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Rajesh Dalal

Ph. D. Research Scholar,
Department of Animal
Nutrition, Lala Lajpat Rai
University of Veterinary and
Animal Sciences, Hisar
Haryana, India

VS Panwar

Ph. D. Research Scholar,
Department of Animal
Nutrition, Lala Lajpat Rai
University of Veterinary and
Animal Sciences, Hisar
Haryana, India

Sony

Ph. D. Research Scholar,
Department of Animal
Nutrition, Lala Lajpat Rai
University of Veterinary and
Animal Sciences, Hisar
Haryana, India

Parveen Kumar Ahlawat

Ph. D. Research Scholar,
Department of Animal
Nutrition, Lala Lajpat Rai
University of Veterinary and
Animal Sciences, Hisar
Haryana, India

BS Tewatia

Ph. D. Research Scholar,
Department of Animal
Nutrition, Lala Lajpat Rai
University of Veterinary and
Animal Sciences, Hisar
Haryana, India

Nancy Sheoran

Ph. D. Research Scholar,
Department of Animal
Nutrition, Lala Lajpat Rai
University of Veterinary and
Animal Sciences, Hisar
Haryana, India

Correspondence

Rajesh Dalal

Ph. D. Research Scholar,
Department of Animal
Nutrition, Lala Lajpat Rai
University of Veterinary and
Animal Sciences, Hisar
Haryana, India

Effect of *Emblica officinalis* fruit powder on fatty acid profile and meat cholesterol of broiler chicken

Rajesh Dalal, VS Panwar, Sonu, Parveen Kumar Ahlawat, BS Tewatia and Nancy Sheoran

Abstract

To study the hypolipidemic effect of dietary supplementation of amla fruit powder on broiler chicken meat, 300 commercial broiler chicks were randomly distributed into six treatments having five replicates consisting of ten birds each. The chicks fed with standard basal diet in two different growth phases i.e. starter (0-28d) and finisher (29-42 d). The first group was kept as control (T₁) and given the basal diet without antibiotic, while second group (T₂) was given basal diet with antibiotic. In third (T₃), fourth (T₄), fifth (T₅) and sixth (T₆) groups, basal diet was supplemented with amla fruit powder @ 0.25%, 0.50%, 0.75% and 1% respectively. The birds were weighed fortnightly to calculate performance parameters viz. feed intake, body weight change and FCR. Meat cholesterol of the experimental birds under different dietary treatments ranged between 47.32 (mg/100g fat) to 50.59 (mg/100g fat). Dietary supplementation of amla fruit powder showed significant reduction in the meat cholesterol of the experimental birds in groups supplemented with 0.75% amla powder (T₅) and 1% amla powder (T₆). Saturated fatty acids content of different dietary treatments was ranged between 37.80 (%) to 38.02 (%) and didn't differ significantly as compared to control group. Mean values for monounsaturated fatty acids content were ranged from 50.34 % to 50.36 % and also didn't differ significantly among the supplemented and control group. Polyunsaturated fatty acid and mean values for conjugated linoleic acid content among different dietary treatments ranged from 9.95% to 9.98% and 62.23 (mg/100g fat) to 62.64 (mg/100g fat) respectively and didn't differ significantly supplemented and control group.

