EFFECTS OF MORINGA (*Moringa oleifera*) LEAF MEAL ON THE PERFORMANCE AND IMMUNE RESPONSE OF BROILERS VACCINATED WITH NEWCASTLE DISEASE VACCINE

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ABSTRACT

The study was conducted to evaluate the effects of moringa (Moringa oleifera) leaf meal (MLM) on the performance and immune response of broilers vaccinated with Newcastle disease vaccine. Ninety-six unsexed two week - old broiler chicks were allotted to four treatments with twenty-four chicks in each treatment and four replicates in each with six birds per replicate. The treatments were 0g (control), 2g, 4g and 6g of dry MLM per Kg of diet respectively. The birds were vaccinated at 3 weeks. Blood samples were taken from one bird per replicate via the wing vein post vaccination at the 4th, 5th and 6th week. Sera were analyzed at the Veterinary Medicine Laboratory of the Veterinary Teaching Hospital, (VTH), University of Nigeria, Nsukka (UNN) using Haemagglutination Inhibition (HI) test. Blood samples were also collected from two birds per treatment at the 7th week and analyzed for haematological parameters. At the starter phase, the final weight and average daily feed intake were significantly affected (P<0.05) ranging from 675.90 – 727.00g and 64.83 – 74.05g respectively. The haematological parameters were not significantly different (P>0.05) at the finisher stage. The final weights at the finisher phase were not significantly affected (P>0.05). It was also discovered that the increase of dry MLM increased the immunity status of birds at weeks 5 and 7 but showed no significant effect (P>0.05) at week 6. The results of this study indicated that the inclusion of dry MLM had significant effect (P<0.05) on the performance and a remarkable immune stimulating effect.

Keywords: Broilers, Immune Response, Moringa Leaves, Newcastle Disease, Vaccination

INTRODUCTION

Newcastle disease (ND) is a serious threat to aviculturists and the poultry industry worldwide and belongs to World Organization for Animal Health listed diseases. It is characterized as a transmissible disease that has the potential for very rapid spread irrespective of national borders; a disease of serious socio-economic and public health consequence and of major importance in the international trade of animals and animal products (OIE, 2005, Facon *et al*., 2005).

The outbreaks of velogenic ND are characterized by high mortality, condemnation of other infected flocks, trade restrictions associated with quarantine and surveillance of affected areas within individual states where outbreaks have been detected (Talebi, 2006; Yongola *et al*.,2006). Newcastle disease is caused by Newcastle disease virus (NDV) which is an Avian Paramyxovirus type 1 (APMV-1) that belongs to the family *Paramyxoviridae* and genus *Avulavirus* (Aldous and Alexander, 2001; Alexander, 2003). The only effective means of preventing NDV is through vaccination commonly given by the oral, ocular and intranasal routes (Wambura, 2009). Despite these vaccinations there have been reports of rampart outbreaks of ND.

All parts of the *M. oleifera* tree are edible and have long been consumed by humans. *M. oleifera* is said to be a natural anthelmintic, mild antibiotic, detoxifier and an outstanding immune builder and is used in many countries to treat malnutrition and malaria (Khesorn, 2009). It is also regarded by water purification experts as one of the best hopes for reducing the incidence of water-borne diseases. Moreover, there has been recently an increased interest in the utilization of *M. oleifera* as a protein source for livestock (Sarwatt *et al*., 2002; Kakengi *et al*. 2007). Yang *et al.,* (2006) reported reduced blood triglycerides and enhanced immune response due to increased peripheral and splenocyte T-cell proliferations in rats as a result of Moringa leaf meal inclusion in the diet. Caceres *et al*. (1991) attributed fast growth in man and livestock to Moringa being able to promote metabolism with its bio-available ingredients and its ability to increase the natural defenses of the body. It is therefore a multipurpose tree of significant economic importance with industrial and medicinal uses (Umar, 1998; Anwar *et al.,* 2007). However, there is paucity of information on the use of the leaves as an immune- modulator especially in reducing the mortality rate in chickens infected by NDV, and also as an adjuvant to vaccination.

Recent studies have demonstrated that Moringa *(Moringa oleifera)* leaf meal provides natural antimicrobials/medicines, vitamins, minerals, and proteins. Dry matter (DM) yield is high-15 tons/ha/year. Sankhyan *et al.* (2013) reported that Moringa leaf contains about 0.82mg/g Vitamin C which is roughly 2-4 times higher than that of most known sources of Vitamin C particularly oranges, lemon and mosambi. Vitamin C is reported to provide protection against immune system deficiencies thus enhancing immune response (Sarwatt *et al.,* 2002). In a study in Nicaragua, fresh Moringa leaves were found to contain 23% crude protein (CP) in the DM, 12.3 MJ ME/kg DM and had an in-vitro DM digestibility of 79.7% (Becker, 1995). The objective of this study was to determine the effect of dry Moringa leaf meal (MLM) on the performance, haematology and immunity of broilers vaccinated with Newcastle disease vaccine (LaSota).

