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HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF BROILER CHICKS FED AT DIFFERENT LEVELS OF *MORINGA OLEIFERA* LEAF MEAL

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ABSTRACT : This study was carried out to determine the influence of *Moringa oleifera* leaf meal (Molm) on the haematological and serum biochemical indices of broiler chickens. A total of 225 day-old broiler chicks (Ross 308) were randomly distributed into five groups contains three replicate (15 birds/pen). Each group was fed on the one of the following experimental diets. Diet 1 was the control diet (with not supplementation). Diets 2, 3, 4 and 5 supplied with *Moringa oleifera* leaf meal (Molm) at a level 0.25, 0.50, 0.75 and 1.0%, respectively. Results showed that red blood cell increased significant (P≤0.05), while packed cell volume, white blood cell, lymphocyte (L), Hetrophil (H) and H/L ratio were similar (P≥0.05) among the groups. Haemoglobin was improved (p≤0.05) by *Moringa oleifera* leaf meal at 0.75 and 1.0% inclusion rate. The serum biochemical indices in birds fed Molm showed significant reductions (p≤0.05) in total protein, albumin (at 0.75 and 1%), globulin, alkaline phosphatase (ALP) activity and uric acid contents. Albumin to Globulin ratio was highest at 0.25 and 0.50% inclusion rate as compared to other groups. Serum total cholesterol, low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) levels were decreased significantly in all treatments compared to control. Additionally, high density lipoprotein cholesterol (HDL-c) and triglycerideslevels were increased (p≤0.05) in all treatments compared to control group.Based on the results obtained, the addition of 0.75% of *Moringa oleifera* leaf meal diet is recommended.

Key words : Broilers, *Moringa oleifera*, haematobiochemistry.

INTRODUCTION

For many years, antibiotics have been used in the poultry industry. However, the misuse or continuous use of antibiotics has led to the emergence of the antibiotics residue and drug resistance. Now a day's use of antibiotics as growth promoter in animal nutrition is facing reduced social acceptance and their use has been banned or curtailed in many countries (Laxman, 2016). The negative impact on consumers of meat or poultry products due to residual effects has led to the ban on the use of antibiotics as growth promoters since 2006 by the European Union. Animal scientists and veterinarians are now turning attention to safe and natural alternatives such as plants (phytobiotic) to replace antibiotics. Plants contain phytonutrients and phytochemicals (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides), which are referred to as secondary metabolites (Ogbe and Affiku, 2012). Plants' secondary metabolites are applied in nutrition and as pharmacologically active agents (Soetan and Oyewole, 2009). Moringa (*Moringa oleifera* Lam. moringaceae) is a highly valued plant that is mostly cultivated in the tropics and subtropics. It is used for food, medication and industrial purposes, plant leaves are rich in nutrients and has potential to be used as a feed additive with multiple purposes (Moyo *et al*, 2011). *Moringa oleifera* is one of the plants that can utilize in the preparation of poultry feeds. Plants leaves act as a good source of vitamins and amino acids of natural antioxidant due to the presence of various antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Sreelatha and Padma, 2009; Moyo *et al*, 2012). Some studies have indicated that different parts of *Moringa* plant (root, bark, gum, leaf, fruit, flowers, seed and seed oil) has medicinal properties, including immune enhancement and blood formation (Olugbemi *et al*, 2010; Adegbite *et al*, 2016). It was used against high blood pressure, diarrhea, inflammation of colon, intestinal worms, skin antiseptic, as a diuretic agent (Lowell, 2002). Leaves of the plant

were offered many important medicinal properties include antioxidant, hepatoprotective, antibacterial and antifungal activities (Bukar *et al*, 2010), antihepatotoxic and hypoglyceridemic (Akpet *et al*, 2014). According to studies by Omolaso *et al* (2016) acute intake of *Moringa oleifera* leaves has been shown to lower blood pressure, body temperature and blood glucose level in human. Concerning the effect of *Moringa oleifera* leaves on poultry performance, Donkor *et al* (2013) indicated that supplementation of *Moringa oleifera* leaves to poultry feed will lead to high output performance in poultry production. Supplementation of *Moringa oleifera* leaf powder also helped in improving immunocompetence and gut health of broilers. From this given background, the aim of this study was to investigate the effects of *Moringa oleifera* leaf meal (Molm) as dietary supplementation on some blood haematological and biochemical parameters of broiler chicks.

