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Impact of Supplementation of Moringa Oleifera in Diet of Broiler Chicks on Their Behavior, Welfare, Performance and Immune Responses

Sameh G.A. Ramadan

Department of Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, University of Sadat City, Egypt

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Correspondence to:

samehgad2001@yahoo.com

ABSTRACT

A total of two hundred and four, one day old Ross broiler chicks were randomly allotted into four dietary treatments floor pen groups (17 birds/ pen, 10 birds/m^2) with three replicates for each treatment. The dietary treatments were designed to feed the chick's basal diet supplemented with graded levels of Moringa oleifera leaves meal, MOLM [3%, 5%, 8% and 0% (control) MOLM]. Behavioral observation was carried out twice daily (AM, 7:30- 10:30 and PM, 13:30- 15:50) two days a week for 6 consecutive weeks. Body weight (BW), Body weight gain (BWG) and Feed intake per pen was measured weekly, from which feed conversion ratio (FCR) was calculated. At 30th day of age blood samples were collected to evaluate anti body titer (HI) against New Castel Diseased Virus (ND). At the end of the experiment, fear responses, differential leucocyte count, Hb and PCV were assayed as well as some carcass traits. Also, mortality rate was estimated. The obtained results showed that higher proportion of birds were engaged in feeding in groups dietary treated with 3% and 5% MOLM, while intermediate responses was shown in birds supplemented with 8% MOLM compared to control ones. The supplemented groups with MOLM showed a lower walking and standing activities than non-supplemented ones. Dietary supplementation with different levels MOLM significantly improved BW, BWG and FCR. In addition, the mortality rates for the dietary treated groups were not affected. The MOLM supplemented groups were less fearful in tonic immobility test, less stressed and had lower heterophil/lymphocyte ratio when compared to un-supplemented ones. MOLM had no effect on Hb concentration and carcass traits. PCV was significantly higher in birds fed 5% MOLM compared to control birds. Birds supplemented with 3%, 5% and 8% MOLM had significant higher antibody titre against ND compared to un- supplemented birds. In conclude, dietary supplementation of broiler chicks with MOLM up to 8% resulted in improved welfare, performance and immune response against ND.

1. INTRODUCTION

Although several studies have been conducted to determine the effect of dietary supplementation with MOLM on the performance of broiler and other livestock's, there was no available report that describes their behavior and fear effects as the behavior of livestock had been strongly influenced by environmental conditions under which they survive.

In broilers industry, feed costs considered a one of the greatest challenge especially in developing countries. It constitutes about 60-80% of the total cost of poultry meat production (Teguia and Beynen, 2005). The high cost and increasing demand of traditional energy source and protein source such as fishmeal and soybean meal have encouraged the search for alternative feeds (Gaia, 2005) not only for economy but also to reduce the direct competition

between human being and the animals for the available traditional feedstuffs (Muriu et al., 2002).

Moringa oleifera Lam belong to family Moringaceae which is well distributed in tropics and subtopics countries and cultivated all over the world due to its various benefits and regarded as a "miracle tree" (Makkar and Becker, 1999). It has an average height of 5 to 12 meters (Paguia et al., 2012). Different parts of the tree are used as nutrition, medicine, spices, natural coagulants, water purifications, fuel and fertilizer. MOLM had a significant high quantity of anti-inflammatory and antioxidant compounds (Yang et al., 2007). The antioxidant compound improved the shelf-life and the quality of animal meat products in the pre slaughtered and post-slaughtered periods (Valeria and Williams, 2011). The protein content of M. Leaves is of high digestibility and of high quality and have significant amount of all the essential amino acids (Foidl and Paull, 2008). Leaves also are a good source of vitamines (C, A, and B), minerals (Fe, Ca and ph), carotenoids and amino acids (Oduro et al., 2008). Ramachandran et al., (1980) and Loren (2007) reported that, MOLM leaves have a calcium content equal to 4 glasses of milk, iron 3 times that of spinach, vit A 4 times that of carrot, protein 2 times that of milk, potassium 3 times of banana and vit C 7 times that of orange. as heavy metals are absent from the leaves of MOLM so it was integrated safely in poultry and ruminant diets (Foidl et al., 2001; Donkor et al., 2013), in pregnant and nursing women's and also, those with malnutrition so it is known as mother`s best friend (Siddhuraju and Becker, 2003).

