

Synergistic role of Sodium Bicarbonate and Vinegar in mitigating heat stress and enhancing thermotolerance in broilers

V. Maurya¹, U.K. Tripathi¹, R. Varshney², A. Kumari¹, R.G. Kadam³

¹Department of Livestock Production Management, ²Department of Veterinary Microbiology, ³TVCC, Faculty of Veterinary and Animal Sciences, IAS, RGSC-BHU, Barkachha, Mirzapur, India
Corresponding author e-mail: vipinmaurya@bhu.ac.in

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Abstract

Heat stress significantly hampers poultry productivity by impairing physiological and metabolic functions. This study evaluated the ameliorative effects of dietary sodium bicarbonate (NaHCO_3) and vinegar, administered individually and in combination, on broiler resilience under cyclic heat stress. A total of 450 Vencobb broilers were allocated into nine groups, receiving 1% or 2% sodium bicarbonate (1 or 2 g/100 mL drinking water) and 2% or 5% vinegar (2 or 5 mL/100 mL drinking water) via drinking water from day 21 to 45. Results indicated that combined supplementation, particularly 2% NaHCO_3 + 5% vinegar (T8), significantly ameliorated heat-induced physiological alterations, reflected by reduced respiration rate, heart rate, and body temperature. The combined treatment notably improved antioxidant status (increased SOD, GSH; decreased MDA), hormonal balance (elevated T_3 , T_4 , GH; reduced TSH), and organ function (improved liver and kidney profiles). Furthermore, combined supplementation improved feed conversion ratio, feed conversion efficiency, and water intake, with the greatest improvements observed in T8. The modulation of gut microflora, characterized by increased *Lactobacillus* spp. and reduced pathogenic loads, further evidenced the beneficial effects. These findings highlight the synergistic potential of sodium bicarbonate and vinegar as effective dietary interventions to alleviate the multifaceted detrimental effects of heat stress in broilers.

Keywords: Heat stress; Broilers; Sodium bicarbonate; Vinegar; Oxidative stress.

Introduction

The rapid growth rate and elevated metabolic activity of modern broiler chickens make them particularly vulnerable to environmental stressors, most notably heat stress (Renaudeau *et al.*, 2012; Taleb *et al.*, 2017). High ambient temperatures, often intensified by elevated humidity, can severely compromise broiler health, welfare, and performance, resulting in considerable economic losses across the global poultry industry (Siddiqui *et al.*, 2024). The physiological impact of heat stress is multifaceted, involving disruptions in acid-base homeostasis, altered metabolic function, immunosuppression, and an increased susceptibility to oxidative damage (Raghebian *et al.*, 2017; Livingston *et al.*, 2022). In India, heat stress is one of the major environmental constraints affecting poultry production, particularly during the summer months when ambient temperatures frequently exceed the thermoneutral zone of broiler chickens. Severe thermal stress reduces feed intake, impairs growth performance, disrupts physiological homeostasis and immunity, and ultimately leads to substantial economic losses to the poultry industry (Lara and Rostagno, 2013; Attia *et al.*, 2014).

Oxidative stress is a central pathological outcome of heat stress, caused by an imbalance between the excessive production of reactive oxygen species (ROS) and the bird's antioxidant defence capacity (Livingston *et al.*, 2022). This imbalance leads to widespread cellular damage, including lipid peroxidation, protein oxidation, and DNA fragmentation, ultimately impairing tissue integrity and overall physiological function (Azad *et al.*, 2010; Raffei-Tari *et al.*, 2018). To counteract rising body temperatures, broilers resort primarily to panting as a thermoregulatory mechanism; however, this response often results in excessive CO₂ loss and respiratory alkalosis, further exacerbating metabolic disturbances (Saker *et al.*, 2020). The endocrine system is also significantly impacted by heat stress. Elevated levels of stress hormones such as corticosterone, coupled with a marked suppression of key metabolic hormones, including triiodothyronine (T₃), thyroxine (T₄), and growth hormone (GH), compromise growth and metabolic efficiency in broilers (Yahav, 2009; Ramezani *et al.*, 2012).

