Int.J.Curr.Microbiol.App.Sci (2020) *9*(12): 3183-3187

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 12 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.912.379

Serum Lipid Profile and Meat Cholesterol Levels as Influenced by Triphala in Commercial Broiler Chicken

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Keywords

Serum lipid profile, Meat cholesterol levels, Triphala

Article Info

Accepted:
22 November 2020
Available Online:
10 December 2020

ABSTRACT

The biological experiment was carried out to determine the serum lipid profile and chicken meat cholesterol levels as influenced by Triphala supplementation in broilers. A total of three hundred commercial one day-old sex separated broiler chicks were randomly grouped into six treatments with five replicates for each treatment containing ten chicks per replicate. The chicks were reared from day-old to 35 days of age in deep litter system under standard management conditions throughout the experimental period. The treatment groups were fed with basal diet without supplementation of antibiotic growth promoter / Triphala (T_1) or with supplementation of Oxytetracycline at 0.004 per cent (T_2), or with supplementation of Triphala at 0.025 per cent (T_3), 0.05 per cent (T_4), 0.075 per cent (T_5) or 0.10 per cent (T_6). At the end of the experiment (35th day), one male and one female bird per replicate, totally ten birds per treatment group were randomly selected. Blood samples were collected for serum lipid profile and meat cholesterol level studies. The experiment revealed that there existed no significant differences in triglycerides and very low density lipoprotein level of serum among the treatment groups. Supplementation of Triphala decreased the serum total cholesterol of broiler chicken in dose dependant fashion with lowest cholesterol recorded in the group supplemented with 0.10 per cent Triphala in diet (T_6). The groups supplemented with Triphala had significantly ($P<0.01$) lowered total cholesterol in serum, breast and thigh meat, and increased ratio of HDL: LDL in serum of broiler chicken in a dose dependent manner with maximum effect at 0.10 per cent Triphala supplementation (T_6) than control (T_1) and antibiotic supplemented group (T_2).

Introduction

Broiler chicken meat has become the most common source of animal protein with low cholesterol and lipid content, because the meat is cheaper and has no ritualistic restrictions for consumption. Cholesterol is a necessary substance, and all of the cholesterol we actually need is produced by our bodies. But cholesterol is also achieved from our foods, namely animal products. Chicken, of course, is one of these animal products, and though it has less cholesterol than other fatty proteins *viz.* bacon or steaks with ribbons of fat and it still contributes to your overall cholesterol levels. In general, Ayurvedic preparations containing more than one ingredient have combined effect on performance of animals including poultry. Triphala is normally ingested for therapeutic purposes in human health and hence, it could be safe to be used in the animal feed. It is proved to have antimicrobial, anti-inflammatory, antimutagenic, immunogenic, antipyretic, free radical scavenging, antioxidant, analgesic, wound healing, antistress, adaptogenic and hypoglycemic activity (Kumar *et al.,* 2016) and hypolipidemic activity (Saravanan *et al.*, 2007). Considering upon the efficacy and beneficial effects of Triphala through dietary supplementation in broiler chicken, an attempt is carried out to assess the serum lipid profile and meat cholesterol levels as influenced by Triphala in broiler chicken.

Materials and Methods

Biological experiment

The biological experiment was carried out with three hundred, sex separated and commercial (Vencobb 400) broiler chicken belonging to single hatch. The whole experimental period was divided into three phases *viz.*, pre-starter (1 to 12 days), starter (13 to 24 days) and finisher phase (25 to 35 days). All the chicks were reared from 0 days to five weeks of age under deep litter in an open sided housing system under standard management conditions. The experimental chicks were wing banded, weighed and randomly allotted into six treatment groups. Each treatment had five replicates with ten chicks per replicate comprising fifty chicks per treatment.

Experimental diet

A basal diet was formulated according to the Vencobb standard, without supplementation of antibiotic growth promoter or Triphala. The experimental diets were prepared by supplementing the basal diet with antibiotic growth promoter (Oxytetracycline @ 0.004 per cent) or Triphala at 0.025, 0.050**,** 0.075 or 0.10 per cent levels. The control group (T_1) was fed with basal diet without any supplementation and the treatment groups were fed with antibiotic growth promoter (T_2) and Triphala at 0.025, 0.05, 0.075 and 0.10 percent ($T_{3,}$ $T_{4,}$ T_5 and T_6) in the basal diet, respectively.

