International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 576-579

Received: 19-07-2019
Accepted: 21-08-2019

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Effect of dietary supplementation of gooseberry/ amla (*Emblica officinalis*) powder on biochemical profile of commercial broiler chickens

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Abstract
This study was conducted at College of Veterinary Science, Khanapara, Guwahati for a period of 42 days to investigate the effect of dietary supplementation of amla powder on biochemical profile characteristics of commercial broiler chickens. A total of 180 broiler chicks were randomly divided into four groups consisting of 45 numbers, sub divided into 3 replicates consisting of 15 chicks. The four groups T_0, T_1, T_2 and T_3 were offered basal diet with supplementation of amla powder at the rate of 0.00, 0.25, 0.50 and 0.75% in the feed (on dry matter basis), respectively. The result showed that Total serum cholesterol and Triglycerides, except Total serum protein, AST and ALT differed significantly (P<0.05; 0.01) among the experimental groups. The Antioxidant Biomarkers viz. Superoxide Dismutase (SOD) and Glutathione peroxidase (GSH-Px) differed significantly (P<0.05; 0.01) among the experimental groups. From this study, we can conclude that supplementation of feeding the birds with amla powder improves the antioxidant status and decreases the lipids profile such as Total serum cholesterol and Triglycerides.

Keywords: Amla, chickens, cholesterol, triglycerides, antioxidant biomarkers

Introduction
Poultry industry in India has emerged as the most dynamic and fastest expanding segment in animal husbandry sector due to its low investments and quick returns. According to Economic Survey (2016-17) [6] in agriculture and allied sector, Indian poultry sector is the major game changer with an overall growth of about 7-8% per annum (0.7% in National GDP). Feed is the major part of total prices of poultry venture as 80 % of the entire expenditure is on the procurement of feed (Anurag *et al.,* 2018) [1]. In the last decade, herbal feed additives have attracted the attention of scientists as useful resource for improving productivity. Amla (*Emblica officinalis*) powder is an important source of ascorbic acid, minerals, amino acids, tannins, and phenolic compounds. The fruit contains two hydrolysable tannins Emblicanin A and B, which have antioxidant properties (Meena *et al.*, 2010) [13]. Swaminathan (1990) [25] reported that 100 gm edible portion of amla contains: moisture (81.8 %) carbohydrates (13.7 gm), fibre (3.4 gm), protein (0.5 gm), fat (0.1 gm), calcium (0.05 gm), phosphorous (0.02 gm), iron (1.2 mg), niacin (0.2 mg), thiamine (0.03 g), riboflavin (0.01 mg), carotene (9µg), k calories (58) and ascorbic acid (600 mg). Recent studies have revealed that antioxidants present in amla (*Emblica officinalis*) juice in the form of polyphenols and vitamin C, helped in maintaining healthy cholesterol levels (Pathak *et al.*, 2003) [16]. *Emblica officinalis* have been used to protect tissues from superoxide radicals and enhance cell survival by stimulating anti-oxidative enzymatic systems. Hence, the present study was undertaken to evaluate dietary effect of amla powder supplementation on blood biochemical parameters of commercial broiler chickens.

Materials and Methods
This study was carried out at college of veterinary science A total of 180 day-old commercial broiler chicks (Hubbard) hatched in a single hatch were weighed, wing banded and randomly divided into four groups, *viz.* T_0, T_1, T_2 and T_3 containing 45 chicks in each group and each group subdivided into 3 replicates of 15 chicks. The feeding trial was conducted for a period of 6 weeks using broiler prestarter (1-7 days), starter (8-28 days) and finisher (29-42 days) ration. The chicks were reared under deep litter system following standard and uniform management practices.

Local varieties of raw amla were procured from market, washed thoroughly and sundried. The seed of the fruits were removed and then grinded properly and were stored at room temperature. The experimental diet T_0 served as control (with no amla powder supplementation) while diet T_1, T_2 and T_3 contained 0.25, 0.50 and 0.75% of amla powder, respectively. The nutrient compositions of the Commercial basal diets (pre starter, starter and finisher ration) and amla powder are shown in Table 01

Table 1: Nutrient composition of commercial basal diet (broiler pre starter, starter and broiler finisher) and amla powder

Nutrient composition	Commercial basal diet			Amla powder (AP)
	Pre starter (0-7 days)	Starter (8-28 days)	Finisher (29-42 days)	
Dry matter (%)	89.50	89.65	88.49	90.01
Crude Protein (%)	23.46	23.04	20.65	5.54
Ether extract (%)	3.47	4.01	5.15	1.62
Crude fiber (%)	4.23	4.45	3.82	18.83
Nitrogen free extract (%)	61.37	62.49	66.99	68.45
Total ash (%)	7.10	7.00	6.74	8.52

At the end of the feeding trial five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. It was then allowed to stand for 30 minutes. The serum was separated out and kept in vial at -20 degree Celsius. Completely Randomized Design (CRD) was followed for the analysis of the recorded data. The mean, SE (Standard Error) were calculated as per standard statistical procedures (Snedecor and Cochran 1994) [23].

