

Immuno-Modulatory Activity of Aqueous Leaf Extract of *Moringa Oleifera* in Broiler Chickens

Faluyi, O.B.; Agbede, J.O.

Department of Animal Production and Health, The Federal University of Technology, Akure, Ondo State, Nigeria.

Abstract— This experiment was conducted to investigate the immuno-modulatory activity of aqueous leaf extract of *Moringa oleifera* on immune response of broiler chickens to Newcastle disease (ND) vaccinations. The performance, blood parameters and serum biochemistry of the birds were also determined. A total of one hundred and twenty (120) day-old commercial broiler chicks were randomly allotted to 3 dietary treatments of 4 replicates each. Each replicate had 10 birds. The treatments: T1 – Control group in which the birds were not given any extract; T2 and T3 – birds in these groups were given the prepared stock solution of *Moringa oleifera* leaf extract at dose rate of 2500mg/kg and 5000mg/kg of body weight in drinking water. The experimental birds were vaccinated with ND vaccines using a stipulated vaccination regime. The *Moringa oleifera* leaf extract exhibited significant ($p \leq 0.05$) influence on final body weight of the experimental broiler chickens with birds in T3 having an average weight of 1947.43g and birds in T1 had 1733.33g. The immune modulating effect of the leaf extract was insignificant ($p \geq 0.05$) though it elicited higher antibody titre of $\text{Log}_2 7$ and $\text{Log}_2 9$ in birds in T3 compared to $\text{Log}_2 6$ and $\text{Log}_2 8$ of birds in control group after the first and second ND vaccinations respectively. The leaf extract caused significant ($p \leq 0.05$) increase in white blood cells and leucocytes count. The study concluded that the plant extract had slight immune stimulatory effects on response to ND vaccinations and improved the growth performance of broiler chickens.

Keywords— Antibody, aqueous, growth, immune response, *Moringa oleifera*.

I. INTRODUCTION

Poultry production provides base for the socioeconomic advancement in the majority of developing countries and this has led to increased demand for poultry products especially broiler meat. This is because consumers perceive that it is a healthy product that contains less fat, predominantly unsaturated fatty acids, and particularly polyunsaturated fatty acids, compared to beef or pork products.

The continuing survival and growth of the broiler chicken industry in developing countries of the world depends on its ability to compete globally, which is largely dependent

on the efficiency of its production system. It is a common practice in the management of poultry to administer antibiotics in drinking water as growth promoters and to prevent or control infectious bacterial diseases. The benefit of such practice is to maintain good health, suppress mortality of birds, and to support maximal growth via improved utilization of nutrients and ultimately improve profit (Zeweil *et al.*, 2006). However, the use of synthetically-produced substances especially antibiotic growth promoters was soon found to have objectionable side-effects (Makanjuola *et al.*, 2014). This has led to antibiotics growth promoter being banned mainly due not only to cross-resistance but also to multiple resistances.

Therefore due to the desire for improved economic status in poultry production, researchers revolutionized the application of feed and water additives by focusing on organic or natural supplements instead of using synthetic medicament (Zeweil *et al.*, 2006). The use of medicinal plant either alone or in group (combination) as possible therapeutic measures has become a subject of active scientific investigation (Oyewole, 2012). Some medicinal plant products are known to enhance natural resistance of host to infection due to the presence of bioactive phytochemicals or phyto-nutrients (Soetan and Oyewole, 2009).

Moringa (*Moringa oleifera* Lam.) is a multipurpose tropical tree and it has been dubbed the "miracle tree" or "tree of life" in popular media (Bosch, 2004; Orwa *et al.*, 2009; Radovich, 2013; FAO, 2014) mainly because it is used for food and has numerous industrial, medicinal and agricultural uses, including animal 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, phenolics and carotenoids (Anwar and Bhangar, 2003). It is one of the herbs containing bioceutical agents that could substitute synthetic growth enhancers and supplements in broiler and other livestock production since it possesses important medicinal properties which include antibacterial and antifungal activities (Nickon *et al.*, 2008).). In Nigeria, leaf preparations of *Moringa oleifera* is widely used in

folklore for the treatment of immune system related disorders (Oyewo *et al.*, 2013).

