RESEARCH ARTICLE

Supplementation of Polyherbal Antistressor Premix as Heat Ameliorating and Immunomodulatory Agent in Broilers during Summer Stress

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Abstract

The present study was conducted in broiler chickens to evaluate the effect of supplementation of polyherbal antistressor products on heat ameliorating activity and immunomodulatory properties. In the current study a total 90, day old broiler chicks were selected and randomly assigned into three groups on the basis of body weight with two replicates consisting of 15 chicks in each replicate and 30 chicks in each group. Group $T_0$was control group whereas T_1 and T_2 were treatment groups. Group T_1 was fed Ayucee premix supplement @ 100 g/tonne for 0-42 days, Group $T_2$was fed Stresomix Premix @ 250g/tonne for 0-42 days. The growth performance was recorded on day 7, 14, 21, 28, 35 and 42 of age. Blood samples were collected on 5th week from 6 representative birds of each replicate. The haemoglobin, PCV, H/L count, TLC, cortisol, immune organ weight, blood glucose, BUN, serum cholesterol, ALP, AST, and LDH were estimated. The individual vital organ weight of liver, spleen, kidney, thymus and bursa were noted. During fifth and sixth week of age, the T_2 group showed significantly ($p<0.05$) higher body weight and higher level of Hb and PCV than other groups. The weights of spleen, bursa and thymus, antibody titer against ND, activities of SOD, CAT, and GPx were significantly higher in T_1 and T_2 supplemented groups compared to control group. The results suggested that the polyherbal antistressors supplementation in diet was helpful to ameliorate heat stress in broilers.

Key words: Antistressors, Broiler chicken, Growth, Heat stress, Polyherbal formulation.

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Introduction

Heat stress is a significant concern in broiler chicken production, particularly during hot weather conditions. During hot weather, broilers may experience heat stress, which can impair their ability to regulate their body temperature resulting in reduced feed intake, decreased growth rates and increased mortality. Nutritional management such as dietary manipulations, nighttime feeding and wet feeding often applied with timely and effective correction of environmental conditions have been proven to ameliorate the effect of heat stress in chicks and adult birds (Onagbesan *et al.,* 2023). Phytogenic substances act as anti-oxidants are being used for optimizing gut health in poultry (Shehata *et al*., 2022). Nano-Se and nanocurcumin were found to be more effective in ameliorating oxidative stress with maintenance of cellular architecture that imparts physiological well-being of broiler chickens (Shwetha *et al*., 2022). Stresomix was found to be efficacious in the amelioration of summer stress associated losses in dairy cows (Prasad *et al*., 2022).This study was undertaken with the objective to evaluate the effect of polyherbal antistressor products supplementation on growth performance, feed conversion ratio, haematological and biochemical parameters, behavior parameters, histological and meat characteristics in broilers under heat stress.

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Materials and Methods

The trial was conducted in the month of March and April with the approval of Institutional Animal Ethical Committee of the Veterinary College, Hassan, Karnataka, India (No. HVC/IAEC/12/2022, Dated 29.10.2022). The experimental birds were reared at the farmer's poultry house and samples analyzed at Veterinary College, Hassan. The experimental birds were randomly allotted into three treatment groups (T_0, T_1 and T_2) with each group having 30 birds and two replicates each of 15 birds in each group.

Treatment Groups

Group T_0 was considered as control group with environment stress and without any antistress supplement, whereas treatment groups were reared under environmental summer stress and fed with basal diet along with supplements, *viz*. Group T_1 with Ayucee premix supplement @ 100 g/tonne for 0-42 days, Group T_2 with Stresomix Premix @ 250 g/tonne for 0-42 days. The T_1 supplement was Ayucee premix (polyherbal formulation) containing natural Vitamin C and bioflavonoids, which are known for their anti-oxidant and free radical scavenging activities. T_2 was Stresomix (Ayurvet, India) which is a scientifically-formulated polyherbal antistressor and immunomodulator containing several herbal ingredients *viz*. *Withania somnifera, Ocimum sanctum, Mangifera indica,* and *Phyllanthus emblica* that are well-known in traditional Indian materia medica for their antistressor properties (Prasad *et al.,* 2022). The temperature was maintained and recorded on daily basis where mean maximum daily temperature of 38.26 °C and minimum temperature of 24 °C was recorded throughout the experiment. The combination of daily temperature and relative humidity 51 % were above the threshold established for poultry which indicates the birds were subjected to heat stress.

