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Effect of garlic and holy basil leaf powder supplementation on chemical composition and cholesterol content of breast and thigh muscles of broiler chicks

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Abstract
The purpose of this research work was to investigate the effect of dietary supplementation with different levels of garlic and holy basil leaf powder on chemical composition and cholesterol content of breast and thigh muscles of broiler chicks. A total of 280 day-old broiler chicks were procured and randomly distributed into 7 treatments, each treatment had 4 replicates with 10 chicks in each replicate. T_1 was served as control and fed a basal diet formulated as per [3] standards. T_2 and T_3 were fed with garlic powder @ 0.5, 1% feed, respectively. T_4 and T_5 were given with holy basil leaf powder @ 0.5, 1% feed, respectively. T_6 was fed with garlic powder and holy basil leaf powder @ 0.5% each. T_7 was treated with garlic powder and holy basil leaf powder @1.0% each. The breast and thigh muscles were analyzed for moisture, crude protein, ether extract and cholesterol content. Present study depicted that the percent moisture, CP and EE contents of thigh and breast muscles in broilers had no significant difference among the mean values of different treatments. Cholesterol content in thigh muscle was lowest (P<0.05) in T_6 & T_7 and highest in T_1 treatment. Similarly, in breast muscle, was lowest (P<0.05) in T_6 & T_7 and highest in T_1 group. Thus supplementation of garlic and holy basil powder @ 0.5% and 1.0% showed the best results in terms of decreasing cholesterol content.

Keywords: Garlic, holy basil, broiler, cholesterol

Introduction
Growth promoters are agents added to poultry feeds in order to enhance the feed conversion efficiency and body growth and broadly can be categorized as Antibiotic growth promoters (AGP) and Non–Antibiotic growth promoters (NAGP). Antibiotic growth promoters have been helpful in improvement of growth performance and feed conversion ratio in poultry [6, 8, and 13]. However, constant treatment of poultry by antibiotic may result in residues of these substances in poultry products and bacteria resistance against treatments in human body. Due to such threats to human health, use of antibiotics in poultry is banned in many countries [1, 4, and 17]. On the other hand use of NAGP is commonly regarded as favourable alternatives to AGP in poultry production. The main advantage of NAGP over AGP is that they usually do not bear any risk regarding bacterial resistance or undesired residues in meat. Addition of NAGP to feeds of poultry may have a number of beneficial effects, including rapid development of a healthy gut microflora and stabilization of digestion along with improved feed efficiency. NAGP include predominantly organic acids, probiotics, prebiotics, synbiotics, phytogenics, feed enzymes and immune stimulants. Among these alternatives, phytogenics are drawing much attention nowadays. Phytogenics are derived from herbs, spices or aromatic plants and have shown antimicrobial, antifungal, antiviral, antioxidant or sedative properties. A complex mixture of bioactive compounds present in them is known for their appetizing effects, since they increase the palatability of the feed and stimulate endogenous digestive enzymes.
Beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions [20].
The name ‘‘*Allium sativum*’’ is derived from the Celtic word ‘‘all’’, meaning burning or stinging, and the Latin ‘‘sativum’’meaning planted or cultivated [10]. Garlic is a member of the family Liliaceae [16]. Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids and minerals such as selenium [15]. The sulphur compounds are responsible both for garlic’s pungent odour and many of its medicinal effects like lowering cholesterol level [5].

Garlic appears to enhance the synthesis of nitric oxide, which accounts for its antihypertensive and coagulant effects [11]. The purpose of this investigation was to find out the effect of dietary supplementation with different levels of garlic and holy basil leaf powder on chemical composition and cholesterol content of breast and thigh muscles of broiler chicks.

Materials and Methods

A total of 280-day-old commercial broiler chicks (Vencobb-400) were procured and randomly distributed into seven treatment groups of 40 chicks each. Each treatment groups was further subdivided into four replicates of ten chicks each. The herbs used in the study were purchased from local market. The experimental diets (Table 1) were formulated to meet the nutrient recommendations [3]. Growth trial of 6 weeks was conducted in a complete randomized design comprising seven dietary treatments. T_1 was served as control and fed a basal diet formulated as per [4] standards. T_2 and T_3 were fed with garlic powder (GP) @ 0.5, 1% feed respectively. T_4 and T_5 were given with holy basil leaf powder (HBLP) @ 0.5, 1% feed respectively. T_6 was fed with 0.5% GP and 0.5% HBLP. T_7 was treated with 1.0% GP and 1.0% HBLP.

The chicks were kept hygienically on floor litter system in separate pens. All the birds were reared adopting uniform management conditions. The chicks were brooded at 35ºC during the first week and thereafter the temperature was reduced by 3ºC every week until the temperature reached 25±1ºC. The birds were vaccinated against prevailing diseases adopting a standard protocol.

