

Growth, antibacterial properties and haematological parameters of broiler chickens fed moringa and neem leaf meals as additives

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Target Audience: Farmers, Researchers

Abstract

The effectiveness and accessibility of broiler feed additives and antibiotics could be challenging to farmers. There is therefore the need to research into the effectiveness of the use of substitute feed additives. Moringa (*Moringa oleifera*) and neem (*Azadirachta indica*) leaves are edible plants to both humans and animals that can be included as feed additives. This work was conducted to determine the effect of different levels of Moringa oleifera leaf meal (MLM) and neem leaf meal (NLM) as substitute feed additives/antibiotics and their effects on the performance of broilers. Two hundred and ten (210) day-old Abor Acres broiler chicks were randomly allotted to seven dietary treatments of three replicates each. The parameters investigated were feed intake, final weight, weight gain, feed conversion ratio, meat quality, sensory evaluation, mortality and bacterial counts. The final weight for broilers fed with MLM and NLM diets were higher than those fed with conventional feed. The bacteria load and mortality recorded for the conventional feed groups were significantly higher ($p < 0.05$) than groups fed moringa and neem. There was no significant difference ($p > 0.05$) in the haematology and serum parameters recorded for all the groups. The organoleptic properties of cooked broiler meat samples for all treatment groups were comparable with those fed with conventional feed. Inclusion of MLM and NLM produced similar results compared to the conventional feed ingredients and may be considered as possible sources of alternative feed additives and promising natural antimicrobial agents for controlling pathogenic bacteria in poultry production.

Key words: Broilers, Neem leaf meal, Moringa leaf meal, Production

Description of Problem

The production of meat and eggs from chickens is one of the most intensive types of livestock production (1). It is an industry that has achieved worldwide success in its primary objective of providing a source of affordable animal protein (2). This success is due to several factors which include the provision of quality feed, prevention of disease, partly achieved by the use of antibiotics (3). Antibiotics are used in human and veterinary medicines to treat and prevent diseases and for other purposes including growth promotion in food animals (4, 5). The use of these

substances may offer possibilities to improve animal performance and increase economic output of livestock producing units (5). Increased productivity of poultry units play important roles especially in developing countries and regions/continents where the availability of land is limited (3, 6). However, the use of synthetic substances especially antibiotic growth promoters was soon found to have objectionable side-effects including antibiotic residues in meat, milk and eggs as well as development and transmission of resistant bacteria (5). The increase usage of antibiotics has led to an increased risk of

bacteria developing antibiotic resistance (4, 7). Increased concern about the potential for antibiotic resistant strains of bacteria has compelled researchers to explore the utility 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 (8).

Compared with antibiotics, phytobiotics have proven to be natural, less toxic, residue-free and ideal feed additives in food animal production (5, 9). 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 (5, 10). According to reports, neem (*Azadirachta indica*) and *Moringa* (*Moringa oleifera*) contain chemicals which have antimicrobial, antihelminth, antioxidant, antifungal, insecticidal and spermicidal activities (11). The active constituent contained in neem include azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, quercetin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol (12). *Moringa oleifera* plant have been reported to contain a rich and rare combination of zeatin, quercetin, kaempferol, niazirin, niazirin, niazininins A and B, benzoic acid, gallic acid, beta benzaldehyde, minerals, vitamins A, C and B (13).

This study evaluated the effect of MLM and NLM as alternative feed additives/antibiotics on the performance of broiler chickens.

Materials and Methods

In a completely randomized design, two hundred and ten (210) day-old Abor Acres broiler chicks were allotted to 7 dietary

treatments of 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 free access to cool clean water. The study was conducted for 56 days. The dietary treatments were; Treatment 1: Positive control diet with 40 g oxytetracycline (Oxytetracycline Hydrochloride^(R) Kepro Deventer, Netherlands) as antibiotic/100 kg feed; Treatment 2: Diet with 600 g of MLM/100 kg of feed; Treatment 3: Diet with 600 g of NLM/100 kg of feed; Treatment 4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; Treatment 5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; Treatment 6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; Treatment 7: Negative control was diet without any additive. *Moringa* and neem leaves sourced from the University of Ibadan botanical garden were morphologically identified according to Gandji et al and Alzohairy (14, 15) while varying quantities of moringa and neem leaves were included in the feed to be administered to the treatment groups to study the effect of their inclusion on desired variables.

