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RESEARCH ARTICLE

Impact of Various Organic and Inorganic Compounds on Production and Welfare Traits under Heat Stress in Commercial Broilers

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ABSTRACT

Heat stress is a well-known ecological stressor that adversely affects the welfare and management of domestic poultry. To mitigate these negative impacts, various organic and inorganic compounds, such as vitamin C, betaine, vinegar, and sodium bicarbonate, have been used for their antioxidant and osmoregulatory properties. The purpose of this research was to investigate the impact of various organic and inorganic compounds during heat stress on growth performance, body conditioning score, physical asymmetry, gait score, feather score, and the novel object test in commercial broilers. Birds were divided into six groups: CON (Control), HS (Heat stress at 37°C), HS+VC (Heat stress with vitamin C supplementation at 250 mg/kg), HS+BT (Heat stress with betaine supplementation at 2 g/kg), HS+VG (Heat stress with vinegar supplementation at 10 ml/liter), and HS+SB (Heat stress with sodium bicarbonate supplementation at 250 mg/kg). Throughout the experimental period, all groups were provided ad libitum feed and water. The results revealed that birds exposed to heat stress (HS group) showed significantly decreased growth performance, welfare traits parameters, physical asymmetry, body conditioning score, feather score, gait score, and novel object test compared to the other experimental groups. However, supplementation with vitamin C, betaine, vinegar, and sodium bicarbonate markedly reduced the adverse effects of heat stress on growth performance and other experimental parameters. Additionally, the vitamin C group was more potent during the experimental period as compared to the other treatment groups. Conclusively, vitamin C, betaine, vinegar, and sodium bicarbonate supported the broiler growth performance and welfare traits and can be included as protective management practices in poultry production to alleviate heat stress.

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INTRODUCTION

The poultry industry is expanding globally to meet rising demand for eggs and meat. Poultry meat is high in protein, minerals, and vitamins and has low saturated fatty acids (Wasti *et al.,* 2020). Considering these facts, the world's consumption of eggs and chicken meat has doubled over the last ten years and is predicted to double by 2050 (Alexandratos and Bruinsma, 2012). To fulfill these demands, chicken genetics has improved significantly over the last 10 years. In the 1950s, broiler chickens weighed about 900 g in 56 days; by 2005, that weight had dropped to about 4202 g (Zuidhof *et al.,* 2014). These enhanced laying hens and broilers have increased metabolic rates and productivity (Tallentire *et al.,* 2016). Attributable to a higher metabolic rate, they produce more body heat and are more vulnerable to heat stress. A high ambient temperature and a higher bird stocking density enhance heat stress propensity (Goo *et al.,* 2019). Heat stress triggers oxidative impairment, acid-base imbalance, reduced feed intake, decreased body weight, poor meat quality, increased disease risk, and a higher mortality rate (Oluwagbenga and Fraley, 2023). Prolonged heat stress could contribute to declined growth and egg production, reduced egg size and weight, decreased eggshell thickness, and increased egg breakage (Oguntunji and Alabi, 2010). In the poultry business, heat stress is a significant issue that affects the health and efficiency of birds. In the poultry sector, heat stress caused yearly losses of $128 to $165 million as of 2003 (St-Pierre *et al.,* 2003). Therefore, to reduce the negative effects of heat stress on broilers, it is essential to consistently improve nutritional practices and implement optimal feeding strategies in line with the rapid growth of these birds (Mohamed *et al.,* 2023).

Dietary intervention is considered to be an effective method to alleviate the detrimental effects of heat stress on bird's production (Gouda *et al.,* 2020). Feed additives are employed in broiler diets to increase digestive efficiency. Nutritional improvements in feed additives comprise synthetic amino acids, ascorbic acid, trace minerals, vitamins, prebiotics, and probiotics (Nomi *et al.,* 2024). Poultry chickens can produce vitamin C (L-ascorbic acid); however, this capability could be deficient under stressful circumstances; therefore, ascorbic acid supplementation is indispensable to maintain the biological development of poultry chickens during heat stress (Ghazi *et al.,* 2015). Influences of ascorbic acid supplement on poultry diets under heat stress conditions have been shown in previous research on performance and blood parameters (albumin, total protein, alkaline phosphatase, glucose, and cholesterol concentrations) has demonstrated a decrease in ascorbic acid due to the harmful effects of heat stress (Kutlu and Forbes, 1993). Ascorbic acid addition to birds' feed or drinking water during periods of pathological, nutritional, and environmental stress seems to reduce many of the negative physical effects of exposure to one or more concurrent stressful stimuli, such as transportation, high environmental temperatures, beak trimming, and coccidiosis challenges (Biswas *et al.,* 2024).

