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Mitigating Effect of Phytogenic Plant Extracts on Heat Induced Stress in Broilers

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ABSTRACT

Background: This study was aimed to evaluate the effectiveness of a phytogenic blend composed of extracts from *Mangifera indica, Citrullus lanatus, Cymbopogon citratus, Allium cepa, Rosmarinus officinalis* and *Allium sativum,* against induced heat stress in commercial broilers. These were studied by examining their effects on growth performance, haematological parameters and stress biomarkers. The biomarkers used were HSP70 and cortisol (COR) in feathers and blood serum.

Methods: A total of 1760 one day-old Cobb 430Y broiler chicks were randomly allocated into four treatment groups, consisting of 440 birds in each group. A phytogenic plant extract, referred as PPE, was used in this study. The experimental groups were as follows: Normal Control (NC, 36.05±0.32°C at 2 PM, no PPE), NC+PPE (36.05±0.32°C at 2 PM, with PPE), Heat Stress (HS, 42.20±1°C at 2 PM, no PPE), HS+PPE (42.20±1°C at 2 PM, with PPE). Heat stress was induced using portable electric heater for the HS groups. After a 14-day acclimatization period, PPE was administered through drinking water. Feed was withdrawn from 12:00 hrs. and 16:00 hrs, while water was provided *ad libitum* for all the birds.

Result: The NC+PPE and HS+PPE groups showed significantly higher ($P<0.05$) body weight and body weight gain compared to their respective control groups. The cumulative weekly mortality percentage was lowest in the NC+PPE group (1.36%, 6 birds). Feather HSP70 levels on day 41 were lowest ($P<0.05$) in the HS+PPE group. No significant differences were observed in the haematological parameters between the treatment groups. The NC+PPE and HS+PPE both groups performed comparatively better than other treatment groups. Both the groups had a comparatively better reduction of stress biomarkers, *viz*., feather HSP70, as compared to the respective control groups.

Key words: Broiler chicks, Growth performance, Haematological parameters, Heat stress, HSP70, Stress biomarkers.

INTRODUCTION

India's poultry industry is among the fastest-growing agricultural sectors, expanding at an annual rate of approximately 8% and currently valued at around USD 7.5 billion (Kumar *et al*., 2024). Global population is projected to reach 9.7 billion by 2050, driving a 60% increase in food demand and a 70% rise in animal-based product consumption (Ritchie *et al*., 2019). As a result, global meat production is expected to reach 347 million tonnes by 2030, with poultry accounting for approximately 52% (OECD-FAO Agricultural Outlook 2021-2030, 2021). Despite this growth, heat stress remains a major constraint, causing losses of up to USD 2.36 billion annually in the U.S. poultry sector (Abbas *et al*., 2025). Heat stress disrupts physiological balance by overwhelming thermoregulation, leading to oxidative damage and reduced performance (Greene *et al*., 2019). Broiler chickens are especially prone to heat stress due to dense feathers and absence of sweat glands, limiting heat loss (Song and King, 2015).

Heat shock proteins (HSPs) function as molecular chaperones that play a crucial role in preserving cellular homeostasis by inhibiting apoptosis and enhancing cell survival mechanisms (Madkour *et al*., 2022). Under stressful conditions, HSPs are induced to synthesize specific proteins that maintain cell viability and stabilize the internal environment, with HSP70 playing a central role in thermotolerance and serving as a key biomarker for heat stress in animals (Song and King, 2015). Feather-expressed HSP70 serves as a reliable, non-invasive biomarker for assessing thermal stress in broilers (Greene *et al*., 2019).