Estimation of serum lipid profile and meat cholesterol

Blood samples collected during the slaughter were allowed to clot and centrifuged for 10 minutes at 2000 rpm to separate the serum sample. These serum samples were stored at -20 ºC for analyzing total cholesterol, HDL and triglycerides. Similarly, muscle cholesterol was estimated from breast and thigh muscle samples collected from the birds during the slaughter for carcass study. The biochemical kits used for these assays were procured commercially.

Serum total cholesterol

Serum cholesterol was reacted with hot solution of ferric per chlorate, ethyl acetate and sulphuric acid (Code no of the kit -71MB100-64) which resulted in lavender coloured complex. The colour intensity was measured at 560 nm (Wybenga *et al.,* 1970).

Serum triglycerides

Triglyceride was estimated as per method of Bucolo and David (1973) using Code no -72LS100-60 kit. The principle of this method involves hydrolysis of triglyceride to glycerol and free fatty acids in the presence of lipase. Subsequently, glycerol was converted to hydrogen peroxide and dihydroxyacetone phosphate using Glycerol 3-Phosphate Oxidase. The H_2O_2 was coupled with 4-Aminoantipyrine (4-AAP) and 4-

chlorophenol to form a red coloured complex, whose absorbance was measured at 505 nm.

Serum HDL cholesterol

HDL cholesterol was estimated as per Seigler and Wu, (1981) using Code no-71MB100-64 kit. About 200 µl of serum and 200 µl of precipitating reagent (polyethylene glycol) were added in a test tube, mixed well, incubated at 37^0 C for 10 minutes followed by centrifugation at 2000 rpm for 15 minutes. The supernatant represented the HDL fraction. This supernatant fraction was estimated for HDL cholesterol as detailed in total cholesterol estimation.

Serum LDL cholesterol

Serum LDL-cholesterol was calculated by Friedewald equation (Friedewald *et al.*, 1972), as LDL cholesterol (mg/dl) = Total cholesterol - (Triglyceride/5) - HDL cholesterol

Serum VLDL cholesterol

Serum VLDL cholesterol was measured by dividing serum triglyceride concentration by five (Khaki *et al.*, 2012).

Meat cholesterol

The breast / thigh muscle samples were chopped and minced with mortar and pestle. The total lipid was extracted from muscle tissue samples as per the method of Folch *et al.*, (1957) using chloroform and methanol (2:1) solutions. The chloroform layer containing cholesterol was separated using separating funnel. The extracted muscle cholesterol was estimated for cholesterol by one-step method of Wybenga *et al.*, (1970). Cholesterol reacts with cholesterol reagent (solution of ferric per chlorate, ethyl acetate and sulphuric acid) which resulted in lavender coloured complex; the absorbance was measured at 560 nm.

Results and Discussion

Serum Lipid profile and Meat Cholesterol

The mean (± S.E.) serum lipid profile and meat total cholesterol (mg %) of broiler chicken at 35^{th} day of age, as influenced by dietary supplementation of graded levels of Triphala are presented in Tables 1 and 2, respectively. Statistical analysis on the serum lipid profile revealed highly significant differences in serum total cholesterol, high density lipoprotein and low density lipoprotein among treatment groups due to supplementation of Triphala. However, there existed no significant differences in triglycerides and very low density lipoprotein level of serum among the treatment groups.

The result on serum total cholesterol (mg%) revealed that the control group (T_1) (227.75) and the group supplemented with antibiotic growth promoter (T_2) had significantly ($P<0.01$) higher level (222.71) of total cholesterol in serum than the groups supplemented with Triphala at all inclusion levels (T_3, T_4, T_5, T_6). Supplementation of Triphala decreased the serum total cholesterol of broiler chicken in dose dependant fashion with lowest cholesterol recorded in the group supplemented with 0.10 per cent Triphala in diet (T_6).

The ratio of HDL: LDL was recorded to be lowest (1.61) in the group supplemented with antibiotic growth promoter (T_2) than the control group (1.84). Supplementation of Triphala was found to increase the ratio of HDL: LDL in dose dependant fashion with highest ratio (4.92) in the group supplemented with 0.10 per cent Triphala (T_6). Statistical analysis of data on the meat total cholesterol exerted highly significant differences in breast and thigh meat, among treatment groups due to supplementation of Triphala. The result on meat total cholesterol revealed that the control group (T_1), the group supplemented with antibiotic growth promoter (T_2) and the group with lowest inclusion level of Triphala (T_3) had significantly (P<0.01) higher level of cholesterol in breast as well as thigh meat than the groups supplemented with Triphala at 0.05, 0.075 and 0.10 per cent levels (T_4, T_5, T_6). Supplementation of Triphala at and above 0.05 per cent in diet decreased the breast and thigh meat cholesterol of broiler chicken in dose dependant fashion with lowest cholesterol recorded in the group supplemented with 0.10 per cent Triphala in diet (T_6).

