



EFFECT OF HERBAL EXTRACT-SUPPLEMENTED FEEDS ON OXIDATIVE STRESS, BIOCHEMICAL MARKERS AND PRODUCTION PERFORMANCE IN JABALPUR COLOUR LAYER BIRDS

S.M. Bashir^{1*}, M. Bilal, M.A. Quadri and A.R. Wani

Department of Veterinary Biochemistry, College of Veterinary Science and Animal, Husbandry, Nanaji Deshmukh Veterinary Sciences University, Jabalpur - 482 001, Madhya Pradesh (India)

¹Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, SK University of Agricultural Sciences and Technology of Kashmir, Shuhama, Srinagar - 190 006, Jammu & Kashmir (India)

*e-mail: showkeen.muzamil82@gmail.com

(Received 2 February, 2014; accepted 25 March, 2014)

ABSTRACT

The present study was aimed to evaluate the impact of ginger, cinnamon bark, chicory and stevia extract-supplemented feeds on serum clinical biochemistry and production performance in Jabalpur colour birds. Fifty four birds of 32 weeks age were randomly divided into 9 groups of 6 birds each. Control group was fed normal basal feed whereas rest groups were fed on feeds supplemented with alcoholic extracts of various herbs separately at 2 levels each [ginger, cinnamon and stevia extract mixed with feed @ 2.5 and 5.0 g kg⁻¹ feed and chicory extract @ 1.25 and 2.5 g kg⁻¹ feed]. Blood samples, collected on 0, 14, 28, 42 and 56th day of treatment, revealed a non-significant decrease in lipid peroxidase and non-significant increase in superoxide dismutase activity in birds of treatment groups. Reduced glutathione showed significant increase in all the treatment groups with higher value in birds fed with chicory (2.5 g kg⁻¹) and cinnamon (5 g kg⁻¹) supplemented feed, respectively. Glucose-6-phosphate dehydrogenase showed significant increase from days 0 to 56 in all the treatment groups. Alanine aminotransferase and aspartate aminotransferase showed a decreasing trend and significant lowering effect was noticed in birds fed with chicory and ginger. The serum creatinine showed significantly low value in chicory (2.5 g kg⁻¹) fed birds. No significant variation was observed in blood urea nitrogen. Increase in egg weight was significant higher in treatment groups with highest increase in ginger @ 5 g kg⁻¹ fed birds. The supplementation of herbal drugs increased body weight significantly as the interval of time increased up to 56th days with highest weight in birds fed with chicory @ 2.5 g kg⁻¹ feed.

Key words: Blood urea nitrogen, chicory, cinnamon, creatinine, ginger, oxidative stress, stevia, superoxide dismutase

INTRODUCTION

During normal metabolism animal body creates highly reactive and toxic by-products often referred to as oxidative molecules. In turn, the body has developed natural defenses to protect against oxidative molecules or even take advantage of them (Fang, 1997). This imbalance between

production of oxidative molecules and body's natural defenses most often leads to a disease inducing process known as "oxidative stress" (Adly, 2010). Oxidative stress is recognized as a cause to the progression of a long list of diseases including cardiovascular disease, cancer, central nervous system disorders, diabetes, inflammatory bowel, rheumatoid arthritis, respiratory problems, autoimmune diseases, liver and kidney diseases, etc. The most well studied oxidative molecules are reactive oxygen species (ROS). ROS play vital role in the initiation and progression of diabetes and cardiovascular disease. When the stress reducing herbal supplementation was consumed it resulted in the normalization of various biomarkers (Das and Kathiriya, 2012) which showed elevated level in stress and also improved growth and production performance in birds (Mansoub, 2011). Dietary supplementation of ginger powder improved laying performance and serum and egg yolk antioxidant status and enhanced dietary oxidation stability in a dose-dependent manner (Zahu *et al.*, 2011). Dietary supplementation with cinnamon in boilers improved growth performance (Balasasirekha and Lakshmi, 2011; Moula *et al.*, 2012). Chicory supplementation in diet of boilers resulted in improved performance of birds (Izadi *et al.*, 2013). So keeping in view the above facts, present study was undertaken to evaluate the effect of herbal supplementation in feeds on the oxidative stress, biochemical markers and production performance in Jabalpur colour birds.

MATERIALS AND METHODS

The study was conducted in September, 2012 in the Department of Poultry Science, Adhartal, Jabalpur, Madhya Pradesh (India). The ginger (*Zingiber officinale* Roscoe) and cinnamon (*Cinnamom verum* Presl.) extracts were prepared by soaking 500 g powdered ginger stem and cinnamon bark, separately, in 2 L 90% ethyl alcohol for overnight and then extracted till complete exhaustion by percolation several times with ethanol as a solvent. The solvent was evaporated at 50°C by using rotavapour apparatus connected to a vacuum pump till a semisolid ethanolic extract was obtained which was finally dried in a vacuum desiccators (Shalaby and Hamowieh, 2010). In case of chichory (*Cichorium intybus* L.) whole plant was used for extract preparation with 80% ethanol, concentrated at 40°C using a rotavapour and dried (Pushpuraj *et al.*, 2007). In case of stevia (*Stevia rebaudiana* Bertoni) dried leaves (1.0 kg) were first defatted with *n*-hexane and then extracted with medium-polar extract using benzene: acetone in 1:1 (v/v) ratio. The extracts were then distilled and simultaneously dried *in vacuo* using rotatory evaporator (Mishra *et al.*, 2011).

