RESEARCH ARTICLE

J Anim Sci Technol 2023;65(1):113-131
https://doi.org/10.5187/jast.2022.e94

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

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

Jaewoo An[1#], Jihwan Lee[2#], Minho Song[3#], Hanjin Oh[1], Yongju Kim[1], Seyeon Chang[1], Youngbin Go[1], Dongcheol Song[1], Hyunah Cho[1], Haeryoung Park[4], Hyeun Bum Kim[5]* and Jinho Cho[1]*

[1]*Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea*
[2]*Department of Poultry Science, University of Georgia (UGA), Athens, GA 30602, USA*
[3]*Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea*
[4]*Korea Agriculture Technology Promotion Agency, Iksan 54667, Korea*
[5]*Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea*

Check for updates

Received: Jul 18, 2022
Revised: Oct 17, 2022
Accepted: Oct 26, 2022

#These authors contributed equally to this work.

***Corresponding author**
Hyeun Bum Kim
Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea.
Tel: +82-41-550-3653
E-mail: hbkim@dankook.ac.kr

Jinho Cho
Division of Animal Science, Chungbuk National University, Cheongju 28644, Korea.
Tel: +82-43-261-2544
E-mail: jinhcho@chungbuk.ac.kr

ORCID
Jaewoo An
https://orcid.org/0000-0002-5602-5499
Jihwan Lee
https://orcid.org/0000-0001-8161-4853
Minho Song
https://orcid.org/0000-0002-4515-5212

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 ± 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

Hanjin Oh
https://orcid.org/0000-0002-3396-483X
Yongju Kim
https://orcid.org/0000-0002-0960-0884
Seyeon Chang
https://orcid.org/0000-0002-5238-2982
Youngbin Go
https://orcid.org/0000-0002-5351-6970
Dongcheol Song
https://orcid.org/0000-0002-5704-603X
Hyunah Cho
https://orcid.org/0000-0003-3469-6715
Haeryoung Park
https://orcid.org/0000-0002-6753-8177
Hyeun Bum Kim
https://orcid.org/0000-0003-1366-6090
Jinho Cho
https://orcid.org/0000-0001-7151-0778

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

Funding sources
This study was supported by Regional Innovation Strategy (RIS) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE) (2021RIS-001).

Acknowledgements
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.
Formal analysis: An J, Go Y, Song D.
Methodology: An J, Lee J.
Software: Kim Y, Song D, Cho H, Park H.
Validation: Oh H, Chang S, Park H.
Investigation: Kim Y, Chang S, Go Y.
Writing - original draft: An J, Lee J, Song M.
Writing - review & editing: An J, Lee J, Song M, Oh H, Kim Y, Chang S, Go Y, Song D, Cho H, Park H, Kim HB, Cho J.

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_2, Al_2O_3, K_2O, Fe_2O_3, Na_2O, TiO_2, respectively, 67.4%, 20.3%, 5.50%, 2.35%, 0.54%, 0.27% and other minerals, provided by YonggungIllite (Seoul, Korea). Zeolite is SiO_2 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

 https://doi.org/10.5187/jast.2022.e94

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℃ (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℃ 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 measuremets

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[1)]	0.20
Mineral premix[2)]	0.20
Analyzed composition	
ME (kcal/kg)	3,100
CP (%)	21

[1)]Contained per kg of diet: vit A, 10,000 IU; vit D_3, 2,000 IU; vit E, 421 IU; vit K, 5 mg; riboflavin, 2,400 mg; vit B_2, 9.6 mg; vit B_6, 2.45 mg; vit B_{12}, 40 ug; niacin, 49 mg; pantothenic acid, 27 mg, biotin, 0.05 mg.

[2)]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_2O_3 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℃. 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℃ 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℃ 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– [(concenration of nutrient in fecal × Cr_2O_3 feed) / (concentration of nutrient in diet × Cr_2O_3 fecal) × 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^{-4} to 10^{-7} 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_3EDTA and a tube not treated with heparin for serum analysis. The collected blood samples were centrifuged at 12,500×g at 4℃ for 20 minutes. Red blood cell, white blood cell, heterophil, lymphocyte, monocyte, and basophil were analyzed using an automatic

 https://doi.org/10.5187/jast.2022.e94

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 Laakkonoen [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℃ 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℃ 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 (Mteeler Delta 340, Mettler-tolede, Leicester, UK) after homogenization using a homogenizer (Bihon seiki, Ace, Osaka, Japan). The meat color was measured using a spectro colormeter (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 Liner 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^{a}	1,334.90^{b}	1,448.30^{a}	1,453.10^{a}	1,411.10^{ab}	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^{a}	1.58^{ab}	1.51^{ab}	1.50^{b}	1.53^{ab}	0.02	0.013
2-4W							
BWG	1,059.70^{a}	928.20^{b}	1,026.10^{a}	1,026.10^{a}	991.60^{ab}	21.30	0.003
FI	1851.70^{a}	1598.20^{b}	1702.10^{b}	1697.70^{b}	1664.60^{b}	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^{a}	1,289.90^{b}	1,403.30^{a}	1,408.10^{a}	1,366.10^{ab}	23.40	0.003
FI	2,429.20^{a}	2,170.90^{b}	2,273.10^{ab}	2,269.10^{ab}	2,239.00^{b}	41.60	0.004
FCR	1.71	1.68	1.62	1.61	1.64	0.02	0.061

