

## Effects of supplemental different clay minerals in broiler chickens under cyclic heat stress

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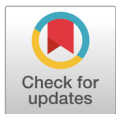
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### Abstract

The objective of this study was to investigate the effect of supplementing clay minerals and organic chromium in feed on broiler chicken under heat stress (HS). A total of 90 one-day-old broiler chicken (Arbor Acres) with an initial body weight of  $45.0 \pm 0.2$  g were assigned to five treatment groups (six replications, three birds each cage): 1) NC group, basal diet under room temperature environment; 2) PC group, basal diet under high temperature (HT) environment; 3) ILT group, basal diet + 1% illite + HT; 4) ZLT group, basal diet + 1% zeolite + HT; 5) OC group, basal diet + 400 ppb/kg organic chromium + HT. The ILT and ZLT groups had significantly higher body weight than the PC group in 4 weeks. Apparent total tract digestibility of gross energy was increased in the ILT, ZLT, and OC groups compared to the PC group. The NC group had lower foot-pad dermatitis score than other groups. *Escherichia coli* population in the cecum and feces was decreased in the ZLT group than in the PC group. *Lactobacillus* in cecum and feces was significantly increased in the ZLT group than in the PC group. Regarding blood profiles, blood cortisol was decreased in the NC and ILT groups compared to the PC group. Water holding capacity and pH were increased in the ZLT group than the PC group. In conclusion, according to the results of growth performance, nutrients digestibility, bacteria counts, and meat characteristics, supplementation of the ZLT in broiler diet can alleviate HS.

**Keywords:** Broiler, Illite, Zeolite, Organic chromium, Heat stress

## INTRODUCTION

Meat production in the poultry industry has gradually increased over three decades through the change of industrialization, consumer lifestyle, development of breeding, and increment of the annual per capita consumption [1,2]. Thus, contemporary researchers in this industry are struggling to maintain increased production from various challenges. One of these challenges is heat stress (HS) exposure. HS is fatal to chicken because they cannot dissipate their body heat outside by having feathers and the lack of

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### Competing interests

No potential conflict of interest relevant to this article was reported.

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Not applicable.

### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

### Authors' contributions

Conceptualization: An J, Lee J, Oh H, Kim HB, Cho J.  
Data curation: Lee J, Kim Y, Chang S.  
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Methodology: An J, Lee J.  
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### Ethics approval and consent to participate

The experimental protocol was approved (CBNUA-1621-21-02) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

sweat glands on the skin [3]. Other effects of HS include immunomodulatory disorders, endocrine disorders, respiratory alkalosis, electrolyte imbalance, and increased mineral excretion [4–7]. HS can also accelerate the production of free radicals (e.g., reactive oxygen, nitrogen, chlorine, etc.), causing oxidative stress beyond optimal antioxidant capacity [8,9]. Additionally, it can stimulate the hypothalamic–pituitary–adrenal axis to increase corticosteroid secretion from adrenal glands [10]. As a result, HS has many negative impacts, such as reducing growth performance, high mortality, and severe immune suppression, leading to physiological changes [11–14].

To mitigate these problems, various approaches have been studied in terms of nutrition such as probiotics, synbiotics, herbal extract, minerals and vitamins [15–19]. Clay minerals (CMs) consisting of aluminosilicate molecules are mainly composed of phyllosilicates that have interlayers, causing increased electronic charge and creating internal void and channels [20,21]. These properties can bind and/or trap toxic materials and heavy metals and decrease the passage rate of digesta [22–24]. Among CMs, zeolites and illites have been studied worldwide in the livestock industry due to their advantageous properties such as ion exchange, adsorption, pollution reduction, catalysis [25,26]. Supplementation of these CMs in diets can improve growth performance, nitrogen retention, and restriction of pollution, and diarrhea in pigs [22,27–29] and enhance the pH of litter, nitrogen digestibility, and growth performance in poultry [30,31]. In addition, there was a report that the addition of silicon dioxide to the feed improved the foot-pad dermatitis (FPD) of turkeys [32]. Chromium possesses antioxidant properties, which participate in the metabolism of carbohydrates, protein, and lipid [33]. It also activates insulin and decreases cortisol concentration in broilers [34]. Dietary chromium supplementation can also improve the growth performance and immunity of broiler [6].

However, studies on CMs under HS have not been reported yet. It is thought that the side effects caused by HS will be improved by protecting the intestinal morphology and improving the intestinal environment and increasing the digestibility due to the intestinal mucosa adsorption of illite and zeolite supplemented in the feed of broilers. In addition, in order to compare with organic chromium, an additional experiment was conducted. Therefore, the purpose of this study was to investigate and compare the effects of illite, zeolite, and organic chromium on growth performance, nutrient digestibility, FPD, intestinal morphology, bacteria counts, blood profiles, subjective and sensory characteristics of broilers under cyclic HS.

