Poultry Science®

Effect of increasing supplementation of dietary glycine on growth performance, meat quality, liver characteristics, and intestinal health in broiler chickens raised under heat stress conditions

Sung Hoon Kwon, Ji Hye Lee, Hyun Woo Kim, Deok Yun Kim, and Dong Yong Kil[1]

Department of Animal Science and Technology, Chung-Ang University, Anseong-si, Gyeonggi-do 17546, Republic of Korea

ABSTRACT The current study aimed to investigate the effect of increasing supplementation of dietary glycine (**Gly**) on growth performance, meat quality, liver characteristics, and intestinal health in broiler chickens raised under heat stress (**HS**) conditions. A total of one thousand six hundred 25-d-old broiler chickens were randomly allotted to 1 of 5 dietary treatments with 8 replicates. Each replicate comprised 20 male and 20 female birds. A negative control (**NC**) diet was prepared to meet or exceed energy and nutrient requirement estimates, whereas a positive control (**PC**) diet was formulated to contain increasing concentrations of AME_n by 50 kcal/kg as well as those of digestible amino acids, total Ca, and available P by 10% compared with the respective concentrations in the NC diet. Three additional diets were prepared by supplementing the NC diet with 0.4, 0.8, or 1.6% Gly. All chickens were raised under cyclic HS conditions at 29°C ± 0.89°C for 10 h/d and 23°C ± 1.45°C for the remaining time over an 18-d feeding trial. Results indicated that broiler chickens fed the NC diet had a greater ($P < 0.05$) FCR than those fed the PC diet under HS conditions. Increasing supplementation of up to 1.6% Gly in diets decreased (linear, $P < 0.001$) FCR in broiler chickens. Increasing supplementation of dietary Gly tended to increase (linear, $P = 0.070$) water holding capacity in the breast meat. Increasing supplementation of dietary Gly decreased (linear, $P < 0.05$) serum aspartate aminotransferase concentrations and tended to decrease blood heterophil:lymphocyte (linear, $P = 0.083$) and liver malondialdehyde concentrations (quadratic, $P = 0.084$). A tendency for increased villus height (linear, $P = 0.086$) and a significant increase in villus height:crypt depth ratio and goblet cell numbers (linear, $P < 0.05$) were identified following increasing Gly supplementation. In conclusion, increasing supplementation of dietary Gly improved feed efficiency, meat quality, liver health, and intestinal morphology possibly by mitigating oxidative stress and stress response in broiler chickens raised under HS conditions.

Key words: broiler chicken, glycine, growth performance, heat stress, intestinal health

2024 Poultry Science 103:104352
https://doi.org/10.1016/j.psj.2024.104352

INTRODUCTION

As global temperatures rise, heat stress (**HS**) is increasingly considered an urgent and emerging threat in broiler production because HS has been consistently reported to exert various detrimental effects on growth performance, product quality, and health in broiler chickens (Zaboli et al., 2019; Liu et al., 2020). These negative effects of HS are highly associated with various physiological and metabolic alterations in broiler chickens (Chowdhury et al., 2014; Ratriyanto and Mosenthin, 2018). One of the important HS-induced metabolic alterations is an increase in energy and nutrient requirements in broiler chickens (Kim et al., 2022; Onagbesan et al., 2023). Therefore, feeding diets containing high amounts of energy and nutrients to broiler chickens under HS conditions is extensively practiced in the commercial situation (Attia and Hassan, 2017; Abdel-Moneim et al., 2021); however, the results are highly variable. Therefore, poultry nutritionists make increasing efforts to develop functional diets as an alternative to broiler diets with high energy and nutrients for hot seasons.

Glycine (**Gly**) is the simplest amino acid (**AA**) and is considered a functional nutrient owing to its involvement in diverse functions; for example, it is a precursor molecule for the synthesis of collagen, creatine, porphyrins, glutathione, bile acids, nucleic acids, and uric acid (Graber and Baker, 1973; Corzo et al., 2004; Li et al., 2017). In addition, Gly also acts as a biological osmolyte and is associated with single-carbon metabolism as a methyl-group donor or acceptor in the body, which largely shares physiological functions of betaine

© 2024 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Received July 16, 2024.
Accepted September 18, 2024.

[1]Corresponding author: dongyong@cau.ac.kr

(Eklund et al., 2005). Therefore, dietary Gly supplementation is implicated to have the potential to modify intestinal development, body protein and lipid metabolism, oxidative status, and immune responses (Eklund et al., 2005; Ratriyanto and Mosenthin, 2018; Won et al., 2023). However, the importance of dietary Gly has often been disregarded in poultry nutrition because Gly can be endogenously synthesized in poultry, and relatively high amounts of Gly are present in typical feed ingredients (Meléndez-Hevia et al., 2009). Nonetheless, under specific circumstances, such as feeding low-CP diets and raising poultry under HS conditions, dietary Gly can be regarded as a semi-essential AA for broiler chickens due to increased Gly requirements as well as limited supplies of Gly in broiler diets (Hejdysz et al., 2022; Deng et al., 2023). Awad et al. (2017) reported that additional Gly supplementation in low-CP diets alleviated impaired performance and abnormal physiological changes in broiler chickens under HS conditions; however, beneficial effects of dietary Gly were limited by decreased concentrations of other AAs in low-CP diets. Notwithstanding, in our previous study, dietary supplementation of 0.79% Gly improved growth performance with decreasing stress response and oxidative stress in broiler chickens under HS conditions when dietary CP concentrations were maintained at the required level (Won et al., 2023). More interestingly, our recent study demonstrated that increasing supplementation of dietary Gly from 0.341% and 0.683% decreased stress response and fatty liver incidence with improving feed efficiency in laying hens under HS conditions (Nam et al., 2023). Furthermore, these positive outcomes elicited by increasing supplementation of dietary Gly were comparable to high-energy commercial diets for laying hens under HS conditions. However, the effect of varying supplemental levels of dietary Gly on growth performance, meat quality, liver characteristics, and intestinal health in broiler chickens under HS conditions is yet to be elucidated. Moreover, to our knowledge, no studies have been conducted to compare the effect of increasing supplementation of dietary Gly with feeding hot season diets containing high energy and nutrients to broiler chickens under HS conditions.

Therefore, the objective of the current study was to investigate the effect of increasing supplementation of dietary Gly on growth performance, meat quality, liver characteristics, and intestinal health in broiler chickens raised under HS conditions.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The protocol for the present experiment was approved by the Institutional Animal Care and the Use Committee (IACUC) at Chung-Ang University. A total of one thousand six hundred 25-d-old Ross 308 growing broiler chickens (initial body weight [**BW**] = 935 ± 10.5 g) were used and raised in conventional floor pens (200 × 230 × 100 cm = width × length × height). Before commencing the experiment, chickens were fed a commercial-type diet adequate in energy and nutrients for broiler chickens. At 25 d of age, all chickens were allotted to 1 of 5 dietary treatments with 8 replicates in a completely randomized design. Each replicate had 40 birds (20 male and 20 female birds) per floor pen. The experiment lasted for 18 d.

