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Amelioration of stress in broiler chickens by feeding amla

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ABSTRACT

An experiment was carried out to test the efficacy of supplementing amla (*Emblica officinalis*) in broiler chickens diet from 1 to 6 weeks of age. Stress was induced by injecting adrenocorticotrophic hormone for 5 days from 22 d of age @ 3 IU / kg body weight. Amla fruits were dried and pulverized. The dietary treatments were T_1 (standard diet with 0.0% amla), T_2 (standard diet with 0.5% amla), T_3 (standard diet with 1.0% amla), T_4 (standard diet with 2.0% amla), T_5 (standard diet with 250 mg/kg diet vitamin C). Blood samples were collected on d 21, 28 and 42 to measure the plasma antioxidants such as glutathione peroxidase (GSH-Px), reduced glutathione (GSH), superoxide dismutase (SOD), malonaldehyde (MDA) and plasma corticosterone and serum thyroxin. Results showed that MDA level was reduced significantly in amla and vitamin C fed groups compared to control. However, GSH-Px, SOD, GSH and thyroxin level did not vary significantly between treatment groups. The plasma corticosterone level was significantly reduced in amla and vitamin C supplemented groups groups compared to control. It can be concluded that supplementing broiler diet with amla powder at 0.5% or at higher level can act as antistress agent in broiler chicken production.

Key words: Amla,Broiler diet, *Emblica officinalis*, Stress reduction

Stress induces deleterious effects in the broiler chickens mainly by reducing the status of cellular defense systems, particularly the antioxidant enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and water or lipid soluble antioxidants such as ascorbic acid and vitamin E (Pigeolet *et al*. 1990). McKee *et al*. (1997) suggested that ascorbic acid supplementation conserved body energy stores that might be used for energy metabolism during stress. Chickens can synthesize vitamin C (Satterlee *et al*. 1989) but the metabolic need for vitamin C is likely to increase during disease and stress conditions. Increased serum concentrations of vitamin C in broiler chickens depressed malondialdehyde (MDA) concentration, indicating prevention of free radical production and consequently cell damage (Sahin *et al*. 2003).

Presently emphasis is directed towards herbal formulations, which can be helpful in the stress management and related disorders. *Emblica officinalis*, commonly known as amla (Indian gooseberry) is extensively cultivated all over India, Sri Lanka, Malaysia, China, Pakistan and Bangladesh. The fruits of the plant are used in *Ayurveda* as a potent rasayana (revitalizers, biological response modifiers), in which the *E. officinalis* was added as an antistress agent. The low molecular tannoid principles of *E. officinalis* were reported to be responsible for the anti-oxidative activity (Bhattacharya *et al.* 2000). The present study was organized with the aim of studying the anti-stress effects of different levels of amla and comparing these effects with vitamin C taken as a standard anti-stress agent in broilers through feeding and to understand the optimal level of inclusion in the diet.

MATERIALS AND METHODS

Day-old Vencobb broiler straight-run chicks (120) were wing banded, weighed and randomly allotted to 5 groups with 3 replicates of 8 chicks each and reared in cages. During the 0 to 42 d trial period, the maximum and minimum temperature and relative humidity (RH) of the poultry house were recorded. During the 42 d study period, the maximum temperature ranged between 33.07 and 35.07°C and the minimum temperature ranged between 31.00 and 33.85°C. The RH of the poultry house ranged from 81.14% in the morning to 31.21% in the evening.

The birds were fed *ad lib.* and water was freely available. The pericarb of locally procured fresh amla was removed, air-dried, pulverized and added to the experimental diet. The experimental standard diet was formulated following Bureau of Indian Standards (BIS 1992) and fed to birds as per the following treatment schedule: T_1, standard diet (control); T_2,

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standard diet + amla 5 g /kg feed (0.5%), T_3, standard diet + amla 10 g / kg feed (1.0%), T_4, standard diet + amla 20 g / kg feed (2.0%), T_5, standard diet + synthetic vitamin C 250 mg/ kg feed. The broiler starter and finisher diets were fed from 1 to 21 and 22 to 42 days age, respectively. Adrenocorticotropic hormone (ACTH) was injected to all the broiler chickens @ 3 IU/kg body weight for 5 d from 22 d of age (Puvadolpirod and Thaxton 2000).