Keywords: Amla, conjugated linoleic acid, hypolipidemic, meat cholesterol

Introduction

Poultry today not only act as an income stabilizer but also provides regular and timely income as compared to livestock and crop farming. The broiler sector has been the most dynamic sector in poultry due to its marginal investments and quick returns. Furthermore, the success of poultry industry depends upon its fast growth and low mortality during first two weeks of its life, which can be managed by good hygienic and feeding conditions. The consumers are now becoming more aware of safety and quality of food products consumed by them. The production of safer poultry products without any chemical and microbial residues is the order of the day. Feed additives are commonly described as non-nutrient substances which accelerate growth, efficiency of feed utilization, beneficial for health or metabolism of the animals [1]. The range of feed additives used in animal production industry is very broad ranging from growth promoters to disease preventing agents. Supplementations of these agents in poultry nutrition are mainly aimed to improve digestibility and bioavailability of various nutrients, thereby, enhancing economic gains by reducing the input costs. The additives that hold great promise in the feeding of poultry comprise of antibiotics, coccidiostats, antioxidants, enzymes, hormones, probiotics, buffers, organic acids, mould inhibitors, herbal products, synthetic micronutrients etc. Recently, the emphasis is being directed towards the search of herbal formulations which could be effective for amelioration of stress and leads to increase in production of birds. Several Indian herbs are reported to possess adaptogenic, antistress and immunomodulatory properties [2]. New additives of phytogetic origin have been proposed to livestock producers. In this view, the plants identified with properties of secondary metabolites became interesting due to their antimicrobial, antioxidant effects and their stimulating effects on animal performance and digestive enzymes. The use of naturally occurring compounds like herbs, herbal preparations and other botanicals are preferred over chemical compounds to satisfy consumer concerns over safety and toxicity [3]. In poultry health management, *Emblica officinalis* has been widely used as growth promoter,

immunomodulator [4] and antioxidant [4, 5]. The key bioactive principles of amla fruit are flavonoids, phyllembin, ascorbic acid, gallic acid, alkaloids and tannins. Vitamin C, tannins and flavonoids are found in maximum concentration and exhibit antioxidant action [6]. The fruit of amla due to presence of saponins, phenols and tannins have potent antimicrobial activity against both Gram positive and Gram negative bacteria.

Materials and methods

Ethical approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee, 12/CPCSEA Dated 6.2.2017 in the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

Experimental design

Completely Randomized Design was used as experimental design at uniform and standard management practices.

Birds and management

A total of 300 commercial broiler chicks were randomly distributed into six treatments having five replicates consisting of ten birds each. The chicks fed with standard basal diet in two different growth phase i.e. starter (0-28d) and finisher (29-42 d). The first group was kept as control (T₁) and given the basal diet without antibiotic, while in second group (T₂) basal diet was given with antibiotic. The diet in third (T₃), fourth (T₄), fifth (T₅) and sixth (T₆) groups were supplemented with amla fruit powder @ 0.25%, 0.50%, 0.75% and 1%, respectively. The birds were weighed fortnightly to calculate growth performance parameters viz. feed intake, body weight gain and FCR.

Extraction of meat lipids

Meat lipids were extracted according to the methods of the AOAC [7]. Meat samples were separated for each replicate, pooled and homogenized. 4 g well mixed sample was weighed into a 100 ml volumetric flask and 25 ml chloroform-absolute alcohol were added (1:1, v/v); the solvent was mixed very slowly from pipette. It was shaken constantly until proteins were coagulated. An additional 60-65 ml mixed solvent was added and left stand for 1 hour, shaken every 5 min. At the end of the time it was diluted to 100 ml with mixed solvent and the mixture was let stand until clear. The mixture was then filtered very slowly and solvent was evaporated and meat lipids were obtained.

Estimation of total conjugated linoleic acid

The total conjugated linoleic acid content in fat was determined as per AOAC. 0.01 gm fat was taken in 25 ml volumetric flask and 5 ml isooctane was added to it. After mixing well volume was made 25 ml with isooctane. Absorbance was read at 233 nm in UV range against reagent blank and CLA content was calculated using the formula given below:

$$\% \text{ CLA} = \frac{(A-0.03) \times DF \times 0.9}{\text{Wt. of sample}}$$

Where, A = Absorbance at 233 nm
DF = dilution factor (25 ml)

Preparation of fatty acid methyl esters and their Fractionation

Fatty acids were converted into fatty acid methyl esters (FAME) according to the method described by [8]. The top layer containing methylated fatty acids was removed carefully and stored in capped glass tubes and used for GC analysis. Methyl esters of fatty acids were separated by gas chromatograph equipped with flame ionization detector. The peak was identified by comparison of its retention time that of standard fatty acid. The area under peak was calculated by triangulation and converted directly into relative percentage.

Evaluation of feed ingredients

Feed ingredients used for ration formulations were evaluated for proximate nutrients [7]. The evaluated and measured values of feed ingredients used in preparing the experimental diets are presented in Table 1.