MATERIALS AND METHODS

Animal husbandry and treatments

An experiment was conducted at the Poultry Research Farm of the Animal Production Department, College of Agriculture, University of AL-Kufa, between 9 October and 19 November 2017. A total of 225 day-old broiler chicks (Ross 308) were randomly distributed into five groups contains three replicate (15 birds/pen).

Each group was fed on the one of the following experimental diets. Diet 1 was the control diet (with not supplementation). Diets 2, 3, 4 and 5 supplied with *Moringa oleifera* leaf meal (Molm) at a level 0.25, 0.50, 0.75 and 1.0%, respectively. Basal diets were formulated to meet the nutrient requirements of the broiler (Commercial recommendation). The formulation of the basal diet is shown in Table 1. The birds were fed a starter diet until 21days of age, followed by a finisher diet from 22 to 42 days. All birds had *ad libitum* access to feed for the 42-day period.

Table 1 : Ingredients and nutrient composition of broiler starter and finisher diets.

Ingredient (%)	Starter diet 1-21 day	Finisher diet 22-42 day
Yellow corn	44.90	49.72
Wheat	10.0	10.0
Soybean meal (47%)	38.0	31.70
Corn oil	2.70	4.50
[1]Premix (20%)	2.5	2.5
Dicalicum phosphate	1.20	1.20
L-Lysine	0.10	0.0
DL-Methionine	0.30	0.08
Sodium chloride	0.30	0.30
Total	**100**	**100**
Calculated composition[2]		
Metabolizable energy(Kcal /Kg)	3003	3178
Crude protein (%)	23.82	20.94
Calorie: protein ratio	126.07	151.77
Calcium (%)	1.00	1.00
Phosphorus available (%)	0.64	0.53
Lysine (%)	1.43	1.23
Methionine + Cysteine (%)	1.07	0.86

[1]Premix (BIRMIX M-25) /kg diet: Vitamin A (400.000 IU), vitamin D3 (160.000 IU), vitamin E (1600 IU), vitamin K (80 mg), Vitamin B_1 (80 mg), Vitamin B_2 (240 mg), Calcium pantothenate (CAL-PA) (5200 mg), niacin (1400 mg), vitamin B_6 (1200 mg), biotin (2 mg), folic acid (40 mg), vitamin B_{12} (0.4 mg), Dicalicum phosphate (120.000 mg), phytase (4,000 mg), Premix content with enzyme (%): protein (20%), ME (3000 kcal/kg), lysine digested (5.71), methionine digested (8.2) and common salt (5.92). [2]Was calculated according to the chemical composition of feedstuff contained in NRC (1994).

Heamatological biochemical parameters evaluation

At 42 day of age blood sample were taken from Brachial vein from three birds from each treatment (chick/ replicate) randomly. Blood sample 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). Packed Cell Volumes (PCV) were measured according to Archer (1965). Hemoglobin was directly calculated depending on the PCV values using the equation described by Campbell (1995)and H/ L ratio were measured according to Varley *et al* (1980). Forserum biochemical indices, blood sample was drawn and allowed to stand for an hour at room temperature (18^0C) to serum collection. Serum was separated by centrifugation and stored at -20^0C for further analysis. Total protein and albumin were analyzed by a colorimetric method using commercial kits (Spinreact, Spain). Serum globulin was calculated by subtraction from total proteins.Blood serum cholesterol, triglycerides concentrations were determined according to the methods of Tietz (1999), uric acid (Ross *et al*, 1978), using commercial kits (Spinreact, Spain). The concentration of high density lipoprotein cholesterol (HDL-c) in the serum was estimated by method (Warnick and Wood, 1995). The low density lipoprotein cholesterol (LDL-c) was estimated as the difference between total cholesterol and high density lipoprotein with triglyceride divide by five as the equations described by Friedewald *et al* (1972), Wilson (1998).

Alkaline phosphatase (ALP) activity was determined according to the methods of Thomas (1995).

Statistical analysis

All data were subjected to analysis of variance

(ANOVA) in accordance with a completely randomized design (CRD) using SPSS software (2012). Significant treatment means were assessed using the Least Significant Difference (L.S.D.) test at P≤0.05 (SPSS, 2012).