Several studies have been conducted to determine the effect of graded levels of MOLM on broiler`s feed intake and productive performance (Olugbemi et al., 2010a; Banjo, 212; El Tazi, 2012; Zanu et al., 2012; Gakuya et al., 2014), also blood parameters (Onu and Aniebo, 2011 & Makanjuola et. al., 2014) and immune responses (Du et al., 2007; Yang et al., 2007; Eze et al., 2013; Younis et al., 2016).

MOLM could be used as a protein source supplementing soya bean meals at 25% to improve broilers growth than traditional commercial foods (Gadzirayi et al., 2012) and up to 15%, with optimum level of 5% in growing Japanese Quail (Muhammd et al., 2016). It has been used as a substitute of fishmeal in broiler diets (Zanu et al., 2012). Studies were conducted to determine its effect on performance and blood indices of weaner rabbits (Nuhu, 2010) and also on productive performance of laying hens (Abou-Elezz et al., 2011). MOLM could be included in cassava based layer diets up to 10% (Olugbemi et al., 2010b) and by 10 % and 20% in sunflower seed meal (Kakengi et al., 2007). Olugbemi et al., (2010c) indicated that, M. oleifera possesses hypocholesterolemic proparties and could be involved in laying hen diets to facilitate production of eggs with low cholesterol content.

Moringa oleifera has a potent antioxidant, antibacterial and antifungal proparties (Nickon et al. 2003) also used in the treatment of cardiac disease and obesity (Gbasi et al., 2000) and displayed anti carcinogenic, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities (Chumark et al., 2008). M. oleifera improves the digestibility of other foods, helping chickens to express their natural genetic potential (Gaia, 2005) and strengthening immune functions and gut health of broiler chicks (Yang, et al., 2007). Makanjuol et al., (2014) concluded that MOLM could be used as alternative to (Enrofloxacin) with most of all the parameters measured found well in MOLM supplemented birds with decreasing the cost of broiler chick's productivity. MOML was a good alternative supplement to oxytetracycline in broilers which resulted in improved percentage of eviscerated weight of breast and drum stick with no effect on carcass qualities (Ologhobo et al., 2014).

The aim of the current study was to investigate the effect of MOLM on the behavior, fear, stress, performance and immune status of broiler chicks.

2. Materials and Methods

2.1. Experimental birds

Two hundred and four, day-old Ross broiler chicks were utilized in this study. Chicks were weighed on arrival and randomly assigned to four dietary treatments. Each treatment was housed in wood shavings bedded floor pen (17 birds/pen, 10 birds /m^2) with three replicates for each treatment. The light regimen in the house was 23 h light: 1 h dark. Temperature was reduced from 32 ^{o}c during the first week of life to 25 ^{o}c at third week and was then kept constant. Feed and water were provided *ad libitum*. Birds fed on commercial ration (International Company for Feed and Animal Production, IFAP). The dietary treatments consists of basal diet supplemented with 3% Moringa oleifera leaves meal,

MOLM (3g Moringa leaves for every 97g of broiler diet), basal diet supplemented with 5% MOLM, basal diet supplemented with 8% MOLM and basal diet without supplementations 0% MOLM (control). Dietary treatment started from day one to day 43 of age (end of the experiment). Moringa oleifera were obtained from the farm of Moringa friends at Sadat city, Menfoia, Egypt as a powder. The proximate analysis of MOLM is presented in Table 1 according to AOAC 2005. A standard vaccination program was applied for all birds.

2.2. Measurements

2.2.1. Behavioral observations

Each pen was directly observed two times per day, AM (7:30- 10:30) and PM (13:30- 15:30) for 15 minutes per time. Behavioral observation was carried out twice per week for six consecutive weeks. The observer stand quietly in front of each pen to get the birds accustomed to his presence before recording. Scanning technique of observations was performed according to (Fraser and Broom, 1990). The percentage of chicks engaged in feeding, drinking, lying, standing, walking, preening, foraging, stretching and pecking behaviors was calculated during all scan samples in each pen at different ages.