[a,b]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^{ab}	77.98^{b}	79.60^{a}	79.96^{a}	79.67^{a}	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^{a}	75.94^{b}	77.32^{ab}	77.59^{ab}	77.03^{ab}	0.46	0.034
DM	76.46^{a}	74.33^{b}	75.56^{ab}	75.85^{ab}	75.30^{ab}	0.45	0.038
CP	74.37	73.39	74.42	75.42	74.25	0.58	0.227

[a,b]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.

 https://doi.org/10.5187/jast.2022.e94

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

Score[1)]	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[2)]	36	100.00	36	100.00	36	100.00	36	100.00	36	100.00		
Average	0.4^{c}		1.6^{ab}		1.3^{b}		1.3^{b}		1.7^{a}		0.6	< 0.001

[1)]Lesion score: Lesion score was dermined as follow : 0, no lesion; 1, lesion covering less than 25% of the sole of the foot large area lesion; 2, covering between 25% and 50% of the sole of the foot; 3, more than 50% of the lesion of the plantar.

[2)]Total values are rounded to one decimal place.

[a–c]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 (μm)	NC	PC	ILT	ZLT	OC	SE	*p*-value
Villus height (VH)	943.8^{a}	851.8^{b}	896.0^{ab}	904.9^{ab}	872.2^{ab}	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

[a,b]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 significant increased ($p < 0.05$) in the PC group compared to the NC group. The number of *Lactobacillus* in the cecum and feces were significant decreased ($p < 0.05$) in the OC group than the NC group. The number of *E. coli* in feces was significant 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 significant 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_{10} CFU/g)	NC	PC	ILT	ZLT	OC	SE	*p*-value
Cecum							
Escherichia coli	7.38^{b}	7.93^{a}	7.82^{ab}	7.39^{b}	7.81^{ab}	0.11	0.002
Lactobacillus	9.26^{a}	8.54^{b}	8.71^{ab}	9.23^{a}	8.49^{b}	0.17	0.003
Salmonella	7.42	7.63	7.47	7.46	7.44	0.07	0.322
Feces							
Escherichia coli	7.40^{b}	7.82^{a}	7.65^{ab}	7.50^{b}	7.66^{a}	0.07	0.005
Lactobacillus	9.03^{a}	8.64^{b}	8.66^{b}	9.00^{a}	8.71^{ab}	0.08	0.002
Salmonella	7.43	7.69	7.62	7.64	7.64	0.10	0.414

^{a,b}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^{6}/ μm)	2.24	2.32	2.30	2.28	2.38	0.05	0.475
WBC (10^{3}/ 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^{b}	0.12^{a}	0.08^{b}	0.09^{ab}	0.09^{ab}	0.01	0.001

^{a,b}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^{ab}	20.04^{ab}	20.38^{ab}	23.07^{a}	20.23^{b}	0.67	0.025
Fat	2.34^{a}	1.20^{b}	1.31^{b}	1.42^{b}	2.29^{a}	0.14	< 0.001
Ash	0.87^{ab}	1.01^{a}	1.13^{a}	1.04^{a}	0.71^{b}	0.07	0.003
Meat quality (%)							
WHC	75.88^{ab}	73.80^{b}	74.20^{b}	77.11^{a}	73.94^{b}	0.66	0.005
DL	2.54^{b}	3.72^{a}	3.40^{a}	2.68^{b}	3.30^{a}	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^{a}	5.86^{c}	5.99^{b}	6.06^{a}	6.00^{b}	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

$^{a–c}$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.

https://doi.org/10.5187/jast.2022.e94

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[a]	1.83[b]	2.67[ab]	3.50[a]	2.67[ab]	0.23	< 0.001
Juiciness	3.67[a]	2.00[b]	2.67[ab]	3.50[a]	2.83[ab]	0.27	0.001
Flavor	3.50[a]	1.50[b]	3.00[a]	3.67[a]	3.17[a]	0.25	< 0.001
Total preference	3.83[a]	2.00[b]	3.17[ab]	3.83[a]	3.17[ab]	0.30	< 0.001

[a,b]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 decreased 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.

 https://doi.org/10.5187/jast.2022.e94

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.

