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Efficacy evaluation of certain herbal products in amelioration of heat stress in broilers

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Abstract

This study was conducted to evaluate the efficacy of certain herbal products (Herbal C @10 g/kg feed, Osmo C @10 g/kg feed, Heat Beat @10 g/kg feed and Immuplus AFS @ 50g/kg feed) in broiler birds in alleviating the heat stress on productive performance. A total of 225 day old chicks were randomly divided into 5 dietary groups *Viz*: Control, Herbal C, Osmo C, Heat Beat and Immuplus AFS, with each group consisting of 9 replicates of 5 chicks. The birds were reared in sheds where the temperature and humidity index ranged from 93.78- 97.16% for a period of 6 weeks. Body weight gain, feed intake and feed conversion ratio were recorded at weekly intervals. Blood samples were collected and assayed for haematological, biochemical, antioxidant enzyme, serum corticosterone and immune indicators. The body weight gain was significantly higher in treated groups compared to control with similar trend in feed consumption. Feed conversion ratio (FCR) values were non-significantly lower over control at the end of the experiment. Among the groups, Osmo C supplementation showed better growth rate and FCR. Herbal supplementation significantly increased the total serum protein values. While, Blood urea nitrogen, glucose, total cholesterol and corticosterone levels were significantly decreased over control. The antioxidant activity of Glutathione reductase (GSHRx), Super oxide dismutase (SOD) and Glutathione peroxidase (GSHPx) in serum significantly increased in all the experimental groups over control. While, Lipid peroxidation (LPO) showed a decreased effect. Humoral immune response in terms of antibody titer was significantly increased in all experimental groups with highest value (6.67 log₂ titre) observed in Immuplus AFS supplemented group.

The percent mortality of the birds during the experimental period were higher in heat stressed control group (6.66%), while low mortality (4.44%) was recorded in treated groups suggestive of improved immune response to heat stress. From the results of the present study, it is concluded that, the herbal products particularly Immuplus AFS and Osmo C may be suggested as natural alternatives in amelioration of heat stress and also in boosting the immunity.

Keywords: Certain herbal products, amelioration, heat stress, broilers

Introduction

India lies in tropical climatic zone where the weather remains reasonably cold in the winter and hot in the summer season. A hot environment along with humidity is one of the important stressors in poultry production. The ideal ambient temperature for optimal performance is likely to be 19 to 22 °C for laying hens and 18 to 22°C for broiler growth ^[1]. When birds are exposed to environmental temperatures above thermo neutral zone i.e., above 30 °C, signs of heat stress are likely to appear ^[2].

Several methods are available to alleviate the negative effects of high environmental temperature on performance of poultry. Because of the high cost and impracticality of cooling animal buildings, interest in dietary manipulations has increased. Studies have shown that antioxidant nutrient supplementation, especially ascorbic acid and tocopherol could be used to attenuate the negative effects of environmental stress ^[3]. Even though poultry can synthesize ascorbic acid, synthesis is inadequate under stressful conditions such as high environmental temperature, high humidity, High egg production rate and parasite infestation if any ^[4]. In recent past large number of studies with dietary supplementation with antioxidants were shown to ameliorate heat stress. Further, use of immunopotentiator products are known to improve productive performance during heat stress. However, most of such synthetic additives besides having expensive are known to cause side effects.

Consumer awareness about the health, safety and quality of food products of animal origin has increased the popularity of natural feed additives to reduce heat stress in livestock production. Some of the herbal antioxidant make a safer alternative by virtue of their composition. *Withania somnifera*, *Asparagus raecemosus*, *Phyllanthus emblica* and *Ocimum sanctum* herbs

have been used to protect tissues from ROS and enhance cell survival by stimulating antioxidative enzymatic systems [5]. Hence, the present study was aimed to assay the ameliorative efficacy of certain herbal products in diets in combating heat stress and improve productivity.