Housing and Management

The diet was a corn-soya-based diet and was formulated to meet the National Research Council (NRC) nutrient requirements for broiler starters and finishers (Table 1) (16). The pen houses were thoroughly cleaned with vinkokill[®] (Chlorophenol 97%) five days prior to the arrival of the chicks. The day-old chicks were offered a vitamin/electrolyte solution (vitalyte[®]) upon arrival. The birds were vaccinated against Infectious Bursal Disease (IBD) on days 7, 14 and 21. Body weight was taken on the first day and then subsequently on a weekly basis till the end of the experiment. Feed intake was measured on a weekly basis as

well. Body weight gain and feed intake were measured while feed conversion ratio (FCR) was obtained by dividing the total amount of feed consumed by the amount of weight gained (17). The average values of these parameters for the treatment groups were determined and recorded.

Blood Collection

At the end of the experiment, 5 ml of blood was collected from 3 randomly selected birds from each replicate via the jugular vein into specimen bottles. Blood samples for haematological analysis were collected into sterilized bottles containing ethylene diamine tetra acetic acid (EDTA) as anti-coagulant, while those used for serum biochemical analysis were collected into tubes without EDTA and were centrifuged before analysis. Packed cell volume (PCV) was determined using the microhaematocrit method (18), while the haemoglobin content was determined using the cyanomethaemoglobin method. Red blood cell count was determined using the Neubauer

haemocytometer, while aspartate transaminase (AST) and alanine transaminase (ALT) were determined with a spectrophotometer (19).

Microbial Analysis

Fresh faecal samples were collected from the cloaca using sterile swab at day-old, 4th week and 8th week and screened for bacterial load (20). Total aerobic and anaerobic bacterial counts were enumerated on Plate Count Agar at 30 °C for 24 and 48 hours. Bacteria counts was expressed as colony-forming units per gram of sample (cfu/g) (21).

Sensory Parameters

Chicken breast meat samples were collected for sensory analysis at the end of the experiment. They were steamed separately with 60 ml of water to a temperature of 100 °C for 10 minutes to produce cooked meat samples which is a modified protocol used by Choi et al. (22). The meat samples were served to 42 trained sensory panellists using sensory evaluation questionnaire.

Sensory Evaluation Questionnaire

Name:

Product:

Panelist no:

Date:

You are provided with coded samples.

Please evaluate them for the quality factors listed; using the scale provided below.

- | | | |
|-----------------------|-----------------------------|--------------------|
| 1. Dislike extremely | 4. Dislike slightly | 7. Like moderately |
| 2. Dislike very much | 5. Neither like nor dislike | 8. Like very much |
| 3. Dislike moderately | 6. Like slightly | 9. Like extremely |

S/No	Sample	Colour	Appearance	Flavour	Texture	Taste	Overall acceptability
T1							
T2							
T3							
T4							
T5							
T6							
T7							

General Comment:

Ethical approval, ACUREC NO: UI-ACUREC/18/0105 was obtained from Animal Care and Use Research Ethics Committee of the University of Ibadan.

Statistical Analysis

Data generated were presented using descriptive statistics and subjected to one-way Analysis of Variance (ANOVA) using the General linear model of SAS (1999), associations between observed variables were considered significant where p values were < 0.05 (Duncan, 1955).

Results and Discussion

There was increase in feed intake and body weight gain among broilers in the 7 treatment groups. At the end of the starter phase, the lowest and highest feed intake were recorded in T1 (1446.13 g) and T2 (1608.43 g) respectively. Broilers in T2 treatment group gained the highest weight (941.03 g) while those in T1 gained the lowest weight (833.23 g). The best feed conversion ratio (FCR) was recorded in T7 (1.83) while the poorest was in T3 (1.75) (Table 2). At the end of the finisher phase, the highest quantity of feed (5843.10 g) was consumed by broilers in treatment group T2 while the lowest quantity was consumed by birds in T1 (1446.13 g). The highest (2295.00 g) and lowest (2047.00 g) body weight were gained by broilers in groups T2 and T6 respectively. Broilers in group T7 had the highest (2.78) feed conversion ratio (FCR) while the lowest was recorded in T3 (2.55) (Table 3).

