

The Effect of *Moringa oleifera* Leaf Meal and their Aqueous and Ethanolic Extracts on Immunological Parameters, and Liver Enzymes of Broiler Chickens

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Abstract: The purpose of this study is to investigate the effect of *Moringa oleifera* leaf meal and their aqueous and ethanolic extracts on immunological parameters, economic results and liver enzymes of broiler chickens. Two hundred and ten unsexed day-old Ross308 broiler chicks were divided into seven experimental diets. Each treatment had three replicates with 10 birds per replicate. Each replicate was fed with an assigned experimental diet for five weeks. The treatments were as follows: T1 was the control without addition, T2 adding 2 g *M. oleifera* Leaf Meal (MOLM)/kg of feed, T3 adding 4 g *M. oleifera* Leaf Meal (MOLM).kg⁻¹ feed, T4 adding 2 ml *Moringa Aqueous* Leaf Extract (MALE) each 1 litre water, T5 adding 4 ml *Moringa Aqueous* Leaf Extract (MALE) each 1 litre water, T6 adding 2 ml *Moringa Ethanolic* Leaf Extract (MELE) each 1 litre water, T7 adding 4 ml (MELE) each 1 litre water. The results showed there was a significant decrease ($P < 0.05$) in the weights of the bursa gland of moringa treatments in a comparison with control group. While the addition of moringa did not have any significant effect on relative weights of spleen and on the ratio of heterophils to lymphocytes (H/L) in the blood of broilers. There was no significant difference in the values of liver enzymes (AST and ALT) among *M. oleifera* treatments and control.

Keywords: *Moringa oleifera*, Aqueous extract, Ethanolic extract, Broiler.

Introduction

Poultry is a popular industry because it enjoys a relative advantage of easy management, higher income, quick returns to capital investment and wide acceptance of its products for human consumption (Haruna & Hamidu, 2004). Poultry plays a very important role in the humans' food supply, income, and employment generation and providing grew materials to some industries. The increase in the prices of conventional feeding redients is a major factor affecting net

return to the poultry business (ElBanna & Atef, 2016). This is because 70-75% of the total cost of poultry operation is incurred on feeding (Mahmood *et al.*, 2005). One of the challenges facing poultry farmers is the issue of diseases and infection. This has caused a high rate of mortality and lack of productivity in poultry production (Alikwe *et al.*, 2016). In developing countries, average daily protein intake of human diets is well below recommended standards, and the poultry production is playing a major role to fill this

gap (Hassan *et al.*, 2017). Plant products have been used for centuries by humans as food and to treat ailments. Natural medicinal products originating from herbs and spices have also been used as feed additives for farm animals (Guo, 2003). Among the ingredients, protein supplements are very expensive; therefore; it is necessary to look for alternative sources available locally for use as a protein supplement in poultry feed. Using leaf sources as a protein ingredient in broiler's diet is getting attention due to availability, abundance and relatively reduced cost (Onyimonyi & Onu, 2009). According to Fasuyi *et al.* (2008) leaf meal does not serve only as protein sources but also provides some necessary vitamins, minerals and also oxy-carotenoid which causes yellow colour of broiler skin, shank and egg yolk.

The use of chemical feeds additives as growth promoters have criticism due to adverse effects on consumers and there is increasing demand for organic meat and egg (Agashe *et al.*, 2017). Some medicinal plant products are known to enhance natural resistance of hosts of infection due to the presence of bioactive phytochemicals or phytonutrients (Soetan & Oyewole, 2009). Antibiotic growth promoters (AGP) have been used as poultry feed additives to enhance gut health, control sub-clinical diseases and improving the growth performance of poultry such as increasing body weight gain and improving feed conversion ratios (Alcicek *et al.*, 2003). Furthermore, medication in water using antibiotics helps birds to recover from diseases (Khalafalla *et al.*, 2010). However, the benefit of the use of antibiotics as growth promoters has some disadvantages such as drug toxicity, residual effects, and development of bacteria resistance (Ogbe & John, 2012).