Materials and Methods

Birds and management

Experimental birds were reared at the housing facility at Poultry Experimental Station, Department of Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad, which is located in the Deccan plateau of Southern India. The mean temperature of the experimental shed ranged between 38.50-41.69 °C and mean relative

humidity was in the range of 60.92-70.27%. Temperature humidity index was calculated on the basis of daily observations recorded for temperature and humidity using the formula as per [6]. The temperature humidity index ranged between 93.78 to 97.16% during the experimental period (Table 1). The chicks were reared in battery brooders (6'6" x 4') with an average floor space of 1sq feet per bird with optimum brooding conditions from day old to 42 days of age. Standard management practices were followed during the entire experimental period. Birds were immunized for Marek's disease at hatchery and Newcastle disease (ND) with Lasota vaccine at 7th day and 28th day of age and Infectious Bursal disease of Georgia strain vaccine at 14th day of age.

Table 1: Average Weekly data on Temperature, Humidity and Temperature Humidity Index in Experimental shed

Week	Temperature (°C)	Humidity (%)	Temperature-Humidity Index (%)
1 st week	38.50 ^b	68.94 ^a	93.78 ^b
2 nd week	40.21 ^a	70.27 ^a	96.69 ^a
3 rd week	40.51 ^a	63.21 ^b	95.25 ^{ab}
4 th week	40.74 ^a	64.23 ^b	95.82 ^a
5 th week	41.69 ^a	64.34 ^b	97.16 ^a
6 th week	41.37 ^a	60.92 ^b	95.89 ^a
SEM	0.28	0.71	0.30
N	7.00	7.00	7.00
P value	0.01	0.00	0.01

Experimental design

The birds were fed with maize and soybean meal based commercial diets containing 21.5% and 19.5% of crude protein and metabolizable energy (ME) of 3050 and 3150 kcal/kg diet respectively during starter (0-21 d) and finisher (22-42d) phases. The experimental period was from day old to 42 days of age. About 225 one day old commercial male broiler chicks were randomly distributed into five dietary treatment groups of nine replicates with five chicks in each replicate. The experimental diets were prepared by supplementation of herbal extracts. Control diet was formulated with basal corn soya bean meal, whereas experimental diets were the basal diet with herbal products viz., Herbal C, Osmo C, Heat Beat and Immuplus AFS supplemented at 10g/kg, 10g/kg, 10g/kg and 50g/kg of feed, respectively.

Materials and Methods

Blood was drawn from the wing vein on 3rd and 6th week of age from 9 birds in each group and were used for the estimation of Haemato-biochemical constituents such as hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), total leukocyte count (TLC), Heterophil/Lymphocyte ratio (using automatic blood analyzer (Mindray BC 2600, China)). Serum samples were separated from the blood by centrifugation at 3000 rpm for 15 min and were assayed for estimation of total protein, glucose, Blood urea

nitrogen, total cholesterol (using commercially available kits (Coral clinical systems Pvt. limited, India)) and antioxidant enzymes viz., Glutathione reductase [7], Superoxide dismutase [8], Glutathione peroxidase [9] and Lipid peroxidation [10] done with serum samples. Immune response was also assessed by Haemagglutination inhibition (HI) activity [11] on 3rd and 6th week while serum corticosterone levels (solid phase enzyme linked immunosorbent assay (ELISA)) were measured from the serum samples obtained at 6th week of age. Mortality percentage was also been observed during the whole experiment period. The statistical analysis was done by using Statistical Package for Social Sciences (SPSS) 15th version.

Results

Growth and performance parameters

In the current study, the body weight gain at the end 6th week was found to be significantly ($P < 0.01$) improved in Heat Beat supplemented birds (556.13g/b) as compared to control group (438.98g/b) and other treated groups Herbal C (499.98g/b), Osmo C (518.56g/b) and Immuplus AFS (490.07g/b). Feed intake was observed to be non-significantly more in Heat Beat supplemented group at the end of experiment as compared to control and other treated group. FCR varied non-significantly between different treatments groups except at 2nd and 5th week. At 6th week of age, feed conversion ratio was found to be non-significantly better in Heat Beat supplemented birds (1.65) as compared to control group (1.86).

Table 2: Effect of dietary inclusion of herbal products on weekly body weight gain (g) in commercial broiler chicken.