This study was done to investigate the effect of aqueous leaf extraction of *Moringa oleifera* in drinking water of broiler chickens on their growth performance and immune response to Newcastle disease vaccinations.

II. MATERIALS AND METHODS

2.1 Study site

This study was approved by the Research Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure (FUTA) Nigeria. The field trial was conducted at the Poultry unit of the Teaching and Research Farm of FUTA, Nigeria. The laboratory analyses were done at the Microbiology Laboratory of the Department of Animal Production and Health and Central Research Laboratory, FUTA.

2.2 Aqueous Leaf Extraction of *Moringa oleifera*

Fresh leaves of the plant were harvested and 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 Whatman's filter paper. The filtrate was collected, the solvent was removed using rotary evaporator and the residue obtained after evaporation was weighed. The concentrated stock solution of *Moringa* leaf extract was prepared by dissolving 500g of the residue in 1 litre of sterile distilled water and stored at 4°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.

2.3 Experimental design and Animal Management

A total of one hundred and twenty (120) day-old broiler chicks of the Abor acre breed purchased from a reputable hatchery in Akure, Ondo State, Nigeria were used for the study which lasted for a period of 6 weeks. The birds were reared on deep litter using routine management procedures as outlined by the Teaching and Research Farm of FUTA and feed and water provided *ad libitum*. The chicks were divided into three treatment groups (T1, T2 and T3) with four replicates of 10 birds each using a completely randomized design. Birds in control group (T1) were not given any extract while birds in T2 and T3 received the prepared stock solution of *Moringa oleifera* leaf extract at dose rate of 2500mg/kg and 5000mg/kg of body weight respectively in their drinking water. The experimental chickens were vaccinated with Newcastle disease vaccines (NDV) - NDV intra-ocular (Hithner B1 strain) at 3 days old and NDV LaSota via the oral route at 28 days old. The study was conducted for a period of 6 weeks.

2.4 Performance criteria measurement:

Initial weight of birds was measured at day old, then on a weekly basis for the final weight. Thereafter weight gain for each week over the trial period was measured as the difference between the initial weight and the final weight. The feed consumption was recorded per replicate and the feed conversion ratio calculated as a ratio of feed consumed to weight gain of birds per replicate.

2.5 Blood and Sera collection:

Samples of blood for the purpose of serum analysis were collected from 3 birds per replicate in each treatment group before the trial commenced via the heart to determine baseline maternal antibody titre levels against Newcastle disease. The birds were sedated using chloroform before the bleeding exercise. Thereafter, in each treatment 12 birds (3 per replicate) were randomly selected and blood was collected 14 days after administering each of the ND vaccines through the jugular vein for serological analysis to determine the antibody titre values. At the end of the 6 weeks experimental period blood was also collected for haematological and serum protein biochemistry analysis from 12 birds in each treatment.

2.6 Laboratory Analysis

2.6.1 Haematological Analysis

Erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB) and white blood cell differentials were analysed as described by (Lamb, 1981). The Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were also calculated accordingly.

2.6.2 Haemagglutination and Haemagglutination Inhibition Test (HA/HI Test)

Serum samples taken from the experimental broiler chickens were analysed using beta (β) micro haemagglutination inhibition technique as described by Thayer and Beard, (1998) to determine the antibody titre levels. The antibody titre is a measure of the humoral immune response elicited in the experimental birds to Newcastle disease vaccinations.

2.6.3 Serum Biochemical Analysis

Diagnostic kits (Randox Laboratories, UK Test Kits) was used to analyse serum biochemical parameters (Total protein, Globulin, Albumin, Alanine transferase, Alanine phosphatase, Aspartate transaminase) of the experimental broiler chickens.

2.7 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA). Where significant differences were observed, the mean were separated using the SAS statistical package (2012).