RESULTS

Hematological traits

Hematological measures [red blood count (RBC), white blood cell (WBC), Packed cell volume (PCV), hemoglobin, lymphocyte (L), Hetrophil (H)] and H/L ratio under different *Moringa oleifera* leaf meal (Molm) treatments is shown in Table 2. The PCV, WBC, L, H and H/L ratio were similar among different Molmgroups (P ≥ 0.05) while there were significant (P≤0.05) increased in RBC count as compared with control. Chicks received 0.75 and 1.0% Molm in their diets had significantly (P≤0.05) higher hemoglobin level as compared with the control one.

Biochemical traits

Serum total protein, globulin, alkaline phosphatase (ALP) activity and uric acid was significantly (P≤0.05) declined in all dietary groups in comparison with the control (Table 2). Broilers that received diets with 0.75% and 1.0% *Moringa oleifera* leaf meal (Molm) had the lowest albumin concentration as compared with other groups.

The results of albumin (A) to globulin (G) ratio revealed that birds supplemented with Molm at levels 0.25% and 0.50% had better (P≤0.05) A/G ratio as compared with other groups, whereas there was no significant difference in A/G ratio between birds fed 0.75% and 1.0% of Molm and control group.

Lipid profile

Serum Total cholesterol (TC), LDL-c and VLDL-c were significantly (P≤0.05) declined in all dietary groups in comparison with the control, whereas, the high density lipoprotein cholesterol (HDL-c) and triglyceride (TG) concentrations were increased significantly (P≤0.05) when *Moringa oleifera* leaf meal was included in the diets of broilers.

Table 2 : Haematological and serum biochemical indices of broiler chicks fed *Moringa oleifera* leaf meal.

Parameter	Dietary Group					SEM
	Control (0.0%)	Molm (0.25%)	Molm (0.50%)	Molm (0.75%)	Molm (1.0%)	
Heamoglobin (g/dl)	9.63^{b}	9.92^{ab}	9.97^{ab}	10.25^{a}	10.19^{a}	0.090
Red Blood Cell (x10^{6}/mm^{3})	2.84^{d}	3.04^{c}	3.18^{b}	3.28^{ab}	3.33^{a}	0.049
Packed Cell Volume (%)	29.07	29.76	29.93	30.77	30.59	0.267
White Blood Cells (x10^{3}/mm^{3})	12.27	13.81	13.97	13.39	13.95	0.030
Hetrophil (%)	5.49	4.76	4.12	4.40	4.44	0.267
Lymphocytes (%)	9.12	8.76	8.79	8.77	9.32	0.105
H/L Ratio	0.59	0.53	0.47	0.50	0.47	0.028
Total Protein (g/dl)	4.62^{a}	4.42^{b}	4.16^{c}	4.01^{d}	3.76^{e}	0.081
Albumin (g/dl)	1.51^{ab}	1.53^{a}	1.45^{b}	1.32^{c}	1.21^{d}	0.033
Globulin (g/dl)	3.11^{a}	2.89^{b}	2.71^{c}	2.69^{d}	2.55^{e}	0.053
Albumin to Globulin ratio	0.48^{b}	0.53^{a}	0.54^{a}	0.49^{b}	0.48^{b}	0.008
ALP (U/l)	34.39^{a}	30.67^{a}	25.39^{b}	19.31^{c}	16.23^{c}	1.887
Uric acid (mg/dl)	4.82^{a}	4.70^{b}	4.59^{c}	4.17^{d}	3.91^{e}	0.092

^{abcd} Means in the same row with no common superscript are different significantly (P≤0.05). HDL-c: High density lipoprotein cholesterol, ALP: Alkaline phosphatase enzyme.

Table 3 : Lipid profile of broiler chicks treated with *Moringa oleifera* leaf meal.

Parameter	Dietary Group					SEM
	Control (0.0%)	Molm (0.25%)	Molm (0.50%)	Molm (0.75%)	Molm (1.0%)	
Cholesterol (mg/dl)	147.00^{a}	137.00^{b}	127.17^{c}	121.83^{d}	110.33^{e}	3.397
Triglyceride(mg/dl)	72.67^{e}	77.60^{d}	78.67^{c}	85.00^{b}	90.80^{a}	1.765
HDL- c (mg/dl)	85.20^{e}	91.64^{d}	95.39^{cd}	116.45^{b}	127.88^{a}	4.354
LDL-c (mg/dl)	23.67^{a}	20.67^{ab}	20.67^{ab}	19.11^{b}	15.73^{c}	0.788
VLDL- c (mg/dl)	20.19^{a}	17.78^{ab}	17.73^{ab}	15.76^{bc}	14.54^{c}	0.642

^{abcde}Means in the same row with no common superscript are different significantly (Pd"0.05). HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol, VLDL- c: Very low density lipoprotein cholesterol.