Group	Starter phase			Finisher phase		
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week
Control	59.97 ^b	113.17 ^b	241.47	397.71	442.98 ^b	438.98 ^c
Herbal C	68.19 ^a	119.51 ^a	246.93	429.20	468.96 ^a	499.98 ^{ab}
Osmo C	66.30 ^a	117.06 ^{ab}	250.93	451.62	472.87 ^a	518.56 ^{ab}
Heat Beat	68.29 ^a	112.66 ^b	245.51	399.71	444.07 ^b	556.13 ^a
Immuplus AFS	65.77 ^a	119.89 ^a	253.20	406.73	480.22 ^a	490.07 ^{bc}
SEM	0.83	0.84	3.72	10.35	3.99	10.32

N	9.00	9.00	9.00	9.00	9.00	9.00
P Value	0.01	0.01	0.89	0.42	0.00	0.00

^{abc} Means with different superscript in a column differ significantly: $P \leq 0.01$, P-value: probability value. Each pen is a replicate of 5 chicks: number of replicates; SEM: Standard Error Mean

Table 3: Effect of dietary inclusion of herbal products on weekly feed intake (g) in commercial broiler chicken

Group	Starter phase			Finisher phase		
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week
Control	72.76 ^b	147.64 ^b	334.00	698.89	734.00	815.55
Herbal C	79.47 ^a	152.02 ^a	344.04	729.79	734.04	870.25
Osmo C	77.96 ^{ab}	150.00 ^{ab}	342.44	733.69	742.44	914.63
Heat Beat	80.88 ^a	150.22 ^a	334.36	714.36	734.36	915.79
Immuplus AFS	79.74 ^a	150.00 ^{ab}	341.58	718.51	741.58	850.47
SEM	0.90	0.41	4.65	8.98	4.65	15.45
N	9.00	9.00	9.00	9.00	9.00	9.00
P Value	0.03	0.01	0.96	0.77	0.96	0.18

^{abc} Means with different superscript in a column differ significantly: $P < 0.05$, $P < 0.01$, P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean

Table 4: Effect of dietary inclusion of herbal products on weekly and phase-wise feed conversion ratio (g) in commercial broiler chicken

Group	Starter phase			Finisher phase		
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week
Control	1.21	1.30 ^{ab}	1.38	1.76	1.66 ^a	1.86
Herbal C	1.17	1.27 ^b	1.39	1.70	1.56 ^b	1.74
Osmo C	1.17	1.28 ^{ab}	1.36	1.62	1.57 ^b	1.76
Heat Beat	1.18	1.33 ^a	1.36	1.79	1.65 ^a	1.65
Immuplus AFS	1.21	1.25 ^b	1.35	1.76	1.54 ^b	1.74
SEM	0.02	0.01	0.01	0.04	0.01	0.03
N	9.00	9.00	9.00	9.00	9.00	9.00
P Value	0.92	0.05	0.87	0.80	0.01	0.30

^{abc} Means with different superscript in a column differ significantly: $P < 0.05$, $P < 0.01$, P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean

Haemato-Biochemical Parameters

The total erythrocyte count at the end of 3rd and 6th week did not show any significant ($P > 0.05$) difference within the experimental groups. The mean values of TEC in different groups ranged from 2.17 to 2.43 ($10^6/\mu\text{l}$) on 3rd week and 2.41 to 2.76 ($10^6/\mu\text{l}$) on 6th week. A non-significant ($P > 0.05$) increase in the packed cell volume (%) was observed at the end of 3rd and 6th week, the values of PCV ranged between 31.55-36.37% and 33.67 – 39.32% respectively.

