HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF BROILERS FED DIET WITH *Moringa oleifera* LEAF MEAL (MOLM)

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ABSTRACT

The present study was carried out to study the effect of dietary inclusion of Moringa oleifera leaf meal (MOLM) on blood and serum parameters of broilers. Day old broilers (n=180) were distributed randomly into six dietary groups each with five replicates of 6 birds. The dietary treatments include Moringa oleifera leaf meal at 0% (T_1; negative control), T_1 + Vit-E, Se, Vit-C (T_2; positive control), 1.5% (T_3), 3.0% (T_4), 4.5% (T_5) and 6.0% (T_6) levels by marginal adjustment of other feed ingredients. PCV, Hb, RBC, WBC and H/L ratio were not influenced by the level of MOLM in diet. Decreased ($p<0.01$) serum cholesterol and increased HI titers ($p<0.05$), serum protein ($p<0.05$), globulin ($p<0.01$) with increasing MOLM in diets. Results showed that MOLM can be included up to 6% without any deleterious effect on health status of the broilers and meat production with low cholesterol.

Key words: Blood parameters, Broilers, Cholesterol, Leaf meal, *Moringa oleifera,* Serum biochemistry

INTRODUCTION

Shift in food consumption pattern was observed in the world with increasing growth rate of the population and also increased food economy. Data available indicates that there is a gap between availability and requirement of animal protein, so to cater the needs of increasing human population, the layer and broiler industry has to be up scaled by 5 and 10 folds, respectively (ICAR VISION 2050 (2013). However, the projected growth of industry correspondingly depends on the availability of feed ingredients to meet the requirement, as well as health status of the flocks.

The non-availability and increasing prices of feed ingredients have compelled researchers in developing countries to direct their attention to non-conventional feeds, with particular emphasis on protein substitutes. Among that alternatives leguminous multipurpose trees and shrubs has been suggested to be a viable source of

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proteins, vitamins and minerals for poultry feeding. *Moringa oleifera* is the most widely cultivated species among plants that used as a cheap protein supplement to improve digestibility of other diets (Gadzirayi *et al*., 2012). The leaves of the tree have been reported to bear an antioxidant activity due to the higher amount of polyphenols (Moyo *et al.,* 2012). Both phenolic and flavonoid compounds affect lipid oxidation potential and fatty acid composition (Sreelatha and Padma, 2009). The result of haematology and serum analysis was considered to assess the health status of an animal. Haematological and serum parameters have been observed as good indicators of the physiological status of animal and their changes are important in assessing the response of such animal to various physiological situations (Khan and Zafar, 2005). This study was carried out to determine the effects of graded levels of *Moringa oleifera* leaf meal (MOLM) on haematological and serum biochemical profile of broiler chickens.

MATERIALS AND METHODS

Ross broiler chicks (n=180) at day old age were distributed randomly in to six treatments with five replicates containing six birds each. *Moringa oleifera* leaves were procured from college, it was shade dried and powdered. The chemical composition of *Moringa oleifera* leaf meal was analysed (Table 1) as per AOAC (2007). Other ingredients utilised for this study were procured from local market, Basal diet was prepared as per BIS (2007) acted as Negative control (T1). The other dietary treatments include Positive control (T2), which was supplemented with Vitamin E, Se & Vitamin C (@150g/ ton) to T1 and the MOLM was incorporated at 1.5, 3.0, 4.5 and 6.0 % levels to the basal diets by replacing maize and SBM as T3, T4, T5 & T6, respectively. All the experimental diets were prepared iso-nitrogenous and iso-caloric. The prestarter, starter and finisher diets were formulated with calorie protein ratio of 130, 141 and 160, respectively.

All the birds were housed in 4-tier battery cages throughout the experiment under uniform management conditions. Feed and water were provided *ad libitum* from 0 to 6 weeks.

