Mitigating Heat Stress in Ross 308 Broilers Through Drinking Water with Different Betaine Forms

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Abstract

Heat stress (HS) remains a critical challenge in poultry production, particularly in tropical regions, causing significant physiological disruptions and economic losses. This study investigated the efficacy of various betaine forms as nutritional interventions to enhance thermos tolerance in Ross 308 broilers. A total of 240 one-day-old chicks were randomly assigned to four treatments for five weeks under heat stress: T1 (control), T2 (1 g/L Betaine Hydrochloride), T3 (1 g/L Betaine Anhydrous), and T4 (1 g/L Betafine S4). Supplementation was administered through drinking water. At the end of the trial Physiological, hematological, and biochemical parameters were analyzed. Results demonstrated that all betaine forms significantly ($P \leq 0.05$) improved the physiological traits of broiler compared to the control. Supplementation effectively reduced the heterophil-to-lymphocyte (H/L) ratio and core body temperature during peak heat periods, indicating diminished stress levels. Specifically, T4 (Betafine S4) and T2 exhibited superior performance in increasing (total protein, albumin, and globulin) concentrations. T4 significantly $P< (0.05)$ enhanced hemoglobin (Hb), packed cell volume (PCV), and erythrocyte indices (MCV, MCH, MCHC). Furthermore, betaine treatments lowered blood glucose and uric acid levels, suggesting improved metabolic efficiency and cellular osmoregulation. In conclusion, supplementing drinking water with betaine, particularly in the Betafine S4 and Hydrochloride forms, serves as a potent strategy to mitigate the adverse effects of heat stress. These findings underscore betaine's role as an osmoprotectant and methyl donor, promoting physiological adaptation and maintaining homeostasis in high-temperature environments.

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Keywords: Ross 308 Broilers, Heat Stress, Betaine Supplementation.

Introduction

The global poultry industry has emerged as one of the most rapidly expanding sectors within animal agriculture, playing a pivotal role in meeting the escalating demand for affordable, high-quality animal-source protein. The confluence of accelerating population growth, rising living standards, and shifting dietary preferences has substantially amplified the consumption of poultry meat and its processed derivatives worldwide [1]. Among commercially available broiler strains, Ross 308 has gained widespread adoption owing to its exceptional growth performance and superior feed conversion efficiency. Nevertheless, intensive and prolonged genetic selection for productive traits has concurrently rendered these birds increasingly susceptible to environmental perturbations, most notably thermal stress, which poses a significant challenge to sustainable broiler production under modern intensive husbandry systems.

Heat stress is considered one of the most critical challenges affecting poultry production in tropical and subtropical regions, including Iraq, where elevated ambient temperatures persist for extended periods annually. Exposure to high temperatures adversely affects feed intake, body weight gain, feed conversion efficiency, immune competence, and survivability, leading to substantial economic losses in the poultry sector [2]. Broiler chickens are especially vulnerable to thermal stress because of their high metabolic heat production and limited ability to dissipate body heat.

Physiologically, heat stress induces several metabolic and cellular disturbances in birds. Increased respiratory activity and oxygen consumption during heat exposure enhance the production of reactive oxygen species (ROS), resulting in oxidative stress and lipid peroxidation, which subsequently damage cellular structures and impair organ function [3]. Consequently, considerable research efforts have focused on developing nutritional and management strategies to improve thermos tolerance and reduce the adverse effects of heat stress in poultry production systems.

Among the nutritional approaches used to alleviate thermal stress, betaine has received considerable scientific attention due to its multifunctional physiological properties. Betaine is a naturally occurring water-soluble trimethyl derivative of glycine found in plants, animals, and microorganisms [4]. It functions primarily as an osmolyte that regulates intracellular water balance and protects cells against dehydration and osmotic disturbances during stressful conditions. In addition, betaine acts as a methyl group donor involved in several metabolic pathways associated with protein synthesis, lipid metabolism, and antioxidant defense mechanisms [5].

Previous studies have demonstrated that betaine supplementation may improve growth performance, antioxidant status, intestinal integrity, and physiological adaptation in heat-stressed broiler chickens. Nevertheless, limited information is available regarding the comparative efficacy of different commercial forms of betaine, particularly when administered through drinking water under daily heat stress conditions. Therefore, the present study aimed to evaluate the effects of supplementing different forms of betaine, namely betaine hydrochloride, anhydrous betaine, and Betafine S4, through drinking water on the physiological parameters of Ross 308 broiler chickens exposed to daily heat stress, as well as to determine the most effective form for improving thermos tolerance and physiological adaptation under elevated environmental temperatures

Materials and Methods

This experiment was conducted at the Poultry Research Field, Department of Animal Production, College of Agriculture, University of Kufa, from September 4 to October 9, 2025, for a period of five weeks. The study aimed to evaluate the effect of supplementing different forms of betaine (Betaine Hydrochloride, Betaine Anhydrous, and Betafine S4) in drinking water on the physiological characteristics of broiler chickens exposed to heat stress.

