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Effect of Different Types of Betaine (Hydrochloride, Anhydrous, Betafine S4) Supplemented with Drinking Water on The Productive Traits of Broilers (Ross 308) Under Heat Stress Conditions

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Abstract

The experiment was conducted in the poultry field of the Department of Animal Production –College of Agriculture – University of Kufa for the period 4/9/2025 until 9/10/2025. The study aimed to determine the effect of different types of Betaine (Hydrochloride, Anhydrous, Betafine S4) supplemented with drinking water on some productive traits of broiler chickens exposed to heat stress. 240 one-day-old Ross 308 unsexed broiler chicks with an average weight of 45g/chick were used. The chicks at one day old were randomly allocated into 4 treatments with 3 replicates per treatment, each replicate containing 20 chicks. The treatments were: T_1, a standard diet without any addition (Control). Treatments T_3, T_2, and T_4 were fed a standard diet, and betaine (Betafine S4, Anhydrous, Hydrochloride) was added to the drinking water at a rate of 1 g betaine/liter of drinking water, respectively. The chicks were fed a starter diet for 1-10 days, a grower diet for 11-24 days, and a finisher diet for 25-35 days. A completely randomized design was used to analyze the data, and significant differences between treatments were tested at significance levels of ($P \leq 0.05$) and ($P \leq 0.01$). The most important results were the following:

- Significant increase in final body weights at 5 weeks of age and weight gain for all treatments, T_4, T_3, and T_2, compared to the control treatment T_1, and the superiority of the treatment T_2 over treatments T_3 and T_4.
- A significant increase in total feed intake was observed in all treatments compared to the control treatment T_1. No significant differences in total feed conversion were observed between all experimental treatments.
- A significant increase in the treatments T_3 and T_2 with respect to the production index and the economic indicator compared to the control treatment T_1 and treatment T_4.
- A significant increase in breast meat weight was observed in treatments T_2, T_3, and T_4 compared to the control treatment T_1. No significant differences were observed in dressing percentage or relative thigh weight for any of the treatments.

Keywords: Heat stress, Broilers, Productive performance, Betaine

Introduction

The world is witnessing an increase in demand for livestock products as a result of the continuous increase in population on the one hand, and the improvement of living standards on the other hand, which has changed eating habits and increased demand for various poultry meat products [(1)] and a decrease in relative cost with the increase in the nutritional value of poultry meat, making it more consumed in global markets [(2)]. Therefore, the great importance of the poultry industry has emerged, which has prompted many researchers to find new ways that, in turn, are sufficient to reduce the most important problems that most countries of the world suffer from, including Iraq, which is the problem of high temperatures for a period exceeding six months of the year. High temperatures cause significant losses in the poultry industry and are the main reason for reduced growth and weak production performance [(3)]. High temperatures also increase the respiratory rate in birds, which leads to the entry of large amounts of oxygen into the body. This, in turn, leads to an increase in the production of free radicals that cause oxidative damage to cells through the occurrence of lipid peroxidation and thus oxidative damage [(4)]. Many research studies have been conducted to learn more about the interactive mechanisms and paths caused by heat stress inside the bird's body for the purpose of confronting it and reducing its negative effects, and using genetic engineering and biotechnology to identify genes related to resistance to harsh heat stress [(5)]. The high temperatures in Iraq cause huge losses that threaten the economy, so it was necessary to use many means and methods to mitigate the negative effects of high temperatures on birds, such as using Betaine, which is a water-soluble nitrogenous compound, a trimethyl derivative of glycine acid, as it is found naturally in plants, animals, and microorganisms [(6)]. Because it has two basic physiological functions: the first is a donor of the methyl group CH_3, and the second is osmotic regulation because it is a bipolar (zwitterion), as it carries two charges, one positive and the other negative [(7)]. The current study aimed to evaluate the effect of the three types of betaine on the productive performance of birds under conditions of heat stress, and in addition to what was mentioned, to determine the optimal type among them for the purpose of using it in conditions of high temperatures.