Another one of the most effective methods for using naturally found phytogenic feed additive ingredients, which is found extensively in various plant and animal tissues, is the derivative betaine classified as the trimethyl glycine extract (amino acid) (Chendrimada *et al.,* 2002). Additionally, pure forms of betaine, like betaine hydrochloride, betaine monohydrate, and anhydrous betaine, are available as feed additives and are frequently included in animal diets (Eklund *et al.,* 2005). Betaine has been classified as a non-toxic feed ingredient and is stable. Because of its significant osmotic qualities, betaine could make nutrients easier to digest (Eklund *et al.,* 2006).

Additionally, as betaine is a methyl group donor, it plays a role in energy and protein metabolism. By substituting part of the additional dietary choline and methionine, feed costs can be reduced. The total amount of bacteria in the crop, which is linked to an increase in gram-positive bacteria like *Enterococci* in poultry, was reduced by the betaine added to the birds' diet. Attributable to its functional significance in preserving cell integrity in conditions with high osmolarity, betaine could help gut bacteria cope with osmotic stress (Saeed *et al.,* 2017). However, betaine hydrochloride might help the stomach's pH drop, which could enhance the digestion of nutrients. It is also recommended as a dietary source of betaine because pure betaine often comes from the extraction of molasses soluble (Eklund *et al.,* 2005). According to research studies, betaine inhibits coccidiosis disorders and improves the intestinal structure and function of poultry birds, hence facilitating the deadly consequences of coccidiosis. Another researcher found that the meat quality of ducks exposed to extremely hot conditions might be improved if betaine 1200 ppm was supplemented with restricted feeding in the morning and afternoon (Park and Park, 2017).

This study was designed to evaluate the physiological, behavioral, and production-related welfare traits affected by heat stress in commercial broilers and to determine the effectiveness of organic (vitamin C, betaine, vinegar) and inorganic (sodium bicarbonate) compounds in improving growth performance and welfare responses under heat stress conditions. It was hypothesized that vitamin C, betaine, vinegar, and sodium bicarbonate would have significant effects on the growth performance and welfare traits of broiler chickens during heat stress.

MATERIALS AND METHODS

Animals and experimental design: The study was conducted at the C Block, MNS- University of Agriculture, Multan, Pakistan. To conduct this experiment, 360 broiler chicks were procured from a marketable hatchery. A total of 360 day-old broiler chicks were raised under standard management conditions. After the first week, the chicks were randomly divided into six treatment groups. In order to sustain consistent pen size, feeding, and drinking facilities, each treatment was divided into three duplicates, each comprising 24 birds. The composition of the basal diet is presented in Table 1.

Animal ethics: All study procedures were conducted with approval from the Animal Ethics Committee of the MNS-University of Agriculture, Multan, and in compliance with the IACUC.

Experimental design: Birds were divided into six groups: 1) CON (Control), 2) HS (Heat stress 37°C was applied), 3) HS+VC (Heat stress+ vitamin C supplemented with 250mg/kg), 4) HS+BT (Heat stress + betaine supplemented with 2g/kg), 5) HS+VG (Heat stress + vinegar supplemented with 10ml/kg), and 6) HS+SB (Heat stress + sodium bicarbonate supplemented with 250m/kg). Feed ingredients inclusion levels and vaccination schedule for broilers are depicted in Table 2 and Table 3.

Table 1: Composition of experimental diets Ross 308 strain

Nutrients	Starter	Grower	Finisher
Metabolizable Energy (Kcal/Kg)	2950	2950	2960
Crude Protein(%)	23.174	20.486	18.385
Arginine (%)	1.464	1.300	1.145
Lysine (%)	1.450	1.150	0.990
Methionine (%)	0.668	0.561	0.495
Met+Cys (%)	1.043	0.890	0.800
Calcium (%)	1.023	1.024	1.000
Available P (%)	0.487	0.512	0.5
Sodium (%)	0.180	0.150	0.180

Table 2: Feed ingredients inclusion level for broiler

Ingredients	Starter	Grower	Finisher
Corn	54.84	60.00	65.90
Soyabean Meal	33.39	31.80	27.33
Fish Meal	5.45	2.00	1.00
Soyabean Oil	2.18	1.70	1.09
Oyster Shell (CaCO3)	1.19	1.19	1.19
Dicalcium Phosphate	1.17	1.71	1.84
Common salt	0.33	0.31	0.40
Vitamin Premix	0.50	0.5	0.5
Mineral Premix	0.50	0.5	0.5
DL-Methionine	0.28	0.22	0.19
L-Lysine HCl	0.168	0,06	0.04
Total	100	100	100

To fulfill the chicken nutrient requirement for growth and maintenance the following ingredients were used to form diet.

