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Research Article

Pre-hatch thermal manipulation and post-hatch guanidinoacetic acid supplementation enhance meat quality under chronic heat stress in broiler chickens

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Abstract This study aimed to evaluate the effects of pre-hatch thermal manipulation (TM) and post-hatch guanidinoacetic acid (GAA) supplementation on meat quality in male broiler chickens exposed to chronic heat stress (CHS). A total of 710 Ross 308 eggs were incubated under two conditions: a control group maintained at 37.8°C with 56% relative humidity and a TM group (39.5°C and 65% RH for 12 h/day, from embryonic days 7 to 16). After hatching, chicks were divided into four experimental groups (n=12 birds/pen; 5 replicates/treatment): control (C), control with 1.2 g/kg GAA supplementation (GAA), pre-hatch TM (TM), and TM with 1.2 g/kg GAA supplementation (GT). All birds were exposed to heat stress conditions (32-36°C, 6 h/day) from days 28 to 42. Experimental treatments significantly influenced the ultimate pH, lightness (L), and redness (a) values of the pectoral ($p \leq 0.05$). The GT and Control groups exhibited the highest and lowest ultimate pH, respectively. In the Texture analysis, the GT group breasts had a higher juiciness compared to the control, ($p=0.0002$), while other sensory parameters remained unaffected. GT and GAA increased the pectoralis major yield (1.58%). No significant differences were observed in myopathy rates among treatments. These findings suggest that pre-hatch TM and post-hatch GAA supplementation improve meat quality attributes, including pectoralis major yield, juiciness, and color, in broilers under CHS.

Keywords broiler, guanidinoacetic acid, chronic heat stress, thermal manipulation, meat quality

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1. Introduction

The poultry industry has recently been regarded as a promising and rapidly growing sector capable of meeting the increasing demand for animal protein in developing countries (Ayssiwede et al., 2009). However, certain limiting factors constrain the development of this industry. Heat stress is among the most significant challenges confronting the poultry industry, especially in broiler production, due to the intensifying effects of climate change driven by greenhouse gas emissions (Renaudeau et al., 2011). This issue is further exacerbated by projections indicating a global surface temperature increase of between 1.4 and 5.8°C from 1990 to 2100 (Houghton, 2001). Moreover, genetic selection and improvements in nutrition and management of broilers for rapid growth in recent decades have increased susceptibility to heat stress, reducing their resilience to high temperatures. While economically important organs, such as the breast and thighs, have enlarged due to a higher metabolic rate, internal organs (including the cardiovascular and respiratory systems) have not grown proportionally, limiting their ability to manage heat stress effectively (Havenstein et al., 2003; Zaboli et al., 2019). Additionally, chickens are highly vulnerable to heat stress (HS) due to their narrow thermoneutral zone (16-25℃), a lack of sweat glands, and insulating feathers (Al Amaz et al., 2024). Heat stress can compromise broiler performance, leading to reduced growth rates, impaired feed conversion efficiency, economic losses and increased mortality (Yahav, 2004). Furthermore, it negatively impacts the quality of broiler meat by altering metabolic processes and muscle physiology. Elevated temperatures lead to increased oxidative stress and reduced muscle glycogen reserves, resulting in pale, soft, and exudative (PSE) meat. Additionally, heat stress impairs protein synthesis and accelerates proteolysis, compromising texture, water-holding capacity, and overall meat quality. It also lowers the pH of meat, affecting its taste and flavor profile (Zaboli et al., 2019). Furthermore, this phenomenon negatively impacts meat quality traits such as tenderness, water-holding capacity, and oxidative stability, resulting in economic losses for producers and reduced consumer satisfaction (Lara and Rostagno, 2013). With the increasing frequency and intensity of heat waves globally, coupled with the growth improvement of modern broilers to heat susceptibility, there is an urgent need for effective strategies to mitigate the adverse effects of thermal stress.

Several strategies have been developed to alleviate the negative impacts of heat stress on broilers. These include genetic, technical, or Feeding and environmental strategies such as improved ventilation, evaporative cooling systems, and shading (Renaudeau et al., 2011). Nutritional interventions have also been widely investigated, focusing on dietary supplementation with antioxidants, vitamins, and minerals to enhance the birds' resilience to heat stress (Zhang et al., 2012). Heat acclimation is an approach that could improve the thermotolerance of fast-growing broilers (Meteyake et al., 2020).

