Research Article

Effects of Cold Water and Vitamin C on Growth Performance and Serum Malondialdehyde Concentration of Broilers Under Natural High Ambient Temperature

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Abstract | In the tropics, broilers raised in open housing systems regularly experience heat stress, which negatively impacts their health and production. Previous studies have shown that providing cold water or vitamin C could alleviate adverse effects of heat stress in broilers. Thus, the objective of this study was to evaluate the effects of cold water and vitamin C on the growth performance, mortality rate, relative weight of visceral organs, and serum malondialdehyde (MDA) concentration of growing broilers in an environment with naturally high temperatures. A total of nine hundred 21-d-old Ross 308 broilers (initial body weight: 607 ± 65.17 g/broiler) were randomly allocated to three treatments. The broilers were given unchilled water (29.5 ± 1°C) as a control (UCW), chilled water (15 ± 1°C) from 9 AM to 16 PM only (CW), and unchilled water enriched with 120 ppm vitamin C (UCWC). There were 10 replicate pens with 30 broilers (15 males and 15 females) in each treatment. The broilers of the CW and UCWC groups had considerably greater final body weight, average daily feed intake, and average daily gain than those of the UCW group over the course of a 3-week research period ($P < 0.01$). The mortality rate of broilers in the CW group was found to be lower than that of broilers in the UCW and UCWC groups ($P = 0.067$). The relative weights of the broilers' gizzard, spleen, liver, and heart did not differ across the three treatments ($P > 0.05$). When compared to the UCW and CW, the UCWC dramatically decreased the broiler's serum MDA concentration ($P < 0.001$). In summary, broilers may benefit from the use of chilled drinking water or vitamin C to mitigate the negative effects of high ambient temperatures without compromising relative organ weights.

Keywords | Broilers, Chilled water, Growth performance, Malondialdehyde (MDA), Visceral organ weight, Vitamin C

Received | July 12, 2025; **Accepted** | October 09, 2025; **Published** | December 17, 2025
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Citation | Tung CM, Khang LT, Nhan NTM (2026). Effects of cold water and vitamin C on growth performance and serum malondialdehyde concentration of broilers under natural high ambient temperature. J. Anim. Health Prod. 14(1): 22-28.
DOI | https://dx.doi.org/10.17582/journal.jahp/2026/14.1.22.28
ISSN (Online) | 2308-2801

INTRODUCTION

Chickens, as homeothermic species, can easily regulate their body temperature within a certain range of the ambient air temperature, known as the thermoneutral zone (Nawaz *et al.*, 2021). Broilers can maintain their body temperature in this zone without expending additional energy. When ambient temperatures rise over the thermoneutral zone, broilers suffer from heat stress due to an imbalance between the amount of heat produced by their metabolism and their ability to transfer body heat into the atmosphere. Heat stress has a deleterious influence on chicken performance, health, and welfare (Altan *et al.*, 2003; Pawar *et al.*, 2016; Brugaletta *et al.*, 2022). Panting is one of the thermoregulatory processes used by broilers to remove heat buildup from their bodies. As a result, broilers will drink more water and eat less feed, resulting in poorer growth performance.

In chicken production, water is necessary for the movement of nutrients, control of body temperature, and general health. In the thermoneutral zone, broilers drink roughly 1.6-2.0 times as much water as feed (Elsaidy *et al*., 2015). However, when exposed to heat stress, broilers require substantially more water than usual. This is because they must regulate their body temperature by evaporative cooling, which results in higher water loss. Abioja *et al*. (2013) and Farghly *et al*. (2018) claim that drinking cold water enhances performance during heat stress and that heat dissipation is made easier when the temperature disparity between body temperature and consumed water increases.