III. RESULTS

3.1 Performance Characteristics

Table 1 presents the performance characteristics of the experimental broiler chickens. The administration of the *Moringa oleifera* leaf extract resulted in significant ($p \leq 0.05$) increase in the FBW and WG of experimental birds. The FBW for birds in treatment 3 (1947.43g) was the highest and significantly ($p \leq 0.05$) different from that of birds in treatment 1 (1733.33g). The WG result followed a similar trend with birds in T3 gaining an average weight of 1911.43g and birds in T1 having 1733.33g. The table also shows that the FBW and WG of birds given the leaf extract (birds in T1 and T2) were not significantly ($p \geq 0.05$) different regardless of the dose. The FI and FCR though not significantly ($p \geq 0.05$) influenced by administration of leaf extract were seen to be highest as the dose increased.

3.2 Immunological Responses

In Table 2 the antibody titre values of the experimental birds in response to ND vaccinations are shown. The table revealed that the birds had a uniform maternal antibody titre value of $\text{Log}_2 4$ across the treatments. However after the ND vaccinations, administration of the leaf extracts elicited higher titre values with no significant ($p \geq 0.05$) effect with increasing dose. The birds in T2 and T3 had similar antibody titre values of $\text{Log}_2 7$ and $\text{Log}_2 9$ while those in T1 had $\text{Log}_2 6$ and $\text{Log}_2 8$ after the first and second ND vaccinations respectively.

3.3 Haematological Parameters

The blood parameters of the experimental broiler chickens are shown in Table 3. In all the indices measured only the WBC and lymphocyte counts were significantly ($p \leq 0.05$) influenced by administration of the leaf extracts. The WBC values of birds in T2 ($2.61 \times 10^6 \text{ mm}^3$) and T3 ($2.66 \times 10^6 \text{ mm}^3$) were similar but significantly ($p \leq 0.05$) different from that of birds in T1 ($2.51 \times 10^6 \text{ mm}^3$). The lymphocyte counts also followed similar trend. The PCV and MCV values though not significantly ($p \geq 0.05$) different amongst treatments decreased with higher doses of the leaf extracts. It was observed that the ESR, RBC, Hb, MCH, MCHC values increased in experimental birds with no significant ($p \geq 0.05$) differences among the doses.

3.4 Serum Biochemistry

The trend of the serum biochemical parameters of the broiler chickens measured following administration of the leaf extract are presented in Table 4. The leaf extracts did not have any significant ($p \geq 0.05$) influence on serum indices of the experimental birds. An increase was observed in protein indices (Total protein, albumin and globulin) of the birds administered the leaf extract which was not dose dependent.

IV. DISCUSSION

The immuno-modulatory effects of aqueous leaf extracts of *Moringa oleifera* in broiler chickens in response to Newcastle disease vaccinations as investigated in this present study is negligible. However, the birds given the *Moringa oleifera* leaf extract reportedly had the highest immune response when compared with the birds in the control group ascribing from the antibody titre values. This result is in line with the work of Olugbemi *et al* (2010) who reported that giving *Moringa oleifera* aqueous extract had been shown to have beneficial effect on immune response, thus improving the health status of broiler chickens. It is also in line with the work of Madubiike *et al* (2006) that reported that supplementation of *Moringa oleifera* resulted in marked improvement of humoral immunity of broiler chickens. This study is in accordance with the work of Oyewo *et al.* (2013) which suggested that the aqueous leaf extract of *Moringa oleifera* has immune modulation activities. This immune enhancing attribute of *Moringa oleifera* may be due to the phytochemical constituents such as alkaloids and saponins present in aqueous extracts of the plant as reported by Oyewo *et al.* (2013). Also the mineral content of the aqueous leaf extracts like selenium, zinc, iron, manganese and magnesium have been known to contribute to its immuno-modulatory effect (Prasad, 2000; Ravalglia *et al.* 2000; Oyewo *et al.* 2013).