2.2.2. Performance

2.2.2.1. Body weight, body weight gain, feed intake and feed conversion ratio.

Body weight (BW) of each chick was measured weekly. Feed intake (FI) per pen was measured weekly, a known weighed amount of ration was supplied to chicks of each pen daily in the morning, and then at the end of each week the remaining amount was weighed and subtracted from the original amount of ration. From the previous data, body weight gain (BWG), and feed conversion ratio (FCR) were calculated.

2.2.2.2 Carcass traits.

At forty three days of age, four birds per pen were randomly chosen, weighed, slaughtered and their liver, gizzard, heart, spleen and bursa weight were calculated as a percentage of live body weight.

2.2.2.3 Mortality rate

Mortality rate was recorded for each dietary treatment.

2.2.3. Hematological parameters:

Differential leukocyte count, hemoglobin concentration (Hb) and Packed cell volume (PCV %).

At the 43th day of age, blood samples were collected from wing vein of four chicks per pen (12 chicks per treatment). The blood collected was received into EDTA anticoagulant for the determination of hemoglobin content (Hb) and packed cell volume (PCV) according to (Wintrobe, 1976). Direct blood smears were prepared and stained using May-Grunewald-Giemsa stain. One hundred leucocytes, including heterophils, esinophils, lymphocytes, and monocytes were counted at ×40 (oil immersion lens) and heterophil to lymphocyte ratio (H/L) was calculated.

2.2.4. Immune response

Antibody titer against Newcastle diseased virus (ND) was determined at 30 days of age. Random blood samples were obtained from 4 chicks per pen from the wing vein without anticoagulant to obtain serum which was kept at -20^{0}c for subsequent analysis. HI test was used to determine the antibody titer which was expressed as the log2.

2.2.5. Tonnic immobility (fear test).

Tonic immobility (TI) reaction was assessed on six birds per pen. Assessment was conducted in a separate room from the rearing pens. Each bird was restrained on its back in a U-shaped wooden cradle covered with several layers of clothing (Jones and Faure 1981) with the head hanging for maximum 5–min test period, by stop watch, the amount of time up to the bird stand on its feet was recorded (Mills and Faure 1991).

2.3. Statistical analysis

Data collected were subject to analysis of variance and where significant differences were observed means were further subjected to Duncan's multiple range, using IBM SPSS, Version 22 for Windows (IBM SPSS Inc., Chicago, IL). The results were considered as significant when P- values less than 0.05 and 0.01. Percentage of birds died was analyzed by Chi Square.

3. RESULTS AND DISCUSSION

Behavioral observation

The effects of dietary supplementation of MOLM on broiler behaviors are presented in Table 2. A significant high proportion of birds supplemented

Table 1.Chemical composition (%) of Moringa leaves according to AOAC 2005.

Dry matter %	Crude protein%	EE %	Ash%	Calcium%	Phosphorus%
89.6	7.25	11.7	12.3	2.10	.77

with 3% and 5% MOLM were engaged in feeding behavior while those fed on 8 % MOLM showed intermediate proportion in comparison with un-supplemented ones, which was in turn reflected on final body weight and other productive performance. This result was in close agreement with El Tazi (2014) who found that, broilers fed diet supplemented with 5% MOLM exhibited significantly higher feed consumption compared to control and other *Moringa oleifera* treated birds. Onu and Aniebo (2011) indicated that broilers chick fed MOLM starting from 7th day of age had significantly higher average feed intake compared to control birds. However, birds fed 2.5% and 5% MOLM had significantly a higher feed intake than those un-supplemented or fed on 7.5% MOLM. Moreover, Melesse et al. (2011) reported that Rhode Island Red chicks fed on 2%, 4% and 6% Moringa Stenopetala leaf meal showed a significantly higher feed consumption than control ones. Furthermore, Banjo (2012) found that broilers supplemented with 1%, 2% and 3% MOLM from the 2nd week of age had a significantly higher feed intake when compared with un-supplemented ones, also birds fed on 1% and 2% consumed more feed than those fed on 3%. However, Gakuya et al. (2014) reported that feed intake of birds fed on 7.5% MOLM was not significantly different from control ones, while increasing level of MOLM to 15% and 30% showed a significant reduction in feed intake. Portugaliza and and Fernandez (2012) indicated that *Moringa oleifera* Aqueous Leaf Extract at 30 mL and 60 mL significantly improved feed intake compared to control diet however, at 90 mL feed intake significantly reduced. Low feed intake in birds supplemented with 8% MOLM compared to other MOLM treated groups could be attributed to presence of some anti- nutritional factors such as mimosine and tannins (Atawodi et al., 2008). However, Makkar and Becker (1997) indicated that, leaves of Moringa are very poor in anti-nutritional factors. Also, low feed consumption may be attributed to high crude fiber content in Moringa which may resulted in decreased palatability (Kakengi et al. 2003).