## MATERIALS AND METHODS

### Ethics

The experimental protocol was approved (CBNUA-1621-21-02) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

### Illite, zeolite, and organic chromium

The composition of illite is SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, K<sub>2</sub>O, Fe<sub>2</sub>O<sub>3</sub>, Na<sub>2</sub>O, TiO<sub>2</sub>, respectively, 67.4%, 20.3%, 5.50%, 2.35%, 0.54%, 0.27% and other minerals, provided by YonggungIllite (Seoul, Korea). Zeolite is SiO<sub>2</sub> 63.23%, and Soma Bond Basic product was provided by Soma (Eumsung, Korea). Organic chromium is chelated with methionine and consists of 1,099.35 ppm of chromium, and Chromium-Aminox 1000 was provided by Soma.

### Animals and dietary design

A total of 90, one-day-old broiler chickens (Arbor Acres) with an initial average weight of 45.0 ± 0.2 g were purchased from a local hatchery (Cherrybro, Jincheon, Korea). Broilers were assigned

to 5 treatment groups in 6 replications, 3 birds each (5 trt × 6 rep × 3 birds). The NC group is composed of basal diet + room temperature at 24°C (RT); the PC group is a basal diet + high temperature (HT), the ILT group is basal diet + 1% illite + HT, and the ZLT group is a basal diet + 1% zeolite + HT. The OC group was basal diet + 400 ppb/kg organic chromium + HT. All treatment groups except for the NC group were reared at RT for 2 weeks and then cyclic HS was given for 9 hours from 2 weeks of age to 34°C or higher from 9:00 to 17:00 (15:9, RT:HT). All diets were formulated to meet or exceed the nutrient requirements for poultry by the NRC [35]. Compositions of basal diets are shown in Table 1. The birds were fed *ad libitum* and they had free access to the water.

## Sampling and measurements

### Growth performance

Growth performance was measured by body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). BW, BWG, FI, and FCR were recorded at the start of the experiment, days 14 and 28. By subtracting the previous week's BW from the current week's BW, BWG was calculated. The residual amount was subtracted from the feed amount to calculate FI. FCR was calculated by dividing FI by BWG.

**Table 1. Compositions of basal diets (as-fed-basis)**

Items	Content
Ingredients (%)	
Corn (USA, No.3)	44.18
Soybean meal (44% CP)	16.50
Wheat	20.00
Wheat bran	4.00
Corn gluten meal	4.00
Fish meal (local)	1.00
Animal fat	3.00
Rapeseed meal	2.00
Meat meal	2.00
Salt	0.23
Choline-HCl	0.01
Methionine	0.12
Lysine	0.66
Calcium carbonate	0.20
Tricalcium phosphate	1.60
Vitamin premix <sup>1</sup>	0.20
Mineral premix <sup>2</sup>	0.20
Analyzed composition	
ME (kcal/kg)	3,100
CP (%)	21

<sup>1</sup>Contained per kg of diet: vit A, 10,000 IU; vit D<sub>3</sub>, 2,000 IU; vit E, 421 IU; vit K, 5 mg; riboflavin, 2,400 mg; vit B<sub>2</sub>, 9.6 mg; vit B<sub>6</sub>, 2.45 mg; vit B<sub>12</sub>, 40 ug; niacin, 49 mg; pantothenic acid, 27 mg, biotin, 0.05 mg.

<sup>2</sup>Contained per mg per kg of diet: Cu 140 mg, Fe 145 mg, Zn 179 mg, Mn 12.5 mg, I 0.5 mg, Co 0.25 mg, Se 0.4 mg. CP, crude protein; ME, metabolizable energy.

### **Nutrient digestibility**

All treatment groups were fed 0.2% Cr<sub>2</sub>O<sub>3</sub> in their feed starting 3 days before the end of the experiment. Broilers were euthanized by cervical dislocation. Feathers and foreign matter were removed from all feces 2 days before the end of the experiment, and after collection, they were stored in sealed packs and stored at -20°C. The ileal digest was carefully squeezed out 1 cm before Meckel's diverticulum, rinsed with saline, and collected in a conical tube. The ileal digest was carefully squeezed out 1 cm before Meckel's diverticulum, rinsed with saline, and collected in a conical tube. The feed, feces, and ileal digests of all treatment groups were dried in an oven at 50°C for 72 hours and then ground to a fine powder. The powdered samples were analyzed for dry matter (DM), crude protein (CP), and gross energy (GE). DM analyzed samples according to the AOAC method [36] in an oven at 105°C for 16 h. CP was calculated by multiplying the sample by 6.25 by titrating N according to the Kjeldahl Method. GE was analyzed using a bomb calorimeter (model 12361, Parr Instrument, Moline, IL, USA). Apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) follow the formula of  $100 - [(concentration\ of\ nutrient\ in\ fecal \times Cr_2O_3\ feed) / (concentration\ of\ nutrient\ in\ diet \times Cr_2O_3\ fecal) \times 100]$ .