Concurrently, heat stress compromises the integrity of the intestinal barrier, leading to "leaky gut" syndrome, which impairs nutrient absorption and increases susceptibility to pathogens (Song *et al.*, 2014; Olayiwola *et al.*, 2025). To mitigate these adverse effects, nutritional interventions using buffering agents and organic acids have shown promise. NaHCO₃ is widely acknowledged for its buffering potential, which helps counteract respiratory alkalosis and stabilize acid-base homeostasis, thereby enhancing performance and electrolyte balance (Siddiqui *et al.*, 2024; Sa'adu *et al.*, 2026). Separately, vinegar, primarily composed of acetic acid, enhances gut health by reducing gastrointestinal pH, inhibiting pathogenic microbial populations, and improving nutrient absorption (Allahdo *et al.*, 2018; Adil *et al.*, 2011).

Although the individual efficacy of sodium bicarbonate and vinegar in poultry has been reported, their combined application, particularly under cyclic heat stress conditions, remains inadequately explored. Therefore, the present study evaluated the individual and synergistic effects of these additives on physiological responses, endocrine profiles, oxidative stress biomarkers, carcass quality, gut morphometry, electrolyte balance, and immune status of broilers subjected to cyclic heat stress.

Materials and Methods

Experimental Site and Design

The experiment was conducted at the Poultry Unit of the Livestock Farm Complex, Faculty of Veterinary and Animal Sciences (FVAS), Rajiv Gandhi South Campus (RGSC), Banaras Hindu University (BHU), Barkachha, Uttar Pradesh, India. The study was carried out during the summer season (June–July), when environmental temperatures are typically associated with heat stress in poultry production systems.

A total of 450 one-day-old Vencobb broiler chicks were procured from a certified commercial hatchery and vaccinated against Marek's disease following standard hatchery protocols. Birds were reared under a deep-litter system in well-ventilated pens (1.5 m × 1.5 m each), ensuring optimal space and hygiene. Standard brooding practices were followed for the first three weeks. On day 21, chicks were randomly allocated into nine experimental groups (n = 50 per group), each replicated across five pens (10 birds/replicate). All birds were provided *ad libitum* access to fresh drinking water and a nutritionally balanced commercial broiler diet formulated as per ICAR (2013) standards.

Treatments

The experimental treatments were administered via drinking water from day 21 to day 45. This period was selected because broilers are more susceptible to heat stress during the grower-finisher phase owing to rapid growth and increased metabolic heat production, making it an appropriate stage for evaluating heat stress mitigation strategies (Lara and Rostagno, 2013; Attia *et al.*, 2014). To mimic cyclic heat stress conditions, the ambient temperature in the poultry house was maintained at 34°C–36°C during the day (10:00 AM to 6:00 PM) and allowed to drop to 28°C–30°C during the night (6:00 PM to 10:00 AM), with relative humidity maintained at 65%–70% throughout the experimental period. The average ambient temperature during the study was 34.4°C (ranging from 28.6°C during nighttime to 36.8°C during daytime), representing typical summer conditions in northern India and sufficient to induce severe heat stress in broilers. This temperature regime resulted in a

calculated Temperature-Humidity Index (THI) of 84.5 ± 1.2 , which significantly exceeds the critical threshold of $\text{THI} > 82$ that defines severe heat stress conditions in poultry (Lara and Rostagno, 2013).

The nine treatment groups were:

- ✓ **C:** Control group (no additive)
- ✓ **T1:** 1% Sodium Bicarbonate (NaHCO_3)
- ✓ **T2:** 2% Sodium Bicarbonate (NaHCO_3)
- ✓ **T3:** 2% Vinegar
- ✓ **T4:** 5% Vinegar
- ✓ **T5:** 1% NaHCO_3 + 2% Vinegar
- ✓ **T6:** 1% NaHCO_3 + 5% Vinegar
- ✓ **T7:** 2% NaHCO_3 + 2% Vinegar
- ✓ **T8:** 2% NaHCO_3 + 5% Vinegar

Food-grade sodium bicarbonate and white distilled vinegar (5% acetic acid) were freshly mixed into drinking water daily to ensure uniform intake. A 1% sodium bicarbonate solution was prepared by dissolving 1 g sodium bicarbonate in 100 mL of drinking water (w/v basis), whereas a 1% vinegar solution was prepared by adding 1 mL vinegar to 100 mL of drinking water (v/v basis). Similarly, 2% and 5% solutions were prepared proportionately.

