



Mitigation of Heat Stress in Broiler Chickens during Hot Summer Seasons Through Betaine Hydrochloride Osmoprotectant Intervention

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ABSTRACT

Background: Modern poultry genotypes produce more body heat due to their greater metabolic activity accompanied with tropical environment often worsens the situation. Nutritional interventions play an important role in ameliorating the negative effects of heat stress which will be more economical and sustainable approach.

Methods: In order to evaluate the role of osmolyte (betaine) as a heat ameliorating agent, an experiment was conducted in broiler chicken with gradient levels of 0, 0.5, 1.0 and 1.5 g/kg for a growing period of 42 days. Each group comprised 48 birds in six replicates of 8 each. Thermo-Humidity Index value ranged 85-87 throughout the experimental period. Production performance, immunity, gut morphometry, hemato-biochemical and stress indicators were studied.

Result: Supplementation of betaine significantly improved production performance as well as breast meat yield ($P<0.05$). Supplementation (1 and 1.5 g/kg) significantly ($P<0.01$) improved both the humoral and cellular immunity. Betaine supplemented groups also depict significant ($P<0.01$) rise in hemoglobin and relative immune organ weight. Addition of betaine to basal diet significantly ($P<0.01$) increased the villus length and also lowered H:L ratio, with better ALT and AST values than the control. Villi structure was more improved in 1 g/kg dose level. The present study confirms that, supplementation of betaine at 1g/kg levels could significantly improve overall growth performance, gut morphology and welfare in heat stressed broiler chickens.

Key words: Betaine, Broilers, Immunity, Production, Stress.

INTRODUCTION

Ameliorating heat stress is the major challenge to the broiler industry especially in tropical regions, because of their rapid growth and conventional open-shed housing. Heat stress is a result of disequilibrium between production and loss of body heat, has deleterious effect on productive performance in poultry which often results in economic losses. The major effects due to heat stress might be increased osmotic disturbances and elevated production of free radicals causing oxidative injuries at cellular level to ultimately imposing stress. In order to maintain homeostasis, physiological and behavioral responses are exhibited by birds, which compromises the performance and immunity (Shakeri *et al.*, 2018). The most cost-effective and efficacious method for reducing heat stress for marginal farmers is nutritional management as opposed to housing management or automation. Efforts are under way to mitigate the negative effects of heat stress through dietary interventions to achieve optimum performance.

Among the myriad of interventions, betaine can be feasible and sound choice in poultry's diet, because of its osmo-protectant, methyl donor and amino acid sparing effects, can be the best strategy to control heat stress losses. As a by-product of sugar beet processing, betaine is commercially available as a feed additive. Betaine is a trimethyl derivative of the amino acid glycine, a metabolite of plant and animal tissues which may provide a strategic solution for these challenges (Akhavan-Salamat and

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Ghasemi, 2016). Betaine protects the intestinal cell proteins and enzymes from high ambient temperature, accumulates inside the cell and facilitates normal functions by retaining ion balance to improve thermotolerance (Abudabos *et al.*, 2016). Consisting of transmethylation process, wherein methyl groups ($-CH_3$) containing a single carbon atom is donated, to synthesize critical metabolites and regulate gene expression through DNA methylation (Chand *et al.*, 2017). Improving hatchability and chick

weight; spare choline and methionine; improve gut health and function; alleviate heat stress; reduce wet litter condition; improve immune response, *etc.* were some of the substantial roles of betaine in poultry (He *et al.*, 2015). To address the complex challenges posed by heat stress in broiler chickens, particularly in tropical regions, a comprehensive understanding of the physiological responses and nutritional interventions is imperative.

From a nutritional standpoint, betaine proves to be a versatile substitute for other costly methyl donors, effectively reduces the requirements for essential nutrients such as methionine, sulphur-containing amino acids and choline. With this backdrop, the present study evaluates efficacy of the multifaceted benefits of betaine supplementation on broiler performances reared under higher environmental temperatures.

MATERIALS AND METHODS

Birds, experimental design and diet

The followed experiment conducted in ICAR-Central Avian Research Institute, Izatnagar in year 2019. Total of 192, day-old broilers (CARIBRO-VISHAL) were divided into four treatments groups and fed with diets contained 0 (control), 0.5, 1.0 and 1.5 g betaine/kg feed. Each group consisted of 48 birds in six replicates (8 birds). The experimental diets were formulated as per the broiler's standard requirement (Table 1). All the birds were raised under uniform standard managemental conditions of brooding and vaccination.

