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Research Article
The Efficacy of a Phytogenic Blend Against Induced Heat Stress in Broilers

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Abstract
Objective: This study aimed to evaluate the efficacy of a phytogenic additive containing extracts of *Mangifera indica*, *Citrullus lanatus*, *Cymbopogon citratus*, *Allium cepa*, *Rosmarinus officinalis* and *Allium sativum* to mitigate heat stress in broilers. The effects of phytogenic additive on growth performance, hematological parameters and stress biomarkers, such as HSP70 and cortisol (CORT) in feathers, intestines and heart tissues, were assessed. **Materials and Methods:** A total of 80 one-day-old Cobb 430 Y broilers (initial weight: 48.2±0.3 g) were divided into four groups, with two replicates per group: Normal Control (NC), NC+API, Heat Stress (HS) and HS+API. The test product termed 'API' used was plant extract blend. Heat stress was induced by exposing birds to 40°C using 100-watt bulbs, while the NC groups were kept at 30°C. In the 3rd week, after a 14-day acclimatization period, API (1 mL/L) was provided via drinking water. Feed was withdrawn between 12:00 and 16:00 hrs and water was available *ad libitum*. **Results:** The NC+API group showed significantly ($p<0.05$) better growth and hematological parameters compared to the other groups. The HS+API group showed a significant ($p<0.05$) increase in body weight compared to the HS group. Cortisol levels were highest in the HS group and lowest in NC+API. The HS group exhibited the highest leukocyte count, HSP70 and CORT levels, indicating maximum stress. **Conclusion:** Overall, the NC+API group performed better than those of the others, while the HS+API group reduced stress biomarkers more effectively than the HS group.

Key words: Broiler chicks, growth performance, heat stress, hematological parameters, HSP70 stress biomarkers

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The production of chicken and turkey meat has doubled over the last two decades, reaching 125.5 million tons in 2020, accounting for about 37% of global meat production. By 2050, the world's population is projected to reach 9.9 billion, resulting in an increase in animal-based food demand of nearly 70%[1]. Heat stress occurs when chickens are unable to maintain a balance of body heat production and heat loss. Heat stress is caused by the interaction of various elements such as high environmental temperature, humidity, radiant heat and airspeed; among them, high ambient temperature plays an important role[2]. Therefore, the poultry industry must adopt technologies and practices to reduce environmental impact and develop production systems that are resilient to rising global temperatures[3].

The gradual rise in Earth's surface temperature is a prominent consequence of climate change. Animals suffer from heat stress when temperatures exceed their thermoneutral zone, resulting in poor performance and poor health. Stress negatively impacts growth performance, output and meat quality.

Figure 1 illustrates the physiological, metabolic and genetic changes amid Heat Stress (HS) and its relation to meat production and quality in chicken[4].

Coordinating and controlling the neuroendocrine and autonomic systems is required to restore and maintain homeostasis. When an animal's central nervous system detects stress, it activates four biological responses: The autonomic nervous system reaction, the neuroendocrine system response, the immune system response and the behavioural response. As a result of stress, the body releases hormones into the bloodstream, which affect a variety of physical activities. The hypothalamic-pituitary-adrenal (HPA) axis is the key mechanism implicated in this reaction and it causes the production of cortisol, the principal glucocorticoid hormone. Elevated cortisol levels suggest physiological stress, whether acute or chronic[5].

Heat shock proteins (HSPs) are stress-indicative proteins found in all living organisms. They protect cells from stressors like heat and also help in protein formation and help repair damaged proteins in the cells. HSPs are produced in cells under high ambient temperatures and play a crucial role in cell recovery after damage caused by heat. Studies have shown that HSP70 and HSP90 are extensively studied families among heat shock proteins, exhibiting various functions from cell tolerance to controlling the cell cycle. In broiler chickens, HSPs play a key role in repairing damaged cells during acute heat stress, with increased expression observed in muscles, liver, heart, kidney and blood vessels[4].

Figure 2 demonstrates the interaction of HSPs and HSF (heat shock factors) in response to heat stress. During stress, the Hsp-heat Shock Factor (HSF) complex separates to become active in the cytosol. After binding to the Heat Shock Element (HSE) in the promoter, the phosphorylated HSF trimer complex moves into the nucleus and activates the HSP gene. As a result, the production of HSPs in the cell increases to repair damaged proteins[3]. Biomarkers such as glucocorticoid cortisol (CORT), Heterophil to Lymphocyte ratio and HSPs are used to assess acute or long-term stress in animals. CORT and HSP70 are released by commercial poultry under stress. These factors can be measured in the blood to indicate acute stress. Additionally, an increase in the Heterophil to Lymphocyte ratio reflects the immune response to ongoing stress caused by high CORT levels[6].