A significant ($P < 0.05$) increase in mean Hb values was observed on 3rd and 6th week. Immuplus AFS fed birds showed significant ($P < 0.05$) increase in Hb values (11.10 and 13.03g/dl) at 3rd and 6th week when compared to control. The total leucocyte count did not show any significant ($P > 0.05$) difference within the experimental groups on 3rd and 6th week. At the end of 3rd and 6th week, the values of TLC ranged between 8.00 – 8.72 $10^3/\mu\text{l}$ and 8.28-9.13 $10^3/\mu\text{l}$ respectively. A non-significant decrease in total leucocyte count was observed on 3rd and 6th week. The mean values of TLC ranged between 8.00 – 8.72 $10^3/\mu\text{l}$ and 8.28-9.13 $10^3/\mu\text{l}$ at 3rd and 6th week. The percent of Heterophil/ Lymphocyte ratio did not show any significance ($P > 0.05$) on 3rd and 6th week. At 3rd and 6th week, the values for Heterophil/ Lymphocyte ratio ranged between 0.08 - 0.11% and 0.12- 0.16% respectively.

The concentration of blood glucose (mg/dl) in stress control group was significantly ($P < 0.01$) raised (123.77 and 213.03) as compared to all other groups at the end of 3rd and 6th week. The lowest concentration was recorded in Immuplus AFS and Herbal C among the treatments at 3rd week and 6th week. The serum protein (g/dl) in control group was significantly ($P < 0.01$) lower (3.90 and 4.52) compared to all other groups at the end of 3rd and 6th week (4.79, 5.11; 4.45, 5.05; 4.81, 5.18; 4.47, 5.00 respectively in Herbal C, Osmo C, Heat Beat and Immuplus AFS). The concentration of Blood urea nitrogen (mg/dl) in Control group was significantly ($P < 0.05$) raised (75.56 and 89.10) compared to all other groups at the end of 3rd and 6th week (24.06, 39.49; 28.89, 44.04; 35.93, 49.15 and 42.56, 47.78, respectively in Herbal C, Osmo C, Heat Beat and Immuplus AFS). The values of total cholesterol at 3rd and 6th week in control group was 194.63 and 206.76 which was significantly ($P < 0.01$) raised as compared to Herbal C, Osmo C, Heat Beat and Immuplus AFS (178.10, 191.06; 178.50, 186.38; 181.33, 187.80 and 165.17, 187.07 respectively). The concentration of serum corticosterone levels revealed a significant ($P < 0.01$) increase in control group with 7.28 $\mu\text{g/dl}$ as compared to other treated groups in the range of 6.40 -6.68 $\mu\text{g/dl}$.

Table 5: Effect of dietary inclusion of herbal products on Haematological parameter (Total Erythrocyte Count, Packed Cell Volume, Haemoglobin, Total leucocyte count and Heterophil/Lymphocyte ratio) at 3rd and 6th week in commercial broiler chicken

Group	Total Erythrocyte Count (10 ⁶ /ul)		Packed Cell Volume (%)		Haemoglobin (g/dl)		Total leucocyte count (10 ³ /ul)		H: L ratio (%)	
	3 rd week	6 th week	3 rd week	6 th week	3 rd week	6 th week	3 rd week	6 th week	3 rd week	6 th week
Control	2.17	2.41	31.55	33.67	9.30 ^b	10.20 ^b	8.72	9.13	0.11	0.16
Herbal C	2.28	2.68	36.37	39.00	11.68 ^a	11.47 ^{ab}	8.70	8.77	0.10	0.14
Osmo C	2.28	2.58	35.23	39.32	10.48 ^{ab}	11.45 ^{ab}	8.00	8.28	0.08	0.12
Heat Beat	2.20	2.44	32.85	34.95	10.20 ^{ab}	10.72 ^b	8.27	8.52	0.09	0.16
Immuplus AFS	2.43	2.76	34.02	38.37	11.10 ^a	13.03 ^a	8.33	8.57	0.08	0.15
SEM	0.061	0.061	0.926	1.048	0.268	0.323	0.145	0.126	0.005	0.005
N	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
P Value	0.711	0.297	0.524	0.316	0.01	0.05	0.498	0.279	0.215	0.192

^{abc} Means with different superscript in a column differ significantly: $P < 0.05$, P-value: probability value. N: number of replicates; SEM: Standard Error Mean

Table 6: Effect of dietary inclusion of herbal products on Biochemical parameters (Glucose, Total protein, Blood Urea Nitrogen and Total Cholesterol) at 3rd and 6th week in commercial broiler chicken