A total of 240 one-day-old unsexed broiler chicks (Ross 308 strain) were used in the experiment, with an average initial body weight of 45 g. The chicks were obtained from Al-Anwar Hatchery located in Babil Province, Al-Muradiyah District, Iraq. Birds were randomly allocated to four experimental treatments, with 60 chicks per treatment and three replicates per treatment, each replicate containing 20 chicks. The birds were housed in floor pens bedded with approximately 5 cm of coarse wood shavings.

Throughout the experimental period, birds were fed three basal diets according to their age phases:

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Starter diet (1–10 days): containing 3015 kcal ME/kg and 23.11% crude protein.
Grower diet (11–24 days): containing 3081 kcal ME/kg and 21.51% crude protein.
Finisher diet (25–35 days): containing 3210 kcal ME/kg and 19.58% crude protein.

The experimental treatments were as follows:

T1: Control group, provided with feed and drinking water without any supplementation throughout the experimental period.
T2: Drinking water supplemented with 1 g Betaine Hydrochloride/L.
T3: Drinking water supplemented with 1 g Betaine Anhydrous/L.
T4: Drinking water supplemented with 1 g Betafine S4/L.

At the end of the fifth week, blood samples were collected from two male birds per treatment. Hematological parameters were analyzed, including red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), hemoglobin concentration (Hb), platelet count (Thrombocytes), and erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), using a Genix Vet 60 hematology analyzer.

Several blood biochemical parameters were also evaluated (Total protein, albumin, and cholesterol) concentrations were determined according to the methods described by Tietz [6]. Globulin concentration was calculated according to the equation described by Al-Omari [7]. Blood glucose concentration was determined following the method of Coles [8], while uric acid concentration was estimated according to the method of Fossati and Prencipe [9].

Statistical Analysis

Experimental data were statistically analyzed using a Completely Randomized Design (CRD) to evaluate the effects of the different treatments on the studied traits. Significant differences among treatment means were determined using Duncan's Multiple Range Test at significance levels of $P \leq 0.05$. Statistical analyses were performed using the SAS software package (2012), according to the following mathematical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

- Y_{ij} = observed value,

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- μ = overall mean,
- Ti = effect of treatment,
- eij = experimental error

Results and Discussion

Table (1) A significant decrease ($P \leq 0.05$) in red blood cell count (RBC) was recorded in birds of treatment T3, and A significant reduction ($P \leq 0.05$) in white blood cell count (WBC) was observed in treatments T3 and T2. Regarding erythrocytic indices (MCH, MCV, and MCHC), a significant increase ($P \leq 0.05$) was observed in treatment T4.

Table 1. Hematological responses of Ross 308 broilers with different Betaine forms under heat stress (Mean ± SE).

Treatment	RBC 10^6/ML	WBC 10^3/ML	MCV Fl	MCH Pg./Cell	MCHC g/dl	Throm 10^3/ML
T1	2.23 a ±0.06	23.67 a ±0.33	124.68 b ±1.91	46.54 c ±0.61	37.32 b ±0.09	33.83 ±9.33
T2	2.21 ab ±0.01	22.36 b ±0.41	127.46 b ±2.87	50.14 bc ±1.75	39.37 ab ±1.57	38.50 ±2.02
T3	2.10 b ±0.01	22.17 b ±0.39	140.45 a ±2.91	56.18 ab ±2.33	39.96 ab ±0.87	37.33 ±4.21
T4	2.14 ab ±0.008	23.88 a ±0.26	143.19 a ±2.82	59.57 a ±2.66	41.56 ±1.03	39.10 ±3.24

* Different letters in a column indicate significant differences between means at a probability level of ($P \leq 0.05$). T1: Control group, T2: 1 g Betaine Hydrochloride/L, T3: 1 g Betaine Anhydrous/L, T4: 1 g Betafine S4/L.

Table (2) A significant increase ($P \leq 0.05$) in PCV and Hb values was observed in birds of treatment T4. A significant decrease ($P \leq 0.05$) in heterophil percentage (H) and H/L ratio, accompanied by a significant increase ($P \leq 0.05$) in lymphocyte percentage (L), was recorded in all betaine-treated groups (T2, T3, and T4) compared with the control group T1.