Temperature-humidity index (THI) measurement

The study was carried out during hot summer season in order to simulate the field conditions of heat stress to broilers. The temperature humidity index (THI) was calculated as per the formula suggested by Zulovich and DeShazer (1990). Ambient temperature and Relative humidity were measured using dry-bulb and wet-bulb temperatures. According to Zulovich and DeShazer, 1990, THI ranges were classified as comfort (THI<70), alert (THI 70-75), danger (THI 76-81) and emergency (THI>81) zones, used to assess the potential indices of heat stress for poultry. The Temperature Humidity Index (THI) was recorded throughout the experimental period (42 days) and were in the ranged from 85 to 87 (Rajalaxmi *et al.*, 2017).

Production performance

Body weight (g) was measured in different growth phases (14-21, 21-42, 14-42 and 0-42 days) during the experimental period to ascertain body weight gain (BWG) during each phase. Weighed quantities of respective diets were offered *adlib* on daily basis and residues were weighed on weekly basis to ascertain weekly as well as overall feed consumed to calculate feed to gain conversion ratio (Chuskit *et al.*, 2021).

Evaluation of immune response

A. Humoral immune response

The microtiter hemagglutination (HA) is performed to determine antibody titre concentration in chickens against

Table 1: Ingredients and nutrient composition of basal diets for broiler chicken.

Ingredients (g/kg)	Pre-starter	Starter	Finisher
Maize	571	579	635
Soybean meal (46% CP)	342	330	288
Fish meal (48% CP)	30	32	20
Vegetable oil	24	29	28
Limestone powder	9.0	9.0	8.0
Di-calcium phosphate	14	12	12
DL-Methionine	2.0	1.0	1.0
L-lysine HCl	1.0	0.0	0.0
Trace mineral Premix	1.0	1.0	1.0
Vitamin Premix	2.0	2.0	2.0
Vitamin B complex	1.5	1.5	1.5
Choline chloride	1.0	1.0	1.0
Salt	3.0	3.0	3.0
Toxin binder	1.0	1.0	1.0
Nutrient composition			
Metabolizable energy (ME) (Kcal/kg)	3000	3050	3100
Crude protein, %	23.0	21.9	20.0
Lysine, %	1.30	1.19	0.99
Methionine, %	0.52	0.49	0.45
Calcium, %	1.00	1.00	1.00
Available phosphorus, %	0.45	0.41	0.41

1% Sheep RBCs (SRBC) by using method of Siegel and Gross 1980 in 12 randomly selected healthy birds per group on 28th day post-hatch.

B. *In vivo* cell mediated immune response (CMIR)

The CMIR was studied at four weeks of age by intra-dermal injection of 0.1 ml (1 mg/ml) of phytohemagglutinin type-P (PHA-P) at 3rd and 4th inter-digital space of the right foot and 0.1 ml of sterile PBS in left foot (control) (Cheng and Lamont, 1988).

C. Relative weight of lymphoid organs

Weights of lymphoid organs such as bursa of Fabricius, spleen and thymus (g) were recorded at 42 days of age and expressed as percentage of pre-slaughter live weight (Bora *et al.*, 2024).

Histomorphometry of intestinal mucosa

Histomorphometry measure *viz.*, villus height (VH), villus width (VW) and ratio of villus height to villus width (VH: VW) were measured at 28 and 42 days of age from 12 birds per treatment. A piece (0.5-1.0 cm) of jejunum was collected and preserved in 10% neutral buffered formalin solution for histological slide preparation. Further, slides were stained with hematoxylin and eosin stain following by mounting with coverslip. Mounted slides were examined under microscope under low power (10×). The measurements were carried out by using Primo Star 3.0 imaging software, Zeiss, Germany.

Hematological attributes and serum enzymes

Blood samples were collected (~1ml) from the jugular vein using 26-gauge needle in K3-EDTA tubes on 2, 4 and 6 weeks of age to determine hematological attributes. Autoanalyzer (Abacus Junior Vet5, Diatron, USA) was used to determine hemoglobin (Hb) levels.