A negative control (**NC**) diet was formulated to meet or exceed the energy and nutrient requirement estimates of Brazilian manuals for growing broiler chickens (Table 1; Rostagno et al., 2011). A positive control (**PC**) diet was formulated to contain increasing concentrations of AME_n by 50 kcal/kg as well as those of digestible AA, total Ca, and available P by 10% as compared to the respective concentrations in the NC diet. The PC diet was prepared to simulate the conventional diet containing high amounts of energy and nutrients for broiler chickens during hot seasons in South Korea (Kim et al., 2017; Nam et al., 2023). Increased energy and nutrient concentrations in the PC diet were achieved by adjusting inclusion levels of feed ingredients, such as corn, soybean meal, corn gluten meal, and soybean oil. Three additional treatment diets were prepared by supplementing 0.4, 0.8, or 1.6% purified Gly (99%, Neo-Cremar Co., Ltd., Seoul, Republic of Korea) to the NC diet. The supplementation levels of Gly in diets were designed to increase standardized ileal digestible (**SID**) Gly+Ser concentrations by 25%, 50%, and 100% compared with those in NC diets (i.e., calculated SID Gly + Ser in NC diets = 1.60%). The calculated concentrations of AME_n and CP were linearly increased by supplementing the NC diet with dietary Gly from 0.0 to 1.6% because AME_n and CP values for supplemental Gly (Rostagno et al., 2011) were considered in diet formulations. The analyzed concentrations of total Gly in NC and PC diets were 0.76 and 0.80%, respectively, whereas those of total Gly in the NC diet supplemented with 0.4, 0.8, and 1.6% Gly were 1.11, 1.49, and 2.40%, respectively. Experimental diets were prepared in mash form. The diets and water were provided ad libitum throughout the experiment. All chickens were exposed to cyclic HS conditions at 29°C ± 0.89°C for 10 h/d and 23°C ± 1.45°C for the remaining time. The average relative humidity was 61.2 ± 5.65% during the experiment. A 24-h lighting schedule was used throughout the experiment. The BW, BW gain (**BWG**), and feed intake (**FI**) were recorded at the end of the experiment. The mortality was recorded daily, and the feed conversion ratio (**FCR**) was calculated as FI divided by BWG after the correction for mortality (Choi et al., 2021).

Sample Collection and Dietary Nutrient Analysis

At the conclusion of the experiment (42 d of age), 1 male bird per replicate with a BW approximating the

Table 1. Composition and nutrient contents of experimental diets (as-fed basis).

Item	Dietary treatments[1]				
	PC	NC	0.4% Gly	0.8% Gly	1.6% Gly
Ingredients					
Corn	50.18	56.99	56.99	56.99	56.99
Soybean meal, 45% CP	32.20	28.99	28.99	28.99	28.99
Corn gluten meal	4.48	3.01	3.01	3.01	3.01
Soybean oil	6.82	5.01	5.01	5.01	5.01
Monodicalcium phosphate	1.28	1.14	1.14	1.14	1.14
Limestone	1.15	1.06	1.06	1.06	1.06
L-Lys HCl, 99%	0.27	0.24	0.24	0.24	0.24
DL-Met, 98.5%	0.28	0.25	0.25	0.25	0.25
L-Thr, 99%	0.09	0.08	0.08	0.08	0.08
L-Arg, 99%	0.08	0.06	0.06	0.06	0.06
L-Ile, 99%	0.02	0.02	0.02	0.02	0.02
Gly, 99%[2]	0.000	0.000	0.400	0.805	1.610
Celite	2.000	2.000	1.600	1.195	0.390
Salt	0.30	0.30	0.30	0.30	0.30
50% Choline	0.30	0.30	0.30	0.30	0.30
$NaHCO_3$	0.30	0.30	0.30	0.30	0.30
Antioxidant	0.05	0.05	0.05	0.05	0.05
Vitamin premix[3]	0.10	0.10	0.10	0.10	0.10
Mineral premix[4]	0.10	0.10	0.10	0.10	0.10
Total sum	100.00	100.00	100.00	100.00	100.00
Calculated energy and nutrient contents[5]					
AME_n, kcal/kg	3,150	3,100	3,106	3,112	3,124
CP, %	21.17	19.25	19.72	20.19	21.14
Digestible Lys, %	1.14	1.04	1.04	1.04	1.04
Digestible Met + Cys, %	0.83	0.76	0.6	0.76	0.76
Digestible Met, %	0.55	0.50	0.50	0.50	0.50
Digestible Thr, %	0.74	0.68	0.68	0.68	0.68
Digestible Trp, %	0.21	0.19	0.19	0.19	0.19
Digestible Arg, %	1.23	1.12	1.12	1.12	1.12
Digestible Ile, %	0.78	0.71	0.71	0.71	0.71
Digestible Val, %	0.90	0.82	0.82	0.82	0.82
Digestible Leu, %	1.83	1.67	1.67	1.67	1.67
Digestible Gly + Ser, %	1.75	1.60	2.00	2.40	3.20
Total calcium, %	0.78	0.71	0.71	0.71	0.71
Available phosphorus, %	0.37	0.33	0.33	0.33	0.33
Analyzed nutrient contents					
Analyzed CP, %	20.93	19.61	19.64	20.04	21.93
Analyzed total Gly, %	0.80	0.76	1.11	1.49	2.40
Analyzed total Gly+Ser, %	1.81	1.71	2.05	2.37	3.37

[1]Dietary treatments: PC, positive control diet (NC + 50 kcal/kg AME_n + 10% higher essential amino acids, calcium, and available phosphorus); NC, negative control diet; 0.4% glycine (Gly), NC + 0.4% Gly; 0.8% Gly, NC + 0.8% Gly; 1.6% Gly, NC + 1.6% Gly.

[2]Supplemental levels of Gly were set to increase Gly supplementation by 0.4%, 0.8%, and 1.6% with consideration of the purity of supplemental Gly (99%, Neo Cremar Co., Ltd., Seoul, Republic of Korea).

[3]Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 13,000 IU; vitamin D_3, 5,000 IU; vitamin E (from $_{DL}$-α-tocopherol acetate), 80 IU; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 10 mg; vitamin B_6, 6 mg; vitamin B_{12}, 20 μg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 200 μg; niacin, 60 mg.

[4]Provided per kilogram of the complete diet: Zn (as ZnO), 100 mg; Mn (as $MnSO_2 \cdot H_2O$), 120 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 60 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 16 mg; Co (as $CoCO_3$), 1,000 μg; I (as $Ca(IO_3)_2 \cdot H_2O$), 1.25 mg; Se (as Na_2SeO_3), 300 μg.

[5]Calculated values from Brazilian tables for poultry and swine 3rd edition (2011).

replicate mean BW was euthanized after 8-h fasting via CO_2 asphyxiation and used to collect tissue samples of breast, blood, liver, and jejunal segments.

The experimental diets were analyzed for CP (method 984.13; AOAC, 2006) using the Kjeldahl method (Kjeltec auto system 2300 Analyzer, FOSS, Hilleroed, Denmark). The concentrations of total Gly and Ser in diets were analyzed using the L8900 Amino Acid Analyzer (HITACHI, Tokyo, Japan) as described by Won et al. (2023).

Breast Meat Quality

The breast muscle was collected to assess meat quality measurements. The right portion of the muscle was used to analyze pH at 1-h and 24-h postmortem and meat color, whereas the left portion was subjected to the analysis of water holding capacity (**WHC**). The detailed procedure of meat quality analysis was reported previously (Choi et al., 2021).