One promising nutritional strategy involves supplementing guanidinoacetic acid (GAA) in broiler diets. GAA, a precursor of creatine, plays a pivotal role in energy metabolism by enhancing cellular energy availability, particularly under stressful conditions (Peng et al., 2022). It is commercially available as a feed additive. It is more efficient than arginine or creatine supplementation because it is less expensive and chemically more stable than creatine. Furthermore, GAA also saves Arg and Gly for other metabolic pathways (Esser et al., 2017). Studies have demonstrated that GAA supplementation can improve growth performance, muscle energy status, and overall breast meat quality in broilers (Michiels et al., 2012; Khajali et al., 2020). The mechanism underlying these benefits includes improved mitochondrial function, reduced oxidative stress, and enhanced intestinal function. Furthermore, GAA's ability to support energy-demanding processes makes it a potential candidate for mitigating heat stress-induced losses in broiler production (Majdeddin et al., 2020). A study by Majdeddin et al. (2020) demonstrated that GAA not only enhances the antioxidant status of birds but also improves meat quality by reducing oxidative stress, a key factor affecting muscle tissue during heat stress. Additionally, Michiels et al. (2012) found that supplementation of the diet with GAA at concentrations of 0.6 and 1.2 g/kg significantly enhances performance and carcass characteristics, specifically in relation to the gain-to-feed ratio and breast meat yield. Oviedo-Rondón and Córdova- Noboa. (2020) demonstrated that supplementing with GAA (600 g/ton) improved breast meat yield and ameliorated WB myopathy in male Ross 708 broilers. Another innovative approach is embryo thermal manipulation (TM) during incubation. ETM involves exposing broiler embryos to mild, controlled thermal stress during critical developmental stages to induce adaptive physiological changes that enhance thermotolerance post-hatch (Piestun et al., 2008). Although variations in the thermal manipulation (TM) protocol are prevalent in the literature, exposure to 39.5℃ for 12 h daily from embryonic day 7 (E7) to embryonic day 16 (E16) consistently yields the most significant improvements in post- hatch thermotolerance without compromising hatchability, chick quality, or bird performance (Collin et al., 2005; Piestun et al., 2008; Piestun et al., 2015). Studies have proposed that pre-hatch thermal manipulation (TM) may improve the long-term thermotolerance of broiler chickens by reducing body temperature through decreased plasma thyroid hormone levels (Morita et al., 2016). Furthermore, TM can change the adrenal threshold response to stress, resulting from lower corticosterone secretion (Piestun et al., 2008). TM can affect epigenetically and change the pituitary-adrenal-hypothalamic threshold. This technique has shown potential for improving growth, feed conversion, and breast development, as well as decreasing mortality (Yahav et al., 2009). Loyau et al. (2013) conducted a study to investigate the effects of thermal manipulation (TM) on broiler chickens. The findings indicated that the application of this technique during the pre-hatch period resulted in a reduction of body temperature and T3 levels, which are indicative of thermotolerance. Additionally, TM was found to enhance breast meat yield and decrease

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abdominal fat; The increase in breast yield reported is likely due to myoblast proliferation, as indicated by Piestun et al. (2015). Yalçin et al. (2022) observed reduced meat drip loss, finding lower drip loss in the breast meat of broilers exposed to 39.6℃ from embryonic day 10 to 18. Meat pH is related to water-holding capacity (Jung et al., 2010), which is positively correlated with the texture, juiciness, and flavor of the meat. Meteyake et al. (2022) demonstrated that TM reduced drip loss, increased the ultimate pH of the meat, and subsequently improved the processing ability of the meat. Moreover, Yalçin et al. (2022) reported that an increase in incubation temperature led to an increase in pH24.

The integration of nutritional strategies, such as GAA supplementation, with innovative techniques like TM represents a novel approach to improving broiler performance and meat quality under chronic heat stress conditions. While each method offers unique benefits, their combined application has the potential to yield synergistic effects. For instance, the enhanced thermotolerance induced by TM may complement the metabolic and muscle quality improvements associated with GAA, resulting in more robust birds that can maintain productivity and meat quality in high-temperature environments. Exploring the combined effects of these strategies could pave the way for a comprehensive solution to one of the poultry industry's most pressing challenges. Based on this theory, a research project was planned as part of a broader study initially presented by Zaboli and Rahmatnejad (2024). While the first article covered performance, blood profile, and intestinal morphology, the present work focuses on meat quality.

Thus, the present study aims to determine the response of pre-hatch thermal manipulation and post-hatch GAA supplementation, and their combination, on meat quality in broilers subjected to chronic heat stress. Key meat quality parameters, including slaughter, sensory, myopathy, and rheological variables, will be assessed to evaluate the efficacy of these interventions. The findings from this research will provide valuable insights into practical strategies for enhancing broiler resilience to heat stress and improving meat quality, thereby supporting the sustainability and productivity of the poultry industry in the face of the challenges posed by global warming.