Procurement of feed ingredients, additives and supplements

All feed ingredients, additives and supplements were procured in one lot before the start of the experiment. The ingredients, additives and supplements used in the diet formulations were maize, soybean meal, rice police, fish meal, ground- nut cake, soybean oil, mineral mixture, vitamin mixtures, coccidiostat, lysine, DL-methionine and choline chloride. The composition, mixing rate and cost of additives /supplements used in ration formulations are presented in Table 1.

Table 1: Composition, rate of mixing and cost of feed additives/supplements.

Sr. No.	Additive/ supplement	Composition	Rate of mixing (g/100 kg)	Cost (Rs/kg)
1	Vitamin mixture- I (Spectromix)	Each gm contained vitamin A-82500 IU, vit. D_3-12000 IU, vit. B_2-50 mg and vit. K-10 mg	10	930.60
2	Vitamin, amino acids and Ca mixture- II (Spectro BE)	Each gm contained vitamin B_1-8 mg, vit. B_6-16 mg, vit. B_{12}-80 mg, niacin-120 mg, calcium pantothenate-80 mg, vit. E-160 mg, L-lysine hydrochloride-10 mg, DL-methionine-10 mg and calcium–260 mg	20	697.50
3	Coccidiostat (Cocciwin)	Dinitro-O–Toluamide 25% (coccidiostat)	50	290.00
4	Choline chloride	60 percent choline chloride	50	130.00
5	Lysine	98 percent lysine	50	271.80
6	DL- methionine	98 percent methionine	80	850.00
7	Chlorotetracycline (CTC)	Powder form	33.5	245.00
7	Mineral mixture	Moisture maximum 3%, calcium minimum 32%, phosphorus minimum 6%, manganese- minimum 0.27%, iodine minimum 0.01%, zinc minimum 0.26%, copper minimum 0.001%, iron minimum 0.001% and fluorine maximum 0.03%	2000	55.00
8	Soyabean oil	Ether extract 99.4%	5000	125.00
9	Garlic powder	Powder form	500 and 1000 as per treatment	1000
10	Holy basil (Tulsi) powder	Powder form	500 and 1000 as per treatment	1020

Cholesterol composition in breast and thigh muscles

Extraction of lipids

Total lipids from sample from breast and thigh muscles were extracted according to the method of [2] with slight modification.

Meat sample (25 g) was homogenized with 100 ml solvent (chloroform: methanol, (3:1) for 5 minutes. It was kept for 10 minutes and then this mixture was put on Buchner suction filter. Residue was homogenized two times again as above. Combined organic filtrates were transferred to separating funnel. Two volumes of 0.88% aqueous potassium chloride were added and the funnel was shaken vigorously. Non lipid material was partitioned to upper aqueous phase by keeping the funnel undisturbed for 12 hours. Lower layer was drawn off and dried over sodium sulphate. Lower phase after removing upper phase, if cloudy was made clear by addition of a few drops of methanol. Lipid extract was dried to a constant weight at 60^0C, first in water bath and then in hot air oven. Cholesterol, HDL and LDL of extracted fat from breast and thigh muscles was estimated by using "ERBA Kit" in Automatic Analyzer from this lipid extract.

Statistical analysis

Data was analysed statistically as described by [19]. Analysis of variance was used to study the differences among treatment means and they were compared by using Duncan's Multiple Range Test (DMRT) as modified by [9].

Result and discussion

Thigh and breast muscle cholesterol (mg/dl)

Data pertaining to cholesterol in thigh and breast muscles of broilers under different dietary treatments at the end of experimental period (42 days of age) are presented in Table 2 and Fig. 1.

Table 2: Mean values of cholesterol in thigh and breast muscles of broilers under different dietary treatments.

Treatments	Thigh muscle cholesterol (mg/dl)	Breast muscle cholesterol (mg/dl)
T_1	$98.50^{e}\pm0.44$	$63.75^{e}\pm0.62$
T_2	$86.75^{b}\pm0.47$	$54.75^{c}\pm0.75$
T_3	$90.25^{c}\pm0.94$	$56.00^{c}\pm0.91$
T_4	$95.25^{d}\pm0.62$	$60.75^{d}\pm0.85$
T_5	$88.50^{bc}\pm0.64$	$51.75^{b}\pm0.75$
T_6	$82.75^{a}\pm0.47$	$46.00^{a}\pm0.70$
T_7	$84.00^{a}\pm0.40$	$47.25^{b}\pm0.85$

Mean bearing different superscripts in a column differ significantly ($P<0.05$)
Each value is a mean of four replicates