Table 3: Vaccination schedule of broiler

Age of birds (days)	Vaccine Name	Route of entry
5	ND+IB	Eye drop
10	Gumboro	Drinking water
14	ND	Drinking water
22	Nd Lasoota	Drinking water

Sample collection: On the 8th day of the experiment, samples were collected from randomly selected birds in each treatment group to assess physical, behavioral, and physiological parameters.

Physical asymmetry: The actual asymmetry of ten birds from each treatment group was measured (Campo *et al.*, 2008). Metatarsal length, metatarsal width, and middle toe length (mm) were recorded for both left and right legs using a vernier caliper.

Feather score: Fifteen birds from each treatment group were evaluated for feather coverage on the 8th day of rearing. The feather score ranged from 1 to 5, where 1 = No feathers, 3 = Average feather coverage, and 5 = Full feather coverage.

Gait score: Fifteen birds per treatment were assessed for walking ability on the 8th day using the U.S. gait scoring system. The gait performance was evaluated according to the Kestin Step Scoring Framework (Kestin *et al.*, 2008) as modified by Dawkins *et al.* (2004). Scores ranged from 0 to 2, where 0 = Normal walking with no visible impairment, 1 = Mild impairment but able to walk at least 5 feet without sitting, and 2 = Severe walking difficulty.

Body conditioning core: Fifteen birds per treatment were evaluated for body condition on the 8th day. Observations included the condition of the hock joint, beak, eyes, nostrils, and feather coverage on the chest. Scores ranged from 0 to 2, where 0 = Normal, 1 = Mild signs, and 2 = Severe signs.

Novel object test: Fifteen birds from each treatment were subjected to the novel object test on the 8th day. A novel object (such as a ball or toy unfamiliar to the birds) was introduced into the pen and placed in a visible and accessible location. The birds' responses were observed at 30, 60, 90, and 120 seconds at a distance of 1-3 meters. The number of birds approaching or pecking the object within each time interval was recorded to evaluate curiosity and behavioral response under heat stress conditions.

Statistical analysis: One-way ANOVA was applied to assess the differences among mean values. Duncan's test was used for multiple comparisons. All data were presented as the mean ± SEM, and the significance level was set at $p < 0.05$ for all measurements. Whole analysis was conducted using IBM SPSS Statistic 22.

RESULTS

Growth performance: Upon arrival, each bird was weighed independently. Feed conversion ratio, weight gain, and feed consumption in each group were noted every week. These records were made on thirty-five days. The significance level was set at P<0.05. On 35 days, the growth performance of six treatment groups, in terms of maximum weight gain, was attained by the vitamin C group, with lower mortality among the control, betaine, vinegar and sodium bicarbonate groups shown in Table 4. Vitamin C is an efficient converter of feed into meat when compared with heat-stressed groups. The heat stress group showed the lowest weight gain and FCR as compared to other groups. These results demonstrated the effect of organic and inorganic compounds as vitamin C, betaine, vinegar and sodium bicarbonate, had significant effect on growth performance of broilers.

Physical asymmetry: The results of physical asymmetry among treatment groups of broilers are shown in Table 5. Metatarsal length, width and center toe length (mm) were determined for both left and right legs by using a Vernier Caliper. The combined inequality score was viewed as taking the amount of the whole worth left short of every trait, then, at that point, dividing by the total number of characteristics.

Gait and feather score: The effects of treatment groups on gait and feather score were summarized in Table 6. The heat stress was recorded as the lowest gait score compared with other groups. The performance of broilers was not affected by the treatment compared to the control group. These results demonstrated that the effect of organic and inorganic compounds had a significant effect on welfare traits of gait and feather score. The significance level was set at P<0.05.

Body conditioning score: The effects of treatment groups on body conditioning score were summarized in Table 7. The heat stress group was recorded the lowest body conditioning score as compared with other groups. The treatment groups significantly improved the body conditioning scores of broilers as compared with the heat stress group.