Moringa Moringa oleifera leaves are reported to have potential prebiotic effects and potentially anti-oxidant phytochemicals, such as chlorogenic acid and caffeic acid (Siddhuraju & Becker, 2003). *M. oleifera* is one of the plants that can be utilized in the preparation of poultry feeds (Makker & Becker, 1999). The plant apart from being a good source of vitamins and amino acids that has medicinal uses (Francis *et al.*, 2005). *M. oleifera*, otherwise regarded as a "miracle trees" has been used in the treatment of numerous diseases (Gbasi *et al.*, 2000). *M. oleifera* leaf meal, widely available in many tropical countries, is also a good source of antioxidant compounds such as ascorbic acid, flavonoids, phenolic, and carotenoids (Teixeira *et al.*, 2014). The leaves of the moringa tree have been reported to have an antioxidant activity due to the higher amount of polyphenols (Sreelatha & Padma, 2009; Moyo *et al.*, 2012).

M. oleifera leaves are a rich source of vitamins. Its leaf meal may be a promising source of natural antioxidants for broiler meat (Makker & Becker, 1996). It also possesses antimicrobial activity due to its principle component which removes free radicals, activates antioxidant enzymes and inhibits oxidases (Luqman *et al.*, 2012). Moringa is a multi-purpose tropical tree and it has been dubbed the "miracle tree" or "trees of life" in popular media (Bosch *et al.*, 2004; Radovich, 2013; FAO, 2014) mainly because it is used for food and has numerous industrial, medicinal and agricultural uses, including animals feeding. Moringa leaves have been reported to be a rich source of β -carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidant compounds such as flavonoids, phenolic, and carotenoids (Anwar & Bhangar, 2003).

It is one of the herbs containing biomedical agents that could substitute synthetic growth enhancers and supplements with broilers and other livestock production since it possesses important medicinal properties which include antibacterial and anti-fungal activities (Nickon *et al.*, 2008). The importance of *M. oleifera* in ethno botany as health remedies the anti-microbial property of crude extracts and anti-nutritional factors particularly saponins can be removed through solvent and aqueous extractions of the petals of *M. oleifera* that has been studied as part of the exploration for new and novel bioactive compounds (Makkar & Becker, 1997; Richter *et al.*, 2003).

The objective of this study was to determine the effect of *M. oleifera* leaf meal and their aqueous and ethanolic leaf extract on immunological parameters, economic results and liver enzymes of broiler chickens.

Materials & Methods

This study was carried out at the farm of The Animal Production Department, Agriculture

College, University of Maysan. Two hundred and ten unsexed day-old Ross308 broiler chicks were divided into seven experimental diets in a Complete Randomized Design (CRD). Each treatment had three replicates with 10 birds per replicate. Each replicate was fed with an assigned experimental diet for a period of five weeks. The birds were purchased from Al- Barakat Hatchery in Al-Amarah City with an average initial body weight (40) g and reared under similar managerial conditions by using the floor breeding system. The experimental diets and drinking water was supplied to the birds *ad libitum*. Throughout the study period, the recommended routine medication and vaccination programs were observed. The lighting system was artificial for 24 hours. The ventilation was naturally achieved by opening and closing windows in addition to using fans for drawing a vicious air. The nutrition was free through experimental periods and the feed intake was recorded daily.

Immunological traits

$$\text{Relative weight of interior bowels} = \frac{\text{Organ weight (gm)}}{\text{Live body weight (gm)}} \times 100 \quad (\text{Alfayaz \& Naji, 1989})$$

$$\text{Account of fibrcus gland index} = \frac{\text{Ratio of gland weight to the body weight of defined treatment}}{\text{Ratio of gland weight to the body weightof control treatment}} \times 100$$

(Naji, 1996)

Account of percentage Heterophils/ Lymphocytes

$$\text{H/ L Ratio} = \frac{\text{No.of Hetrophils in 100 White cell}}{\text{No.of Lymphocytes in 100 White cell}} \times 100 \quad (\text{Shen \& Patterson, 1983})$$

Liver enzymes

Measurement activity of AST enzyme

The activity concentrating of the enzyme which carrier to the AST (GOT) Amine in blood plasma by Kit supply from Bio lab.

technical support. The analysis were action according to the an information of prepared company. The absorbency sample and the standard solution were measure by spectrophotometer on the wave length 500 nanometre (Reitman & Frankel, 1975).

