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ORIGINAL ARTICLE **OPEN ACCESS**

Antioxidant and Immunomodulatory Activity of Polyherbal Antistress formulations in Commercial Broilers under Summer Stress

Puthuru Naresh[1], M.Usha Rani[2],Vijay Kumar.M[3],Sunil Chandra.U[4] and A.Gopala Reddy[6]
[1]Post Graduate Scholar,Department of Veterinary Pharmacology and Toxicology
College of Veterinary Science,PVNRTVU,Rajendranagar,Hyderabad (TS) India-500030
Email:Nareshp728@gmail.com M:+918977512242
2Associate Professor, Department of Veterinary Pharmacology and Toxicology College of Veterinary Science,PVNRTVU,Koratla (TS),India-505326
Email: matukumalliusha@gmail.com M:+919848747678
[3]Assistant Professor, Department of Veterinary Pharmacology and Toxicology Veterinary College,KVAFSU,Bidar (KS)-585401
Email:vijaymatham@gmail.com M:+919886154400
*corresponding author
4Assistant Professor, Department of Veterinary Pharmacology and Toxicology Veterinary College,KVAFSU,Bidar (KS)-585401
Email:sunilvet@gmail.com M:+919448501496
[6]Professor and Head, Department of Veterinary Pharmacology and Toxicology
College of Veterinary Science,PVNRTVU,Rajendranagar,Hyderabad (TS) India- 500030
Gopalareddy123@rediffmail.com M:+919440323340

ABSTRACT
Heat Stress not only causes suffering and death in the birds, but also results in reduced or lost production that adversely affects the profit from the enterprise. A comparative study was conducted to evaluate comparative effects of supplementation of poly herbal formulation and synthetic vitamin C (supplied by M/S Ayurvet Ltd., Baddi, H.P., India) on the performance of environmental heat stressed broilers birds.160 day-old unsexed broiler chicks of Vencobb strain exposed to environmental heat were randomly divided into four groups each having four replicates of ten birds. Group-T0: Untreated control, Group- T1: stress roak @ 1kg/ton of feed, Group- T2: supplemented with Ayucee premix @100gms/tone of feed and group-T3 synthetic Vit. C @ 100g/ton of feed The birds were fed standard ration throughout the experiment. The sero biochemical data, Anti oxidant defense profiles,pre and post vaccination phytohaemagglutinine (PHA) revealed the stress control group T1 had a significantly ($p<0.05$) reduced activity of SOD,Catalas and GPx. The concentration of TBARS and the activity of ALT, AST, ALP were significantly ($p<0.05$) increased.Antibody titer was significantly higher in the treated groups as compared to untreated control. From the results it can be concluded that broiler birds under heat stress exhibited low anti oxidant defenses and sero bio chemistry as compared to treated groups. In view of these results, it is prudent to use herbal anti oxidants in test in poultry feeds to boost the anti oxidant defenses, immune status and over all performance of broilers under environmental heat stress.
Keywords: *Polyherbal antistressor formulation , heat stress, immunosuppression, Broilers*

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INTRODUCTION

Heat stress is of major concerns for poultry production. Biochemical and physiological changes associated with heat stress can potentially promote reactive oxygen species (ROS) formation and impaired muscle membrane integrity in breast muscle of heat- stressed broiler chickens [1] was also considered to be related with the changed redox balance because broiler chickens that were exposed to acute heat stress exhibited more than a 2-fold increase of MDA as an indicator for lipid peroxidation, in the skeletal muscle [2-3]. High ambient temperature negatively influences the performance of broilers. An ambient

temperature above 30°C is considered to have an adverse effect on the performance of broiler chicks. Earlier findings have suggested that reduced feed intake, body weight, and feed conversion efficiency is caused by high environmental temperatures [4-5]. During the periods of heat stress, most of the production energy is diverted to thermoregulatory adaptations which results in oxidative stress induced immunosupression, predisposing birds to various infectious diseases and high mortality rates [6-7]. Several methods are available to alleviate the effect of high environmental temperature on the performance of poultry. Dietary modifications are among the most preferred and practical ways to alleviate the effect of high environmental temperature in poultry [8]. Many polyherbal products containing different immunomodulator, antistressor and adaptogenic herbs have been used to protect tissues from superoxide radicals and enhance cell survival by stimulating antioxidative enzymatic systems [9-10]. Supplementing the diet with vitamins and minerals can alleviate some of these adverse effects on growth performance, attributed to high ambient temperatures [3, 8]. Several studies indicated that heat stress reduces the bodyweight [11], immune response and also causes mortality [12] and different therapeutic measures are used to minimize the harmful effects of heat stress on performance of broiler chick such as ascorbic acid [12], vitamin E [3], acetylsalicylic acid [13], potassium chloride [14], sodium bicarbonate, acetic acid and organic and inorganic chromium [16]. Poultry have the ability to synthesize ascorbic acid, but this ability is inadequate under stress conditions, such as high environmental temperatures, high humidity, a high productive rate, and parasitic infestation. Particular environmental stressors can alter the use or synthesis of ascorbic acid in poultry [17]. Therefore present study was conducted to evaluate comparative effects of supplementation of poly herbal formulations and synthetic Vitamin C.supplements diet and water on the performance of heat- exposed broiler.