Materials and Methods

This study was conducted in a poultry farm, Department of Animal Production, College of Agriculture, University of Kufa, for the period 4/9/2025 until 9/10/2025, over a period of 5 weeks. The study investigated the effect of adding different types of betaine (Betafine s4, Anhydrous, Hydrochloride) to drinking water on certain productive traits of broiler chickens exposed to heat stress. In the experiment, 240 unsexed broiler chicks of Ross308 hybrids, one day old and with an initial weight of 45 grams, were used. They were prepared from the Al-Anwar hatchery located in the Al-Muradiya area of Babylon Governorate. The chicks were distributed among the treatments, with 60 chicks per treatment and three replicates (20 chicks/replicate). Each replicate represents a Pen. A 5 cm layer of coarse wood shavings was placed in the Pen. The birds were fed three diets (Starter, Grower, Finisher). The starter diet was for chicks aged 1-10 days with an energy and protein content of 3015 kcal and 23.11%, respectively. The grower diet was for chicks aged 10-24 days with an energy and protein content of 3081 kcal and 21.51%, respectively. The finisher diet was for chicks aged 25-35 days with an energy and protein content of 3210 kcal and 19.58%, respectively. The treatments were as follows: (T_1): Control, which received feed and water throughout the experiment without any additions. (T_2): Standard diet supplemented with 1 g of betaine hydrochloride per liter of drinking water. (T_3): Standard diet supplemented with 1 g of betaine anhydrous/liter of drinking water. (T_4): Standard diet supplemented with 1 g of Betafine S4/liter of drinking water. Live body weight, weight gain, feed intake [(8)], and feed conversion ratio [(9)] were measured for each treatment weekly from week 1 to week 5, as well as for the total for all weeks. The production index, economic figure [(10)], dressing percentage, and carcass cut percentage were also measured [(8)].

Statistical Analysis:

The data for the experiment were analyzed statistically using a complete randomized design (CRD) to determine the effect of different treatments on the studied traits. Significant differences between treatments were tested using Duncan's multiple range test (1955) at significance levels of 0.05 and 0.01 using the SAS statistical software (2012) and according to the following mathematical model.

$$Yij = \mu + Ti + eij$$

Results and Discussion

Productive Characteristics

1. Body Weight and Weight Gain

Table (1) shows the effect of different treatments on weekly body weight. No significant differences were observed in any of the initial weight treatments. During the first week, treatment (T_3) significantly surpassed the control treatment (T_1) ($P \leq 0.05$), while no significant difference was found between treatments (T_1, T_2, and T_4) or between (T_2, T_3, and T4). In the second week, treatments (T_2 and T_4) surpassed the control treatment (T_1). In the third week, treatments (T_2, T_3) significantly surpassed the control treatment T_1 and treatment T_4. In the fourth and fifth weeks, all treatments (T_2, T_3, T_4) surpassed the control treatment; also, treatment T_2 surpassed treatments T_3 and T_4.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
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Table (1) Effect of different types of betaine supplemented with drinking water on the weekly and total live body weight (g/bird) of broiler chickens exposed to periodic daily temperature stress[1] for ages (1-5) weeks

Mean ± Standard Error						
Treatments	Day 1	2	3	4	5	Total
Control (T_1)	0.83±45.8	b 1.69±175.1	b 2.72±475.6	b1.22±897.6	d4.14±1427.0	d3.59±2010.6
T_2	0.28±46.0	ab0.44±177.3	a 4.24±488.1	a2.17±916.0	a2.67±1515.7	a1.26±2188.5
T_3	0.44±46.1	a 1.03±180.3	ab 1.07±481.3	a3.13±910.5	b4.68±1493.7	b3.07±2148.2
T_4	0.16±46.3	ab1.78±178.1	a 1.93±485.1	c1.85±885.8	c1.33±1438.5	c1.97±2068.5
Significance	N. S	*	*	*	*	*

*Different letters within the column indicate significant differences between treatments under a probability level of (P≤0.05), N.S. indicates no significant differences. 1- Periodic daily temperature for weeks (3, 4, 5) was (31-36-31°C), (28-35-28°C), and (26-35-26°C), respectively. 2- Treatments T_1 (control without addition), T_2, T_3, and T_4 (adding 1 g of betaine/L of drinking water) for the hydrochloride, anhydrous, and betafin s4 species, respectively.

Table (2) shows the effects of adding different betaine treatments on the weekly and total weight gain of broiler chickens exposed to heat stress. In the first week, treatment T_3 exceeded the control treatment T_1, while no significant differences were observed between treatments T_1, T_2, and T_4. In the second week, treatment T_2 exceeded the control treatment T_1, while no significant differences were observed between treatments T_2, T_3, and T_4, as well as between treatments T_1, T_3, and T_4.