Blood samples were collected from two birds/ replicate on 42nd day @ 2 ml of blood for serum biochemical tests and 1ml of blood in vials with 2mg ethylene-diamine tetra-acetic acid (EDTA) for haematology from the brachial vein. Blood samples were used for fresh blood count. Red Blood Cell (RBC) and White Blood Cell (WBC) were measured according to the method of Natt and Herrick (1952). PCV was measured in plain capillary tube using a microhaematocrit centrifuge. Haemoglobin (Hb) was estimated by cyanomethaemoglobin method using Drabkins solution and H/L ratio were measured according to Varley *et al*. (1980).

Serum cholesterol was estimated calorimetrically by using diagnostic kit (M/s. ERBA) by enzymatic method of Allain *et al.* (1974) for *in vitro* estimation. Serum total proteins were estimated by using diagnostic kit. Albumin concentration was estimated photometrically by bromocresol green (BCG) method (Doumas *et al.,* 1971) using Cormay albumin kits. Globulin

concentration was calculated by subtracting the concentration of albumin from total protein concentration.

The humoral immunity was estimated in birds by measuring antibody titre in serum to Newcastle disease (ND) vaccine (antibody production against ND virus).

Statistical analysis of the data was carried out by one-way ANOVA. Difference between means was tested at the 5% probability level using Duncan's LSD test (Duncan, 1955).

RESULTS AND DISCUSSION

Haematological parameters

Inclusion of MOLM up to 6.0% in the diet had no effect ($p>0.05$) on packed cell volume in broilers (Table 2). But the values fall within normal range (23-58%) of healthy birds (Mabruk*et al.,* 2013). These findings are similar to the results of Paul *et al.* (2018) Liaqat *et al.* (2016) in broilers. In contrary, Oghenebrorhie and Oghenesuvwe (2016) reported that PCV showed significantly increasing values in birds fed with 8 and 10% MOLM than control. On other hand, Aderinola *et al.* (2013) reported that the mean PCV values were significantly ($p<0.05$) decreased with increasing levels of MOLM in diet.

Haemoglobin concentration was not affected by the level of MOLM in diet (Table 2). These fluctuations are within normal range in the blood and are a normal phenomenon and associated with the physiological status of the birds. Similarly, Paul *et al.* (2018) and Elbashier and Ahmed (2016) also reported (15-16.2% Hb and 39.6 to 42.7 PCV) in broilers by incorporation of MOLM up to 5%. In contrary, Abbas *et al.* (2018) reported that inclusion of MOLM at 0.75 and 1% levels in diets had higher ($p<0.05$) Hb level as compared with the control. This might be due to level tested (0.25 to 1%) was lower than the lowest (1.5%) incorporation level in the current study.

The present study revealed that inclusion of MOLM up to 6.0% in the diet had no effect ($p>0.05$) on RBC count in broilers (Table 2). These values are within normal range. These findings are similar to the results of Elbashier and Ahmed (2016); Liaqat *et al.* (2016); Oghenebrorhie and Oghenesuvwe (2016). In contrary, Abbas *et al.* (2018) reported that inclusion of MOLM at 0.25, 0.5, 0.75 and 1% levels in diets had significantly ($p<0.05$) increased RBC count as compared with the control.

The present study revealed that inclusion of MOLM up to 6.0% in the diet had no effect on WBC count, H/L Ratio in broilers (Table 2). These findings are similar to the results of Abbas *et al.* (2018) who reported that inclusion of MOLM at 0.25, 0.5, 0.75 and 1% levels in diets had no significant effect on WBC count and H/L ratio as compared with the control.

Haemagglutination inhibition test

The $\log_2$ HI titers (Table 3) were increased ($p<0.05$) with increase in the level of MOLM. The response of body towards antibody titers is attributed to bioactive compounds like antioxidants, vitamins, minerals and amino acid profile of MOLM might be responsible for improved immune status of birds. Similarly, Rao *et al.* (2019),

Ahmad *et al.* (2018) Mousa *et al.,* (2017), Liaqat *et al.* (2016) also reported improved HI titers by feeding Moringa based diets to broilers.