It is extremely important to collect a more convenient sample to analyse stress biomarkers. Researchers can use non-invasive samples like hair, faeces, or feathers for sampling. Alternative measures provide a more detailed view of an animal's physiology over different time frames: hours (faeces), weeks, or months (feathers and hair)[7]. Long after birds have died, feathers can be used to evaluate stress biomarkers, revealing information about their stress levels. Monitoring CORT and HSP70 levels in feathers can help determine long-term stress without requiring specialist training or creating further stress to the birds. This method is useful since feather samples are simple to collect, can be maintained at room temperature and offer precise assessments of stress indicators. CORT is thought to be deposited into feathers during growth and keratinization, possibly from the blood or by diffusion from the skin around the feather[5]. Heat stress reduces body weight, glucose levels, protein, CORT levels and the heterophil-to-lymphocyte ratio in broiler chickens, which can lead to higher mortality. To mitigate the negative impact of heat stress on the productivity and well-being of poultry, current studies are focusing on phytogenic feed additives (PFAs). These additives have shown significant effectiveness in improving poultry health and productivity, bolstering immune resistance, enhancing the gut environment and increasing feed intake under heat-stress conditions[8,9].

The API is a phytogenic blend for poultry and other animal species, with a combination of extracts, namely *Mangifera Indica*, *Citrullus lanatus*, *Cymbopogon citratus*, *Allium cepa*, *Rosmarinus officinalis* and *Allium sativum*. These plants have been found to have significant antioxidant properties. Mango leaf supplementation in broiler feed enhances antioxidant defenses, reduces oxidative stress and improves overall cellular health. It increases the activity of key antioxidant enzymes and total antioxidant capacity while reducing levels

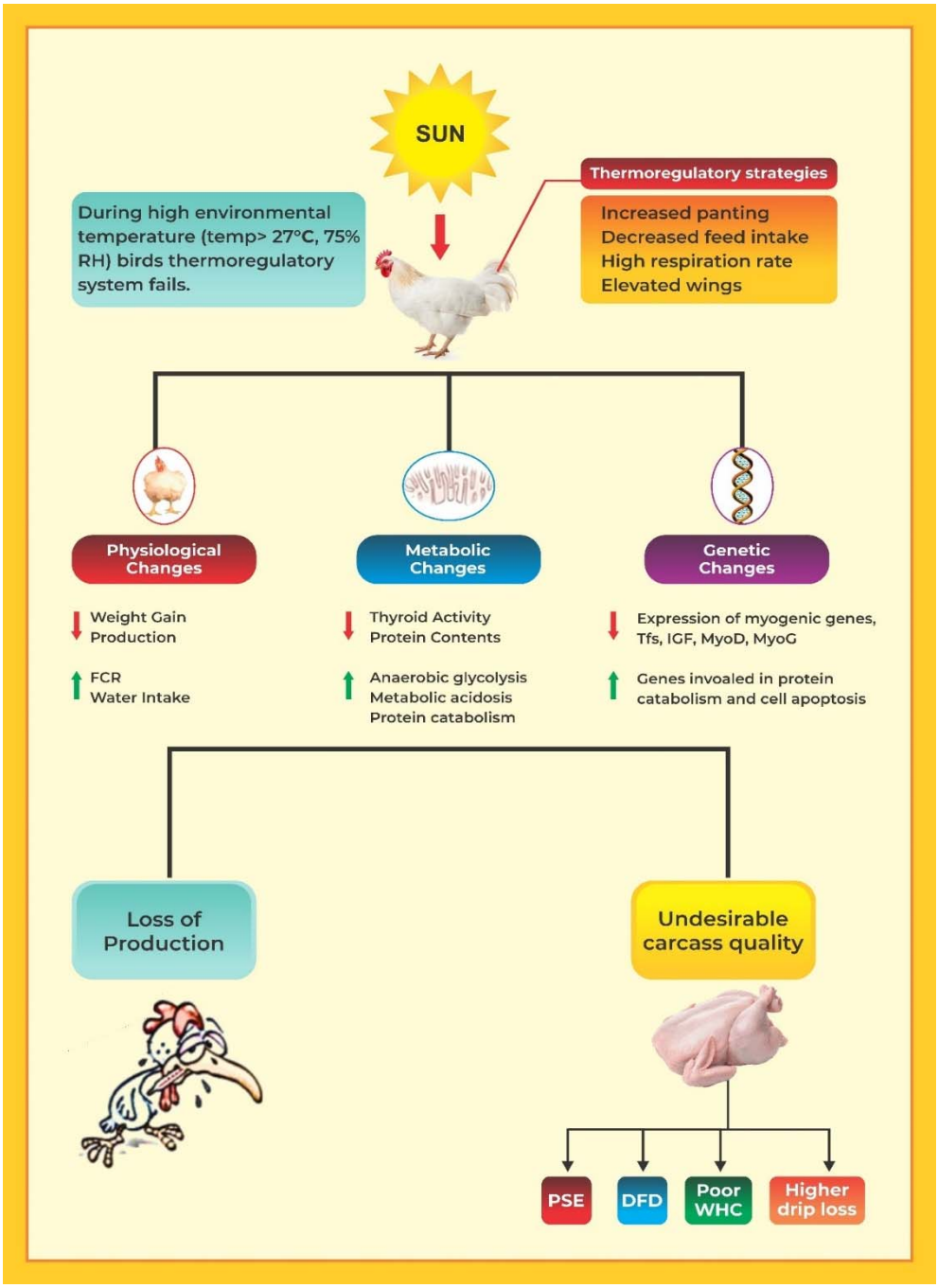

Fig. 1: Physiological, metabolic and genetic changes amid Heat Stress (HS) and its relation to meat production and quality in chicken