Phytogenic compounds from various plant parts act as bioactive agents that promote health, food safety and shelf-life. *Mangifera indica* provides mangiferin, a potent

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antioxidant (Okwu and Ezenagu, 2008), while *Citrullus lanatus* (watermelon) is rich in antioxidants like beta-carotene, vitamins C and E, arginine and citrulline, which help protect cells, DNA and tissues from oxidative damage (Collins *et al*., 2007). Lemongrass (*Cymbopogon citratus*) contains region-specific phytochemicals, including antimicrobial myrcene and flavonoids like luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol and apigenin (Shah *et al*., 2011). Onion *(Allium cepa)* naturally contains allicin and sulfur compounds such as S-methylcysteine sulfoxide and S-allylcysteine sulfoxide (Kothari *et al*., 2019).

Rosemary (*Rosmarinus officinalis*) contains bioactives like camphor, ursolic acid, carnosol, carnosic acid and caffeine (Ulbricht *et al*., 2010). Garlic (*Allium sativum*) is rich in organosulfur compounds such as allicin and diallylsulfide, contributing to anti-inflammatory and immune-enhancing effects (Jang *et al*., 2018). However, evidence on the role of phytogenic in reducing heat stress in broilers remains limited (Chiwaya *et al*., 2025). Therefore, our current objective is to investigate the potential of phytogenic plant extracts (PPE), comprising blends of *Mangifera indica, Citrullus lanatus, Cymbopogon citratus, Allium cepa, Rosmarinus officinalis* and *Allium sativum,* in alleviating heat stress in broilers and to evaluate their synergistic effects.

MATERIALS AND METHODS

The research trial was conducted following ethical guidelines at Dr. B.V. Rao Institute of Poultry Management and Technology (IPMT), Pune, using 1,760 one-day-old Cobb 430Y broiler chicks (mixed sex) procured from Venkateshwara Hatcheries, Pune. The 42-day trial was conducted from 17 January to 27 February 2025. It involved four groups: NC (normal temperature, no PPE), NC+PPE (normal temperature with PPE), HS (heat stress at 42°C, no PPE) and HS+PPE (heat stress with PPE). Each group consisting of 440 birds, subdivided into four replicates of 110 birds each. Groups HS and HS+PPE were housed in shed C1, while NC and NC+PPE were in shed C2.

An acclimatization period (day 0-14) without heat stress or test treatments was provided. Venky's Aminovit Gold was supplemented via drinking water for the first five days. Heat stress was induced from day 15 to 42 using a portable electric heater from 12:00 to 16:00 hrs daily. Recorded average temperatures in the HS shed were 26.71±0.50 °C at 08:00, 42.20±0.10 °C at 14:00 and 34.31 ±0.39 °C at 17:00.

Vaccination was performed as follows: Newcastle disease (Super LaSota™) intranasally on day 7, booster orally on day 21; IBD vaccine on day 14 and day 28 (intermediate strain). Birds were fed in-house formulated mash diets: pre-starter (weeks 1-2), starter (weeks 3-4) and finisher (weeks 5-6) with *ad libitum* water. Proximate analysis was performed by Envirocare Labs Pvt. Ltd., Thane, as presented in Table 1. Feed was restricted daily from 12:00 to 16:00 hrs. Growth performance (body weight, weight gain, feed intake and FCR) was recorded weekly from 20 randomly selected birds per replicate.

The heat stress-relieving agent (PPE) was a blend of plant extracts: *Rosmarinus officinalis*, *Cymbopogon citratus*, *Mangifera indica*, *Citrullus lanatus*, *Allium sativum* and *Allium cepa*, administered *via* drinking water at 0.25 mL/L from day 15 to 42 for 12 hours daily (6 AM-6 PM).

