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Anti-stress properties of *Citrus limon* in chickens

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Stress, a major concern for poultry industry especially in hot regions of the world reduces feed intake, body weight gain, carcass yield, increase morbidity and mortality and susceptible to changes in behavioural and physiological response in broilers (McGeehin and Mirabelli 2001, Altan *et al*. 2003). Heat stress increases oxygen consumption resulting into increased production of free radicals/reactive oxygen species (ROS) possibly by disruption of the electron transport assemblies of the membrane (Valko *et al.* 2007). Increased production of free radicals/ROS may be responsible for molecular changes in DNA, proteins, lipids and other biological molecules (Bruskov *et al.* 2002). Lemon (C*itrus limon* L.) is a good source of vitamins and important macro- and micro-minerals, carbohydrates, proteins, lipids and beta -carotene (Paul and Shaha 2004). Poultry has an ability to synthesize vitamin C, but this ability is inadequate under stress conditions (Padayatty *et al*. 2003). Vitamin C supplementation increases the feed conversion ratio and immune status in birds (Takahashi *et al*. 1991). It also alleviates the negative side effects of stress (Schwedhelm *et al.* 2003). Decrease in plasma ascorbate levels also reduces the plasma concentration of vitamin E (Padayatty *et al.* 2003). Therefore present study was planned to elucidate the anti-stress effects of lemon water supplementation in Vanaraja chicks during summer.

Experimental protocol and animals: Day-old chicks (120) of Vanaraja were procured from the Department of Animal Husbandry, J&K. After 2 weeks of brooding, chicks were randomly divided into 2 groups of 40 chicks with 4 replicates of 10 chicks each. Group 1 chicks were kept as control and offered normal diet whereas, group 2 chicks were offered lemon juice 2 ml/litre (0.2% v/v) in drinking water continuously from 2 to 8 weeks. The study was conducted during summer (May and June 2008), and during entire study period environmental temperature and humidity were recorded. Temperature humidity index (THI) was calculated as per Mc Dowell (1972).

Biochemical assays: Blood was collected directly from the heart using 18 gauge needle in clean sterile heparinised tubes from 2 chicks of each replicates and 8 chicks from each group after 2 weeks of supplementing lemon water (4 weeks of age). A 8 however weeks, blood was collected from the tarsal vein. Whole blood was used for the estimation of blood glutathione (GSH) and haemoglobin. Erythrocyte lysate (1%) was used for the catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) whereas, 33% lysate was used for the determination of lipid peroxidation (LPO) activity as per Ohkawa *et al.* (1979). The activity of SOD and catalase was measured according to Marklund and Marklund (1974) and Aebi *et al.* (1987) respectively. The GSH-Px and GSH activity in erythrocyte lysate were assayed by the methods of Hafeman *et al.* (1974) and Beutler *et al.* (1975) respectively. Total protein, albumin, total cholesterol, creatinine, total bilirubin and plasma calcium concentration were estimated by standard kits using chemistry analyzer. Globulin was estimated by subtracting albumin from the total

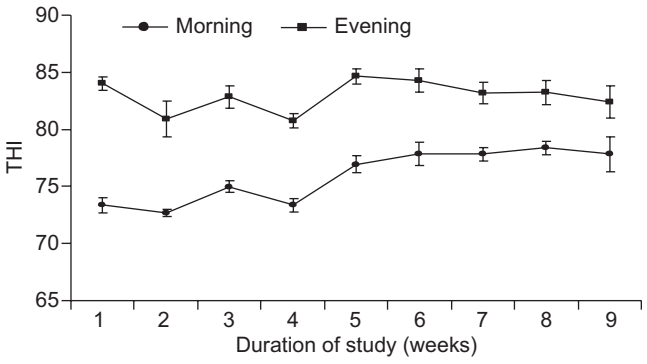

Fig. 1. Alterations in the temperature humidity index (THI) of morning and evening during entire periods of the study period.

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Table 1. Effect of lemon juice supplementation in drinking water on different stress parameters at different age groups in Vanaraja chickens