Vietnam is located in a tropical region with high ambient temperatures of more than 30 °C during the hot, dry months. Fast-growing broilers raised in this hot, humid climate are regularly subjected to heat stress. Elevated temperatures not only affect broiler growth performance but also often raise blood malondialdehyde (MDA), which is a key indicator of lipid peroxidation, a process where cell membranes are damaged by reactive oxygen species (Gawel *et al*., 2004; Bai *et al*., 2019). Therefore, vitamin C has been widely employed as an effective technique for reducing the negative effects of heat stress in broilers (De Souza *et al*., 2016; Saiz del Barrio *et al*., 2020). In addition to vitamin C, previous studies have demonstrated that giving cold water to broilers and layers under heat stress improves feed intake, growth performance, immunity, and egg output (Gutierrez *et al*., 2009; Abioja *et al*., 2011; Eltahan *et al*., 2023). However, to date, limited research has been conducted in Vietnam to compare the effects of cold water and vitamin C on broiler growth performance under high ambient temperatures. Thus, the objective of this study was to evaluate the effects of cold water and vitamin C on the growth performance, mortality rate, relative weight of visceral organs, and serum MDA concentration of growing broilers in an environment with naturally high temperatures, which will reveal effective practices for managing heat stress.

MATERIALS AND METHODS

All experimental protocols in this study were reviewed and approved by the Animal Ethics Committee (AEC) of Nong Lam University, Ho Chi Minh City, Vietnam (Approval No.: NLU-250525).

Experimental design and animals

Day-old broiler chicks (Ross 308) were purchased from a commercial hatchery and brooded for 3 weeks. They were given fresh water and fed the same diet during this brooding period. At 21 days of age, a total of nine hundred 21-d-old Ross 308 broilers (initial body weight (BW): 607 ± 65.17 g/broiler) were randomly allocated to three treatments in a completely randomized design. The broilers were given unchilled water (UCW, 29.5 ± 1°C) as a control (UCW), chilled water (CW, 15 ± 1°C) from 9:00 AM to 16:00 PM only (CW), and UCW enriched with 120 ppm vitamin C (UCWC). There were 10 replicate pens with 30 broilers (15 males and 15 females) in each treatment (Che *et al*., 2021).

Animal housing and management

Broilers were kept in floor pens with bedding made of rice husk. Each pen was 2.5 m long by 1.2 m wide and was positioned inside an 8 × 36 m open-sided house with curtains on both sides. Every pen in a treatment had an insulated water line that was connected to a chiller (for chilled water) or a water tank (for unchilled water). Each pen had 4 automatic nipple drinkers. Daily readings of the water temperature were taken at 10:00, 12:00, 14:00, and 16:00 h (Abioja *et al*., 2011). In addition, pre-measurements of the chilled water temperatures were also taken every hour prior to the start of the experiment to guarantee that they were kept within the target range (15 ± 1°C) during the experimental period. The average ambient temperature and relative humidity in the broiler house were recorded daily at 9:00, 11:00, 14:00, and 17:00 h. Throughout the trial, the broilers were fed the same feed (Table 1). They always had unrestricted access to feed and water. Antibiotics were not included in the mash-based diet.

Climatic conditions and water temperature

During the 3-week rearing period, the average ambient temperature and relative humidity of the broiler house were 31.1°C and 66.1%, respectively (Table 2). The highest average temperature recorded at 14:00 h was 32.8°C, and the lowest average temperature was 28.4°C at 9:00 h. The ambient temperature during the experimental period was high enough to induce heat stress in broilers. The average temperature of water given to the broilers is shown in Table 3. The average unchilled water temperature was 29.5°C (ranging from 29.3°C at week 4 to 29.8°C at week 6), while the average chilled water temperature was 15.1°C (ranging from 15.0°C at week 4 to 15.3°C at week 6). These results confirm that the target water temperatures were successfully maintained throughout the experimental period.

Assessment of growth performance, mortality, and relative organ weight

The initial BW of 21-day-old broilers in each pen was recorded at the beginning of the experiment. The subsequent measurement of the broiler weights was determined at the end of the experiment (42 days old). The quantity of feed and residue in each pen was recorded weekly for calculation of average daily feed intake (ADFI). The average daily gain (ADG), ADFI, and feed conversion ratio (FCR) were calculated on a per-pen basis (Che *et al.*, 2021). The number of dead/culled broilers from each pen was recorded daily to calculate the mortality rate.

Table 1: Composition of the diet fed to broilers during the experimental period.