REFERENCES

1. Resurreccion AVA. Sensory aspects of consumer choices for meat and meat products. Meat Sci. 2004;66:11-20. https://doi.org/10.1016/S0309-1740(03)00021-4
2. Aydemir E, Altın E, Bilge İ. The relationship between white meat consumption and consumption psychology during COVID-19 pandemic period. World J Adv Res Rev. 2021;9:14-9. https://doi.org/10.30574/wjarr.2021.9.3.0076
3. Zhang C, Zhao XH, Yang L, Chen XY, Jiang RS, Jin SH, et al. Resveratrol alleviates heat stress-induced impairment of intestinal morphology, microflora, and barrier integrity in broilers. Poult Sci. 2017;96:4325-32. https://doi.org/10.3382/ps/pex266
4. Teeter RG, Smith MO, Owens FN, Arp SC, Sangiah S, Breazile JE. Chronic heat stress and respiratory alkalosis: occurrence and treatment in broiler chicks. Poult Sci. 1985;64:1060-4. https://doi.org/10.3382/ps.0641060
5. Sohail MU, Ijaz A, Yousaf MS, Ashraf K, Zaneb H, Aleem M, et al. Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poult Sci. 2010;89:1934-8. https://doi.org/10.3382/ps.2010-00751
6. Bahrami A, Moeini MM, Ghazi SH, Targhibi MR. The effect of different levels of organic and inorganic chromium supplementation on immune function of broiler chicken under heat-

 https://doi.org/10.5187/jast.2022.e94

stress conditions. J Appl Poult Res. 2012;21:209-15. https://doi.org/10.3382/japr.2010-00275

7. Lara LJ, Rostagno MH. Impact of heat stress on poultry production. Animals. 2013;3:356-69. https://doi.org/10.3390/ani3020356
8. Lin H, Decuypere E, Buyse J. Acute heat stress induces oxidative stress in broiler chickens. Comp Biochem Physiol A Mol Integr Physiol. 2006;144:11-7. https://doi.org/10.1016/j.cbpa.2006.01.032
9. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol. 2004;142:231-55. https://doi.org/10.1038/sj.bjp.0705776
10. Quinteiro-Filho WM, Rodrigues MV, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Sá LRM, et al. Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: role of acute hypothalamic-pituitary-adrenal axis activation. J Anim Sci. 2012;90:1986-94. https://doi.org/10.2527/jas.2011-3949
11. Xu Y, Lai X, Li Z, Zhang X, Luo Q. Effect of chronic heat stress on some physiological and immunological parameters in different breed of broilers. Poult Sci. 2018;97:4073-82. https://doi.org/10.3382/ps/pey256
12. Hosseini-Vashan SJ, Raei-Moghadam MS. Antioxidant and immune system status, plasma lipid, abdominal fat, and growth performance of broilers exposed to heat stress and fed diets supplemented with pomegranate pulp (Punica granatum L.). J Appl Anim Res. 2019;47:521-31. https://doi.org/10.1080/09712119.2019.1676756
13. Ma D, Liu Q, Zhang M, Feng J, Li X, Zhou Y, et al. iTRAQ-based quantitative proteomics analysis of the spleen reveals innate immunity and cell death pathways associated with heat stress in broilers (Gallus gallus). J Proteomics. 2019;196:11-21. https://doi.org/10.1016/j.jprot.2019.01.012
14. Awad EA, Najaa M, Zulaikha ZA, Zulkifli I, Soleimani AF. Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora, and meat quality in two broiler strains. Asian-Australas J Anim Sci. 2020;33:778-87. https://doi.org/10.5713/ajas.19.0208
15. Mohammed AA, Jacobs JA, Murugesan GR, Cheng HW. Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. Poult Sci. 2018;97:1101-8. https://doi.org/10.3382/ps/pex421
16. Abo Ghanima MM, Bin-Jumah M, Abdel-Moneim AME, Khafaga AF, Abd El-Hack ME, Allam AA, et al. Impacts of strain variation on response to heat stress and boldo extract supplementation to broiler chickens. Animals. 2019;10:24. https://doi.org/10.3390/ani10010024
17. Hassan AA, Asim RA. Effect of vitamin C and acetylsalicylic acid supplementation on some hematological value, heat shock protein 70 concentration and growth hormone level in broiler exposed to heat stress. Iraqi J Vet Sci. 2020;34:357-63. https://doi.org/10.33899/ijvs.2019.125950.1195
18. Hassan RA, Soliman ES, Hamad RT, El-Borady OM, Ali AA, Helal MS. Selenium and nano-selenium ameliorations in two breeds of broiler chickens exposed to heat stress. S Afr J Anim Sci. 2020;50:215-32. https://doi.org/10.4314/sajas.v50i2.5
19. Jiang S, Yan FF, Hu JY, Mohammed A, Cheng HW. Bacillus subtilis-based probiotic improves skeletal health and immunity in broiler chickens exposed to heat stress. Animals. 2021;11:1494. https://doi.org/10.3390/ani11061494
20. Vondruskova H, Slamova R, Trckova M, Zraly Z, Pavlik I. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: a review. Vet Med. 2010;55:199-224.