2. Materials and methods

2.1. Experimental design and sample collections

A total of 710 fertile Ross 308 eggs, each weighing an average of 65±2 g, were obtained from a commercial hatchery in Mazandaran, Iran. These eggs were sourced from a single breeder flock of hens at 35 weeks of age, during their optimal period of egg production. All eggs were randomly placed into a single incubator (Nowin Sanat Khazar Company, Iran) and incubated at a standard temperature of 37.8℃ and 56% relative humidity (RH) for the first 7 embryonic days (ED). On ED 7, infertile and undeveloped eggs were identified and removed through candling. According to Zaboli et al. (2017), on ED 7, the remaining eggs were divided into two incubation groups: 1) control (37.8℃ and 56% RH), and 2) TM group (39.5℃ and 65% RH, 12 h/day, from ED 7 to 16). From ED 16 to 21, all eggs were transferred to the same hatcher at 37.8℃ and 56% RH. After hatching, male day-old chicks were equally divided into two primary groups: 1) control and 2) TM. Four treatments were administered, with two subgroups from each control and TM group at hatch. The post-hatch treatments were: 1) control: birds receiving basal diets with eggs incubated under standard conditions, 2) GAA (control supplement): control group supplemented with GAA (1.2 g/kg), 3) TM (thermal manipulation): birds receiving basal diets with eggs incubated under pre-TM conditions, and 4) GT (thermal manipulation supplement): TM group supplemented with GAA (1.2 g/kg).

A total of 240 male chicks were individually weighed and randomly placed into 20 pens, with 12 birds per pen. This setup yielded 5 replicates for each treatment group, comprising 60 birds per treatment. The control and TM groups were fed the same corn-soybean meal diets, including starter (days 1-10), grower (days 11-24), and finisher (days 25-42) diets, as recommended by the Ross Management Guide (Table 1). The GAA and GT groups were fed basal diets supplemented with GAA at 1.2 g/kg of diet. To induce heat stress (HS) from days 28 to 42, all birds in the experimental units were exposed to temperatures of 32 to 36℃ and 55% RH between 10:00 AM and 4:00 PM, with overnight temperatures maintained at 28±2℃ and 46±5% RH. The transition from 28 to 32℃ took approximately 30 min on average. Temperature and RH were measured three times a day at 8:00 AM, 3:00 PM, and 7:00 PM. The chicks were reared in a litter floor

Table 1. Ingredient and nutrient composition of basal diets for the starter (d 0-10), grower (d 11-25), and finisher (d 25-42) phases[1)]

Item	Strater	Grower	Finisher
Ingredient (g/kg)			
Corn	565.0	585.8	635.0
Soybean meal	372.4	348.2	295.2
Corn oil	16.6	27.8	33.9
Dicalcium phosphate	18.3	16.3	14.6
Limestone	8.7	7.8	7.4
Sodium chloride	3.0	3.0	3.0
Sodium bicarbonate	1.0	1.0	1.0
L-Lysine-HCl	2.3	1.5	1.6
dl -Methionine	3.4	2.8	2.6
L-Threonine	1.3	0.8	0.7
Vitamin and mineral premix[2)]	5.0	5.0	5.0
Calculated nutrient composition (g/kg)			
ME (kcal/kg)	2,900	3,000	3,100
Crude protein	220	208	189
Calcium	9.3	8.4	7.8
Available phosphorus	4.6	4.2	3.8
Sodium	1.6	1.6	1.6
Lysine	12.4	11.1	10.0
Methionine	6.3	5.6	5.2
Methionine+cystine	9.2	8.4	7.8
Threonine	8.3	7.5	6.7
Arginine	10.3	10.2	10.1

[1)] The basal diet was supplemented with guanidinoacetic acid (GAA) at 1.2 g/kg of diet to make the respective experimental diets (Zaboli and Rahmatnejad, 2024).

[2)] Suppliedas retinyl acetate, 9,000 IU; cholecalciferol, 2,000 IU; dl-α-tocopherylacetate, 12.5 IU; menadione sodium bisulfite, 1.76 mg; choline chloride, 320 mg; nicotinic acid, 28 mg; calcium d-pantothenate, 6.4 mg; riboflavin, 3.2 mg; pyridoxine, 1.97 mg; thiamine, 1.2 mg; folic acid, 0.38 mg; biotin, 0.12 mg; cyanocobalamin, 0.01 mg; $FeSO_4 \cdot 7H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 60 mg; ZnO, 51.74 mg; $CuSO_4 \cdot 5H_2O$, 8 mg; iodized NaCl, 0.8 mg; Na_2SeO_3, 0.2 mg/kg ofdiet.

pen system following the Ross 308 broiler rearing and management guidelines. Water and feed were provided ad libitum, and standard lighting was used (23 h light: 1 h dark until day 7, followed by 20 h light: 4 h dark from day 8 to 42). On day 42, two birds from each replicate were randomly selected, stunned by an electrical stunner (40 V alternating current, 400 Hz for 5 seconds), and slaughtered within 5 min via exsanguination from the left jugular vein. Within 15 min postmortem, the entire left pectoralis major (breast) was sampled for Sensory Evaluation and stored at 4℃ for determination of pH, meat color, cook loss, and shear force value. The right breast was sampled to determine the moisture, protein, ash, and ether extract content. Additionally, after feathering, the slaughter yield, as well as the yields of the pectoralis major, wings, thighs, drumsticks, and breast, were measured.