Group	Glucose (mg/dl)		Total protein (g/dl)		Blood Urea Nitrogen (mg/dl)		Total Cholesterol (mg/dl)	
	3 rd week	6 th week	3 rd week	6 th week	3 rd week	6 th week	3 rd week	6 th week
Control	123.77 ^b	213.03 ^a	3.90 ^c	4.52 ^b	75.56 ^a	89.10 ^a	194.63 ^a	206.76 ^a
Herbal C	81.66 ^b	94.85 ^b	4.79 ^a	5.11 ^a	24.06 ^b	39.49 ^b	178.10 ^b	191.06 ^b
Osmo C	70.06 ^b	107.10 ^b	4.45 ^b	5.05 ^a	28.89 ^b	44.04 ^b	178.50 ^b	186.38 ^b
Heat Beat	87.06 ^b	103.43 ^b	4.81 ^a	5.18 ^a	35.93 ^b	49.15 ^b	181.33 ^b	187.80 ^b
Immuplus AFS	68.08 ^{ab}	108.61 ^b	4.47 ^b	5.00 ^a	42.56 ^b	47.78 ^b	165.17 ^c	187.07 ^b
SEM	6.43	10.56	0.073	0.066	4.927	5.145	2.02	1.71
N	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
P Value	0.03	0.00	0.01	0.01	0.01	0.01	0.00	0.00

^{abc} Means with different superscript in a column differ significantly: $P < 0.01$, P-value: probability value. N: number of replicates; SEM: Standard Error Mean

Table 7: Effect of dietary inclusion of herbal immunopotentiator products on serum Corticosterone ($\mu\text{g}/\text{dl}$) at 6th week in commercial broiler chicken

Group	Serum Corticosterone ($\mu\text{g}/\text{dl}$)
Control	7.28 ^a
Herbal C	6.68 ^b
Osmo C	6.60 ^{bc}
Heat Beat	6.40 ^c
Immuplus AFS	6.55 ^{bc}
SEM	0.063
N	9.00
P Value	0.001

^{abc} Means with different superscript in a column differ significantly: $P < 0.01$, P-value: probability value. N: number of replicates; SEM: Standard Error Mean

Antioxidant enzyme activity

Glutathione reductase (GSHRx) activity was lower in control group (1338.98 and 1362.74 units/ml) at 3rd and 6th week respectively. Dietary inclusion of herbal products significantly ($P < 0.01$) improved GSHRx values at both stages. Similarly, the mean values of SOD was significantly lower in control group at 3rd and 6th week. Supplementation with Herbal C, Osmo C, Heat Beat and Immuplus AFS improved the values (7.18, 6.29, 4.33 and 7.31 units/mg protein) at 3rd week and (7.89, 6.94, 5.09 and 7.86 units/mg protein) at 6th week. A significant ($P < 0.05$) increase in Glutathione peroxidase was observed at 3rd and 6th week. Though dietary supplementation could significantly ($P < 0.05$)

improve GSHPx values at 3rd and 6th week, the Immuplus AFS improved the enzyme activity to significantly ($P < 0.05$) higher values of 952.81 units/ml followed by Heat Beat (837.49 units/ml) and Osmo C (775.11 units/ml), while Herbal C showed similar concentration of enzyme activity (725.96 units/ml) as that of control (712.72 units/ml). A significant ($P < 0.01$) decrease in the values of Lipid peroxidation (6.10, 10.40; 6.25, 16.58; 8.12, 7.99 and 5.70, 5.09 nmol MDA level per mg protein) was observed in Herbal C, Osmo C, Heat Beat and Immuplus AFS when compared to control group (14.18, 17.00 nmol MDA level per mg protein) at 3rd and 6th week.