MATERIALS AND METHODS

The study was conducted at the Poultry Unit of the Teaching and Research Farm of Kogi State University, Anyigba. The study site is located on the latitude 7^0 09'N and 6^0 43'E and with an average altitude of 420m above sea level. The area falls within tropical wet and dry climate region of the derived savanna, with average rainfall of 1600mm. The daily temperature range is between 25°C-35°C (Ifatimehin *et al*., 2011).

Moringa leaves were plucked from different places within the staff quarters of the Kogi State University Anyigba, Kogi State. The leaves were plucked, dried at room temperature, milled and a portion of it was taken to the laboratory for proximate analysis.

Ninety - six two weeks - old broiler chicks were used. The birds were randomly assigned to four (4) dietary treatments of 24 birds each. Each treatment was randomly subdivided into four replicates of six birds. Starting at the 3rd week of life, the birds were fed with compounded feed at different level of moringa inclusion. The moringa meal was added to the different treatment groups as follows: Group T1 (control), was fed diet containing 0g inclusion of Moringa leaf extract (MLE) while T2, T3 and T4 feeds contained graded levels of of 2g, 4g, and 6g MLE respectively.

The gross composition of the starter, and finisher phases of the experimental feed are as shown in Tables 1 and 2.

Lasota vaccine was used as a test vaccine and a vial of vaccine was reconstituted in (2) two litres of drinking water and served to the birds at 3 weeks of age. Routine vaccination against Gumboro disease was administered at the 2^{nd} and 4^{th} week of age.

Table 1: Gross composition of on-farm experimental diet at the starter phase

Ingredient (%)	T1	T2	T3	T4
Maize	32.75	32.75	32.75	32.75
Soybean meal	36.00	36.00	37.00	37.00
Maize offal	6.00	6.00	6.00	6.00
Bone meal	3.40	3.40	3.40	3.40
Salt	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30
Moringa leaf meal	0.00	2.00	4.00	6.00
Lysine	0.10	0.10	0.10	0.10
Brukutu waste	21.00	19.00	16.00	14.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
Crude protein (%)	23.12	22.90	22.79	22.57
Ether extract (%)	9.45	9.30	9.26	9.11
Crude fiber (%)	5.38	5.15	4.85	4.61
M.E.(kcal/kg)	2956.98	2932.78	2916.58	2892.39

M.E. = Metabolizable energy.

Table 2: Gross Composition of on –farm experimental diet at the finisher phase

Ingredients (%)	T1	T2	T3	T4
Maize	45.00	45.00	45.00	45.00
Soybean meal	41.85	39.85	37.85	35.85
Maize offal	9.00	9.00	9.00	9.00
Bone meal	3.40	3.40	3.40	3.40
Salt	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30
Moringa leaf meal	0.00	2.00	4.00	6.00
Total	100.00	100.00	100.00	100.00
Calculated Analysis				
Ether extract (%)	9.49	9.20	8.92	8.64
Crude fiber (%)	3.67	3.73	3.79	3.85
M.E.(kcal/kg)	3105.45	3099.77	3094.09	3088.41
Crude protein (%)	20.65	20.29	19.92	19.56

M.E. = Metabolizable energy.

Parameters collected were weight gain (WG), feed intake (FI) and feed conversion ratio (FCR). The birds were weighed at the beginning (initial weight) and subsequently on weekly basis. The initial live weight was subtracted from the final live weight to determine the weight gain (WG) of the birds. Weighed quantities of feed were provided daily and the leftover of the feed (ort) was weighed weekly to obtain the weekly feed intake. An average of the weekly feed intake determined the daily feed intake (FI). The efficiency with which each bird converted feed to body weight was computed as the ratio of the average daily feed consumed to the average daily weight gain. This was the feed conversion ratio (FCR).

Blood samples were collected from two birds per treatment through the wing vein into a set of well - labeled sterile bottles containing Ethylene Diamine Tetra-Acetic Acid (EDTA) and were evaluated for haematological parameters such as; PCV, Hb, WBC and RBC while MCH, MCHC, and MCV were calculated at the finisher stage. In addition, blood sample was taken from one bird per replicate via the wing vein post vaccination at the 4th, 5th and 6th week. The sera samples were subjected to Haemagglutination inhibition (HI) test to determine the immune response as described by OIE (2002).

