

# Effects of dietary betaine supplementation on growth performance of broilers during heat stress

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**Abstract:** As an environmental stressor due to global warming, heat stress (HS) is considered as a major challenge in poultry industries in tropical countries like Bangladesh for substantial economic loss because of its severe effects on poultry health and productivity. Dietary betaine is thought to be an effective solution for reducing such losses. An experiment was conducted on 250 Ross 308 broilers for 35 days to see if betaine supplementation could improve performance under stressful conditions. During the trial, broiler birds were exposed to artificial HS of 30°C for 8 hours (in 18,19 and 20 days) and for 11h (in 26,27,28 days) during the day. The treatments are, G1: control feed without HS; G2: control feed with HS; G3: control +HS+1 g/kg betaine; G4: control + HS +1.5 g/kg betaine and G5: control +HS+2 g/kg betaine from day 11 to day 33 and average weight (AW), daily weight gain (DWG), feed intake (FI), FCR were analysed. HS had a lower effect on average weight and daily weight gain for the chickens whereas in total FI and FCR, betaine treated feed have some significant effect ( $p<0.05$ ) for FI and ( $p<0.05$ ) and FCR. Overall, supplementation of the betaine did not show any adverse effect on the zootechnical performances of experimental species and seems to support the birds during stressful condition.

**Keywords:** Broilers, Heat Stress, Growth Performances, Betaine.

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## 1. INTRODUCTION

The modern poultry industry is growing day by day due to the increase in population, demand for poultry products and market growth, especially in hot countries. With the increase in demand, the environmental stress caused by global warming is an obstacle to the development of the poultry industry. As a result of global warming, poultry are often raised under stressful conditions related to increased ambient temperature, known as HS (Nawab et al., 2018). HS has been considered as a critical obstacle that affects poultry industry specially in tropical regions or hot climate areas (Abdel-Moneim et al., 2021). Poultry in tropical countries are reared in open side sheds with natural ventilation and thus they are often raised under stressful conditions (FAO, 2013). As an important environmental determinant HS affects the performance of poultry like egg production, meat production worldwide (Kumar et al., 2021). These losses of production due to exposure of high ambient temperature leads to substantial economic losses for poultry industry (Abdel-Moneim et al., 2021).

A direct strategy for eliminating HS in poultry is to reduce the temperature of the house to the thermoneutral zone, and the thermoneutral zone can optimise growth potential (Nawab et al., 2018). However, for small-scale commercial poultry farmers, it is difficult to spend money on building house with mechanical ventilation or expensive cooling system (Liu et

al., 2019). Therefore, nutritional solution along with different nutritional feeding strategies can be an alternative to reduce the negative effects of high ambient temperature in poultry (Farghly et al., 2018). The use of betaine as a feed supplement in broiler diets is one such nutritional strategy to reduce stress in broilers (Chand et al., 2017).

Betaine contains a neutral methyl derivative of the amino acid glycine with a positively charged trimethylammonium group and a negatively charged carboxyl group (Craig, 2004; Attia et al., 2009). As a methyl group donor, betaine is involved in transmethylation reactions for methyl group donors and, being an osmo-protectant, it helps maintain cell osmolarity (Ratriyanto et al., 2009). In order to exert osmo-protective effects under osmotic or ionic stress, betaine accumulates in cells to replace inorganic ions (e.g. K<sup>+</sup>) (and prevents inactivation of enzymes and cell membranes by inorganic ions (Petronini et al., 1992; Klasing et al., 2002). Thus, betaine can help maintain cell integrity and hydration under HS through its osmoregulatory (Kidd et al., 1997) and anti-stress functions (Saunderson & Mackinlay, 1990). Betaine also contributes to nutrient digestibility by controlling osmotic pressure within the intestinal epithelial cells and helping the gut microbes to cope with osmotic variations in the digestive tract (Eklund et al., 2005). However, few studies have been conducted on betaine as a dietary supplement to prevent the adverse effects of HS. Therefore, the current experiment was conducted to investigate the adverse effect of HS on growth performance and to evaluate whether feeding of betaine could ameliorate HS-induced impairment of growth parameters.

## 2. MATERIALS AND METHODS

The trial ran from June 6 to July 8, 2020 and total experimental duration was for 5 weeks.

### The zootechnical research farm

The trial was carried out on the chicken associated with research in Alam poultry farm, Dinajpur. Total area of the farm measures about 200 square metres with static ventilation.

