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Effect of supplementation of *Tinospora cordifolia* (Thunb.) Miers and ascorbic acid either alone or in combinations on serum protein profile and AG ratio of broiler chickens

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Abstract

A 42 day feeding trial was carried out to investigate the effect of *Tinospora cordifolia* (at graded levels) or ascorbic acid alone and in combinations on Leucogram and H/L ratio using 360 one-day-old broiler chicks in 5×2 factorial experiment within a completely randomized design and divided into ten (10) dietary treatments groups (T₁-T₁₀) in triplicate of twelve chicks per replicate. The average temperature (31 °C) during the research trial was higher than the recommended normothermia zone *i.e.* 18-24 °C established for poultry birds, which indicated that poultry birds were in chronic heat stress. Improvement in serum albumin, serum globulin, total protein and A/G ratio of broilers was observed in the present trial due to supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) alone and in combinations. Supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) may be an indicator of immune response in broilers during chronic heat stress.

Keywords: Serum albumin, serum globulin, total protein, A/G ratio, ascorbic acid, *Tinospora cordifolia*

Introduction

Poultry industry of India has a significant contribution in discharging the increasing demand of quality protein in human diet in the form of meat and eggs. Presently, the broiler and egg production in the country is booming at the rate of 15% and 5%, respectively (Yamuna and Thangavel, 2011) [13]. Various types of feed additives such as herbal products, enzymes, prebiotics, probiotics, antibiotics, hormones etc., are being used as growth stimulants in poultry production. Stress such as heat remains a perpetual challenge for the poultry production in tropical climate of India, which increases the poultry need for vitamin C. During heat stress, the poultry is not able to synthesize adequate vitamin C to meet physiological demands, hence the need for Vitamin C. Various herbal preparations have been proved to exert positive effect in heat stressed broilers (Maini *et al.*, 2007; Sanjaykumar *et al.*, 2008) [6, 9]. A significant effect had been reported on protein content and blood cholesterol level in broilers if fed herbal growth promoters (Vidarthi *et al.*, 2008) [12]. *Tinospora cordifolia* (Geloi) grow in the tropical and subtropical regions of India (Sengupta *et al.* 2011) [10], which are well known for its antioxidant, immunomodulatory, antiviral properties and antibacterial (Srinivasan *et al.* 2008) [11]. Therefore, experiment was conducted to evaluate the effects of supplementation of *Tinospora cordifolia* or ascorbic acid alone or in combinations in poultry birds on serum protein profile and AG ratio in arid zone of Rajasthan (India).

2. Material and Methods

A 42 days feeding trial was carried out at the poultry farm of College of Veterinary and Animal Science (CVAS), Bikaner, Rajasthan. The three hundred sixty (360) experimental day-old-broiler chicks were equally and randomly divided into ten (10) dietary treatments groups (T₁-T₁₀) and each dietary group was replicated to three (3) sub-groups (R₁-R₃) to make sure uniformly in various treatment groups. Rations included: T₁-basal ration with no supplementation; T₂-basal ration supplemented with 0.25% *Tinospora cordifolia*; T₃-basal ration supplemented with 0.50% *Tinospora cordifolia*; T₄-basal ration supplemented with 0.75% *Tinospora cordifolia*; T₅-basal ration supplemented with 1.0% *Tinospora cordifolia*; T₆-basal ration supplemented with 0.025% ascorbic acid; T₇-basal ration supplemented with

0.25% *Tinospora cordifolia* plus 0.025% ascorbic acid; T₈-basal ration supplemented with 0.50% *Tinospora cordifolia* plus 0.025% ascorbic acid; T₉-basal ration supplemented with 0.75% *Tinospora cordifolia* plus 0.025% ascorbic acid and T₁₀-basal ration supplemented with 1.0% *Tinospora cordifolia* plus 0.025% ascorbic acid. Good quality of *Tinospora cordifolia* (Geloi) stem was procured from reputed firm of Bikaner (Rajasthan). Thereafter, it was identified and authenticated by the Department of Botany, Government Dungar College, Bikaner (Rajasthan). The commercially available ascorbic acid (99.99% pure) was used. The broiler starter and finisher feed contained 21.37% and 20.32% crude protein (CP), respectively. Broilers were maintained under standard managerial practices regarding brooding, watering, feeding and disease control throughout the research period. During the study period, digital thermohygrometer was used to record temperature and relative humidity thrice a day at 08:00, 14:00 and 22:00 hours, respectively. Further, daily maximum and minimum values of temperature and relative humidity were also recorded. THI values were calculated from the recorded measurement as described below by (Kibler, 1964) [5].