2.2. Rheological properties, lipid oxidation, and chemical composition

The muscles were stored at 4℃ for 24 h postmortem, then cooked in a water bath at 80℃ in plastic bags until they reached an internal temperature of 72℃ for 30 min. The cooked samples were cooled under cold running tap water for 20 minutes before being blotted and weighed. Mass changes were expressed as a percentage of the initial mass (Zhang et al., 2012). Samples used for cook loss analysis were also utilized to measure shear force. The shear force value was determined using a digital meat tenderness meter (model C-LM3, Northeast Agricultural University, Harbin, China), as described by Chen et al. (2007). Each sample was measured three times, and the average of these readings was taken as the shear force value, expressed in Newtons. The moisture, protein, Ash and ether extract contents of the samples were analyzed according to the official standard methods of analysis (AOAC, 1990). The moisture content was determined by heating the samples in an oven at 85℃ until a constant weight was achieved. Protein content was measured using a micro-Kjeldahl apparatus, and total ether extract content was determined by ether extraction for 16 h.

Muscle pH was measured using a portable pH meter (Testo 205, Germany). The pH meter was calibrated using a two-point method with standard buffer solutions at pH levels of 4.0 and 7.0. The pH value was expressed as the average of three measurements (Hao et al., 2014). At 24 h postmortem, meat color was measured with a Chroma meter CR-410 (Minolta, Suita-shi, Japan). Color measurements were taken on the dorsal side of the breast fillet at three locations. Color was reported in the CIE-LAB trichromatic system as L*, a*, and b* values (Zhang et al., 2012).

Thiobarbituric acid reactive substances (TBARS) were

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measured according to the method suggested by the Turkish Standards Institute 2409 (2007). In this regard, 10 g of the homogenised sample was broken into pieces and mixed with 50 mL of distilled water. This mixture was washed with 47.5 ml of distilled water, poured into a bowl, and 2.5 mL of hydrochloric acid was added to adjust the pH to 1.5. This bowl was then placed on a distillation unit, and 50 mL of distillate was produced. 3 distillates of 5 mL were prepared. Five mL of a 0.02 M thiobarbituric acid standard solution, prepared with 90% glacial acetic acid, was added to each tube. Then the tubes were placed in a double boiler for 35 s. Five mL of distilled water and 5 mL of TBA (Thiobarbituric acid) standard solution were prepared as a blank and underwent the same procedures. The tubes were then cooled. The optic density of the solution in each of the three tubes was measured with a spectrometer adjusted to a wavelength of 538 nanometres against the blank. Averages were calculated as follows. The TBA amount in the sample is expressed as micrograms of malondialdehyde per gram (μg MA/g).

$$TBA = a \times 7 : 8$$

where, a: absorbance of the sample measured by a spectrophotometer.

2.3. Breast myopathies

Carcasses were evaluated through gross examination to assess the presence and severity of breast myopathies, including white striping (WS), wooden breast (WB), and spaghetti meat (SM). The degree of WS on the *pectoralis major* muscle was classified as normal, moderate, or severe based on established criteria (Kuttappan et al., 2012). Additionally, the presence or absence of WB and SM was determined following the methodologies described by Sihvo et al. (2014) and Baldi et al. (2018), respectively.