Data Collection

Performance Parameters

Body weight and feed intake were recorded weekly for each pen. Weight gain was calculated as the difference between consecutive weekly average body weights. Daily water intake was determined by measuring the volume offered and subtracting the residual amount. Feed Conversion Ratio (FCR) was calculated as the total feed consumed (g) per gram of body weight gain. Mortality was monitored and recorded daily per pen.

Physiological Parameters

From day 21 to 45, physiological responses were assessed bi-weekly. Ten birds per group (two birds per replicate) were randomly selected for measurement. Respiration rate (breaths/min) was determined by counting thoracoabdominal movements for 30 seconds and doubling the value. Heart rate (beats/min) was measured using a veterinary stethoscope placed over the chest. Cloacal and surface temperatures (comb and shank) were recorded using a digital thermometer with $\pm 0.1^\circ\text{C}$ accuracy. The mean values over the 21–45 day period were calculated and presented.

Blood Sampling and Analysis

All hematological, biochemical, hormonal, and oxidative stress analyses were carried out at the VLD Laboratory, FVAS, RGSC-BHU, Barkachha, Mirzapur, Uttar Pradesh, India. At two-week intervals starting from day 21, blood samples were collected from the brachial vein of five randomly selected birds per group ($n = 5/\text{group}$) using a 23-gauge needle and 3 mL syringe. Samples for hematological analysis were collected into EDTA-coated vacutainer tubes, while those for serum separation were collected into plain tubes. Serum samples were allowed to clot at room temperature for 1–2 hours and then centrifuged at 3000 rpm for 10 minutes. The separated serum was aliquoted and stored at -20°C until further biochemical and hormonal analyses.

Hematological Parameters

Complete blood count (CBC) was performed to measure red blood cell (RBC) and white blood cell (WBC) counts, packed cell volume (PCV), and mean corpuscular volume (MCV). Differential leukocyte counts were conducted manually on Giemsa-stained blood smears (200 cells per slide) to determine neutrophil percentages. Hemoglobin (Hb) concentration was estimated spectrophotometrically using the cyanmethemoglobin method (Drabkin's solution).

Oxidative Stress Markers

Serum samples were analyzed for key oxidative stress indicators using commercially available assay kits. Superoxide dismutase (SOD) activity was determined using a Randox kit (Cat. No. SD 125), based on inhibition of pyrogallol auto-oxidation (McCord et al, 1969). Malondialdehyde (MDA), a marker of lipid peroxidation, was measured via the thiobarbituric acid reactive substances (TBARS) assay following Ohkawa et al. (1979). Reduced glutathione (GSH), a vital non-enzymatic antioxidant, was assessed using Ellman's reagent (DTNB).

Hormonal Analysis

On day 45, serum samples were analyzed for triiodothyronine (T_3), thyroxine (T_4), thyroid-stimulating hormone (TSH), and growth hormone (GH) using avian-specific ELISA kits (e.g., Cusabio Biotech Co., Ltd., China), following the manufacturers' protocols. Serum Biochemistry (Liver and Kidney Function Tests). Liver function tests (LFTs) included serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), and total bilirubin. Kidney function tests (KFTs) included urea, creatinine, and uric acid. All parameters were assessed using standard assay kits on an automated clinical chemistry analyzer (Erba EM 200).

Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) in SPSS (IBM SPSS Statistics, Version 26.0). When significant differences were observed ($P < 0.05$), group means were compared using Duncan's Multiple Range Test. Results are expressed as mean $\pm$ standard error (SE).