MATERIALS AND METHODS

The present study was conducted at a poultry house at Kamthana, Bidar (KS), India under the Department of Veterinary Pharmacology and Toxicology, Veterinary College, KVAFSU, Bidar, Karnataka, India during hot-dry season (April-June, 2016). India. The climate of this experimental site was hot and dry humid. The relative humidity and temperature of the experimental location was 82% and 38^0C.

Experimental Design

160 day-old unsexed broiler chicks of strain Vencobb were purchased from a local hatchery for this study and were randomly allotted to four treatment groups with each treatment having three replicates and each replicate having ten birds. Group- T0: Untreated control, Group- T1: stress roak (*Withania somnifera, Ocimum sanctum, phyllanthus eblica* and shilajit) @ 1kg/ton of feed, Group- T2: supplemented with Ayucee premix(*Withania somnifera, Phyllantus emblica and Terminalia chebula*) @100gms/ton of feed and group-T3 synthetic Vit. C @ 100g/ton of feed and water given to birds. shown in (Table 1 & 2). The birds were floor-brooded on wood-shaving in the experimental pens where they were allowed a week for adjustment. Additional source of heat was provided during the brooding period. Water at ambient temperature was supplied ad libitum throughout the period of the experiment in plastic drinkers. Birds of all the groups were vaccinated with Marek's Disease (day 1) New castle disease (ND) vaccine on 7th and 21st and 31st day and infectious bursal disease (IBD) vaccine on 14th day.

Parameters studied

Serum samples were separated from the blood. These samples were used for the estimation of Aspartate aminotransferase (AST), Alanine amino transferase (ALT), Alkaline Phosphatase (ALP), 4th and 6th weeks using standard diagnostic kits of Qualigens Pvt. Ltd. Hepatic tissue was collect and estimation of TBARS, GPx, SOD and Catalase Cell mediated (PHA assay) and humoral immune response (SRBC and NDV) was estimated as per standard procedure.

Statistical design and analyses

All the results were analyzed statistically by analysis of variance to determine the means and standard error [18].

RESULTS AND DISCUSSION

The present study was conducted to evaluate the antistressor activity of herbal products stress roak, Ayucee and vitamin C in broilers under heat stress. Record of temperature was maintained on daily basis where mean maximum daily temperature of 38°C and minimum temperature of 28.6 °C was recorded throughout the experiment. The combination of daily temperature of and relative humidity 82% were above the threshold established for poultry [19-20] indicates that the birds were subjecte to heat stress

Haemato-Biochemical Parameters

The results of haemato- biochemical estimations are tabulated in Table 2. An increased level of liver marker enzymes is indication of extent of liver damage due to impact of heat. The levels of ALT (IU/L), AST (IU/L), and ALP (IU/L) were significantly high ($p \leq 0.05$) in untreated control group T0 at 4th and 6th

week of trial as compared to treated groups stressroak T1,AyuceeT2 and vit-C T3 .The activity of ALT (IU/L) in serum revealed a significant ($p<0.05$) rise in the end of 4th and 6th wk in stress control group 1 (13.57±0.790 and 11.13±0.329, respectively) as compared to groups 2, 3 and 4 (9.12±0.781, 11.13±0.822 and 10.37±0.472, and 8.95±0.429, 10.43±0.872 and 9.85±0.472, respectively at the end of 4th and 6th wk). The activity of ALT (IU/L) in serum revealed a significant ($p<0.05$) rise in the end of 4th and 6th wk in stress control group 1 (13.57±0.790 and 11.13±0.329, respectively) as compared to groups 2, 3 and 4 (9.12±0.781, 11.13±0.822 and 10.37±0.472, and 8.95±0.429, 10.43±0.872 and 9.85±0.472, respectively at the end of 4th and 6th wk). The activity of AST (IU/L) in serum revealed a significant ($p<0.05$) raise in the end of 4th and 6th wk in group 1 (201.50±2.36 and 249.00±3.48, respectively) as compared to groups 2, 3 and 4 (176.00±3.55, 191.00±2.86 and 181.00±2.44, and 209.00±2.33, 230.00±2.98 and 206.80±4.20, respectively at the end of 4th and 6th wk) decreased levels of serum enzymes after antistressor supplementation was also reported earlier [29-30].