Fifty four birds of Jabalpur colour breed (32 weeks old) were divided into 9 groups with 6 birds in each group. All the birds were provided basal diet having 2700 ME k cal kg⁻¹ feed and 17% protein. The treatments comprised of four herbal extract viz., ginger, chicory, cinnamon and stevia added separately @ 2.5 and 5.0 g extract kg⁻¹ feed, except in case of chicory where the extract was supplemented @ 1.25 and 2.5 g kg⁻¹ feed. Control group birds were fed on basal diet without any supplementation. All the birds were kept in individual cages under standard management conditions. Blood samples were collected from all experimental birds from wing vein in a sterile vial with all aseptic precautions on 0, 14, 28, 42 and 56th day of experiment. Half the blood sample was kept for oxidative indices and rest sample for serum separation for biochemical estimations.

Membrane peroxidative damage (lipid peroxidise, LPO) in erythrocytes was determined in terms of malondialdehyde production (MDA) according to the method of Rehman (1984). For this, 1 mL 33% packed erythrocytes was incubated at 37°C for 2 hr and 1 mL 10% TCA added. After thorough mixing, the reaction mixture was centrifuged at 2000 rpm for 10 min. To each 1 mL supernatant 1 mL 0.67% TBA was added and kept in boiling water bath for 10 min., cooled and diluted with 1 mL distilled water. Blank was prepared by adding all the reagents, except packed erythrocytes. The absorbance was read at 535 nm. Reduced glutathione was estimated by 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB] method suggested by Prins and Loos (1969). In this

method, RBC packed (0.2 mL) [33% dilution in PBS] was added to 4 mL 0.08 N H_2SO_4 and mixed carefully. After 10 min. standing at room temperature, 0.5 mL tungstate solution was added to clear the brown haemolysate. The tube was stoppered and the mixture shaken vigorously for 5 min. in order to avoid bubble formation. The suspension was centrifuged for 15 min. at 2000 rpm at room temperature; 2.5 mL TRIS buffer and 0.2 mL DTNB reagent added to 2 mL supernatant and mixed well. Absorbance was measured immediately at 412 nm against blank (distilled water). Superoxide dismutase (SOD) activity was determined in erythrocyte haemolysate as per Madesh and Balasubramanian (1998). In a set of test tubes 650 μL of phosphate buffer saline was taken. To this, 30 μL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was added. The sample (10 μL) was added to only one of the test tubes. Then 75 μL pyrogallol was added to both the tubes and mixture incubated for 5 min. at room temperature. To stop this reaction, 750 μL DMSO (dimethyl sulfoxide) was added to both the tubes and finally 10 μL sample added to the second tube to notice the percentage of reduction of MTT formation. The colour development was read at 570 nm. The results were expressed in units g^{-1} haemoglobin. Glucose-6-phosphate dehydrogenase was estimated by using diagnostic kit. Alanine aminotransferase, aspartate aminotransferase, creatinine and blood urea nitrogen were estimated by using diagnostic kit (Erba). Egg production, egg weight and body weight was measured fortnightly basis. Data was analyzed using hierarchical analysis of variance (Steel and Torrie, 1992).

RESULTS AND DISCUSSION

The lipid peroxidase in birds showed highly significant variation between treatments and periods. A significant increase was noticed in all treatment groups as compared to control. Ginger and chicory significantly reduced LPO at 56th day. The cinnamon and stevia showed no significant variation among intervals. The SOD activity showed increase as the feeding day progressed from 0 day to 56 day, except the treatment group fed with stevia. Significantly high SOD activity was observed in treatment groups fed with chicory. The reduced glutathione (GSH) showed significant increase in all the treatments and intervals upto day 56. The significant higher value was recorded in chicory (2.5 g kg^{-1} feed) and cinnamon (5 g kg^{-1} feed) groups, respectively, while the groups treated with ginger (5 g kg^{-1} feed) and stevia (5 g kg^{-1} feed) showed raised GSH as compared to 0 day. Glucose-6-phosphate dehydrogenase (G6PD) assay revealed no change in control but a significant increase from day 0 to 56 in all treatment groups. Significant increase was recorded in higher dose groups of herbal supplementation. Ginger extract possesses antioxidative characteristic since it can scavenge superoxide anion and hydroxyl radicals (Bhandari *et al.*, 2005).

Hassan and EL-Gendy (2003) reported that antioxidant enzymes viz., glutathione-S-transferase, superoxide dismutase, catalase activities and reduced glutathione decreased in the liver of CCl_4 toxicity group but got reversed when treated with ginger (1% in diet) for one month. Hassan and Yousef (2010) reported a promising role of chicory in ameliorating the oxidative stress and hepatic injury induced by nitrosamine compounds. Further, supplementation of chicory induced reduction in thiobarbituric acid reactive substances (TBARS) and, in turn, improved various biochemical and antioxidant parameters. Milala *et al.* (2009) reported that the lyophilized extract from chicory seeds was rich in polyphenol compounds, contained over 10% total phenolics, including 71% dicaffeoylquinic acids, and was characterized by highest antioxidant activity (0.513 m mol TAEC g^{-1} preparation). Kannappan and Anuradha (2008) found that cinnamon bark extract with total phenolic content of 1.32 $\text{m meugenol equivalents}$ reduced the formation of lipid peroxidation products and lipid hydroperoxides (LHPs) in the plasma and liver, and replenished the enzymatic and non-enzymatic antioxidant system in blood and liver. Ciftci *et al.* (2010) reported that cinnamon oil supplementation significantly decreased serum malondialdehyde (MDA, n mol g^{-1}

Table 1: Changes in blood LPO, SOD, GSH and G6PD in Jabalpur colour birds fed with different doses of ginger, chicory, cinnamon and stevia at various time intervals