of oxidative stress markers. The antioxidant effects are attributed to rich phytochemical composition of *Mangifera indica* leaf, including compounds like mangiferin, known for their antioxidant properties[10]. Rosemary (*Rosmarinus officinalis* L.) is categorized within the Lamiaceae botanical family. It contains a variety of bioactive compounds, such as camphor, caffeine, ursolic acid, carnosol and carnosic acid. Studies have shown that rosemary treatment significantly reduces cardiac damage markers, including lactate dehydrogenase (LDH), Creatine Kinase (CK) and CKMB. It was also indicated that rosemary helps mitigate cardiac damage caused by heat stress[11].

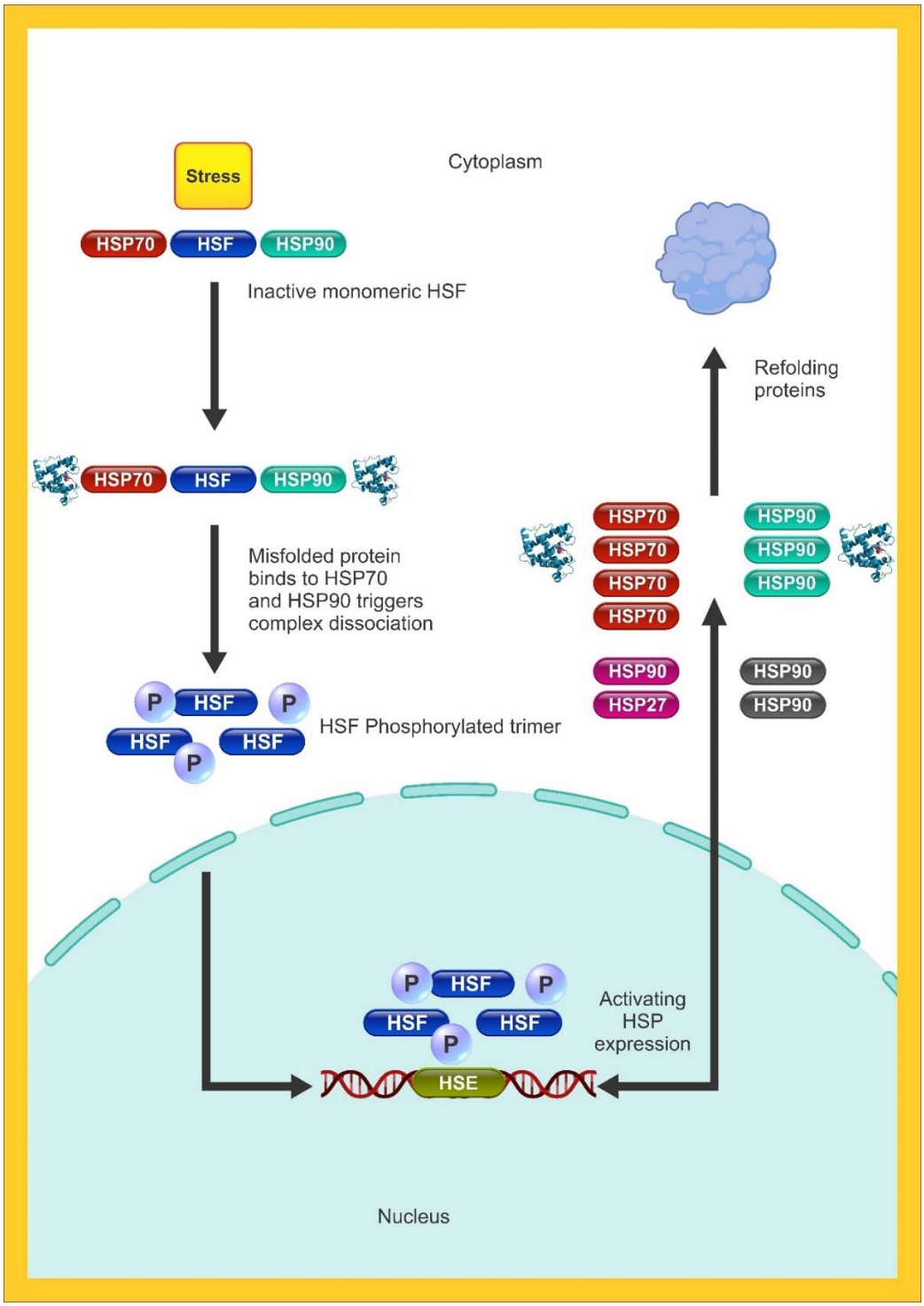

Fig. 2: The interaction of HSPs and HSF (heat shock factors) in response to heat stress