Sample collection and analysis

On day 13 and 41, blood and feather samples were collected for cortisol and HSP70 estimation. Feather samples were collected twice daily morning (9:30-10:30 AM) and afternoon (2:30 PM), while blood was sampled only in the morning. Eight birds per replicate were marked; serum was collected from three birds/replicate without EDTA. Feather samples from all eight birds were pooled. Morning sampling: HS and HS+PPE (9:30-10:30 AM), then NC and NC+PPE (10:45–11:45 AM). On day 13, feather collection was done outside the shed; on day 41, inside. Sampling followed shed order: C1 then C2. Cortisol and HSP70 were quantified using chicken-specific sandwich ELISA kits (Cortisol: Cat. No. EA0017Ch; HSP70: Cat. No. E0326Ch; BT LAB, China). Tests were done according to the manufacturer's protocol. For haematological analysis (day 41), 2 mL blood was collected from three birds per replicate (n=48) into 4% EDTA vacutainers. Hematological parameters were assessed using an automated veterinary hematology analyser (MicroCC Vet, High Technology Inc., USA). Differential leukocyte counts were performed on Field stain. Field Stains A (Molychem 31680) and B (Molychem 31720) processed blood smears.

Statistical analysis

One-way ANOVA (Quirk, 2012) was used to analyze treatment effects followed by Tukey's multiple comparison test (Abdi and Williams, 2010). All analyses were performed using GraphPad Prism (Version 10.3.0).

RESULTS AND DISCUSSION

Growth performance parameters and mortality

The results of various growth performance parameters are presented in the Table 2,3,4,5 and 6, respectively. The average live body weight (g) and cumulative body weight gain (g) were significantly ($p<0.05$) higher in the NC+PPE and HS+PPE groups supplemented with PPE, compared

Table 1: Nutrient composition of pre-starter, starter and finisher broiler diets.

	Pre-starter	Starter	Finisher
ME (Kcal/kg)	3020	3098	3202
Moisture (%)	12.5	12.6	11.1
CP %	21.4	21.7	16.3
Total Ash	6.9	6	6.7
EE %	3.5	4.7	8
Soluble dietary fibre %	1.7	BLQ	1.6
Insoluble dietary fibre %	17.9	15.1	13.9

to their respective control groups, NC and HS, which did not receive PPE. Throughout the research trial, the NC+PPE group exhibited a significantly ($p<0.05$) higher LBW and BWG compared to the other three groups. No significant ($p>0.05$) difference was observed in the 5th and 6th weeks between the NC and HS+PPE groups, indicating that both groups was relatively performed similarly in terms of LBW and BWG. At the end of the experimental period, all four groups showed no significant ($p>0.05$) difference in average cumulative feed consumption (g). After one week of PPE administration, in the 3rd week, both the NC+PPE and HS+PPE groups showed a significantly ($p<0.05$) better average cumulative feed conversion ratio compared to their respective control groups. In the 4th and 5th weeks, the HS+ PPE group continued to show a better FCR compared to the HS group. In the 6th week the average cumulative mortality was lowest in normal control group (Table 4) supplemented with PPE and second lowest seen in the heat stress group supplemented with PPE.

Lemongrass essential oil may improve broiler growth under heat stress through its antioxidant, digestive and antimicrobial properties (Alagawany *et al*., 2021). Thymol enhances trypsin and pancreatic enzyme activity (Lee *et al*., 2003). Supplementation with garlic, lemon and their combination (200 mg/kg) significantly increased body weight gain, improved FCR and mortality, without affecting feed intake (Elbaz *et al*., 2022). Similarly, rosemary extract at 50 mg/kg improved ABW and BWG without affecting feed intake, likely due to carnosic acid, a potent antioxidant derived from ferruginol, scavenging ROS and reducing lipid peroxidation. Conversely, 100 mg/kg under heat stress significantly improved FCR, while 50 mg/kg showed no such effect (Madkour *et al*., 2024). Similarly, the supplementation of a mixture of 1.5 g/kg lemongrass leaf and wormwood powders significantly improved live weight gain in heat-stressed birds compared to the control group (Al-Gburi, 2025). In contrast, Rokade *et al.* (2017) reported improved FCR ($p<0.001$) in broilers under hot summer conditions with mannan oligosaccharide (3% and 5%) supplementation. The mitigation of heat-induced reductions in broiler performance with PPE supplementation may be due to lemongrass essential oils' antioxidant, digestive enzyme-stimulating and antimicrobial effects (Alagawany *et al*., 2021). The results are consistent with our findings, supplementation with 40% watermelon juice (WJ) resulted in 0% mortality (100% survival rate) compared to the control

Table 2: Average weekly live body weight (g) of experimental birds from different groups.

