# THE USE OF PHYTOBIOTICS (NEEM [*AZADIRACHTA INDICA*] AND MORINGA [*MORINGA OLEIFERA*]) AS ALTERNATIVES TO ANTIBIOTICS IN BROILER PRODUCTION

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**ABSTRACT**
***An experiment was conducted to determine the effect of different levels of Moringa oleifera leaf meal (MLM) and Neem leaf meal (singly or combined) as alternatives to antibiotics on the growth performance and blood profile of broiler chickens. Two hundred and ten (210) day-old broiler chicks were randomly allotted to 7 dietary treatments of 3 replicates each. Each replicate had 10 birds. The treatments were Treatment 1- Positive control (diet with an antibiotic {oxytetracycline: dosage was as recommended by the manufacturer - 1g to 3kg of feed}), Treatment 2-diet with 600g of Moringa leaf meal (MLM)/100kg of feed, Treatment 3-diet with 600g of Neem leaf meal (NLM)/100kg of feed, Treatment 4-diet with 300g MLM and 300g NLM in 100kg of feed, Treatment 5-diet with 450g MLM and 150g NLM in 100kg of feed, Treatment 6-diet with 150g MLM and 450g NLM in 100kg of feed and Treatment 7-Negative control (diet without any additive). The experiment lasted 56 days. Birds on treatment 2 had significantly ($p < 0.05$) higher final body weight than birds on the other dietary treatments. Significant ($p < 0.05$) differences were also observed in the levels of haemoglobin, lymphocytes and heterophils in birds on the different treatments. Glucose levels were also significantly ($p < 0.05$) lower in birds on treatments 2 and 3 than those on the other treatments. Cost incurred per kg weight gain was lowest for birds on treatment 2. The results suggest that Neem and Moringa could be effectively used as alternatives to antibiotics to boost the growth and immune response of broilers. These feed additives could therefore be introduced as safe and natural alternatives to antibiotics in broiler diets.***

**Keywords**: *Antibiotics, moringa, neem, oxytetracycline phytobiotics, alternatives*

## INTRODUCTION

The production of meat and eggs from chickens is one of the most intensive types of animal agriculture. It is an industry that has achieved worldwide success in its primary objective of providing a source of affordable animal protein. This success is as a result of several factors which include the prevention of disease and improvement of gut health which are partly achieved by the use of antibiotics. Antibiotics are used in human and veterinary medicine to treat and prevent disease and for other purposes including growth promotion in food animals (Phillips *et al.,* 2004). The use of these substances offered possibilities to improve animal performance and increased economic output of livestock producing units. The increased productivity of those units played an important role especially in regions and continents where the availability of land is limited. This resulted in a higher output per livestock unit hence cheaper livestock produce without having to invest in additional land

(Reimensperger 2011). However, the frequent and indiscriminate use of synthetically-produced substances, especially antibiotic growth promoters, was soon found to have objectionable side-effects (Castanon, 2007). The use of antibiotics has led to an increased risk of bacteria developing antibiotic resistance and at the same time leaving antibiotic residues in animal products (Phillips *et al.,* 2004). The increased concern about the potential for antibiotic-resistant strains of bacteria and the health risk associated with the consumption of animal products with antibiotic residues have compelled researchers to explore the use of other non-therapeutic alternatives like enzymes, probiotics, prebiotics, immune stimulants, organic acids and phytobiotics (a wide range of plants and spices and their derivatives) as feed additives in animal production (Vidanarachchi *et al.,* 2013).

Compared with antibiotics, phytobiotics have proven to be natural, less toxic, residue-free and are thought to be ideal feed additives in food animal production (Wang and Bourne 1998). Antimicrobial activity and immune enhancement probably are the two major mechanisms by which phytobiotics exert positive effects on the growth performance and health of animals (Yang *et al.,* 2008a). According to reports by Elangovan *et al.* (2000), neem (*Azadirachta indica*) contains phyto-chemicals which have antimicrobial, antihelminth, antioxidant, antifungal, insecticidal and spermicidal activities. Makanjuola *et al.* (2014) reported that *Moringa oleifera* leaf meal (fed to broilers at the rate of 600g in 100kg of feed) could be a promising natural antimicrobial agent for controlling pathogenic bacteria in livestock production. This present study evaluated the effect of replacing antibiotics with *Moringa oleifera* leaf meal and Neem leaf meal (singly or combined) on the performance of broiler starters.