Table 8: Effect of dietary inclusion of herbal products on Antioxidant enzyme activity (GSHRx, SOD, GSHPx and LPO) at 3rd and 6th week in commercial broiler chicken

Group	Glutathione reductase (GSHRx) (units/ml)		Super oxide dismutase (units/mg protein)		Glutathione peroxidase (GSHPx) (units/ml)		Lipid peroxidation (LPO) (nmol MDA/mg protein)	
	3 rd week	6 th week	3 rd week	6 th week	3 rd week	6 th week	3 rd week	6 th week
Control	1338.98 ^b	1362.74 ^b	3.14 ^b	3.79 ^b	447.42 ^b	712.72 ^b	14.18 ^a	17.00 ^a
Herbal C	1778.33 ^a	2018.74 ^a	7.18 ^a	7.89 ^a	680.58 ^a	725.96 ^b	6.10 ^b	10.40 ^{ab}
Osmo C	1743.86 ^a	2005.19 ^a	6.29 ^a	6.94 ^a	570.30 ^a	775.11 ^{ab}	6.25 ^b	16.58 ^a

Heat Beat	1490.97 ^b	1911.93 ^a	4.33 ^b	5.09 ^b	631.43 ^a	837.49 ^{ab}	8.12 ^{ab}	7.99 ^b
Immuplus AFS	1729.81 ^a	1865.49 ^a	7.31 ^a	7.86 ^a	633.95 ^a	952.81 ^a	5.70 ^b	5.09 ^b
SEM	42.25	59.07	0.40	0.39	21.42	29.34	1.05	1.30
N	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
P Value	0.001	0.001	0.001	0.001	0.002	0.05	0.04	0.01

^{abc} Means with different superscript in a column differ significantly: $P < 0.05$, $P < 0.01$, P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean

Humoral immune response

At 3rd and 6th week, humoral immune response in Control group was 3.00 and 3.83 log₂ titer and these values were significantly ($P < 0.01$) improved with dietary herbal products. Highest (5.67 and 6.67 log₂ titer at 3rd and 6th week) immune

response was achieved in Immuplus AFS followed by Osmo C (4.33 and 5.33 log₂ titer). While, immune response in terms of HI titers recorded with Heat Beat, Herbal C supplemented diets were comparable to control group.

Table 9: Effect of dietary inclusion of Herbal products on Immune Response at 3rd and 6th week in commercial broiler chicken

Group	3 rd Week	6 th Week
Control	3.00 ^b	3.83 ^b
Herbal C	3.67 ^b	4.17 ^b
Osmo C	4.33 ^{ab}	5.33 ^{ab}
Heat Beat	3.33 ^b	4.67 ^b
Immuplus AFS	5.67 ^a	6.67 ^a
SEM	0.26	0.283
N	9.00	9.00
P Value	0.01	0.005

^{abc} Means with different superscript in a column differ significantly: $P < 0.01$, P-value: probability Value. N: number of replicates; SEM: Standard Error Mean

Mortality

Mortality recorded during the experimental period of six weeks is presented in Table.10 Mortality in different groups were observed at starter and finisher phase when the average highest temperature was 38.50^c to 41.69^c. In the present

experiment, highest mortality (6.66%) was found in Control group and there was no mortality observed in Osmo C and Immuplus AFS groups. While, other treated groups Herbal C and Heat Beat group recorded 4.44% mortality, respectively.

Table 10: Percentage of mortality during starter phase (0-3 weeks) and finisher phase (4-6 weeks) of age as influenced by different dietary treatments

Group	No. of birds housed	No. of birds died	Percent mortality (age in weeks)		
			Starter phase(0-3wks)	Finisher Phase (4-6wks)	Overall (0-6wks)
Control	45	3	6.66	-	6.66
Herbal C	45	2	-	4.44	4.44
Osmo C	45	-	-	-	-
Heat Beat	45	2	4.44	-	4.44
Immuplus AFS	45	-	-	-	-

Discussion

Performance parameters

High temperature induces physiological changes in birds such as reduced feed consumption, insufficient digestion due to reduced activity of digestive enzymes [12] which reduces body weight. The products used in the present study such as Herbal C, Osmo C and Heat Beat have naturally occurring vitamin C. Various investigators who used synthetic vitamin C also reported an increase in body weight gain by feeding it [13]. Vitamin C is known to facilitate utilization of nutrients [14] by better digestion which might have improved weight gains in birds. Panting is increased in heat stressed birds to dissipate heat. This requires higher expenditure of energy resulting in higher FCR in heat stressed birds.