Serum biochemical parameters

Serum cholesterol (mg/dl) content (Table 4) was decreased ($p<0.01$) with increasing the MOLM in diet. Similar findings were noticed by Abbas *et al*. (2018); Ahmad *et al.* (2018) Mousa *et al.* (2017); Abousekken (2015) in broilers and Elkloub *et al.* (2015) in Japanese quails. The significant reduction of cholesterol due to supplementation of MOLM in feed might be attributed to presence of phytosterols in the Moringa plant tissues, which decreases the absorption of cholesterol from the intestine with an immediate release in feces.

Work of Nobakht and Moghaddam (2013), Srinivasan (2005) inferred that antioxidant properties of Moringa with flavanoids and phenolic compounds (Hussain *et al.,* 2014) potentiates the production of bile salts, which results in emulsification of fats and decrease in the absorption of lipids so decreasing the levels of cholesterol. Besides, bioactive compound flavonoids (quercetin) and carotenoids (b-carotene) positively affected and reduced the levels of cholesterol in the serum (Elkloub *et al.,* 2015; Melesse *et al.,* 2013). Whereas, Jiwuba *et al.* (2016) reported that low cholesterol levels by incorporation MOLM in diet were may be due to decline in lipid mobilization and suggest that *Moringa* leaf meal diet were capable in reducing serum cholesterol, hence assisting in the reduction and deposition of cholesterol in the muscle, thus production of lean meat.

Increased ($p<0.05$) serum protein (g/dl) with increase in MOLM in diet was noticed (Table 4). These results were in corroborated with the findings of Mousa *et al.* (2017); Hassan *et al.* (2016) and Abousekken (2015) in broilers and Elkloub *et al.* (2015) in Japanese quail. Contradicting the present findings Divya *et al.* (2014) reported that feeding of MOLM up to 1.5% level had decreased serum total protein concentration in broilers when compared with control. On other hand inclusion of MOLM in the diet had no effect on serum total protein (g/dl) content in broilers (Aderinola *et al.,* 2013; Tijani *et al.,* 2016), in growing rabbits (Jiwuba *et al.,* 2016).

Serum albumin content (g/dl) of broilers was not influenced by level of MOLM in diet as compared to the control (Table 4). This was coinciding with Hassan *et al.* (2016) who observed by incorporation of 0.2% MOLM in diet when compared to control. Whereas, Divya *et al.* (2014) in broilers, Elkloub *et al.* (2015) in quails noticed significant ($p<0.05$) decrease in albumin levels.

Serum globulin values of birds fed with MOLM in diets were significantly increased ($p<0.01$) when compared with control groups (Table 4). Globulin level has been used as indicator of immune responses and sources of antibody production. It infers that the potential effect of MOLM in improvement of immunity. Several researchers (Mousa *et al.,* 2017; Abousekken, 2015) reported that inclusion of *Moringa oleifera* leaf meal in the diet had no effect on serum globulin content in broilers and in Japanese quail (Elkloub *et al.,* 2015).

The present study revealed that inclusion of MOLM up to 6.0% in the diet had no effect on A/G ratio in broilers (Table 4). The results of present study were in line with the findings of Elkloub *et al.* (2015) in quails. In contrary, Abousekken (2015) reported that Albumin/globulin ratio were significantly ($p<0.05$) improved with extract of Moringa leaf when supplemented through drinking water @50ml and 100ml/L compared to control and powder forms in broilers. This might be due to immunomodulatory effect of *Moringa oleifera* was more effective through drinking water than through feed form.