Determination of H/L ratio

Immediately after blood collection, thin blood smear was prepared on clean glass slides. After drying, the smears

were fixed with methanol and stained with Giemsa stain. After drying, smears were examined under microscope at 100× objective with oil immersion. 100 cells, *i.e.*, heterophils and/or lymphocytes counted in each smear to calculate H/L ratio (Gross and Siegel 1983).

A. Estimation of serum ALT and AST levels

The serum ALT (alanine amino transferase) and AST (aspartate amino transferase) values were determined for twelve healthy birds per treatment using standard kits (Reitman and Frankel, 1957) from M/s Coral Clinical Systems Ltd. India.

B. Carcass traits

After starvation for 12 h, twelve birds per treatment were weighed and sacrificed following standard practice of throat slitting for different carcass trait parameters.

Statistical analysis

Data generated from different treatments were analyzed for their statistical significance by the method of using SPSS 20.0 software package. The data obtained as percentage were subjected to arcsine transformation before analysis. Variables having different observations were analyzed following Duncan's multiple range test.

RESULTS AND DISCUSSION

Production performance

The results obtained for growth for the period 14-21, 21-42, 14-42-and 0-42-day's growth phase is presented in Table 2. Body weight gain was significantly ($P<0.01$) higher in betaine supplemented groups than control. In the present study, significant positive impact ($P<0.001$) of betaine was observed on feed intake in all phases including overall period, except during 21-42 days of age. Supplemental betaine significantly ($P<0.001$) improved the FCR (1.88) compared with the rest of the treatments.

Table 2: Effect of betaine supplementation on production performance of broiler chickens reared under heat stress.

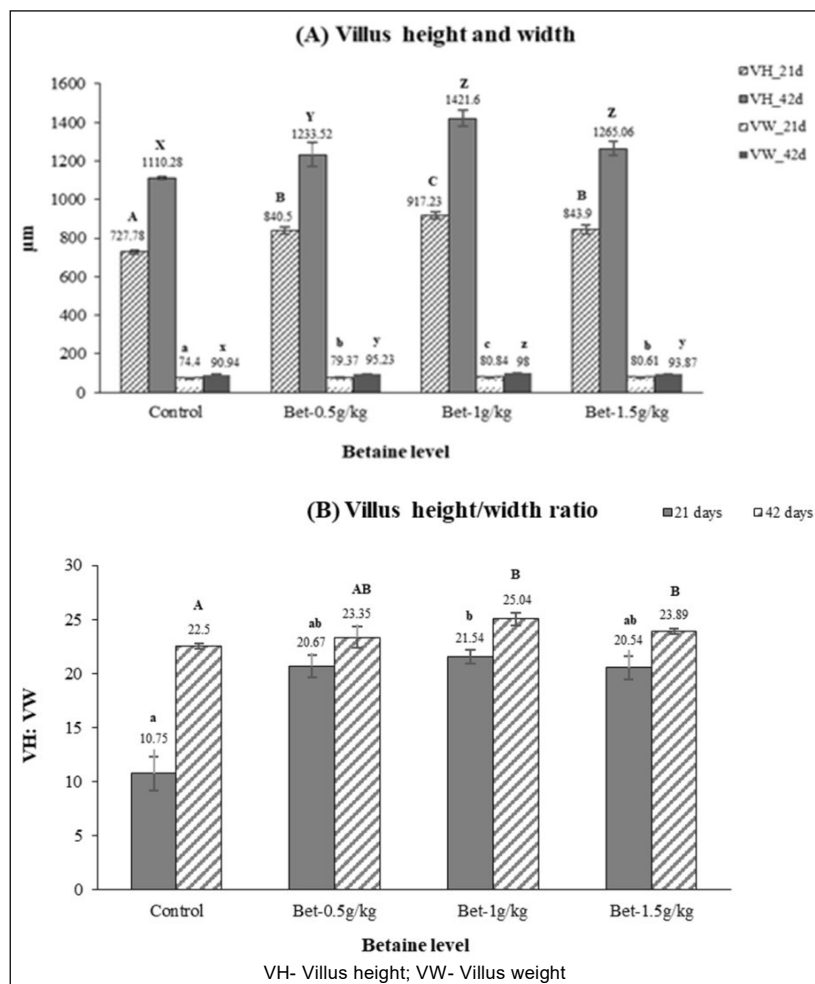
Parameter	Phase (Days)	Control	0.5 g	1.0 g	1.5 g	SEm	Significance
Body weight gain (g)	14-21	171.6 ^a	249.7 ^b	260.2 ^b	261.1 ^b	13.191	$P<0.001$
	21-42	1097.7	1126.4	1097.9	1099.0	6.927	NS
	14-42	1269.3 ^a	1376.1 ^b	1358.1 ^b	1360 ^b	14.967	$P<0.001$
	0-42	1475.7 ^a	1582.8 ^b	1564.8 ^b	1570 ^b	14.747	$P<0.001$
Feed intake (g)	14-21	287.6 ^a	415.8 ^b	432.6 ^b	434.5 ^b	21.682	$P<0.01$
	21-42	2211.1	2256.1	2172.3	2175	16.323	NS
	14-42	2498.0 ^a	2671.9 ^b	2604.8 ^b	2610 ^b	24.364	$P<0.01$
	0-42	2837.1 ^a	3010.8 ^c	2942.7 ^b	2945 ^b	23.795	$P<0.001$
FCR	14-21	1.67 ^b	1.66 ^a	1.66 ^a	1.66 ^a	0.004	$P<0.01$
	21-42	2.01 ^b	2.00 ^b	1.98 ^a	1.98 ^a	0.005	$P<0.01$
	14-42	1.97 ^c	1.94 ^b	1.92 ^a	1.91 ^a	0.007	$P<0.001$
	0-42	1.92 ^c	1.90 ^b	1.88 ^a	1.88 ^a	0.006	$P<0.001$