Results

Health and Oxidative Stress Markers

Hematological parameters (Hb, Neutrophils, Cortisol) showed no significant differences ($P > 0.05$) across all groups, with values remaining within physiological norms, indicating that neither heat stress nor the treatments significantly influenced these specific indicators of acute stress or overall hematological status. Cortisol levels exhibited a modest decline in treated groups, particularly under combined treatments (T5–T8), suggesting a potentially stress-mitigating effect while remaining within the normal physiological range.

In contrast, oxidative stress parameters exhibited significant differences ($P < 0.05$). Superoxide dismutase (SOD) activity was markedly higher in all treated groups compared to the control (C). Notably, T2 (2% NaHCO₃) and T4 (5% vinegar) showed significantly greater SOD activity than T1 (1% NaHCO₃) and T3 (2% vinegar), respectively, while the highest levels were recorded in T5–T8, with T8 showing the peak activity (70.2 ± 1.0 U/mg), significantly higher than all other groups ($P < 0.05$).

Malondialdehyde (MDA) levels, a key indicator of lipid peroxidation, were markedly reduced in all treated groups relative to the control. The most pronounced declines were noted in T5–T8, with T8 recording the lowest value (4.8 ± 0.2 nmol/mg), reflecting the strongest protective effect against oxidative damage.

Reduced Glutathione (GSH) concentrations followed a similar trend to SOD activity, with significantly higher levels in all treated groups compared to the control. T2 and T4 showed higher GSH than T1 and T3, respectively. The highest concentrations were recorded in T5–T8, with T8 reaching the peak value (27.0 ± 0.4 nmol/mg), demonstrating enhanced antioxidant capacity (Sahin et al, 2003).

Complete Blood Count (CBC)

Complete blood count parameters, including red blood cell (RBC) count, white blood cell (WBC) count, packed cell volume (PCV), and mean corpuscular volume (MCV), remained within physiological norms across all treatment groups, with no significant differences observed ($P > 0.05$). While RBC counts ranged from $2.50 \pm 0.05 \times 10^6/\mu\text{L}$ in the control group to $2.72 \pm 0.04 \times 10^6/\mu\text{L}$ in T8, and WBC counts ranged from $30.0 \pm 1.0 \times 10^3/\mu\text{L}$ to $26.5 \pm 0.8 \times 10^3/\mu\text{L}$, these variations were not statistically significant. Similarly, PCV values ($32.0 \pm 0.5\%$ to $35.5 \pm 0.4\%$) and MCV values (60 ± 1.5 fL to 63.5 ± 1.3 fL) showed no treatment-related differences. These findings indicate that neither cyclic heat stress nor the dietary interventions induced major hematological changes, reflecting stable health and physiological resilience across all groups.

Liver and Kidney Function Tests

Liver function tests (LFTs) remained unaffected by the initial treatments (T1–T4), whereas combined treatments (T5–T8) produced significant improvements ($P < 0.05$). SGPT, SGOT, ALP, and Total Bilirubin were markedly reduced in T5–T8 compared to the control, with T8 showing the lowest values (SGPT: 27 ± 0.8 U/L, SGOT: 32 ± 0.9 U/L, ALP: 235 ± 4 U/L, Total Bilirubin: 0.66 ± 0.02 mg/dL), highlighting a significant hepatoprotective effect under heat stress.

Kidney function test (KFT) parameters remained unaffected by the initial treatments (T1–T3), whereas T4 (5% vinegar) significantly reduced urea, creatinine, and uric acid compared to the control and lower single-additive groups ($P < 0.05$). Combined treatments (T5–T8) further enhanced this effect, with group T8 showing the lowest levels (Urea: 22 ± 0.8 mg/dL, Creatinine: 1.04 ± 0.03 mg/dL, Uric Acid: 4.6 ± 0.1 mg/dL), highlighting renal resilience and improved metabolic homeostasis under heat stress.

Table 1. Health and oxidative stress markers (Mean $\pm$ SE) of broilers across different treatment groups.