The building is composed of 25 cages with 5 square metres for each, separated by metal grids. Each cage is equipped with manual drinker and feeder which is filled manually with bags distributed by the breeder. The neon lights are lit in the building from the first to the 32nd day with a light intensity of 20 lx. The maximum and minimum temperatures of the building, water and feed consumption are recorded daily.

### Distribution of the chickens

250 ROSS-308-day-old chicks (100% male) were of same weight and randomly distributed in 25 cages with 10 chickens per cage with 5 repetitions for each treatment. Before arrival, the chicks were vaccinated against infectious bronchitis and a vaccine against Gumboro disease was done at about 17 days of age in the rearing pens.

**Table 1: Grouping of chickens on the research farm**

	Group 1	Group 2	Group 3	Group 4	Group 5
0-35 days	Standard diet (no HS)	SD + HS	SD + HS+ 1g betaine per kg feed	SD+ HS+ 1.5g betaine per kg feed	SD+ HS + 2g betaine per kg feed

SD=Standard Diet, HS= Heat Stress

### Feed

5 groups were considered according to the diet supplied and these feeds consisted of cereals and different premixes depending on the diet, each of which met the needs of the chickens. The feeds are made up of wheat, maize, soya, sunflower, and premixes containing minerals, vitamins and different amino acids. Group-1 (G1) means control group fed up only with Standard diet (table 1), Group-2(G2) was heat treated group having standard diet and betaine with different concentrations were added with the feed in the treatment groups i.e. Group- 3 (G3), Group-4 (G4) and Group-5 (G5), The broiler feed was divided into three phases: the start-up phase from 1 day to 10 days, the rearing phase from 11 days to 22 days and the finishing phase from 23 days to 33 days. The feeds were formulated with Excel and the formulation was done including the basics of feeding chickens under HS. Feed and drinking water were supplied ad libitum.

### Heat stress protocol

During the trial, HS was artificially created in 3 periods to observe the thermo-tolerance capacity and zootechnical performance in broilers. The starting temperature of the building was 33°C for day-old chicks. From day 5 at 9 a.m. to at 9 a.m. of day 6, the temperature in the building was gradually increased to 35°C for 24 hours to create an acclimatisation phase. Early acclimatisation has indeed been shown to be the most effective method to improve the thermal tolerance of broilers in later periods of heat (Yahav and Hurwitz, 1996). A first HS condition was created during days 18, 19 and 20 with a temperature of 30°C between 11:00 and 19:00, followed by a return to 24°C at night. The chickens were not fasted during the hours of HS.

A second HS condition was created during days 26, 27 and 28 by artificially raising the building temperature to 30°C between 9am and 8pm and then returning the set points to 24°C at night. The chickens were fed on an empty stomach between 11am and 9pm during the HS.

### Statistical treatments

The statistical analysis of the data was carried out using the software R. The calculation of means, standard deviations, analysis of variance and comparison of means (ANOVA) was carried out using R software. The zootechnical performances such as AW, DWG, FCR, mortality rate, etc. were subjected to a two-factor analysis of variance. In case of significance of the treatments group, a post-hoc pairwise comparison was conducted. 5% alpha threshold is used that means the probability of error on a single test is 5%.

## 3. RESULTS

Assessment of overall growth performance at 35 days are shown in table 2. The result presents that supplementation of betaine in different doses with broiler daily intake have some effects in overall growth parameters of the chicken at 35 days. A very significant ( $p < 0.05$ ) variation was observed in case of total FI and also the FCR was significantly differed among the betaine treated groups but in case of AW and DWG, the variation among the treatment groups were not statistically significant ( $p > 0.05$ ).

AW of all the chicken of G1 was recorded about 1909 g which was slightly decreased to 1898 g in G2 population. The AW were seen of about 1988 g for G3, 1971 g for G4 and the highest weight was observed of 2032 g for G5 with about 2.0g betaine/kg body weight. The variation of AW within the betaine treated groups was not statistically significant ( $p > 0.05$ ). Simultaneously the addition of betaine in the feed did not show any significant alteration ( $p > 0.05$ ) in the DWG of various groups, although the initial DWG was recorded of 54.6 g in G1 which was declined very minutely at the tempered group of chicken, that was 54.2 g. DWG in the betaine treated groups explored the small increase for G3, G4 and G5 with 56.8 g, 56.3 g and 58.0 g respectively and highest DWG was found for G5.