Blood Measurements

Blood samples were obtained from euthanized chickens via heart puncture and collected in 6-mL serum tubes (BD Vacutainer Serum Tube, BD, Franklin Lakes, NJ). The concentrations of alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), creatinine, and uric acid in the serum were analyzed using a Hitachi Automatic Analyzer 7020 (Hitachi Ltd., Tokyo, Japan). Blood heterophil to lymphocyte ratio (**H:L ratio**) as a stress biomarker was measured using the method of Lentfer et al. (2015) with a minor modification. The detailed procedure was reported in our previous study (Nam et al., 2023).

Liver Characteristics and Antioxidant Status

The liver was pictured to determine the subjective fatty liver score using a scale from 1 to 5 (1 = dark red; 5 = yellowish red; Han et al., 2023). In addition, the objective CIE color scale for lightness (**L***), redness (**a***), and yellowness (**b***) was also determined using a colorimeter (model CR-10, Konica Minolta Optics Inc., Tokyo, Japan). Hemorrhagic status in the liver was subjectively scored from 0 to 5, with 0 indicating normal liver and 5 indicating extensive hemorrhages (Diaz et al., 1999). A portion of the middle part of the liver was used to analyze the concentrations of acid-hydrolyzed ether extract (**AEE**) in the liver (method 996.01; AOAC, 2006). A portion of liver samples was also subjected to the analysis of antioxidant status, such as malondialdehyde (**MDA**), total antioxidant capacity (**TAC**), and reactive oxygen species (**ROS**) in the liver. The MDA, TAC, and ROS levels were determined using commercially available kits according to the respective manufacturers' protocols. Protein concentrations were also analyzed using a commercial Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). The detailed procedures for the analysis of antioxidant status were described in our previous study (Yu et al., 2021).

Table 2. The sequence of the primers used for quantitative RT-PCR.

Primer name	Forward	Reverse	Product size (bp)	GenBank accession number
GAPDH	5′- TGCTGCCCAGAACATCATCC-3′	5′-ACGGCAGGTCAGGTCAACAA -3′	142	NM_204305
JAM-2	5′-AATTTACAGTTCCTCCCACT-3′	5′-GTCTTTTCCAGTAAGGCAAC-3′	151	NM_001006257
OCLN	5′-TCGTGCTGTGCATCGCCATC-3′	5′-CGCTGGTTCACCCCTCCGTA-3′	178	NM_205128
ZO-1	5′-CACACTGTGACCCCAAAA-3′	5′-AAGGTCCATCTCAGTTTCAC-3′	151	XM_040680632
MUC2	5′-CAGAAGGAACTTCTTCGTACA-3′	5′-AGAGGATGCTCCTGTAGATT-3′	137	XM_040673077

Abbreviations: *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *JAM-2*, Junctional adhesion molecule-B; *MUC2*, Mucin 2; *OCLN*, Occludin; *ZO-1*, Zonula occludens-1.

Jejunal Morphology and Gene Expression

Jejunal segments measuring 2 cm in length were collected from the middle of the jejunum for the analysis of jejunal morphology and goblet cell counts. Jejunal segments were flushed and fixed with 10% neutral-buffered formalin solutions. Villus height (**VH**), villus width (**VW**), crypt depth (**CD**), and VH:CD ratio were analyzed by the method of Nari et al. (2020) with a minor modification. The detailed procedure was reported in our previous study (Won et al., 2023). Goblet cell counts in the jejunal villus were determined using the method of Dos Santos et al. (2019) with a minor modification. An average of 10 villi per sample was calculated by screening a 200-μm area from the central part of the villi.

Total RNA were obtained from the jejunal mucosa. Expression levels of genes related to tight junction proteins and mucin were determined via a quantitative real-time PCR (Won et al., 2023). Target genes included junctional adhesion molecule-B (***JAM-2***), occludin (***OCLN***), zonula occludens-1 (***ZO-1***), and mucin 2 (***MUC2***). The primers of selected genes were designed using available sequences from public databases and synthesized by Genotech Co. Ltd. (Daejeon-si, Republic of Korea). The primer sequences are listed in Table 2. The specificity of primers was confirmed by PCR amplification as demonstrated by Aznar and Alarćon (2002). The relative quantification of gene-specific expression was conducted using the $2^{-\Delta\Delta Ct}$ method after normalization to glyceraldehyde-3-phosphate (***GAPDH***; Thomsen et al., 2010). More detailed procedure to analyze gene expression levels was reported previously (Won et al., 2023).

Statistical Analysis

All data were analyzed by ANOVA as a completely randomized design using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). The replicate was considered as an experimental unit. Outlier data were identified by the UNIVARIATE procedure of SAS, but no outliers were detected. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate means if the difference was significant. In addition, the unequal-spaced polynomial contrast test was performed to determine linear and quadratic effects of increasing Gly supplementation in NC diets without the data for PC treatment (Nam et al., 2023). A probability of $P < 0.05$ was considered significant and $0.05 \leq P \leq 0.10$ was considered a tendency.

RESULTS

Growth Performance and Breast Meat Quality

The BW, BWG, and mortality were not influenced by dietary treatments under HS conditions (Table 3). However, birds fed the NC diet had a greater ($P < 0.05$) FCR than those fed the PC diet. Increasing supplementation of dietary Gly up to 1.6% decreased (linear, $P < 0.001$) FCR in broiler chickens. Birds fed the diet supplemented with 1.6% Gly yielded a similar FCR to those fed the PC diet. No differences in FI were found between birds in PC and NC treatments; however, a tendency (linear, $P = 0.059$) for a decrease in FI was observed by increasing supplementation of dietary Gly.

Table 3. Effects of increasing supplementation of dietary glycine (Gly) on growth performance in broiler chickens under heat stress conditions.[1]

	Dietary treatments[2]						*P*-value		
Items	PC	NC	0.4% Gly	0.8% Gly	1.6% Gly	SEM	Total[3]	Gly (linear)[4]	Gly (quadratic)[4]
BW, g	2,628	2,587	2,601	2,561	2,598	20.2	0.247	0.900	0.409
BWG, g	1,691	1,652	1,666	1,628	1,663	21.7	0.367	0.901	0.481
FI, g	2,497	2,559	2,521	2,491	2,481	28.4	0.319	0.059	0.385
FCR, g/g	1.48[d]	1.55[a]	1.51[bc]	1.53[ab]	1.49[cd]	0.007	<0.001	<0.001	0.879
Mortality, %	0.63	1.25	1.88	0.94	0.63	0.526	0.426	0.241	0.651

Abbreviations: BW, body weight; BWG, BW gain; FI, feed intake; FCR, feed conversion ratio.
[a-d]Means within a row with no common superscript differ significantly ($P < 0.05$).
[1]Data are least squares means of 8 observations per treatment.
[2]Dietary treatments: PC, positive control diet (NC + 50 kcal/kg AME_n + 10% higher digestible essential amino acids, total calcium, and available phosphorus); NC, negative control diet; 0.4% Gly, NC + 0.4% Gly; 0.8% Gly, NC + 0.8% Gly; 1.6% Gly, NC + 1.6% Gly.
[3]Overall effects of treatments.
[4]Linear and quadratic effects of increasing supplementation of Gly in diets without the data for PC treatment.