### **Foot-pad dermatitis**

FPD was scored according to the type of lesion according to Eichner [37] method: no lesion (score 0), lesion covering less than 25% of the sole (score 1), large area lesion, covering between 25% and 50% of the sole (score 2), more than 50% of the plantar (score 3). Scores were assessed on both paws of the birds, and the raters were independently conducted by three observers. The average score for foot lesions was performed by turning the statistics.

### **Intestinal morphology**

At the end of the experiment, all broilers were excised 2 cm towards the junction of the small intestine Meckel's diverticulum and cecum. For intestinal morphology analysis, the excised samples were fixed in 10% neutral buffered formalin (NBF; Sigma-Aldrich, St Louis, MO, USA). After installing the sample on the slide, it was treated with paraffin and stained with hematoxylin and eosin. Field morphology was observed using an Olympus IX51 inverted phase-contrast microscope. The villus height (VH), crypt depth (CD), and VH : CD ratio are all things to look at when examining intestinal morphology.

### **Bacteria counts**

At the end of the experiment, cecum samples were collected in conical tubes after the broilers were slaughtered. Fecal samples were collected before the end of the experiment and analyzed immediately. From the sample, 0.1 g was suspended in distilled water, homogenized, and diluted from 10<sup>-4</sup> to 10<sup>-7</sup> to count the number of bacteria. Evenly spread 100 µL of the diluted solution on the agar. *Escherichia coli* (*E. coli*), *Lactobacillus*, and *Salmonella* were analyzed for bacteria, and MacConkey agar was used for *E. coli*, MRS agar was used for *Lactobacillus*, and BG Sulfa agar was used for *Salmonella*. *E. coli* and *Salmonella* were cultured for 24 hours and *Lactobacillus* was cultured for 48 hours.

### **Blood profiles**

Before slaughter, 2 mL of blood samples were taken from the wing veins of all broilers and collected in a vacuum tube containing K<sub>3</sub>EDTA and a tube not treated with heparin for serum analysis. The collected blood samples were centrifuged at 12,500×g at 4°C for 20 minutes. Red blood cell, white blood cell, heterophil, lymphocyte, monocyte, and basophil were analyzed using an automatic

hematology analyzer (XE2100D, Sysmex, Kobe, Japan). Cortisol was analyzed by ECLIA (Cobas 8000 e801, Roche Diagnostics, Mannheim, Germany).

### **Meat characteristics**

Broilers were slaughtered and breast meat was removed and stored in vacuum packs. Analysis of general components of breast meat Moisture, protein, fat and ash content were measured according to the AOAC method. Water holding capacity (WHC) was analyzed according to the method of Laakkonen [38]. By measuring the drip loss (DL) generated while shaping a 2 cm thick breast into a circular shape, placing it in a polypropylene bag, vacuum-packing it, and storing it in a refrigerator at 4°C for 24 hours, DL was calculated as the weight ratio (%) of the initial sample. The weight of a 3 cm thick chicken breast after molding it into a circular form, heating it to a core temperature of 70°C in a hot water heater, and allowing it to cool for 30 minutes was used to compute the cooking loss. Shearing force was evaluated using a Rheometer (Compac-100, Sun Scientific, Tokyo, Japan) for shear force cutting test. The pH was measured with a pH meter (Mettler Delta 340, Mettler-toledo, Leicester, UK) after homogenization using a homogenizer (Bihon seiki, Ace, Osaka, Japan). The meat color was measured using a spectro colorimeter (Model JX-777, Color Techno. System, Tokyo, Japan) optimized on a white plate (Lightness;  $L^*$ , 94.04; Redness;  $a^*$ , 0.13; Yellowness;  $b^*$ , -0.51).

### **Subjective and sensory evaluation**

Subjective evaluation and sensory evaluation were performed to determine the palatability of broiler breast meat. For the subjective evaluation, water dispersible, color, and hardness were evaluated, and very bad (score 1), bad (score 2), normal (score 3), good (score 4), and very good (score 5) were evaluated, respectively. The sensory evaluation items were texture, juiciness, flavor, and total preference were evaluated, and each was evaluated as very bad (score 1), bad (score 2), normal (score 3), good (score 4), and very good (score 5), respectively. Subjective evaluation and sensory evaluation were performed through 5 panels.

### **Statistical analysis**

Data from all studies were caged as an experimental unit and the data collected were analyzed using SAS software (Statistical Analysis System Software, 2012) of the General Linear Model procedure. All statistical analysis differences were analyzed using Tukey's multiple range test for  $p < 0.05$ .

## **RESULTS**

### **Growth performance**

The growth performance data are shown in Table 2. At the 4th week of the experiment, BW had significant increased ( $p < 0.05$ ) in the NC, ILT and ZLT groups compared to the PC group. The ZLT group showed a significant decreased ( $p < 0.05$ ) in FCR compared to the NC group at 0-2 weeks. In Body weight gain, the NC, ILT and ZLT groups showed significantly higher ( $p < 0.05$ ) than the PC group at 2-4 weeks from the and for the entire experimental period. Also, the NC group showed a significant increased ( $p < 0.05$ ) in FI compared to the other groups at 2-4 weeks. During the entire experimental period, FI had significant decreased ( $p < 0.05$ ) in the PC and OC groups compared to the NC group.