## MATERIALS AND METHODS

### Experimental site

The experiment was conducted at the poultry unit of the Institute of Agricultural Research and Training, Ibadan, Nigeria. Ibadan is in South-western Nigeria and it lies on the geographical coordinates of 7° 23′ 16″N and 3° 53′ 47″ E. Ibadan has a mean annual rainfall of 1382mm, annual mean temperature range of 21.3 – 31.2° above sea level.

### Management of experimental birds

Two hundred and ten (210) day-old broiler Abor Acres broiler chicks were allotted C, relative humidity of 60 – 90% and is 160m to 7 dietary treatments with 3 replicates each. Each replicate had 10 birds. The birds were reared in a well ventilated and illuminated poultry house on deep litter. Routine management procedures were followed while fresh feed was supplied *ad libitum* and the birds had access to cool clean water.

### Preparation of leaf meals

Moringa leaves were harvested and air-dried for 5 days at which point they were dry and crisp. The leaves were then milled and kept an air-tight container until the meal was ready to be used. Neem leaf meal was prepared in a similar way.

### Experimental diets

The diets were formulated to meet the NRC (1994) nutrient requirements for broiler starters and finishers. The gross composition of the diets are shown in Table 1.

The dietary treatments are as listed as follows:

**Table 1: Gross composition of experimental starter and finisher diets (%)**

| Ingredients | Starter diet | Finisher diet |
|---|---|---|
| Maize | 53.00 | 59.00 |
| Groundnut cake | 15.30 | 13.30 |
| Soyabean meal | 18.00 | 15.00 |
| Wheat bran | 7.00 | 6.00 |
| Fish meal (72%) | 1.00 | 1.00 |
| Limestone | 1.00 | 1.00 |
| Bone meal | 3.00 | 3.00 |
| Lysine | 0.85 | 0.85 |
| Methionine | 0.35 | 0.35 |
| Salt | 0.25 | 0.25 |
| Broiler premix | 0.25 | 0.25 |
| **Total** | 100.00 | 100.00 |
| **Calculated analysis** | | |
| Crude protein (%) | 21.14 | 19.34 |
| Metabolizable energy (kcal/kg) | 2.76 | 2.81 |
| Crude fibre (%) | 3.61 | 3.34 |

### Feeding trial

The study lasted 56 days which comprised the starter phase (day 1 – day 28) and the finisher phase (day 29 – day 56) and the diet was a corn/

**Table 2: Performance characteristics of broilers fed Moringa leaf meal and Neem leaf meal as alternatives to antibiotics**

**Dietary Treatments**

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 | T7 | SEM |
|---|---|---|---|---|---|---|---|---|
| Initial wt.(g) | 38.37 | 38.5 | 38.07 | 38.53 | 38.23 | 38.06 | 38.03 | ±0.13 |
| Final wt. (g) | 2161.5$^{ab}$ | 2295.4$^{a}$ | 2182.8$^{ab}$ | 2192.4$^{ab}$ | 2191$^{ab}$ | 2047.7$^{b}$ | 2107$^{ab}$ | 30.08 |
| Wt. gain (g) | 2123.1$^{ab}$ | 2256.9$^{a}$ | 2144.7$^{ab}$ | 2143.9$^{ab}$ | 2152.8$^{ab}$ | 2009.6$^{b}$ | 2069$^{ab}$ | 29.93 |
| Feed intake (g) | 5036.3 | 5250 | 5018.7 | 5246.3 | 5088.7 | 4918.7 | 5058.5 | 52.24 |
| FCR | 2.38 | 2.33 | 2.34 | 2.43 | 2.36 | 2.45 | 2.45 | 0.02 |

*$^{ab}$ Means along the same row with different superscripts differ significantly ($p < 0.05$)*
*FCR – Feed conversion ratio*

**Table 3: Serum metabolites of broilers fed Neem and Moringa leaf meals (used singly and combined) as alternatives to antibiotics**

**Dietary Treatments**

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 | T7 | ±SEM |
|---|---|---|---|---|---|---|---|---|
| Glu (mg/dl) | 245.67$^{ab}$ | 182.57$^{b}$ | 191.09$^{b}$ | 288.05$^{a}$ | 213.11$^{ab}$ | 256.24$^{ab}$ | 229.27$^{ab}$ | 12.09 |
| AST (IU/L) | 114.05 | 110.91 | 110.99 | 105.31 | 109.00 | 106.02 | 112.39 | 1.55 |
| ALT (IUL) | 12.13 | 10.43 | 12.15 | 11.91 | 11.25 | 11.00 | 11.23 | 0.38 |
| TP (g/dl) | 3.53 | 3.70 | 3.95 | 3.78 | 3.99 | 3.74 | 3.5 | 0.08 |