Values bearing different superscripts within a row differed significantly ($P<0.05$); FCR- Feed conversion ratio; SEm- Standard error mean; NS- Non-significant.

Table 3: Effect of betaine supplementation on immunity and immune organs weight (g) of broiler chickens reared under heat stress.

Parameter	Sex	Control	0.5 g	1.0 g	1.5 g	Mean	SEm	Prob.
CMI (foot web index in mm)	Male	0.27	0.37	0.41	0.42	0.35 ^N	0.020	NS ^S
	Female	0.25	0.34	0.38	0.37	0.32 ^M	0.016	NS ^I
	Mean	0.26 ^a	0.35 ^b	0.39 ^c	0.39 ^c	0.33	0.013	P<0.001 ^T
HIR (Log ₂ HA Titer)	Male	7.25	9.00	9.50	9.45	8.58	0.398	NS ^S
	Female	7.00	8.50	9.25	9.30	8.25	0.411	NS ^I
	Mean	7.13 ^a	8.75 ^b	9.38 ^b	9.37 ^b	8.42	0.282	P<0.01 ^T
Thymus	Male	0.28	0.30	0.26	0.25	0.28	0.008	NS ^S
	Female	0.27	0.30	0.29	0.30	0.29	0.010	NS ^I
	Mean	0.27	0.30	0.28	0.28	0.28	0.006	NS ^T
Bursa of fabricius	Male	0.16	0.19	0.31	0.31	0.22	0.024	NS ^S
	Female	0.16	0.17	0.30	0.30	0.21	0.024	NS ^I
	Mean	0.16 ^a	0.18 ^b	0.31 ^c	0.31 ^c	0.22	0.016	P<0.001 ^T
Spleen	Male	0.17	0.23	0.21	0.22	0.21	0.011	NS ^S
	Female	0.16	0.22	0.19	0.22	0.19	0.013	NS ^I
	Mean	0.17 ^a	0.22 ^b	0.20 ^{ab}	0.22 ^b	0.20	0.008	P<0.05 ^T

Values bearing different superscript viz. ^{abc}(Treatment) and ^{MN}(Sex), differed significantly (P<0.05); NS= Non significant; mm- Millimeter.

**Fig 1:** (A) and (B). Effect of betaine supplementation on gut morphometry of broiler chickens reared under heat stress.