Table 4. Effects of increasing supplementation of dietary glycine (Gly) on breast meat quality in broiler chickens under heat stress conditions.[1]

		Dietary treatments[2]						*P*-value		
Items		PC	NC	0.4% Gly	0.8% Gly	1.6% Gly	SEM	Total[3]	Gly (linear)[4]	Gly (quadratic)[4]
WHC, %		66.9	67.0	70.0	69.1	71.5	1.42	0.135	0.070	0.747
Meat color (CIE Lab value)	L*	47.1	46.0	46.1	45.3	45.8	0.56	0.274	0.585	0.531
	a*	2.9	3.0	2.1	2.7	2.5	0.37	0.439	0.617	0.311
	b*	14.7	13.7	13.5	13.6	13.9	0.66	0.728	0.764	0.703
Postmortem pH	1 h	6.43	6.29	6.48	6.40	6.44	0.073	0.417	0.317	0.304
	24 h	5.63	5.64	5.63	5.75	5.67	0.061	0.593	0.566	0.408

Abbreviations: a*, redness; b*, yellowness; CIE, Commission Internationale de l'Eclairage; L*, lightness; WHC, water holding capacity.
[1]Data are least squares means of 8 observations per treatment.
[2]Dietary treatments: PC, positive control diet (NC + 50 kcal/kg AME_n + 10% higher digestible essential amino acids, total calcium, and available phosphorus); NC, negative control diet; 0.4% Gly, NC + 0.4% Gly; 0.8% Gly, NC + 0.8% Gly; 1.6% Gly, NC + 1.6% Gly.
[3]Overall effects of treatments.
[4]Linear and quadratic effects of increasing supplementation of Gly in diets without the data for PC treatment.

Increasing supplementation of dietary Gly tended to increase (linear, $P = 0.070$) WHC in the breast meat of broiler chickens under HS conditions (Table 4). No differences in WHC were observed between birds in PC and NC treatments. Meat color values for L*, a*, and b*, and postmortem pH were not affected by dietary treatments.

Blood Measurements

No differences in serum ALT, AST, creatinine, uric acid, and blood H:L ratio were observed between birds in PC and NC treatments under HS conditions (Table 5). However, increasing supplementation of dietary Gly decreased (linear, $P < 0.05$) serum AST concentrations in broiler chickens, whereas there was a tendency ($P = 0.090$) for a quadratic relationship between serum ALT concentrations and increasing supplementation of dietary Gly with supplementation of 0.4% Gly exhibiting the least serum ALT concentrations. Likewise, a tendency ($P = 0.058$) for a quadratic relationship was found between serum uric acid concentrations and increasing supplementation of dietary Gly with dietary supplementation of 0.4% Gly yielding the least uric acid concentrations. Increasing supplementation of dietary Gly tended (linear, $P = 0.083$) to decrease blood H:L ratio in broiler chickens under HS conditions.

Liver Characteristics and Antioxidant Status

The liver characteristics, including liver color, hemorrhagic score, and AEE concentrations, were not affected by dietary treatments (Table 6). However, there was a tendency ($P = 0.051$) for greater MDA concentrations in birds fed the NC diet than in birds fed the PC diet. Moreover, increasing supplementation of dietary Gly tended to decrease (quadratic, $P = 0.084$) MDA concentrations in the liver. However, TAC and ROS levels in the liver were not influenced by dietary treatments.

Jejunal Morphology and Gene Expression

Birds fed the NC diet had less ($P < 0.05$) VH than those fed the PC diet, but showed no difference in CD, resulting in a tendency for less ($P = 0.080$) VH:CD ratio in birds fed the NC diet than those fed the PC diet (Table 7). A tendency (linear, $P = 0.086$) for increased

Table 5. Effects of increasing supplementation of dietary glycine (Gly) on blood measurements in broiler chickens under heat stress conditions.[1]

	Dietary treatments[2]						*P*-value		
Items	PC	NC	0.4% Gly	0.8% Gly	1.6% Gly	SEM	Total[3]	Gly (linear)[4]	Gly (quadratic)[4]
Serum measurements									
AST, U/L	368[a]	336[ab]	306[ab]	288[b]	270[b]	23.8	0.049	0.043	0.513
ALT, U/L	2.48[a]	2.75[a]	2.08[b]	2.56[a]	2.50[a]	0.137	0.024	0.798	0.090
Creatinine, mg/dL	0.22	0.21	0.21	0.19	0.21	0.014	0.428	0.979	0.338
Uric acid, mg/dL	6.67	6.49	3.94	4.49	7.13	1.226	0.267	0.428	0.058
Blood H:L ratio	0.39	0.42	0.36	0.38	0.32	0.034	0.313	0.083	0.840

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; H:L ratio, heterophil to lymphocyte ratio.
[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).
[1]Data are least squares means of 8 observations per treatment.
[2]Dietary treatments: PC, positive control diet (NC + 50 kcal/kg AME_n + 10% higher digestible essential amino acids, total calcium, and available phosphorus); NC, negative control diet; 0.4% Gly, NC + 0.4% Gly; 0.8% Gly, NC + 0.8% Gly; 1.6% Gly, NC + 1.6% Gly.
[3]Overall effects of treatments.
[4]Linear and quadratic effects of increasing supplementation of Gly in diets without the data for PC treatment.

Table 6. Effects of increasing supplementation of dietary glycine (Gly) on the liver characteristics and antioxidant status in broiler chickens under heat stress conditions.[1]

Items		Dietary treatments[2]					SEM	*P*-value		
		PC	NC	0.4% Gly	0.8% Gly	1.6% Gly		Total[3]	Gly (linear)[4]	Gly (quadratic)[4]
Liver characteristics										
Subjective color score		1.2	1.2	1.5	1.2	1.3	0.11	0.121	0.538	0.709
Liver color (CIE Lab value)[5]	L*	26.9	26.8	27.1	27.3	26.6	0.49	0.889	0.724	0.369
	a*	15.4	16.5	15.9	16.7	15.6	0.73	0.673	0.480	0.707
	b*	6.5	7.4	7.2	7.4	6.9	0.41	0.495	0.397	0.645
Hemorrhagic score		0.2	0.4	0.4	0.4	0.5	0.13	0.460	0.476	0.882
AEE concentrations (% DM)		18.6	14.1	26.2	17.1	21.6	2.95	0.063	0.375	0.388
Liver antioxidant status										
TAC, μmol/mg protein		948	975	924	923	948	32.0	0.769	0.723	0.260
MDA, μmol/mg protein		1.9	2.8	2.3	2.1	2.3	0.21	0.051	0.261	0.084
ROS, mM		20.5	21.9	20.2	20.3	20.3	1.70	0.945	0.592	0.583

Abbreviations: AEE, acid-hydrolyzed ether extract; a*, redness; b*, yellowness; CIE, Commission Internationale de l'Eclairage; L*, lightness; MDA, malondialdehyde; ROS, reactive oxygen species; TAC, total antioxidant capacity.

[1]Data are least squares means of 8 observations per treatment.

[2]Dietary treatments: PC, positive control diet (NC + 50 kcal/kg AME_n + 10% higher digestible essential amino acids, total calcium, and available phosphorus); NC, negative control diet; 0.4% Gly, NC + 0.4% Gly; 0.8% Gly, NC + 0.8% Gly; 1.6% Gly, NC + 1.6% Gly.