https://doi.org/10.17221/2998-VETMED
21. Almeida JAS. Identification of mechanisms of beneficial effects of dietary clays in pigs and chicks during an enteric infection. [Ph.D. dissertation]. Champaign, IL: University of Illinois, Urbana-Champaign; 2013.
22. Papaioannou DS, Kyriakis CS, Alexopoulos C, Tzika ED, Polizopoulou ZS, Kyriakis SC. A field study on the effect of the dietary use of a clinoptilolite-rich tuff, alone or in combination with certain antimicrobials, on the health status and performance of weaned, growing and finishing pigs. Res Vet Sci. 2004;76:19-29. https://doi.org/10.1016/j.rvsc.2003.08.006
23. Yu DY, Xu ZR, Yang XG. Effects of lead and particulate montmorillonite on growth performance, hormone and organ weight in pigs. Asian-Australas J Anim Sci. 2005;18:1775-9. https://doi.org/10.5713/ajas.2005.1775
24. Thieu NQ, Ogle B, Pettersson H. Efficacy of bentonite clay in ameliorating aflatoxicosis in piglets fed aflatoxin contaminated diets. Trop Anim Health Prod. 2008;40:649-56. https://doi.org/10.1007/s11250-008-9144-3
25. Armbruster T. Clinoptilotite-heulandite: applications and basic research. In: Galarneau A, Fajula F, Di Renzo F, Vedrine J. editors. Studies in surface science and catalysis. Vol. 135. Elsevier; 2001. p. 13-27.
26. Schneider AF, Zimmermann OF, Gewehr CE. Zeolites in poultry and swine production. Cienc Rural. 2017;47. https://doi.org/10.1590/0103-8478cr20160344
27. Kondo N, Wagai B. Experimental use of clinoptilolite-tuff as dietary supplements for pigs. Yotonkai. 1968; May: 1-4.
28. Poulsen HD, Oksbjerg N. Effects of dietary inclusion of a zeolite (clinoptilolite) on performance and protein metabolism of young growing pigs. Anim Feed Sci Technol. 1995;53:297-303. https://doi.org/10.1016/0377-8401(94)00744-T
29. Leung S, Barrington S, Wan Y, Zhao X, El-Husseini B. Zeolite (clinoptilolite) as feed additive to reduce manure mineral content. Bioresour Technol. 2007;98:3309-16. https://doi.org/10.1016/j.biortech.2006.07.010
30. Schneider AF, De Almeida DS, Yuri FM, Zimmermann OF, Gerber MW, Gewehr CE. Natural zeolites in diet or litter of broilers. Br Poult Sci. 2016;57:257-63. https://doi.org/10.1080/00071668.2016.1150962
31. Chung YH, Choi IH. Comparison of bentonite and illite on the growth performance and litter quality of duck. Adv Anim Vet Sci. 2019;7:522-5. https://doi.org/10.17582/journal.aavs/2019/7.6.522.525
32. Tran ST, Bowman ME, Smith TK. Effects of a silica-based feed supplement on performance, health, and litter quality of growing turkeys. Poult Sci. 2015;94:1902-8. https://doi.org/10.3382/ps/pev158
33. Haq Z, Jain RK, Khan N, Dar MY, Ali S, Gupta M, et al. Recent advances in role of chromium and its antioxidant combinations in poultry nutrition: a review. Vet World. 2016;9:1392-9. https://doi.org/10.14202/vetworld.2016.1392-1399
34. Herran J, Peña H, Latorre S, Calderon C. Characteristics of the channels and blood parameters of chickens complemented with chromium and their productive acting Resumen. Rev Colomb Cienc Pecu. 2011;4:15-9.
35. NRC [National Research Council]. Nutrient requirements of poultry. 9th ed. Washington, DC: National Academies Press; 1994.
36. AOAC [Association of Official Analytical Chemists]. Official method of analysis of AOAC International. 16th ed. Washington, DC: AOAC Intenational; 2000.
37. Eichner G, Vieira SL, Torres CA, Coneglian JLB, Freitas DM, Oyarzabal OA. Litter moisture

