

Effect of *Tinospora cordifolia* and Ascorbic Acid Supplementation on Biochemical Parameters of Broiler Chickens

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Abstract

A 42 days feeding trial was carried out to investigate the effect of *Tinospora cordifolia* (at graded levels) or ascorbic acid alone and in combinations on biochemical parameters using 360 one-day-old broiler chicks in 5x2 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 heat stress. Improvement in various biochemical 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 exert positive response on health status of broilers during chronic heat stress.

Keywords: ALT, Ascorbic Acid, AST, *Tinospora cordifolia*, Triglyceride

Introduction

Broilers have been chosen for higher growth rates, better feed conversion (FCR) and improved meat yield for many decades. To gain 1850 g body weight in broiler it was required more than 12 weeks before fifty years back, however, through advances in genetic selection and dietary interventions, a 2268 g broiler can now be raised in 6 to 7 weeks. Genetic and dietary improvement in broiler production is extremely important in terms of efficiency of meat production; however, full genetic potential of broilers cannot be obtained unless proper environment is maintained in the broiler house. Stress creates physical and chemical changes in broilers by affecting the homeostatic balance in broilers. One of the most important stress factors in poultry farming is temperature. The thermo neutral zone varies from 13-24°C for poultry. Beyond that, temperature on either side of the critical zone (24-38°C and 0-13°C) may cause negative effect and it may be detrimental for broilers. Many research studies confirmed that when the house temperature exceeds 30°C, mortality and morbidity rates was increased. At an average humidity, poultry birds start panting, when the ambient temperature reaches 29.4°C (North and Bell, 1990). To this end, various feed additives have been served to prevent stress and better broiler health. Antibiotics have been used for this purpose for over 50 years, which adversely affect the human health due to residual effect of antibiotic in meat and meat byproduct.

Tinospora cordifolia (Geloi) grows in the tropical and subtropical areas in India (Sengupta *et al.*, 2011). The stem of *T. cordifolia* (Geloi) has potential application in food systems as a biologically potent nutraceutical and as an antioxidant, because it can decrease oxidative stress with consequent health benefits (Bhawya and Anilakumar 2010). Under normal conditions, poultry can synthesize ascorbic acid (Vitamin C) within their body but endogenous synthesis may not be enough to provide the full need of poultry during heat stress, thus, the need of this vitamin may increase during heat stress (Lin *et al.*, 2006). Ascorbic acid supports to save cells from oxidative damage. Ascorbic acid supplementation augmented productivity, immune responses and survivability under nutritional stress (Zulkifli *et al.*, 1996). The addition of ascorbic acid to the diet of bird improves the immune response during heat stress (Zahraa, 2008). Thus, we can say that both geloi and ascorbic acid have their role to combat against heat stress. Their effect on broilers to combat heat stress particularly in combination has not much studied. The heat stress can be judged indirectly by study of blood biochemical parameters like ALT, AST, Creatinine, glucose etc. Therefore, present study was designed to evaluate the effect of supplementation of *Tinospora cordifolia* or ascorbic acid alone and in combinations in poultry birds on various biochemical parameters in arid zone of Rajasthan (India).

Materials and Methods

A 42 days feeding trial was carried out from September, 2016 to October, 2016 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 uniformity in various treatment groups. Two levels of ascorbic acid (0 and 0.025% of Ration) and five levels of geloi (0, 0.25, 0.50, 0.75 and 1.0% of Ration) were considered as per factorial design. Treatment include interaction treatments consisted of : T₁-basal ration (BS) with no supplementation; T₂- BS+ 0.25% Geloi (*Tinospora cordifolia*) (G₁) T₃-BS+ 0.50% Geloi (G₂) ; T₄- BS+ 0.75% Geloi (G₃); T₅- BS + 1.0% Geloi (G₄); T₆- BS+ 0.025% ascorbic acid (A); T₇-BS+ G₁ +A ; T₈- BS+G₂+A T₉- BS+G₃+A and T₁₀- BS+G₄+A. Good quality of *Tinospora cordifolia* (Geloi) stem were 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 were fed. 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).

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

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 serum samples were analysed for aspartate transaminase (AST), alanine transaminase (ALT), glucose, creatinine, triglyceride and cholesterol by the Vet Test Chemistry Analyzer using kits supplied by Idexx laboratories, as per the manufacturer's subscribed procedure. Effect of geloi and ascorbic acid at different level was calculated by working means and anova by considering one supplement only. For example, geloi @ 0.25% was supplemented in T₂ and T₇, therefore, both this group were considered for study of main effect of geloi at 0.25% level. Likewise, main effect of other levels and ascorbic acid were calculated. The means of various treatments in which both supplements were given were considered as interactive effect.