### **Nutrient digestibility**

The nutrient digestibility data are shown in Table 3. GE was significantly higher ( $p < 0.05$ ) in the

**Table 2. Effects of supplemental different minerals on growth performance in broiler chickens under cyclic heat stress**

Items (g)	NC	PC	ILT	ZLT	OC	SE	p-value
BW							
Initial	45.00	45.00	45.00	45.00	45.00	0.10	0.973
2W	408.60	406.70	422.20	427.00	419.50	10.50	0.597
4W	1,468.30 <sup>a</sup>	1,334.90 <sup>b</sup>	1,448.30 <sup>a</sup>	1,453.10 <sup>a</sup>	1,411.10 <sup>ab</sup>	23.30	0.003
0-2W							
BWG	363.60	361.70	377.20	382.00	374.50	10.50	0.598
FI	577.50	572.70	571.00	571.50	574.40	13.20	0.997
FCR	1.59 <sup>a</sup>	1.58 <sup>ab</sup>	1.51 <sup>ab</sup>	1.50 <sup>b</sup>	1.53 <sup>ab</sup>	0.02	0.013
2-4W							
BWG	1,059.70 <sup>a</sup>	928.20 <sup>b</sup>	1,026.10 <sup>a</sup>	1,026.10 <sup>a</sup>	991.60 <sup>ab</sup>	21.30	0.003
FI	1851.70 <sup>a</sup>	1598.20 <sup>b</sup>	1702.10 <sup>b</sup>	1697.70 <sup>b</sup>	1664.60 <sup>b</sup>	35.10	0.001
FCR	1.75	1.72	1.66	1.65	1.68	0.04	0.356
0-4W							
BWG	1,423.30 <sup>a</sup>	1,289.90 <sup>b</sup>	1,403.30 <sup>a</sup>	1,408.10 <sup>a</sup>	1,366.10 <sup>ab</sup>	23.40	0.003
FI	2,429.20 <sup>a</sup>	2,170.90 <sup>b</sup>	2,273.10 <sup>ab</sup>	2,269.10 <sup>ab</sup>	2,239.00 <sup>b</sup>	41.60	0.004
FCR	1.71	1.68	1.62	1.61	1.64	0.02	0.061

<sup>a,b</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400 ppb of organic chromium. SE, standard error; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

**Table 3. Effects of supplemental different minerals on nutrient digestibility in broiler chickens under cyclic heat stress**

Items (%)	NC	PC	ILT	ZLT	OC	SE	p-value
ATTD							
GE	78.78 <sup>ab</sup>	77.98 <sup>b</sup>	79.60 <sup>a</sup>	79.96 <sup>a</sup>	79.67 <sup>a</sup>	0.48	0.044
DM	79.03	77.78	78.86	78.71	78.38	0.34	0.105
CP	74.66	73.85	74.31	73.84	73.75	0.36	0.367
AID							
GE	78.08 <sup>a</sup>	75.94 <sup>b</sup>	77.32 <sup>ab</sup>	77.59 <sup>ab</sup>	77.03 <sup>ab</sup>	0.46	0.034
DM	76.46 <sup>a</sup>	74.33 <sup>b</sup>	75.56 <sup>ab</sup>	75.85 <sup>ab</sup>	75.30 <sup>ab</sup>	0.45	0.038
CP	74.37	73.39	74.42	75.42	74.25	0.58	0.227

<sup>a,b</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400 ppb of organic chromium. SE, standard error; ATTD, apparent total tract digestibility; GE, gross energy; DM, dry matter; CP, crude protein; AID, apparent ileal digestibility.

ILT, ZLT and OC groups than the PC group in the ATTD. The AID of GE and DM showed significantly higher ( $p < 0.05$ ) the NC group than the PC group.

### Foot-pad dermatitis

The FPD data are shown in Table 4. The NC group showed a significantly lower ( $p < 0.05$ ) average FPD score than the other groups. In addition, the ILT and ZLT groups showed significantly lower ( $p < 0.05$ ) average FPD score than the OC group.

### Intestinal morphology

The intestinal morphology data are shown in Table 5. In the case of VH in the ileum, the PC group showed significantly lower ( $p < 0.05$ ) VH than the NC group.

**Table 4. Effects of supplemental different minerals on foot-pad dermatitis in broiler chickens under cyclic heat stress**

Score <sup>1)</sup>	NC		PC		ILT		ZLT		OC		SE	p-value
	N	%	N	%	N	%	N	%	N	%		
0	26	72.22	9	25.00	10	27.78	10	27.78	8	22.22		
1	7	19.44	5	13.89	12	33.33	12	33.33	7	19.44		
2	3	8.33	12	33.33	8	22.22	7	19.44	9	25.00		
3	0	0.00	10	27.78	6	16.67	7	19.44	12	33.33		
Total <sup>2)</sup>	36	100.00	36	100.00	36	100.00	36	100.00	36	100.00		
Average		0.4 <sup>c</sup>		1.6 <sup>ab</sup>		1.3 <sup>b</sup>		1.3 <sup>b</sup>		1.7 <sup>a</sup>	0.6	< 0.001

<sup>1)</sup>Lesion score: Lesion score was determined as follows: 0, no lesion; 1, lesion covering less than 25% of the sole of the foot; 2, covering between 25% and 50% of the sole of the foot; 3, more than 50% of the lesion of the plantar.