Contrary to results of this study, Zanu et al. (2012) found that no significant difference in feed intake of Cobb broilers chicks fed on 0, 5, 10 and 15% MOLM leaf meal as partial replacement for fish meal at 2-6 weeks of age. Also, Gadziray et al. (2012) found no difference in mean feed intake of Hubbard chicks fed soyabean diets supplemented up to 25% MOLF as protein source compared to control birds. In the same trend, Juniar et al. (2008) found no differences in feed consumption between birds fed 2.5, 5, 7.5 and 10% MOLF compared to control birds. Moreover, Aderinola et al. (2013) revealed that control diet had significantly higher average daily feed intake in broiler chicks compared to MOLM diet (0.5%, 1%, 1.5 and 2%).

Results from behavioral observations in the present study indicated that dietary supplementation of MOLM significantly affects the proportion of birds engaged in standing and movement behaviors (Table 2). Birds in control group were exhibited significantly higher walking and standing activities compared to birds fed MOLM groups. Increased walking behaviors in control birds may be related to low feeding behavior. Hocking et al. (1997) reported that pacing was negatively related to rate of consumption. However, other behavioral patterns including drinking, lying, foraging, preening, stretching and feather pecking was not significantly affected by the dietary treatment with MOLM leaves as shown in Table 2.

3.1. Productive performance:

Results summarized in Table 3. revealed that, birds supplemented with 5% MOLM had significantly increased BW ($p< 0.05$) compared to control birds at the end of the 2ed week, while BW of birds fed 3% and 8% MOLM being intermediary but not different from them. At the end of the 3ed and the 4th weeks birds supplemented with 3% and 5% MOLM had significantly higher body weight compared to control birds. Birds supplemented with 5% MOLM were significantly heavier than birds fed 8% MOLM. BW of birds fed diet contain 8% MOLM not significantly differed from control birds or birds fed 3% MOLM. BW at end of the 5th week and final body weight (43th day) as well as BWG were significantly higher in all MOLM treated groups compared to control birds. Birds fed 5% MOLM had significantly higher BW and BWG compared to birds fed 8% MOLM. FCR was significantly better in all MOLM supplemented birds compared to control birds. The improved productive performance in MOLM supplemented birds may be attributed to high amount of amino acids, vitamins and minerals content as well as antioxidant, growth stimulating factors, immunostimulants and antimicrobial compounds, while, very low antinutritional factors (Makkar and Becker, 1997; Fahey, 2005; Foidl et al., 2001; Anwar et al., 2007). The decreased BW and BWG of birds supplemented with 8% MOLM compared to birds fed

Table 2. The effect of MOLM on behavior of broiler chicks. .

	3%MOLM	5%MOLM	8%MOLM	0%MOLM	p-value
Feeding	16.13^{a}±1.15	17.67^{a}±0.87	14.46^{ab}±1.15	12.63^{b}±1.37	<0.05
Drinking	3.02±0.68	2.34±0.56	2.07±0.59	2.29±.063	ns
Lying	58.64±2.24	57.37±1.89	59.78±1.71	54.49±2.38	ns
Walking	4.44^{b}±0.91	4.53^{b}±0.67	5.20^{b}±0.72	7.99^{a}±1.01	<0.01
Standing	5.89^{b}±0.76	5.77^{b}±0.85	6.75^{b}±0.89	11.14^{a}±1.41	<0.01
Foraging	6.43±1.22	5.70±0.90	5.01±.82	4.79±1.02	ns
Preening	4.18±0.86	5.06±0.93	4.82±0.83	6.51±0.88	ns
Stretching	1.58±0.42	1.75±0.44	1.63±0.45	1.63±0.45	ns
Pecking	.00±0.00	.27±0.15	23±0.16	22±0.22	ns

Means with the same row carry same letters are non-significantly different. ns: non significant.