and footpad dermatitis as affected by diets formulated on an all-vegetable basis or having the inclusion of poultry by-product. J Appl Poult Res. 2007;16:344-50. https://doi.org/10.1093/japr/16.3.344
38. Laakkonen E, Wellington GH, Sherbon JN. Low-temperature, long-time heating of bovine muscle 1. changes in tenderness, water-binding capacity, pH and amount of water-soluble components. J Food Sci. 1970;35:175-7. https://doi.org/10.1111/j.1365-2621.1970.tb12131.x
39. Nawaz AH, Amoah K, Leng QY, Zheng JH, Zhang WL, Zhang L. Poultry response to heat stress: its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. Front Vet Sci. 2021:8:699081. https://doi.org/10.3389/fvets.2021.699081
40. Patra AK, Kar I. Heat stress on microbiota composition, barrier integrity, and nutrient transport in gut, production performance, and its amelioration in farm animals. J Anim Sci Technol. 2021;63:211-47. https://doi.org/10.5187/jast.2021.e48
41. Qin S, Li J, Huang W, Wang H, Qin S, Pei W, et al. Effects of montmorillonite on the growth performance, immunity, intestinal morphology and caecal microflora of broilers. Anim Prod Sci. 2021;61:1546-52. https://doi.org/10.1071/AN20663
42. Kim YJ. Effect of dietary supplemention with probiotics, illite, active carbon and hardwood vinegar on the performance and carcass characteristics of broiler. Korean J Poult Sci. 2007;34:165-72. https://doi.org/10.5536/KJPS.2007.34.3.165
43. Tang ZG, Wen C, Wang LC, Wang T, Zhou YM. Effects of zinc-bearing clinoptilolite on growth performance, cecal microflora and intestinal mucosal function of broiler chickens. Anim Feed Sci Technol. 2014;189:98-106. https://doi.org/10.1016/j.anifeedsci.2013.12.014
44. Tang Z, Wen C, Li P, Wang T, Zhou Y. Effect of zinc-bearing zeolite clinoptilolite on growth performance, nutrient retention, digestive enzyme activities, and intestinal function of broiler chickens. Biol Trace Elem Res. 2014;158:51-7. https://doi.org/10.1007/s12011-014-9900-3
45. Sahin K, Sahin N, Kucuk O. Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). Nutr Res. 2003;23:225-38. https://doi.org/10.1016/S0271-5317(02)00513-4
46. Jahanian R, Rasouli E. Dietary chromium methionine supplementation could alleviate immunosuppressive effects of heat stress in broiler chicks. J Anim Sci. 2015;93:3355-63. https://doi.org/10.2527/jas.2014-8807
47. Afrin S, Sultana F, Chowdhury SD, Roy BC. Response of heat-stressed commercial broilers to dietary supplementation of organic chromium. IOSR J Agric Vet Sci. 2016;9:65-9. https://doi.org/10.9790/2380-0909016569
48. Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Sakai M, Sá LRM, et al. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult Sci. 2010;89:1905-14. https://doi.org/10.3382/ps.2010-00812
49. de Souza LFA, Espinha LP, de Almeida EA, Lunedo R, Furlan RL, Macari M. How heat stress (continuous or cyclical) interferes with nutrient digestibility, energy and nitrogen balances and performance in broilers. Livest Sci. 2016;192:39-43. https://doi.org/10.1016/j.livsci.2016.08.014
50. Yi D, Hou Y, Tan L, Liao M, Xie J, Wang L, et al. N-acetylcysteine improves the growth performance and intestinal function in the heat-stressed broilers. Anim Feed Sci Technol. 2016;220:83-92. https://doi.org/10.1016/j.anifeedsci.2016.07.014.
51. Song Z, Cheng K, Zhang L, Wang T. Dietary supplementation of enzymatically treated