Growth Performance Parameters

For each group of birds (T_0, T_1 and T_2), live body weight/growth rate and cumulative weight gain were recorded. The observation was taken at the time of grouping on day 0 and further recordings were done on day 7, 14, 21, 28, 35 and 42 of age. Average body weight gains at weekly intervals were calculated. Daily feed intake and leftover (average voluntary feed intake) was recorded and feed conversion efficiency/ratio were calculated.

Haemato-Biochemical Parameters

Blood samples were collected on 5th week from 6 representative birds of each replicate. The haemoglobin (Hb), packed cell volume (PCV), heterophil/lymphocyte ratio (H/L) Count and total leukocyte count (TLC) were estimated by manual methods. The serum/plasma were separated from the collected blood samples for the analysis of biochemical parameters, *viz*. blood glucose, blood urea nitrogen (BUN), serum cholesterol, alkaline phosphatase (ALP), aspartate amino transferase (AST) and lactic dehydrogenase (LDH) by using assay kits and biochemistry analyzer (Model No:Rose-0194740A supplied by Rapid Diagnostic PVT.LTD).

Anti-Oxidative and Immunological Parameters

Serum cortisol, Glutathione Peroxidase (GPx) and Superoxide dismutase (SOD) were estimated in the trial on representative 10 birds per group during 5th week using ELISA kit.Post-vaccine immune response for Newcastle disease virus (NDV) vaccine was observed.

Pathological and Carcass Studies

Representative six birds were sacrificed from each replicate at the end of experimental study and examined for microscopic and macroscopic pathological lesions including fatty liver syndrome. The individual vital organ weight of liver, spleen, kidney, thymus and bursa were noted. The carcass traits (carcass yield, dressing %, livability, giblet weight, abdominal fat percentage, tender & fillet weight) were recorded to the nearest 0.001 g using a monopan balance.

Microbiological Parameters

Microbiological parameters were assessed in terms of the *E. coli* count plus Salmonella counts. The fecal samples/intestinal contents from caeco-colon junction were taken aseptically to study the *bacterial* counts. Samples were used for enumeration of bacteria as per spread plate method. Specific media such as MacCankey agar was used for *E. coli* and salmonella counts. To assess bacterial count, tenfold serial dilution of intestinal contents was made. The bacterial counts were expressed as lakhs/gm of the sample from intestinal content.

Statistical Analysis

The experiment was in complete randomized design (CRD). The data obtained was subjected to statistical analysis and summarized in tabular form for each individual group obtained by General linear model with Duncan post hoc test using SPSS 16.0 software.

Results and Discussion

Growth Production Parameters

The body weight, weekly weight gain, internal organs weight, meat quality and carcass traits were studied.

Body weight: The weekly body weight gain is represented in Table 1. At second and third week, Group T_1 showed significantly ($p<0.05$) higher weight than control group. At fourth week of age, T_1, and T_2 groups showed significantly ($p<0.05$) higher body weight than T_0 group. During fifth and sixth week of age, T_2 group showed significantly ($p<0.05$) higher weight than other groups. Non-significant ($p>0.05$) changes were observed during first week of the study on weekly body weight gain between different treatment groups and control group (Table 2). At sixth week, T_1 supplemented

group showed significantly higher (p<0.05) body weight gain than other groups. The results of body weight gain after sixth week corroborated with those of Sapcota *et al.* (2006) and Maini *et al.* (2007), who reported increase in body weight gain when *Phyllanthus emblica* was fed to the broilers at the end of 6th week. Similar results were reported by Sharma *et al.* (2008) who investigated the efficacy of herbal liver tonic in broiler chickens. Diet supplementation with 300 mg/kg complex antioxidants containing tertiary butylhydroquinone (TBHQ) and tea polyphenols (TP) could improve growth performance and meat quality at 42 day and the average daily gain (ADG) from 22 to 42 day by increasing the antioxidant capacity of broilers (Chen *et al.*, 2024). Bortoluzzi *et al.* (2021) found that the addition of 150 mg/kg of P (BF+AOX) improves growth performance of broiler chickens undergoing early life stress. Sahin *et al.* (2003) and Njoku (1986), in their studies found increased body weight gain in ascorbic acid supplemented group than the control broilers under heat stress.