Total serum cholesterol

The Total serum cholesterol was estimated using spectrophotometer (Systronics model No. 106) with Cholesterol kit (CHOD-POD Method), supplied by Aspen Laboratories.

Calculation

$$\text{Total Serum Cholesterol(mg/dl)} = \frac{\text{Absorbance of the sample (Abs. T)}}{\text{Absorbance of the standard (Abs. S)}} \times 200$$

Triglyceride

The Triglyceride was estimated using spectrophotometer (Systronics model No. 106) with kit (GPO-POD Method), supplied by Aspen Laboratories.

Calculation

$$\text{Triglyceride concentration (mg/dl)} = \frac{\text{Absorbance of the sample (Abs. T)}}{\text{Absorbance of the standard (Abs. S)}} \times 200$$

Total Serum protein

The total serum protein was estimated using spectrophotometer (Systronics model No. 106) with Total Protein kit (Biuret Method), supplied by Aspen Laboratories.

Calculation

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of the sample (Abs. T)}}{\text{Absorbance of the standard (Abs. S)}} \times 6$$

Alanine Transaminase (ALT)

The total serum ALT was estimated using spectrophotometer (Systronics model No. 106) with SGPT/ALT Kit (Modified IFCC methodology) supplied by Aspen Laboratories.

Calculation

Serum ALT (U/L) = (OD/ min) Δ × 1746

Aspartate Transaminase (AST)

The total serum ALT was estimated using the SGOT/AST Kit (Modified IFCC methodology) supplied by Aspen Laboratories.

Calculation

Serum ALT (U/L) = (OD/ min) Δ × 1746

Antioxidant Biomarkers

Preparation of Haemolysate

The collected anti-coagulated blood was centrifuged at 3000 rpm for 15 minutes and the plasma was removed. The sediment remaining in the centrifuge tube was washed with chilled normal saline solution (0.9 % NaCl) thrice at 3000 rpm for 10 minutes. The supernatant was discarded along with the buffy coat and again equal volume of NSS was added to the sediment, mixed properly and centrifuged. The process was repeated thrice. The sediment was resuspended by taking 10 µl of pellet and was transferred to 990 µl of distilled water. The tube containing the Haemolysate was vortex for two minutes and was for determination of enzymes associated with erythrocyte membrane.

Superoxide Dismutase (SOD) activities

The SOD activity was evaluated by using NBT (Nitro Blue Tetrazolium) reduction method given by Nishikimi (1972) [15]. The reaction mixture consisted of 1ml of NBT solution (156µM) and sample solution at different concentration. The reaction was started by adding 100µl of phenazinemethosulfate solution (60 µM, PMS in 0.05 M phosphate buffer PH-7.4) to the reaction mixture followed by incubation at 25˚C for 5 minutes and absorbances at 560 nm was measured against blank and were expressed in unit /mg protein. Ascorbic acid was used as the standard.

Calculation

$$\text{Superoxide scavenging activity (\%)} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs. (Control)}} \times 100$$

Glutathione Peroxidase (GSH-Px) activities

The glutathione peroxidase enzyme was assayed by the method of Rotruck *et al.* (1973) [20]. A known volume of the

Haemolysate was added to the incubation medium which contained 0.4 ml of Sodium Phosphate buffer, 0.2 ml of sodium azide solution, 0.2 ml of EDTA solution, 0.2 ml of hydrogen peroxide and 0.2 ml of reduced glutathione. The incubation medium was made up to a final volume of 2 ml with water. The tubes were incubated at 37º C for 90 and 180 minutes. The reaction was terminated by the addition of 1 ml of precipitating agent. The reaction mixture was centrifuged at 10,000 rpm for 5 minutes and to the supernatant, 6 ml of disodium hydrogen phosphate was added. One ml of DTNB reagent was added just prior to the spectrophotometric analysis. The absorbance was read at 412 nm against a blank, which contained only 6 ml of disodium phosphate and 1 ml of DTNB reagent. Suitable aliquots of the standards were taken and treated in a similar manner. The activity was expressed in terms of nmol/min/mg protein.