Group	Hb (g/dL)	Neutrophils (%)	Cortisol (ng/mL)	SOD (U/mg)	MDA (nmol/mg)	GSH (nmol/mg)
C	10.5 ± 0.3^a	38 ± 1.2^a	5.5 ± 0.3^a	50.0 ± 1.2^a	8.0 ± 0.3^a	20.0 ± 0.5^a
T1	11.2 ± 0.3^a	36 ± 1.1^a	4.8 ± 0.3^a	55.2 ± 1.1^b	7.0 ± 0.2^b	22.0 ± 0.4^b
T2	11.0 ± 0.3^a	37 ± 1.2^a	5.0 ± 0.3^a	60.1 ± 1.0^c	6.0 ± 0.2^c	24.0 ± 0.4^c
T3	11.1 ± 0.3^a	35 ± 1.1^a	4.6 ± 0.3^a	53.0 ± 1.3^b	7.5 ± 0.3^b	21.0 ± 0.5^b
T4	10.8 ± 0.3^a	35 ± 1.2^a	4.9 ± 0.3^a	$58.3 \pm 1.2^{b,c}$	$6.5 \pm 0.2^{b,c}$	23.0 ± 0.4^c
T5	12.0 ± 0.3^a	33 ± 1.0^a	3.9 ± 0.3^a	68.0 ± 1.0^d	5.2 ± 0.2^d	26.0 ± 0.4^d
T6	11.9 ± 0.3^a	32 ± 1.0^a	4.0 ± 0.3^a	69.0 ± 1.0^d	5.1 ± 0.2^d	26.5 ± 0.4^d
T7	12.1 ± 0.3^a	31 ± 1.0^a	3.8 ± 0.3^a	69.5 ± 1.0^d	5.0 ± 0.2^d	26.8 ± 0.4^d
T8	12.2 ± 0.3^a	30 ± 1.0^a	3.7 ± 0.3^a	70.2 ± 1.0^d	4.8 ± 0.2^d	27.0 ± 0.4^d

*Different superscripts within a column indicate significant differences at $P < 0.05$ for oxidative stress parameters (SOD, MDA, GSH). Hematological parameters (Hb, Neutrophils, Cortisol) remained within physiological ranges and showed no significant differences (same superscript "a").

Hormonal Profile

Thyroid hormones (T₃ and T₄) and Growth Hormone (GH) increased progressively with treatment, with significant elevations in combined groups (T5–T8; $P < 0.05$), while TSH showed a reciprocal decline. Group T8 recorded the most favorable profile (T₃: 1.78 ± 0.04 ng/mL, T₄: 5.5 ± 0.1 µg/dL, GH: 3.2 ± 0.1 ng/mL, TSH: 0.58 ± 0.02 µIU/mL), indicating enhanced thyroid axis activity and growth hormone secretion, typically suppressed under heat stress.

Physiological Parameters

All physiological parameters (respiration rate, heart rate, cloacal and surface temperature) were lower in treated groups than in the control, indicating reduced heat stress. Groups T5–T8 showed significant reductions ($P < 0.05$), with T8 recording the lowest values (Respiration: 48 ± 1.4 /min, Heart Rate: 265 ± 4 /min, Cloacal Temp: 41.0 ± 0.2 °C, Surface Temp: 38.2 ± 0.2 °C), aligning more closely with the normal physiological range and minimizing the adverse consequences of heat stress (Lin et al., 2006).

Feed and Water Efficiency

Feed Conversion Ratio (FCR) was reduced in all treated groups, with significant improvement in combined treatments (T5–T8) compared to the control ($P < 0.05$). Feed Conversion Efficiency (FCE) followed an opposite trend, being significantly higher in the same groups. The best performance was recorded in T8 (FCR: 1.71 ± 0.02 g feed/g gain; FCE: 0.59 ± 0.01 g gain/g feed). Water intake was significantly greater in treated groups, peaking in T8 (5600 ± 80 mL), reflecting an adaptive thermoregulatory response under heat stress.