Weekly Feed Consumption and Feed Conversion Ratio (FCR): In the study, the weekly feed consumption during 1st week did not vary among groups (Table 3). At second and sixth week, Group T_1 showed significantly (p<0.05) higher feed intake than other groups. In the study, FCR varied non-significantly between different treatment groups at 1st week (Table 4). At 3rd week FCR was better in T_1 and T_2 group compared to other groups. At 6th week, the T_2 group showed significantly (p<0.05) lower FCR than other groups. Feed supplementation with dietary complex of bio-factors and antioxidants (BF+AOX) had no significant effect on final body weight (BW), body weight gain (BWG), average feed intake or feed conversion ratio (FCR) of broiler chickens. However, differences were found in feed conversion ratio and thawed weight loss (Azzam *et al.,* 2023).

Table. 4: Weekly feed conversion ratio (FCR) of broilers in different groups

Groups	Age (weeks)					
	1st	2nd	3rd	4th	5th	6th
T_0	1.16	1.46 [a]	1.53	1.68[a]	1.84 [a]	2.21[a]
T_1	1.15	1.39	1.48[a]	1.57	1.78	2.04[b]
T_2	1.12	1.33	1.45[a]	1.51	1.75[b]	1.98[c]

Means with common superscripts did not differ significantly (p> 0.05)

Haemato-Biochemical Parameters

In the study, at 5th week, Hb and PCV level were significantly higher in T_2 group as compared to other groups (Table 5). The TLC was significantly higher in T_1 supplemented group as compared to control group. The H:L ratio was significantly (p<0.05) lower in T_1 supplemented group as compared to other groups. In the study, at 5th week, BUN, AST, LDH, Alkaline Phosphatase and serum cholesterol levels were

Table. 1: Body weight (gm) per bird of broilers at weekly interval in different groups

Groups	Age (weeks)						
	0	1st	2nd	3rd	4th	5th	6th
T_0	46.00±0.05	133.00±2.04	258.00±2.21[a]	610.00±3.31[a]	971.00±5.11[a]	1410.00±8.23[a]	1594.00±6.25[a]
T_1	45.00±0.03	140.00±2.11	369.00±2.35[b]	746.00±3.54[b]	1120.00±5.67[b]	2060.00±10.21[b]	2290.00±4.31[b]
T_2	46.00±0.50	136.00±2.06	335.00±2.46[a]	723.00±3.87 [b]	1140.00±5.51[b]	2198.00±10.04[c]	2410.00±8.22[c]

Means with different superscripts in a column differ significantly (p<0.05).

Table. 2: Weekly body weight gain (gm) per bird of broilers at weekly interval in different groups

Groups	Age (weeks)					
	1st	2nd	3rd	4th	5th	6th
T_0	87.00±0.01	125.00±1.11[a]	352.00±2.45	361.00±1.43	439.00±2.34[a]	184.00±3.14[a]
T_1	95.00±0.01	229.00±1.16[b]	377.00±2.92	374.00±1.11	940.00±2.11[b]	230.00±3.33[b]
T_2	90.00±0.02	199.00±.1.4	414.00±1.54[a]	391.00±1.06	1060.00±2.45[c]	212.00±3.58

Means with common superscripts did not differ significantly (p>0.05)

Table. 3: Weekly feed consumption (gm) per bird of broilers at weekly interval in different groups