Allium cepa extract contains sulfur compounds such as allicin and other organosulfur compounds (like S-Methyl cysteine sulfoxide and S-allyl cysteine sulfoxide) that have antioxidant effects. These compounds help neutralize free radicals and oxidative stress, which can be exacerbated by high-temperature exposure. In addition to its antioxidant

properties, the extract benefits poultry by improving lipid metabolism, reducing blood cholesterol and LDL levels, influencing behavioural responses to heat stress and lowering feed intake while maintaining body weight gain and improving feed conversion efficiency. Additionally, onion (*Allium cepa*) supplementation can help reduce rectal temperature in birds, contributing to better thermoregulation[12]. *Allium sativum* extract is known for its strong antioxidant properties. It contains the compounds allicin, diallyl disulfide and sulfur compounds that help neutralize free radicals and reduce oxidative stress. It has been shown to reduce inflammation caused by heat stress[13]. The bioactive compounds in garlic, particularly allicin, inhibit the production of pro-inflammatory cytokines and enzymes such as cyclooxygenase-2 (COX-2), thereby reducing inflammation and improving overall health[14]. Both *Citrullus lanatus* (watermelon) and *Cymbopogon citratus* (lemongrass) extracts, enhance growth rates and overall development in heat-stressed animals also improve the immune response in animals, support electrolyte balance and improve hydration and nutrient utilization[15-17].

The study aimed to induce heat stress using external heat source (100-watt bulb) and examine the efficacy of API supplementation on the growth performance of broiler chickens. The study will measure stress biomarkers such as HSP70 and cortisol (CORT) in the heart, intestines and feathers, as well as the heterophil: lymphocyte ratio and other haematological parameters to understand the impact of heat stress on different groups.

MATERIALS AND METHODS

Housing and vaccination: One-day old Vencobb 430 Y broiler chicks of mixed sexes (n = 80) were obtained from Venkateshwara Hatcheries Pvt. Ltd. in Pune, Maharashtra, India. The birds were housed in a non-environmentally controlled shed. Birds were maintained in separate pens with 2 sq. ft. of area per bird. Two to three inches of rice husk litter were evenly distributed on a cement floor as bedding. Upon arrival at the farm, the birds were given jaggery water and multivitamins for three days. Their weight and feed consumption were recorded every week from the day they arrived (day 0) until the end of the trial (day 42). Humidity was not controlled but was measured daily and ranged between 68-91%. Additionally, the chicks underwent a two-week acclimatization period after being brought to the farm.

The birds were vaccinated with the Super LaSota™ vaccine against Newcastle disease on day 7, followed by a booster dose on day 21. The vaccination for Infectious Bursal Disease (IBD-intermediate strain) was given on day 14 and booster on day 28. Both the vaccines were administered-intranasally and their boosters were dosed through oral consumption in drinking water.

Feed and water: The diet of the birds was in accordance with the Bureau of Indian Standards (BIS) regulations, 2007[18]. Commercial feed from Baramati Agro Ltd., Pune, based primarily on maize and soybeans, was provided to the birds. The proximate analysis of the feed was carried out at Omega Laboratories, Lonand. The birds were fed a standard starter diet for the first 21 days, followed by a standard finisher diet until the end of the experiment. The detailed formulation of the diet is given in Table 1 and nutrient composition in Table 2. After the acclimatization period, the feed was withdrawn daily between 12:00 and 16:00 hrs to prevent the generation of metabolic heat. This step was crucial to standardize and understand the effect of induced heat stress on birds. Water was available *ad libitum* for all the birds. Each bird consumed approximately 9.5 liters of water throughout the trial.

API formulation: The API tested in this study was plant extract blend provided by Nutranovel Additives Pvt. Ltd.

Table 1: Formulation of starter and finisher diets

Ingredient	Starter	Finisher
Maize Red (Moisture max 10 -11%)	57	63
Broken Rice	3.7	0
Soyabean Meal 46%	27	23.98
Maize Gluten Meal 60%	3	2
Meat and Bone meal Allana	3.5	3.5
Veg. Oil	2.4	3.7
DCP	0.75	0.55
LSP	0.95	0.7
De Oiled Rice Bran	0	0.9
Vitamin Mix-Miavit Viamin blend+c	0.05	0.05
Breeplex Inorganic minerals Venkeys	0.1	0.1
Organic Minerals Eg Bioplex	0.05	0.05
Lysine	0.31	0.29
Methionine	0.32	0.27
Threonine	0.11	0.08
Choline Chloride 60%	0.11	0.15
Salt Powder	0.25	0.24
Sodium Bicarbonate	0.1	0.1
Axtra XAP 101 Enzyme DuPont	0.01	0.01
Axtra PHY 5000 Phytase DuPont	0.01	0.01
Bargapur/Burgapur	0.05	0.05
MasterSorb Gold EWNutrition/TBinder	0.1	0.1
Hepatocare/Liver Tonic	0.05	0.05
Proviguard/ Antioxidant	0.01	0.01
BMD	0.02	0.05
Anticoccidial Gromax	0.05	0.06
Total	100	100

Table 2: Proximate compositions % (DMB) of starter and finisher diets

Nutrient	Starter	Finisher
C Protein (%)	21.8	20.2
M E kcal	3115	3207
Fat (%)	5.35	4.42
Calcium (%)	0.96	0.98
Available Phos (%)	0.44	0.47
Lysine (%) Av	1.16	0.98
Methionine (%) Av	0.61	0.63
Threonine (%) Av	0.77	0.68
Na	0.16	0.13

It is a plant-based formulation aimed at alleviating heat stress in poultry. It consists of *Allium cepa* (15000 mg/L), *Mangifera indica* (10000 mg/L), *Citrullus lanatus* (10000 mg/L), *Rosmarinus officinalis* (5000 mg/L), *Allium sativum* (10000 mg/L) and *Cymbopogon citratus* (7000 mg/L). API was administered at a dosage of 1mL/L with drinking water.