Parameters	Feed supplementation		Duration (days)				
	(g kg ⁻¹ feed)		0	14	28	42	56
Blood LPO ($\mu\text{M L}^{-1}$)	Control		3.21 $\pm$ 0.2	3.22 $\pm$ 0.2	3.23 $\pm$ 0.2	3.24 ² $\pm$ 0.23	3.26 ² $\pm$ 0.2
	Ginger	2.5	3.28 $\pm$ 0.2	3.25 $\pm$ 0.2	3.22 $\pm$ 0.2	3.18 ^{1,2} $\pm$ 0.22	3.16 ² $\pm$ 0.2
		5.0	3.13 ^{b;1} $\pm$ 0.2	3.10 ^b $\pm$ 0.2	3.03 ^b $\pm$ 0.2	3.00 ^{ab;1} $\pm$ 0.22	2.82 ^{a;1} $\pm$ 0.2
	Chicory	1.25	3.23 $\pm$ 0.2	3.21 $\pm$ 0.2	3.18 $\pm$ 0.2	3.16 ^{1,2} $\pm$ 0.23	3.14 ² $\pm$ 0.2
		2.5	3.21 ^b $\pm$ 0.2	3.17 ^b $\pm$ 0.2	3.12 ^{ab} $\pm$ 0.2	3.14 ^{ab;1,2} $\pm$ 0.22	2.96 ^{a;1,2} $\pm$ 0.2
	Cinnamon	2.5	3.16 $\pm$ 0.2	3.14 $\pm$ 0.2	3.12 $\pm$ 0.2	3.11 ^{1,2} $\pm$ 0.23	3.10 ² $\pm$ 0.2
		5.0	3.15 $\pm$ 0.2	3.18 $\pm$ 0.2	3.12 $\pm$ 0.2	3.11 ^{1,2} $\pm$ 0.22	3.10 ² $\pm$ 0.2
	Stevia	2.5	3.18 $\pm$ 0.2	3.15 $\pm$ 0.2	3.13 $\pm$ 0.2	3.10 ^{1,2} $\pm$ 0.24	3.09 ² $\pm$ 0.2
		5.0	3.17 $\pm$ 0.2	3.15 $\pm$ 0.2	3.12 $\pm$ 0.2	3.08 ^{1,2} $\pm$ 0.22	3.05 ² $\pm$ 0.2
Blood SOD (units g ⁻¹ Hb)	Control		292.50 $\pm$ 6.2	291.50 $\pm$ 6.2	290.67 $\pm$ 6.3	289.33 ¹ $\pm$ 6.0	288.50 ¹ $\pm$ 6.3
	Ginger	2.5	297.17 $\pm$ 6.0	298.33 $\pm$ 6.0	298.33 $\pm$ 6.5	301.33 ^{1,2} $\pm$ 6.7	302.50 ^{1,2} $\pm$ 6.4
		5.0	298.67 $\pm$ 7.4	300.17 $\pm$ 7.4	302.17 $\pm$ 7.4	303.17 ^{1,2} $\pm$ 7.4	304.17 ^{1,2} $\pm$ 7.4
	Chicory	1.25	305.83 $\pm$ 6.8	306.83 $\pm$ 6.8	307.83 $\pm$ 6.8	308.83 ^{1,2} $\pm$ 6.8	309.83 ² $\pm$ 6.8
		2.5	306.83 $\pm$ 6.4	307.83 $\pm$ 6.4	309.50 $\pm$ 6.5	311.17 ² $\pm$ 6.3	312.17 ² $\pm$ 6.3
	Cinnamon	2.5	297.50 $\pm$ 7.0	298.5 ² $\pm$ 7.0	299.50 $\pm$ 7.0	300.50 ^{1,2} $\pm$ 7.0	301.50 ^{1,2} $\pm$ 7.0
		5.0	297.83 $\pm$ 7.8	298.83 $\pm$ 7.8	299.83 $\pm$ 7.8	301.83 ^{1,2} $\pm$ 7.8	302.83 ^{1,2} $\pm$ 7.8
	Stevia	2.5	301.00 $\pm$ 7.9	302.00 $\pm$ 7.9	302.67 $\pm$ 8.0	303.50 ^{1,2} $\pm$ 7.8	304.50 ^{1,2} $\pm$ 7.8
		5.0	306.83 $\pm$ 7.5	301.33 $\pm$ 7.5	302.33 $\pm$ 7.5	303.33 ^{1,2} $\pm$ 7.5	304.33 ^{1,2} $\pm$ 7.5
Blood GSH ($\mu\text{M L}$)	Control		16.37 ^{a1} $\pm$ 0.55	16.40 ^{a1} $\pm$ 0.4	15.93 ^{a1} $\pm$ 0.7	16.40 ^{b1} $\pm$ 0.6	16.78 ^{b;1} $\pm$ 0.6
	Ginger	2.5	16.83 ^{a;1} $\pm$ 1.1	25.00 ^{b;2} $\pm$ 1.0	33.5 ^{c;2} $\pm$ 1.0	48.17 ^{d;2} $\pm$ 0.7	58.00 ^{e;2} $\pm$ 1.0
		5.0	21.67 ^{a;2} $\pm$ 0.8	33.83 ^{b;3} $\pm$ 1.1	47.17 ^{c;4} $\pm$ 0.6	55.33 ^{d;2} $\pm$ 0.6	70.33 ^{e;3} $\pm$ 0.5
	Chicory	1.25	18.33 ^{a;1,2} $\pm$ 0.8	27.83 ^{b;2,3} $\pm$ 0.8	36.45 ^{c;2} $\pm$ 1.1	46.17 ^{d;3} $\pm$ 0.8	57.83 ^{e;2} $\pm$ 1.4
		2.5	22.67 ^{a;2} $\pm$ 0.8	34.67 ^{b;3,4} $\pm$ 0.9	45.83 ^{c;3,4} $\pm$ 1.1	56.67 ^{d;3} $\pm$ 0.9	76.33 ^{e;4} $\pm$ 0.8
	Cinnamon	2.5	30.67 ^{a;3} $\pm$ 1.3	40.33 ^{b;4} $\pm$ 0.9	49.83 ^{c;4,5} $\pm$ 0.7	57.67 ^{d;3} $\pm$ 0.3	66.00 ^{e;3} $\pm$ 0.3
		5.0	29.50 ^{a;3} $\pm$ 1.3	41.67 ^{b;4} $\pm$ 1.9	53.00 ^{c;5} $\pm$ 1.6	64.50 ^{d;4} $\pm$ 1.4	78.33 ^{e;4} $\pm$ 1.2
	Stevia	2.5	23.50 ^{a;2,3} $\pm$ 1.3	31.67 ^{b;3} $\pm$ 1.4	41.17 ^{c;3} $\pm$ 1.0	49.67 ^{d;2} $\pm$ 1.5	60.83 ^{e;2} $\pm$ 1.4
		5.0	28.00 ^{a;3} $\pm$ 1.2	38.50 ^{b;4} $\pm$ 1.5	48.83 ^{c;4,5} $\pm$ 1.3	58.83 ^{d;3} $\pm$ 1.5	70.00 ^{e;3} $\pm$ 1.5
Blood G6PD (IU L ⁻¹)	Control		36.91 ² $\pm$ 0.5	36.93 ^{1,2} $\pm$ 0.6	37.03 ^{1,2} $\pm$ 0.6	37.00 ¹ $\pm$ 0.6	37.07 ¹ $\pm$ 0.6
	Ginger	2.5	37.52 ^{a;2} $\pm$ 0.5	38.37 ^{ab;2} $\pm$ 0.5	39.28 ^{ab;2} $\pm$ 0.5	40.23 ^{b;2} $\pm$ 0.4	41.66 ^{b;2} $\pm$ 0.5
		5.0	35.88 ^{a;1,2} $\pm$ 0.8	36.91 ^{ab;1,2} $\pm$ 0.6	38.4 ^{b;1,2} $\pm$ 0.7	40.23 ^{bc;2} $\pm$ 0.6	41.51 ^{c;2} $\pm$ 0.6
	Chicory	1.25	35.02 ^{a;1,2} $\pm$ 0.5	35.94 ^{a;1} $\pm$ 0.5	36.71 ^{a;1} $\pm$ 0.5	39.52 ^{b;2} $\pm$ 0.6	40.40 ^{b;2} $\pm$ 0.6
		2.5	35.54 ^{a;1,2} $\pm$ 0.6	37.25 ^{ab;1,2} $\pm$ 0.6	38.92 ^{b;2} $\pm$ 0.6	40.55 ^{bc;2} $\pm$ 0.6	41.65 ^{c;2} $\pm$ 0.6
	Cinnamon	2.5	35.56 ^{a;1,2} $\pm$ 0.6	36.37 ^{ab;1,2} $\pm$ 0.6	37.28 ^{b;1,2} $\pm$ 0.6	39.63 ^{bc;2} $\pm$ 0.5	40.53 ^{c;2} $\pm$ 0.6
		5.0	35.49 ^{a;1,2} $\pm$ 0.6	36.91 ^{b;1,2} $\pm$ 0.6	37.65 ^{b;1,2} $\pm$ 0.6	39.73 ^{bc;2} $\pm$ 0.7	41.10 ^{c;2} $\pm$ 0.6
	Stevia	2.5	34.78 ^{a;1} $\pm$ 0.6	35.76 ^{ab;1} $\pm$ 0.6	37.01 ^{b;1,2} $\pm$ 0.6	39.72 ^{c;2} $\pm$ 0.7	40.13 ^{c;2} $\pm$ 0.6
		5.0	35.55 ^{a;1,2} $\pm$ 0.6	36.60 ^{ab;1,2} $\pm$ 0.6	37.72 ^{c;1,2} $\pm$ 0.6	39.88 ^{bc;2} $\pm$ 0.6	40.98 ^{c;2} $\pm$ 0.6