[3]Overall effects of treatments.

[4]Linear and quadratic effects of increasing supplementation of Gly in diets without the data for PC treatment.

VH and a significant increase in VH:CD ratio (linear, $P < 0.05$) was observed by increasing supplementation of dietary Gly. Likewise, birds fed the NC diet had less ($P < 0.05$) goblet cell numbers than those fed the PC diet. Increasing supplementation of dietary Gly increased (linear, $P < 0.05$) goblet cell numbers. Birds fed the diet supplemented with 1.6% Gly displayed comparable goblet cell numbers to those fed the PC diet. However, the expression levels of tight junction-related genes (i.e., *ZO-1, OCLN, JAM-2*) and *MUC2* in the jejunal mucosa did not differ among dietary treatments.

DISCUSSION

The most common response of poultry to HS is a reduction in FI owing to an attempt to reduce heat loads from the body (He et al., 2018; Liu et al., 2020). This reduced FI results in decreased energy and nutrient intake, leading to an impairment in productive performance and health in poultry (He et al., 2018; El-Naggar et al., 2019). Therefore, in commercial situations, it is commonly practiced to increase the concentrations of energy and nutrients in poultry diets as a way to compensate for reduced FI during hot seasons (Kim et al., 2017; Nam et al., 2023). In the present study, the PC diet was prepared by increasing energy and nutrient concentrations to simulate a conventional diet for broiler chickens raised under HS conditions. As expected, broiler chickens fed the PC diet exhibited an improvement in growth performance, particularly for FCR, and intestinal morphology compared with those fed the NC diet, suggesting that broiler chickens under HS

Table 7. Effects of increasing supplementation of dietary glycine (Gly) on jejunal morphology and tight junction protein-related gene expression in the jejunal mucosa of broiler chickens raised under heat stress conditions.[1]

Items	Dietary treatments[2]					SEM	*P*-value		
	PC	NC	0.4% Gly	0.8% Gly	1.6% Gly		Total[3]	Gly (linear)[4]	Gly (quadratic)[4]
Jejunal morphology[5]									
VH, μm	1,820[a]	1,577[bc]	1,521[c]	1,628[bc]	1,654[b]	41.0	<0.001	0.086	0.711
VW, μm	209	208	213	196	196	7.9	0.461	0.180	0.870
CD, μm	231	224	209	218	215	6.8	0.256	0.636	0.487
VH:CD ratio	7.87	7.08	7.30	7.50	7.69	0.207	0.080	0.047	0.666
Goblet cell, cells / 200 μm	16.36[a]	13.89[bc]	13.68[c]	13.94[bc]	15.11[ab]	0.471	0.001	0.029	0.228
Gene expression in the jejunal mucosa									
JAM-2	1.24	2.00	1.42	1.26	1.76	0.473	0.742	0.849	0.259
OCLN	0.89	1.03	1.02	0.91	1.01	0.211	0.980	0.899	0.738
ZO-1	1.20	1.23	1.10	1.08	1.67	0.242	0.432	0.191	0.239
MUC2	1.45	1.26	1.38	1.31	1.06	0.383	0.961	0.608	0.657

Abbreviations: CD, crypt depth; *JAM-2*, Junctional adhesion molecules-B; *MUC2*, Mucin 2; *OCLN*, Occludin; *ZO-1*, Zonula occludens-1; VH, villus height; VH:CD ratio, villus height to crypt depth ratio; VW, villus width.

[a-c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 8 observations per treatment.

[2]Dietary treatments: PC, positive control diet (NC + 50 kcal/kg AME_n + 10% higher digestible essential amino acids, total calcium, and available phosphorus); NC, negative control diet; 0.4% Gly, NC + 0.4% Gly; 0.8% Gly, NC + 0.8% Gly; 1.6% Gly, NC + 1.6% Gly.

[3]Overall effects of treatments.

[4]Linear and quadratic effects of increasing supplementation of Gly in diets without the data for PC treatment.

conditions require more energy and nutrients than those raised under thermoneutral conditions.

Additional Gly supplementation in poultry diets has recently gained an increasing attention despite Gly being classified as a non-essential AA for poultry. The possible reason is that Gly is required for the synthesis of vital biomolecules, such as collagen, creatine, porphyrins, glutathione, bile acids, nucleic acids, and uric acid (Graber and Baker, 1973; Corzo et al., 2004). Gly can also serve as a biological osmolyte and a donor of methyl groups, similar to the functions of dietary betaine (Eklund et al., 2005; Ratriyanto and Mosenthin, 2018). More importantly, the requirement of Gly in poultry may increase under stress conditions due to both increased demand for specific Gly functions and inadequate endogenous Gly synthesis (Nam et al., 2023; Won et al., 2023). Previous studies have reported that increasing Gly supplementation in diets increased BWG and decreased FCR in broiler chickens under HS conditions (Awad et al., 2017; Won et al., 2023). Consistently, the current study also revealed a linear decrease in FCR of broiler chickens under HS conditions in response to increasing supplementation of up to 1.6% Gly in diets. The reason for this beneficial effect may be that Gly requirement of broiler chickens is increased by HS conditions, such that additional Gly supplementation in diets may satisfy increased Gly requirement. Furthermore, additional Gly supplementation may preclude the use of Ser and Thr for endogenous Gly synthesis, thereby promoting the proper utilization of Ser and Thr for protein synthesis or other body functions (Siegert and Rodehutscord, 2019). In addition, similar to dietary Met, betaine, and choline, dietary Gly may also enhance methylation potentials for regulating lipid metabolism, inflammatory responses, and antioxidant systems in the body (Arumugam et al., 2021). However, it should be emphasized that decreased FCR in broiler chickens was primarily achieved by FI reduction in this study, which is inconsistent with previous findings (Awad et al., 2017; Won et al., 2023). However, in our previous study, increasing supplementation of dietary Gly decreased FCR, along with decreased FI and similar egg production in laying hens under HS conditions (Nam et al., 2023). Therefore, the reason for inconsistent results for FI in poultry as affected by dietary Gly supplementation remains unclear; nonetheless, it may be related to variations in the age and breeds of birds, the duration and extent of HS conditions, and other dietary components. Interestingly, the positive effect of dietary supplementation of 1.6% Gly on FCR was close to the effect of the PC diet, indicating that dietary supplementation of 1.6% Gly may be a potential alternative to conventional broiler diets with high energy and nutrients during hot seasons.

