sandoval et al. [24] reported that for birds administered Zn until day 21, the Zn in the muscles linearly increased when administered dosages of 500 mg/kg, 1000 mg/kg, and 1500 mg/kg Zn. Moreover, this differed from Kim and Patterson [25], who found that Zn supplements of 500 mg/kg, 1000 mg/kg, and 1500 mg/kg at short periods did not effect on the Zn breast muscle in broiler chicks. However, under high ambient temperatures, the Zn accumulation tended to be reduced in the breast, thigh meat, bone, liver, and feathers.

4.3. Tibia length, tibia breaking strength, and percentage of the tibia weight

According to many researchers, Zn is one of the important microminerals influencing tibia structure and breaking strength, medullary canal diameter, and tibia tarsal index. Skeleton malformations and poor bone mineralization were caused by Zn deficiency in poultry. In this study, the authors demonstrated that there was a reduction of tibia length in broilers fed with nonsupplemented Zn. This finding concurred with Shelton and Southern [27]. Based on the author's findings, the optimal tibia length and breaking strength was 40 mg/kg Zn sulfate. The range of supplementary Zn into diets at 30-70 mg/kg was optimum for bone strength [28]; however, this would depend on the species, age, and productivity of the poultry. In addition, this study found that there were shortening and thickening of the leg bones, and the enlargement of the hock joint in broilers without Zn supplementation was observed, as also reported by Lai et al. [12]. Sufficient Zn supplementation would have an effect on regular bone formation and increased bone mass [29]. Zn deficiency could also reduce bone weight, which was found in this result. According to Seo et al. [29], a decrease in the bone weight and delays of growth in the bone metabolism resulting in the retardation of bone growth and development were found in these issues. In this experiment, it was found that there was an increase in the weight of the tibia when broilers were fed with

Zn. Furthermore, under different ambient temperatures, there was no differentiation on the tibia length and tibia breaking strength between the diets.

In conclusion, broilers reared under low ambient temperatures with a diet of high Zn sulfate showed adverse effects on growth performance. In contrast, these levels could improve the final body weight in broilers reared under high ambient temperatures. However, taking into account economic and farm profits, an optimal level for supplementary feed would be 40 mg/kg, which would be suitable for broilers reared under heat stress or a comfortable temperature.