Table 2. Liver and kidney function test results of broilers under different treatment groups

Group	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)
C	35 ± 1.0^a	40 ± 1.2^a	250 ± 5^a	0.80 ± 0.02^a	30 ± 1.0^a	1.20 ± 0.05^a	5.5 ± 0.1^a
T1	33 ± 0.9^a	39 ± 1.1^a	248 ± 5^a	0.78 ± 0.02^a	29 ± 0.9^a	1.18 ± 0.04^a	5.4 ± 0.1^a
T2	32 ± 0.9^a	38 ± 1.1^a	247 ± 5^a	0.77 ± 0.02^a	28 ± 0.9^a	1.17 ± 0.04^a	5.3 ± 0.1^a
T3	32 ± 0.8^a	37 ± 1.0^a	245 ± 5^a	0.75 ± 0.02^a	27 ± 0.8^a	1.15 ± 0.04^a	5.2 ± 0.1^a
T4	31 ± 0.9^a	36 ± 1.0^a	244 ± 4^a	0.74 ± 0.02^a	26 ± 0.8^b	1.12 ± 0.04^b	5.1 ± 0.1^b
T5	30 ± 0.8^b	35 ± 0.9^b	242 ± 4^b	0.72 ± 0.02^b	25 ± 0.8^b	1.10 ± 0.03^b	5.0 ± 0.1^b
T6	29 ± 0.8^b	34 ± 0.9^b	240 ± 4^b	0.70 ± 0.02^b	24 ± 0.8^b	1.08 ± 0.03^b	4.9 ± 0.1^b
T7	28 ± 0.8^b	33 ± 0.9^b	238 ± 4^b	0.68 ± 0.02^b	23 ± 0.8^b	1.06 ± 0.03^b	4.8 ± 0.1^b
T8	27 ± 0.8^b	32 ± 0.9^b	235 ± 4^b	0.66 ± 0.02^b	22 ± 0.8^c	1.04 ± 0.03^c	4.6 ± 0.1^c

Values are expressed as Mean $\pm$ SE. Different superscripts (a, b, c) within a column indicate significant differences ($P < 0.05$).

Table 3: Hormonal profiles of broilers at day 45.

Group	T ₃ (ng/mL)	T ₄ (µg/dL)	TSH (µIU/mL)	GH (ng/mL)
C	1.45 ± 0.04^a	4.4 ± 0.1^a	0.82 ± 0.02^a	2.1 ± 0.1^a
T1	1.52 ± 0.04^a	4.6 ± 0.1^a	0.78 ± 0.02^a	2.2 ± 0.1^a
T2	1.50 ± 0.05^a	4.7 ± 0.1^a	0.76 ± 0.02^a	2.3 ± 0.1^a
T3	1.55 ± 0.04^a	4.8 ± 0.1^a	0.74 ± 0.02^a	2.4 ± 0.1^a
T4	1.58 ± 0.04^a	4.9 ± 0.1^a	0.72 ± 0.02^a	2.5 ± 0.1^a
T5	1.70 ± 0.04^b	5.1 ± 0.1^b	0.66 ± 0.02^b	2.8 ± 0.1^b
T6	1.72 ± 0.04^b	5.2 ± 0.1^b	0.64 ± 0.02^b	2.9 ± 0.1^b
T7	1.74 ± 0.04^b	5.3 ± 0.1^b	0.62 ± 0.02^b	3.0 ± 0.1^b
T8	1.78 ± 0.04^c	5.5 ± 0.1^c	0.58 ± 0.02^c	3.2 ± 0.1^c

*Values are presented as Mean $\pm$ SE. Different superscripts (a, b, c) within a column denote significant differences ($P < 0.05$). Reference ranges are based on standard poultry physiology literature.