Groups	Age (weeks)					
	1st	2nd	3rd	4th	5th	6th
T_0	100.92±6.34	182.50±6.68[a]	538.56±8.54	606.48±6.43	807.76±10.04[a]	406.64±10.33[a]
T_1	109.25±8.21	318.31±5.21[b]	557.96±6.32	587.18±5.49	1,6732±8.56[b]	469.20±14.21[b]
T_2	100.8±5.43	264.67±4.96	600.30±5.31[a]	590.41±5.02	1,855±10.55[c]	419.76±8.48

Means with common superscripts did not differ significantly (p>0.05)

significantly ($p<0.05$) lower in T_1, T_2 supplemented groups as compared to T_0 group. The total protein, albumin and globulin levels were significantly ($p<0.05$) higher in T_1, and T_2 supplemented groups as compared to T_0 group (Table 5). According to Bianchi and Antunes (1999), the combined use of antioxidants can reduce the effects of stress due to its oxido-reduction properties, which may have an important role in free radical quenching and neutralization. Ascorbic acid (AA) supplementation showed positive effects on the reduction of corticosterone plasma levels and on the heterophil/ lymphocyte (H:L) ratio, as evaluated by Mckee and Harrison (1995) in birds submitted to heat stress (Pena *et al.,* 2008). The state of heat stress during the study period was evident from the continuing increase in serum cortisol levels of the non-supplemented control group (Prasad *et al.,* 2022). The ability of the constituent herbal ingredients of the polyherbal antistressor premix to diminish stress induced increase in serum cortisol as evident from our results, may be one of the important mechanisms by which losses in yield were restored (Prasad *et al.,* 2022; Sivajothi *et al.,* 2018).

Immune and Antioxidant Profile

The weights of spleen, bursa and thymus were significantly higher ($p<0.05$) in T_1, and T_2 supplemented groups as compared to control group at fifth week of age. The antibody titre against ND vaccine was significantly ($p<0.05$) higher in T_1, T_2 supplemented groups (Table 6). The TBARS level was significantly ($p<0.05$) lower in T_1 and T_2 supplemented groups as compared to T_0 at fifth week. The activities of SOD, CAT, GPx were significantly ($p<0.05$) higher in T_1, and T_2 groups (Table 6). Chen *et al.* (2024) found that supplementing with 300 mg/kg Complex Antioxidants (CA) significantly increased

Table. 5: Effect of antistressor products on haemato-biochemical parameters in broilers

Parameters	Groups		
	T_0	T_1	T_2
Hb (g%)	7.50±3.21[a]	9.80±2.67[b]	10.04±1.34[c]
PCV (%)	25.40±4.55[a]	33.24±4.78	35.80±5.21[b]
H:L Count	0.75[a]	0.52[b]	0.59[c]
TLC (millions/mL)	1.91±10.04a	2.95±15.67b	2.55±13.21[c]
Serum glucose (mg/dL)	203.00±3.89	196.70±8.21	191.00±5.76
BUN (mg/dL)	5.40 ± 0.07[a]	5.98 ± 0.15[b]	6.01 ± 0.14[b]
Serum cholesterol (mg/ dL)	128 ± 0.07	121 ± 0.60	124 ± 0.90
Alkaline phosphatase (µ/L)	629.72 ± 1.43[a]	560.78 ± 1.07[b]	548.00 ± 1.04[b]
AST (µ/L)	178.19 ± 4.59[a]	133.32 ± 3.61[b]	126.23 ± 5.98[b]
LDH (IU/L)	1444 ± 4.29[a]	1369 ± 4.20[b]	1357 ± 4.11[b]
Serum cortisol (nmol/L)	5.15 ± 0.14[a]	3.42 ± 0.20[b]	3.39 ± 0.25[b]
Total protein (g/ dL)	2.60 ± 0.06[a]	2.94 ± 0.01[b]	3.03 ± 0.87[b]
Albumin (g/ dL)	1.15 ± 0.02[a]	1.35 ± 0.04[b]	1.38 ± 0.93[b]
Globulin (g/ dL)	1.45 ± 0.04[a]	1.59 ± 0.03[b]	1.65 ± 0.07[b]

Means with common superscripts did not differ significantly ($P < 0.05$)