The mortality record showed that 20 (9.5% N=210) birds died after a period of 8 weeks in all the treatments groups. The least mortality, 1 (0.48%) was recorded in T3 while T7 had the highest, 6 (2.86%) mortality (Table 4). After 24 hours incubation, the total bacteria counts of the faecal samples of birds from groups T1-T7 at day old were highest (0.12 cfu/ml) in groups T7 and T4 while the lowest (0.10 cfu/ml) bacteria counts were recorded in groups T1, T5 and T6. At the end of the starter stage (4 weeks) the highest (5.20 cfu/ml) and lowest (2.40 cfu/ml) bacteria counts were recorded in treatment groups T7 and T3

respectively. The highest (0.12 cfu/ml) total bacteria counts at the end of the finisher phase (8 weeks) were recorded from T4 while T1, T2, T3, T5, T6 recorded the lowest (0.10 cfu/ml) counts. At the end of 48 hours incubation, faecal samples from day old chicks had bacteria counts that were highest (0.13 cfu/ml) in T2, T4 and T7 while the lowest (0.11 cfu/ml) were recorded in T5. At 4 weeks (the end of starter phase), T7 and T5 yielded the highest (5.70 cfu/ml) and lowest (2.80 cfu/ml) bacteria counts respectively. Treatment groups T7 and T5 recorded the highest (5.10 cfu/ml) and lowest (2.50 cfu/ml) bacteria count from the faecal samples after 8 weeks (the end of finisher phase) (Table 4).

The mean total faecal bacteria counts of the starter phase were 2.28×10^3 cfu/ml and 3.72×10^3 cfu/ml after 24 and 48 hours incubation respectively while the mean total faecal bacteria count for the finisher phase were 2.62×10^3 cfu/ml and 3.14×10^3 cfu/ml respectively after 48 hours incubation (Table 4). The bacteria load and mortality recorded for the conventional feed groups were significantly higher ($p < 0.05$) than groups fed moringa and neem.

The basal haematological parameters of broilers in the treatment groups at day old were presented in Table 5. There was statistically significant difference between values of glucose recorded across the treatment groups. At 4 weeks, there was significant difference in the values of monocytes recorded from T1 to T7 (Table 6). The haematological picture at 8 weeks revealed that recorded parameters were within normal range except the variation from normal in the values of haemoglobin in T6 (8.92 g/dl) and T7 (8.73 g/dl), glucose in both T2 (188.57 mg/dl) and T3 (191.09 mg/dl) and aspartate amino transferase (AST) were lower than normal range in all the treatment groups (Table 7).

The highest (6.5) meat colour intensity was recorded in treatment group T1 while T3

was the lowest (5.5). The best meat appearance was recorded in group T1 (6.7), T7 was the lowest (5.6), the best in meat texture was recorded in T1 (6.8) while the lowest was from T6 (6.0). Treatment group T7 was highest in flavour (7.2), taste (8.0) and overall acceptability with the lowest ranking recorded for the 3 parameters in T3 (Figure 1).

Table 1: Gross composition (g/100g) of experimental broiler starter and finisher diets

Ingredients	Starter	Finisher
Maize	53.00	59.00
Groundnut cake	15.00	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

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Table 2: Weight gain and feed conversion ratio (FCR) of broilers fed oxytetracycline, moringa and neem leaf meals at the end of the starter phase (1-4 weeks)

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
Initial weight (g)	38.37	38.50	38.03	38.53	38.23	38.08	38.03	0.12
Final weight (g)	838.23	941.03	878.17	884.30	904.20	878.70	874.10	11.31
Weight gain (g)	799.80	902.53	840.10	845.77	865.97	840.63	836.07	11.34
Feed intake (g)	1446.13	1608.43	1464.90	1527.83	1546.70	1497.17	1526.77	15.31
FCR	1.82	1.78	1.75	1.82	1.79	1.79	1.83	0.02

T1: Positive control diet with oxytetracycline 40 g/100 kg feed as antibiotic; T2: Diet with 600 g of MLM/100 kg of feed; T3: Diet with 600 g of NLM/100 kg of feed; T4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; T5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; T6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; T7: Negative control was diet without any additive; FCR: Feed conversion ratio

Table 3: Performance characteristics of broilers fed moringa and neem leaf meals as alternative to oxytetracycline at the end of the finisher phase

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
Initial weight (g)	38.37	38.50	38.03	38.53	38.23	38.08	38.03	0.13
Final weight (g)	2160.70	2295.30	2258.50	2192.40	2191.00	2047.70	2107.00	31.13
Weight gained (g)	2122.33	2256.80	2220.47	2153.87	2152.77	2009.63	2068.97	29.12
Feed intake (g)	5776.80	5843.10	5652.20	5662.40	5655.70	5431.20	5748.50	43.15
FCR	2.72	2.59	2.55	2.63	2.63	2.70	2.78	3.15