Measurement activity of ALT enzyme

The activity concentrating of the enzyme which carrier to the ALT Amine in blood plasma by Kit supply from Bio meri Eux. The analysis were action according to the an information of prepared company. The absorbency sample and the standard solution were measure by spectrophotometer on the wave length 500 nanometre (Kind & King, 1954).

Nutrition

It was used two diets throughout the experimental period. First, the starter diets from 7 to 17 days. The metabolic energy was (2936) kcal/kg feed and the crude protein percentage (22.26%). The second was a finisher diet and it's given to birds from 18 to the end of this study at 35 days. It contains (3118) kcal/kg feed as metabolic energy and (20.20%) the percentage of the crude protein. The feed ingredient purchased from the local market, and broilers were given the experimental diets from the end of the first week (table 1).

Table (1) Percentage composition of the starter experiment and broiler diets.

Feed ingredient	Starter diet %(7-17)days	Finisher diet % (18-35) days
Maize	54	58
Wheat bran	9	0
Wheat	0	10
Soya bean meal 44%	25	22
Protein concentrate*	10	8
Vegetable oil	1	1
Lime stone	0.50	0.50
Salt	0.25	0.25
Premix	0.25	0.25
Total	100	100
Calculating chemical analysis **		
Metabolic energy (k cal/kg)	2936	3118
Crude protein %	23.26	0.821
ME/ CP Ratio	131.90	154.36
Crude Fat %	4.90	4.78
Crude Fiber %	4.13	3.30
Ca %	1.02	0.88
Available P %	0.50	0.35
Lysine %	1.32	1.14
Methionine + Cystine %	0.80	0.73

*□Cp 40%, ME 2000 kcal/kg, C. fiber 3%, EE 3%, Ash 34%, Ca 8%, Av. P 1.38%, Lysine 12%, Methionine 3%, Methionine + Cystine 3.5%. Vitamin A 250000 IU/kg, Vitamin D3 50000 IU/kg, Vitamin E 500 mg/kg, Vitamin K3 60 mg/kg, Vitamin B1/Thiamin 20 mg/kg, Vitamin B2/Riboflavin 100 mg/kg, Niacin Vitamin PP 600 mg/kg, Pantothenic acid/Vitamin B3 160 mg/kg, Vitamin

B6/Pyridoxine 40 mg/kg, Vitamin B12 300 mcg/kg, Biotin/Vitamin H 2000 mcg/kg, Choline 10000 mg/kg, Vitamin C 4000 mg/kg, Folic Acid 30 mg/kg, Iron 800 mg/kg, Manganese 1400 mg/kg, Copper 120 mg/kg, Zinc 1000 mg/kg, Iodine 6 mg/kg, Cobalt 12 mg/kg, Selenium 3 mg/kg

** According to the chemical analysis (NRC, 1994) .

Experimental treatments

The study included seven treatments and each treatment had three replicate with 10 birds per replicating as follows:- T1 (controls without addition), T2 adding 2g *M. oleifera* leaf meal (MOLM). kg⁻¹ of feed, T3 adding 4 g (MOLM). kg⁻¹ of feed, T4 adding 2 ml *Moringa* Aqueous Leaf Extract (MALE) each 1 litre water, T5 adding 4 ml (MALE) each 1 litre water, T6 adding 2 ml *Moringa* Ethanolic Leaf Extract (MELE) each 1 litre water, T7 adding 4 ml (MELE) each 1 litre water.