Artemisia annua could alleviate the intestinal inflammatory response in heat-stressed broilers. J Therm Biol. 2017;69:184-90. https://doi.org/10.1016/j.jtherbio.2017.07.015
52. He X, Lu Z, Ma B, Zhang L, Li J, Jiang Y, et al. Chronic heat stress damages small intestinal epithelium cells associated with the adenosine 5′-monophosphate-activated protein kinase pathway in broilers. J Agric Food Chem. 2018;66:7301-9. https://doi.org/10.1021/acs.jafc.8b02145
53. Zhou P, Tan YQ, Zhang L, Zhou YM, Gao F, Zhou GH. Effects of dietary supplementation with the combination of zeolite and attapulgite on growth performance, nutrient digestibility, secretion of digestive enzymes and intestinal health in broiler chickens. Asian-Australas J Anim Sci. 2014;27:1311-8. https://doi.org/10.5713/ajas.2014.14241
54. Ly J, Grageola F, Lemus C, Castro M. Ileal and rectal digestibility of nutrients in diets based on leucaena (Leucaena leucocephala (Lam.) de Wit) for pigs. Influence of the inclusion of zeolite. J Anim Vet Adv. 2007;6:1371-6.
55. Xia MS, Hu CH, Xu ZR. Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. Poult Sci. 2004;83:1868-75. https://doi.org/10.1093/ps/83.11.1868
56. Wu QJ, Wang LC, Zhou YM, Zhang JF, Wang T. Effects of clinoptilolite and modified clinoptilolite on the growth performance, intestinal microflora, and gut parameters of broilers. Poult Sci. 2013;92:684-92. https://doi.org/10.3382/ps.2012-02308
57. Shepherd EM, Fairchild BD. Footpad dermatitis in poultry. Poult Sci. 2010;89:2043-51. https://doi.org/10.3382/ps.2010-00770
58. Al-Homidan A. Effect of temperature and light regimen on ammonia, dust concentrations and broiler performance. Br Poult Sci. 2004;45:S35-6. https://doi.org/10.1080/00071660410001698164
59. Mayne RK. A review of the aetiology and possible causative factors of foot pad dermatitis in growing turkeys and broilers. Worlds Poult Sci J. 2005;61:256-67. https://doi.org/10.1079/WPS200458
60. Mayne RK, Else RW, Hocking PM. High litter moisture alone is sufficient to cause footpad dermatitis in growing turkeys. Br Poult Sci. 2007;48:538-45. https://doi.org/10.1080/00071660701573045
61. Youssef IMI, Beineke A, Rohn K, Kamphues J. Effects of litter quality (moisture, ammonia, uric acid) on development and severity of foot pad dermatitis in growing turkeys. Avian Dis. 2011;55:51-58. https://doi.org/10.1637/9495-081010-Reg.1
62. Berg C. Pododermatitis and hock burn in broiler chickens. In: Weeks C, Butterworth A, editors. Measuring and auditing broiler welfare. Wallingford: CABI International; 2004. p.37-49.
63. Summers JD. Effect of choline or betaine supplementation on broilers exposed to different temperature treatments. [Master's thesis]. Knoxville, TN: University of Tennessee; 2013.
64. Karamanlis X, Fortomaris P, Arsenos G, Dosis I, Papaioannou D, Batzios C, et al. The effect of a natural zeolite (clinoptilolite) on the performance of broiler chickens and the quality of their litter. Asian-Australas J Anim Sci. 2008;21:1642-50. https://doi.org/10.5713/ajas.2008.70652
65. Katouli MS, Boldaji F, Dastar B, Hassani S. Effect of different levels of kaolin, bentonite and zeolite on broilers performance. J Biol Sci. 2010;10:58-62. https://doi.org/10.3923/jbs.2010.58.62
66. de Jong I, van Harn J. Management tools to reduce footpad dermatitis in broilers [Internet]. Aviagen. 2012 [cited 2021 Dec 11]. http://www.gohardanehco.com/en/wp-content/