Experimental design: The birds were randomly distributed into four groups, with twenty birds in each group. Each experimental group consisted of two replicates, with ten birds in each replicate. The four experimental groups were as follows- 1) Normal Control (NC): This group was maintained at 30°C ±1°C without the API. 2) Positive Control (PC): This group was maintained at 40°C±1°C without the API. 3) Test 1: The group was maintained at 30°C±1°C with API administration. 4) Test 2: The group was maintained at 40°C±1°C with API administration. For heat exposed groups, the heat was generated using a 100-watt bulb for each pen.

Evaluation of zootechnical characteristics: The individual weights of the birds were recorded at the end of each week. Their total feed consumption was calculated by subtracting the amount of feed left over from the original amount of feed given. The Feed Conversion Ratio (FCR) was calculated for each week using the following formula as:

$$\text{FCR} = \frac{\text{Total feed consumed in a week (g)}}{\text{Weight gained in a week (g)}}$$

Sample extraction from bird tissues: At the end of the experimental period on day 42, six birds from each experimental group, i.e., three birds from each replicate, were randomly selected and slaughtered for HS biomarker and hematological testing. The feather, heart and intestine tissue samples were collected for cortisol and HSP70 estimations. Feather cortisol and HSP70 were estimated at day 0 of the experimental study, i.e., day 14 after the birds were brought. The extraction procedures for each tissue are listed below:

Feather tissue extraction: The feather samples were cleaned with tissues to get rid of any debris. The vanes of the feathers were separated using sterile scissors and chopped into <5 mm pieces. The samples, along with extraction buffer, i.e., 10 mL of HPLC-grade methanol for cortisol and 10 mL of 100 mM Tris buffer containing 1% Triton X-100, were placed in a shaking water bath at room temperature for 30 min, followed by an overnight shaking bath at 50°C. For the extraction of HSP70, the overnight shaker temperature was 37°C. The insoluble portion was then separated using vacuum filtration and washed twice with 2.5 mL of extracting solvent to extract any remaining protein. The solvent was evaporated at 50°C in a fume hood. The dried extract was resubmerged in a 0.05 M phosphate buffer system at pH 7.6. The solution was stored at -20°C.

Heart and intestinal tissue extraction: The same procedures were used for the extraction of both cortisol and HSP70. About 50 g of tissue was collected from each sample. The tissues were dissected using clean tools on an ice slab and transferred to round-bottom microfuge tubes containing liquid nitrogen to snap freeze. These samples were stored at -80°C until further analysis. For every 5 mg piece of tissue, 300 µL of 100 mM Tris buffer containing 1% Triton X-100 was used. The sample, along with the extraction buffer, was homogenized and maintained at constant agitation at 4°C for 2 hrs. The supernatant containing the protein of interest was collected by centrifugation at 13,000 rpm at 4°C. The extract was stored at -80°C until further analysis.

ELISA test for cortisol and HSP70 estimation: The Cortisol HSP70 levels were estimated by sandwich ELISA method using Chicken HSP70 (Heat shock protein 70) ELISA Kit, RealGene, USA (Cat. No. 3154696) and Chicken Cor (Cortisol) ELISA Kit, RealGene, USA (Cat. No. 3158733). The test was carried out according to the protocol prescribed by the commercial kits. A total of eight standard solutions of HSP70 and cortisol were prepared, excluding the test samples. The data from these samples was used to determine the concentration of HSP70 and cortisol in various tissues. At the end of the protocol, the plates were analysed using Epoch 2, Microplate reader, BioTek (Agilent), USA.

Test for hematological parameters: After this, 2 mL of blood was collected from the wing veins of each bird. It was collected using sterile syringes and needles in sterile tubes containing EDTA. The cells were analysed microscopically

using an Olympus X-21 microscope. Differential Leucocyte Count (DLC) was estimated using Leishman stain[19] and the Total Leucocyte Count (TLC) was estimated using Nattas and Harris fluid. The following cell counts were performed:

- RBC
- Hemoglobin
- RBC
- Leukocytes
- Heterophils
- Lymphocytes
- Monocytes
- Eosinophils

Statistical analysis: The data was processed and presented as the Mean±standard error (SE). For analyzing the growth parameters, the data for each week was processed using one-way Analysis of Variance (ANOVA)[20] and two-way Analysis of Variance (ANOVA)[21] for heat stress markers and hematological parameters. Tukey's multiple comparison test[22] was used to analyze the significant differences among the treatment groups. The significance level was maintained at $p<0.05$. All the statistical tests and graphs were performed using GraphPad Prism Version 10.3.0.