Variables measured

At 21, 28 and 42 d of age, 6 broilers from each group were selected and blood samples were collected to measure plasma malonaldehyde (Yagi 1976), glutathione peroxidase (Rotruk *et al*. 1973), reduced glutathione -GSH (Moron *et al*. 1979) and superoxide dismutase (Kono 1978). Plasma corticosterone and serum thyroxin levels were measured as per the standard radioimmunoassay methods using commercial kits. All the data collected were analyzed by completely randomized block design and two factor analysis as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The MDA level was significantly reduced in amla and vitamin C fed groups compared to control even prior to stress induction (III week) which might be due to the continued effect of amla and vitamin C against lipid peroxidation (Table 1). Maini *et al.* (2007) also observed lower MDA level at third week of age in broiler chicks consuming amla in diet from day 1. The MDA level was significantly lower (P<0.01) at fourth week of age in T_2 to T_5 compared to control. At sixth week of age also, the group T_4 had the lowest MDA level compared to other groups. The results of our study is in close agreement with Ramnath *et al.* (2008) who reported lower MDA level in heat stressed broilers fed rasayana containing *Emblica officinalis.* Also, Belge *et al*. (2003) and Sahin *et al*. (2003) reported that vitamin C supplementation to broiler chickens reduced lipid peroxidation. Similarly, Bhattacharya *et al*. (1999) and Anila and Vijayalakshmi (2003) had also reported that administration of amla to rats lead to reduction in MDA level in various tissues. The reduced level of plasma MDA in the amla and vitamin C fed groups indicated the protective role of amla even at 0.5% level in diet in reducing lipid peroxidation and this effect was comparable to vitamin C.

The plasma SOD level did not show significant difference among the treatment groups. The natural cellular antioxidant enzymes include SOD, which scavenges superoxide radicals by speeding up their inactivation. It is possible that, in stress, the increased level of SOD lead to increased generation of peroxides which lead to reduction in GSH-Px level resulting in non-effective scavenging activity. *Emblica officinalis* appears to mitigate these stress induced effects, tending to normalize SOD activity and increasing the activity of GSH-Px (Bhattacharya *et al*. 2000). On the contrary, elevated SOD activity was reported in broiler chicks fed amla (Maini *et al*. 2007, Ramnath *et al*. 2008).

The plasma GSH-Px level was not significantly influenced by the treatments but there was a highly significant (P<0.01) variation between week intervals (Table 1). The GSH-Px activity was not increased after ACTH administration from pretreatment levels in all the groups of birds. However, at 6th week of age the plasma GSH-Px activity increased in all the groups of birds which might be due to increase in environmental temperature above 34°C during the study period (Altan *et al*. 2003). Belge *et al*. (2003) and Ramnath *et al.* (2008) indicated that rise in GSH-Px activity may be a

Table 1. Mean (±SE) plasma malondialdehyde, superoxide dismutase, glutathione peroxidase and reduced glutathione level before and after adrenocorticotropic hormone injection in broilers fed amla and vitamin C from 1 to 6 weeks of age

Treatment	MDA (nmol/ml)			SOD (U/ml)			GSH-Px (U/g protein)			GSH (mg/dl)		
	Age in weeks											
	III	IV	VI	III	IV	VI	III	IV	VI	III	IV	VI
T_1 0% amla	2.10^{a} ±0.09	1.68^{A}± 0.08	1.18^{a}± 0.03	0.86± 0.14	0.75± 0.11	0.73± 0.05	18.81^{Y}± 0.53	19.06^{Y}± 0.51	21.89^{X}± 0.53	73.73^{Z}± 1.29	78.85^{Y}± 1.76	86.13^{X}± 7.13
T_2 0.5% amla	1.50^{b}± 0.11	1.24^{B}± 0.16	1.04^{bc}± 0.03	0.83± 0.09	0.73± 0.24	0.86± 0.07	18.52^{Y}± 0.56	18.82^{Y}± 0.62	21.80^{X}± 0.80	82.13^{Y}± 2.80	82.49^{Y}± 4.82	90.10^{X}± 4.81
T_3 1.0% amla	1.78^{ab}± 0.15	1.19^{B}± 0.03	1.09^{abc}± 0.06	0.78± 0.14	0.90± 0.14	0.95± 0.20	19.58^{Y}± 0.80	19.67^{Y}± 0.82	21.55^{X}± 0.66	84.62^{Y}± 3.15	86.54^{X}± 3.35	86.20^{X}± 8.34
T_4 2.0% amla	1.67^{b}± 0.14	1.22^{B}± 0.09	0.97^{c}± 0.05	1.22± 0.07	0.87± 0.13	0.93± 0.23	19.24^{Y}± 0.81	19.37^{Y}± 0.78	21.05^{X}± 0.67	87.48^{Y}± 4.60	88.16^{Y}± 2.71	90.23^{X}± 1.82
T_5 vitamin C 250 mg/kg	1.69^{b}± 0.12	1.23^{B}± 0.02	1.17^{ab}± 0.05	0.82± 0.12	1.06± ±0.18	0.75± 0.09	17.02^{Y}± 0.41	17.02^{Y}± 0.74	19.68^{X}± 0.66	84.70^{Z}± 3.55	90.59^{Y}± 4.03	93.18^{X}± 4.78