uploads/2014/03/AviaTech-FoodpadDermatitisSept2012.pdf
67. Nikolakakis I, Dotas V, Kargopoulos A, Hatzizisis L, Dotas D, Ampas Z. Effect of natural zeolite (clinoptilolite) on the performance and litter quality of broiler chickens. Turk J Vet Anim Sci. 2013;37:682-6. https://doi.org/10.3906/vet-1212-9
68. Cheng YF, Chen YP, Chen R, Su Y, Zhang RQ, He QF, et al. Dietary mannan oligosaccharide ameliorates cyclic heat stress-induced damages on intestinal oxidative status and barrier integrity of broilers. Poult Sci. 2019;98:4767-76. https://doi.org/10.3382/ps/pez192
69. Song J, Xiao K, Ke YL, Jiao LF, Hu CH, Diao QY, et al. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult Sci. 2014;93:581-8. https://doi.org/10.3382/ps.2013-03455
70. Abdelqader A, Al-Fataftah AR. Effect of dietary butyric acid on performance, intestinal morphology, microflora composition and intestinal recovery of heat-stressed broilers. Livest Sci. 2016;183:78-83. https://doi.org/10.1016/j.livsci.2015.11.026
71. Liu G, Zhu H, Ma T, Yan Z, Zhang Y, Geng Y, et al. Effect of chronic cyclic heat stress on the intestinal morphology, oxidative status and cecal bacterial communities in broilers. J Therm Biol. 2020;91:102619. https://doi.org/10.1016/j.jtherbio.2020.102619
72. Kim CH, Lim KC, Hwang JH, Ra CS, Pak JI. Effect of Bio-Silverlite(R) on performance, weight of organ, intestinal villus and intestinal microbial in broiler chicks. Korean J Poult Sci. 2006;33:33-9.
73. Saçakli P, Calik A, Bayraktaroğlu AG, Ergün A, Şahan Ö, Özaydin S. Effect of clinoptilolite and/or phytase on broiler growth performance, carcass characteristics, intestinal histomorphology and tibia calcium and phosphorus levels. Kafkas Univ Vet Fak Derg. 2015;21:729-37. https://doi.org/10.9775/kvfd.2015.13283
74. Hayat K, Bodinga BM, Han D, Yang X, Sun Q, Aleya L, et al. Effects of dietary inclusion of chromium propionate on growth performance, intestinal health, immune response and nutrient transporter gene expression in broilers. Sci Total Environ. 2020;705:135869. https://doi.org/10.1016/j.scitotenv.2019.135869
75. Wu QJ, Zhou YM, Wu YN, Wang T. Intestinal development and function of broiler chickens on diets supplemented with clinoptilolite. Asian-Australas J Anim Sci. 2013;26:987-94. https://doi.org/10.5713/ajas.2012.12545
76. Qu H, Cheng Y, Chen Y, Li J, Zhao Y, Zhou Y. Effects of dietary zeolite supplementation as an antibiotic alternative on growth performance, intestinal integrity, and cecal antibiotic resistance genes abundances of broilers. Animals. 2019;9:909. https://doi.org/10.3390/ani9110909
77. Čík G, Bujdáková H, Šeršeň F. Study of fungicidal and antibacterial effect of the Cu(II)-complexes of thiophene oligomers synthesized in ZSM-5 zeolite channels. Chemosphere. 2001;44:313-9. https://doi.org/10.1016/S0045-6535(00)00306-4
78. Varasteh S, Braber S, Akbari P, Garssen J, Fink-Gremmels J. Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. PLoS One. 2015;10:e0138975. https://doi.org/10.1371/journal.pone.0138975
79. Chen YP, Cheng YF, Li XH, Zhang H, Yang WL, Wen C, et al. Dietary palygorskite supplementation improves immunity, oxidative status, intestinal integrity, and barrier function of broilers at early age. Anim Feed Sci Technol. 2016;219:200-9. https://doi.org/10.1016/j.anifeedsci.2016.06.013
80. Su Y, Chen Y, Chen L, Xu Q, Kang Y, Wang W, et al. Effects of different levels of modified palygorskite supplementation on the growth performance, immunity, oxidative status and intestinal integrity and barrier function of broilers. J Anim Physiol Anim Nutr.

2018;102:1574-84. https://doi.org/10.1111/jpn.12974
81. Nochi T, Jansen CA, Toyomizu M, van Eden W. The well-developed mucosal immune systems of birds and mammals allow for similar approaches of mucosal vaccination in both types of animals. Front Nutr. 2018;5:60. https://doi.org/10.3389/fnut.2018.00060
82. Wang LC, Zhang TT, Wen C, Jiang ZY, Wang T, Zhou YM. Protective effects of zinc-bearing clinoptilolite on broilers challenged with Salmonella pullorum. Poult Sci. 2012;91:1838-45. https://doi.org/10.3382/ps.2012-02284
83. Safwat AM, Elnaggar AS, Elghalid OA, El-Tahawy WS. Effects of different sources and levels of dietary chromium supplementation on performance of broiler chicks. Anim Sci J. 2020;91:e13448. https://doi.org/10.1111/asj.13448
84. Altan Ö, Altan A, Çabuk M, Bayraktar H. Effects of heat stress on some blood parameters in broilers. Turk J Vet Anim Sci. 2000;24:145-8.
85. Gross WB, Siegel HS. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 1983:972-9. https://doi.org/10.2307/1590198
86. McFarlane JM, Curtis SE. Multiple concurrent stressors in chicks.: 3. effects on plasma corticosterone and the heterophil: lymphocyte ratio. Poult Sci. 1989;68:522-7. https://doi.org/10.3382/ps.0680522
87. Sugiharto S, Yudiarti T, Isroli I, Widiastuti E, Kusumanti E. Dietary supplementation of probiotics in poultry exposed to heat stress – a review. Ann Anim Sci. 2017;17:591-604. https://doi.org/10.1515/aoas-2016-0062
88. Mittchell MA. Indicators of physiological stress in chicken chickens during road transportation. Anim Welf. 1992;1:91-103.
89. Lee J, Song M, Yun W, Liu S, Oh H, An J, et al. Effects of silicate derived from quartz porphyry supplementation in the health of weaning to growing pigs after lipopolysaccharide challenge. J Appl Anim Res. 2020;48:440-7. https://doi.org/10.1080/09712119.2020.1817748
90. Van Laack RLJM, Liu CH, Smith MO, Loveday HD. Characteristics of pale, soft, exudative broiler breast meat. Poult Sci. 2000;79:1057-61. https://doi.org/10.1093/ps/79.7.1057
91. Feng J, Zhang M, Zheng S. The effect of cyclic high temperature on mitochondrial ROS production, Ca2+-ATPase activity and breast meat quality of broilers. Acta Vet Zootech Sin. 2006;37:1304.
92. Zhang ZY, Jia GQ, Zuo JJ, Zhang Y, Lei J, Ren L, et al. Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. Poult Sci. 2012;91:2931-7. https://doi.org/10.3382/ps.2012-02255
93. Lu Z, He X, Ma B, Zhang L, Li J, Jiang Y, et al. Chronic heat stress impairs the quality of breast-muscle meat in broilers by affecting redox status and energy-substance metabolism. J Agric Food Chem. 2017;65:11251-8. https://doi.org/10.1021/acs.jafc.7b04428
94. Banaszak M, Biesek J, Bogucka J, Dankowiakowska A, Olszewski D, Bigorowski B, et al. Impact of aluminosilicates on productivity, carcass traits, meat quality, and jejunum morphology of broiler chickens. Poult Sci. 2020;99:7169-77. https://doi.org/10.1016/j.psj.2020.08.073
95. Mir NA, Rafiq A, Kumar F, Singh V, Shukla V. Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol. 2017;54:2997-3009. https://doi.org/10.1007/s13197-017-2789-z
96. Zhao J, Wang T, Xie J, Xiao Q, Cheng J, Chen F, et al. Formation mechanism of aroma compounds in a glutathione-glucose reaction with fat or oxidized fat. Food Chem. 2019;270:436-44. https://doi.org/10.1016/j.foodchem.2018.07.106