Preparation materials using in experimental treatments

M. oleifera Leaf Meal

The green leaves of *M. oleifera* were purchased from a local orchard in Abu Al-

Khaseeb city, Basrah Governorate at early flowering stages. Branches were cut from the *Moringa* trees, spread out and dried under the shade for a period of 4 to 5 days. Thereafter, branches were threshed carefully to separate leaves from twigs before milling and also removed by hand. The dried leaves were ground with hammer mills to make a leaf meal. The leaf meals were stored in the plastic bags during entire periods of the study. A small amount of meal was dried by putting it in electric oven for 4 hours at 105C° in the nutrition laboratory of Animal Production Department, College of Agriculture, University of Basrah. Then the percentage of humidity, dry matter, ash, and other parameters were evaluated according to special methods. The results showed in table (2).

Table (2): The composition of *M. oleifera* leaf meal.

Parameters	Percentage composition (%)
Moisture	06.26
Dry matter(DM)	93.74
Crude protein	26.31
Ether extract	2.440
Total ash	.7008
Crude fiber	16.08
Nitrogen free extract	40.21

Aqueous leaf extract

Fresh leaves of the plant were air-dried under normal environmental conditions. The air-dried leaves were ground before extraction and soaked in distilled water for 24 hours using ratio 1:2 (weight/volume). The preparation was then filtered to separate the debris and filtrate using Whitman's filter

paper. The filtrate was collected, the solvent was removed using a rotary evaporator and the residue obtained after evaporation was weighed. The concentrated stock solution of *Moringa* leaf extracts was prepared by dissolving 50 g of the residue in 1 litre of sterile distilled water and stored at 40 °C. The

concentrated extract at calculated doses was administered in fresh drinking water which was served to the birds on a daily basis during the period of study (Pandit *et al.*, 1979).

Ethanollic Leaf Extract

Extraction of the dried leaves was performed by soaking the plant material in ethanolic alcohol (70%) for 24 h in bath water (37°C) then put the mixture in electric stirrer for 1 h next, the solution filtration by using Whitman's filter paper. The filtered distribute in tubes of centrifuge for 15 minutes. The clear liquid was putting in small glasses dishes and then set into the oven at (37 °C) for dried. after drying the extraction scrape off and dissolving in sterile distilled water. The product was kept in closed flasks and put in the icebox for the time of using (Harbone, 1973).

Statistical analysis

All data collected were subjected to one-way analysis of variance (ANOVA). Based on the Completely Randomized Design (CRD) using statistical package for the social science (SPSS, 2009). They were separated using Duncan's Multiple Range Test at 5% level of probability ($P < 0.05$).

Results & Discussion

Immunological parameters:

The primary organs of immune system are bursa of fabricious and thymus, reached their maximum size of chicks about four weeks after hatching and then undergo gradual involution (Tizard, 1995). The finding obtained in this study (Table 3) showed that the birds fed them moringa treatments had a

reduction in bursa relative weights which recorded the lowest weights of this glands among (0.067- 0.073) % in comparison with the control group that had the higher relative weight of bursa gland (0.077) %. This reduction may reflect the good health and natural condition of birds and also for the reason of low mortality percentage or as a result effect of addition *M. oleifera* as natural growth promoter (Guo, 2003). This result was in agreement with the study of Nkukwana *et al.* (2014) who found significant differences ($P < 0.05$) in bursa relative weights. Also, the present study were in line with Kumar *et al.* (2018) which notice a significant decrease in the weight of bursa gland reached 1.60 gm when increasing the level of MOLM in diets to 20 %. However, it was not agreed with the findings of Khan *et al.* (2017). The present results showed no significant differences in spleen relative weights. This result was similar to other studies that not found any significant influence on the relative weights of spleen such as Ayo-Ajasa *et al.* (2016) and Alabi *et al.* (2017); while it was inconsistent with Onunkwo & George (2015) who reported variance between moringa treatments and control group. The addition of MOLM, MALE, and MELE to the diets and drinking water of birds showed no significant effect on the percentage of heterophils to lymphocytes (H/L) between all study treatments. This result was not compatible with the study of Kwari *et al.* (2017) which observed significant differences between all groups of birds which bred in a semi-arid environment and fed *M. oleifera* leaf meal and Baobab as replacement for synthetic premix.