<sup>2)</sup>Total values are rounded to one decimal place.

<sup>a-c</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400 ppb of organic chromium.

SE, standard error.

**Table 5. Effects of supplemental different minerals on ileal morphology in broiler chickens under cyclic heat stress**

Items ( $\mu\text{m}$ )	NC	PC	ILT	ZLT	OC	SE	p-value
Villus height (VH)	943.8 <sup>a</sup>	851.8 <sup>b</sup>	896.0 <sup>ab</sup>	904.9 <sup>ab</sup>	872.2 <sup>ab</sup>	19.7	0.032
Crypt depth (CD)	119.9	139.5	122.4	122.2	123.2	8.3	0.464
VH : CD	7.9	6.1	7.3	7.4	7.1	0.5	0.134

<sup>a,b</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400 ppb of organic chromium.

SE, standard error.

### Bacteria counts

The bacteria counts data are shown in Table 6. The number of *E. coli* in the cecum was significantly increased ( $p < 0.05$ ) in the PC group compared to the NC group. The number of *Lactobacillus* in the cecum and feces were significantly decreased ( $p < 0.05$ ) in the OC group than the NC group. The number of *E. coli* in feces was significantly increased ( $p < 0.05$ ) in the PC and OC groups than the NC group. *Salmonella* showed no significant difference ( $p > 0.05$ ) in the all treatment groups in cecum and feces.

### Blood profiles

The blood profiles data are shown in Table 7. The blood cortisol content was significantly decreased ( $p < 0.05$ ) in the NC and ILT groups than the PC group. The NC group tends to have decreased ( $p = 0.054$ ) in basophil content compared to the PC group.

### Meat characteristic

The meat characteristic data are shown in Table 8. In the case of fat content, the NC and OC groups showed significantly higher ( $p < 0.05$ ) than the other groups. Also, the OC group showed a significantly lower ( $p < 0.05$ ) ash content than the ILT and ZLT groups. In meat quality, the PC group showed lower ( $p < 0.05$ ) WHC than the NC group. The ZLT group showed significantly lower ( $p < 0.05$ ) DL compared to the PC group. In the case of pH, the NC and ZLT groups showed significantly higher ( $p < 0.05$ ) values than the other groups.

### Subjective and sensory evaluation

The subjective and sensory evaluation data are shown in Table 9. In sensory evaluation items such as

**Table 6. Effects of supplemental different minerals on bacteria counts in cecum and feces in broiler chickens under cyclic heat stress**

Items (Log <sub>10</sub> CFU/g)	NC	PC	ILT	ZLT	OC	SE	p-value
Cecum							
<i>Escherichia coli</i>	7.38 <sup>b</sup>	7.93 <sup>a</sup>	7.82 <sup>ab</sup>	7.39 <sup>b</sup>	7.81 <sup>ab</sup>	0.11	0.002
<i>Lactobacillus</i>	9.26 <sup>a</sup>	8.54 <sup>b</sup>	8.71 <sup>ab</sup>	9.23 <sup>a</sup>	8.49 <sup>b</sup>	0.17	0.003
<i>Salmonella</i>	7.42	7.63	7.47	7.46	7.44	0.07	0.322
Feces							
<i>Escherichia coli</i>	7.40 <sup>b</sup>	7.82 <sup>a</sup>	7.65 <sup>ab</sup>	7.50 <sup>b</sup>	7.66 <sup>a</sup>	0.07	0.005
<i>Lactobacillus</i>	9.03 <sup>a</sup>	8.64 <sup>b</sup>	8.66 <sup>b</sup>	9.00 <sup>a</sup>	8.71 <sup>ab</sup>	0.08	0.002
<i>Salmonella</i>	7.43	7.69	7.62	7.64	7.64	0.10	0.414

<sup>a,b</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400 ppb of organic chromium.

SE, standard error.

**Table 7. Effects of supplemental different minerals on blood profiles in broiler chickens under cyclic heat stress**

Items	NC	PC	ILT	ZLT	OC	SE	p-value
RBC (10 <sup>6</sup> /μm)	2.24	2.32	2.30	2.28	2.38	0.05	0.475
WBC (10 <sup>3</sup> /μm)	27.19	24.16	26.96	31.53	28.11	2.52	0.374
Heterophil (%)	5.32	6.42	7.28	6.57	6.70	0.90	0.646
Lymphocyte (%)	94.50	93.08	92.28	93.08	92.80	0.95	0.570
Monocyte (%)	0.15	0.32	0.32	0.23	0.35	0.11	0.713
Basophil (%)	0.03	0.18	0.12	0.12	0.15	0.03	0.054
Cortisol (ug/dL)	0.07 <sup>b</sup>	0.12 <sup>a</sup>	0.08 <sup>b</sup>	0.09 <sup>ab</sup>	0.09 <sup>ab</sup>	0.01	0.001

<sup>a,b</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400ppb of organic chromium.