Table 4: Comparison between treatment groups of broiler on 35 days

Groups	FI (g/bird)	WG (g)	FCR
Control	160±0.02[b]	1950±0.03[e]	1.90±0.03 [b]
Heat Stress	130±0.2[c]	1745±0.36[f]	2.10±0.11 [c]
Heat Stress+ Vit C	170±0.01[a]	2150±0.03[d]	1.70±0.03[a]
Heat Stress+ Bet	165±0.01 [a]	2084±0.03[b]	1.76±0.03[a, b]
Heat Stress+ Vine	166±0.01 [a]	2060±0.03[c]	1.80±0.03[a, b]
Heat Stress+ $NaHCO_3$	167±0.01 [a]	2015±0.03[a]	1.82±0.03[a, b]

Values are represented as the mean± SEM. HS, heat stress; Vit C, vitamin C; Bet, betaine; Vine, vinegar; $NaHCO_3$, Sodium Bicarbonate.

Table 5: Physical asymmetry among treatment groups

Age (Week)	Control	HS	HS + Vit C	HS + Bet	HS + Vine	HS + $NaHCO_3$
Week 1	1.10 ± 0.19[a]	0.12 ± 0.11[c]	0.90 ± 0.19[b]	0.80 ± 0.13[c]	0.70 ± 0.17[d]	0.63 ± 0.19[d]
Week 2	1.21 ± 0.10[b]	0.23 ± 0.16[d]	1.59 ± 0.17[a]	0.61 ± 0.11[c]	0.36 ± 0.19[d]	1.21 ± 0.13[b]
Week 3	2.35 ± 0.12[b]	1.41 ± 0.13[d]	3.76 ± 0.18[a]	2.49 ± 0.13[c]	2.54 ± 0.16[b],[c]	2.59 ± 0.15[b]
Week 4	4.56 ± 0.13[d]	3.63 ± 0.14[e]	5.74 ± 0.16[a]	3.83 ± 0.15[c]	4.94 ± 0.12[b]	4.00 ± 0.17[d]
Week 5	3.67 ± 0.10[c]	2.79 ± 0.12[f]	4.90 ± 0.19[a]	3.96 ± 0.10[b]	3.08 ± 0.16[d]	3.60 ± 0.18[c]

Values are represented as the mean± SEM. HS, heat stress; Vit C, vitamin C, Bet, betaine; Vine, vinegar; $NaHCO_3$, Sodium Bicarbonate.

Table 6: Gait and father score among treatment groups.

Age (Week)	Control	HS	HS + Vit C	HS + Bet	HS + Vine	HS + $NaHCO_3$
Week 1	0.65±0.02[c]	0.30±0.08[d]	0.98±0.02[a]	0.72±0.06[b,c]	0.75±0.05[b]	0.79±0.01[b]
Week 2	0.52±0.01[d]	0.42±0.06[e]	0.96±0.05[a]	0.82±0.04[b]	0.65±0.01[c]	0.79±0.06[b]
Week 3	0.82±0.06[c]	0.50±0.08[d]	1.78±0.02[a]	0.87±0.01[c]	0.90±0.07[c]	0.99±0.09[b]
Week 4	0.84±0.09[c]	0.60±0.07[d]	0.94±0.01[b]	1.10±0.06[a]	1.14±0.06[a]	1.17±0.06[a]
Week 5	0.94±0.07[d]	0.70±0.09[e]	1.80±0.09 [a]	1.16±0.12[c]	1.20±0.06[b,c]	1.26±0.01[b]

Values are represented as the mean± SEM. HS, heat stress; Vit C, vitamin C, Bet, betaine; Vine, vinegar; $NaHCO_3$, Sodium Bicarbonate.

Table 7. Body conditioning score among treatment groups

Age	Control	HS	HS+Vit C	HS+Bet	HS+Vine	HS+ $NaHCO_3$
Week 1	0.65±0.19[c]	0.49±0.15[d]	0.96±0.13[a]	0.70±0.16[b]	0.73±0.13[b]	0.76±0.15[b]
Week 2	0.69±0.16[b]	0.56±0.08[b]	0.90 ±0.18[a]	0.82±0.11[a]	0.85±0.17[a]	0.89±0.13[a]
Week 3	0.75±0.17[c]	0.65±0.12[d]	0.98 ±0.16[a]	0.84±0.18[b]	0.89±0.12[b]	0.81±0.16[b,c]
Week 4	0.92±0.14[c]	0.60±0.12[d]	1.64±0.11[a]	1.10±0.09[b]	1.14±0.19[b]	1.17±0.18[b]
Week 5	1.10±0.15[d]	0.85±0.19[e]	1.50±0.15[a]	1.16±0.15[c,d]	1.20±0.18[b,c]	1.26±0.16[b]

Values are represented as the mean± SEM. HS, heat stress; Vit C, vitamin C, Bet, betaine; Vine, vinegar; $NaHCO_3$, Sodium Bicarbonate.