The observed changes in the control group, including lower lymphocyte percentage and higher heterophil percentage and H/L ratio, are consistent with previous studies [10,11,12], which reported that heat stress activates the hypothalamic–

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pituitary–adrenal axis, leading to increased secretion of stress hormones, particularly corticosterone, resulting in lymphocyte reduction (lymphopenia) and alteration of the H/L ratio.

Table 2. Leukocyte profile and hematological indices of Ross 308 broilers with different Betaine forms under heat stress (Mean ± SE).

Treatment	PCV %	Hb g/100 ml	Heterophils – H %	Lymphocytes – L %	H/L
T1	27.86 b ±0.60	10.40 b ±0.40	22.96 a ±0.17	66.17 b ±0.66	0.35 a ±0.003
T2	28.11 b ±0.80	11.06 a ±0.44	21.87 b ±0.30	70.25 a ±0.72	0.31 b ±0.005
T3	29.50 ab ±0.40	11.80 ab ±0.52	21.80 b ±0.52	69.25 ab ±0.89	0.31 b ±0.011
T4	30.60 a ±0.80	12.73 a ±0.62	22.10 b ±0.17	69.55 a ±0.03	0.32 b ±0.006

* Different letters in a column indicate significant differences between means at a probability level of ($P \leq 0.05$). T1: Control group, T2: 1 g Betaine Hydrochloride/L, T3: 1 g Betaine Anhydrous/L, T4: 1 g Betafine S4/L.

These findings are in agreement with [13,11,12] who reported that betaine supplementation in feed or drinking water of heat-stressed broilers reduces stress indicators, particularly by decreasing the H/L ratio. Bedanova et al. [14] reported that the normal H/L ratio is approximately 0.4, but it may increase up to eight times under heat stress conditions, and therefore can be used as an indicator of chronic stress in poultry.

The significant increase ($P<0.05$) in lymphocyte percentage in betaine-treated groups may be associated with the role of betaine in maintaining cellular homeostasis and membrane stability, as well as its function as a methyl group donor (CH_3), which enhances metabolic efficiency and promotes proliferation and differentiation of immune cells, particularly lymphocytes [15].

Table (3) A significant increase ($P \leq 0.05$) in total protein, albumin, and globulin was observed in all treated groups (T2, T3, and T4) compared with the control group (T1). Additionally, T2 and T4 showed significantly higher ($P \leq 0.05$) values than T3 for total protein and globulin.

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Table 3. Serum biochemical and antioxidant responses of Ross 308 broilers with different Betaine forms under heat stress (Mean ± SE).

Treatment	TP g/dl	ALB g/dl	GLB g/dl	TC. mg/dl	Glu. mg/dl	UA mg/dl	Sod u/ml	T anti. Mmol fe^{+2}/l
T1	3.04 c ±0.02	1.59 b 0.02	1.45 c 0.006	124.33 a 5.41	281.67 a 4.36	7.17 a 0.34	350.74 b 3.75	143.05 b 9.19
T2	3.94 a 0.02	1.72 a 0.05	2.22 a 0.04	121.00 a 4.39	275.00 ab 2.60	7.20 a 0.26	370.44 a 10.13	187.78 a 11.39
T3	3.59 b 0.03	1.74 a 0.03	1.84 b 0.0008	124.33 a 1.33	273.00 b 0.73	6.63 b 0.06	356.51 b 1.85	156.94 b 8.70
T4	4.03 a 0.06	1.74 a 0.02	2.28 s 0.04	111.33 b 0.95	269.47 c 2.48	7.73 a 0.32	363.07 a 4.38	186.38 a 5.13

* Different letters in a column indicate significant differences between means at a probability level of ($P \leq 0.05$). T1: Control group, T2: 1 g Betaine Hydrochloride/L, T3: 1 g Betaine Anhydrous/L, T4: 1 g Betafine S4/L.

Regarding blood glucose levels, T3 and T4 exhibited a significant reduction ($P \leq 0.05$) compared with T1. T3 acid concentration showed a significant decrease ($P \leq 0.05$) compared with all other groups. SOD activity and total antioxidant capacity were significantly higher ($P \leq 0.05$) in T2 and T4.

El-Husseiny et al. [16] and Alshukri [12] reported similar effects of dietary or drinking-water betaine supplementation on serum protein fractions in heat-stressed broilers.

The observed increases in total protein, albumin, and globulin may be associated with the role of betaine as a methyl group (CH_3) donor, particularly under heat stress conditions where methyl group demand is elevated. This may reduce the requirement for methionine in methylation reactions, thereby sparing methionine for protein synthesis. Betaine is involved in methylation pathways that regenerate methionine from homocysteine, which may support protein deposition [12].