2.4. Meat sensory evaluation

Sensory Evaluation of Poultry Meat: The pectoralis major muscles were evaluated for sensory attributes using a quantitative descriptive analysis (QDA) methodology. The sensory evaluation followed the protocol outlined by Huerta et al. (2023) and was conducted by a trained panel under controlled conditions. The evaluation focused on 11 sensory descriptors, assessed on a structured, continuous scale ranging from 0 (not intense) to 10 (very intense). Twelve individuals (6 men and 6 women, aged 23 to 60) were selected from the trained panel in accordance with ISO standards (ISO 8586; ISO 3972; ISO 5496). Prior to testing, panelists completed a comprehensive 1-month training program. All experimental procedures were reviewed and approved by the Ethics Committee of the University of Zabol under the protocol number IR.UOZ.REC.1398.4. In the initial phase of training, the panelists were familiarized with the product by testing chicken breasts prepared through various cooking methods. This phase allowed the panelists to identify and agree upon key sensory attributes, providing exposure to a wide range of variations for each descriptor. Once the group had established a common vocabulary and refined the evaluation protocol, the second phase of training emphasized quantitative assessments using the intensity scale (0-10). To ensure unbiased sensory evaluations, panelists adhered to strict guidelines, including refraining from smoking, eating, or drinking for at least 1 h prior to tasting sessions. Over two weeks, the panelists conducted evaluations during four separate sessions. All evaluations took place at 10:00 AM in the sensory testing facility at DAFNAE. During each session, panelists assessed two sets of three chicken breast samples, representing different experimental groups, with a 15-min break between the two sets. By the end of the four sessions, each panelist had evaluated a total of 24 samples (2 samples per experimental group per session). The evaluation sequence began with odor attributes (e.g., brothy, chickeny/meaty, wet feathers), followed by taste (e.g., sweetness, saltiness), and texture (e.g., cohesiveness, hardness, juiciness, chewiness, toothpack). Finally, panelists assessed overall pleasantness. To minimize carryover effects, the panelists cleansed their palates between samples using a combination of an apple slice, an unsalted cracker, and water, followed by a two-min pause before proceeding to the next sample. Each tested sample consisted of a cooked chicken breast piece measuring 3 × 2 × 1 cm. Sample Preparation for Sensory Evaluation: Chicken breast samples were prepared following standardized protocols. Frozen chicken breasts were thawed overnight at 4°C before processing. Samples were vacuum-sealed in bags and cooked *sous vide* at a constant temperature of 85°C for 25 min. Post-cooking, each meat portion was divided into four equal pieces (3 × 2 × 1 cm) and immediately served to the panelists.

2.5. Statistical analysis

The experimental data were assessed for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and for homogeneity of variances using Levene's test. Data analysis was conducted using the GLM procedure of SAS (SAS Institute, 2013), in accordance with a completely randomized design. Duncan's multiple comparison test was applied to compare the treatment means at a significance level of 5%. The results are reported as mean values with their respective standard errors. The occurrence of myopathies was analyzed with the PROC CATMOD (SAS Institute Inc., 2013).

3. Results and discussion

3.1. Rheological properties, lipid oxidation, and chemical composition

As presented in Table 2, The pH value of the pectoralis major muscle exhibited a significant increase in all treatment groups compared to the control group (p=0.0202), with GAA and GT demonstrating increases of 1.62% and 3.60%, respectively. The L* values revealed notable differences among the groups (p=0.0552), with a numerical increase observed in all groups. Furthermore, the a* values showed a significant effect (p<0.0001), with the lowest values recorded in the Control group and the highest in the GT group. Cooking losses and TBARS were not significantly affected by the treatments (p>0.05), indicating a stable lipid oxidation status. Additionally, no significant differences were detected in the chemical composition (dry matter, crude protein, ether extract, or ash) across the groups (p>0.05).

3.2. Slaughter results and carcass characteristics

Table 3 presents a summary of the effects of various treatments on slaughter parameters. The cold carcass weight, slaughter yield, and breast yield did not exhibit significant differences (p>0.05). However, the percentage of Pectoralis major was significantly influenced by the treatments, with the highest percentage observed in the GT group and the lowest in the control group. Furthermore, the percentage of Pectoralis major was significantly greater in the GAA and GT groups compared to the control group (p=0.0091). No significant differences were noted for wings, thighs, drumsticks, or hind legs (p>0.05). These findings indicate that most parameters remained unaffected, with the exception of the *Pectoralis*

Table 2. Rheological properties, lipid oxidation (TBARS), and chemical composition of the pectoralis major muscle in male broiler chickens at 42 days of age, according to treatment[1)]

Variables	Control	GAA	TM	GT	p-value	SEM[2)]
pH	5.46±0.003[3)a4)]	5.64±0.03[b]	5.55±0.12[b]	5.75±0.05[b]	0.02	0.02
L*	53.00±0.71	50.50±2.06	51.00±0.71	51.33±21.59	0.05	0.31
a*	0.75±0.02[c]	0.92±0.02[b]	0.88±0.01[b]	1.10±0.06[a]	<0.001	0.001
b*	13.73±0.15	13.40±1.07	13.75±0.05	13.00±0.97	0.69	0.16
Cooking losses (%)	41.88±1.02	43.50±1.12	42.50±1.12	43.33±1.11	0.06	0.24
Shear force (kg/g)	4.80±0.58	4.48±0.37	4.85±0.54	4.70±0.32	0.53	0.10
TBARS (mg MDA/kg)	0.16±0.02	0.14±0.03	0.13±0.01	0.14±0.01	0.17	0.001
Dry matter (%)	22.75±0.28	23.00±1.48	22.50±2.12	22.47±1.19	0.83	0.32
Water (%)	77.28±1.48	77.25±1.22	77.00±1.12	77.53±0.63	0.98	0.25
Crude protein (%)	19.48±0.28	19.38±0.19	19.58±0.19	19.50±0.36	0.66	0.05
Ether extract (%)	2.24±0.01	2.27±0.03	2.23±0.02	2.25±0.04	0.12	0.001
Ash (%)	1.06±0.03	1.07±0.03	1.04±0.04	1.05±0.01	0.450	0.007