Table (2) Effect of adding betaine on weekly and total weight gain (g/bird) of broiler chickens exposed to periodic daily temperature stress[1] for ages (1-5) weeks

Mean ± Standard Error						
Treatments	1	2	3	4	5	Total
Control (T_1)	b0.89±129.32	b4.38±300.50	a3.65±421.94	d4.48±529.40	d2.83±583.62	d2.81±1964.78
T_2	ab0.17±131.33	a3.86± 310.83	a4.85±427.84	a2.22±599.75	a1.43±672.75	a0.98±2142.50
T_3	a0.65±134.16	ab0.90±301.00	a2.27±429.17	b3.37±583.20	b7.75±654.52	b3.52±2102.05
T_4	ab1.84±131.82	ab0.41 ±307.00	b0.53±400.67	c0.55±552.68	c0.66±630.04	c1.99±2022.21
Significance	*	*	*	**	**	**

*Different letters within the column indicate significant differences between treatments under a probability level of (P≤0.05), N.S. indicates no significant differences. 1- Periodic daily temperature for weeks (3, 4, 5) was (31-36-31°C), (28-35-28°C), and (26-35-26°C), respectively. 2- Treatments T_1 (control without addition), T_2, T_3, and T_4 (adding 1 g of betaine/L of drinking water) for the hydrochloride, anhydrous, and betafin s4 species, respectively.

Tables 1 and 2 show that the significant improvement in body weight and weight gain observed in the betaine treatments compared to the control group is consistent with the findings of [11,12,13]. This contrasts with the findings of [12,13], who did not observe any significant differences between the betaine hydrochloride and control treatments in terms of body weight and weight gain, despite the significant improvement observed in the betaine anhydrous treatments compared to the control treatment. The significant superiority achieved by the three betaine treatments compared to the control treatment T_1 may be due to the effectiveness of betaine in reducing the negative effects of heat stress on birds [14,15]. Moreover, [13], observed that adding betaine to broiler diets reduced the panting rate of 7 and 10-week-old Arbor Acres birds and rectal temperature. Panting is an important strategy for regulating the bird's temperature and usually involves muscular activity that requires energy expenditure [16,17]. Thus, losing energy directed towards body building, and therefore dietary betaine works to preserve the energy spent on panting, thereby improving the bird's health and productive performance [18]. The improvement in live body weight and weight gain of betaine treatments compared to the T_1 treatment may be due to the role of betaine in regulating the gene expression of muscle-generating genes and the insulin-like growth factor-1 signaling pathway to increase muscle protein deposition [12]. Betaine also plays a distinct role in providing essential amino acids important in the formation of body proteins, especially sulfur-containing amino acids such as methionine and cysteine, thus improving productive performance, represented by body weight and weight gain [19,20]. The improvement in body weight may be due to betaine's role in improving the digestive tract environment through its osmotic function and quantity in intestinal cells, increasing the water-binding capacity of those cells, and enhancing changes in the structure of the intestinal epithelium. [21] stated that adding betaine improves the height and width of the small intestinal villi, which positively affects the digestion and absorption of nutrients.

2. Feed intake

Table (3) illustrates the effect of different treatments on the weekly and total feed intake of broiler chickens exposed to heat stress. During the first week of age, treatment T_2 showed a significant increase (P<0.05) compared to the control T_1 and treatments T_3 and T_4. In the second week, treatment T_2 showed a significant superiority (P<0.05) over the control treatment T_1, with no significant differences between treatments T_2, T_3, and T_4. In the third week, there were no significant differences between all treatments. In the fourth week, all treatments surpassed the control treatment, and treatment T_2 also surpassed treatments T_3 and T_4. In the fifth week, treatments T_2 and T_3 surpassed the control treatment T_1. In terms of total feed intake, all treatments significantly exceeded the control treatment T_1 (P<0.05), and there were no significant differences between T_2, T_3, and T_4. Treatment T_2 surpassed treatment T_4, while there was no significant difference between T_2 and T_3, nor between T_3 and T_4. The results showed a significant superiority achieved in feed intake for the treatments adding betaine, especially in the last weeks of the experiment T_2, T_3, T_4, compared to the control treatment, is consistent with [22,12], who found a significant superiority in feed intake in favor of the anhydrous betaine treatments compared to the control for birds exposed to heat stress, and differs with [21,13], as they did not observe any significant differences between the betaine treatments and the control. The significant superiority in feed intake observed in birds of T_2, T_3, and T_4 treatments compared to birds in the control treatment T_1 may be attributed to Betaine's osmotic function, which reduces dehydration by facilitating water retention within the body. This, in turn, helps dissipate heat through evaporation, consequently lowering body temperature under conditions of heat stress [21]. Furthermore, [23] reported a decrease in feed intake of approximately 1.27% for every 1°C increase in ambient temperature within the range of 18-32°C, with the decrease in feed intake increasing at temperatures within the range of 32-38°C.