RBC, red blood cell; WBC, white blood cell; RBC, red blood cell; WBC, white blood cell.

SE, standard error.

**Table 8. Effects of supplemental different minerals on meat characteristic in broiler chickens under cyclic heat stress**

Item	NC	PC	ILT	ZLT	OC	SE	p-value
Meat content (%)							
Moisture	76.39	77.75	77.18	74.47	76.77	0.77	0.088
Protein	20.40 <sup>ab</sup>	20.04 <sup>ab</sup>	20.38 <sup>ab</sup>	23.07 <sup>a</sup>	20.23 <sup>b</sup>	0.67	0.025
Fat	2.34 <sup>a</sup>	1.20 <sup>b</sup>	1.31 <sup>b</sup>	1.42 <sup>b</sup>	2.29 <sup>a</sup>	0.14	< 0.001
Ash	0.87 <sup>ab</sup>	1.01 <sup>a</sup>	1.13 <sup>a</sup>	1.04 <sup>a</sup>	0.71 <sup>b</sup>	0.07	0.003
Meat quality (%)							
WHC	75.88 <sup>ab</sup>	73.80 <sup>b</sup>	74.20 <sup>b</sup>	77.11 <sup>a</sup>	73.94 <sup>b</sup>	0.66	0.005
DL	2.54 <sup>b</sup>	3.72 <sup>a</sup>	3.40 <sup>a</sup>	2.68 <sup>b</sup>	3.30 <sup>a</sup>	0.11	< 0.001
CL	21.58	22.48	22.40	20.21	22.76	0.68	0.087
SF (g)	1,134.00	1,563.00	1,186.00	1,083.00	1,411.00	195.00	0.384
pH	6.06 <sup>a</sup>	5.86 <sup>c</sup>	5.99 <sup>b</sup>	6.06 <sup>a</sup>	6.00 <sup>b</sup>	0.01	< 0.001
Color							
L*	61.46	65.22	60.59	59.81	57.97	2.02	0.167
a*	10.25	7.56	10.55	8.60	12.39	1.16	0.061
b*	8.30	10.80	11.46	10.36	7.80	0.85	0.131

<sup>a-c</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400 ppb of organic chromium.

SE, standard error; WBC, white blood cell; DL, drip loss; CL, cooking loss; SF, shearing force.



**Table 9.** Effects of supplemental different minerals on subjective and sensory evaluation in broiler chickens under cyclic heat stress

Items	NC	PC	ILT	ZLT	OC	SE	p-value
Subjective							
Water	2.08	1.83	2.00	2.17	1.92	0.25	0.884
Color	2.50	2.33	2.83	2.17	2.83	0.22	0.157
Hardness	3.00	2.83	3.17	2.67	2.67	0.29	0.684
Sensory							
Texture	3.17 <sup>a</sup>	1.83 <sup>b</sup>	2.67 <sup>ab</sup>	3.50 <sup>a</sup>	2.67 <sup>ab</sup>	0.23	< 0.001
Juiciness	3.67 <sup>a</sup>	2.00 <sup>b</sup>	2.67 <sup>ab</sup>	3.50 <sup>a</sup>	2.83 <sup>ab</sup>	0.27	0.001
Flavor	3.50 <sup>a</sup>	1.50 <sup>b</sup>	3.00 <sup>a</sup>	3.67 <sup>a</sup>	3.17 <sup>a</sup>	0.25	< 0.001
Total preference	3.83 <sup>a</sup>	2.00 <sup>b</sup>	3.17 <sup>ab</sup>	3.83 <sup>a</sup>	3.17 <sup>ab</sup>	0.30	< 0.001

<sup>a,b</sup>Means within column with different superscripts differ significantly ( $p < 0.05$ ).

NC, basal diet; PC, NC and start of heat stress at the 2nd week; ILT, PC + 1% of illite; ZLT, PC + 1% of zeolite; OC, PC + 400 ppb of organic chromium.

SE, standard error.

texture, juiciness and flavor, the NC and ZLT groups were significantly higher ( $p < 0.05$ ) than the PC group. In total preference, the NC group showed significantly higher ( $p < 0.05$ ) than the PC group.