DISCUSSION

The haematobiochemical parameters studied on feeding diet supplemented with different levels of *Moringa oleifera* leaf meal (Molm) (Table 1). With the exception ofred blood cells (RBC) and hemoglobin (Hb), the other blood parameters (PCV, WBC, lymphocyte (L), Hetrophil (H) and H/L ratio) did not change significantly with inclusion of *Moringa oleifera* leaf meal in broiler diets at 42 day of age. The absence of a response to the dietary inclusion of Molm on these parameters similar to findings of Zanu *et al* (2012), who found no significant effect when 15% *Moringa oleifera* leaf meal subtitled fish meal in broiler diets on the hematological indices [PCV, WBC, Hb, HCT, mean corpuscular hemoglobin concentration (MCHC) and lymphocytes] and with Anthony and Ashawe (2014), who found no significant effect of 15% *Moringa oleifera* leaf meal supplement level on the PCV, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), (MCHC), WBC, lymphocytes, neutrophils, monocytes, basophils and eosinophil of rabbits after the 8 wks. of feeding period, also, Adejumo *et al* (2016) stated that no significant effect ofraw *Moringa oleifera* leaf meal and raw and cooked *M. oleifera* seed meal as a replacement for synthetic antibiotic (tetracycline) on PCV and WBC of broilers.

The current study showed an elevation in red blood cells count and heamoglobin concentration (in 0.75 and 1.0% groups) as a results of inclusion different levels of Molmto broilers diet. This increase may be due to iron contains (23mg/100g) in *Moringa oleifera* leaves. In this regard, Lutz and Prytulski (2008); Elbashier and Ahmed (2016) reported that iron is necessary for many functions in the body including the formation of hemoglobin and myoglobin. According to Olugbemi *et al* (2010) red blood cells are responsible for the transportation of oxygen and carbon dioxide in the blood as well as the manufacture of haemoglobin, hence higher values indicates a greater potential for these function and a better state of health. A marked improvement in the number of RBC and Hb value may be attributed to the influence of *Moringa oleifera* protein content, which is rich in nutrients such as protein and minerals (Elbashier and Ahmed, 2016). Jiwuba *et al* (2016) proposed that haemoglobin improvement by *Moringa oleifera* leaf meal inclusion was due to the higher quality of the protein in leaves, a view confirmed by Fuglie (2009) and with Elbashier and Ahmed (2016), who suggested that *Moringa oleifera* has a blood boosting effect because for the high protein content, with significant quantities of most essential amino acids (Foidland Paull, 2008). Conversely, the higher inclusion level of *Moringa oleifera* leaf meal (20%) caused adverse effects on the haematological and serum biochemical profile (Tijani *et al*, 2016). Besides, Aderinola *et al* (2013), showed that feeding 0, 0.5, 1.0, 1.5 and 2.0% *Moringa oleifera* leaf caused a significant reduction in hematological parameters of broiler chicks. Total serum protein, albumin (in 0.75 and 1.0%), globulin, alkaline phosphatase (ALP) enzyme activity and uric acid were significantly decreased in all supplemented groups with *Moringa oleifera* leaf meal as compared with the control one (Table 2). Generally, total plasma protein (TPP) values in birds range from (3.5–6.0 g/dl) and birds with TPP levels below 3.5 g/dl would have less chance of recovery from illness than birds with higher TPP levels (Amand, 1986). The values of serum total protein (3.76-4.62 g/dl) obtained in current study however fell within the normal range (2.5 and 4.5 g/dl) for healthy broilers as reported by Campbell (2004), an indication of nutritional adequacy of the dietary proteins in this experiment. Additionally, the globulin values (2.55-3.11 g/dl) obtained in this trial fell within the normal range (2-4 g/dl) as noted by Ross (1976). Similar results were recorded by Jiwuba *et al* (2016) in his study on growing rabbits and by Tijani *et al* (2016) on broilersin term of total serum protein, albumin and globulin. Contrary to the current findings, Makanjuola *et al* (2014) reported that 0.2%, 0.4% and 0.6. Moringa leaf meal did not influence the serum total protein, albumin, globulin and AST of broilers and with Elkloub *et al* (2015), who found that 0.2% or 0.4% and 0.6%. Moringa leaf meal in quail diets was significant increase in total protein and globulin, while, albumin /globulin ratio in all dietary treatments appeared to be decreased in Molm supplemented birds. In respect with, serum alkaline phosphatase (ALP) activity, Akpet *et al* (2014) indicated that no significant changes in ALP activities at 20% inclusion rate of *Moringa oleifera* leaf, however, there was a significant increase in serum ALP activity at 10% Molm inclusion rate in broiler diets. Zilvaand Pannall (1984) reported that ALP enzyme is widely distributed to many organs and tissues of the body such as the bone, hepatic biliary tract, kidney, spleen, pancreas and lactating mammary gland. This increase in ALP serum activity may indicate injuries to any of these organs and if the liver is involved, it is localized to the biliary tract (Akpet *et al*, 2014). The uric acid value in this study was significantly reduced by the *Moringa oleifera* leaf meal treatment diets.This observation was disagreed with the result of Tijani *et al* (2016), who reported that uric acid contents were similar in birds fed 5%, 10% and 15% *Moringa* leaves - based diets, whereas, significant increased in uric acid and creatinine content when broilers fed 20% Molm. Uric acid is the primary catabolic end product of protein, non-