Table 3. The influence of MOLM on productive performance of broiler chicks

Group	BW W0	BW W1	BW W2	BW W3	BW W4	BW W5	BW W6	BWG (g/bird)	FCR
3%MOLM	44.9± 1.13	163.06 ±1.85	380.80^{ab} ±12.13	670.74^{ab}± 9.40	1085.15^{ab} ±14.15	1610.28^{ab} ±19.74	1988.81^{ab} ±19.77	1943.84^{ab} ±19.84	2.05^{b} ±.020
5%MOLM	45.02± 0.79	165.40 ±1.99	393.53^{a}± 11.79	692.64^{a} ±10.93	1121.48^{a}± 14.13	1655.89^{a} ±22.65	2044.09^{a} ±20.95	1999.06^{a} ±21.24	2.00^{b} ±0.02
8%MOLM	45.46± 1.31	160.93 ±1.55	373.80^{ab}± 9.59	649.98^{bc}± 12.85	1053.47^{bc} ±13.08	1579.28^{b} ±17.38	1941.86^{b} ±26.64	1896.40^{b} ±26.27	2.05^{b} ±0.02
0%MOLM	45.62± 1.29	159.73 ±1.46	348.26^{b}± 10.50	625.06^{c} ±12.11	1020.96^{c}± 15.34	1524.65^{c} ±16.59	1871.92^{c} ±22.48	1826.30^{c} ±22.20	2.13^{a} ±0.02
p-value	ns	Ns	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Means with the same column carry same letters are non-significantly different. ns: non significant.

Table 4. Effect of MOLM on some carcass traits and mortality rates of broiler chicks.

	3%MOLM	5%MOLM	8%MOLM	0%MOLM	p-value
Carcass%	76.72±2.05	77.37±1.90	77.85±0.76	74.68±0.47	ns
Gizzard%	1.63±0.01	1.29±0.23	1.72±0.05	1.58±0.12	ns
Heart%	.46±0.03	.53±0.07	.43±0.02	.48±0.02	ns
Liver%	2.91±0.34	2.30±0.22	2.37±0.36	2.90±0.23	ns
Spleen %	0.14±0.01	0.16±0.01	0.13±0.01	0.13±0.01	ns
Bursa %	0.10±0.02	0.10±.02	0.10±0.01	0.06±0.01	ns
Mortality rate	1.96	0.00	1.96	3.92	ns

Means with the same row carry same letters are non-significantly different. ns: non significant.

Table 5. Effect of MOLM on haematological parameters of broiler chicks at 43 days of age.

	3%MOLM	5%MOLM	8%MOLM	0%MOLM	p-value
Heterophile	8.80^{b}±1.157	7.20^{b}±0.86	9.20^{b}±0.37	13.80^{a}±0.58	<0.01
Lymphocyte	81.80^{a}±1.71	85.00^{a}±1.67	83.00^{a}±1.84	75.80^{b}±1.42	<0.01
Monocyte	7.00±1.00	5.60±0.74	5.40±1.28	7.80±1.15	ns
Eosinophil	2.40±0.50	2.20±0.58	2.40±0.51	2.60±0.67	ns
H/L	0.10^{b}±0.016	0.08^{b}±0.011	0.11^{b}±0.006	0.18^{a}±0.009	<0.01
Hb	0.48±0.062	0.54±0.059	0.57±0.020	0.45±0.081	ns
PCV	29.33^{ab}±1.70	34.16^{a}±1.40	28.16^{b}±1.40	25.33^{b}±2.60	<0.05

Means with the same row carry same letters are non-significantly different. ns: non significant.

Table 6. Effect of MOLM on Tonnic Immobility (TI) duration of broiler chicks (43 days of age).

	3%MOLM	5%MOLM	8%MOLM	0%MOLM	p-value
Tonnic Immobility (sec)	$119.00^{b}\pm$ 8.83	$103.17^{b}\pm$ 15.16	$121.33^{b}\pm$ 14.63	$178.16^{a}\pm$ 10.18	<0.05

Means with the same row carry same letters are non-significantly different.

Table 7. Effect of MOLM on antibody titer (log2 HI) against ND.