Means with different alphabets and numbers in superscripts vary significantly ($P < 0.05$) in a row or in a column respectively.

protein). Cinnamon extract reduced melonaldehyde production in rabbits as compared to those fed diets without cinnamon (Jawad *et al.*, 2011). Cinnamon extract treatment to STZ induced diabetic rats improved G6PD activity (Hassan *et al.*, 2012). Similarly, decrease in LPO activity and increase in SOD, GSH and G6PD was noticed in birds fed with *Aloevera* (Sinha, 2012).

The ALT showed no significant variation in control and treatment groups, whereas AST showed highly significant difference between treatments and intervals. No significant variation was found in control, ginger (2.5 g kg⁻¹ feed), chicory (1.25 g kg⁻¹ feed) and stevia (2.5 g kg⁻¹ feed) at different intervals; however, significantly lower activity was recorded in chicory (1.25 g kg⁻¹ feed), cinnamon (5 g kg⁻¹ feed), stevia (2.5 g kg⁻¹ feed) and minimum in ginger (5 g kg⁻¹ feed) on 56th day. As the time progressed, the ALT activity reduced significantly. The lowest ALT activity was found

Table 2: Changes in serum AST (mg dL⁻¹), serum ALT (mg dL⁻¹), serum creatinine (mg dL⁻¹) and BUN (mg dL⁻¹) in Jabalpur colour birds fed different doses of ginger, chicory, cinnamon and stevia at various time intervals