Week	Experimental groups			
	NC	NC + PPE	HS	HS + PPE
Day old	43.72±0.24	43.81±0.06	43.66±0.29	43.37±0.09
1	207.25±1.70	207.75±0.95	204.25±1.80	208.00±0.91
2	509.50±3.01	516.25±6.25	509.25±2.53	515.25±3.12
3	909.00^{b}±6.14	977.50^{a}±3.12	908.75^{b}±7.89	976.75^{a}±6.47
4	1445.50^{b}±8.91	1531.00^{a}±17.35	1448.25^{b}±6.02	1513.75^{a}±6.42
5	2002.75^{b}±4.09	2082.00^{a}±11.01	1990.25^{b}±33.34	2048.75^{ab}±18.49
6	2603.25^{bc}±17.45	2694.25^{a}±12.66	2549.25^{c}±20.99	2649.75^{ab}±32.84

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

Week	Experimental groups			
	NC	NC + PPE	HS	HS + PPE
1	163.53±1.94	163.94±1.01	160.60±2.04	164.63±0.93
2	465.78±3.05	472.44±6.24	465.60±2.50	471.88±3.08
3	865.28^{b}±6.37	933.69^{a}±3.08	865.10^{b}±7.95	933.38^{a}±6.47
4	1401.78^{b}±8.85	1487.19^{a}±17.40	1404.60^{b}±5.85	1470.38^{a}±6.45
5	1959.03^{b}±4.16	2038.19^{a}±11.03	1946.60^{b}±33.23	2005.38^{ab}±18.43
6	2559.53^{bc}±17.65	2650.44^{a}±12.66	2505.60^{c}±20.85	2606.38^{ab}±32.88

Table 4: Average cumulative weekly feed consumption (g/bird) of experimental birds from different groups.

Week	Experimental groups			
	NC	NC + PPE	HS	HS + PPE
1	173.32±1.52	173.09±2.05	170.21±3.58	172.49±3.01
2	552.37±2.79	557.88±3.67	563.38±2.37	566.42±6.80
3	1126.84^{b}±8.25	1188.40^{a}±5.64	1122.56^{b}±3.93	1180.08^{a}±2.03
4	1948.90^{b}±10.30	2052.63^{a}±17.43	1979.83^{b}±6.51	2030.68^{a}±6.95
5	3123.37±13.74	3212.59±19.32	3206.45±88.37	3160.43±17.50
6	4404.74±6.65	4429.94±18.79	4387.01±119.55	4377.77±42.11

group (Jimoh *et al*., 2018). Similarly, provision of Amla juice, Aloe vera gel and rough lemon juice (2% each) through drinking water reduced mortality in broilers during summer (Gowri *et al*., 2022).

Hematological parameters

PPE significantly ($p<0.05$) increased red blood cell counts under heat stress and improved ($P<0.05$) the heterophil-to-lymphocyte ratio (H:L) in the NC+PPE group compared to the NC group. While all other hematological parameters were showed no significant ($p>0.05$) difference (Table 7). The higher H:L ratio in the NC group may be due to differences in blood collection timing, as noted in the materials and methods. Similarly, Rabadan (2022) reported no significant ($p>0.05$) changes in total leukocyte counts, hemoglobin, packed cell volume, or mean corpuscular volume in broilers given various concentrations of lemongrass hydrosol extract. Likewise, El-Sahn *et al*., (2024) observed that 200-300 mL/L lemongrass extract significantly ($P<0.05$) improved RBC count. On contrary, they also reported significantly ($P<0.05$) improved haemoglobin, lymphocytes, PCV, MCV and MCH in broilers.