^{abc}Mean within the same row with different superscript letters, were significantly different (P<0.05)* T1: Positive control diet with oxytetracycline 40 g/100 kg feed as antibiotic; T2: Diet with 600 g of MLM/100 kg of feed; T3: Diet with 600 g of NLM/100 kg of feed; T4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; T5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; T6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; T7: Negative control was diet without any additive; FCR: Feed conversion ratio of faecal samples of birds from (T1-T7) after 24 and 48 hours of culture of the faecal samples at the end of both the starter (1-4 weeks) and finisher phases (4-8 weeks).

Table 4: Number of mortality (%), total bacteria counts and the mean total bacteria counts (cfu/ml)

	T1	T2	T3	T4	T5	T6	T7	Total
Number of mortality (%) N=210	4 (1.90) ^a	3 (1.43)	1 (0.48)	2 (0.95)	2 (0.95)	2 (0.95)	6 (2.86) ^a	20 (9.52)
Sample (x10 ³ cfu/ml) 24 hour culture	SEM							
Faeces (day-old Chicks)	0.10 ^a	0.11	0.10	0.12	0.10	0.10	0.12 ^a	0.01 ^a
Faeces (starter/4th week)	3.00 ^b	2.80	2.40	2.90	2.70	2.50	5.20 ^b	0.39
Faeces (finisher/8th week)	0.10 ^a	0.10	0.10	0.12	0.10	0.10	0.12 ^a	0.06
Sample (x10 ³ cfu/ml) 48 hour culture	SEM							
Faeces (day-old Chicks)	0.12 ^a	0.13	0.12	0.13	0.11	0.12	0.13 ^a	0.01
Faeces (4 th week)	3.70 ^a	3.20	3.20	3.10	2.80	2.90	5.70 ^b	2.07
Faeces (8 th week)	3.50 ^a	3.10	2.70	2.90	2.50	2.70	5.10 ^b	2.11
Mean total bacteria count (x10 ³ cfu/ml)	SEM							
Time	Starter (1-4 weeks)		Finisher (4-8 weeks)				SEM	
After 24 hours of culture	2.88		2.62				0.13	
After 48 hours of culture	3.27		3.14				0.27	

^a p<0.05

T1: Positive control diet with oxytetracycline 40 g/100 kg feed as antibiotic; T2: Diet with 600 g of MLM/100 kg of feed; T3: Diet with 600 g of NLM/100 kg of feed; T4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; T5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; T6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; T7: Negative control was diet without any additive

Table 5: Haematological parameters of broilers fed moringa and neem leaf meals at day-old

Dependent Variables	Normal range	T1	T2	T3	T4	T5	T6	T7	SEM
PCV (%)	29-44	29.756	27.567	27.667	28.056	28.667	28.667	29.556	1.05
Hb (g/dl)	9-13	9.2333	8.111	11.223	4.333	9.667	9.001	10.456	0.50
RBC ($\times 10^6\text{mm}^{-3}$)	1.6-4.1	3.001	3.011	3.000	3.445	3.333	3.123	3.300	0.65
WBC ($\times 10^6\text{mm}^{-3}$)	9.2-31.0	17.556	18.500	18.327	19.711	19.700	16.811	18.111	1.05
Lymph ($10^3/\text{L}$)	47.2-85.0	68.723	62.223	63.711	63.333	62.973	58.127	59.971	1.25
Heterophils		34.533	33.100	31.000	31.001	31.633	31.973	36.859	0.75
Monocytes ($\times 10^3/\text{L}$)	0.06-5.0	3.001	3.1773	3.9133	3.7771	3.9111	4.1333	3.7111	0.66
Eosinophils		3.1010	3.331	4.511	3.5331	3.3551	4.7333	4.7333	0.91
Basophils		0.37111	0.3733	0.0000	0.6773	0.177	0.0000	0.3771	0.07
Platelets		875333	135133	125311	145151	145100	131133	130555	7156.20
SERUM									
Glucose (mg/dl)	245	256.31	215.11	239.51	251.13	217.33	233.71	277.11	6.75
AST(u/l)	131-486	117.13	119.51	123.71	117.23	129.53	117.33	113.10	1.45
ALT (u/l)	2-20	15.00	15.311	17.13	17.93	16.76	16.33	16.96	2.85
TP (g/dl)	5-7	2.93	2.73	2.75	2.73	2.98	2.75	2.75	1.25