Parameters	Feed supplementation		Duration (days)				
	(g kg ⁻¹ feed)		0	14	28	42	56
Serum AST (mg dL ⁻¹)	Control		165.61±7.0	165.49±7.1	165.75±7.1	166.02±4.6	165.98±7.0
	Ginger	2.5	165.11±6.8	164.94±6.8	164.63±6.8	164.45±6.8	164.28±6.8
		5.0	164.80±7.0	164.49±6.8	164.14±6.9	163.77±7.0	163.44±6.9
	Chicory	1.25	165.73±6.7	165.47±6.6	165.24±6.6	164.99±6.7	164.73±6.7
		2.5	165.98±6.6	165.69±6.4	165.10±6.5	164.70±6.5	164.32±6.5
	Cinnamon	2.5	165.26±6.6	165.00±6.6	164.73±6.6	164.41±6.6	164.14±6.6
		5.0	166.15±7.0	165.70±6.9	165.33±6.9	164.95±6.9	164.55±6.9
	Stevia	2.5	165.91±6.6	165.65±6.6	165.49±6.66	165.32±6.64	165.09±6.62
		5.0	167.17±7.1	166.84±7.0	166.53±7.09	166.13±7.09	165.80±7.07
Serum ALT (mg dL ⁻¹)	Control		35.92±1.3	35.04±1.3	34.29±1.3	34.15±1.2	34.07±1.3
	Ginger	2.5	36.11 ^b ±1.4	34.88 ^{ab} ±1.4	33.58 ^{ab} ±1.2	32.50 ^{ab} ±1.2	31.53 ^a ±1.1
		5.0	36.05 ^b ±1.7	35.07 ^b ±1.7	33.56 ^{ab} ±1.5	32.36 ^{ab} ±1.4	30.90 ^a ±1.3
	Chicory	1.25	35.91 ^b ±1.6	34.65 ^{ab} ±1.5	33.65 ^{ab} ±1.4	32.56 ^{ab} ±1.5	31.55 ^a ±1.5
		2.5	36.08 ^b ±1.4	34.97 ^{ab} ±1.4	33.83 ^{ab} ±1.5	32.88 ^{ab} ±1.7	31.90 ^a ±1.7
	Cinnamon	2.5	36.04±1.1	34.93±1.1	34.46±1.3	33.92±1.3	33.31±1.3
		5.0	36.06±1.3	35.18±1.4	34.34 ^{ab} ±1.5	33.75±1.3	33.18±1.3
	Stevia	2.5	36.17±1.1	35.43±1.1	34.64 ^{ab} ±1.0	34.17±1.1	33.47±1.0
		5.0	35.03±1.3	34.13±1.3	33.36 ^{ab} ±1.3	33.02±1.3	32.42±1.2
Serum creatinine (mg dL ⁻¹)	Control		0.90 ¹ ±0.1	0.91 ¹ ±0.1	0.90 ¹ ±0.1	0.89 ¹ ±0.1	0.88 ¹ ±0.01
	Ginger	2.5	0.95 ² ±0.1	0.93 ^{1,2} ±0.1	0.92 ^{1,2} ±0.01	0.90 ^{1,2} ±0.1	0.89 ^{1,2} ±0.1
		5.0	0.98 ² ±0.1	0.97 ^{1,2} ±0.1	0.95 ² ±0.01	0.93 ^{1,2} ±0.1	0.92 ^{1,2} ±0.1
	Chicory	1.25	1.00 ² ±0.1	0.99 ² ±0.1	0.98 ² ±0.01	0.97 ² ±0.1	0.96 ² ±0.1
		2.5	0.97 ^{b,2} ±0.1	0.95 ^{ab,2} ±0.1	0.93 ^{ab,1,2} ±0.1	0.92 ^{ab,1,2} ±0.1	0.90 ^{a,1,2} ±0.1
	Cinnamon	2.5	0.99 ² ±0.01	0.98 ² ±0.1	0.98 ² ±0.01	0.97 ² ±0.1	0.96 ² ±0.1
		5.0	0.93 ² ±0.1	0.92 ^{1,2} ±0.1	0.92 ^{1,2} ±0.1	0.91 ^{1,2} ±0.1	0.90 ^{1,2} ±0.1
	Stevia	2.5	1.00 ² ±0.01	0.99 ² ±0.1	0.98 ² ±0.1	0.97 ² ±0.1	0.97 ² ±0.1
		5.0	0.97 ² ±0.1	0.96 ² ±0.1	0.95 ² ±0.1	0.94 ² ±0.1	0.94 ^{1,2} ±0.1
BUN (mg d L ⁻¹)	Control		3.77±0.3	3.79±0.3	3.84±0.3	3.83±0.3	3.88±0.4
	Ginger	2.5	3.80±0.3	3.7±0.3	3.62±0.3	3.53±0.3	3.47±0.3
		5.0	3.90±0.3	3.8±0.3	3.73±0.3	3.58±0.3	3.43±0.3
	Chicory	1.25	3.73±0.4	3.63±0.4	3.55±0.4	3.47±0.4	3.38±0.4
		2.5	3.95±0.4	3.87±0.4	3.77±0.4	3.72±0.4	3.62±0.4
	Cinnamon	2.5	3.95±0.4	3.87±0.4	3.77±0.4	3.72±0.4	3.62±0.4
		5.0	3.85±0.1	3.80±0.4	3.68±0.4	3.57±0.4	3.45±0.4
	Stevia	2.5	3.62±0.3	3.55±0.3	3.47±0.3	3.38±0.3	3.33±0.3
		5.0	3.80±0.4	3.72±0.4	3.62±0.4	3.38±0.4	3.33±0.4

Means with different alphabets and numbers in superscripts vary significantly (P<0.05) in a row or in a column, respectively.

in birds fed ginger (5 g kg⁻¹ feed) and chicory (2.5 g kg⁻¹ feed) supplemented feed. Hassan and EL-Gendy (2003) while using ginger for treatment of liver disorders reported its capability of reversing the elevated levels of AST, ALT and bilirubin due to the induced nitrosamine toxicity. Rasheeduz and Mujahid (1998) found that CCl₄ intoxication increased the levels of ALT and AST enzymes in rat serum while treatment with root and root callus extracts of chicory reduced these enzyme levels.