Statistical Analysis

The data obtained in the research trial were analyzed statistically for main effect of *Tinospora cordifolia* (Geloi) or ascorbic acid alone as well as interaction (Geloi x Ascorbic acid) in factorial design (5X2) as per Snedecor and Cochran (2004) and significance of mean differences was tested by Duncan's New Multiple Range Test (DNMRT) as modified by Kramer (1957).

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). The average temperature (31°C) during the experimental trial was higher than the recommended normothermia zone *i.e.* 22-28°C (Donkoh, 1989) established for poultry in the tropical regions, which indicated that birds in experimental trial were in heat stress.

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

Period (weeks)	Temperature (°C)				Relative humidity (%)				THI			
	8:00 AM	2:00 PM	10:00 PM	Avg.	8:00 AM	2:00 PM	10:00 PM	Avg.	8:00 AM	2:00 PM	10:00 PM	Avg.
I	32.24	35.54	31.44	33.08	53.3	42.09	53.21	49.53	81.66	83.68	80.58	82.06
II	31.13	33.4	33.83	32.79	50.86	42.71	39	44.19	79.76	81.18	80.98	80.7
III	30.59	32.87	32.84	32.1	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	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	43.9	74	77.66	76.32	76.01
VI	27.45	33.31	26.4	29.05	46.03	27.3	42.94	38.76	74.31	78.13	72.62	75.26
Mean	29.58	32.79	30.65	31	53.63	44.52	47	48.38	78.21	80.7	78.49	79.21

The various biochemical values observed in various treatment groups have been presented in Table 2. The mean serum glucose (mg/dl) were found 220.83, 200.50, 214.00, 212.50, 206.83, 182.67, 170.17, 171.33 174.33 and 174.83 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 201.75, 185.33, 192.67, 193.42 and 190.83 (mg/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 210.93 (mg/dl) in non-supplemented group and 174.67(mg/dl) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect due to geloi supplementation and interaction (Geloi x Ascorbic acid). With respect to ascorbic acid supplementation, serum glucose (mg/dl) was significantly ($P < 0.01$) decreased in ascorbic acid supplemented group. The findings of ascorbic acid supplementation on serum glucose are in line with the observations of Dhore *et al.* (2014), who reported that ascorbic acid supplementation inhibits the gluconeogenesis by exerting the effect of adrenal secretion. The results observed with respect to effect of geloi supplementation in present study are contrary to Khobragade (2003) who reported significant effect on serum glucose. Heat stress is generally thought to increase the glucocorticoids secretion which further, increases gluconeogenesis (Webster, 1983) which might be a one reason for increase in serum glucose concentration in non-supplemented group. The overall mean values of serum triglycerides (g/dl) were 118.33, 99.33, 103.17, 103.83, 104.17, 87.67, 83.50, 85.00, 84.17 and 85.17 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 103.00, 91.42, 94.08, 94.00 and 94.67 (g/dl) in 0%, 0.25%, 0.50%, 0.75% and 1% geloi supplementation, respectively.

Table 2: Effect of supplementation of geloi and ascorbic acid on biochemical parameters

Treatment groups	Glucose (mg/dl)	Triglyceride (g/dl)	Cholesterol (g/dl)	AST (IU/L)	ALT (IU/L)	Creatinine (g/dl)
T ₁	220.83	118.33	136.5	210.83	24.33	0.363
T ₂	200.5	99.33	117.83	197.33	19.5	0.35
T ₃	214	103.17	116.5	204.83	20	0.352
T ₄	212.5	103.83	118.33	196.5	19.67	0.353
T ₅	206.83	104.17	117.17	193.67	19.83	0.352
T ₆	182.67	87.67	111.83	173.33	17.17	0.35
T ₇	170.17	83.5	105.17	166.17	15	0.347
T ₈	171.33	85	107.17	167.83	15.5	0.35
T ₉	174.33	84.17	106.67	169.83	16	0.348
T ₁₀	174.83	85.17	106.67	165.67	16.17	0.348
SEM	11.455	9.622	10.018	11.774	2.779	0.0144
Effect of Geloi						
0%	201.75	103	124.17	192.08	20.75	0.357
0.25%	185.33	91.42	111.5	181.75	17.25	0.348
0.50%	192.67	94.08	111.83	186.33	17.75	0.351
0.75%	193.42	94	112.5	183.17	17.83	0.351
1%	190.83	94.67	111.92	179.67	18	0.35
SEM	8.1	6.804	7.083	8.326	1.965	0.01
Effect of Ascorbic acid						
0%	210.93 ^b	105.77 ^b	121.27 ^B	200.63 ^b	20.67 ^B	0.354
0.03%	174.67 ^a	85.10 ^a	107.50 ^A	168.57 ^a	15.97 ^A	0.349
SEM	5.123	4.303	4.48	5.266	1.243	0.006

Means with different superscripts (a,b) in a column differ significantly ($p < 0.01$), Means with different superscripts (A,B) in a column differ significantly ($p < 0.05$)