[1)]Control, birds receiving basal diets with eggs incubated under standard conditions; GAA (control supplement), control group supplemented with GAA (1.2 g/kg); TM (thermal manipulation), birds receiving basal diets with eggs incubated under pre-TM conditions; and GT (thermal manipulation supplement), TM group supplemented with GAA (1.2 g/kg). All treatments were subjected to chronic heat stress from 28 to 42 days of age.
[2)]SEM, standard error of the mean.
[3)]All values are mean±SD (n=40).
[4)]Different superscript letters in the same row indicate significant differences at (p<0.05).

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Table 3. Slaughter results and carcass traits in male broiler chickens at 42 days of age, according to treatment[1)]

Variables	Control	GAA	TM	GT	p-value	SEM[2)]
Cold carcass weight (g)	1956.25±14.74[3)]	1951.25±18.83	1953.75±37.67	1947.50±28.61	0.86	5.93
Slaughter yield (%)	73.75±3.03	74.00±3.94	73.88±1.80	73.25±2.77	0.92	0.67
Breast yield (% CC[4)])	37.50±2.29	38.25±1.09	37.88±2.92	37.00±4.06	0.87	0.63
Pectoralis m (% CC)	27.25±0.11^{b}	27.68±0.15^{a}	27.46±0.46^{ab}	27.86±0.04^{a}[5)]	0.01	0.05
Wings (% CC)	9.38±0.41	9.33±0.15	9.35±0.11	9.40±0.16	0.85	0.05
Thighs (% CC)	15.38±0.74	15.25±0.78	15.31±0.53	15.23±0.15	0.95	0.14
Drumsticks (% CC)	13.35±0.42	13.25±0.23	13.30±0.23	13.28±0.18	0.67	0.06
Hind legs (% CC)	28.73±0.68	28.50±1.10	28.61±0.51	28.50±0.22	0.83	0.16

[1)]Control, birds receiving basal diets with eggs incubated under standard conditions; GAA (control supplement), control group supplemented with GAA (1.2 g/kg); TM (thermal manipulation), birds receiving basal diets with eggs incubated under pre-TM conditions; and GT (thermal manipulation supplement), TM group supplemented with GAA (1.2 g/kg). All treatments were subjected to chronic heat stress from 28 to 42 days of age.
[2)]SEM, standard error of the mean.
[3)]Values are mean±SD (n=40).
[4)]% CC, percentage of cold carcass weight
[5)]Different superscript letters in the same row indicate significant differences at ($p<0.05$).

major.

3.3. Breast myopathies

The data presented in Table 4 indicate that no significant differences were observed among the experimental groups (Control, GAA, TM, and GT) regarding the incidence of breast myopathies, which include moderate and severe white striping, wooden breast, and spaghetti meat ($p>0.05$). These findings suggest that the treatment manipulation and GAA supplmentation did not have a measurable impact on these parameters.

3.4. Sensory analysis of broiler breast meat

The sensory characteristics of broiler breast (Pectoralis major) were assessed with respect to texture-related parameters, which included cohesiveness, hardness, juiciness, chewiness, and toothpack scores. Additionally, flavor-related attributes were evaluated, encompassing brothy, chickeny/meaty, wet feathers, sweet, salty, and overall pleasantness. The findings of this analysis are detailed in Table 5.

No significant differences were observed in the majority of flavor and texture attributes across the various treatments, except for juiciness. The analysis of variance indicated that the treatment had a statistically significant effect on juiciness ($p<0.0002$). All treatment groups showed enhanced juiciness scores compared to the control group. Specifically, the control treatment recorded the lowest juiciness score (5.32), whereas the GT group achieved the highest score (5.73).

In this study, it was shown that thermal treatment and supplementation with GAA acid together led to an increase in the pH level of the meat, as well as an increase in the a*, compared to the control group. The GT exhibited the highest pH level and redness a* index. However, these improvements were observed in both TM and GAA treatments, with the maximum improvements occurring in the GT group, which used post-hatch supplementation of GAA combined with thermal manipulation during the pre-hatch period. In the current study, the GAA-supplemented group exhibited greater redness, which may be attributed to the GAA-induced enhancement of muscle pH.