Table (3): Effect of *M. oleifera* leaf meal and their aqueous and ethanol leaf extracts on immunological parameters of broiler chickens (Mean± SE).

Parameters	T1	T2	T3	T4	T5	T6	T7	S.L
Bursa glandweight (%)	0.077 a ± 0.003	0.070 bc ± 0.004	0.071bc ± 0.071	0.072bc ±0.002	0.073ab ±0.002	0.067±c 0.003	0.068±c 0.002	*
Bursa gland index	1.000a±0.000	0.911b±0.077	0.922ab ±0.011	0.936 ab ±0.055	0.949ab ± 0.032	0.871b±0.054	0.881b± 0.024	*
Spleen (%)	0.147±0.027	0.117±0.005	0.171±0.047	0.158± 0.027	0.142± 0.017	0.155±0.051	0.136 ± 0.023	NS
H / L	0.550±0.135	0.520± 0.161	0.540± 0.095	0.460± 0.089	0.470± 0.082	0.490±0.098	0.500± 0.108	NS

^{abc} Means in the same row with different superscripts were significantly different (P<0.05).

T1 (control without addition) , **T2** adding 2g *M. oleifera* leaf meal (MOLM)/ kg of feed, **T3** adding 4 g (MOLM) \ kg of feed , **T4** adding 2 ml Aqueous Leaf Extract (MALE) each 1 litre water , **T5** adding 4 ml (MALE) each 1 litre water, **T6** adding 2 ml Ethanolic Leaf Extract (MELE) each 1 litre water , **T7** adding 4 ml (MELE) each 1 litre water

Liver enzymes

The liver enzymes (AST & ALT) are playing essentially role in the biotic process by transfer amino group from amino acids to ketone acids inversely in most of a live organism (Ibrahim & Saleh, 2005). If the values of these enzymes were reduced that mean the birds in a good health because the livers were not in the case of stress. The values of (AST & ALT) will be high in case of death of cells and damage of tissues (Aldaraji *et al.*, 2008). The findings of the table (4) indicated that there was no significant difference in values of liver enzymes (AST and ALT) among groups of

bird treated with *M. oleifera* and the control which free from any addition. This maybe reflects the natural function of intestines and liver of the birds (Melesse *et al.*, 2013). The reason maybe as result to the addition a few levels of leaf meal , aqueous and ethanolic extracts, and thus not happen any impact on liver excretions from enzymes. This result was in agreement with the study of Olugbemi *et al.* (2010). Also, the values of AST and ALT were natural and without any negative effects on the experiments birds which added to their diets graded levels of cassava and moringa leaves (Aderemi & Alabi, 2013).

Table (4): Effect of *M. oleifera* leaf meal and their aqueous and ethanol leaf extracts on liver enzymes of broiler chickens (Mean± SE).

Parameters	T1	T2	T3	T4	T5	T6	T7	S.L
AST IU/L	25.50±0.53	25.29± 0.80	26.50±0.83	25.74±0.80	26.42±0.53	24.90±0.67	26.46±0.47	NS
ALT IU/L	13.92±0.86	12.68±0.80	13.17±0.75	12.93± 0.64	13.23±0.84	12.82±0.78	13.12±0.80	NS

T1: control without addition, **T2:** adding 2g *M. oleifera* leaf meal (MOLM) \ kg of feed, **T3:** adding 4 g (MOLM) \ kg of feed , **T4** adding 2 ml Aqueous Leaf Extract (MALE) each 1 litre water , **T5** adding 4 ml (MALE) each 1 litre water, **T6** adding 2 ml Ethanolic Leaf Extract (MELE) each 1 litre water , **T7** adding 4 ml (MELE) each 1 litre water .

Conclusions

From the results obtained in this study, we can conclude that the *Moringa olivera* plant meal 2 g. Kg⁻¹ can be used of feed and leaf aqueous and ethanol extracts at the level of 2 ml of drinking water in broiler meals and drinking water without harmful effects on the immune system and liver enzymes.

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