$$\text{THI} = 1.8 \times \text{DBT} - (1 - \text{RH}) \times (\text{DBT} - 14.3) + 32$$

Where: THI = Temperature-humidity index

DBT = Dry-bulb temperature (°C)

RH = Relative humidity as fraction of a unit (%)

Blood was collected at 42nd day from 2 birds per replicate (6 birds/treatment) at the end of experiment for the estimation of various biochemical parameters. The blood samples were transferred to non-EDTA tubes for separation of serum. Subsequently, the serum was harvested through centrifugation of sample at 3000 rpm for 15 min and stored at -20 °C until analysis. The serum samples were analysed for serum albumin, serum globulin and total protein by the Vet Test Chemistry Analyzer using kit supplied by Idexx laboratories, as per the manufacturer's subscribed procedure.

3. Results and Discussion

The mean values of temperature (°C), relative humidity (%) and temperature humidity index (THI) recorded during different weeks have been presented in Table 1. The calculated THI for different weeks was obtained to be in range from 75.26 to 82.06; a value above the THI threshold of 70, established for poultry (Karaman *et al.*, 2007) [3]. The average temperature (31 °C) during the experimental trial was higher than the recommended normothermia zone *i.e.* 22-28 °C (Donkoh, 1989) [1] established for poultry in the tropical regions, which indicated that birds in experimental trial were in chronic heat stress.

The data of serum albumin, serum globulin, total protein and A/G ratio recorded in various treatment groups have been presented in Table 2. The overall mean values of albumin (g/dl) were found to be 1.17, 1.18, 1.20, 1.18, 1.18, 1.20, 1.18, 1.18, 1.20 and 1.18 in T₁ (Control), T₂, T₃, T₄, T₅, T₆, T₇, T₈, T₉ and T₁₀ treatment groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 1.18, 1.18, 1.19, 1.19 and 1.18 (g/dl) in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplementation, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 1.18 (g/dl) in non-supplemented group and 1.19 (g/dl) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect

of geloi and ascorbic acid supplementation as well as interaction. The overall mean values of globulin (g/dl) were found to be 2.02, 2.23, 2.23, 2.22, 2.18, 2.23, 2.23, 2.27, 2.27 and 2.25 in T₁ (Control), T₂, T₃, T₄, T₅, T₆, T₇, T₈, T₉ and T₁₀ treatment groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 2.13, 2.23, 2.25, 2.24 and 2.22 (g/dl) in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplementation, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 2.18 (g/dl) in non-supplemented group and 2.25 (g/dl) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect due to supplementation of geloi and ascorbic acid as well as interaction.

The overall mean values of total protein (g/dl) were found to be 3.18, 3.42, 3.43, 3.40, 3.37, 3.43, 3.42, 3.45, 3.47 and 3.43 in T₁ (Control), T₂, T₃, T₄, T₅, T₆, T₇, T₈, T₉ and T₁₀ treatment groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 3.31, 3.42, 3.44, 3.43 and 3.40 (g/dl) in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplementation, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 3.36 (g/dl) in non-supplemented group and 3.44 (g/dl) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect due to geloi and ascorbic acid supplementation as well as interaction.

The overall mean values of A/G ratio were found to 0.58, 0.53, 0.54, 0.54, 0.55, 0.54, 0.53, 0.52, 0.54 and 0.53 in T₁ (Control), T₂, T₃, T₄, T₅, T₆, T₇, T₈, T₉ and T₁₀ treatment groups, respectively. Regarding effect of geloi supplementation, the mean values were recorded to be 0.56, 0.53, 0.53, 0.54 and 0.54 in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplementation, respectively. With respect to effect of ascorbic acid supplementation, the mean values were recorded to be 0.55 in non-supplemented group and 0.53 in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect of geloi and ascorbic acid supplemented groups as well as interaction.