Belal (2011) reported that feeding chicory leaf powder to rats through basal diet @ 10% concentration decreased the elevating of liver enzyme (AST, ALT and ALP) levels in their serum as compared to control. Water extract of chicory (10%), given orally, decreased AST and ALT enzyme activities as compared to control (Ahmed *et al.*, 2009). Similarly, stevia supplement to diet decreased AST, ALT, GPT and bilirubin levels in rats (Das and Kathiriyai, 2012).

Serum creatinine concentration in colour birds fed with different herbal supplementation showed highly significant variation between treatments and days. However, only chicory feeding (2.5 g kg⁻¹ feed) significantly reduced serum creatinine by 7%. On 56th day significantly low values was recorded in chicory, cinnamon and stevia supplemented groups. Blood urea nitrogen (BUN) showed no significant variation between control and treatment groups. BUN showed highly significant variation between treatments and intervals. Significantly low values were observed in all treatment groups with lowest value in chicory (2.5 g kg⁻¹ feed) at day 56. Singh (2012) reported similar results in treatment groups fed with turmeric. Egg weight increased continuously upto 56 days. Increase in egg weight was significantly higher in treatment groups ginger (5g kg⁻¹ feed), chichory (2.5 g kg⁻¹ feed), cinnamon (5 g kg⁻¹ feed) and stevia (5 g kg⁻¹ feed) with increase in egg weight ranging between 19 and 20%, respectively. The increase in egg weight was also significantly higher than control in treatment groups chichory (1.25 g kg⁻¹ feed), cinnamon (2.5 g kg⁻¹ feed) and stevia (2.5 g kg⁻¹ feed). Increase in egg weight has previously been reported soon after herbal supplementation like aloe vera (Sinha, 2012), fenugreek and banaba and turmeric in diet (Singh, 2012). The egg production showed progressive increase with increase in treatment period. Significantly higher number of eggs was recorded in all treatment groups. The increase in egg weight was also significantly higher than control in treatment groups chichory (1.25 g kg⁻¹ feed), cinnamon (2.5 g kg⁻¹ feed) and stevia (2.5 g kg⁻¹ feed). The highest increase in eggs was noticed in ginger (5 g kg⁻¹ feed). Significantly higher number of eggs was observed in all treatment groups as compared to control. Inclusion of chichory supplementation in diet reportedly resulted in increase in egg production and feed efficiency of layers without impairing the egg quality (Chen *et al.*, 2005). Similar results were noticed in birds with supplementation of aloe vera (Sinha, 2012), fenugreek,

Table 3: Changes in egg weight (g) and egg production in Jabalpur colour birds fed different doses of ginger, chicory, cinnamon and stevia at various time intervals

Parameters	Feed supplementation		Duration (days)			
	(g kg ⁻¹ feed)		14	28	42	56
Egg weight (g)	Control		41.07 ^a ±0.6	42.77 ^{ab} ±0.7	43.05 ^{b;1} ±0.8	45.82 ^{b;1} ±1.0
	Ginger	2.5	40.58 ^a ±0.9	42.72 ^b ±0.5	43.49 ^{b;1,2} ±0.7	45.28 ^{b;1} ±0.8
		5.0	40.65 ^a ±0.7	42.78 ^a ±0.7	47.79 ^{a;2} ±0.7	48.67 ^{b;2} ±1.6
	Chicory	1.25	40.50 ^a ±0.6	41.76 ^{b;} ±0.7	45.57 ^{c;2} ±0.6	46.94 ^{c;1,2} ±0.9
		2.5	40.82 ^a ±0.5	42.92 ^b ±0.7	47.61 ^{c;2} ±0.7	48.63 ^{c;2} ±0.8
	Cinnamon	2.5	40.45 ^a ±0.6	41.58 ^a ±0.7	45.58 ^{b;2} ±0.7	46.79 ^{b;1,2} ±0.7
		5.0	40.49 ^a ±0.6	42.58 ^{b;} ±0.7	47.65 ^{c;2} ±0.7	48.48 ^{c;2} ±0.5
	Stevia	2.5	40.40 ^a ±0.6	42.20 ^a ±0.6	45.60 ^{b;2} ±0.6	46.63 ^{b;1,2} ±0.8
		5.0	40.44 ^a ±0.7	42.60 ^b ±0.7	47.68 ^{c;2} ±0.7	48.58 ^{c;2} ±0.8
	Control		33.17±1.1	34.00±1.2	34.00 ¹ ±1.1	33.67 ¹ ±0.9
Egg production (Number of eggs)	Ginger	2.5	33.50 ^a ±0.9	36.33 ^{ab} ±1.0	38.50 ^{b;2} ±1.0	41.00 ^{b;2} ±1.1
		5.0	32.83 ^{a;} ±1.1	36.00 ^b ±1.0	38.83 ^{c;2} ±1.1	42.17 ^{c;2} ±0.9
	Chicory	1.25	33.33 ^a ±1.5	35.67 ^a ±1.3	37.83 ^{b;2} ±1.3	40.17 ^{b;2} ±0.2
		2.5	34.83 ^a ±1.1	37.33 ^a ±1.3	40.17 ^{b;2} ±1.1	42.83 ^{b;2} ±1.2
	Cinnamon	2.5	34.67 ^a ±1.1	36.50 ^{ab} ±1.1	38.33 ^{b;2} ±1.1	40.50 ^{b;2} ±1.1
		5.0	34.83 ^a ±1.1	36.33 ^{ab} ±0.8	38.83 ^{b;2} ±0.7	42.17 ^{b;2} ±1.0
	Stevia	2.5	34.83 ^a ±1.1	36.67 ^{ab} ±1.2	38.83 ^{b;2} ±0.7	41.00 ^{b;2} ±1.3
		5.0	34.83 ^a ±1.3	37.00 ^{ab} ±1.2	39.83 ^{b;2} ±1.3	42.50 ^{b;2} ±1.3

Means with different alphabets and numbers in superscripts vary significantly (P<0.05) in a row or in a column, respectively