Table 4: Physiological Parameters (Average of Day 21–45)

Group	Respiration Rate (/min)	Heart Rate (/min)	Cloacal Temp (°C)	Surface Temp (°C)
C	62 ± 2.0^a	290 ± 5^a	42.2 ± 0.2^a	39.5 ± 0.2^a
T1	60 ± 1.8^a	288 ± 5^a	42.0 ± 0.2^a	39.3 ± 0.2^a
T2	59 ± 1.8^a	285 ± 5^a	41.9 ± 0.2^a	39.2 ± 0.2^a
T3	58 ± 1.7^a	282 ± 4^a	41.8 ± 0.2^a	39.1 ± 0.2^a
T4	57 ± 1.6^a	280 ± 4^a	41.7 ± 0.2^a	39.0 ± 0.2^a
T5	53 ± 1.5^b	275 ± 4^b	41.5 ± 0.2^b	38.8 ± 0.2^b
T6	52 ± 1.5^b	272 ± 4^b	41.4 ± 0.2^b	38.7 ± 0.2^b
T7	51 ± 1.5^b	270 ± 4^b	41.3 ± 0.2^b	38.5 ± 0.2^b
T8	48 ± 1.4^c	265 ± 4^c	41.0 ± 0.2^c	38.2 ± 0.2^c

*Values are expressed as Mean $\pm$ SE. Different superscripts (a, b, c) within columns indicate significant differences ($P < 0.05$).

Table 5: Feed and Water Efficiency Parameters (Day 21–45).

Group	FCR (g feed/g gain)	FCE (g gain/g feed)	Water Intake (ml)
C	1.82 ± 0.02 ^a	0.55 ± 0.01 ^a	5000 ± 100 ^a
T1	1.78 ± 0.02 ^a	0.56 ± 0.01 ^a	5200 ± 95 ^a
T2	1.80 ± 0.02 ^a	0.56 ± 0.01 ^a	5300 ± 90 ^a
T3	1.77 ± 0.02 ^a	0.56 ± 0.01 ^a	5150 ± 90 ^a
T4	1.76 ± 0.02 ^a	0.57 ± 0.01 ^a	5100 ± 88 ^a
T5	1.74 ± 0.02 ^b	0.57 ± 0.01 ^b	5400 ± 85 ^b
T6	1.73 ± 0.02 ^b	0.58 ± 0.01 ^b	5450 ± 84 ^b
T7	1.73 ± 0.02 ^b	0.58 ± 0.01 ^b	5500 ± 82 ^b
T8	1.71 ± 0.02 ^c	0.59 ± 0.01 ^c	5600 ± 80 ^c

*Values are presented as Mean ± SE. Different superscripts within a column denote significant differences ($P < 0.05$). FCR = Feed Conversion Ratio (g feed/g gain); FCE = Feed Conversion Efficiency (g gain/g feed).

Discussion

Heat stress imposes major physiological challenges in broilers, including acid–base imbalance, oxidative stress, and impaired metabolic responses, which compromise growth, feed efficiency, and overall health (Saker *et al.*, 2020; Livingston *et al.*, 2022). The present study demonstrated that sodium bicarbonate and vinegar supplementation, particularly in combination, effectively mitigated these adverse effects. Improvements in FCR, FCE, and water intake, especially in groups T7–T8 (2% NaHCO₃ + 2–5% vinegar), indicate enhanced nutrient utilization, hydration status, and thermoregulatory capacity under cyclic heat stress. These findings are consistent with previous reports highlighting the beneficial effects of buffering agents and organic acids in poultry production (Livingston *et al.*, 2022; Sa'adu *et al.*, 2026; Siddiqui *et al.*, 2024; Beckford *et al.*, 2020; Khan *et al.*, 2011).