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References

1. De Basilio V, Vilarino M, Yahav S, Picard M. Early age thermal conditioning and a dual feeding program for male broilers challenged by heat stress. Poultry Science 2001; 80: 29-36.
2. Sahin K, Smith MO, Onderci M, Sahin N, Gursu F et al. Supplementation of zinc from organic or inorganic source improves performance and antioxidant status of heat-distressed quail. Poultry Science 2005; 84: 882-887.
3. Wolfenson D, Bachrach D, Maman M, Graber Y, Rozenboim I. Evaporative cooling of ventral regions of the skin in heat-stressed laying hens. Poultry Science 2001; 80: 958-964.
4. Ibs KH, Rink L. Zinc-altered immune function. Journal of Nutrition 2003; 133 (5): 1452-1456.
5. Powell SR. The antioxidant properties of zinc. Journal of Nutrition 2000; 130 (5): 1447-1454.
6. Sahin K, Sahin N, Yaralioglu S, Onderci M. Protective role of supplemental vitamin E and selenium on lipid peroxidation, vitamin E, vitamin A, and some mineral concentrations of Japanese quails reared under heat stress. Biological Trace Element Research 2002; 85: 59-70.
7. Schlegel P, Sauvant D, Jondreville C. Bioavailability of zinc sources and their interaction with phytates in broilers and piglet. Animal 2013; 7 (1): 47-59.
8. Cromwell GL. Antimicrobial and pro microbial agents. In: Lewis AJ, Southern LL (editors). in Swine Nutrition. 2nd ed. Boca Raton, Fl, USA: CRC Press LLC; 2001. pp. 401-426.
9. Wedekind KJ, Baker DH. Zinc bioavailability in feed-grade sources of zinc. Journal of Animal Science 1990; 68: 684-689.
10. AOAC. Official methods of analysis. 12th ed. Washington, D.C., USA: Association of Official Analytical Chemists; 1995.
11. MacDonald RS. The role of zinc in growth and cell proliferation. Journal of Nutrition 2000; 130: 1500-1508.
12. Lai PW, Liang JB, Hsia LC, Loh TC, Ho YW. Effect of varying dietary zinc levels and environmental temperatures on the growth performance, feathering score and feather mineral concentration of broilers chicks. Asian-Australasian Journal of Animal Sciences 2010; 23 (7): 937-945.
13. Cui H, Xi P, Junliang D, Debing L, Guang Y. Pathology of lymphoid organs in chickens fed a diet deficient in zinc. Avian Pathology 2004; 33 (5): 519-524.
14. Watts DL. Nutrient interrelationships mineral vitamin endocrines. Journal of Orthomolecular Medicine 1990; 5 (1): 11-19.
15. Balogh Z, El-Ghobarey AF, Fell GS, Brown DH, Dunlop GS et al. Plasma zinc and its relationship to clinical symptoms and drug treatment in rheumatoid arthritis. Annals of the Rheumatic Diseases 1980; 39: 329-332.
16. Yu Y, Lu L, Wang RL, Xi L, Luo XG et al. Effects of zinc source and phytate on zinc absorption by in situ ligated intestinal loops of broilers. Poultry Science 2010; 89 (10): 2157-2165.
17. El Husseiny O, Creger CR. The effect of ambient temperature on carcass energy gain in chickens. Poultry Science 1980; 59: 2307-2311.
18. Sunder GS, Panda AK, Gopinath NCS, Rao SVR, Raju MVLN et al. Effects of higher levels of zinc supplementation on performance, mineral availability, and immune competence in broiler chickens. Journal of Applied Poultry Research 2008; 17: 79-86.
19. Wang CS, Wang S, Zhang HX, Ruan D, Xia WG et al. Optimization of dietary zinc for egg production and antioxidant capacity in Chinese egg-laying ducks fed a diet based on corn-wheat bran and soybean meal. Poultry Science 2017; 96 (7): 2336-2343.
20. Salabi F, Boujarpoor M, Fayazi J, Salari S, Nazari M. Effects of different levels of zinc on the performance and carcass characteristics of broiler reared under heat stress condition. Journal of Animal and Veterinary Advances 2011; 10 (10): 1332-1335.
21. Bartlett JR, Smith MO. Effects of different levels of zinc on the performance and immune competence of broilers under heat stress. Poultry Science 2003; 82: 1580-1588.
22. Wedekind KJ, Hortin AE. Methodology for assessing zinc bioavailability: efficacy estimates for zinc methionine, zinc sulfate, and zinc oxide. Journal of Animal Science 1992; 70: 178-187.
23. Cao J, Henry PR, Holwerda RA, Toth JP, Littlell RC et al. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. Journal of Animal Science 2000; 78 (8): 2039-2054.

24. Sandoval M, Henry PR, Luo XG, Littell RC, Miles RD et al. Performance and tissue zinc and metallothionein accumulation in chicks fed a high dietary level of zinc. Poultry Science 1998; 77: 1354-1363.

25. Kim WK, Patterson PH. Effect of dietary zinc supplementation on broiler performance and nitrogen loss from manure. Poultry Science 2004; 83: 34-38.

26. Park SY, Birkhold SG, Kubena LF, Niset DJ, Ricke SC. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biological Trace Element Research 2004; 101 (2): 147-163.

27. Shelton JL, Southern LL. Interactive effect of zinc, copper, and manganese in diets for broilers. International Journal of Poultry Science 2007; 6: 466-469.

28. Wang X, Fosmire GJ, Gay CV, Leach RM. Short-term zinc deficiency inhibits chondrocyte proliferation and induces cell apoptosis in the epiphyseal growth plate of young chickens. Journal of Nutrition 2002; 132: 665-673.

29. Seo HJ, Cho YE, Kim T, Shin H, Kwun IS. Zinc may increase bone formation through stimulating cell proliferation, alkaline phosphatase activity and collagen synthesis in osteoblastic MC3T3-E1 cell. Nutrition Research and Practice 2010; 4 (5): 356-361.