RESULTS

Growth performance: The data concerning the average weekly live body weights (g) of birds from day-old to six weeks of age across different groups are outlined in Table 3. The average weekly live body weight of birds in the NC+API group was significantly ($p<0.05$) higher than those of the other three groups. Additionally, birds in the HS+API group showed a significant ($p<0.05$) increase in live body weight compared to the HS group. However, there was no significant ($p>0.05$) difference in live body weight between the Normal Control and HS+API groups. The average weekly body weight gains of birds from various groups over the first to sixth weeks of age are summarized in Table 4. In the 3rd, 4th and 6th weeks of the experimental period, the live body weight gain of birds in the NC+API group was significantly ($p<0.05$) higher than that of the NC group, whereas birds in the HS+API group showed a significant ($p<0.05$) increase in live body weight gain compared to the HS group. However, in the 5th week of the experimental period, only NC+API group showed significantly ($p<0.05$) higher weight gain compared to the NC group and there was no significant difference ($p>0.05$) in the HS+API and HS groups.

The average weekly feed intake data for various groups from the first to sixth week of age is shown in Table 5. There was no significant ($p>0.05$) difference in feed consumption from the 1st to the 5th week between birds in the NC+API group and the NC group, as well as between birds in the HS+API group and the HS group. On the contrary, at the end of the 6th week of the experimental period, there was a significant difference ($p<0.05$) in the birds from the NC+API group compared to the NC group.

The average weekly Feed Conversion Ratios (FCR) from day-old to 42 days for all experimental groups are shown in Table 6. There was no significant ($p>0.05$) difference in Feed Conversion Ratio (FCR) from the 1st to the 4th week

Table 3: Average weekly live body weight (g) of experimental birds from different groups

	Experimental groups			
Weeks	NC	NC+API	HS+API	HS
1	210.90±2.02^{a}	209.80±1.25^{a}	211.60±1.34^{a}	211.90±2.08^{a}
2	507.90±1.17^{a}	507.85±1.03^{a}	509.25±1.32^{a}	507.85±1.12^{a}
3	1000.30±1.78^{b}	1030.25±1.43^{a}	992.60±2.45^{b}	965.45±3.38^{c}
4	1593.00±4.21^{b}	1621.80±1.92^{a}	1587.65±3.50^{b}	1567.50±3.00^{c}
5	2002.10±2.98^{b}	2112.45±10.68^{a}	2019.85±2.32^{b}	1958.65±2.45^{c}
6	2595.25±10.44^{b}	2825.85±5.14^{a}	2600.75±15.04^{b}	2356.3±15.99^{c}

Means marked with distinct superscripts (a-c) in the same row exhibit statistically significant differences ($p<0.05$)

Table 4: Average weekly live body weight gain (g) of experimental birds from different groups

	Experimental groups			
Weeks	NC	NC+API	HS+API	HS
1	161.05±1.6^{a}	163.20±1.6^{a}	166.05±2.6^{a}	166.55±2.85^{a}
2	294.25±2.7^{a}	299.83±1.78^{a}	300.67±3.03^{a}	297.13±13^{a}
3	498.00±5.65^{b}	525.65±3.2^{a}	480.98±2.38^{b}	459.50±1.9^{c}
4	595.05±2.3^{b}	597.68±6.13^{a}	597.38±2.33^{b}	597.83±4.22^{c}
5	446.00±3.3^{b}	497.13±6.48^{a}	436.90±4.7^{bc}	411.70±1.9^{c}
6	599.68±6.53^{b}	717.45±4.05^{a}	584.15±3.2^{b}	403.18±5.53^{c}

Means marked with distinct superscripts (a-c) in the same row exhibit statistically significant differences ($p<0.05$)

Table 5: Average weekly feed consumption (g) of experimental birds from different groups

	Experimental groups			
Weeks	NC	NC+API	HS+API	HS
1	189.0±2.0^{a}	184.5±1.5^{a}	188.0±1.0^{a}	187.5±2.5^{a}
2	308.0±3.0^{a}	315.0±2.0^{a}	303.0±2.0^{a}	312.0±3.0^{a}
3	725.5±2.5^{a}	731.0±2.0^{a}	698.0±5.0^{b}	704.0±2.0^{b}
4	929.5±1.5^{b}	931.5±1.5^{b}	975.0±3.0^{a}	966.5±2.5^{a}
5	1178.0±9.0^{a}	1175.5±2.5^{a}	1193.0±2.0^{a}	1158.0±9.0^{a}
6	1078.5±5.5^{a}	1036.5±3.5^{b}	993.5±6.5^{c}	958.5±9.5^{c}

Means marked with distinct superscripts (a-c) in the same row exhibit statistically significant differences (p<0.05)

Table 6. Average weekly feed conversion ratio of experimental birds from different groups

	Experimental groups			
Weeks	NC	NC+API	HS+API	HS
1	1.174^{a}±0.024^{a}	1.131±0.002^{a}	1.132±0.012^{a}	1.126±0.004^{a}
2	1.047^{a}±0.20^{a}	1.051±0.0005^{a}	1.008±0.017^{a}	1.050±0.006^{a}
3	1.457^{ab}±0.022^{a}	1.391±0.005^{b}	1.451±0.018^{ab}	1.532±002^{a}
4	1.562^{b}±0.004^{b}	1.559±0.018^{b}	1.632±0.011^{a}	1.617±0.007^{ab}
5	2.642^{b}±0.040^{b}	2.365±0.026^{c}	2.731±0.034^{ab}	2.813±0.009^{a}
6	1.799^{b}±0.010^{b}	1.445±0.013^{c}	1.701±0.021^{b}	2.378±0.056^{a}