protein nitrogen and purines in birds. It represents 80-90% of the total nitrogen excreted by the kidneys (Frye, 1991). The normal blood uric acid concentration for most birds is less than 10 mg/dl (Campbell, 2004). The low serum uric acid observed in current study, may be attributed to good quality protein utilization in *M. oleifera* leaf meal. The serum lipid profile of broiler chicks treated with *Moringa oleifera* leaf meal (Molm) presented in Table 3. The result shows a significant reduction in total serum cholesterol (TC), LDL-c and VLDL-c concentrations, whereas, the HDL-c and TG concentrations were increased significantly (P≤0.05) as a results of inclusion Molmto broiler diet. This result is consistent with the result of Akpet *et al* (2012), Dey and De (2013), Aderinola *et al* (2013), Gakuya *et al* (2014) on broiler, Anthonyand Ashawe (2014) on rabbits, Elkloub *et al* (2015) on Japanese quailand Ahmad *et al* (2017) on layer hens, who stated significant decreased in the TC and LDL-c concentration in those fed different levels of *Moringa oleifera* leafmeal. The reduction in the levels of TC, LDL-c and VLDL-c when the birds supplemented with *Moringa oleifera* may reflect the beneficial effects of *M. oleifera* in the diets, as *M. oleifera* has a high content of phytosterols, which decrease the cholesterol levels of serum and eggs (Hussain *et al*, 2014). On other hands, Hussian *et al* (2014) reported that *M. oleifera* plant riches in flavanoids and phenolicscompounds, that are accountable for antioxidant activity of plant. Previous studies Srinivasan (2005), Nobakhtand Moghaddam (2013) indicated that antioxidants potentiate 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 (β-carotene) positively affected and reduced the levels of cholesterol in the serum (Melesse *et al*, 2013; Elkloub *et al*, 2015). As well as,may be attributed that low cholesterol levels due to the decline in lipid mobilization and maybe suggest that *Moringa* leaf meal diet were capable of reducing serum cholesterol, hence assisting in the reduction and deposition of cholesterol in the muscle, thus production of lean meat (Jiwuba *et al*, 2016). Differently from the present study, Zenu *et al* (2011) noticed that no influenced in serum total cholesterol, HDL-C of broiler chickens treated with *Moringa oleifera* leaf as partial replacement of fishmeal. Also, result is not in agreement with the work reported by Akpet *et al* (2012), who observed that LDL-c concentration significantly elevated at levels 10 and 20% of inclusion of *Moringa oleifera* leaf meal in broiler diets.

CONCLUSION

It could be concluded that *Moringa oleifera* leaf meal with levels of 0.25, 0.50, 0.75 and 1% have beneficiate effect on some heamatological and serum biochemical indices. The best level occurred by using 0.75% Molm in broiler chickens diets.

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