Ingredients, %	Composition
Corn, ground	60.82
Soybean meal, 46%	33.40
Soybean oil	2.30
DL-methionine, 99%	0.13
Lysine, 78.8%	0.18
Salt	0.30
Limestone	1.40
MCP (15, 23)	1.12
Vitamin and mineral premix[1]	0.25
Pigment	0.10
Calculated nutrient composition	
Metabolizable energy, kcal/kg	3050
Dry matter, %	87.95
Crude protein, %	20.00
Lysine, %	1.15
Methionine, %	0.44
Calcium, %	0.92
Available phosphorus, %	0.41

[1]Supplied per kg of feed: vitamin A (10000 IU), vitamin D3 (3000 IU), vitamin E (25 mg), vitamin K (3.13 mg), vitamin B1 (3.13 mg), vitamin B2 (5 mg), niacin (44 mg), vitamin B5 (12.5 mg), vitamin B6 (3.13 mg), vitamin B12 (11 µg), folic acid (1.0 mg), vitamin H (125 µg), Fe (60 mg), Cu (15 mg), Zn (70 mg), Mn (90 mg), I (1.5 mg), Se (0.25 mg), Co (0.3 mg).

Table 2: Average ambient temperature and humidity of the broiler house during the experimental period.

Week	Temperature (°C)					Humidity (%)
	9:00 h	11:00 h	14:00 h	17:00 h	Average	
4	28.1	31.6	32.8	32.1	31.1	61.1
5	28.3	31.3	32.8	31.3	30.9	66.9
6	28.9	30.9	32.8	32.3	31.2	70.4
Average	28.4	31.3	32.8	31.9	31.1	66.1

Feed was withdrawn 10 hours before the broilers were slaughtered, but they were allowed free access to water. Two broilers (one male and one female) were selected randomly for slaughter. The broilers were manually cut by severing the left carotid artery and jugular vein, then bled out for 1.5 minutes. Following this, they were scalded at 60°C for 1.5 minutes and defeathered using automatic equipment. After the slaughtering and evisceration processes, the weights of the gizzard, heart, liver, and spleen were recorded. The relative weights of the organs were calculated by dividing the organ weight by the slaughter weight and multiplying by 100 (Erensoy *et al.*, 2020).

Blood collection and measurement of malondialdehyde

At 21 days of age, prior to the experiment's initiation, two broilers (one male and one female) per pen were randomly selected for blood sample collection from a wing vein. Additional blood samples were taken from the same broilers at 42 days of age. Two milliliters of blood from each broiler were collected into tubes without anticoagulant. The blood was allowed to clot at room temperature and was subsequently stored at 4°C before serum separation through centrifugation (3000 x g for 10 minutes at room temperature) (Che *et al.*, 2021). Serum concentrations of MDA were determined using a specific commercial kit (MyBiosource, CA, USA; detection range: 1.56 nmol/mL-100 nmol/mL, sensitivity: 0.5 nmol/mL) with an autoanalyzer (Thermo Fisher Scientific, MA, USA).

Statistical analysis

Data were analyzed as a completely randomized design by ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC). The Shapiro-Wilk test and Levene's test were used to confirm the normality and homogeneity of variances, respectively. The pen was considered the experimental unit for live BW, ADFI, ADG, and FCR, whereas one broiler was considered the experimental unit for MDA concentration and mortality rate. When a significant F value for treatment means was observed in the analysis of variance, the treatment means were compared using Tukey's test (Che *et al.*, 2021). The mortality rate among treatments was compared by the Chi-square test. Treatment effects were considered significant at $P < 0.05$, whereas a trend for a treatment effect was noted when $P < 0.1$.

Table 3: Average temperature of water given to the broilers from 21 to 42 days of age.

Weeks	Unchilled water				Average	Chilled water				Average
	10:00 h	12:00 h	14:00 h	16:00 h		10:00 h	12:00 h	14:00 h	16:00 h	
4	28.1	29.0	30.5	29.5	29.3	14.8	15.1	14.8	15.0	15.0
5	28.5	29.5	30.5	29.4	29.5	15.1	15.2	14.9	14.9	15.0
6	29.3	29.7	30.5	29.7	29.8	15.2	15.5	15.1	15.1	15.3
Average	28.6	29.4	30.5	29.5	29.5	15.0	15.3	14.9	15.0	15.1