The non-significant results observed in present study of geloi are in agreement with the findings of Khobragade (2003) [4] who reported non-significant effect on serum albumin, serum globulin and total protein on inclusion of geloi herb in broilers. With respect to ascorbic acid, the non-significant improvement in albumin and total protein in present study is in partial agreement with Rindhe *et al.* (2016) [8] who reported significant improvement in albumin and total protein on supplementation of ascorbic acid in broilers while the non-significant decrease in serum globulin is in partial agreement with findings of Majekodunmi *et al.* (2013) [7]. Gudev *et al.* (2007) [2] reported plasma total protein level tended to be lower during heat stress caused by initial haemoconcentration followed by haemodilution. Higher non-significant values of total protein in geloi at the graded levels and ascorbic acid either alone or in combinations may be due to the antioxidant property of geloi and ascorbic acid which stimuli protein synthesis by bird's enzymatic system. Increased level of serum globulin and low albumin: globulin ratio indicate better immune response in geloi or ascorbic acid supplementation alone as well as their combinations, which could be further explained that serum globulin is considered as an positive indicator of immune response and source of antibody production.

Table 1: Mean temperature (°C), relative humidity (%) and THI range observed during different weeks

Period (weeks)	Temperature (°C)				Relative humidity (%)				THI			
	8 am	2 pm	10 pm	Avg.	8 am	2 pm	10 pm	Avg.	8 am	2 pm	10 pm	Avg.
I	32.24	35.54	31.44	33.08	53.30	42.09	53.21	49.53	81.66	83.68	80.58	82.06
II	31.13	33.40	33.83	32.79	50.86	42.71	39.00	44.19	79.76	81.18	80.98	80.70
III	30.59	32.87	32.84	32.10	58.43	52.71	44.71	51.95	80.28	82.39	80.87	81.23
IV	28.94	31.14	29.87	29.99	66.71	59.00	60.14	61.95	79.22	81.15	79.56	80.01
V	27.16	30.46	29.53	29.05	46.43	43.29	42.00	43.90	74.00	77.66	76.32	76.01
VI	27.45	33.31	26.40	29.05	46.03	27.30	42.94	38.76	74.31	78.13	72.62	75.26
Mean	29.58	32.79	30.65	31.00	53.63	44.52	47.00	48.38	78.21	80.70	78.49	79.21

Table 2: Effect of supplementation of geloi and ascorbic acid on serum protein profile and A/G ratio

Treatment groups	Albumin (g/dl)	Globulin (g/dl)	Total protein (g/dl)	A/G Ratio
Geloi X Ascorbic acid				
T ₁	1.17	2.02	3.18	0.58
T ₂	1.18	2.23	3.42	0.53
T ₃	1.20	2.23	3.43	0.54
T ₄	1.18	2.22	3.40	0.54
T ₅	1.18	2.18	3.37	0.55
T ₆	1.20	2.23	3.43	0.54
T ₇	1.18	2.23	3.42	0.53
T ₈	1.18	2.27	3.45	0.52
T ₉	1.20	2.27	3.47	0.54
T ₁₀	1.18	2.25	3.43	0.53
SEM	0.052	0.0707	0.089	0.029
Effect of Geloi				
0%	1.18	2.13	3.31	0.56
0.25%	1.18	2.23	3.42	0.53
0.50%	1.19	2.25	3.44	0.53
0.75%	1.19	2.24	3.43	0.54
1%	1.18	2.22	3.40	0.54
SEM	0.037	0.050	0.0631	0.020
Effect of Ascorbic acid				
0%	1.18	2.18	3.36	0.55
0.025%	1.19	2.25	3.44	0.53
SEM	0.0231	0.0316	0.040	0.013

4. Conclusion

Broilers reared under chronic heat stress impacted improvement in serum albumin, serum globulin, total protein and A/G ratio in various treatment groups as compared to control group. Improvement in serum albumin, serum globulin, total protein and A/G ratio parameters of broilers was observed due to supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) alone and in combination. Supplementation of ascorbic acid or *Tinospora cordifolia* (geloi) may be a positive indicator of immune response in broilers during chronic heat stress.

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