Meat quality is frequently compromised in broiler chickens exposed to HS because of changes in muscle glycogen metabolism and oxidative status (Lara and Rostagno, 2013; Wang et al., 2017). Therefore, a feasible solution to mitigate the negative impact of HS on broiler meat quality should be developed. Dietary Gly supplementation may be a candidate solution because Gly exhibits an osmoregulatory activity to prevent cellular water losses (Eklund et al., 2005; Ratriyanto and Mosenthin, 2018). Moreover, Gly may promote the synthesis of creatine, which has been reported to prevent rapid postmortem pH reduction (Young et al., 2004) and to control osmotic water losses in the muscle (Hultman et al., 1996). Dietary Gly supplementation has also been reported to exert an antioxidant effect, possibly through glutathione metabolism (Wang et al., 2013; Won et al., 2023) as well as improving methylation potentials (Arumugam et al., 2021). In this study, improved WHC in the breast meat was observed by increasing Gly supplementation in diets, suggesting that dietary Gly may increase water retention in the muscle of broiler chickens under HS conditions. However, our previous study demonstrated that dietary supplementation of 0.79% Gly had little impact on pH, WHC, and meat colors in the breast meat of broiler chickens under HS conditions (Won et al., 2023). Therefore, the influence of dietary Gly supplementation on meat quality in broiler chickens under HS conditions appears to be inconclusive. These variable results may be due to the relatively small impact of dietary functional nutrients on meat quality in broiler chickens because meat quality values measured in previous studies fell within normal ranges observed in the conventional broiler meat.

Blood AST and ALT concentrations are typically measured to analyze liver health status because those concentrations are elevated when liver cells are damaged or liver functions are impaired (Goorden et al., 2013). The HS has been reported to increase serum AST and ALT concentrations in broiler chickens (Kanani et al., 2016; Chen et al., 2020). In the current study, increasing supplementation of dietary Gly decreased serum AST and ALT concentrations, indicating that additional Gly supplementation may aid in maintaining liver health and function. Wang et al. (2019) also reported that mice fed diets supplemented with Gly had lower serum AST and ALT concentrations than those fed the control diet. Provided that Gly may display similar physiological functions to betaine (Eklund et al., 2005), dietary Gly supplementation may improve liver health by reducing lipid accumulations, enhancing anti-inflammatory responses, and up-regulating antioxidant systems (Arumugam et al., 2021). Moreover, additional Gly supply may facilitate glutathione synthesis because Gly is a precursor molecule for glutathione synthesis (Wang et al., 2013). Glutathione is an endogenous antioxidant required for the maintenance of intracellular homeostasis (McCarty et al., 2018). The presence of high amounts of glutathione has been reported to protect the liver against oxidative stressors by augmenting the activities of superoxide dismutase and catalase in the liver (Wang et al., 2013). Our findings that MDA concentrations in the liver were decreased by increasing supplementation of dietary Gly may also support a decrease in hepatic lipid peroxidation. In addition, osmoprotectants, such as betaine, sarcosine, and glycerophosphocholine were found to be decreased in birds exposed to HS

(Ratriyanto and Mosenthin, 2018). Those osmoprotectants are all precursor molecules for endogenous Gly synthesis (Grimble et al., 1992). Therefore, it can be inferred that additional Gly supplementation in diets may save the utilization of those osmoprotectants for endogenous Gly synthesis in broiler chickens under HS conditions. This saving may increase the availability of osmoprotectants in the liver exposed to HS. In addition, it appears that Gly itself can function as an osmolyte that mitigates cellular osmotic stress (Yancey, 2001).

Blood H:L ratio is the most broadly used as a stress indicator in poultry (Lee et al., 2022). An increase in blood H:L ratio has been observed in poultry raised under HS conditions (Nam et al., 2023; Won et al., 2023). Our results indicated that increasing supplementation of dietary Gly decreased blood H:L ratio in broiler chickens under HS conditions, reflecting the anti-stress effect of additional Gly supplementation in diets. A similar reduction in blood H:L ratio was also observed in our previous studies following dietary supplementation of 0.79% and 0.683% Gly for broiler chickens (Won et al., 2023) and laying hens (Nam et al., 2023), respectively, under HS conditions. Moreover, those previous studies also reported that feeding diets supplemented with Gly to broiler chickens and laying hens decreased feather corticosterone levels although significance was not detected (Nam et al., 2023; Won et al., 2023).

The Gly is a structural AA for uric acid synthesis in poultry (Siegert and Rodehutscord, 2019). Increasing oxidation of AA in broiler chickens under HS conditions is expected to facilitate ammonia production, eliciting a concomitant increase in uric acid production (Kim et al., 2022). Therefore, additional supply of dietary Gly may be required for broiler chickens under HS conditions. Interestingly, serum concentrations of uric acid exhibited a quadratic association with increasing supplementation of dietary Gly in this study. Specifically, serum concentrations of uric acid were decreased by 0.4% and 0.8% Gly supplementation, but then increased by 1.6% Gly supplementation. The clear reason is unknown; however, it appears that high supply of dietary Gly may augment uric acid production in the liver because of excess oxidation of Gly itself to ammonia.

Poultry exposed to HS often show a high occurrence of fatty liver due to increased fat synthesis and decreased fat removal in the liver (Inoue et al., 2006; Lu et al., 2019). In addition, poultry exposed to HS have been reported to experience inefficient fat utilization for energy production, resulting in increasing hepatic fat accumulations (Lu et al., 2019). This observation is likely due to up-regulation of genes related to fat synthesis and down-regulation of genes related to fat oxidation in the liver (Kim et al., 2022). Nam et al. (2023) reported that increasing supplementation of dietary Gly from 0.341% to 0.683% linearly decreased fatty liver incidence, hemorrhagic score, and total fat concentrations in the liver, revealing lipotropic effects of dietary Gly in laying hens. The reason for this result may be associated with methylation potentials of Gly because the sufficient supply of methyl groups in the liver is essential for synthesizing phosphatidylcholine and apolipoprotein B, which are required for lipid transfers from the liver to extrahepatic tissues (Eklund et al., 2005). In addition, Gly is the end-product of choline and betaine oxidation, such that additional Gly supply may enhance lipotropic effects by improving choline and betaine availability (Nam et al., 2023). However, the current study revealed no beneficial effects of increasing supplementation of dietary Gly on fatty liver-related characteristics in broiler chickens. Won et al. (2023) also found no significant effects of dietary supplementation of 0.79% Gly on liver characteristics of broiler chickens under HS conditions. The reason for this inconsistent result may be that broiler chickens are less susceptible to fatty liver development than laying hens (Zaefarian et al., 2019). Therefore, it is suggested that lipotropic effects of dietary Gly may be relatively smaller in broiler chickens compared with laying hens under HS conditions.

Increased intestinal permeability and impaired intestinal morphology are frequently observed in animals exposed to HS (Goo et al., 2019; Nanto-Hara et al., 2020). This negative outcome is linked to increasing diversion of blood flow from the intestine to the skin, which hinders the supply of nutrient and oxygen to intestinal cells (Lambert, 2009). A previous study reported that dietary Gly supplementation increased VH and goblet cell numbers in the jejunum of broiler chickens under HS conditions (Won et al., 2023). The similar positive effect of increasing supplementation of dietary Gly on VH, VH:CD, and goblet cell numbers was also observed in this study. The clear reason why additional Gly supplementation can improve intestinal morphology, especially for increased VH and goblet cell numbers, in broiler chickens under HS conditions is currently lacking. Given the fact that Gly is a major component of intestinal mucins and digestive secretions (Lensing et al., 2007), it can be inferred that providing additional Gly may facilitate mucin synthesis in the intestinal mucosa, thereby increasing VH and goblet cell numbers in broiler chickens under HS conditions (Won et al., 2023; Kim et al., 2024). However, the current study showed that none of Gly-supplemented diets exhibited a similar effect on intestinal morphology to PC diets with high energy and nutrients.