RESULTS

Growth performance

The initial BW of broilers did not differ among the three treatments at 21 days old (*P* = 0.653; Table 4). However, at 42 days old, the final BW of broilers in the UCW group (1731.1 g/broiler) was significantly lower (*P* = 0.005) compared to that of broilers in the CW group (1850.6 g/broiler) and the UCWC group (1884.0 g/broiler). Broilers in the CW and UCWC groups exhibited greater ADG than those in the UCW group during the experimental period (*P* = 0.003). Additionally, the ADFI of broilers in the CW and UCWC groups was higher than that of broilers in the UCW group (*P* = 0.003). However, there were no significant differences in the FCR among the three treatments (*P* = 0.453).

Table 4: Effects of cold water and vitamin C supplementation on growth performance of broilers from 21 to 42 days of age.

Parameters	Treatments[1]			SEM	P
	UCW[2]	CW[3]	UCWC[4]		
Initial BW, g/broiler	628.08	629.65	627.46	4.19	0.653
Final BW, g/broiler	1731.1[b]	1850.6[a]	1884.0[a]	30.48	0.005
ADG, g/d	52.67[b]	58.21[a]	59.93[a]	1.35	0.003
ADFI, g/d	99.56[b]	108.55[a]	112.02[a]	2.30	0.003
FCR	1.90	1.87	1.87	0.02	0.453

[1]10 replicate pens/treatment, 30 broilers/pen. [2]UCW: Unchilled water (29.5 ± 1°C). [3]CW: Chilled water (15.0 ± 1°C) given to broilers from 9:00 AM to 16:00 PM. [4]UCWC: Unchilled water (29.5 ± 1°C) + 120 ppm vitamin C. [a-b]Within a row, means without a common superscript differ (*P* < 0.05).

Table 5: Effects of cold water and vitamin C supplementation on the relative organ weights of broilers at 42 days of age.

Parameters	Treatments[1]			SEM	P
	UCW[2]	CW[3]	UCWC[4]		
Heart, %	0.43	0.43	0.44	0.013	0.578
Gizzard, %	1.60	1.49	1.48	0.051	0.194
Liver, %	2.00	2.00	1.96	0.073	0.897
Spleen, %	0.12	0.12	0.10	0.008	0.119

[1]10 replicate pens/treatment, 30 broilers/pen. [2]UCW: Unchilled water (29.5 ± 1°C). [3]CW: Chilled water (15.0 ± 1°C) given to broilers from 9:00 AM to 16:00 PM. [4]UCWC: Unchilled water (29.5 ± 1°C) + 120 ppm vitamin C.

Relative organ weights and mortality

The results indicated no significant differences (*P* > 0.05) in the relative weights of the heart, liver, and spleen of broilers among the three treatments (Table 5). The relative gizzard weight for broilers in the CW group (1.49%) and the UCWC group (1.48%) was lower than that of broilers in the UCW group (1.60%); however, this difference was not statistically significant (*P* = 0.194). Additionally, there was a tendency for the mortality rate of broilers in the CW group (1.0%) to be lower (*P* = 0.067; Figure 1) compared to that in the UCW (4.0%) and UCWC (3.0%) groups.

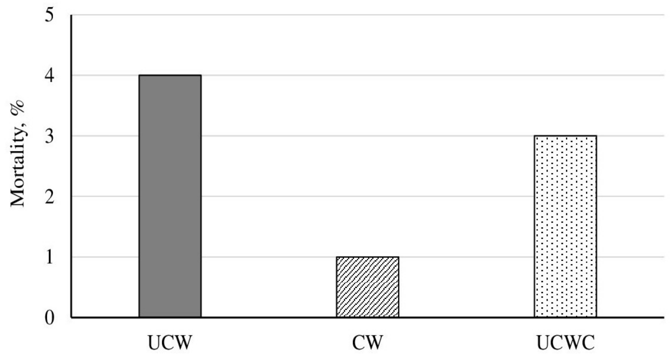

Figure 1: The mortality rate of broilers during the experimental period. There was a tendency that broilers in the CW group had a lower mortality rate than those in the UCW and UCWC groups (P= 0.067). There were 300 birds/treatment. UCW: Unchilled water (29.5 ± 1°C); CW: Chilled water (15.0 ±1°C) given to broilers from 9:00 AM to 16:00 PM.; UCWC: Unchilled water (29.5 ± 1°C) + 120 ppm vitamin C.