With respect to effect of ascorbic acid supplementation, the mean values were recorded 105.77 (g/dl) in non-supplemented group and 85.10 (g/dl) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect due to geloi supplementation and interaction (GeloI x Ascorbic acid). With respect to ascorbic acid supplementation, serum triglycerides (g/dl) was significantly ($P < 0.01$) decreased in ascorbic acid supplemented group. The overall mean values of serum cholesterol (g/dl) were found to be 136.50, 117.83, 116.50, 118.33, 117.17, 111.83, 105.17, 107.17, 106.67 and 106.67 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 124.17, 111.50, 111.83, 112.50 and 111.92 (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 121.27 (g/dl) in non-supplemented group and 107.50 (g/dl) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect due to geloi supplementation and interaction (GeloI x Ascorbic acid). With respect to ascorbic acid supplementation, serum cholesterol (g/dl) was significantly ($P < 0.05$) decreased in ascorbic acid supplemented group.

The findings of study in text are in accordance with the findings of Rindhe *et al.* (2016) who reported significant decrease in serum cholesterol due to supplementation of Vitamin C in broilers. The findings are in partial support with Adenkola and Angani (2017) who reported non-significant decrease in serum cholesterol by supplementation of ascorbic acid in broilers. Majekodunmi *et al.* (2013) reported non-significant increases in serum triglycerides @ 300ppm ascorbic acid in water of broilers. Lower level of cholesterol in geloi at graded levels and ascorbic acid either alone or in combination might be due to reduction of lipid peroxidation and enhancement of clearance of endogenous cholesterol.

The overall mean values of serum AST (IU/L) were found to be 210.83, 197.33, 204.83, 196.50, 193.67, 173.33, 166.17, 167.83, 169.83 and 165.67 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 192.08, 181.75, 186.33, 183.17 and 179.67 (IU/L) 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 200.63 (IU/L) in non-supplemented group and 168.57 (IU/L) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect

due to geloi supplementation and interaction (Geloj x Ascorbic acid). With respect to ascorbic acid supplementation, AST (IU/L) was significantly ($P < 0.01$) decreased in ascorbic acid supplemented group. The overall mean values of serum ALT (IU/L) were found 24.33, 19.50, 20.00, 19.67, 19.83, 17.17, 15.00, 15.50, 16.00 and 16.17 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 20.75, 17.25, 17.75, 17.83 and 18.00 (IU/L) 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 20.67 (IU/L) in non-supplemented group and 15.97 (IU/L) in ascorbic acid supplemented group. The statistical analysis of data revealed non-significant effect due to geloi supplementation and interaction (Geloj x Ascorbic acid). With respect to ascorbic acid supplementation, ALT (IU/L) was significantly ($P < 0.05$) decreased in ascorbic acid supplemented group.

The findings with respect to ascorbic acid supplementation, are in line with the findings of Adenkola and Angani (2017) who reported significant improvement in serum AST and ALT by supplementation of ascorbic acid in broilers and in partial agreement with findings of Majekodunmi *et al.* (2013) who reported non-significant improvement with supplementation of ascorbic acid @ 300 ppm in water of broiler chicken. Jadhav *et al.* (2014) and Naresh (2016) also reported significant improvement in ALT and AST by supplementation of polyherbal feed in broilers, which was on contrary to findings with geloi supplementation. The level of ALT is used as a liver function test and elevated levels monitored liver malfunction (Murray *et al.*, 1990). Lower levels of AST and ALT on supplementation of geloi and ascorbic acid either alone or in combination in broilers could be due to antioxidant activity of both which may act together to scavenge the free radicals during stress *i.e.* chronic heat stress. Presence of various phytochemicals in extracts of geloi indicates antioxidant property of geloi, which was earlier supported by phytochemical study of geloi in the present study. The overall mean values of serum creatinine (g/dl) were found to be 0.363, 0.350, 0.352, 0.353, 0.352, 0.350, 0.347, 0.350, 0.348 and 0.348 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 0.357, 0.348, 0.351, 0.351 and 0.350 (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 0.354 (g/dl) in non-supplemented group and 0.349 (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 and indicated that there was no adverse effect on kidney by supplementation of geloi and ascorbic acid either alone or in combination.

Conclusion

Broilers reared under chronic heat stress impacted improvement in glucose, triglyceride, cholesterol, creatinine, AST and ALT in various treatment groups as compared to control group. Biochemical parameters such as glucose, triglyceride, cholesterol, AST and ALT were found to be significant due to ascorbic acid supplementation. Improvement in various biochemical of broilers was observed due to supplementation of ascorbic acid or *Tinospora cordifolia* (geloj) alone and in combination. Supplementation of ascorbic acid or *Tinospora cordifolia* (geloj) may exert positive response on health of broilers during chronic heat stress.

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Conflict of Interests

There is no conflict of interest.

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