## DISCUSSION

### Growth performance

In this study, the reduction of FI in heat-stressed chicks may be due to the natural mechanism to minimize heat production during HS [39,40], which may be responsible of reduced BWG. However, in this study, supplementing illite and zeolite significantly improved BWG up to the NC group level. It has been reported that the average daily gain (ADG) of broilers supplemented with 0.1% montmorillonite, which has a similar composition to illite, is significantly improved [41]. Qin et al. [41] also reported that montmorillonite supplementation has no significant effect on feed : gain (F : G). Such growth performance is improved due to illite for non-antibiotic and antibacterial [42]. Additives bearing clinoptilolite with zinc, such as zeolite, can improve ADG and F : G of broilers [43,44]. The reason why the BW was significantly improved in the treatment group supplemented with CM might be that has the ability to reduce the passage rate of digested matter in the gastrointestinal tract, thereby allowing nutrients to be more thoroughly digested with a longer digestion time [20]. In this study, a treatment group supplemented with organic chromium was also added to alleviate chromium deficiency caused by HS. HS has a major adverse effect on broilers by increasing chromium excretion, resulting in impaired carbohydrate and protein metabolism, decreased insulin sensitivity of peripheral tissues, and decreased growth performance [45]. Supplementation with organic chromium in this study did not show significant BWG or FCR improvement. In a previous study, supplementation of chromium showed a significant effect on the growth performance in broilers [46,47]. In contrast to this study, several studies, the supplementation of chromium in feed was shown to be helpful in improving growth performance, but this is thought to be different results due to the experimental environment and organic chelation of chromium. Therefore, additional research is needed on the appropriate level and the exact mechanism involved in the effect of organic chromium.

### Nutrient digestibility

HS has several detrimental effects on broiler growth, metabolism, and physiology that are clearly

observable in digestion [7]. It also has a negative effect on digestion due to intestinal damage caused by HS [48]. HS not only causes intestinal damage, but also decreases the digestibility of carbohydrates, lipids, and proteins by altering the activity of enzymes such as amylase, maltase, lipase, trypsin, and chymotrypsin [49–52]. Zhou et al. [53] reported that the ATTD of GE is significantly increased when a mixture of zeolite and attapulgite is supplemented at 2% in feed for broilers. The AID of energy digestibility in pigs is significantly higher in a 5% zeolite-treated group than in the control group [54]. Montmorillonite and zeolite supplementation has a beneficial effect on intestinal development. It can significantly increase in the activity of digestive enzymes including protease, chymotrypsin, trypsin, lipase and amylase in the small intestine [55,56]. Tang et al. [44] reported that supplementation with the zinc-bearing zeolite can increase in the activity of digestive enzymes such as amylase and lipase in the pancreas. Studies on the mechanism involved in the effect of organic chromium supplementation on HS are insufficient. Thus, more research is needed.

### Foot-pad dermatitis

Foot lesion is now a major welfare problem in the poultry industry. Foot lesion has complex causes. Foot lesions are intertwined as a result of various factors related to litter moisture, nutrition, and heredity [57]. The incidence of FPD is associated with litter moisture and ammonia. AL-Homidan [58] reported that higher concentrations of ammonia can occur in high-temperature rooms. In Europe, it has been reported that the wetter the litter, the higher the risk of FPD in broilers and turkeys [59–61]. The combination of high ammonia concentration and moisture with HS causes hock burns, a factor in FPD [62]. In a previous study, there was no significant difference in foot lesion score according to temperature, although the average score was higher at a higher temperature [63]. Supplementation of illite, zeolite, and organic chromium to broilers under HT did not have a significant effect on the FPD score in this study. Similar to results of this study, a previous study has reported that supplementation of silicon dioxide in turkey diet did not significantly affect litter moisture, although it tended to decrease the FPD score [32]. However, other studies have reported that the supplementation of feed with zeolite could reduce the moisture and organic content of the litter by improving intestinal water absorption, thus drying the feces [64–67].

### Intestinal morphology

HS can disrupt the physiological homeostasis of broilers and lead to the production of reactive oxygen species and inflammatory cytokines, thereby increasing intestinal permeability and impairing intestinal function [10, 51, 68]. This damage has a devastating effect on intestinal morphology. Also, it can damage the intestinal epithelium, resulting in decreasing VH and CD [69–71]. Kim et al. [72] reported that supplementation of illite combined with silver in broiler diets can improve the VH more than antibiotics. Similar to results of this study, supplementation of zeolite and chromium propionate did not significantly affect VH, CD, and VH:CD ratio in previous studies [73,74]. However, some studies have shown that zeolite or natural clinoptilolite supplementation has a significant effect on the VH and VH:CD ratios of broilers [75,76]. The difference in these studies might be due to the difference in zeolite content and the age of broilers. The mechanism involved in the effect of zeolite on intestinal morphology is that zeolite can protect the intestinal mucosa by attaching to the mucus at the VH, helping epithelial cell regeneration and reducing the intestinal colonization and infection process, thus affecting the intestinal morphology [77]. Due to these effects, the VH was the highest in broilers supplemented with zeolite in HS treatment groups in this study. However, studies on mechanisms involved in the effect of illite and organic chromium on intestinal morphology are incomplete. Thus, additional studies are needed.