Dietary betaine supplementation has been observed to increase the live weight of the broiler chickens (Sayed and Downing, 2015) by augmenting the stress through up scaling the levels of short-chain fatty acid (SCFA) and the blood electrolytes (Akhavan-Salamat and Ghasemi, 2016). The thermal stress affects satiety center in brain which suppresses feed intake in stressed birds, which negatively affects intestinal development leading to poor nutrient absorption and growth performance (Farag and Alagawany, 2018). However, in the present study, the feed consumption was higher in the betaine supplemented groups when compared to the un supplemented control. Studies have indicated significantly higher feed intake in birds upon betaine feeding (1.5 g/kg) as compared to control group (Awad *et al.*, 2014). Improvement in weight gain and feed intake of broilers might be attributed to methyl donating activity of betaine. Besides this, betaine protects the intestinal epithelia from dehydration and osmotic disorders,

to enhance the growth and feed consumption (Chand *et al.* 2017). The osmo-protective role of betaine could be a possible underlying cause for improving of FCR in heat stressed Cobb broilers (Sakomura *et al.*, 2013, El-Husseiny *et al.*, 2007). Feeding 1.5 and 2.0% of dietary betaine increases the feed consumption, BWG and reduced the FCR of fast-growing broilers under thermal stress (Chand *et al.*, 2017).

Immune response and immune organs weight

Betaine supplemented groups exhibited significantly ($P<0.001$) better immunity for CMI (0.39 mm) and HA titer (9.38) than control group (Table 3). Betaine supplementation significantly ($P<0.05$) influenced the immune organs (spleen and bursa) weight at 42 days of age. The release of nitric oxide from heterophils and macrophages and cytokine regulation play a crucial role in enhancing immune response in betaine supplemented birds (Zhang *et al.*, 2016). Betaine

Table 4: Effect of betaine supplementation on hematology and serum biochemical parameters of broiler chickens reared under heat stress.

Parameter	Sex	Control	0.5 g	1 g	1.5 g	Mean	SEm	Prob.
Hemoglobin (g%)	Male	13.33	14.81	15.25	15.35	14.47	0.396	NS ^s
	Female	13.13	14.15	14.63	14.70	13.97	0.289	NS ⁱ
	Mean	13.23 ^a	14.48 ^b	14.94 ^b	15.02 ^b	14.22	0.245	$P<0.01^T$
H:L ratio	Male	0.42	0.34	0.34	0.34	0.37	0.014	NS ^s
	Female	0.42	0.35	0.34	0.34	0.37	0.013	NS ⁱ
	Mean	0.42 ^b	0.35 ^a	0.34 ^a	0.34 ^a	0.37	0.009	$P<0.001^T$
AST (IU/L)	Male	146.39	158.86	170.53	160.10	158.59 ^N	4.948	$P<0.001^s$
	Female	46.45 ^X	97.11 ^Y	112.86 ^Y	110.25	85.47 ^M	9.012	$P<0.01^i$
	Mean	96.42 ^a	127.99 ^b	141.69 ^b	135.18 ^b	122.03	9.132	$P<0.001^T$
ALT (IU/L)	Male	5.16 ^X	8.63 ^Y	9.53 ^Z	9.55 ^Z	7.77 ^N	0.572	$P<0.001^s$
	Female	1.09 ^X	1.52 ^Y	1.74 ^Z	1.70 ^Z	1.45 ^M	0.084	$P<0.001^i$
	Mean	3.12 ^a	5.07 ^b	5.63 ^c	5.62 ^c	4.61	0.717	$P<0.001^T$

Values bearing different superscript viz. ^{abc}(Treatment), ^{MN}(Sex), ^{XYZ}(Interaction) differed significantly ($P<0.05$); NS= Non significant; IU- International Unit.

Table 5: Effect of betaine (Bet) supplementation on carcass yield (g) of broiler chickens reared under heat stress.

Parameter	Control	0.5 g	1.0 g	1.5 g	Mean	SEm	Prob.
Breast	14.95 ^a	15.84 ^{ab}	17.36 ^b	17.36 ^b	16.05	0.411	$P<0.05$
Drumstick	10.52	10.74	10.31	10.41	10.52	0.162	NS
Thigh	9.92	10.01	10.11	10.00	10.01	0.134	NS
Back	16.23	16.98	16.62	16.60	16.61	0.346	NS
Wings	9.06	9.10	8.90	8.92	9.02	0.226	NS
Neck	3.95	4.42	5.04	5.12	4.47	0.224	NS
Intestine	5.23	5.22	5.22	5.30	5.22	0.126	NS
Liver	2.30	2.34	2.20	2.30	2.28	0.084	NS
Gizzard	2.57	2.70	2.56	2.66	2.61	0.065	NS
Heart	0.50	0.48	0.53	0.53	0.50	0.015	NS
Ready to cook yield	71.95	73.78	74.94	75.04	73.56	0.827	NS
Eviscerated yield	66.58	68.26	69.64	69.44	68.16	0.798	NS
Blood loss	3.31	3.30	3.32	3.32	3.31	0.147	NS