Sera samples were stored at - 20^0C and later taken for HI analysis at the Veterinary Medicine Laboratory of the Veterinary Teaching Hospital (VTH), UNN, Nsukka, Nigeria. 0.6% chicken red blood cell used for the Haemagglutination inhibition (HI) test was prepared as described by Wosu (1984). The Haemagglutination inhibition (HI) test was done as described by OIE (2002).

All data obtained from the study (performance and HI Titres) were subjected statistically to One-way Analysis of Variance (ANOVA) using SAS statistical software and difference between treatment means was separated using Duncan's Multiple Range Test (SAS 2002).

RESULTS AND DISCUSSION

Performance of broilers fed dry moringa leaf-meal from week 3 to 4 (starter phase) is presented in Table 3. The result indicated that the inclusion of dry moringa leaf meal in the diet had significant effect (P<0.05) on the final weight and average daily feed intake. The increase in the moringa level led to increase in the final weights of T2 and T3 but there was a decrease in the final weight of T4, although there was no significant difference (P>0.05) between T1, T3 and T4 with T2 having the highest final weight gain. There was a significant difference (P<0.05) in the average daily feed intake with T3 having the lowest feed intake. The feed intake of T1, T2 and T4 were similar. From the result, it was shown that there was no significant difference (P>0.05) in the average weight gain and feed conversion ratio. The feed conversion ratio was similar among the treatments. The feed conversion efficiency in this study disagrees with that reported by Etela *et al.* (2007) who got between 3.28 to 3.311 when the authors supplemented commercial feed of broilers with moringa and other green vegetables.

Table 3: Performance of broilers fed with dry moringa leaf meal (MLM) at the starter phase (3- 4 weeks).

Parameters	T1 0g/kg	T2 2g/kg	T3 4g/kg	T4 6g/kg	SEM	LOS
Initial weight (g)	356.25	300.00	306.25	306.25	0.09	NS
Final weight (g)	694.00^{ab}	727.00^{a}	700.25^{ab}	675.90^{b}	0.06	*
ADWG(g/day)	24.13	30.50	28.13	26.40	0.01	NS
ADFI(g/day)	71.58^{a}	74.05^{a}	64.83^{b}	73.2^{a}	0.01	*
FCR	2.97	2.50	2.36	2.92	0.03	NS

SEM: Standard error of means, a, b, c, d: means the same row with different superscript differ significantly. NS: No Significance (P>0.05), *: significance (P<0.05), LOS: level of significance. ADWG: Average daily weight gain, ADFI: Average daily feed intake. FCR: feed conversion ratio

The inclusion of moringa leaf meal in feed had no significant effect (P>0.05) on the weight gain, and feed conversion efficiency but showed significant increase (P<0.05) in final weight and average daily feed intake as seen in Table 3 above. Table 4 shows the effect of dry moringa leaf meal based diets given to broilers from 5 to 8 weeks on their performance. The results in Table 4 indicated that the inclusion of dry moringa leaf in the diet showed no significant difference (P>0.05) in the final weight, average daily feed intake, average weight gain and feed conversion ratio. However, the increase in the level of dry moringa leaf meal led to increase in the final weight gain of the birds with T4 (6 g/kg) having the highest weight gain. The average weight gain of the birds in all the treatment was similar with T4 (6g/kg) having the highest average weight gain. The average feed intake was similar with T3 (4 g/kg) having the lowest feed intake, also the feed conversion efficiency was similar with T4 (6 g/kg) having the best feed conversion ratio. The inclusion of dry moringa leaf had no significant difference (P>0.05) on the final live weight, average daily weight gain, and average daily feed intake and feed conversion ratio.