Materials used in diet formulation

All feed ingredients, additives and supplements used in the experiment were procured in one lot before the start of the experiment. The ingredients, additives and supplements used in the diet formulation were maize, soybean meal, vegetable oil, fish meal, mineral mixture, vitamins, coccidiostat, lysine, DL-methionine and amla fruit powder. The sources, composition and mixing rate of additives/supplements used in ration formulations are presented in Table 2.

Figuring and composition of diets

Basal ration was formulated as per BIS [9] to fulfil the metabolizable energy (ME) and crude protein requirements of birds. Level of crude protein in starter (0-4weeks) and finisher (4-6weeks) ration was 22 percent and 20 percent, respectively. The respective ME content was 3000 and 3200 KCal/kg are presented in Table 1.

Housing and brooding

The experimental chicks were reared under deep litter system. The floor of the pens was thoroughly cleaned, disinfected before scattering of the bedding material. Well chopped dry wheat straw was used as bedding material to form the litter. The straw was evenly spread upto 5 cm thickness. The litter was regularly raked to avoid any lump formation. Wooden brooders fitted with bulb in the centre were used in each pen for brooding.

Feeding and watering

During the initial period of growth extra care was taken to assure efficient feeding and watering of the chicks so that they could be well introduced and acclimatized. The feeding programme consisted of a starter diet until 28 days and a finisher diet from 29 to 42 days of age. Weighed amount of feed was offered on paper sheets for first 3 days and thereafter, in the automatic feeders up to 28 days of age. Afterwards, the feeds were offered through hanging feeders maintained at appropriate heights. The chicks were provided *ad libitum* clean drinking water through the plastic waterers during first two weeks of the experiment. Thereafter, bigger plastic waterers were used till the end of the experiment.

Table 1: Chemical composition of feed ingredients used in ration formulation

Ingredient	CP (%)	CF (%)	EE (%)	TA (%)	Lysine* (%)	Methionine*(%)	ME*(kcal/kg)
Maize	9.11	2.44	3.44	2.25	0.18	0.15	3300
Soybean meal	45.15	3.93	3.16	8.47	2.57	0.76	2230
Fish meal	47.40	1.79	5.16	26.62	1.42	1.42	2210

*Calculated values ^[10]**Table 2:** Ingredient composition of experimental diets during different phases of growth

Ingredient (kg /100 kg of feed)	0-4wks	4-6 wks
Maize	58	60
Soybean meal	30	25
Fish meal	7	7
Vegetable oil	3	6
Mineral mixture	2	2
Feed additives (g/100 kg feed)		
Spectromix ¹	10	10
Spectromix BE ²	20	20
Veldot ³	50	50
Choline chloride ⁴	50	50
Lysine ⁵	50	50
DL-methionine ⁶	150	150

Composition, sources and rate of mixing of feed additives/supplements

1. Spectromix: Powder (Ranbaxy Animal Health, New Delhi). Each gm. contained Vitamin A-82,500 IU, Vit D3-12000 IU, Vit B2-50 mg and Vit.K-10mg. Mixing rate: 10 g/100Kg of feed.
2. Spectromix BE: Powder (Ranbaxy Animal Health, New Delhi). Each gm. Contained Vit.B1- 8mg, Vit.B6- 16mg, Vit.B12- 80mg, niacin-120mg, calcium pantothenate-80mg, Vit. E-160 mg, Lysine hydrochloride-10 mg, DL-

methionine-10 mg and calcium 260 mg. Mixing rate: 20g/100kg of feed.

3. Veldot: Venkeys- Dinitro-O-Toluamide (Coccidiostat). Mixing rate: 50g/100kg of feed.
4. Choline chloride: Contain 60 percent choline. Mixing rate: 50g/100kg of feed.
5. Lysine: Contained 98% lysine. Mixing rate: 50g/100kg of feed.
6. DL-methionine: Contained 98% methionine. Mixing rate: 150g/100kg of feed.

Statistical analysis

Data was analysed statistically as described by ^[11]. Analysis of variance was used to study the differences among treatment means and they were compared by using Duncans Multiple Range Test (DMRT) as modified by ^[12].