The increase in pH observed in our study alligns with the findings of Yalçin et al. (2022) and Meteyake et al. (2020), who reported that thermal manipulation during the pre-hatch period resulted in an increase in pH at 24 h post-mortem. Some researchers have, however, reported no changes in meat quality characteristics as a result of thermal manipulation (Zhang et al., 2012; Loyau et al., 2013). Given the increase in pH and improvement the redness index observed in the GT group, it can be hypothesized that the synergistic effect of guanidine and thermal manipulation may be responsible for these incidences. These findings are consistent with the results of both Wang et al. (2022) and Liu et al. (2015), who

Table 4. Myopathy rates (means) in male broiler chickens at 42 days of age, according to treatment[1)]

Variables	Control	GAA	TM	GT	p-value	SEM[2)]
Moderate white striping (%)	24±3.92[3)]	25±3.81	22±4.14	24±2.97	0.58	0.66
Severe white striping (%)	14±3.31	15±6.74	13±4.70	16±3.81	0.22	0.85
Total white striping (%)	38±2.88	37±3.89	37±3.01	39±3.78	0.43	0.60
Wooden breast (%)	21±6.67	18±7.30	17±5.31	14±4.87	0.16	1.08
Spaghetti meat (%)	16±4.77	12±5.23	14±4.33	13±4.30	0.44	0.83

[1)]Control, birds receiving basal diets with eggs incubated under standard conditions; GAA (control supplement), control group supplemented with GAA (1.2 g/kg), TM (thermal manipulation), birds receiving basal diets with eggs incubated under pre-TM conditions; and GT (thermal manipulation supplement), TM group supplemented with GAA (1.2 g/kg). All treatments were subjected to chronic heat stress from 28 to 42 days of age.
[2)]SEM, standard error of the mean.
[3)]Values are mean±SD (n=40).

Table 5. Sensory mean scores (0–10 scale) of descriptive attributes of cooked breast fillets (*pectoralis major*) in male broiler chickens at 42 days of age, according to treatment[1)]

Variables	Control	GAA	TM	GT	p-value	SEM[2)]
Texture						
Cohesiveness	3.44±0.31[3)]	3.33±0.13	3.22±0.12	3.29±0.16	0.75	0.07
Hardness	4.42±0.29	4.27±0.34	4.47±0.36	4.07±0.39	0.71	0.13
Juiciness	5.32±0.03[c]	5.52±0.08[b]	5.39±0.11[bc]	5.73±0.12[a4)]	0.01	0.02
Chewiness	4.36 ±0.38	4.18±0.46	4.12±0.41	4.53±0.38	0.78	0.15
Toothpack	3.80±0.76	3.72±0.76	3.80±0.84	3.90±0.82	0.98	0.18
Flavor/taste						
Brothy	3.84±0.54	3.71±0.46	3.90±0.014	3.72±0.45	0.88	0.09
Chickeny/meaty	5.00±0.94	4.87±0.94	5.60±0.94	5.47±0.58	0.24	0.14
Wet feathers	5.40±0.37	5.38±0.17	5.50±0.15	5.61±0.33	0.76	0.08
Sweet	5.87±0.29	6.05±0.33	6.07±0.51	5.87±0.61	0.70	0.07
Salty	4.9±0.30	5.12±0.25	5.32±0.41	5.20±0.49	0.68	0.11
Pleasantness	5.57±1.16	5.65±1.19	5.67±1.15	5.53±1.12	0.99	0.25

[1)]Control, birds receiving basal diets with eggs incubated under standard conditions; GAA (control supplement), control group supplemented with GAA (1.2 g/kg); TM (thermal manipulation), birds receiving basal diets with eggs incubated under pre-TM conditions; and GT (thermal manipulation supplement), TM group supplemented with GAA (1.2 g/kg). All treatments were subjected to chronic heat stress from 28 to 42 days of age.
[2)]SEM, standard error of the mean.
[3)]Values are mean±SD (n=40).
[4)]Different superscript letters within a column indicate significant differences at ($p<0.05$).

reported that dietary supplementation of GAA for finishing pigs could increase meat pH value.

The supplementation of GAA likely enhances glucose utilization and energy metabolism in poultry, resulting in reduced mortality and improved growth (Zaboli and Rahmatnejad, 2024). and pH (Ren et al., 2023). Additionally, GAA increases glycogen reserves and facilitates energy recovery in muscles, helping to preserve muscle tissue and minimize protein breakdown (Zaboli and Rahmatnejad, 2024). Moreover, dietary supplementation of GAA may reduce hexokinase activity and lactic acid production, which consequently leads to an increase in meat redness (Liu et al., 2015). This compound also boosts myoglobin activity, improving oxygen delivery to muscles and contributing to an increase in meat a* and an increase in pH (Khajali et al., 2020). Consequently, GAA, when combined with thermal

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treatments, can have significant positive effects on growth (Zaboli and Rahamatnejad, 2024). Ringel et al. (2008) reported a decrease in a* in GAA-fed broilers, which contrasts with our findings. This discrepancy may be explained by the prolonged exposure to chronic heat stress in our study, which likely created conditions more conducive to improving meat quality attributes such as redness.