Table 1. Nutrient composition of MOLM

Nutrient	Value (%)
Dry matter	92.42
Crude protein	25.13
*ME (kcal/kg)	2800
Ether extract	8.42
Crude fiber	12.68
Total ash	14.29
NFE	39.48
Acid insoluble ash	1.33
Calcium	3.25
Phosphorus	0.49

*ME Values (Kumar *et al.,* 2018)

Table 2: Effect of dietary inclusion of *Moringa oleifera* leaf meal in broiler diets at varying levels on haematological parameters

Treatment / (MOLM%)	PCV (%)	Hb(g/dl)	RBC($\times10^6$ /µl)	WBC($\times10^3$ /µl)	H/L ratio
T_1 (-C)	27.25±0.66	10.18±0.90	2.13±0.25	29.90±0.57	0.35±.00957
T_2 (+C)	28.25±0.42	9.66±1.13	2.07±0.18	29.50±0.54	0.34±.00400
T_3(1.5% MOLM)	27.70±1.02	10.10±0.94	2.14±0.20	30.1±0.82	0.33±.00745
T_4(3% MOLM)	28.60±0.69	9.73±0.57	1.91±0.12	29.70±0.65	0.33±.00476
T_5(4.5% MOLM)	27.60±0.64	9.10±0.92	1.89±0.05	29.40±1.07	0.33±.00581
T_6(6% MOLM)	27.35±0.57	9.03±0.68	1.86±0.07	29.60±0.69	0.32±.01027
SEM	0.28	0.35	0.06	0.29	0.01
N value	6	6	6	6	6
p-value	0.713	0.904	0.696	0.987	0.72

Table 3: Effect of dietary inclusion of *Moringa oleifera* leaf meal in broiler diets at varying level on mean log_2 values (±S.E) of haemagglutination inhibition test

Treatment	HI
T_1 (-C)	19.51^{c}±7.22
T_2 (+C)	26.97^{b}±14.88
T_3(1.5% MOLM)	30.16^{ab}±14.45
T_4(3% MOLM)	33.23^{ab}±14.11
T_5(4.5% MOLM)	35.88^{a}±15.00
T_6(6% MOLM)	37.08^{a}±14.57
SEM	5.39
N value	6
P value	0.016

Values in column bearing different superscripts differ significantly (P<0.05).

Table 4: Effect of dietary inclusion of *Moringa oleifera* leaf meal in broiler diets at varying levels on serum biochemical profile

Treatment/ (MOLM%)	Cholesterol (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
T_1 (-C)	210.22^{a}±8.45	4.99^{c}±0.16	2.08±0.12	2.91^{c}±0.12	0.71±0.10
T_2 (+C)	198.08^{ab}±7.14	5.38^{bc}±0.20	2.11±0.11	3.26^{bc}±0.16	0.64±0.17
T_3(1.5% MOLM)	176.57^{b}±20.20	5.52^{abc}±0.08	2.13±0.15	3.41^{ab}±0.14	0.62±0.15
T_4(3% MOLM)	167.66^{bc}±7.67	5.85^{ab}±0.17	2.21±0.19	3.64^{ab}±0.15	0.60±0.22
T_5(4.5% MOLM)	158.09^{c}±10.19	5.90^{ab}±0.34	2.25±0.30	3.67^{ab}±0.10	0.61±0.23
T_6(6% MOLM)	151.64^{c}±7.95	6.20^{a}±0.39	2.43±0.38	3.77^{a}±0.18	0.64±0.30
SEM	5.16	0.11	0.07	0.09	0.08
N	6	6	6	6	6
p-value	0.001	0.017	0.899	0.001	0.527

Values in a column bearing different superscripts differ significantly.

CONCLUSION

The haematological parameters of broilers revealed that inclusion of MOLM from 0 to 6.0% in the diet had no effect (P>0.05) on PCV, Hb, RBC, WBC and H/L ratio, albumin, A/G ratio. Significant increase in HI titers, proteins, globulins and decreased cholesterol with increase MOLM in diet. It can be concluded that MOLM can be included up to 6% dietary level without any deleterious effect on health of birds.

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