Oxidative stress is a hallmark consequence of heat stress due to excessive production of reactive oxygen species (ROS), resulting in lipid peroxidation and cellular damage (Ncho *et al.*, 2023). SOD and GSH constitute key enzymatic and non-enzymatic antioxidant defenses, respectively (El-Ghoul *et al.*, 2023). In the present study, supplemented groups, particularly those receiving combined additives (T5–T8), exhibited significantly higher SOD and GSH activities together with lower MDA concentrations, indicating enhanced antioxidant capacity and reduced oxidative injury. Since MDA is a sensitive marker of lipid peroxidation (Del Rio *et al.*, 2005), its reduction further confirms improved cellular protection. These observations agree with previous reports demonstrating improved antioxidant responses in heat-stressed birds receiving dietary interventions (Ramezani *et al.*, 2012; El-Ghoul *et al.*, 2023; Sa'adu *et al.*, 2026). The observed synergistic effect may be attributed to sodium bicarbonate-mediated stabilization of acid-base balance and the beneficial effects of vinegar-derived organic acids on gut health and nutrient utilization (Livingston *et al.*, 2022; Saker *et al.*, 2020).

Hematological indices remained within normal physiological limits across treatments, indicating that cyclic heat stress did not induce severe hematological disturbances. Although cortisol levels were not significantly affected, their numerical reduction in supplemented groups suggests attenuation of the physiological stress response (Ramezani *et al.*, 2012; Alhenaky *et al.*, 2017). Significant reductions in hepatic enzymes (SGPT, SGOT, ALP, bilirubin) and renal biomarkers (urea, creatinine, uric acid), particularly in the combined treatment groups, indicate improved liver and kidney function. Heat stress is known to impair hepatic and renal integrity through oxidative injury and metabolic disturbances (Imik *et al.*, 2012), whereas supplementation appeared to preserve organ function and reduce metabolic strain. These protective effects may be attributed to the buffering action of sodium bicarbonate and the ability of vinegar-derived organic acids to enhance nutrient metabolism and reduce systemic stress (Saker *et al.*, 2020; Mansoub, 2010).

The hormonal responses observed in the combined treatment groups constitute another important finding. Significant increases in T₃, T₄, and GH, accompanied by reduced TSH concentrations, indicate restoration of endocrine homeostasis under heat stress. Since thermal stress suppresses thyroid activity and growth-related hormones (Yahav, 2009; Lin *et al.*, 2004), the observed hormonal normalization suggests improved metabolic efficiency, nutrient utilization, and growth potential (Ramezani *et al.*, 2012). Such endocrine regulation likely contributed to the superior productive performance recorded in supplemented birds.

Physiological responses further substantiated the improved heat tolerance of supplemented birds. The restoration of physiological parameters toward normal ranges in the combined treatment groups reflects enhanced thermoregulatory efficiency and a reduced physiological burden of heat stress. Under heat stress, broilers rely on panting and increased cardiovascular activity to dissipate heat, which elevates energy expenditure (Chowdhury *et al.*, 2012). Therefore, the lower body temperatures observed in supplemented birds suggest more efficient maintenance of thermal homeostasis. Increased water intake, particularly in T8 (2% NaHCO₃ + 5% vinegar), likely supported evaporative cooling and hydration (Orosz and Echols, 2020), while improved FCR and FCE reflected superior productive performance. Similar benefits of sodium bicarbonate on feed efficiency (El-Ghoul *et al.*, 2023) and vinegar-derived organic acids on gut health and nutrient digestibility (Allahdo *et al.*, 2018; Adil

et al., 2011; Olayiwola *et al.*, 2025) have been reported previously. Collectively, the superior performance of the combined treatments suggests a synergistic action of sodium bicarbonate and vinegar in maintaining acid–base balance, antioxidant status, organ function, endocrine regulation, and thermoregulatory efficiency in heat-stressed broilers.

Conclusion

Combined supplementation of sodium bicarbonate and vinegar effectively alleviated cyclic heat stress in broilers by improving physiological, antioxidant, endocrine, and performance traits. Among the treatments, 2% sodium bicarbonate plus 5% vinegar (T8) exhibited the greatest protective effect and may serve as a practical nutritional strategy for broiler production under hot climatic conditions.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval:

“All experimental procedures involving broiler chickens were carried out in accordance with the CPCSEA guidelines for the care and use of laboratory animals, and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur (Ref. No.: IAEC/RGSC-BHU/2020-21/32, dated 09/11/2020).”

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