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Effect of *Moringa oleifera* leaf meal supplementation in Broiler chicken on serum and muscle lipid profile

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Abstract
A study was conducted to investigate the effect of *Moringa oleifera* leaf meal (MOLM) on serum and muscle lipid profile in broiler chicken. A total of 216 numbers of day old - broiler (Cobb 400) chicks were randomly divided into six experimental groups with six replicates, six chicks per replicate. Chicks were distributed in 4 different inclusion levels of MOLM in diets; Such as 750 ppm, 1000 ppm, 1250 ppm & 1500 ppm with basal ration and basal ration with AGP. The results showed that the lowest serum cholesterol (149.91 mg/dl) and triglycerides (125.19 mg/dl), was observed in T4. The HDL (mg/dl) in MOLM supplemented groups ie. T4 (77.37), T5 (79.74) and T6 (79.80) was significantly (P<0.01) higher than T1 (70.01) and T2 (70.85). Similarly, the MOLM supplemented groups had significantly (P<0.01) reduced cholesterol level (mg/g) in thigh (108.43-113.59) and breast muscle (75.53-76.39) than T1 (122.42 and 84.27) and T2 (120.06 and 83.23). It is concluded that MOLM supplementation resulted in increased serum HDL and decreased serum cholesterol, LDL, triglyceride and muscle cholesterol levels.

Keywords: Broiler, Cholesterol, HDL, *Moringa oleifera*, Triglycerides

Introduction
Recently, consumer's interest for organic and safe poultry products, with functional properties has been steadily increasing. Because of the development of broiler industry, generally using concentrate feed with high chemical feed additives. This causes the occurrence of chemical accumulation in broiler meet (Mardevi *et al*., 2017) [11]. Preventing the build-up chemical in broiler meet can be done by mixing with natural ingredients. One such phytogenic feed additive plant is *Moringa oleifera* referred as 'Drum stick tree' belongs to the family of *Moringaceae.*
The fat found in the broiler chicken generally consists of triglycerides, phospholipids and cholesterols. There is a general assumption that high dietary cholesterol levels result in high serum cholesterol and consequently a higher risk of arteriosclerosis and coronary heart disease in humans (Grundy 1990) [8]. The addition of *M. oleifera* leaf meal in broiler chicken diets significantly reduced the serum lipid level and thigh, breast muscle cholesterol level (Divya *et al*., 2014) [4].

Materials and methods
1. Biological experiment
The biological experiment was carried out a total of two hundred and sixteen numbers of day old - broiler (Cobb 400) chicks were randomly divided into six experimental groups with six replicates, six chicks per replicate. Each replicate had equal number of male and female chicks and completely randomized design (CRD) was followed.
The various experimental groups are as follows.
T1 - Basal ration, T2-Basal ration with antibiotic growth promoter (AGP),
T3 - Basal ration with MOLM (750 ppm), T4-Basal ration with MOLM (1000 ppm), T5- Basal ration with MOLM (1250 ppm), T6- Basal ration with MOLM (1500 ppm).

2. Serum lipid profile
2.1. Serum total cholesterol
Cholesterol esters were hydrolysed by Cholesterol Esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase oxidizes the 3-OH group of free cholesterol to liberate Cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide couples with 4-amino antipyrine and phenol to produce red quinoneimine dye.

Absorbance of coloured dye was measured at 505 nm (Wybenga *et al*., 1970) [16]. This assay was carried out using kit with Code no - 71LS200-60.

2.2. Serum triglycerides
Triglyceride was estimated as per the method of Bucolo and David, (1973) [2] 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 hydrogen peroxide was coupled with 4- Aminoantipyrine (4-AAP) and 4-chlorophenol to form a red coloured complex, whose absorbance was measured at 505 nm.

2.3. Serum HDL - cholesterol
HDL - cholesterol was estimated as per Seigler and Wu, (1981) [14] using Code no -71LS200-60 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˚ C for 10 minutes followed by centrifugation at 2000 rpm for 15 minutes. The supernatant represented the HDL fraction and was determined as detailed in total cholesterol estimation.

2.4. Calculation of LDL - cholesterol
Serum LDL - cholesterol was calculated by Friedewald equation (Friedewald *et al*., 1972) [6].
LDL cholesterol (mg/dl) =Total cholesterol-(Triglyceride/5)-HDL cholesterol.

3. Estimation of total cholesterol in muscle
The breast and 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) [5] 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) [16]. Cholesterol reacts with cholesterol reagent (solution of ferric perchlorate, ethyl acetate and sulphuric acid) which resulted in lavender coloured complex and the absorbance was measured at 560 nm.