Serum MDA concentrations

At 21 days old, there were no significant differences (*P* = 0.149) in serum MDA concentrations among the three treatments (Table 6). However, at 42 days old, the serum MDA concentration of broilers in the UCWC group (5.48 nmol/mL) was significantly lower (*P* < 0.001) compared to the UCW group (10.87 nmol/mL) and the CW group (12.91 nmol/mL).

Table 6: The serum malondialdehyde (MDA) concentration of broilers at 21 and 42 days of age.

MDA (nmol/mL)	Treatments[1]			SEM	P
	UCW[2]	CW[3]	UCWC[4]		
21 d old	2.94	4.65	3.23	0.64	0.149
42 d old	10.87[a]	12.91[a]	5.48[b]	1.20	< 0.001

[1]20 broilers/treatment (2 broilers per pen were sampled). [2]UCW: Unchilled water (29.5 ± 1°C). [3]CW: Chilled water (15.0 ± 1°C) given to broilers from 9:00 AM to 16:00 PM. [4]UCWC: Unchilled water (29.5 ± 1°C) + 120 ppm vitamin C. [a-b]Within a row, means without a common superscript differ (*P* < 0.05).

DISCUSSION

Today, poultry farming has become an important activity worldwide. However, due to climate change, heat stress has become a significant concern in the poultry industry, particularly in hot climate regions of Asia and South America. Over the past few decades, genetic modifications aimed at rapid growth in broilers have increased their susceptibility to heat stress (Brugaletta *et al*., 2022). In Vietnam, modern, fast-growing chickens raised in open-sided housing systems are frequently exposed to high ambient temperatures, which can lead to reduced feed intake, poor growth, and high mortality rates. Research has shown that environmental temperatures exceeding 25°C have considerable negative effects on the performance of broilers (Oke *et al*., 2024). In the current experiment, the average ambient temperature recorded from 9:00 h to 17:00 h during the experimental period ranged from 28.4°C to 32.8°C, with an average temperature of 31.1°C (Table 2). This temperature level was sufficient to induce heat stress in broilers. According to Pawar *et al*. (2016) and Kpomasse (2021), the ideal environmental temperature for growing broilers is between 18°C and 22°C; temperatures above this range are classified as thermal stress. When subjected to heat stress, broilers undergo changes in their behavioral, physiological, and immunological responses, adversely affecting their overall productivity, particularly during hot seasons (Pawar *et al*., 2016).

In the present study, both chilled drinking water (administered for 7 hours per day during the hottest periods) and vitamin C (provided continuously for 24 hours per day) effectively alleviated the adverse effects of heat stress in broilers. Our results indicated that the ADFI and ADG of broilers in the CW and UCWC groups were significantly higher than those of broilers in the UCW group (Table 4). Previous studies have also demonstrated that cold water positively influences feed intake, weight gain, and egg production in poultry (Abioja *et al*., 2011; Farghly *et al*., 2018; Kim *et al*., 2025). When the temperature of the water consumed is lower than the broilers' body temperature, it helps lower metabolic temperature and increases their appetite for both water and feed (Puma *et al*., 2001; Farghly *et al*., 2018). In contrast to cold water, vitamin C known for its antioxidant properties has been widely used to maintain poultry performance during heat stress. Previous research by Sun *et al*. (2023) and Li *et al*. (2024) has shown that vitamin C supplementation enhances growth rates and feed intake, which aligns with our findings. However, we observed that feed conversion ratios were not affected by either cold water or vitamin C supplementation. This study is consistent with the findings of Abioja *et al*. (2011), Erensoy *et al*. (2020), and Tavakolinasab and Hashemi (2025), suggesting that these interventions primarily influence growth through increased feed intake rather than improved feed efficiency.