DISCUSSION

The recent research findings showed the influence of various organic and inorganic compounds on growth performance and welfare traits in commercial broilers. Dietary supplementation with ascorbic acid restricted and mitigated heat stress, further developed growth performance and welfare traits, upgraded immunological status, and diminished mortality (Gouda *et al.,* 2020). Broilers require between 200 and 500 mg/kg of vitamin C to ensure optimal feed efficiency, meat production, and quality under heat stress (Attia *et al.,* 2017).

Betaine is involved in several biological processes, including osmoprotection, lipid metabolism, and antioxidation (Arumugam *et al.,* 2021). Vinegar could also serve as a chemotherapeutic agent due to its ability to limit microorganism and toxin colonization in the avian intestine while improving nutrient digestibility (Al-Shammari and Batkowska, 2021).

Birds fed 250 mg/kg sodium bicarbonate play a crucial role in maintaining performance, carcass yield, and physiological stability of heat-stressed broilers (Attia *et al.*, 2017). The current interventions to improve the deleterious impacts of heat stress on poultry by using inorganic and organic compounds. Furthermore, ongoing research into genetic and breeding approaches aims to develop heat-resistant poultry breeds by using these nutritional and mineral compounds, enhancing the birds' tolerance to high temperatures while maintaining productivity levels.

The current interventions to improve the deleterious impacts of heat stress on poultry by using inorganic and organic compounds. Factors that may explain the decrease in weight gain and feed conversion ratio (FCR) of heat-stressed broilers include feed intake reduction, reduced nutrient utilisation and higher oxidative stresses. High ambient temperatures cause a disturbance of normal physiological functions and reduce productive performance. Vitamin C, betaine, vinegar and sodium bicarbonate supplementation were beneficial to the growth performance of the birds subjected to heat stress in the present study. The better response seen in the vitamin C group could be related to its antioxidant effect and its role in reducing cellular damage when exposed to heat stress. This has been observed in broiler chickens subjected to chronic heat stress, where heat stress had a significant negative effect on growth performance and feed efficiency when the environmental temperature was increased (Lara and Rostagno, 2013).

Gait scores, feather scores, body conditioning scores and physical asymmetry scores were improved in the supplemented groups indicating improved welfare and physiological stability during the heat stress. The effect of heat stress has been documented to cause oxidative damage, adversely affecting skeletal growth and locomotion and affecting feather quality. Antioxidant supplementation can help to decrease the oxidative stress and enhance the welfare related parameters of the poultry challenged by the environment (Akbarian *et al.*, 2016). An increase in physical asymmetry scores in the treated groups can be interpreted as a decrease in developmental instability and increasing adaptation to stressful conditions. In addition, birds that were fed the supplements had a better response in the novel object test which indicated better behavioural welfare and less stress burden. Decreased exploratory behaviour is observed in chronic stress, and increased interactions with novel stimuli are associated with increased adaptability and welfare. The nutritional interventions that help tackling heat stress have been found to enhance the resilience, behavioural response and overall productivity of broiler chickens raised in hot climatic conditions (Nawab *et al.*, 2018).

Conclusion

In conclusion, heat stress markedly impaired growth performance, welfare indicators, and behavioral responses in broiler chickens, as evidenced by reductions in body condition, gait quality, feather condition, physical symmetry, and exploratory behavior. The inclusion of organic and inorganic supplements effectively mitigated these adverse effects, demonstrating their protective role under thermal stress conditions. Among the tested treatments, vitamin C exhibited the most pronounced improvement, indicating its superior efficacy in enhancing resilience to heat stress. Therefore, supplementation with vitamin C, along with betaine, vinegar, and sodium bicarbonate, can be recommended as a practical and cost-effective nutritional strategy to improve the productivity and welfare of broilers reared under high-temperature environments.

Authors' contribution: HZ, AR and MF Conceptualization, Methodology, Formal analysis, Drafted manuscript, Writing-original draft, Writing-review & editing. MTA, MZY, SB, AAS and HZ Provided assistance in experimentation, Data analysis, Data curation, and statistical guidance. SAR and MAR. Project administration, Funding acquisition, Resources, Supervision. All authors have read and agreed to publish the final version of the manuscript.

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