Table (3) Effect of adding different types of betaine to drinking water on the weekly and total feed intake rate (g/bird) of broiler chickens exposed to periodic daily temperature stress[1] for ages (1-5) weeks

Mean ± Standard Error						
Treatments	1	2	3	4	5	Total
Control (T_1)	±150.33b1.42	b4.40 ±361.16	10.32±620.73	c2.85±856.66	b22.95±1111.82	32.55±3100.70c
T_2	a1.39±157.33	a3.28±373.77	2.57±632.00	a3.41±910.77	a30.91±1214.11	a 29.31±3287.98
T_3	b0.82±149.77	ab2.20±365.81	7.11±626.50	b3.46±892.51	a7.96±1200.71	ab 13.53±3234.98
T_4	b2.07±150.64	ab3.48±369.18	7.56±614.77	b3.49±885.64	ab0.96±1165.06	b 9.43±3185.29
Significance	*	*	NS	*	*	*

*Different letters within the column indicate significant differences between treatments under a probability level of (P≤0.05), N.S. indicates no significant differences. 1- Periodic daily temperature for weeks (3, 4, 5) was (31-36-31°C), (28-35-28°C), and (26-35-26°C), respectively. 2- Treatments T_1 (control without addition), T_2, T_3, and T_4 (adding 1 g of betaine/L of drinking water) for the hydrochloride, anhydrous, and betafin s4 species, respectively.

3. Feed Conversion Ratio (FCR)

Table (4) shows the effect of different treatments on the feed conversion ratio; no significant differences (P>0.05) were observed among all treatments during the second, fifth, and total weeks. However, a significant improvement (P<0.05) was recorded for treatments T_3 and T_4 compared to treatment T_2, and there was no significant difference between T_1, T_3, and T_4 in the first week of life. In the third week, there was a significant improvement in treatments T_1 and T_3 compared to treatment T_4, and there was no significant difference between T_1, T_2, and T_3. In the fourth week, there was a significant improvement (P<0.05) in the feed conversion ratio of birds in treatments T_2 and T_3 compared to T_1 and T_4. The significant improvement in the feed conversion ratio in favor of the betaine treatments compared to the control treatment at the fourth week of life, and the numerical superiority of the same treatments in the total feed conversion, may be due to the

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
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Vol. 36 Issue 2, 2026, Pages 211-214

osmotic function of betaine, which increases cell size and accelerates the building process, thus having a direct role in the safety and health of the digestive system, improving the height of the villi and maintaining the structure of the intestinal villi, which increases the absorption of nutrients [24]. These results are similar to those reported by [1,3,25], indicating that adding betaine to feed improves feed conversion ratio at all levels, stating that whenever the percentage of betaine is higher in the feed, the feed conversion ratio is better. The results also agree with [26], who reported that betaine improved the feed conversion ratio in broiler chickens under heat stress.

Table (4) Effect of adding different types of betaine on the weekly and total feed conversion ratio (g weight/g feed) of broiler chickens exposed to periodic daily temperature stress[1] for ages (1-5) weeks

Mean ± Standard Error						
Treatments	1	2	3	4	5	Total
Control (T_1)	1.16±0.011ab	0.017±1.20	b0.029±1.46	a0.017±1.62	0.040±1.90	0.018±1.57
T_2	a0.012±1.19	0.012±1.20	ab0.013±1.47	b0.007±1.52	0.041±1.80	0.013±1.53
T_3	b0.006±1.11	0.006±1.21	b0.020±1.46	b0.008±1.52	0.008±1.83	0.005±1.54
T_4	b0.026±1.14	0.010±1.20	a0.018±1.53	a0.005±1.60	0.003±1.84	0.008±1.57
Significance	•	NS	*	*	NS	NS

*Different letters within the column indicate significant differences between treatments under a probability level of (P≤0.05), N.S. indicates no significant differences. 1- Periodic daily temperature for weeks (3, 4, 5) was (31-36-31°C), (28-35-28°C), and (26-35-26°C), respectively. 2- Treatments T_1 (control without addition), T_2, T_3, and T_4 (adding 1 g of betaine/L of drinking water) for the hydrochloride, anhydrous, and betafin s4 species, respectively.