Haemato- biochemical profile

There was a significant raise in haemoglobin values in all supplemented groups. The present results with improved haemoglobin values were in agreement with earlier reports of [15] who observed similar increase in the haemoglobin in broilers with dietary inclusion of ascorbic acid. Contrary to the present observation, Investigator [16] reported that dietary

supplementation of 150 and 300 ppm of ascorbic acid showed reduction of Heterophil/ Lymphocyte ratio in heat stressed birds. There was a significant decrease in blood glucose in all the supplemented groups when compared with control. Part of this effect may be due to dehydration in heat stressed control birds compared to stress protected experimental group. Further, increased glucose in heat stressed broilers can also be due to increased gluconeogenesis as a consequence to high glucocorticoid secretion [17]. Author [18] reported that, addition of vitamin C to the basal diet reduced blood glucose in broilers under heat stressed conditions. Vitamin C possibly reduced blood glucose due to decreased synthesis and secretion of glucocorticoids [18].

There was a significant increase in total protein levels in all the experimental groups over control. Increase in stress induces sympatho adrenal activity which lead to catabolism of proteins and fat [19] which in turn may have effected plasma protein concentration. Earlier investigator [20] who have also observed an increase in protein levels by feeding herbal mixture and Ashwagandha respectively. The raise in BUN in control birds may be attributed to increased catabolism of proteins in heat stressed birds and partly due to dehydration

with haemo concentration due to heat stress. There was a significant decrease in total cholesterol values in the experimental groups as against to control group. Heat stress induces mobilisation of fats to the sympathetic activity which in turn may have elevated plasma cholesterol concentration in control group. This reduction in cholesterol levels of herbal supplemented broilers is also in agreement with earlier findings of other reporters [21] using various immuno potent herbal supplementation in broiler birds.

Serum Corticosterone concentration

High ambient temperature has a profound effect on glucocorticoid synthesis and secretion [22]. Hence, serum corticosterone concentration was estimated at the end of 6th week of experimental period. The corticosterone in control group was high with a value of 7.28µg/dl. The higher corticosterone in thermal stressed birds might be mediated through enhanced CRH-ACTH corticosteroid activity acting through hypothalamo-pituitary Adrenal cortex axis. A significant decrease in corticosterone levels were regarded from all experimental group suggestive of reduced heat stress. Similar observation was reported by earlier workers [22] with supplemented diets in heat stressed birds. Similar lowering effect of herbal supplemented diets on serum cortisol was also reported by other investigators [22].

Serum Antioxidant enzyme activity

The supplementation of herbal products in diet have significantly increased the activity of GSHRx, SOD, GSHPx except LPO when compared to controls. Among the supplemented groups, Immuplus AFS showed a non-significant higher activity of SOD and GSHPx indicating it as a better immunopotentiator over the other supplements. The present results are in agreement with those of other works [23] who also observed an increase in GSH, SOD and reduced LPO by supplementation of 0.1% Ashwagandha. Vitamin C supplementation may have helped in preventing peroxidation through quenching the free radicals and thus improved the antioxidant activity [24].

Immune parameters

In the present study, the immune response in terms of antibody titer was significantly higher in Immuplus AFS supplemented group over other supplemental groups during 3rd and 6th week. Similar observations were made by earlier investigators [25] who reported higher HI titer values when supplemented with Ashwagandha and attributed these results to the presence of the active principle glycowithanolides.

Mortality

There was no mortality of birds from group treated with Osmo C and Immuplus AFS. Reduced mortality or no mortality in these supplemented groups may be due to antistress activity of ashwagandha which contains glycowithanolides which are known to have antistress properties [20]. Further, *Tinospora cordifolia* which is present in Immuplus AFS with antioxidative, antistress and immunomodulatory properties may have protected from heat induced mortality.

Conclusion

In conclusion, herbal products may be considered as safer alternative in place of synthetic antioxidants in combating heatstress induced oxidative changes and in boosting

immunity and production performance during summer.

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