	3%MOLM	5%MOLM	8%MOLM	0%MOLM	p-value
Titer	$3.83^{a}\pm0.30$	$4.16^{a}\pm0.30$	$4.00^{a}\pm0.25$	$2.83^{b}\pm0.31$	<0.05

Means with the same row carry same letters are non-significantly different.

3% and 5% despite high crude protein may be attributed to high crude fiber content which may reduce digestibility and absorption of the nutrients (Otuma and Onu, 2008).

Results from productive performance in the current study were in close agreement with Onu and Aniebo (2011) who found that birds supplemented with 2.5%, 5% and 7.5% MOLM had significantly higher final BW and BWG at 35 days of age compared to control birds however, birds fed 2.5% and 5% diets recorded significantly ($P < 0.05$) the highest BWG. They added that, FCR was significantly better in birds fed MOLM supplemented diet compared to control birds. In the same trend, Portugaliza and Fernandez (2012) indicated that birds fed Moringa oleifera Aqueous Leaf Extract at 30, mL 60 mL or 90 mL had significantly heavier BW and significantly better FCR compared to birds fed control diet. Also, Banjo (2012) indicated that, broilers fed 1%, 2% and 3% MOLM had significantly higher final BW and BWG compared to birds fed control diet. However birds fed 2% were significantly heavier than birds fed 3% MOLM, while birds fed 1% or 2% significantly gained more than birds fed 3% MOLM. They added that FCR was significantly superior in all MOLM supplemented groups compared to control birds. Moreover, Melesse et al. (2011) found that the average BW and BWG was significantly higher in Rhode Island Red chicks fed 2%, 4% or 6% Moringa stenopetala leaf meal (MSLM) compared to control birds however, birds fed 6% had significantly heavier BW and higher BWG than birds fed 2% and 4% Moringa meal. Moreover, FCR was better in birds fed MSLM compared to control birds.

On contrary to productive performance results in this study, Zanu et al. (2012) found that final weight and total weight gain were not significantly differed between control birds and those fed 5% and 10% MOLF, however, birds fed 15% had significantly lowered BW and BWG. Feed conversion efficiency significantly ($p<0.05$) declined with increasing level of MOLF. Also, Oludoyi and Toye (2012) observed a significant difference in body weight at 4th week of Hubbard broiler chicks fed (0% MOLM, 10% MOLM > 5% MOLM) thus they concluded that inclusion of MOLM in the diet of broilers from 0-4 weeks suppressed performance compared to control birds at age 4 weeks and thereafter when discontinuation of MOLM in the diet from 4 to 7 weeks age. Gakuya et al. (2014) reported that BW and BWG were significantly reduced in birds fed 7.5%, 15% and 30% MOLM compared to control birds. However, FCR was not differed from control birds up to 7.5% MOLM over which FCR was significantly worst. Olugbemi et al. (2010a) reported that 5% MOLM supplement to cassava-based broilers' diet had no effect ($P > 0.05$) on BW, BWG and FCR compared to control however, supplementation of diet with MOLM above 5% resulted in reduced bird's performance. Moreover, Limcangco-Lopez and Devendra (1989) indicated that Moringa fed 7.5 and 10% to one-week old chicks resulted in decreased growth. Furthermore, Tesfaye et al. (2012) reported that birds fed on 5, 10, 15 and 20 % MOLM had a lower BW and BWG at 56 days of age when compared to control birds. However, Juniar et al. (2008) found no differences in BW, FCR and production efficiency factor between birds fed 2.5, 5, 7.5 and 10% MOLF compared to control birds. Makanjuola et al. (2014) reported that BWG of birds supplemented with 2, 4 and 6% MOLM was not differed significantly compared to birds fed control

diet. In addition, Aderinola et al. (2013) revealed that broiler chicks fed control diet had significantly higher BWG and FCR compared to birds fed 0.5%, 1%, 1.5% and 2% MOLM diets.

3.2. **Carcass traits**

Carcass traits represented as percentage from live body weight of broiler chicks supplemented with graded levels of MOLM at 43 days of age presented in Table 4, showed that percentage of carcass weight, heart, liver, spleen, gizzard and bursa was not significantly differed from birds fed control diet. Similarly, Ayssiwede et al. (2011) found that MOLM up to 24% had no effect on carcass traits and organs characteristics of growing indigenous Senegal chickens. Also, Zanu et al. (2012) found no effect of MOLM on all carcasses traits. In the same trend, Juniar et al. (2008) reported that the inclusion of MOLM up to 10% did not produce significant ($P > 0.05$) effects on carcass weight.