Stress biomarkers

HSP70 (ng/mL) and COR (ng/mL) levels in feathers and serum are presented in Table 8. No significant ($P>0.05$) difference was observed in blood serum HSP70 (ng/mL) and COR (ng/mL) levels in different experimental groups, on both 13th and 41st days. On day 41 (morning and afternoon), significantly lower ($P<0.05$) feather HSP70 levels (ng/mL) were observed in the NC+PPE and HS+PPE groups compared to their respective controls. Additionally, the HS+PPE group recorded significantly ($P<0.05$) lower levels than the other three treatment groups.

Serum HSP70 and COR

Heat stress was applied from 12:00 PM to 4:00 PM, allowing birds overnight recovery, which may have helped maintain

Table 5: Average cumulative weekly feed conversion ratio (FCR) of experimental birds from different groups.

Week	Experimental groups			
	NC	NC + PPE	HS	HS + PPE
1	1.060±0.01	1.056±0.01	1.060±0.02	1.048±0.02
2	1.186±0.00	1.181±0.01	1.210±0.01	1.200±0.01
3	1.302^{a}±0.00	1.273^{bc}±0.01	1.298^{ab}±0.01	1.264^{c}±0.01
4	1.390^{b}±0.00	1.380^{b}±0.01	1.410^{a}±0.01	1.381^{b}±0.00
5	1.594^{b}±0.01	1.576^{b}±0.01	1.647^{a}±0.02	1.576^{b}±0.01
6	1.721±0.01	1.671±0.01	1.751±0.04	1.680±0.13

Table 6: Average cumulative weekly mortality (%) of birds from different experimental groups.

Week	Experimental groups			
	NC	NC + PPE	HS	HS + PPE
1	0.00	0.23 (1)	0.68 (3)	0.45 (2)
2	0.68 (3)	0.91 (4)	0.91 (4)	0.91 (4)
3	0.68 (3)	1.14 (5)	1.36 (6)	0.91 (4)
4	0.91 (4)	1.14 (5)	1.59 (7)	0.91 (4)
5	1.59 (7)	1.14 (5)	2.95 (13)	0.91 (4)
6	2.73 (12)	1.36 (6)	5.23 (23)	1.59 (7)

Table 7: Average haematological parameters of birds from different experimental groups.

Parameters	Experimental groups			
	NC	NC + PPE	HS	HS + PPE
Hb (g)	9.85±0.09	10.10±0.18	9.91±0.07	9.98±0.17
RBC (million)	2.38^{ab}±0.01	2.42^{a}±0.04	2.29^{b}±0.03	2.43^{a}±0.02
PCV (%)	27.25^{b}±0.17	27.40^{ab}±0.23	27.42^{ab}±0.07	28.12^{a}±0.29
TLC (10^3/cumm)	7.59±0.24	6.87±0.31	7.27±0.36	6.62±0.48
Eosinophils (10^3/µl)	0.13±0.01	0.12±0.01	0.13±0.01	0.11±0.02
Monocytes (10^3/µl)	0.14±0.01	0.13±0.01	0.14±0.01	0.13±0.02
Heterophils (10^3/µl)	1.89^{a}±0.08	1.43^{b}±0.10	1.39^{b}±0.04	1.25^{b}±0.08
Lymphocytes (10^3/µl)	5.45±0.16	5.20±0.21	5.62±0.31	5.13±0.36
Blood serum H:L	0.35^{a}±0.01	0.28^{b}±0.01	0.25^{b}±0.01	0.25^{b}±0.00
MCV (fl)	113.77^{b}±0.55	113.25^{b}±1.97	120.20^{a}±1.87	116.01^{ab}±0.80
MCH (pg)	41.43±0.46	41.78±1.07	43.48±0.84	41.14±0.55

Table 8: Average HSP70 and COR testing (ng/ml) of birds of various experimental groups.