The haematological parameters of broilers fed dry moringa leaf at the finisher phase as shown in the Table 5 below shows that there was no significant difference (P>0.05) in Packed cell volume (PCV), Haemoglobin (Hb),White Blood Cell (WBC), Red Blood Cell (RBC), Neutrophils, Lymphocytes, Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) among the treatments. The values obtained in this study were similar to what was reported for broilers by Sjaastad, *et al.*, (2005) and the values fell within the normal ranges for healthy broilers in earlier works stated as follows: PCV = 23-58%, RBC = 2.5 – 3.2 millions/mm3; Hb = 6.5 - 9.0 g/100 mL; WBC = 20-30 thousands/mm^3; MCV = 115-125fl; MCH = 25-27pg and MCHC = 21-23% (Gilbert, 1962; Maxwell *et al*., 1990). Based on the haematological parameters obtained in this study, the broilers can be said to be in health status. Table 6 shows the result of Haemagglutination Inhibition (HI) test titres for broilers fed moringa leaf meal. The results showed significant increase (P<0.05) in HI titres at weeks 5 and 7, T3 had high titres (448 and 24, respectively). However, in week 6, there was no significant difference (P>0.05) in the immunity of the different groups T1, T2, T3 and T4. The result of this finding is in conformity with that reported by Yang *et al.*, (2006) who confirmed the immune stimulating effect of dry moringa leaf meal. Furthermore, the observation in this study is in agreement with the findings of Khesorn (2009) who said that dry moringa leaf is a natural anthelmintic, mild antibiotic, detoxifier and an outstanding immune builder and the observation of Sarwatt *et al*. (2002) who reported that dry moringa leaf contained vitamin C that provides protection against immune system deficiencies thus enhancing immune response.

Table 4: Performance of broilers fed with dry moringa leaf at the finisher phase (5 – 8 weeks)

Parameters	T1 0g/kg	T2 20g/kg	T3 40g/kg	T4 60g/kg	SEM	LOS
Initial weight (g)	694.00^{ab}	727.00^{a}	700.25^{ab}	675.90^{b}	0.06	*
Final weight (g)	2565.00	2552.50	2625.00	2717.80	0.45	NS
ADWG(g/day)	66.83	65.20	68.75	7.95	0.02	NS
ADFI(g/day)	140.90	138.38	132.40	133.05	0.01	*
FCR	2.13	2.14	1.96	1.85	0.02	NS

SEM: Standard error of means, a, b, c, d: means on the same row with different superscript differ significantly. NS: No Significance (P>0.05), *; significance (P<0.05), LOS: level of significance. ADWG: Average daily weight gain, ADFI: Average daily feed intake. FCR: feed conversion ratio

Table 5: Haematological analysis of birds fed dry moringa leaf at the finisher stage

Parameters	T1	T2	T3	T4	SEM	LOS
PCV (%)	32.00	29.50	33.50	32.00	0.26	NS
Hb (g/dl)	10.30	9.35	11.30	10.35	0.12	NS
WBC (x 09/1)	252.45	246.65	256.90	248.10	0.80	NS
RBC (x 106/1)	2.55	2.45	2.65	2.54	0.02	NS
MCV (Fl)	127.75	122.75	128.70	127.25	0.67	NS
MCH (pg)	40.50	38.10	40.10	40.80	0.48	NS
MCHC (g/dl)	31.70	31.00	32.35	32.05	0.16	NS
NEUT (%)	3.50	3.50	3.50	4.00	0.12	NS
LYMPH (%)	96.50	96.50	96.50	96.00	0.12	NS

PCV =Packed cell volume, Hb = Haemoglobin, WBC = White Blood Cell, RBC =Red blood cell, MCV = Mean Cell Volume, MCH = Mean Corpuscular Haemoglobin,MCHC = Mean Cell Haemoglobin Concentration, Neut = Neutrophils, Lymph = Lymphocytes, SEM: Standard error of means, a, b, c, d means the same row with different superscript differ significantly. NS: No Significance (P>0.05), * = significance (P<0.05), LOS: level of significance.

Table 6: Mean HI titres of Newcastle disease virus in vaccinated broilers given dry moringa leaves

Parameters	T1	T2	T3	T4	SEM	LOS
Week 5	144.00^{bc}	96.00^{c}	448.00^{a}	336.00^{ab}	8.36	*
Week 6	320.00	80.00	224.00	256.00	15.91	NS
Week 7	14.00^{b}	56.00^{a}	24.00^{b}	22.00^{b}	0.70	*

a,b,c,d: means on the same row with different superscript differs significantly
SEM: Standard Error of Mean
LOS: Level of Significance

CONCLUSION AND RECOMMENDATION

In conclusion, the moringa leaf meal significantly increased the final weight and average daily weight gain at the starter phase but average daily feed intake and feed conversion ration were not affected. At the finisher phase, however, the final weight, average daily weight gain, average daily feed intake and feed conversion ratio were not significantly affected. Likewise, the haematological parameters were not affected. On the other hand, the moringa leaf meal significantly enhanced the immunity of ND vaccinated broilers with very high HI titres. It is therefore recommended that moringa leaf meal be included in poultry feed to enhance weight gain at the starter phase and overall immunity of ND vaccinated birds.

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