97. Zha LY, Zeng JW, Chu XW, Mao LM, Luo HJ. Efficacy of trivalent chromium on growth performance, carcass characteristics and tissue chromium in heat-stressed broiler chicks. J Sci Food Agric. 2009;89:1782-6. https://doi.org/10.1002/jsfa.3656
98. Untea AE, Panaite TD, Oancea A, Turcu RP, Saracila M. Camelina meal and chromium picolinate effects on broiler thigh meat nutritional properties. Arch Zootech. 2021;24:96-104. https://doi.org/10.2478/azibna-2021-0016
99. Bowker B, Zhuang H. Relationship between water-holding capacity and protein denaturation in broiler breast meat. Poult Sci. 2015;94:1657-64. https://doi.org/10.3382/ps/pev120
100. Arshad MS, Anjum FM, Asghar A, Khan MI, Yasin M, Shahid M, et al. Lipid stability and antioxidant profile of microsomal fraction of broiler meat enriched with α-lipoic acid and α-tocopherol acetate. J Agric Food Chem. 2011;59:7346-52. https://doi.org/10.1021/jf2002393
101. Brenes A, Viveros A, Goñí I, Centeno C, Sáyago-Ayerdy SG, Arija I, et al. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poult Sci. 2008;87:307-16. https://doi.org/10.3382/ps.2007-00297
102. Font-i-Furnols M, Guerrero L. Consumer preference, behavior and perception about meat and meat products: an overview. Meat Sci. 2014;98:361-71. https://doi.org/10.1016/j.meatsci.2014.06.025
103. Kim HJ, Kim D, Song SO, Goh YG, Jang A. Microbiological status and guideline for raw chicken distributed in Korea. Korean J Poult Sci. 2016;43:235-42. https://doi.org/10.5536/KJPS.2016.43.4.235
104. Makarski M, Niemiec T, Łozicki A, Pietrzak D, Adamczak L, Chmiel M, et al. The effect of silica-calcite sedimentary rock contained in the chicken broiler diet on the overall quality of chicken muscles. Open Chem. 2020;18:215-25. https://doi.org/10.1515/chem-2020-0022
105. Hcini E, Ben Slima A, Kallel I, Zormati S, Traore AI, Gdoura R. Does supplemental zeolite (clinoptilolite) affect growth performance, meat texture, oxidative stress and production of polyunsaturated fatty acid of Turkey poults? Lipids Health Dis. 2018;17:177. https://doi.org/10.1186/s12944-018-0820-7
106. Mallek Z, Fendri I, Khannous L, Ben Hassena A, Traore AI, Ayadi MA, et al. Effect of zeolite (clinoptilolite) as feed additive in Tunisian broilers on the total flora, meat texture and the production of omega 3 polyunsaturated fatty acid. Lipids Health Dis. 2012;11:35. https://doi.org/10.1186/1476-511X-11-35