Results and discussion

Fatty acids profile and cholesterol content of meat

Data pertaining to meat cholesterol of the experimental birds under different dietary treatments are presented in Table 3 and ranged between 47.32 (mg/100g fat) to 50.59 (mg/100g fat). Dietary supplementation of amla fruit powder showed significant reduction in the meat cholesterol of the experimental birds in groups supplemented with 0.75% amla powder (T₅) and 1% amla powder (T₆).

Table 3: Fatty acids and cholesterol content of meat of the experimental birds under different dietary treatments

Treatments	SFA %	MUFA %	PUFA %	Cholesterol (mg/100g fat)	CLA (mg/100g fat)
T ₁	37.80±.15	50.35±00	9.96±.03	50.36 ^{ab} ±.08	62.23±.38
T ₂	38.01±.02	50.34±00	9.96±.03	50.59 ^a ±.25	62.38±.82
T ₃	37.87±.03	50.34±00	9.96±.02	49.42 ^{bc} ±.33	62.57±.77
T ₄	37.83±.09	50.36±01	9.94±.02	48.96 ^{cd} ±.64	62.64±.87
T ₅	38.02±.07	50.34±00	9.98±.02	48.11 ^{de} ±.27	62.30±.78
T ₆	37.94±.09	50.35±01	9.95±.03	47.32 ^e ±.40	62.49±.75

a, b, c means bearing different superscripts in a column differ significantly (P<0.05)

Saturated and unsaturated fatty acids

Saturated fatty acids content of different dietary treatments ranged between 37.80 (%) to 38.02 (%) and didn't differ significantly as compared to control group. Mean values for monounsaturated fatty acids content ranged from 50.34 % to 50.36 % and also didn't differ significantly among the supplemented and control group. Polyunsaturated fatty acid and mean values for conjugated linoleic acid content among different dietary treatments ranged from 9.95% to 9.98% and 62.23 (mg/100g fat) to 62.64 (mg/100g fat), respectively and didn't differ significantly between supplemented and control group. Dietary supplementation of amla fruit powder showed significant reduction in the meat cholesterol of the experimental birds in groups supplemented with 0.75% amla powder and 1% amla fruit powder and proves the hypolipidemic property of amla fruit. In contrary to our study ^[13] observed that breast and thigh muscle cholesterol in broilers showed no significant difference among treatment groups due to dietary inclusion of polyherbs. Rest of the parameters like saturated fatty acids PUFA and MUFA and conjugated linoleic acid content showed no significant change

in amla supplemented group as compared to the control group. In contrary to our study, ^[14] retorted that dietary supplementation of phytobiotic turmeric rhizome powder depressed the plasma triglyceride concentration which may be due to lowering of hepatic lipogenesis, triglycerides are produced in the liver by hepatic lipogenesis and secreted into the plasma ^[15,16]. As a result, decreased hepatic lipogenesis possibly affected the thigh meat triglyceride. Poultry meat contains low lipid content and lipids of muscle meat are rich in saturated and monounsaturated fatty acids and found in the intra muscular adipocytes located in the perimysium ^[17]. Adipocytes number and size increase with the total lipid content of the muscle ^[18, 19]. The predominant lipids in thigh meat are triglycerides ^[20]. SFA and MUFA are the results of fatty acid synthesis de novo, whereas polyunsaturated fatty acids originate exclusively from the diet ^[15, 16]. Along with myristic acid (C14:0), palmitic acid is the responsible fatty acid to raise LDL serum cholesterol ^[21, 22].

Conclusion

Based upon the above study, it can be concluded that amla

fruit powder can be effectively supplemented as natural growth promoter as well as effective in decreasing the cholesterol content of meat. Cholesterol can be both good and bad for food consumers. Abnormally high levels of cholesterol and abnormal proportions of low-density lipoproteins and high-density lipoproteins are associated with cardiovascular diseases. Anti-lipidemic effect of amla fruit powder might be due to increase in the 3-hydroxy-3-methylglutaryl coenzyme reductase inhibitor activity thereby, the reduction of 3-hydroxy- 3-methylglutaryl coenzyme reductase resulted in a decrease in total cholesterol biosynthesis.

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Competing interests

The author has no competing interests to declare.

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