*$^{ab}$ Means along the same row with different superscripts differ significantly ($p < 0.05$)*
*Glu – Glucose, AST – Aspartate Amino Transferase, ALT – Alanine Transaminase, TP – Total protein*

**Table 4: Haemtological indices of broilers fed Neem and Moringa leaf meals (used singly and combined) as alternatives to antibiotics**

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 | T7 | ±SEM |
|---|---|---|---|---|---|---|---|---|
| PCV (%) | 28.33 | 29.00 | 29.17 | 29.50 | 31.17 | 28.17 | 27.83 | 0.44 |
| Hb (g/L) | 8.9$^{ab}$ | 9.58$^{ab}$ | 9.35$^{ab}$ | 9.47$^{ab}$ | 10.32$^{a}$ | 8.9$^{ab}$ | 8.73$^{b}$ | 0.18 |
| RBC ($10^6$/L) | 3.26 | 3.25 | 3.24 | 3.38 | 3.54 | 3.36 | 3.16 | 0.05 |
| WBC ($10^3$/L) | 17.61 | 19.43 | 19.17 | 18.33 | 18.01 | 18.97 | 20.08 | 0.30 |
| Lymph ($10^3$/L) | 59.00$^{b}$ | 63.5$^{ab}$ | 71.33$^{a}$ | 61.83$^{ab}$ | 62.83$^{ab}$ | 61.83$^{ab}$ | 65.83$^{ab}$ | 1.24 |
| Hetero ($10^3$/L) | 34.67$^{a}$ | 29.33$^{ab}$ | 23.83$^{b}$ | 32.33$^{ab}$ | 30.5$^{ab}$ | 32.17$^{ab}$ | 29.00$^{ab}$ | 1.18 |

*$^{ab}$ Means along the same row with different superscripts differ significantly ($p < 0.05$)*
*PCV – Packed cell volume, Hb – Haemoglobin, RBC – Red blood cell, WBC – White blood cell, Lymph – Lymphocytes, Hetero - Heterophils*

**Table 5: Organ (liver) histology of broilers fed Neem and Moringa leaf meals (used singly and combined) as alternatives to antibiotics**

| Dietary Treatments | Description |
|---|---|
| T1 | There is moderate to severe periportal cellular infiltration by mononuclear cells. |
| T2 | There is a mild diffuse vacuolar degeneration of hepatocytes |
| T3 | There is a mild to moderate portal congestion |
| T4 | There is a moderate diffuse vacuolar degeneration and periportal cellular infiltration by mononuclear cells |
| T5 | There is severe diffuse vacuolar degeneration of hepatocytes and also a severe periportal cellular infiltration |
| T6 | There is a severe diffuse vacuolar degeneration of hepatocytes, also a severe periportal cellular infiltration by mononuclear cells |
| T7 | There is a severe periportal cellular infiltration by mononuclear cells |

**Table 6: Cost analysis of producing broilers using Moringa and Neem leaf meals (used singly and combined) as alternatives to antibiotics**

| Parameter | Dietary Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
| Cost of feed (₦/kg) | 98.59 | 97.22 | 96.92 | 97.07 | 97.14 | 96.99 | 95.72 |
| Feed intake/bird (kg) | 5.04 | 5.25 | 5.02 | 5.25 | 5.09 | 4.92 | 5.06 |
| Cost of feeding a bird (₦) | 496.89 | 510.41 | 486.54 | 509.62 | 494.44 | 477.19 | 484.34 |
| Weight gain (kg) | 2.12 | 2.26 | 2.14 | 2.14 | 2.15 | 2.01 | 2.07 |
| Cost/weight gain (₦/kg) | 234.38 | 225.85 | 227.36 | 238.14 | 229.97 | 237.41 | 233.98 |

soya-based diet. The birds were weighed at the beginning of the experiment and subsequently on a weekly basis. Data on feed intake were taken daily and pooled on a weekly basis. Weight gain, feed intake and feed conversion ratio (feed intake divided by weight gain) were calculated from the data collected.

**Blood collection and analysis**

At the end of the feeding trial, 5 mls of blood was collected (via the jugular vein) from each of 2 randomly selected birds from each replicate. Blood samples were collected before the birds were offered feed for the day. Blood samples for haematological analysis were collected into sample bottles containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant while the blood samples for serum biochemical analysis were collected into plain sample bottles (without EDTA) and centrifuged before they were subjected to analysis. Packed cell volume (PCV) was determined using the microhaematocrit method while haemoglobin concentrations were determined using the cyanomethaemoglobin method (Mitruka and Rawnsley, 1981). Red blood cell (RBC) and white blood cell (WBC) counts were determined using the neubrauer haematocytometer. Total protein was determined using the biuret method while glucose levels were determined using the method of Bonder and Mead (1974). Aspartate amino transferase (AST) and Alanine aminotransferase (ALT) activities were determined using spectrophotometric methods described by Rej and Hoder (1983).