Table. 6: Effect of antistressor products on immune organs and serum anti-oxidant profile in broilers

Parameters	Groups		
	T_0	T_1	T_2
Spleen weight (g)	2.0b	5.0a	4.0a
Bursa weight (g)	15.0b	18.0a	18.0 a
Thymus weight (g)	2.7b	4.5a	4.1a
ND titre (Log 10 Titer)	2.87c	3.61a	3.17b
TBARS (nmoles MDA/mg protein)	4.26 ± 0.15[a]	2.51 ±0.14[b]	2.45 ±0.11[b]
SOD (U/mg protein)	25.45 ±1.10[a]	36.42 ±1.14[b]	37.21 ±1.20[b]
Catalase (U/mg protein)	73.62 ±2.20[a]	98.71 ±2.11[b]	101.21 ±2.07[b]
GPx (Units/mg protein)	24.80 ±0.50[a]	30.40 ±0.14[b]	32.70 ±0.29[b]

Means with common superscripts did not differ significantly ($p>0.05$)

($p<0.05$) serum and liver T-SOD activity as well as jejunual GST activity whereas liver MDA content significantly decreased ($p<0.05$) when compared with the control group. The results of immune organ weight gain after sixth week corroborated with those of Chen *et al.* (2024) and Vijaykumar *et al.* (2020). According to Vijaykumar *et al.* (2020), the meat quality and carcass traits of the birds were not varied with either Ayucee® and AV/LAP/19 or vitamin C supplemented birds.

Carcass Traits

The dressing percentage, fillet weight and liver weights were significantly ($p<0.05$) higher in T_1 and T_2 supplemented groups as compared to T_0 group at fifth week (Table 7). In the current study, pathological lesions of internal organs were not observed in any group. The addition of graded AA and citric flavonoids (quercitin and rutin) levels to the broiler diets did not affect commercial cut yield, carcass yield or meat quality trait parameters (Pena *et al.*, 2008). Superliv had no significant influence on the physicochemical properties of meat, except pH, which increased slightly in response to the herbal formula (Lipinski *et al.*, 2019).

Table. 7: Effect of antistressor products on Carcass characters in broilers

Parameters	Groups		
	T0	T_1	T_2
Dressing (%)	67.30 ± 3.10^a	74.20 ± 2.11^b	74.33 ± 1.45^b
Giblet weight (g)	140.00 ± 1.23^a	162.00 ± 0.91^b	170.00 ± 1.03^b
Abdominal fat (g)	26	26	26
Fillet weight (g)	83^a	184^b	192^b
Liver (g)	36^a	58^b	60^b

Means with common superscripts did not differ significantly ($p> 0.05$)

Behavioral and Microbiological Parameters

Nevertheless, as shown in Table 8, at 5th week, the number of birds feeding per minute was significantly ($p<0.05$) higher in T_2 followed by $T_1$and T_0. Crouching behavior was significantly ($p<0.05$) lower in T_2 supplemented group compared to the other groups. The microbial load in the fecal content showed significant ($p<0.05$) differences at fifth week of the experiment. The T_1, and T_2 groups showed significantly lower microbial counts as compared to T_0 (Table 8).

Conclusion

The present study evaluated the efficacy of herbal feed supplements on growth performance, serum biochemical parameters and meat quality, including antioxidant capacity of serum in broilers. It was found that broilers fed with a diet supplemented with polyherbal antistressors presented a better growth performance, feed conversion ratio, meat quality and antioxidant capacity than birds fed with control diet during summer stress.

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Table. 8: Effect of antistressor products on behavioral characters and microbial count in broilers

Parameters	Groups		
	T_0	T_1	T_2
Inappetence (Birds/min)	2.11 ± 0.80^a	4.02 ± 0.16^b	4.52 ± 0.21^c
Crouching (0 to 5 scoring)	5.0^b	4.0^b	2.0^a
Total Enterobacteriaceae (lakhs/gm)	32.00 ± 4.67^a	28.56 ± 5.13^b	28.71 ± 8.26^b

Means with common superscripts did not differ significantly ($p> 0.05$)

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