3.3. **Haematological parameters**

The effect of dietary supplementation of different levels of MOLM on differential leucocytic count was presented in Table 5. Supplemented birds had significantly ($p<0.05$) more lymphocyte number compared to control birds, however, heterophil count was significantly higher ($p<0.05$) in the birds of control group compared with chicks in all MOLM supplemented diet groups. Also, results of the current study denoted that, broilers fed the control diet had a significant increase ($p<0.05$) in H/L ratio when compared with chicks in all MOLM supplemented diet groups. This means control chicks were more stressed than chicks in MOLM supplemented groups. In accordance with these results, Gross and Siegel (1983) indicated that, H/L ratio considered the more appropriate measure of long term stress, where stressed birds shown an increase in H/L ratio.

Results from (Table 5) indicated that Hb concentration (gm%) not significantly affected by MOLM supplementation. Chicks fed on 5% MOLM had significantly more PCV% when compared to un-supplemented ones and those supplemented with 8% MOLM, while birds fed on 3% MOLM was intermediate and not different from others. These results were in agreement with Onu and Aniebo (2011) who found that PCV was significantly higher in chicks fed 2.5, and 5% MOLM compared to control birds and those fed 7.5% MOLM however, PCV of birds fed MOLM 7.5% not significantly differed from control birds. They also, found no effect of graded levels of MOLM on Hb concentration. Moreover, Makanjuola et al. (2014) found no effect of 2, 4 and 6% MOLM on PCV and Hb concentration of broiler chicks. In addition, Zanu et al. (2012) found no differences in PCV and Hb concentration of control birds and those fed 5, 10 and 15% MOLM.

3.4.**Tonnic immobility, TI (Fear test)**

Dietary supplementation with 3, 5 and 8% of MOLM had significant effect on the duration of tonic immobility as shown in (Table 6). The control birds were more fearful and showed significantly longer duration in TI test compared to MOLM supplemented groups. Jones (1986) noted that duration of TI is positively correlated with degree of fearfulness, and vice versa. The decreased level of fearfulness in MOLM groups may be attributed to high level of Vitamine C in Moringa leavs. Satterlee et al. (1993) reported that Pretreatment with vitamine C resulted in decreased neophobia in Japanese quail and shortened TI duration in stressed 24 day old broiler chicks (Satterlee et al., 1994). Moreover, Zulkifli (2003) found that broiler chickens pre-treatment with 1,200 ppm L-ascorbic acid in drinking water for 24 hr were significantly less fearful in TI test compared to untreated birds. Furthermore, Toghyani et al. (2015) found that 1000 ppm of L-ascorbic acid for 36 hr in drinking water resulted in decreased fearfulness and improved preslaughter poultry welfare.

3.5. Immune response:

Effect of MOLM on antibody titer against ND at 30 days of age was presented in Table 7. Birds fed 3%, 5% and 8% MOLM had significantly higher antibody titer against ND compared to birds fed control diet. This finding was in close agreement with Younis et al. (2016) who indicated that Ross 308 breed fed 2% and 3% MOLM showed an increased immunity against ND than Cobb 500 breed and control birds. Also, Eze et al. (2013) indicated that methanolic extract of M. oleifera at 200 mg/kg body weight increased ND HI titer in the vaccinated and un-vaccinated chicken groups and it could be recommended as an immune-booster treatment against ND in non-vaccinated birds. In the same trend, Chollom et al. (2012) revealed that M. oleifera seed extract had a strong antiviral activity against ND in ovo. Du et al. (2007) reported that supplementation of MOLM resulted in significantly increased immune

response of broilers. In addition, Yang et al. (2007) revealed that M. oleifera significantly improved duodenum traits, as increased Lactobacillus counts in ileum thus decreased E. coli and improved immune system of broilers.

In conclusions, dietary supplementation of broiler chicks with MOLM up to 8% resulted in reduced fear and stress, and improved performance and immune response.

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