Values bearing different superscript within a row differed significantly ($P<0.05$); NS= Non significant; SEm= Standard error mean.

supplementation improved overall immunocompetence and antibody titer against NDV, respectively upon betaine supplementation during heat stress (Chand *et al.*, 2017). Consistent thermal stress reduces the weight of immune organs and subsequently compromises the immunocompetence. However, our findings revealed that the weight of lymphoid organs was significantly greater in betaine supplemented birds (1.0 and 1.5 g) than control groups. This reveals that betaine can combat the adverse physiological responses originated from thermal stress.

Gut morphology

The morphometrics of the villus height and width and their ratios at 21 and 42 days of post-hatch is presented in Dietary betaine supplementation resulted in significantly ($P<0.05$) higher villus height and better VH: VW ratio, therefore, positively altered intestinal epithelial morphometry, whilst, simultaneous reduction in the intestinal villus height and villus height to crypt depth ratio was observed in the control group (Fig 1 A and B). The deterioration effect of heat stress on the intestinal mucosa in broiler chickens has been well documented (Liu *et al.*, 2019). It was further hypothesized that the mechanism of homeostasis in animals fails when the ambient temperature exceeds its thermoneutral zone, resulting in vasodilation and increased peripheral blood flow as a response to heat stress. As a result, very little blood is supplied to the intestine, causing cellular hypoxia in intestine resulting epithelial shedding, which further leads to a deeper crypts and shorter villi (Liu *et al.*, 2019).

The methyl donating ability of betaine could exhibit positive response in heat stressed broilers through proliferation of gut epithelia (Wang *et al.*, 2020). Besides, its osmo-protective and antioxidant effect, betaine improves the gut environment, promoting nutrient digestibility and absorption in broilers and alleviates heat stress and intestinal oxidative damage respectively (Eklund *et al.*, 2005). Overall, betaine favorably impacted the morphometry and functioning of intestine and boosted the overall performance of birds.

Hematological attributes and serum enzymes

Dietary betaine supplementation significantly ($P<0.001$) altered the blood hematology and serum enzyme profile at 42 days (Table 4). Hemoglobin levels were significantly ($P<0.001$) higher (14.44 g%) in betaine supplemented groups than control. On the contrary, H/L ratio was significantly ($P<0.001$) reduced upon betaine supplemented groups (0.34) than the control group at 42nd day of post-hatch. ALT and AST values were significantly ($P<0.001$) higher in betaine supplemented birds compare to the control groups. Elevated H/L ratio in control group (0.43) at 42nd day may be a consequence of hyper-activation of the hypothalamic-pituitary-adrenal (HPA) axis. Under chronic thermal stress, H/L ratio may get elevated owing to hyper secretion of inflammatory cytokines that stimulates the hypothalamic production of corticotrophin releasing hormones, which consequentially declines growth rate and immune-competence (Ghasemi *et al.*, 2014). Declined H/L ratio

upon dietary inclusion of betaine in broiler chickens has been reported (Ghasemi and Nari, 2020).

Carcass traits

Betaine supplemented groups exhibited significantly higher ($P<0.05$) breast muscle weight (17.36 g) than the other groups, presented in the Table 5. Dietary betaine linearly influenced semi-eviscerated carcass weight, eviscerated carcass weight and breast muscle yield in broilers (Shakeri *et al.*, 2018). The findings indicated that supplementary betaine has a great potential in improving the breast muscle yield by stimulating skeletal muscle deposition and water holding. Other carcass traits such as eviscerated carcass yield, ready to cook yield and cut-up parts were not influenced by the supplementation of betaine in heat stress broiler chickens.

CONCLUSION

On the basis of our results, it can be concluded that betaine supplementation at the level of 1 g/kg feed can be incorporated in the basal diet of the broiler birds to ameliorate the negative effects heat stress without compromising the production performance of the broiler birds

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Ethics approval

The present study was carried out at the ICAR- Central Avian Research Institute, Izatnagar, U.P. India, with Institute's Animal Ethical Committee Approval No. 452/01/ab/CPCSEA.

Conflict of interest

The authors declare no conflict of interest related to this research manuscript.

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