Expression levels of genes related to tight junction proteins in the jejunal mucosa were analyzed to determine the effect of increasing supplementation of dietary Gly on intestinal barrier functions. However, no significant changes in the expression levels of all measured genes were identified. Similar results for no benefits of dietary Gly supplementation on intestinal barrier functions in broiler chickens under HS conditions were observed in our previous study (Won et al., 2023), indicating little positive effects of additional Gly supplementation in diets on intestinal barrier functions. However, it should be noted that the current study only measured the expression levels of tight junction-related genes instead of directly measuring intestinal permeability. This limitation restricts the extent of further interpretation. Further studies are necessary to investigate the

effect of dietary Gly supplementation on intestinal barrier functions, particularly based on a direct assessment of intestinal permeation levels, in broiler chickens under HS conditions.

CONCLUSIONS

Increasing supplementation of dietary Gly up to 1.6% improved feed efficiency in broiler chickens raised under HS conditions. Furthermore, increasing supplementation of dietary Gly improved WHC in breast meat, liver health, and intestinal morphology possibly by mitigating oxidative stress and stress response. Compared with conventional diets containing high energy and nutrients for broiler chickens during hot seasons, dietary supplementation of 1.6% Gly exerted similar positive effects on feed efficiency in broiler chickens, but showed no comparable benefits on meat quality, liver characteristics, and intestinal morphology in broiler chickens raised under HS conditions.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Science, ICT, and Future Planning (NRF- 2019R1A2C4070556).

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Abdel-Moneim, A. M. E., A. M. Shehata, R. E. Khidr, V. K. Paswan, N. S. Ibrahim, A. A. El-Ghoul, S. A. Aldhumri, S. A. Gabr, N. M. Mesalam, A. M. Elbaz, M. A. Elsayed, M. M. Wakwak, and T. A. Ebeid. 2021. Nutritional manipulation to combat heat stress in poultry − a comprehensive review. J. Therm. Biol. 98:102915.

AOAC (Association of Official Analytical Chemists). 2006. Official methods of analysis of AOAC international. . 18th ed AOAC Int., Gaithersburg, USA.

Arumugam, M. K., M. C. Paal, T. M. Donohue Jr., M. Ganesan, N. A. Osna, and K. K. Kharbanda. 2021. Beneficial effects of betaine: a comprehensive review. Biology 10:456.

Attia, Y. A., and S. S. Hassan. 2017. Broiler tolerance to heat stress at various dietary protein/energy levels. Europ. Poult. Sci. 81:171.

Awad, E. A., I. Zulkifli, A. F. Soleimani, and A. Aljuobori. 2017. Effects of feeding male and female broiler chickens on low-protein diets fortified with different dietary glycine levels under the hot and humid tropical climate. Ital. J. Anim. Sci. 16:453–461.

Aznar, R., and B. Alarćon. 2002. On the specificity of PCR detection of *Listeria monocytogenes* in food: a comparison of published primers. Syst. Appl. Microbiol. 25:109–119.

Chen, Y., Y. Cheng, C. Wen, and Y. Zhou. 2020. Protective effects of dietary mannan oligosaccharide on heat stress−induced hepatic damage in broilers. Environ. Sci. Pollut. Res. Int. 27:29000–29008.

Choi, W. J., J. H. Kim, G. P. Han, C. H. Kwon, and D. Y. Kil. 2021. Effects of dietary hatchery by-products on growth performance, relative organ weight, plasma measurements, immune organ index, meat quality, and tibia characteristics of broiler chickens. Anim. Biosci. 34:1181–1192.

Chowdhury, V. S., S. Tomonaga, T. Ikegami, E. Erwan, K. Ito, J. F. Cockrem, and M. Furuse. 2014. Oxidative damage and brain concentrations of free amino acid in chicks exposed to high ambient temperature. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 169:70–76.

Corzo, A., M. T. Kidd, D. J. Burnham, and B. J. Kerr. 2004. Dietary glycine needs of broiler chicks. Poult. Sci. 83:1382–1384.

Deng, C., J. Zheng, H. Zhou, J. You, and G. Li. 2023. Dietary glycine supplementation prevents heat stress-induced impairment of antioxidant status and intestinal barrier function in broilers. Poult. Sci. 102:102408.

Diaz, G. J., E. J. Squires, and R. J. Julian. 1999. The use of selected plasma enzyme activities for the diagnosis of fatty liver-hemorrhagic syndrome in laying hens. Avian. Dis. 43:768–773.

Dos Santos, T. T., S. C. S. Baal, S. A. Lee, F. R. O. e Silva, M. Scheraiber, and A. V. F. da Silva. 2019. Influence of dietary fibre and betaine on mucus production and digesta and plasma osmolality of broiler chicks from hatch to 14 days of age. Livest. Sci. 220:67–73.

Eklund, M., E. Bauer, J. Wamatu, and R. Mosenthin. 2005. Potential nutritional and physiological functions of betaine in livestock. Nutr. Res. Rev. 18:31–48.

El-Naggar, K., S. El-Kassas, S. E. Abdo, A. A. K. Kirrella, and R. A. Al wakeel. 2019. Role of gamma-aminobutyric acid in regulating feed intake in commercial broilers reared under normal and heat stress conditions. J. Therm. Biol. 84:164–175.

Goo, D., J. H. Kim, G. H. Park, J. B. Delos Reyes, and D. Y. Kil. 2019. Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals 9:107.

Goorden, S. M., T. E. Buffart, A. Bakker, and M. M. Buijs. 2013. Liver disorders in adults: ALT and AST. Ned. Tijdschr. Geneeskd. 157:A6443.

Graber, G., and D. H. Baker. 1973. The essential nature of glycine and proline for growing chickens. Poult. Sci. 52:892–896.

Grimble, R. F., A. A. Jackson, C. Persaud, M. J. Wride, F. Delers, and R. Engler. 1992. Cysteine and glycine supplementation modulate the metabolic response to tumor necrosis factor α in rats fed a low protein diet. J. Nutr. 122:2066–2073.

Han, G. P., D. Y. Kim, K. H. Kim, J. H. Kim, and D. Y. Kil. 2023. Effect of dietary concentrations of metabolizable energy and neutral detergent fiber on productive performance, egg quality, fatty liver incidence, and hepatic fatty acid metabolism in aged laying hens. Poult. Sci. 102:102497.

He, S. P., M. A. Arowolo, R. F. Medrano, S. Li, Q. F. Yu, J. Y. Chen, and J. H. He. 2018. Impact of heat stress and nutritional interventions on poultry production. Worlds Poult. Sci. J. 74:647–664.

Hejdysz, M., J. Bogucha, E. Ziółkowska, K. Perz, Ł. Jarosz, A. Ciszewski, S. Nowaczewski, P. Ślósarz, and S. A. Kaczmarek. 2022. Effects of low crude protein content and glycine supplementation on broiler chicken performance, carcass traits, and litter quality. Livest. Sci. 261:104930.

Hultman, E., K. Soderlund, J. A. Timmons, G. Cederblad, and P. L. Greenhaff. 1996. Muscle creatine loading in men. J. Appl. Physiol. 81:232–237.

Inoue, H., N. Sameshima, T. Ishida, A. Tsuji, K. Kudo, and N. Ikeda. 2006. Vulnerability of experimentally induced fatty liver to heat stress in rats. J. Gastroenterol. 41:55–61.