Immune profile

Antibody titre was significantly higher in the treated groups as compared to untreated control. The value of antibody titres in heat stressed control birds (1:32), while in the treated groups the titres were increased in groups 2, 3 and 4 to 1:64. The titre in post-vaccinated heat stressed birds was 1:4, which was significantly ($P<0.01$) improved in groups 2, 3 and 4 to 1:64,1:16 and 1:128, respectively These results indicate that the antistressor products enhanced the immune response of the birds. The findings are in congruence with earlier findings that heat stress reduces immune response of the birds [34]. Impairment of immunological function in heat stress.(Figure -1,2)

Tissue antioxidant profiles

During heat stress because of panting there could be possibilities for oxidative stress, respiratory alkalosis and thus an overproduction of free radicals in the body. The concentration of TBARS (nmoles MDA/mg protein) in Liver at 6th week revealed a significant ($P\leq 0.05$) rise in group T0 1 (9.94±0.13) at the end of 6th wk as compared to groups 2, 3 and 4 (8.73±0.15, 8.96±0.10 and 8.94±0.13, respectively) Broiler chickens exposed to heat stress exhibited more than 2 fold increase in malonaldehyde (MDA) as an indicator of lipid peroxidation in the skeletal muscles [2-3]. The results are consistent with previous studies [35] indicating disturbance of equilibrium and hence increased oxidative stress [36].

The concentration of SOD (U/mg protein) in Liver at 6th week revealed a significant ($p \leq 0.05$) decrease in group T0 (0.67 ± 0.007) as compared to other groups 2, 3 and 4 (0.93 ± 0.007, 0.79 ± 0.003 and 0.75 ± 0.003, respectively) SOD is an important member of antioxidant system, which removes superoxide free radical [27]. Increased concentration of SOD is an indicator of better free radical scavenging with antistressor products stress roak,ayucee and Vit. C.

The concentration of Catalase (U/mg protein) in Liver at 6th week revealed a significant ($p \leq 0.05$) decrease in group T0 (0.054±0.003) as compared to groups 2, 3 and 4 (0.236±0.011, 0.308±0.016 and 0.133±0.010, respectively)

The concentration of GPx (mg/ protein) in Liver at 6th week revealed a significant ($p \leq 0.05$) decrease in group T01 (1.75±0.05) as compared to treated groups 2, 3 and 4 that showed a significant ($p<0.05$) increase in GP_X activity. Glutathione is considered to be the master antioxidant of the body and is found in almost all living cells. Diminished content of GSH in cells ultimately results in cell death [37]. Glutathione protects structural integrity of cell membrane from free radicals [35]. Increase value of GPx in antistressor supplemented group T1 and T2 indicate antioxidative effect of these products.

The altered value of MDA, SOD and GPx indicates the extent of cell membrane damage by free radicals. The values of these parameters in stress roak,ayucee and Vitamin C supplemented groups as compared to untreated control indicates efficacy of antistressor products in combating oxidative stress imposed by heat. Because of increased activity and concentration of enzymatic and non-enzymatic antioxidants resulted by supplementation ofStressroak,Ayucee and Vitamin C and through diet and water, birds could remained healthy, maintained body weight and were acclimated to the heat stressor quicker than untreated group. (Table-3)

CONCLUSION

Heat stress is major welfare problem in the poultry industry leading to huge economic losses every year because of mortality and decreased production. Dietary supplementation of stressroak, Ayucee and synthetic vit. C with bioflavonoids ameliorated the heat stress. From the results of the present study it is observed that significant improvement in oxidative stress ameliorative response, immune response with normalization of sero biochemical parameters. The results may be attributed to immunomodulatory, antioxidant, antistressor, free radical scavenging and hepatoprotective action of the constituent herbs of Stress roak and Ayucee preparations On par with Synthetic Vitamin C.

Table-1 Composition of diets for broiler (starter and finisher phases

Ingredients	Starter phase (%)	Finisher phase (%)
Maize	46.00	50.00
Soybean meal	18.50	12.00
Groundnut cake	15.00	11.00
Fishmeal	2.00	2.00
Wheat offal	12.45	19.05
Bone meal	2.00	2.00
Oyster shell	3.00	3.00
Salt	0.25	0.25
Premix	0.25	0.25
Methionine	0.30	0.25
Lysine	0.25	0.20
	100	**100**
M.E (Kcal/kg)	2816	2809.6
Ether extract (%)	3.93	3.89
Crude fibre (%)	3.67	3.79
Calcium (%)	1.75	1.74
Phosphorus (%)	0.43	0.41