**Table 2: Effect of different treatment on growth performance of broilers**

Specification	Treatment					P value
	Group 1	Group 2	Group 3	Group 4	Group 5	
n	50	50	50	50	50	
AW (g)	1909 <sup>a</sup>	1898 <sup>a</sup>	1988 <sup>a</sup>	1971 <sup>a</sup>	2032 <sup>a</sup>	0.0513
DWG (g/day/bird)	54.6 <sup>a</sup>	54.2 <sup>a</sup>	56.8 <sup>a</sup>	56.3 <sup>a</sup>	58 <sup>a</sup>	0.0513
FI (g/day/bird)	2781 <sup>b</sup>	2750 <sup>b</sup>	2824 <sup>ab</sup>	2833 <sup>ab</sup>	2958 <sup>a</sup>	0.0089 **
FCR	1.46 <sup>ab</sup>	1.52 <sup>a</sup>	1.42 <sup>b</sup>	1.43 <sup>b</sup>	1.46 <sup>ab</sup>	0.0109 *

AW= average Weight, DWG= Daily Weight Gain, FI=Feed Intake, FCR=Feed Conversion Ratio, g=Gram, Superscript <sup>a,b</sup> showed a significant difference at  $p < 0.05$

However, a statistically significant effect ( $p < 0.05$ ) was found in the total FI where 2781 g of feed were fed by the control chickens and was decreased for the G2 group to 2750 g. The betaine-treated groups showed better FI with 2824 g, 2833 g and 2958 g for G3, G4 and G5 respectively and there were significant statistical results found between G5 and G2 ( $p < 0.05$ ) by further post hoc analysis (tukey test).

There was much reduction of the FCR value from the heat-treated group where the FCR value was 1.52 for G2 and the value reduced to 1.42 for G3, 1.43 for G4 and 1.46 for G5 which were very similar to FCR value of control group G1 (1.46). There were significant statistical results ( $p < 0.05$ ) found between G2-G3 and G2-G4 after post hoc analysis (tukey test).

#### 4. DISCUSSION

The result of the current study showed that the growth performance of broilers was affected by high temperature, while betaine supplementation generally improved these factors. Addition of 2 g betaine per kg feed of broiler presented some significant effect upon the total FI of different groups. This statement is also supported by the result of Amer et al. (2018), who stated that betaine supplementation (2 g/kg diet) significantly increased ( $p < 0.05$ ) FI because betaine act as methyl group donor and organic osmolyte and has the ability to improve growth performance in animals.

The addition of betaine at doses of 1 g and 1.5 g/kg feed had a significant effect on the FCR value in the treated groups compared to the control with heat-stressed groups (G2) and this finding is in agreement with the result found by Chand et al. (2017) who concluded that the dietary supplementation of 1.5% and 2% betaine improve the FCR of fast-growing broilers exposed to heat stress. Addition of betaine in poultry ration could partially alleviate the stress of heat and improve the FCR in poultry as compared to the negative treatment (Wahid et al., 2021).

On the other hand, some improvement in AW And DWG were found with the supplementation of betaine with different doses in the feed but the result were not statistically significant ( $p > 0.05$ ). These findings were in an agreement with Esteve-Garcia & Mack, (2000) reported as the effects of betaine were small and not significant ( $p > 0.05$ ) although they were in the direction of improving body weight. Zulkifli et al. (2004), also stated that supplemental betaine in diet has no significant effect on body weight gain. In contrast, Hassan, (2005) and Liu et al. (2019) stated that, betaine improves the structural and functional characteristics of intestinal epithelial resulting better nutrient absorption as well as improved body weight gain.

However, there were no significant increase in AW and DWG between treatments in this study. This might be because the number of observations was insufficient or there may be limitation in the measurement of different criteria. Increasing the sample size of the experiment along with other investigating parameters related to the mentioned zootechnical terms are essential to understand the basic mechanism and effect of betaine on broiler performance.

#### 5. CONCLUSION

Overall better performance was achieved at 1.5 g and 2g betaine mixed ration with improved FCR and FI respectively as compared to no betaine supplementation. The heat-stressed group consumed less feed and gave lower growth performance but betaine supplementation during heat challenge reduced the negative impact on performance, as an antistress agent but the actual activities of betaine in bird's bodies should be addressed in further research works. Difference between the experimental results suggested that this could be due to different levels of betaine used, animal species and environmental conditions and composition of the experimental diets and proper study on other parameters along with pathological observation will be needed in future to escape the experimental gap.

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