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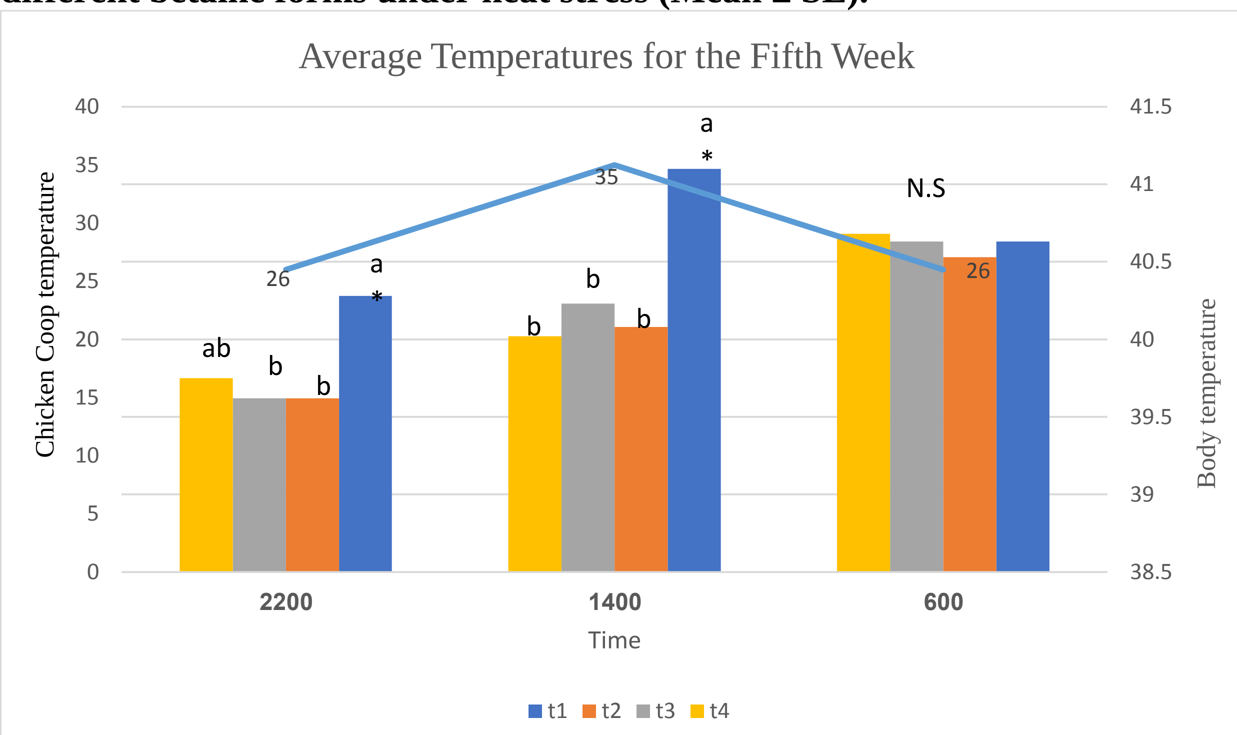
In addition, betaine supplementation is associated with reduced heat stress indicators, as evidenced by the significantly lower H/L ratio (Table 2), indicating reduced corticosterone-related stress responses and consequently reduced protein catabolism.

The significant (P<0.05) reduction in blood glucose levels may be related to the osmoprotective properties of betaine, which contribute to improved water balance and reduced dehydration through enhanced water retention and evaporative heat loss regulation [11].

A significant decrease (P≤0.05) in body temperature was recorded in betaine-treated groups at 14:00 h during the fifth week of age Figure (1). This reduction in body temperature was associated with a lower thermal load during the peak heat period, which coincided with reduced physiological stress indicators and consequently lower stress-related hormonal secretion involved in elevating blood glucose levels.

Figure 1. Body temperature of Ross 308 broilers with different betaine forms under heat stress (Mean ± SE).



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The significant reduction in body temperature observed in may be associated with the osmoprotective properties of betaine. This function contributes to improved cellular water retention, reduced dehydration, and enhanced evaporative heat dissipation, ultimately leading to lower body temperature [11]. This reduction in thermal load was accompanied by a significant decrease in the H/L ratio (Table 2), indicating reduced physiological stress under heat challenge conditions.

Conclusion

The study concluded that supplementing the drinking water of broiler chickens (Ross 308 strain) with various forms of betaine, particularly the preparation (Betafine S4), represents an effective physiological and nutritional strategy for alleviating heat stress. Betaine acts as an osmotic regulator and methyl group donor, which clearly led to a reduction in body temperature and stress indicators (H/L ratio), while enhancing the immune response (lymphocytes) and improving protein metabolic efficiency. The study recommends the adoption of betaine supplementation in production systems in hot regions to enhance physiological adaptation and ensure the sustainability of poultry production performance under thermal stress.

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