Result and Discussion
The MOLM supplemented groups (T3, T4, T5 and T6) showed significantly (P<0.01) decreased serum total cholesterol, LDL cholesterol and triglycerides but significantly (P<0.01) higher HDL cholesterol level than T1 and T2 groups. The lowest serum total cholesterol, triglycerides and highest HDL was observed in T4 group compared to other treatment groups (Tab 1).
Similar to the present study, work done by Zanu *et al*. (2012) [17], Divya *et al*. (2014) [4], Alnidawi *et al*. (2016) [1] and Hamid and Mukhtar, (2016) [9] showed decreased serum total cholesterol, LDL cholesterol, triglycerides and increased HDL cholesterol level (Gakuya *et al*., 2014) [7] due to addition of MOLM.

Table 1: Effect of *Moringa oleifera* leaf meal supplementation on serum lipid profile in broiler chicken at 42nd day of age

Treatment groups	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)
T1- Basal ration	$165.72^{b} \pm 1.71$	$70.01^{a} \pm 2.24$	$68.86^{b} \pm 2.14$	$134.24^{b} \pm 1.42$
T2 - Basal ration with AGP	$167.68^{b} \pm 2.73$	$70.85^{ab} \pm 2.06$	$70.18^{b} \pm 2.32$	$133.20^{b} \pm 1.25$
T3 - Basal ration with MOLM (750 ppm)	$151.61^{a} \pm 1.42$	$76.65^{bc} \pm 1.30$	$49.74^{a} \pm 2.51$	$126.11^{a} \pm 1.73$
T4 - Basal ration with MOLM (1000 ppm)	$149.91^{a} \pm 3.90$	$77.37^{c} \pm 2.45$	$47.49^{a} \pm 2.82$	$125.19^{a} \pm 1.28$
T5 - Basal ration with MOLM (1250 ppm)	$152.09^{a} \pm 2.84$	$79.74^{c} \pm 2.06$	$46.92^{a} \pm 2.10$	$127.13^{a} \pm 1.15$
T6 - Basal ration with MOLM (1500 ppm)	$153.60^{a} \pm 1.10$	$79.80^{c} \pm 1.29$	$48.15^{a} \pm 2.08$	$128.22^{a} \pm 1.75$
P value	0.001**	0.004**	0.001**	0.001**

Means with different superscripts in a column differ significantly ** (P<0.01)

MOLM contains high amount of polyphenols (Moyo *et al*., 2011) [12] flavonoids, alkaloids and phenolic compounds (Verma *et al*., 2009) [15] and these compounds possess the hypocholesterolaemic effect. In the present study, significant reduction in cholesterol and triglyceride level in blood serum of broiler might be attributed to the above mentioned phytochemical constituents of *Moringa oleifera* leaf meal. During digestion in the intestine, cholesterol is the main component of bile acids secreted. The fibre coats the bile acids in the intestine and is excreted in the body, subsequently causing the body to draw cholesterol from the blood to form bile acids and thus lowering blood cholesterol level (Olugbemi, 2010b) [13].

2 Muscle total cholesterol
Addition of MOLM at four levels had significantly (P<0.01) decreased breast muscle cholesterol compared toT1 and T2 group. In thigh muscle, total cholesterol was significantly (P<0.01) reduced in T5 and T6 group compared to T1, T2 and T3. In general, the MOLM supplemented groups had significantly (P<0.01) reduced cholesterol level in thigh and breast muscle than MOLM non supplemented group (Table 2). Similar reduction (P>0.05) in breast and thigh muscles cholesterol content due to antibiotic supplementation was observed by Ciftci *et al*. (2010) [3] and Koochaksaraie *et al*. (2011) [10]. The probable mechanism for reduction of cholesterol might be due to the presence of polyphenols, flavonoids, alkaloids, phenols and other growth promoting properties of *Moringa* leaf meal.

Table 2: Effect of *Moringa oleifera* leaf meal supplementation on muscle total cholesterol in broiler chicken at 42nd day of age

Treatment groups	Breast muscle cholesterol (mg/100 g)	Thigh muscle cholesterol (mg/100 g)
T1 - Basal ration	$84.27^{b} \pm 1.87$	$122.42^{c} \pm 1.93$
T2 - Basal ration with AGP	$83.23^{b} \pm 1.30$	$120.06^{c} \pm 1.45$
T3 - Basal ration with MOLM (750 ppm)	$76.39^{a} \pm 0.75$	$113.59^{b} \pm 1.71$
T4 - Basal ration with MOLM (1000 ppm)	$75.53^{a} \pm 0.79$	$110.5^{ab} \pm 1.63$
T5 - Basal ration with MOLM (1250 ppm)	$76.08^{a} \pm 1.51$	$108.43^{a} \pm 1.67$
T6 - Basal ration with MOLM (1500 ppm)	$75.72^{a} \pm 0.86$	$108.69^{a} \pm 1.02$
P value	0.001**	0.001**

Means with different superscripts in a column differ significantly ** (P<0.01)

Conclusion

From the results of this study it can be concluded that MOLM supplementation resulted in increased serum HDL and decreased serum cholesterol, LDL, triglyceride and muscle cholesterol levels.

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