Table 4: Changes in body weight (g) in Jabalpur colour birds fed with ginger, chicory, cinnamon and stevia at different time intervals

Parameters	Feed supplementation		Duration (days)				
	(g kg ⁻¹ feed)		0	14	28	42 d	56 d
Body weight (g)	Control		1739 ^a ±43.3	1778.3 ^{a;2} ±40.9	1810 ^{a;1} ±37.8	1861 ^{ab;1} ±40.5	1866 ^{b;1} ±48.1
	Ginger	2.5	1763 ^a ±51.5	1916.5 ^{b;2} ±45.5	1947 ^{c;2} ±44.3	1969 ^{cd;2} ±41.5	1984 ^{d;1,2} ±42.6
		5.0	1753 ^a ±47.3	1764.1 ^{a;1} ±44.1	1826 ^{b;1,2} ±44.0	1867 ^{c;1,2} ±46.3	1965 ^{d;1,2} ±60.0
	Chicory	1.25	1767 ^a ±42.1	1911.3 ^{b;2} ±42.7	1952 ^{c;2} ±41.6	1974 ^{cd;2} ±42.4	1994 ^{d;2} ±45.0
		2.5	1747 ^a ±49.5	1765.6 ^{a;1,2} ±46.2	1833 ^{b;1,2} ±44.1	1881 ^{c;1,2} ±44.1	1983 ^{d;1,2} ±44.1
	Cinnamon	2.5	1774 ^a ±43.4	1917.8 ^{b;2} ±43.6	1948 ^{c;2} ±43.4	1968 ^{cd;2} ±41.2	1984 ^{d;1,2} ±42.8
		5.0	1748 ^a ±48.5	1768.3 ^{a;1,2} ±43.2	1843 ^{b;1,2} ±43.4	1883 ^{c;1,2} ±44.1	1961 ^{d;1,2} ±35.8
	Stevia	2.5	1780 ^a ±41.8	1919.1 ^{b;2} ±43.8	1949 ^{c;2} ±42.9	1971 ^{cd;2} ±41.9	1985 ^{d;1,2} ±43.8
		5.0	1757 ^a ±44.9	1773.5 ^{a;1,2} ±44.1	1848 ^{b;1,2} ±40.6	1968 ^{c;2} ±39.7	1985 ^{c;1,2} ±45.3

Means with different alphabets and numbers in superscripts vary significantly (P<0.05) in a row or in a column, respectively

banaba and turmeric in diet (Singh, 2012). Feeding of chicory in diet had shown increased feed conversion efficiency, body weight and carcass weight in poultry birds (Yusrizal and Chen, 2003). Body weight showed highly significant difference among treatments and days. The supplementation of herbal drugs increases the body weight significantly as the interval of time increases upto 56 days. The highest weight was observed in chichory (2.5 g kg⁻¹ feed) followed by lower dose of chichory (1.25 g kg⁻¹ feed) and Stevia (5 g kg⁻¹ feed). The increase in body weight was significant in all treatments as compared to control. Saeid *et al.* (2010) indicated that groups receiving ginger infusion @ 0.4 and 0.6% of drinking water showed better physiological performance. Mohamed *et al.* (2012) reported that body weight, weight gain, FCR and feed intake showed a significant difference between 0.1% ginger and 0.2% ginger and control. Meat quality and growth performance parameters showed improvement in boilers, supplemented with cinnamon powder in diet (Oh *et al.* 2013). The increase in bodyweight has also been noticed in birds with herbal supplementation by Shrivastava *et al.* (2012, 2013) and Sinha (2012).

Conclusion: The herbal supplements such as ginger, cinnamon, chichory and stevia can be used to reduce the oxidative stress and can be beneficial lipid profile parameters in serum as well as in egg yolk of birds in order to design the low cholesterol eggs for health benefits in human beings.

REFERENCES

- Adly, A. 2010. Oxidative stress and disease: An updated review. *Research Journal of Immunology*, **3**: 129-145.
- Ahmed, L.M., Rehman, R.S. and Rehman, M.A. 2009. Biochemical and histopathological studies on the water extract of marjoram, chichory herbs and their mixture on obese rats. *Pakistan Journal of Nutrition*, **8**: 1581-1587.
- Balasasirekha, R. and Lakshmi, U.K. 2011. Effect of cinnamon and garlic on hyperlipidemics. *International Journal of Nutrition and Metabolism*, **3**(7): 77-89.
- Belal, N.M. 2011. Hepatoprotective effect of feeding celary leaves mixed with chichory leaves and barley grains to hypercholestromic rats. *Asian Journal of Clinical Nutrition*, **3**: 14-24.
- Bhandari, U., Kanojia, R. and Pillai, K.K. 2005. Effect of ethanolic extract of ginger on dyslipidemia in diabetic rats. *Journal of Ethanopharmacology*, **97**: 227-230.