Additionally, the highest percentage of pectoralis major was observed in the GAA and GT groups compared to the other groups. This effect on improving pectoralis major has been well-documented in numerous studies related to TM (Loyau et al., 2013; Zaboli et al., 2017). The latter authors suggested that the heat treatment of embryos altered the rate of myoblast proliferation, thereby enhancing the diameter or number of myofibers in the pectoralis major muscle. In future experiments, it would be valuable to verify whether an increase in the proliferative activity of satellite cells, as previously observed in the case of early postnatal (Halevy et al., 2001) or late embryonic (Piestun et al., 2009) thermal manipulations, occurs.

In this study, not only could TM improve Pectoral major, but GAA supplementation could also enhance this effect. This finding is linked to other notable achievements. In two separate experiments with male broilers, Oviedo-Rondón and Córdova-Noboa (2020) demonstrated that supplementing with GAA (600 g/ton) improved breast meat yield in male Ross 708 broilers. Similarly, Nasiroleslami et al. (2018) reported higher creatine kinase (CK) activity in GAA-supplemented broilers, which resulted in improved breast meat yield and increased plasma and muscle creatine levels compared to dietary creatine alone. The improvements associated with GAA supplementation in the diet may be partially attributed to elevated physiological levels of creatine, which benefits cellular energy status and, consequently, protein synthesis both directly and indirectly, for example, through arginine sparing (Dao and Swick, 2021; Khajali et al., 2020). Majdeddin et al. (2020) documented that GAA supplementation in broilers under chronic heat stress improved performance and breast meat yield.

Meat quality can be perceived by its sensory attributes, including taste (e.g., sweetness, saltiness), and texture (e.g., cohesiveness, hardness, juiciness, chewiness, toothpack). Sensory analysis is a critical tool in the meat industry for evaluating product quality, ensuring consumer satisfaction, and driving innovation by systematically assessing sensory. The observed improvement in juiciness values in the post-hatch groups supplemented with guanidine and pre-hatch thermal manipulation subjected to chronic heat stress suggests a synergistic effect between these two factors. This enhancement may be attributed to an increase in meat pH, which potentially contributes to preserving this quality trait. Additionally, guanidine likely supported cellular energy supply under heat stress conditions. The increase in pectoralis major weight, accompanied by improved meat redness, was consistent with the observed enhancement in juiciness. It can be concluded that thermal resistance induced by heat treatment, as reported in Zaboli and Rahmatnejad (2024), combined with cellular energy provision by guanidine, contributed to the increased juiciness of broiler meat in this study.

In this project, it was reported that thyroid hormones decreased in TM-treated broilers (Zaboli and Rahamtnejad, 2024) in which could improve meat quality and enhance juiciness. Chiang et al. (2008) support our findings, reporting that reducing thyroid hormones leads to improved meat quality. It appears that the decrease in thyroid hormones also lowers body temperature, thereby mitigating the negative effects of heat stress, which depletes cellular energy resources. Consequently, this reduction may contribute to the improvement of meat quality, including enhanced juiciness. Moreover, Connelly et al. (1994) reported that a decline in thyroid hormones decreases intracellular Ca^{2+} levels in skeletal muscle through their direct action on the ryanodine receptor (RYR), which, in turn, improves meat quality, including attributes such as juiciness. Overall, it can be hypothesized that pre-hatch thermal treatment combined with post-hatch supplementation under conditions of prolonged chronic heat stress may improve meat quality by slightly increasing pH, enhancing pectoral muscle weight, and improving redness and juiciness.

4. Conclusions

This study demonstrates that the combination of GAA supplementation and thermal manipulation (TM) has a positive impact on broiler meat quality. Improvements were observed in key parameters, including pH, redness (a*), and juiciness, indicating enhanced meat characteristics. Among the experimental groups, the GT group showed the most substantial benefits, reflecting a likely synergistic effect between the pre-hatch thermal treatment and post-hatch GAA administration. These outcomes may be related to improved energy metabolism, increased glycogen storage, and enhanced muscle development. In particular, the increase in pectoralis major weight suggests the potential of these interventions to support higher breast meat yield. Overall, applying GAA supplementation in combination with thermal manipulation is a practical and effective strategy for enhancing broiler production, especially under chronic heat stress conditions.

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Conflict of interests

Gholamreza Zaboli has served as an editorial board member of Food Science and Preservation since 2023, but was not involved in the review process or decision-making for this manuscript. Otherwise, no relevant conflicts of interest have been reported.

Author contributions

Conceptualization; Data curation; Formal analysis; Methodology; Validation; Writing: Zaboli G.

Ethics approval

This experiment was conducted in accordance with the guidelines of the Research Ethics Committee of University of Zabol. All procedures were approved to ensure compliance with ethical standards for animal research (Approval Code: IR.UOZ.REC.1398.4).

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