Parameters	Average HSP70 testing (ng/ml)				Average COR testing (ng/ml)			
	NC	NC+PPE	HS	HS+PPE	NC	NC+PPE	HS	HS+PPE
ELISA feather, 13th day, morning	25.86^{a}±0.34	23.52^{b}±0.42	25.18^{ab}±0.67	21.52^{c}±0.37	186.79^{a}±4.21	125.18^{b} ± 6.41	179.42^{a}±11.51	119.08^{b}±5.97
ELISA feather, 13th day afternoon	27.38^{a}±0.47	22.57^{b}±0.29	28.11^{a}±0.54	21.92^{b}±0.33	146.31^{a}±12.10	106.44^{b}±5.93	122.68^{ab}±5.21	90.79^{b}±5.22
ELISA feather, 41st day, morning	25.25^{a}±0.39	22.51^{b}±0.45	24.30^{ab}±0.47	19.60^{c}±0.46	116.84±7.63	108.87±7.04	111.26±2.44	115.53±3.53
ELISA feather, 41st day, afternoon	25.60^{a}±0.48	20.91^{b}±0.36	21.99^{b}±0.64	17.50^{c}±0.36	111.59±7.74	86.86±5.11	105.99±3.24	86.25±7.67
ELISA serum, 13th day, morning	121.08±9.86	124.65±12.78	134.34±7.12	131.54±16.34	82.92±2.47	70.74±2.94	46.50±12.10	46.93±2.74
ELISA serum, 41st day, morning	50.48±1.20	54.80±2.98	53.92±8.18	48.87±1.53	21.91±1.77	6.46±0.68	6.31±1.78	4.22±0.55

physiological stability and contributed to the non-significant differences in serum HSP70 and COR levels. Sarma *et al*., (2022) reported higher HSP70 in 13-day-old broilers compared to 41-day-olds, suggesting age-related regulation, while developed feathers in older birds may stabilize HSP70 during winter. Their study also reported no significant ($p>0.05$) difference in COR levels across agroclimatic zones in Assam during winter, corroborated with our findings. In contrast, Jimoh *et al*. (2024) observed a significant ($p<0.05$) reduction in HSP70 levels when garlic essential oil (30% v/v) was administered via drinking water for 49 days. In divergence, supplementation with antioxidants such as vitamin E and selenium significantly ($p<0.001$) lowered serum HSP70 levels (ng/mL) in broilers during heat stress (Bora *et al*., 2024).

Feather HSP70 and COR

In this study, ELISA was used to quantify HSP70 and cortisol (COR) levels (ng/mL). Heat stress increased HSP70 expression. On day 41, both HSP70 and COR levels were lower in the afternoon than in the morning. This may be due to sampling conditions as explained in materials and methods: morning samples were collected outdoors at ~37°C, while afternoon samples were taken indoors at ~42.5°C. Both temperatures exceeded the thermoneutral range. On the contrary, Greene *et al*. (2019) reported significantly ($p<0.01$) higher HSP70 levels, both in mRNA expression and protein content, in growing feathers collected in the afternoon compared to the morning from the same birds. Feather HSP70 expression reflects systemic and cellular stress, serving as a reliable, non-invasive biomarker for longitudinal stress monitoring. Sampling is quick, low-stress and suitable for repeated measures (Greene *et al*., 2019).

CONCLUSION

Based on the findings of the present study, it is concluded that inclusion of phytogenic plant extracts under both normal and heat-stressed conditions improved growth performance and reduced mortality in broilers. The NC+PPE and HS+ PPE groups showed greater reductions in stress biomarkers, such as feather HSP70, compared to their respective control groups. PPE supplementation shows potential in mitigating the adverse effects of heat stress in current fast growing commercial broilers raised under high-temperature conditions.

Conflict of interest

All authors declare that they have no conflict of interest.

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