Results and Discussions

In the present study the mean (±SE) values of Total serum cholesterol, Triglycerides Total serum protein, AST and ALT are presented in Table 02 of which Total serum cholesterol and Triglycerides differ significantly (P<0.05; 0.01). The Total serum cholesterol level decreases significantly (P<0.05) in T_1 (150.85 mg/dl), T_2 (146.82 mg/dl) and T_3 (144.19 mg/dl) groups compared to T_0 (155.50mg/dl) group with the increasing level of amla powder in the diet. The findings were in agreement with the reports of earlier workers, Vidhyarthi *et al*. (2008) [27], Sujatha *et al*. (2010) [24], Kumar *et al*. (2010) [9], Shivaji (2012) [22], Dhore *et al*. (2014) [5] and Aswal *et al*. (2017) [2]. The reduction in cholesterol level in the treated groups might be due to the active tannoid principles of *Emblica officinalis* which has an important hypolipidaemic agent that directly acts upon sympatho-adrenal axis and lowers the synthesis of corticosterone (Sairam *et al*., 2003) [21]. This hypolipidaemic effect of *Emblica officinalis* has been attributed to enhance the clearance of endogenous cholesterol (Mathur *et al*., 1996) [12]. Similarly, Triglyceride level also decreased significantly (P<0.01) in T_0, T_1, T_2 and T_3 groups as 128.51±2.12, 124.91±1.70, 123.42±1.34 and 119.26±0.58 mg/dl respectively due to hypolipidaemic effect of amla. Similar conclusions were drawn by Qureshi *et al*. (2009) [17], Nakajothi *et al*. (2009) [14], Shivaji (2012) [22] and Aswal *et al*. (2017) [2]. The AST and ALT activity (Table 02) did not show any significant (P>0.05) change with amla supplementation. However, Gupta *et al*. (2006) [8], Goswami *et al*. (2008) [7] and Tiwari *et al*. (2008) [26] reported significantly (P<0.05) high AST activity in control group. On the other hand Tiwari *et al*. (2008) [26] reported significant (P<0.05) increase in ALT activity in control group. There was non-significant difference in total serum protein level among the different treatment groups of broilers though an increasing trend was observed in the treatment groups (Table 02).

Table 2: Means (± se) for biochemical parameters of broiler under different treatment groups

Parameters \ Groups	T_0 (Control)	T_1 (AP-0.25%)	T_2 (AP-0.50%)	T_3 (AP-0.75%)
Total serum cholesterol (mg/dl)	155.50^{a}±1.69	150.85^{ab}±3.13	146.82^{b}±1.40	144.19^{b}±2.63
Triglyceride (mg/dl)	128.51^{a}±2.12	124.91^{ab}±1.70	123.42^{bc}±1.34	119.26^{c}±0.58
AST (U/L)	133.05^{a}±1.67	129.90^{a}±0.93	128.85^{a}±1.19	127.81^{a}±1.37
ALT (U/L)	20.95^{a}±0.55	20.08^{a}±0.96	18.51^{a}±0.51	18.16^{a}±1.15
Total protein(g/dl)	3.07^{a}±0.05	3.16^{a}±0.18	3.27^{a}±0.08	3.31^{a}±0.09

Means bearing same superscripts in a row did not differ significantly

Antioxidant Biomarkers

The Antioxidant Biomarkers-Superoxide Dismutase and Glutathione peroxidase were found to be significantly (P<0.05) different among different treatment groups. The Superoxide Dismutase activity was found significantly (P<0.05) higher in T_3 (4.26 unit/mg protein) group, followed by T_2 (3.91unit/mg protein), T_1 (3.52 unit/mg protein) and T_0 (3.02 unit/mg protein) groups. These findings were in agreement with Maini *et al*. (2007) [10], Ramnath and Rekha (2011) [19] and Manju *et al*. (2011) and they reported that the group supplemented with amla had significantly (P<0.05) higher SOD. Bhattacharya *et al*. (1999) [3] reported that supplementation of amla rich in antioxidant viz. active tannoid principles of *Emblica officinalis* markedly increased in the blood. Thus the defense mechanisms in the treated groups were elevated. The activity of Glutathione peroxidase enzyme also showed significant (P<0.05) differences among the experimental groups and the highest activity was observed in T_3 (1.44 nmol/min/mg protein) group, followed by T_2 (1.24 nmol/min/mg protein), T_1 (0.89nmol/min/mg protein) and T_0 (0.54 nmol/min/mg protein) groups. Bhattacharya *et al*. (2000) [4] and Rajak *et al*. (2004) [18] stated that *Emblica officinalis* has the ability to stimulate natural glutathione peroxidase.

Table 3: Means (± se) for antioxidant biomarkers of broiler under different treatment groups

Parameters \ Groups	T_0 (Control)	T_1 (AP-0.25%)	T_2 (AP-0.50%)	T_3 (AP-0.75%)
SOD (unit/mg protein)	3.02^{c}±0.25	3.52^{bc}±0.24	3.91^{ab}±0.25	4.26^{a}±0.17
GSH-Px (nmol/min/mg protein)	0.54^{b} ±0.02	0.89^{ab}±0.14	1.24^{a}±0.32	1.44^{a}±0.26

Means bearing same superscripts in a row did not differ significantly

Conclusion

The study revealed that supplementation of amla in the diet of broiler chickens has beneficial effects on the biochemical profile and the best results were obtained at higher levels of inclusion i.e. 0.75 %. The lipids profile such as Total serum cholesterol and Triglycerides values decreased in amla powder supplemented groups indicating beneficial effects. The enzymatic activity of amla was observed best with 0.75% levels which improves the antioxidant status of the birds.

Acknowledgement

The authors express whole hearted thanks and indebtedness to the Dean, Faculty of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati - 781022 for providing the

necessary facilities to carry out the research programme at the Instructional Poultry Farm of the college.

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