**Organ histopathology studies and gut pH measurement**

At the end of the feeding trial, 2 randomly selected birds from each replicate were slaughtered and samples of the liver were taken from the birds. The specimens were fixed in 10% formalin after which they were dehydrated in 100% ethanol. The specimens were then cleared

with xylene and embedded in paraffin. A microtome was used to make 4µm cuts that were mounted on glass slides and stained using the H and E (Haematoxyline and Eosin) method. Pictures of the slides were taken at x100 magnification using a microscope (Acuscope®) with a TS view software for imaging which was used to check for any lesions, alterations and any other abnormalities or otherwise of the tissues.

**Statistical design and data analysis**

The experimental design was Completely Randomized Design. All data generated were subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM of SAS 1999) and means, where significant, were separated using the Duncan Multiple Range Test (Duncan 1955). The following mathematical model was used for the parameters taken in the study: $Y_{ij} = \mu + T_{ij} + E_{ij}$

where

$Y_{ij}$ = Observation on the jth bird on the ith treatment
$\mu$ = General mean effect
$T_i$ = Effect due to the ith dietary treatment
$E_{ij}$ = Random error

## RESULTS AND DISCUSSION

**Performance characteristics of birds fed the experimental diets**

The performance characteristics of the broilers on the different dietary treatments are shown in Table 2. Significant differences were observed in the final body weight and weight gain of the birds with birds on treatment 2 having significantly ($p < 0.05$) higher values than birds on the other diets. This agrees with the report of Fuglie (1999) who reported high performance of broilers fed *Moringa*-based diets. The rich nutrient content of Moringa (Sarwatt *et al.,* 2004) and its antimicrobial properties (Fahey *et al.,* 2001) may be responsible for these findings. Onu and Aniebo (2011) also reported an improved weight gain of broilers fed Moringa-based diets. The authors attributed this to higher protein content of the diets which was efficiently metabolized for growth. Dhama *et al.* (2015) reported that herbs which are proven as excellent growth promoters in poultry include the horseradish (moringa).

Although various researchers have observed that neem exhibits growth-promoting characteristics (Onyimonyi *et al.,* 2009 and Jawad *et al.,* 2014), others have reported that it exhibits growth-depressing characteristics (Shivappa Nayata *et al.,* 2013). In this present study, however, neem did not seem to have exerted a growth-depressing effect. This probably is due to the fact that the amount of neem leaf meal added to the diet was not high enough to depress growth. These authors who reported growth depression supplemented neem leaf meal to the diet at 800g/100kg of feed. Although birds on the Moringa-based diet had significantly ($p < 0.05$) the highest final body weight, the final body weight of the neem-treated birds still compared well with those of birds on the antibiotic-based diet and the other diets. In this study, all the birds reached more than 2kg live weight by the end of the feeding trial irrespective of the dietary treatments.

**Serum metabolites of birds fed the experimental diets**

Serum biochemical indices of the birds are shown in Table 3. No significant differences were observed in the values of the total protein (TP), aspartate transaminase (AST) and the alanine transaminase (ALT) of the birds. Although the total protein values of the birds did not differ significantly, the values fell within the normal range for poultry. This suggests nutritional adequacy of the dietary proteins for broilers. The values of ALT and AST also fall within the normal range for poultry and therefore the absence of significant differences among the values reported in this present study would reflect normal liver functions of the birds on the different treatments. Glucose levels however differed significantly with glucose levels of birds on Diets 2 and 3 being significantly ($p < 0.05$) lower than those of birds on the other diets. This probably suggests that Moringa and Neem possess glucose-lowering properties. This observation is similar to that of Onu and Aniebo (2013) who reported that broilers fed diets supplemented with neem leaf extract showed significant reduction in glucose. This was suggested to be attributable to the presence of bioactive compounds contained in neem leaves which have the ability to block the energy metabolic pathway

(Chattopadhyay 1996). Halim (2003) also reported that aqueous neem root and leaves reduced blood glucose levels in rats. Chakraborty and Pal (2012) reported that neem exhibits a wide range of pharmacological activities which include anti-hyperglycaemic properties without showing any adverse effects. Aderemi and Alabi (2013) investigated the effects of graded levels of cassava peels fortified with Moringa on the performance and blood profile of broilers and reported that the glucose levels of the broilers were reduced. These authors suggested that there was possibly no inhibition of glycolysis by the diets thus, there was no adverse effects on regulation of insulin and/or blood sugar. Makonnen *et al.* (1997) also reported that an infusion of Moringa leaf juice reduced glucose levels of rabbits.