Means marked with distinct superscripts (a-c) in the same row exhibit statistically significant differences (p<0.05)

Table 7. Average hematological parameters of birds from different experimental groups

	Experimental groups			
Parameters	NC	NC+API	HS+API	HS
Hb (g)	8.45±0.06^{b}	9.3±0.05^{a}	8.40±0.03^{b}	7.53±0.07^{c}
RBC (million)	4.49±0.05^{b}	4.86±0.04^{a}	4.46±0.03^{c}	3.98±0.03^{d}
PCV (%)	28.00±0.37^{b}	29.50±0.43^{a}	29.00±0.26^{a}	28.83±0.31^{a}
TLC (10^3/cumm)	7.85±0.19^{c}	7.24±0.11^{d}	10.96±0.23^{b}	13.63±0.16^{a}
Eosinophils (%)	7.33±0.42^{a}	7.17±0.95^{ab}	5.50±0.85^{ab}	3.67±1.17^{b}
Monocytes (%)	6.67±0.67^{ab}	7.50±0.43^{a}	8.00±0.73^{a}	4.67±0.49^{b}
Heterophils (%)	0.55±0.76^{c}	0.48±0.76^{c}	0.89±0.70^{b}	1.52±1.05^{a}
Lymphocytes (%)	55.5±0.62^{a}	57.83±0.48^{a}	45.67±0.56^{b}	36.50±0.76^{c}
Heterophil-to-lymphocyte ratio	0.55±0.02^{c}	0.48±0.01^{c}	0.89±0.02^{b}	1.52±0.04^{a}

Means marked with distinct superscripts (a-c) in the same row exhibit statistically significant differences (p<0.05)

between birds in the NC+API group and the NC group, as well as between birds in the HS+API group and the HS group. At the 5th week of the experimental period, the FCR was significantly lower (p<0.05) in birds from the NC+API group compared to the NC group but non-significant (p>0.05) in the HS+API group compared to the NC group. This indicated that the FCR was similar for birds in normal temperatures and heat-stressed birds with API. In the 6th week of the experimental period, FCR was significantly (p<0.05) lower in the NC+API group and the NC group, as well as between birds in the HS+API group and the HS group.

Haematological profile: The average hematological parameters of birds at the end of the 42-day experimental period across different groups are presented in Table 7, with the average hemoglobin values depicted. The values of hemoglobin were significantly (p<0.05) higher in the NC+API group and significantly lower (p<0.05) in the HS group. There was no significant (p>0.05) difference between the NC and the HS+API group.

The values of packed cell volume (PCV) were significantly higher (p<0.05) in the NC+API, HS and HS+API groups and significantly lower in the NC group. The values of RBC were significantly (p<0.05) higher in the NC +API group and significantly lower in the HS group. The total leukocyte count (TLC) was significantly higher (p<0.05) in the HS group and significantly lower (p<0.05) in the NC+API group.

The heterophilic count was significantly higher (p<0.05) in the HS group and significantly lower (p<0.05) in the NC+API group. The lymphocyte count was significantly higher (p<0.05) in the NC+API group and significantly lower (P<0.05) in the HS group. The heterophil to lymphocyte ratio was significantly (p<0.05) lower in the HS+API group compared to the HS group.

The monocytic count was significantly (p<0.05) higher in the NC+API group and significantly lower (p<0.05) in the HS group. Though there were significant (p<0.05) differences present in monocytic counts, monocytes were within the normal physiological range. The eosinophilic count was significantly higher (p<0.05) in the NC, NC+API group and

Table 8: Average cortisol testing (ng/ml) of birds of various experimental groups

	Experimental groups			
Parameters	NC	NC+API	HS+API	HS
ELISA feather, Day 42 (ng/mL)	49.10±4.43^{cd}	41.012±1.83^{d}	69.75±4.69^{b}	76.86±5.64^{a}
ELISA heart (ng/mL)	122.51±3.09^{cd}	107.48±6.66^{d}	147.66±2.99^{b}	166.63±2.97^{a}
ELISA Intestines (ng/mL)	23.72±1.89^{cd}	22.48±3.37^{d}	37.06±5.42^{b}	61.75±5.90^{a}
Cortisol testing ELISA feather: from feather day 14th of total trail (before initiation of heat stress), (ng/mL)				
Normal control			11.97±0.53	

Means marked with distinct superscripts (a-d) in the same row exhibit statistically significant differences (p<0.05)

Table 9: Average HSP70 testing (ng/ml) of birds of various experimental groups

	Experimental groups			
Parameters	NC	NC+API	HS+API	HS
ELISA feather, Day 42 (ng/mL)	15.99±0.26^{c}	13.50±0.33^{d}	16.05±0.66^{b}	23.43±0.76^{a}
ELISA heart (ng/mL)	128.74±4.64^{c}	82.63±3.32^{d}	142.04±4.91^{b}	171.09±3.45^{a}
ELISA Intestines (ng/mL)	49.103±4.43^{bc}	41.01±1.83^{c}	69.79±4.36^{a}	76.86±5.64^{a}
HSP70 testing ELISA feather: from feather day 14th of total trail (before initiation of heat stress), (ng/mL)				
Normal control			12.84±0.90	

Means marked with distinct superscripts (a-d) in the same row exhibit statistically significant differences (p<0.05)

HS+API group and significantly lower in the HS group. Though there were significant (p<0.05) differences present in monocytic counts, monocytes were within the normal physiological range.