*1kg of premix contains:
Vitamin A-10,000,000 IU; Vitamin D3-2,000,000; Vitamin E-20,000 IU ;Vitamin K-2,250mg; Thiamine B1-1,750mg; Riboflavin B2- 5,000mg; Pyridoxine B6- 2,750mg; Niacin-27,500mg; Vitamin B12-15mg; Pantothenic acid- 7,500mg; Folic acid-7500mg; Biotin-50mg; Choline chloride-400g; Antioxidant-125g; Magnesium-80g; Zinc-50mg; Iron-20g; Copper-5g; Iodine-1.2g; Selenium-200mg; Cobalt-200mg

Table 2.Experimantal Design

Sl.NO	Treatment group	No. of birds/pen/ replication	No. of replicates	Total number of birds/ treatment	Dose (kg/tone feed)
1	Group- T1: stress Control	10	4	40	No treatment
2	Group- T2	10	4	40	Stressrok premix 1kg/ton of feed
3	Group- T3	10	4	40	Ayucee premix @100 g/ton of feed
4	Group- T4	10	4	40	Synthetic Vit. C(AA)in diet@ 100 gm/ton of feed

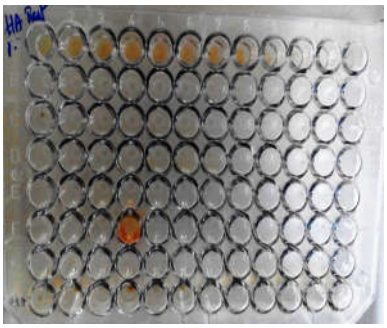
Table 3: Serum Biochemical Profiles in different groups of broiler chicks.

Groups parameters	ALT		ALP		AST	
	4^{th}	6^{th}	4^{th}	6^{th}	4^{th}	6^{th}
T_0	13.57 ± 0.790^{A}	11.13 ± 0.329^{A}	2768 ± 35.894^{A}	2312 ± 49.279^{A}	201.50 ± 2.36^{A}	249.00 ± 3.48^{A}
T_1	9.12 ± 0.781^{B}	8.95 ± 0.429^{B}	2537 ± 76.890^{B}	2032 ± 32.394^{BC}	176.00 ± 3.55^{C}	209.00 ± 2.33^{C}
T_2	11.13 ± 0.822^{B}	10.43 ± 0.872^{B}	2545 ± 33.611^{B}	2088 ± 41.468^{B}	191.00 ± 2.86^{B}	230.00 ± 2.98^{B}
T_3	10.37 ± 0.472^{B}	9.85 ± 0.472^{B}	2340 ± 76.172^{C}	1961 ± 35.728^{C}	181.00 ± 2.44^{C}	206.80 ± 4.20^{C}

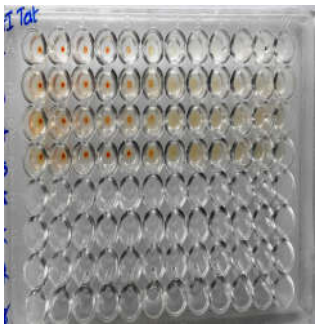
Table 4 Antioxidant Profiles of different groups of broiler chicks

Group parameter	TBARS(nmol/mg protein)	SOD U/mg protein)	CATALASE U/mg protein)	GPx U/mg protein)
T_0	9.94 ± 0.17^{A}	0.67 ± 0.007^{D}	0.054 ± 0.003^{D}	1.75 ± 0.05^{C}
T_1	8.73 ± 0.15^{B}	0.93 ± 0.007^{A}	0.236 ± 0.011^{B}	4.26 ± 0.02^{A}
T_2	8.96 ± 0.10^{B}	0.79 ± 0.003^{B}	0.308 ± 0.016^{A}	5.12 ± 0.01^{A}
T_3	8.94 ± 0.13^{B}	0.75 ± 0.003^{C}	0.133 ± 0.010^{C}	3.69 ± 0.02^{B}

Figure 1: Photograph showing pre vaccination Humoral immunity

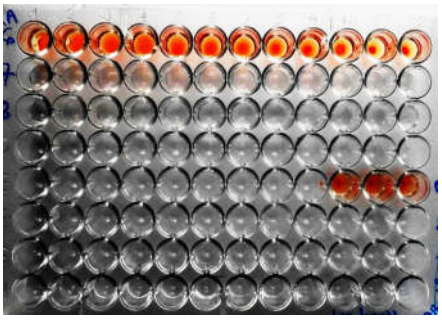


Haemagglutination test

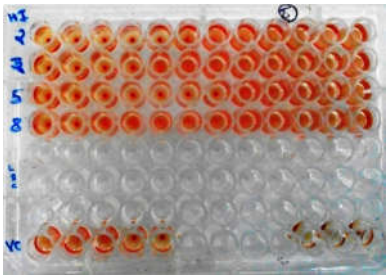


Haemagglutination Inhibition test

Figure 2: Photograph showing post vaccination Humoral immunity



Haemagglutination test



Haemagglutination Inhibition test

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