Stress parameters (units)	Four weeks of chickens		Eight weeks of chickens	
	Control Group-I	Lemon supplemented Group-II	Control Group-III	Lemon supplemented Group-IV
Catalase (µmole of H_2O_2 decomposed min^{-1} $mgHb^{-1}$)	42.15^{B}±6.64	33.61^{B}±3.61	24.08^{B}±4.34	35.10^{B}±3.19
SOD ($mgHb^{-1}$)	0.068^{aB}±0.004	0.100^{aB}±0.020	0.165^{bA}±0.005	0.200^{bB}±0.013
GSH-Px ($mgHb^{-1}$)	3.42^{aA}±0.48	5.17^{aA}±0.16	11.92^{Bb}±0.42	6.05^{bB}±0.16
GSH (n moles ml^{-1})	47.34^{aB}±3.29	62.344^{aA}±8.13	112.85^{bB}±1.79	110.71^{bB}±6.11
LPO (n moles MDA produced g Hb^{-1} h^{-1})	3.26^{a}±0.26	2.14^{b}±0.34	3.97^{b}±0.59	3.07^{b}±0.15
Hb (g %)	9.78^{a}±0.37	10.05^{a}±0.37	10.72^{B}±0.35	11.40^{B}±0.25
Total protein (g %)	6.56^{a}±0.21	6.99^{a}±0.18	7.64^{b}±0.19	8.40^{b}±0.24
Albumin (g %)	1.32^{a}±0.098	1.27^{a}±0.089	2.08^{b}±0.052	2.12^{b}±0.096
Globulin (g %)	5.67^{a}±0.139	5.72^{a}±0.117	5.82^{a}±0.096	5.71^{a}±0.135
A/G ratio	0.23^{a}±0.015	0.22^{a}±0.014	0.37^{b}±0.014	0.36^{b}±0.006
Creatinine (mg/dl)	1.18^{aA}±0.029	0.89^{aA}±0.137	1.35^{B}±0.060	1.12^{A}±0.247
Total bilurubin (g/litre)	0.29^{a}±0.02	$0.26^{c\pm}$0.04	0.56^{bB}±0.06	0.40^{A}±0.02
Total cholestrol (mg %)	235.85^{a}±9.18	218.92^{a}±7.29	328.36^{b}±32.00	308.8^{b}±26.69
Plasma Ca^{+2} conc. (g/litre)	6.99^{a}±0.22	6.79^{a}±1.86	12.45^{b}±1.87	16.00^{b}±1.25

Values are expressed as mean±SE of 8 birds; Capital alphabets: different superscript differs significantly (P<0.05) between groups with in a time period (4 weeks); small alphabets: different superscript differs significantly (P<0.05) between time periods (48 weeks of age within a group).

protein. The data were expressed as mean±SE. Statistical analyses were done by one-way ANOVA followed by Dunnet's test with P 0.05 as a limit of significance.

Various stress parameters estimated during study at pre-determined time intervals are presented in the Table 1 whereas changes in the THI of morning and evening during entire study is presented in Fig. 1. During stress, excess energy generation in mitochondria served as the primary source of excess superoxide production in tissues (Andreyev *et al.* 2005). In present study, the SOD activity increased significantly (P<0.05) in lemon water chickens at 8 weeks of age. Increased erythrocyte SOD activity in birds in lemon water supplementation indicates increased ability of the tissues to handle excessive free radicals generated during oxidative stress (Andreyev *et al.* 2005). Catalase activity decreased significantly in lemon water supplemented group as compared to control. Reduced activity suggested that the supplementation helps in scavenging the excess H_2O_2 production during stress, which is responsible for several degenerative diseases (Sen *et al.* 2006). The activity of GSH-Px was significantly high (P<0.05) in lemon water group at 4 weeks of age and increase was more in control as compared to treated group at 8 weeks. This may be due to increased ability of cell to remove/scavenge excess inorganic and organic peroxides thus help in protecting cell membrane (Sen *et al.* 2006). In present study, GSH was significantly high (P<0.05) in lemon water supplemented group as compared to control. The increased GSH level with age indicates either increase of GSH level synthesis or decrease utilization of GSH (Singh *et al.* 2001). Excess free radicals attack the thiol group of cysteine residue of proteins and polyunsaturated fatty acid (PUFA) chain of phospholipids of biological membrane and increase in free radical-induced LPO deteriorates the activity of biological membranes (Sen *et al.* 2006). Significant reduction in LPO in group 2 as compared to Group I at 4 weeks of age, suggests either reduced free radicals production or increased scavenging activity in birds due to supplementation of lemon water.

Non-significant increase in total protein, albumin and haemoglobin was observed in group 2 birds as compared to their respective control. However, increase was significant as age increases (McDowell 1989). Similarly Sahin *et al.* (2002) and Gursu *et al.* (2004) reported significant increase in total protein and albumin in vitamin C supplemented Japanese quails reared in heat stress. Nonsignificant decrease in globulin and A : G ratio was observed in group 3 birds as compared with control birds at 8 weeks and significant increase in A: G ratio was observed in both the groups as the age progresses. Creatinine concentration increased significantly in group 4 birds as age progress whereas increase was nonsignificant in lemon water offered birds. Similar findings were also reported by Rao *et al.* (2005) in rats. Total bilirubin concentration decreased significantly (P<0.05) at 4 weeks and nonsignificantly at 8 weeks in lemon offered group as compared to control. Total cholesterol decreased non-significantly during the study in treated birds, but increased significantly as the age progresses in both the groups. Similar results were also reported in Japanese quails (Gursu *et al.* 2004) and broilers (Takahashi *et al.* 1991).

SUMMARY

Significant reduction in lipid peroxidation, catalase and increased activity of SOD, GSH-Px and GSH was observed in chicks of lemon water offered non-significantly birds. Total protein, albumin and haemoglobin were increased whereas concentration of globulin was not changed in lemon water offered chickens when compared with control in both. Alterations in different parameters suggests that supplementation of lemon water (0.2% v/v) reduces stress in chickens during summer either by reducing free radicals production or increasing the scavenging ability.

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