Kanani, P. B., M. Daneshyar, and R. Najafi. 2016. Effects of cinnamon (Cinnamomum zeylanicum) and turmeric (Curcuma longa) powders on performance, enzyme activity, and blood parameters of broiler chickens under heat stress. Poult. Sci. J. 4:47–53.

Kim, D. Y., B. Lim, J.-M. Kim, and D. Y. Kil. 2022. Integrated transcriptome analysis for the hepatic and jejunal mucosa tissues of broiler chickens raised under heat stress conditions. J. Anim. Sci. Biotechnol. 13:79.

Kim, H. W., J. H. Kim, G. P. Han, and D. Y. Kil. 2024. Increasing concentrations of dietary threonine, tryptophan, and glycine improve growth performance and intestinal health with decreasing stress responses in broiler chickens raised under multiple stress conditions. Anim. Nutr. 18:145–153.

Kim, M. C., J. H. Kim, F. M. Pitargue, D. Y. Koo, H. S. Choi, and D. Y. Kil. 2017. Effect of dietary b-mannanase on productive performance, egg quality, and utilization of dietary energy and nutrients in aged laying hens raised under hot climatic conditions. Asian-Australas. J. Anim. Sci. 30:1450–1455.

Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J. Anim. Sci. 87:E101–E108.

Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. Animals 3:356–369.
Lee, C., J. H. Kim, and D. Y. Kil. 2022. Comparison of stress biomarkers in laying hens raised under a long-term multiple stress condition. Poult. Sci. 101:101868.
Lensing, M., J. D. Van Der Klis, L. L.e Bellego, and M. Rovers. 2007. The Threonine Requirement of Broiler Chickens During Subclinical Intestinal Infection 343–346.
Lentfer, T. L., H. Pendl, S. G. Gebhardt-Henrich, E. K. F. Fröhlich, and E. Von Borell. 2015. H/L ratio as a measurement of stress in laying hens − methodology and reliability. Br. Poult. Sci. 56:157–163.
Li, P., and G. Wu. 2017. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino acids 50:29–38.
Liu, L., M. Ren, K. Ren, Y. Jin, and M. Yan. 2020. Heat stress impacts on broiler performance: a systematic review and meta-analysis. Poult. Sci. 99:6205–6211.
Lu, Z., X. F. He, B. B. Ma, L. Zhang, J. L. Li, Y. Jiang, G. H. Zhou, and F. Gao. 2019. Increased fat synthesis and limited apolipoprotein B cause lipid accumulation in the liver of broiler chickens exposed to chronic heat stress. Poult. Sci. 98:3695–3704.
McCarty, M. F., J. H. O'Keefe, and J. J. DiNicolantonio. 2018. Dietary glycine is rate-limiting for glutathione synthesis and may have broad potential for health protection. Ochsner J 18:81–87.
Meléndez-Hevia, E., P. de Paz-Lugo, A. Cornish-Bowden, and M. L. Cárdenas. 2009. A weak link in metabolism: the metabolic capacity for glycine biosynthesis does not satisfy the need for collagen synthesis. J. Biosci. 34:853–872.
Nam, J. H., G. P. Han, D. Y. Kim, C. H. Kwon, and D. Y. Kil. 2023. Effect of dietary glycine supplementation on productive performance, egg quality, stress response, and fatty liver incidence in laying hens raised under heat stress conditions. Poult. Sci. 102:103101.
Nanto-Hara, F., M. Kikusato, S. Ohwada, and M. Toyomizu. 2020. Heat stress directly affects intestinal integrity in broiler chickens. J. Poult. Sci. 57:284–290.
Nari, N., H. A. Ghasemi, I. Hajkhodadadi, and A. H. Khaltabadi Farahani. 2020. Intestinal microbial ecology, immune response, stress indicators, and gut morphology of male broiler chickens fed low-phosphorus diets supplemented with phytase, butyric acid, or *Saccharomyces boulardii*. Livest. Sci. 234:103975.
Onagbesan, O. M., V. A. Uyanga, O. Oso, K. Tona, and O. E. Oke. 2023. Alleviating heat stress effects in poultry: updates on methods and mechanisms of actions. Front. Vet. Sci. 10:1255520.
Ratriyanto, A., and R. Mosenthin. 2018. Osmoregulatory function of betaine in alleviating heat stress in poultry. J. Anim. Physiol. Anim. Nutr. 102:1634–1650.
Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. de Oliveria, D. C. Lopes, A. S. Ferreira, S. L. T. Barreto, and R. F. Euclides. 2011. Brazilian Tables for Poultry and Swine: Composition of Feedstuffs and Nutritional Requirements. 3rd ed. Universidade Federal de Vicosa, Departamento de Zootecnia, Vi cosa MG, Brazil.
Siegert, W., and M. Rodehutscord. 2019. The relevance of glycine and serine in poultry nutrition: a review. Br. Poult. Sci. 60:579–588.
Thomsen, R., C. A. E. Sølvsten, T. E. Linnet, J. Blechingberg, and A. L. Nielsen. 2010. Analysis of qPCR data by converting exponentially related Ct values into linearly related X0 values. J. Bioinform. Comput. Biol. 8:885–900.
Wang, M., F. Yuan, H. Bai, J. Zhang, H. Wu, K. Zheng, W. Zhang, M. Miao, and J. Gong. 2019. SHMT2 promotes liver regeneration through glycine-activated Akt/mTOR pathway. Transplantation 103:e188–e197.
Wang, R. H., R. R. Liang, H. Lin, L. X. Zhu, Y. M. Zhang, Y. W. Mao, P. C. Dong, L. B. Niu, M. H. Zhang, and X. Luo. 2017. Effect of acute heat stress and slaughter processing on poultry meat quality and postmortem carbohydrate metabolism. Poult. Sci. 96:738–746.
Wang, W., Z. Wu, Z. Dai, Y. Yang, J. Wang, and G. Wu. 2013. Glycine metabolism in animals and humans: implications for nutrition and health. Amino acids 45:463–477.
Won, S. Y., G. P. Han, C. H. Kwon, E. C. Lee, and D. Y. Kil. 2023. Effect of individual or combination of dietary betaine and glycine on productive performance, stress response, liver health, and intestinal barrier function in broiler chickens raised under heat stress conditions. Poult. Sci. 102:102771.
Yancey, P. H. 2001. Water stress, osmolytes and proteins. Am. Zool. 41:699–709.
Young, J. F., A. H. Karlsson, and P. Henckel. 2004. Water-holding capacity in chicken breast muscle is enhanced by pyruvate and reduced by creatine supplements. Poult. Sci. 83:400–405.
Yu, D. G., N. Namgung, J. H. Kim, S. Y. Won, W. J. Choi, and D. Y. Kil. 2021. Effects of stocking density and dietary vitamin C on performance, meat quality, intestinal permeability, and stress indicators in broiler chickens. J. Anim. Sci. Technol. 63:815–826.
Zaboli, G., X. Huang, X. Feng, and D. U. Anh. 2019. How can heat stress affect chicken meat quality? − a review. Poult. Sci. 98:1551–1556.
Zaefarian, F., M. R. Abdollahi, A. Cowieson, and V. Ravindran. 2019. Avian liver: the forgot organ. Animals 9:63.