Stress biomarkers: CORT levels of feathers, heart muscles and intestines were significantly higher in the HS group and significantly lower (p<0.05) in the NC+API group, As shown in Table 8.

HSP70 levels in feather and heart muscles were significantly higher in the HS group and significantly lower (p<0.05) in the NC+API group. HSP70 levels in the intestines were significantly higher (p<0.05) in the HS group and the HS+API group and significantly lower (p<0.05) in the NC + API group. HSP70 levels in the feathers, heart and intestines are represented in Table 9.

Overall, the hematological parameters of the NC+API group were superior to those of the other treatment groups. TLC will be a considerable marker for stress and it was found that the HS group had a higher TLC, which indicates the maximum level of stress in the HS group. However, the HS+API group showed significantly lower (p<0.05) TLC as compared to the HS group and the values of TLC were found within the normal physiological range for this group. Overall, the NC+API group showed less release of stress biomarkers in different tissues, viz., the feather, heart and intestines. Whereas, the HS group showed comparatively higher levels of stress biomarkers, viz., CORT and HSP70.

DISCUSSION

Several studies have examined nutritional supplements to mitigate the adverse effects of heat stress in broiler chickens. Heat stress disrupts the oxidation system, alters intestinal microbial composition and affects metabolic mechanisms, leading to reduced performance in birds. Our research paper investigates the potential benefits of adding the studied API to the drinking water of birds exposed to high temperatures, focusing on their impact on growth performance, haematological profile and stress biomarkers such as HSP70 and CORT in feathers, intestines and heart tissues. Although few studies have shown that HSP70 is cardioprotective in nature, the increase in serum levels were associated with heat stress.

In line with the results of the current study, significant (p<0.05) differences were observed in live body weight, body weight gain and feed conversion ratio (FCR) in experimental birds fed diet supplemented with 200 mg/kg of garlic essential oil (GEO), 200 mg/kg of lemon essential oil (LEO), a 200 mg/kg mixture of both (GLO) and lemongrass oil (LGO) at 50, 100 and 150 mg/kg of feed, respectively[14,23].

In divergence, throughout the whole experiment, a non-significant difference (p>0.05) was found in body weight gain (BWG), feed intake (FI), or feed conversion ratio (FCR) of birds fed diet supplemented with 1, 2 and 4 g per kg fermented garlic powder[24]. On the contrary, administering rosemary leaf extract at a dose of 100 mg/kg increased the feed conversion ratio (FCR) among the experimental groups[8].

Results of the present study agree with findings of Shahr-e Babak[25] who fed the diet supplemented with various levels of rosemary powder at doses of 5, 10 and 15 g per kg and rosemary extract at doses of 3.5, 7 and 10.5 g per kg and observed significant (p<0.05) effect on the final live weight of chicks and the heterophil to lymphocyte ratio. However, these treatments did not significantly (p>0.05) influence the feed conversion ratio[25].

Diet supplemented with lemongrass hydrosol extract (LGHE) had a significant ($p<0.05$) effect on the hematological parameters of broiler chickens[26,27] Likewise, birds fed a diet supplemented with 0.1 and 0.2% garlic powder had significantly ($p<0.05$) higher total white blood cells, neutrophils, eosinophils, monocytes and lymphocytes compared to the control group[28].

On the contrary, a non-significant ($p>0.05$) effect was observed on the hematological parameters of broiler chickens fed with a dilution of lemongrass hydrosol extract (LGHE) mixed in a litre of water containing 250, 500 and 750 mL and pure LGHE[29].

As compared to the control group, an 80% reduction ($p\leq0.05$) in HSP70 mRNA expression was observed when rosemary leaf extract was added to broiler diets at a concentration of 50 mg/kg[8]. In the current study, a significant ($P < 0.05$) decrease was observed in HSP70 levels in the feather and heart tissues of the NC group and the NC +API group.

On the other hand, administration of a 3% rosemary emulsion liquid through drinking water significantly ($p>0.05$) increased HSP70 levels in the chicken heart under both normal and heat stress conditions. Notably, this treatment exhibited a protective effect on the chicken heart during heat stress. Consequently, purified rosemary extract may be effective in mitigating heat stress in broiler chickens[11].

CONCLUSION

The NC+API group was found to be the most effective compared to other treatment groups. HS+API exhibited similar performance to NC group. The HS+API group showed a comparatively better reduction of stress biomarkers, viz., CORT and HSP70, than the HS group.

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