4. Production Index and Economic Indicator

Results in Table (5) illustrate the effect of different treatments on the production index and the economic indicator. A significant difference (P≤0.05) was observed between treatments T_2 and T_3 for the production index and the economic indicator compared to the control treatment T_1 and treatment T_4. No significant difference was found between treatments T_1 and T_4 and between treatments T_2 and T_3. The reason for the superiority of treatments T_2 and T_3 over the other experimental treatments is attributed to the increased average body weight of these treatments on the one hand and the improved feed conversion ratio, on the other hand, which positively impacted the production index and economic indicators. This may be because the use of betaine reduced heat stress, allowing the birds to express their genes more effectively. These results are consistent with [24], which indicated that the use of betaine in poultry diets led to improved production traits, which in turn increased the economic index and production index.

Table (5) Effect of adding different types of betaine to drinking water on the production index and economic indicator of broiler chickens exposed to periodic daily temperature stress[1] for ages (1-5) weeks

Mean ± Standard Error		
Treatments	Production index	Economic indicator
Control (T_1)	±364.464.99b	±364.46 9 b4.9
T_2	a3.78±406.97	a3.78±406.97
T_3	a0.95±398.56	a0.95±398.56
T_4	b2.45±374.87	b2.45±374.87
Significance	*	*

*Different letters within the column indicate significant differences between treatments under a probability level of (P≤0.05), N.S. indicates no significant differences. 1- Periodic daily temperature for weeks (3, 4, 5) was (31-36-31°C), (28-35-28°C), and (26-35-26°C), respectively. 2- Treatments T_1 (control without addition), T_2, T_3, and T_4 (adding 1 g of betaine/L of drinking water) for the hydrochloride, anhydrous, and betafin s4 species, respectively.

5. Dressing percentage and relative weight of carcass cuts:

The results in Table (6) show the effect of different treatments on dressing percentage and relative weight of carcass cuts. It observes no significant differences in dressing percentage and relative weight of the thighs among all treatments. However, a significant increase (P<0.05) was observed in the relative weight of the breast cut for treatments (T_2, T_3, T_4) compared to the control (T_1). While no significant differences were observed between the treatments (T_2, T_3, T_4). Birds in all treatments (T_2, T_3, T_4) showed a significant decrease (P<0.05) compared to the control treatment (T_1), while no significant difference was found between treatments T_2, T_3, and T_4. As for the relative neck weight, a significant decrease (P<0.05) was observed in birds in treatments T_2 and T_4 compared to the control treatment (T_1). However, no significant difference was found between treatments T_1 and T_3, as well as between treatments T_2 and T_4. Additionally, the significant superiority of the breast cuts in birds of treatment T_2, T_3, T_4 compared to birds of treatment T_1 may be due to betaine's ability to regulate gene expression of muscle-generating genes and the relative growth factor signaling pathway and increase the deposition of muscle proteins, most importantly the breast cut [27], or betaine may have a role in increasing the formation of essential amino acids, methionine and cysteine, and thus increasing protein deposition in the body's muscles, especially the breast muscle [19]. The results are in agreement with the findings of [28], that a significant increase was observed in breast cut in favor of the treatment in which betaine was used in drinking water under heat stress conditions. Furthermore, the findings align with those of [19], who observed a significant increase in breast cut in favor of the betaine treatment when used in the diet of broiler chickens exposed to heat stress.

Table (6) Effect of adding different types of betaine to drinking water on the dressing percentage and relative weight of carcass cuts of broiler chickens exposed to periodic daily temperature stress[1] at the age of (1-5) weeks

Mean ± Standard Error						
Treatments	Dressing percentage Excluding edible parts %	Relative weight of breast %	Relative weight of thighs %	Relative weight of wings %	Relative weight of neck %	Relative weight of the back %
Control (T_1)	1.772±70.25	b0.729± 35.04	0.994±28.90	a0.283±10.33	a0.016±5.91	a0.853±19.60
T_2	0.084±71.44	a0.164±36.92	0.246±30.51	b0.035±9.71	c0.265±4.96	b0.343±17.52
T_3	0.049±71.96	a0.346±37.82	0.225±29.26	b0.195±9.60	ab0.110±5.71	b0.253±17.53
T_4	0.446±71.44	a0.144±38.15	0.308±29.39	b0.057±9.70	bc0.173±5.30	b0.008±17.20
Significance	N. S	•	N. S	*	*	*

*Different letters within the column indicate significant differences between treatments under a probability level of (P≤0.05), N.S. indicates no significant differences. 1- Periodic daily temperature for weeks (3, 4, 5) was (31-36-31°C), (28-35-28°C), and (26-35-26°C), respectively. 2- Treatments T_1 (control without addition), T_2, T_3, and T_4 (adding 1 g of betaine/L of drinking water) for the hydrochloride, anhydrous, and betafin s4 species, respectively.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
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Vol. 36 Issue 2, 2026, Pages 211-214

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