Key: PCV – Packed Cell Volume, Hb – Haemoglobin, RBC – Red Blood Cell, WBC – White Blood Cell, Lymph – Lymphocyte, AST- Aspartate Aminotransaminase, ALT-Alanine Transaminase, TP-Total Protein. T1: Positive control diet with oxytetracycline 40 g/100 kg feed as antibiotic; T2: Diet with 600 g of MLM/100 kg of feed; T3: Diet with 600 g of NLM/100 kg of feed; T4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; T5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; T6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; T7: Negative control was diet without any additive.

Normal Range Source: Mitruka et al. (16).

Table 6: Haematological parameters of broilers fed moringa and neem leaf meals at the end of the 4th week.

Dependent Variables	Normal range	T1	T2	T3	T4	T5	T6	T7	SEM
PCV (%)	29-44	29.50	33.17	30.83	31.17	27.67	30.17	30.00	0.50
Hb (g/dl)	9-13	9.75	11.07	10.08	10.25	9.30	10.20	10.03	0.17
RBC ($\times 10^6\text{mm}^{-3}$)	1.6-4.1	3.49	3.55	3.39	3.27	3.50	3.14	3.20	0.05
WBC ($\times 10^6\text{mm}^{-3}$)	9.2-31.0	18.50	18.54	16.68	16.25	16.59	17.86	18.80	0.44
Lymph ($10^3/\text{L}$)	47.2-85.0	59.00	62.00	65.67	60.83	62.50	63.33	16.80	1.01
Heterophils		34.17	32.00	30.33	33.67	31.50	30.0	56.83	1.00
Monocytes ($\times 10^3/\text{L}$)	0.06-5.0	3.00	3.17	1.83	4.00	3.00	1.67	4.17	0.26
Eosinophils		3.83	2.50	2.17	3.00	2.67	4.33	2.33	0.26
Basophils		0.00	0.33	0.00	0.33	0.33	0.50	0.33	0.66
Platelets		152.67	175.00	184.00	137.00	160.50	182.50	182.50	8885.24
SERUM									
Glucose (mg/dl)	245	156.80	179.38	175.51	152.50	165.83	158.96	147.96	6.55
AST(u/l)	131-486	113.43	111.25	135.44	116.05	123.27	121.18	116.33	1.36
ALT (u/l)	2-20	6.75	5.99	6.85	8.053	7.89	5.31	6.21	0.40
TP (g/dl)	5-7	2.51	2.25	2.12	2.44	2.55	2.50	2.82	0.07

Keys: PCV – Packed Cell Volume, Hb – Haemoglobin, RBC – Red Blood Cell, WBC – White Blood Cell, Lymph – Lymphocyte, AST- Aspartate Aminotransaminase, ALT-Alanine Transaminase, TP-Total Protein. T1: Positive control diet with oxytetracycline 40 g/100 kg feed as antibiotic; T2: Diet with 600 g of MLM/100 kg of feed; T3: Diet with 600 g of NLM/100 kg of feed; T4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; T5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; T6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; T7: Negative control was diet without any additive.

Normal Range Source: Mitruka et al. (23).