### Bacteria counts

The gut acts as a barrier against toxins and infectious agents. With the temperature-humidity change, several types of pathogenic bacteria can multiply and disrupt the intestinal environment in the gut. HS can impair intestinal health by impairing the function of the immune system in the intestinal wall [48], leading to poor growth performance, increased disease incidence, and high mortality rates for broilers [78]. The number of *E. coli* seems to be decreased in broiler feces, because it is thought that the addition of minerals affects the number of bacteria under the influence of palygorskite and zeolite [44,79,80]. Palygorskite and zeolite can promote secretory immunoglobulin A and immunoglobulin G, both of which are important immunoglobulins in the intestinal mucosa, which are thought to affect the number of bacteria in this study. With this improvement in immunity, the body's high level of immunity can affect the number of microorganisms to resist pathogens, including bacteria [81]. Although different from our results, other studies have shown that chromium methionine and zinc-bearing clinoptilolite and formic acid modified clinoptilolite can significantly affect numbers of *E. coli*, *Lactobacillus*, and *Salmonella* [82–83]. The reason for the different research results might be due to the difference in the amount of minerals added and the difference in the chelate of minerals.

### Blood profiles

Blood corticosteroid concentration has been used as a measure of environmental stress in birds [84]. Chicken leukocytes have been found to be a reliable indicator than corticosterone levels because leukocyte levels change less with stress [85,86]. The leukocyte response is an indicator of heat or cold stress in poultry. HS can reduce lymphocyte counts and affect phagocytosis of phagocytes [7,87]. Sufficiently HT can alter the white blood cell compositions of broilers [84,88]. However, in this study, HS did not show a significant effect on white blood cell count. Among white blood cell components, basophils tended to be improved with mineral supplementation. In pigs, silicate supplementation did not significantly affect basophils. However, it significantly improved cortisol levels [89]. There is a lack of research on the mechanism by which illite reduces cortisol in broilers. Research about the effect of zeolite and organic chromium on cortisol in broilers is also lacking. Thus, more research is needed.

### Meat characteristic

Many researchers have reported that the molecular mechanism by which HS reduces meat quality is that mitochondrial dysfunction can reduce aerobic metabolism of fat and glucose in chest and thigh muscles, increase anaerobic glycolysis and intramuscular fat deposition, and lower pH due to accumulation of  $H^+$  and lactic acid [90–93]. As a result, WHC and shear force are reduced. In the study conducted by Banaszak et al. [94], the fat content was increased due to the effect of zeolite. The increase in fat content is not a negative characteristic, similar to results of the present study. In previous studies, the fat content in breast meat of broilers was generally less than 3 g, and since fat was treated as a flavor carrier, the results of this experiment were within the appropriate range, so it is not a negative result [95,96].

Main components of ash are oxides, sulfates, phosphates, and silicates in many cases. According to the results of this study, it is considered that the content of ash in the meat was increased by supplementing the ILT and ZLT groups with CM composed of silicon. As a result of this contrast study, supplementation of various types of chromium did not show a significant difference in the ash content of thigh muscle or breast meat [97,98]. These differences are suggested to be due to differences in broiler breeds and differences in chelation, and further studies are needed.

Bowker et al. [99] studied the correlation between WHC, DL, and pH. WHC and pH have a

positive correlation, whereas WHC and DL have a negative correlation. In this study, the zeolite supplement, which showed the highest pH value among HS treatment groups, showed the highest value in WHC. Poultry meat contains polyunsaturated fatty acids, making it susceptible to free radicals and oxidative deterioration [100]. Oxidative reactions caused by HS can disrupt the normal structure of muscles, affecting poultry meat quality [93,101]. Supplementation of zeolite to the feed of broilers has significant antioxidant effects [76]. Due to the antioxidant effect of zeolite, it seemed that the meat quality of the zeolite supplementation treatment group in this study was improved by reducing the oxidative reaction under HS.

### Subjective and sensory evaluation

Sensory characteristics of meat, such as tenderness, juiciness, and muscle shape, play an important role in consumer evaluation of overall meat quality [102,103]. In a previous study, supplementing of illite also resulted in higher scores for juiciness and flavor than the control [42]. Makarski et al. [104] reported that hardness and chewiness are increased in the leg meat of beef fed with opoka, which is similar to zeolite. In addition, it has been reported that when zeolite is added, the protein gel network is decreased and the water binding force is increased, making the meat softer [105]. Mallek et al. [106] also reported that the zeolite-muscle protein interaction can cause changes in the texture and microstructure of the formulated thigh. These studies could support the finding of the present study that the zeolite supplementation group had the highest WHC value.

## CONCLUSION

Exposure of broilers to HS showed a negative effect on all results. However, supplementation of the ZLT in the broiler diet showed the greatest positive effect in HS-exposed broilers. The effects of supplemental the ZLT improved growth performance, nutrient digestibility, bacteria counts, meat characteristics, and sensory evaluation. These results suggested that supplementation of the ZLT in diet from HS is an effective alleviate.

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