**Haematological indices of birds fed the experimental diets**

Results of the haematological indices of the birds are shown in Table 5. Packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts were similar among the birds on the different diets whereas variations in haemoglobin, lymphocyte and heterophil counts were significant ($p < 0.05$). The PCV of birds on Diets 1, 6 and 7 fell slightly below the normal range for healthy broilers (29 – 44%) as reported by Mitruka and Rawnsley (1981). The same trend was also observed for the haemoglobin concentrations of the birds. Although the Hb concentration of birds on Diet 5 was significantly ($p < 0.05$) higher than that of birds on the other diets, the Hb concentration of birds on Diets 1, 6 and 7 fell below the normal range for healthy broilers (9.1 – 13.9g/dl) as reported by Mitruka and Rawnsley (1981). Although just slightly lower than normal PCV and Hb concentrations for healthy broilers, the values recorded for the treatments could be indicative of anaemic state of the birds. Togun *et al.* (2007) reported that when haematological values fall within the normal range reported for the animal, it is an indication that the diets do not have any adverse effects on the haematological parameters. Low values of hematological values as reported by Bawala *et al.* (2007) could be due to the harmful effects of some of the dietary components. Red blood cell (RBC) and white blood cell (WBC) counts of the birds fall within the normal range for healthy broilers so it means that the diets did not affect the birds' immune systems adversely. Lymphocytes are specialized white blood cells whose function is to destroy invading organisms such as bacteria and viruses. Lymphocyte counts of the birds varied significantly with that of birds on diet 3 (600g NLM) having significantly ($p < 0.05$) higher counts than birds on the other diets. This suggests that neem leaf meal could have some bioactive principle(s) that boosts the immune system of the birds. This is in line with the report of Chauhan (2010) that neem selectively activates the cell-mediated immune response by activating macrophages and lymphocytes. Birds on diet 1 had significantly ($p < 0.05$) higher heterophil counts than birds on the other diets. Heterophils are bacteria-destroying lymphocytes. Lymphocyte and heterophil counts for all the treatments however fell within the normal range. This suggests that the diets fed were adequate for the birds.

**Organ histology of broilers fed Neem and Moringa leaf meals (used singly and combined) as alternatives to antibiotics**

Results of the histology of the liver samples of the birds on the different dietary treatments are shown in Table 5. In all the 7 treatments, some liver samples were healthy (without lesions) while some showed varying levels of degeneration. The liver samples that showed some level of degeneration can be described as follows: birds on dietary treatments 1, 2, 3 and 4 generally showed mild to moderate cellular degeneration while those on dietary treatments 5, 6 and 7 generally showed severe cellular degeneration. The purpose of investigating the histology of the liver samples was to assess their cellular structure in response to the dietary treatments. The levels of aspartate transaminase (AST) of the birds in this present fall within the normal range for broilers so it suggests that there was no injury or damage to the liver and/or other internal organs. The results of the liver histology in this present study probably suggest that though the cells showed varying degrees of degeneration, the birds were still able to cope with such stress. Some other factors according to Bolu *et al.* (2013) that can affect the structure of organs include nutrition, poor management, environmental stress and physical stress. Other factors

other than the dietary treatments may have been responsible for the trend observed in this present study.

**Cost of production**

Results of the economy of raising broilers using MLM and NLM as alternatives to antibiotics are shown in Table 6. Cost of feed was highest for Diet 1 and lowest for Diet 7. It was lowest for Diet 7 because it had no additive. The additives in the other diets came at some cost. The cost of feeding a bird was highest for birds on Diet 2 while it was lowest for birds on Diet 6. The cost incurred per unit kg of weight gained was lowest for birds on Diet 2 while it was highest for birds on diet 4. The data obtained showed that using MLM as an alternative to oxytetracyline in raising broilers is more economical in terms of obtaining maximum profitability.

## CONCLUSION

Results of most of the parameters measured in this study showed that neem leaf meal and *Moringa oleifera* leaf meal (used singly) compared well with the antibiotic (oxytetracycline). Either of the two alternatives (used singly) could replace antibiotics in broiler production. Further studies would be carried out to investigate the effects of combining the alternatives in this study with other natural growth promoters e.g. acidifiers, synbiotics, enzymes etc. on broiler performance. Microbial load of blood and digesta samples would also be evaluated so as to ascertain the degree of efficacy of these alternatives to antibiotics in combating disease and promoting health.

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