In this study, inclusion of *Moringa oleifera* leaf extract in water of broiler chickens influenced the performance characteristics. The leaf extracts caused improved productive performance of the experimental broiler chickens judging from the trend of the growth performance traits. This was made obvious, in that birds given the highest dose of *Moringa oleifera* extract exhibited the best performance characteristics which reflected in their body weights. This could be attributed to complete amino acids, considerable amount of vitamins, and mineral content; antioxidant, immuno-stimulant and antibacterial properties of *Moringa oleifera* leaf (Makkar and Becker, 1997; Fahey, 2005; Anwar *et al.*, 2007). It could also be related to the effect of the available nutritional components and some growth stimulating constituents of *Moringa oleifera* which probably resulted in the improvement of live body weight of the chickens (Kakengi *et al.*, 2007). In addition, the minute quantity of anti-nutritional factors that affect palatability of feeds were not implicated to compromise the bioavailability of nutrients and growth stimulating compounds present in *Moringa oleifera* leaves (Foidl *et al.*, 2001). It has also being reported that the crude extract of *Moringa oleifera* like other herbal drug may contain digestion enhancing properties which stimulates favorable growth of good bacteria while decreasing harmful microorganisms (Hernandez *et al.*, 2004). However, the

mechanism by which this herbal product influences the growth performance and gut micro flora of poultry are poorly understood (Hernandez *et al.*, 2004).

The aqueous leaf extract of *Moringa oleifera* led to a boost in the total white blood cell and lymphocytes counts in experimental broiler chickens. This further reiterates its immunomodulation capability, since white blood cells are involved in fighting infection and clearing off injured or dead cells and tissues in body (Jeremy *et al.*, 2001; Oyewo *et al.*, 2013). The trend in antibodies produced in response to ND vaccinations in experimental birds also supports the result of the total white blood cell and lymphocyte counts. The other haematological parameters were not influenced by the leaf extract and values obtained fell within the normal range for healthy chickens as described by Animashahun *et al.* (2006). This indicates that aqueous extract of *Moringa oleifera* leaves did not have any detrimental effect on physiological indices measured. This is however different from the study of Oyewo *et al.*, (2013) where it was reported that the administration of aqueous leaf extract of *Moringa oleifera* induced anaemia in wistar rats which might have been caused by haemolysis of erythrocytes, due to the levels of saponins and heavy metals (lead) in the aqueous leaf extract. A note of caution was therefore suggested in the use of the crude extract of *Moringa oleifera* in high doses because of the levels of heavy metals in the aqueous leaf extract.

Evaluation of serum biochemical parameters may provide useful information in the assessment of health status of birds and serves to reflect many metabolic alterations of organs and tissues when feeding unconventional feed sources (Kudair and Al-Hussary 2010). In this study, the serum biochemical parameters of the experimental chickens was not influenced by leaf extracts of *Moringa oleifera*. This result of the serum biochemical profile is not in line with the previous work of Adedapo *et al.* (2009) who reported significant effect of *Moringa oleifera* extracts on serum ALT, AST and ALP which led him to conclude that high doses of the aqueous extract could lead to liver damage. It is however supported by the work of (Kudair and Al-Hussary 2010) that reported that *Moringa oleifera* leaf has no significant effect on the serum biochemical indices of broiler chickens.

V. CONCLUSION

It can be concluded in this study that *Moringa oleifera* leaf extracts served to moderately boost immunological responses to ND vaccinations, though increasing the dose did not enhance the suggested immunomodulatory activity. It also achieved improved productive performance in the broiler chickens. However, there is need for further investigation to reiterate the capability of *Moringa oleifera* aqueous leaf extract as a substitute to

synthetic supplements such as antibiotics in improving broiler production.