Providing chilled drinking water to broilers at a high ambient temperature may potentially reduce the heat accumulation in the body, leading to higher livability. Abioja *et al*. (2013) found that cold water given to broilers reduced the respiratory rate. It was also reported that cold water increased heat dissipation and enhanced cellular and humoral immunity in heat-exposed laying hens (Eltahan *et al*., 2023). In our study, broiler death was lowest in the CW group, underscoring the positive impact of lower water temperatures on the mortality rate. This finding is consistent with the observations of Harris *et al*. (1975), Farghly *et al*. (2018) and Kim *et al*. (2025), reinforcing the potential benefits of cold water in poultry management. However, Abioja *et al*. (2011) and Erensoy *et al*. (2020) found no effects of cold water application on the mortality rate of broilers.

The effect of water temperature on the relative weight of visceral organs and serum MDA level was not significant, and the results were consistent with earlier studies. Erensoy *et al*. (2020) reported no significant impact of water temperature on the relative weights of the heart, liver, and gizzard. Additionally, cold water did not influence the percentages of other internal organs, with the exception of the spleen (Abioja *et al*., 2011). The reason for the increased relative weight of the spleen observed in the study by Abioja *et al*. (2011) remains unclear; however, it may be partially related to the effects of corticosteroids under stress conditions (Gross and Siegel, 1981; Rosales, 1994) and to enhanced immunity associated with cold water (Eltahan *et al*., 2023). This result indicates a complex relationship among water temperature, stress, and immune function, highlighting the need for further investigation into the specific mechanisms involved.

Under heat stress, increased levels of reactive oxygen species can overwhelm the body's antioxidant defenses, resulting in enhanced lipid peroxidation and elevated MDA levels (Brugaletta *et al*., 2022; Tang *et al*., 2022). In this study, the serum MDA levels of broilers in the UCWC group were significantly lower compared to those in the UCW and CW groups (Table 4), suggesting that vitamin C supplementation was more effective than cold water in reducing serum MDA levels. This finding indicates that while cold water primarily serves as a cooling agent, vitamin C offers additional antioxidant benefits by reducing fatty acid peroxidation. Vitamin C quickly scavenges reactive oxygen and nitrogen species, which helps protect cellular components from oxidative stress damage. Furthermore, vitamin C supplementation significantly lowered MDA concentrations during heat stress, as reported by Jena *et al*. (2013) and Ismail *et al*. (2015). Broilers received vitamin C continuously for 24 hours a day, whereas cold water was administered only for 7 hours a day during the hottest part of the day. This discrepancy may explain why cold water was less effective in lowering serum MDA levels. Koç Yildirim *et al*. (2023) noted that broilers raised in a temperature range of 24-26°C exhibited no change in MDA concentrations in their hearts, livers, or Bursa of Fabricius when provided with cold water. Thus, further research is needed to investigate the impact of the duration of cold water provision on blood MDA levels in broilers, especially in environments with high ambient temperatures throughout the day.

CONCLUSIONS

Providing cold water and supplementing with vitamin C improved the growth performance of broilers raised under heat stress, primarily by increasing feed intake. While neither treatment significantly affected the relative weights of visceral organs, cold water showed potential to enhance broiler livability, and vitamin C effectively reduced serum MDA levels, indicating lower oxidative stress. These findings support the practical use of cold water and vitamin C as effective strategies to mitigate the adverse effects of heat stress and promote better health and productivity in broiler chickens.

ACKNOWLEDGEMENTS

The authors thank Nong Lam University Ho Chi Minh City (Vietnam) for the financial support of this research (the research project code: CS-CB23-CNTY-07).

NOVELTY STATEMENT

This study evaluates the effects of cold water and vitamin C on the growth performance, mortality rate, relative weight of visceral organs, and serum malondialdehyde (MDA) concentration of growing broilers in an environment with naturally high temperatures. Its findings support the use of cold water and vitamin C as feasible techniques for mitigating the detrimental effects of heat stress and improving broiler production and health overall.

AUTHOR'S CONTRIBUTION

Che Minh Tung: Conceptualization, supervision, methodology, writing-review and editing, validation.
Le Tuan Khang: Investigation, formal analysis, data curation, writing-original draft.
Nguyen Thi My Nhan: Data curation, writing-review and editing .

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI-assited technology was used in data analysis, writing, editing, and manuscript analysis.

Conflict of interest

The authors have declared no conflict of interest.

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