The values of cholesterol in thigh and breast muscles were 98.50, 86.75, 90.25, 95.25, 88.50, 82.75, 83.00 and 63.75, 54.75, 56.00, 60.75, 51.75, 46.00, 46.15 in treatments T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. The contents of cholesterol in thigh muscle was lowest ($P<0.05$) in T_6 & T_7 and highest in T_1 treatment. Similarly, in breast muscle, was lowest ($P<0.05$) in T_6 & T_7 and highest in T_1 group. The perusal of data showed that concentrations of cholesterol in thigh muscle and breast muscle were highest ($P<0.05$) in T_1 and lowest in treatment T_6 & T_7. Cholesterol concentrations were found to be much higher in the thigh than in the breast muscle. A possible explanation for this may be that cholesterol is usually associated with adipose tissue, which is more abundant in thigh than in breast muscle.

The reduction in cholesterol might be attributed to the organic tellurium compounds that are found in high concentration in garlic buds, which may be inhibiting squalene epoxidase, the penultimate enzyme in the synthetic pathway of cholesterol [18]. Reduction in cholesterol with herbal supplementation has also been reported previously by [7, 12, 18]

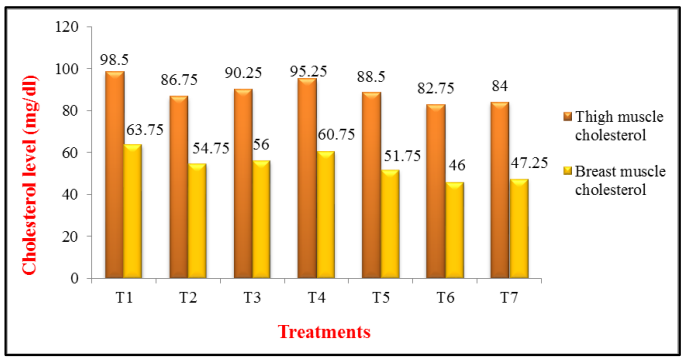

Fig. 1: Cholesterol level in thigh and breast muscles of under different dietary treatments.

Composition of breast and thigh muscles

Data pertaining to the average moisture, protein and ether extract of breast and thigh muscles of experimental birds are presented in Table 3.

Table 3: Mean values of chemical composition (% DM basis) of breast and thigh muscles of experimental birds under different dietary treatments

Treatments	Thigh muscles			Breast muscles		
	Moisture	Crude protein	Ether Extract	Moisture	Crude protein	Ether Extract
T_1	73.07±0.62	20.00±0.25	8.38±0.64	73.49±0.52	21.71±0.65	8.29±0.18
T_2	73.11±1.49	20.25±0.25	8.42±0.50	73.51±0.67	22.48±0.51	8.10±0.50
T_3	73.14±1.25	20.30±0.07	8.33±0.55	73.23±0.81	22.55±0.87	8.17±0.38
T_4	73.27±1.65	20.45±0.21	8.38±0.31	73.26±0.50	21.91±0.54	8.19±0.41
T_5	72.83±0.56	20.48±0.18	7.99±0.61	73.13±0.57	22.64±0.34	8.07±0.26
T_6	73.27±1.65	20.55±0.25	8.18±0.31	73.16±0.25	22.74±0.54	8.15±0.41
T_7	73.13±0.56	20.51±0.48	8.19±0.41	73.15±0.57	22.71±0.44	8.21±0.26

Mean bearing different superscripts in a column differ significantly ($P<0.05$)
Each value is a mean of four replicates

The moisture, CP and EE contents of thigh muscle were 73.07, 73.11, 73.14, 72.27, 72.83, 73.27, 73.13; 20.00, 20.25, 20.30, 20.45, 20.48, 20.55, 20.51 and 8.38, 8.33, 8.38, 7.99, 8.18 and 8.19% and in breast muscle were 73.49, 73.51, 73.23, 73.26, 73.13, 73.16, 73.15; 21.71, 22.48, 22.55, 22.64, 22.74, 22.71 and 8.29, 8.10, 8.17, 8.19, 8.07, 8.15 and 8.21% in treatments T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. There was no particular trend observed in the composition of muscles and the values were statistically similar among the various dietary treatments. Perusal of the data indicated that the percent moisture, CP and EE contents of thigh and breast muscles in broilers had no significant difference among the mean values of different treatments. The present findings are in line with the results of [14] who observed that levels of encapsulated garlic and *Phyllanthus niruri* L. mixture in the diet did not significantly improve the CP percent.

Conclusion

Thus from this study we can conclude that the supplementation of garlic powder and holy basil leaf powder @ 0.5% and 1.0% showed best result in terms of decreasing the cholesterol content of breast and thigh muscles. Whereas there is no significant effect of supplementation on moisture, crude protein and ether extract content of both types of muscles.

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