Each value is mean of 6 observations; Means bearing different lower case (P<0.05) and uppercase (P<0.01) in MDA values within a column differ significantly. Means bearing different superscripts in GSH-Px and GSH values within a row differ significantly (P<0.01).

Table 2. Mean (±SE) plasma corticosterone (*ng/ml*) and thyroxin (mg/ml) before and after adrenocorticotrophic hormone injection in broilers fed amla and vitamin C from 1 to 6 weeks of age

Treatments	Corticosterone			Thyroxin		
	Age in weeks					
	III	IV	VI	III	IV	VI
T_1 0% amla	17.29±0.54	17.76^{a}±0.26	16.68±0.24	2.57±0.05	2.43±0.10	2.42±0.10
T_2 0.5% amla	15.52±0.62	14.09^{c}±0.28	17.38±1.44	2.42±0.10	2.46±0.11	2.48±0.16
T_3 1.0% amla	14.47±0.54	15.09^{bc}±0.87	16.13±0.26	2.38±0.06	2.31±0.02	2.34±0.05
T_4 2.0% amla	14.97±0.81	15.14^{bc}±0.70	17.75±0.72	2.48±0.18	2.51±0.13	2.51±0.12
T_5 vitamin C 250 mg/kg	16.68±0.61	16.25^{ab}±0.57	16.32±0.58	2.48±0.09	2.67±0.01	2.61±0.04

Each value is mean of 6 observations: Means bearing different superscripts in column differ significantly (P<0.05).

response to the need for further enzymatic capacity to deal with the production of H_2O_2 in heat stress.

The plasma GSH level did not vary significantly between treatment groups but varied significantly (P<0.01) between different week intervals (Table 1). The increased level of GSH is the first line of defense against pro-oxidant stress and GSH system may have the ability to manage oxidative stress with adaptational changes in enzymes regulating GSH metabolism (Ahmed *et al*. 2000). Intracellular mechanisms exist which can regenerate vitamin C from its inactive metabolite dehydroascorbate by reduced glutathione (Bhattacharya *et al*. 1999). The increase in level of reduced glutathione observed in the present study after ACTH injection may be due to increased demand to maintain membrane stability (Robinson *et al*. 1993), which was indicated by reduced MDA level in amla fed group. On the contrary, Ramnath *et al.* (2008) observed elevated GSH level plasma of broiler chicks fed *Brahma Rasayana* containing *Emblica officinalis.*

The experiment was conducted during higher environmental temperature ranging from a minimum of 31.0°C and maximum of 35.07°C and the RH was very low reaching up to 31.21%. This could be one of the factors leading to a non-significant difference in the plasma anti-oxidant enzymes in amla and vitamin C supplemented birds compared to control group, since the enzyme activity can undergo adaptive changes during continuous exposure to stress.

The plasma corticosterone level decreased significantly in amla and vitamin C fed groups during fourth week of age (Table 2). However, there was no variation between treatments during third week of age. This indicated the ameliorative effect of amla during the period of stress. The vitamin C has a restrictive action on adrenal steroidogenesis and inhibits adrenal steroid hydroxylating enzymes thereby depressing the plasma corticosterone level (Satterlee *et al*. 1989, Mahmoud Kamel *et al.* 2004).

The serum thyroxin level did not show any difference among the treatment groups (Table 2). Vitamin C was known to elevate thyroxin by influencing thyroid activity which may be responsible for attenuating the negative responses of stressors in poultry (Sahin *et al*. 2003). However, in our study amla and vitamin C did not elevate thyroxin in birds stressed with ACTH injection.

The effects of feeding 0.5% or higher levels of amla to broiler chickens on anti-oxidant enzymes system and on plasma corticosterone levels were comparable to feeding of synthetic vitamin C @ 250 mg / kg diet during higher environmental stress conditions.

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