- Chen, Y.C., Nakhthong, C. and Chen, T.C. 2005. Improvement of laying hen performance by dietary prebiotic chicory oligofructose and inulin. *International Journal of Poultry Science*, **4**: 103-108.
- Ciftci, M., Simsek, U.G., Yuce, A., Yilmaz, O. and Dalkilic, B. 2010. Effects of dietary antibiotic and cinnamon oil supplementation on antioxidant enzyme activities, cholesterol levels and fatty acid compositions of serum and meat in broiler chickens. *Acta Veterinaria Brno*, **79**: 33-40.
- Das, K. and Kathiriya, A.K. 2012. Hepatoprotective activity of *Stevia rebaudiana* Bertoni leaves against thioacetamide induced toxicity. *Turkish Journal of Pharmacology Science*, **9**: 343-352.
- Fang, F. 1997. Host/pathogen interactions: Mechanisms of nitric oxide-related antimicrobial activity. *Journal of Clinical Investigation*, **99**: 2818-2825.
- Hassan, H.A. and El-Gendy, A.M. 2003. Evaluation of silymarin and/or ginger effect on induced hepatotoxicity by carbon tetrachloride in male albino rats. *The Egyptian Journal of Hospital Medicine*, **12**: 101-112.
- Hassan, S., Barthwal, R., Nair, M.S. and Syed, H.S. 2012. Aqueous bark extract of *Cinnamomum zeylanicum*: A potential therapeutic agent for streptozotocin-induced type 1 diabetes mellitus (T1DM) rats. *Tropical Journal of Pharmaceutical Research*, **11**: 429-435.
- Hassan, H.A., and Yousef, M.I. 2010. Ameliorating effect of chicory supplemented diet against nitrosamine precursors-induced liver injury and oxidative stress in male rats. *Food Chemistry and Toxicology*, **48**: 2163-2167.
- Izadi, H., Arshami, J., Golian, A. and Reza, M. 2013. Effects of chicory root powder on growth performance and histomorphometry of jejunum in broiler chicks. *Veterinary Research Forum*, **4**: 169-174.
- Jawad, A.H., Rikaby, A.A. and Kassim, F.A.K. 2011. Antioxidant, antidiabetic and lipid lowering effects of cinnamon and vitamin C in hyperglycemic rabbits. *Basic Journal of Veterinary Research*, **10**(2): 33-48.
- Kannappan, S. and Anuradha, C.V. 2008. Antiradical property of cinnamon reduces fructose-induced oxidative stress in rat liver. *Journal of Food Biochemistry*, **32**: 216-233.
- Madesh, M. and Balasubramaniam, K.A. 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian Journal of Biochemistry and Biophysics*, **35**: 184-188.
- Mansoub, N.H. 2011. Evaluation of herbal plants on different parameters of laying hens. *Annals of Biological Research*, **2**: 510-515.
- Milala, J., Grzelak, K., Kroll, B., Juskiewicz, J. and Zdunczyk, Z. 2009. Composition and properties of chicory extract rich in fructans and polyphenols. *Polish Journal of Food Nutrition Science*, **59**: 35-43.
- Mishra, H., Soni, M., Silawat, N., Mehta, D., Mehta, B.K. and Jain, D.C. 2011. Antidiabetic activity of medium polar extract from the leaves of stevia on alloxan-induced diabetes. *Journal of Pharmacology and Biological Science*, **3**: 242-248.
- Mohamed, A.B., Mohammed, A.M., Rubaee, A. and Jalil, A.Q. 2012. Effect of ginger (*Zingiber officinale*) on performance and blood serum parameters of broiler. *International Journal of Poultry Science*, **11**: 143-146.
- Moula, M.R., Rahman, M.M., Akter, F. and Mostofa, M. 2012. Effects of nishyinda, black pepper and cinnamon extract as growth promoter in broilers. *The Bangladesh Veterinarian*, **29**: 69-77.
- Oh, P.S., Min, R.C., Sung, P.B. and Jung, H.B. 2013. The meat quality and growth performance in boiler chickens fed diet with cinnamon powder. *Journal of Environmental Biology*, **34**: 127-133.

- Prins, H.K. and Loos, J.A. 1969. *Biochemical Methods in Red Cell Genetics*. Academic Press, New York, USA.
- Pushparaj, P.N., Low, H.K., Manikandan, J., Tan, B.K. and Tan, C.H. 2007. Anti-diabetic effects of *Cichoriumintybus* in streptozotocin-induced diabetic rats. *Journal of Ethanopharmacology*, **111**: 430-434.
- Rasheeduz, Z. and Mujahid, S.A. 1998. Anti-hepatotoxicity effect of root and root callus extract of *Chichory intybus*. *Journal of Ethanopharmacology*, **63**: 227-231.
- Rehman, S.U. 1984. Lead induced regional lipid peroxidation in brain. *Toxicology Letters*, **21**: 333-337.
- Shalaby, M.A. and Hamowiech. A.R. 2010. Safety and efficacy of *Zingiber officinale* roots on fertility of male diabetic rats. *Food and Chemical Toxicology*, **48**: 2920-2924.
- Shrivastava, S. Gautam, V.N., Gehlaut, B.S. and Quadri, M.A. 2012. Effect of garlic oil supplementation on egg yolk lipid profile and performance of Jabalpur colour birds. *Applied Biological Research*, **14**: 145-149.
- Shrivastava, S., Gautam, V.N., Gehlaut, B.S. and Quadri, M.A. 2013. Effect of garlic oil supplementation on egg yolk lipid profile and performance of Jabalpur colour birds. *J.N.K.V.V Research Journal*, **46**: 87-89.
- Singh, S. 2012. *Effect of Fenugreek (Trigonella foenumgraecum) and Banaba (Lagerstroemia speciosa) Extracts on Lipid Profile and Performance of Jabalpur Colour Layer Birds*. M.V.Sc. & AH thesis, Madhya Pradesh Pashu Chikitsa Vishwavidyalaya, Jabalpur, India.
- Sinha, S. 2012. *Hypoglycemic and Hypocholesteremic Effect of Aloe vera Supplementation*. M.V.Sc. & AH. thesis, Madhya Pradesh Pashu Chikitsa Vishwavidyalaya, Jabalpur, India.
- Steel, R.G.D. and Torrie, J.H. 1992. *Principles and Procedures of Statistics: A Biometrical Approach*. McGraw Hill, New York, USA.
- Yusrizal, C. and Chen, T.C. 2003. Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length. *International Journal of Poultry Science*, **2**: 214-219.
- Zahu, X., Yang, Z.B., Yang, W.R., Wang, Y., Jiang, S.Z. and Zhang, G.G. 2011. Effects of ginger root (*Zingiber officinale*) on laying performance and antioxidant status of laying hens and on dietary oxidation stability. *Poultry Science*, **90**:1720-1727.