Table.1 Mean (± S.E.) Serum Lipid profile (mg%) of Broiler chicken as influenced by dietary supplementation of graded levels of Triphala

Treatment	Total Cholesterol	Triglycerides	HDL	LDL	VLDL
T_1	227.75^{D} ± 3.43	203.72 ± 2.81	121.24^{D} ± 1.89	65.77^{D} ± 4.10	40.74 ± 0.56
T_2	222.71^{D} ± 3.86	202.69 ± 3.59	112.25^{C} ± 0.70	69.92^{D} ± 4.01	40.53 ± 0.71
T_3	206.04^{C} ± 5.03	197.56 ± 2.70	110.07^{BC} ± 0.48	56.46^{BC} ± 5.25	39.51 ± 0.53
T_4	199.46^{BC} ± 3.04	195.38 ±2.35	105.73^{B} ± 1.15	54.66^{BC} ± 2.54	39.07 ± 0.47
T_5	186.85^{B} ± 2.81	195.12 ± 2.01	98.95^{A} ± 1.28	48.88^{B} ± 2.68	39.03 ± 0.40
T_6	155.58^{A} ± 3.78	196.41 ± 2.47	96.66^{A} ± 0.54	19.64^{A} ± 3.73	39.28 ± 0.49

Value given in each cell is the mean of 6 observations
$^{A-D}$ Means within a column with no common superscript differ significantly (P<0.01)

Table.2 Mean (± S.E.) Meat Total Cholesterol (mg%) of broiler chicken as influenced by dietary supplementation of graded levels of Triphala

Treatment	Breast meat cholesterol	Thigh meat cholesterol
T_1	120.18^{C} ± 2.17	159.54^{D} ± 1.76
T_2	118.49^{C} ± 2.10	163.20^{D} ± 2.54
T_3	121.78^{C} ± 2.38	166.93^{D} ± 1.76
T_4	109.16^{B} ± 1.83	156.18^{CD} ± 4.16
T_5	103.29^{B} ± 1.52	144.36^{BC} ± 3.47
T_6	93.24^{A} ± 2.08	132.89^{A} ± 3.69

Value given in each cell is the mean of 6 observations
$^{A-D}$ Means within a column with no common superscript differ significantly (P<0.01)

The results of present study are in agreement with Saravanan *et al.*, (2007), Maruthappan and Sakthi Shree (2010) and Fatma Eid *et al.*, (2011). Their experiments in rats showed that Triphala induced reduction in the levels of serum total lipids. Similar results were also found in broiler birds by Sujatha *et al.*, (2010) on inclusion of polyherbal feed supplement in the diet.

The result clearly establishes that supplementation of Triphala in broiler diet exerted significant positive influence on serum lipid profile and meat cholesterol, with maximum effect at 0.10 per cent dietary inclusion. The results also have shown that Triphala supplementation increases the ratio of HDL: LDL in broiler chicken. The result further indicates that antibiotic growth promoter has either negative effect or no effect on serum and meat cholesterol and on the ratio of HDL: LDL in broiler chickens.

Based on result of the present study, it could reasonably be concluded that supplementation of Triphala at and above 0.05 per cent in diet decreased the breast and thigh meat cholesterol of broiler chicken in dose dependant fashion with lowest cholesterol recorded in the group supplemented with 0.10 per cent Triphala in diet (T_6). Hence, Triphala could be utilized to reduce the serum and meat cholesterol with improved ratio of HDL: LDL in broiler chicken.

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How to cite this article:

Madhupriya, V., P. Shamsudeen, G. Raj Manohar and Senthilkumar, S. 2020. Serum Lipid Profile and Meat Cholesterol Levels as Influenced by Triphala in Commercial Broiler Chicken. *Int.J.Curr.Microbiol.App.Sci.* 9(12): 3183-3187. doi: https://doi.org/10.20546/ijcmas.2020.912.379