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Table.1: Growth Performance of Broiler Chickens birds given varying doses of *Moringa oleifera* leaf aqueous extract

Parameters	Treatment 1	Treatment 2	Treatment 3	±SEM
Initial weight(g)	35	35	36	0.17
FBW(g)	1768.33 ^b	1863.57 ^a	1947.43 ^a	0.05
WG(g)	1733.33 ^b	1828.57 ^a	1911.43 ^a	0.04
FI(g)	2140.48	2192.86	2259.52	27.15
FCR	1.23	1.20	1.18	0.12

FBW- Final Body Weight; WG- Weight Gain; FI- Feed Intake; FCR- Feed Conversion Ratio

Treatment 1- Birds not given leaf extract of *Moringa oleifera*

Treatment 2- Birds given 2500mg/kg dose of *Moringa oleifera* leaf extract

Treatment 3- Birds given 5000mg/kg dose of *Moringa oleifera* leaf extract

Table.2: Antibody titre values of the broiler chickens given varying doses of *Moringa oleifera* aqueous extract following Newcastle disease vaccinations

Treatments	Baseline antibody titre values	Antibody titre Values after 1 st NDV	Antibody titre values after 2 nd NDV
Treatment 1	Log ₂ 4	Log ₂ 6	Log ₂ 8
Treatment 2	Log ₂ 4	Log ₂ 7	Log ₂ 9
Treatment 3	Log ₂ 4	Log ₂ 7	Log ₂ 9

NDV- Newcastle disease vaccinations

Treatment 1- Treatment 1- Birds not given leaf extract of *Moringa oleifera*

Treatment 2- Birds given 2500mg/kg dose of *Moringa oleifera* leaf extract

Treatment 3- Birds given 5000mg/kg dose of *Moringa oleifera* leaf extract

Table.3: Haematological variables of broiler chickens given *Moringa oleifera* leaf extracts

PARAMETERS	Treatment 1	Treatment 2	Treatment 3	+SEM
ESR(mm/hr)	3.66	3.80	4.16	0.65
PCV (%)	26.83	26.66	26.16	1.04
RBC($\times 10^6$ mm ³)	256.33	258.13	259.41	10.53
HB(g/100 ml)	8.71	8.89	8.95	0.34
WBC($\times 10^6$ mm ³)	2.51 ^b	2.61 ^a	2.66 ^a	0.05
MCV(μ^3)	104.66	103.28	100.84	0.33
MCHC (%)	32.46	33.34	34.21	0.40
MCH (Pg)	33.97	34.44	34.50	0.13
LYM (%)	62.26 ^b	64.00 ^a	64.26 ^a	0.04
HETE (%)	19.30	19.33	19.83	1.19
MONO (%)	12.43	12.66	12.50	1.05
BASO (%)	3.33	3.33	3.50	0.22
EOS (%)	1.50	1.66	1.66	0.02

ESR –Erythrocyte Sedimentation rate, PCV – Packed Cell Volume, RBC – Red Blood Cell, HB - Haemoglobin, MCV - Mean Cell Volume, MCHC - Mean Cell Haemoglobin concentration, MCH - Mean Cell Haemoglobin, LYM – lymphocytes, HETE – M0NO – Monocyte, BASO – Basopils, EOS – Eosinophil

Treatment 1- Treatment 1- Birds not given leaf extract of *Moringa oleifera*

Treatment 2- Birds given 2500mg/kg dose of *Moringa oleifera* leaf extract

Treatment 3- Birds given 5000mg/kg dose of *Moringa oleifera* leaf extract

Table.4: Serum metabolites of broiler chickens administered varying doses of *Moringa oleifera* leaf aqueous extract

TREATMENT	TP(g/dl)	ALB(g/dl)	GLO(g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
T1	30.79	14.50	16.29	79.31	129.50	109.37
T2	36.76	14.12	22.64	78.03	123.00	114.75
T3	31.27	14.61	16.66	71.67	112.83	118.24
+SEM	4.54	0.83	14.59	0.98	21.75	14.59

TP-Total protein; ALB- Albumin; GLO- Globulin; ALT- Alanine transferase;

AST- Aspartate Transaminase; ALP: Alanine Phosphatase