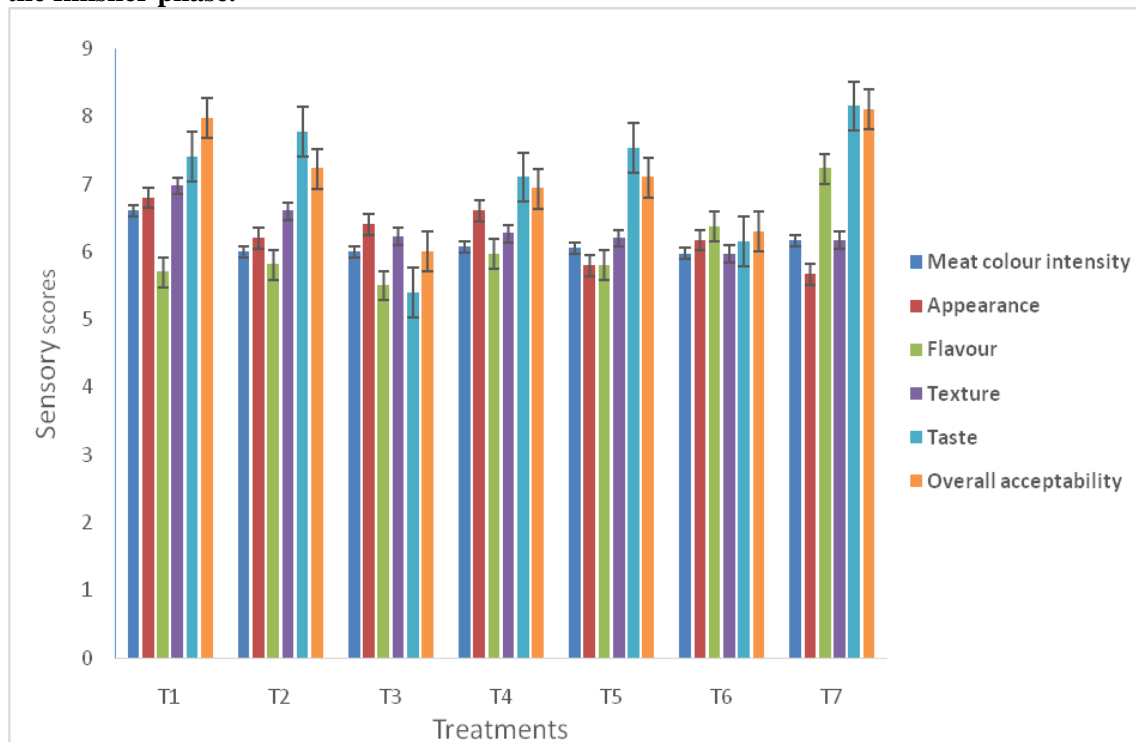
Table 7: Haematological parameters of broilers fed moringa and neem leaf meals at the end of the 8th week.

Dependent Variables	Normal range	T1	T2	T3	T4	T5	T6	T7	SEM
PCV (%)	29-44	28.33	29.00	29.17	29.50	31.17	28.17	27.83	0.44
Hb (g/dl)	9-13	8.87	9.55	9.35	9.47	10.32	8.92	8.73	0.22
RBC ($\times 10^6 \text{mm}^{-3}$)	1.6-4.1	3.26	3.24	3.24	3.38	3.54	3.36	3.16	0.06
WBC ($\times 10^6 \text{mm}^{-3}$)	9.2-31.0	21.61	19.29	19.17	18.33	18.01	18.96	23.10	0.60
Lymph ($\times 10^3/\text{L}$)	47.2-85.0	59.00	63.50	71.33	61.83	62.83	61.83	65.83	1.16
Heterophils		34.67	29.33	23.83	32.33	30.50	33.00	29.00	1.24
Monocytes ($\times 10^3/\text{L}$)	0.06-5.0	3.17	2.50	2.67	2.83	2.50	2.67	2.50	0.20
Eosinophils		3.33	3.33	2.00	2.67	3.57	3.50	2.17	0.29
Basophils		0.00	0.33	0.17	0.33	0.33	0.00	0.17	0.06
Platelets		139.83	128.16	142.00	142.83	140.67	105.05	142.33	84.66
SERUM									
Glucose (mg/dl)	245	245.67	182.57	191.09	288.04	213.11	256.24	229.27	12.10
AST(u/l)	131-486	114.05	110.82	110.99	105.15	109.00	106.02	112.39	1.56
ALT (u/l)	2-20	12.16	10.43	12.15	11.91	11.25	11.00	11.23	0.38
TP (g/dl)	5-7	3.53	3.70	3.95	3.78	3.66	3.74	3.50	0.41

Key: PCV – Packed Cell Volume, Hb – Haemoglobin, RBC – Red Blood Cell, WBC – White Blood Cell, Lymph – Lymphocyte, AST- Aspartate Aminotransaminase , ALT-Alanine Transaminase, TP-Total Protein. T1: Positive control diet with oxytetracycline 40 g/100 kg feed as antibiotic; T2: Diet with 600 g of MLM/100 kg of feed; T3: Diet with 600 g of NLM/100 kg of feed; T4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; T5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; T6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; T7: Negative control was diet without any additive.

Normal Range Source: Mitruka et al. (23).

Figure 1: Sensory/organoleptic meat qualities of broiler breast meat samples at the end of the finisher phase.



T1: Positive control diet with oxytetracycline 40 g/100 kg feed as antibiotic; T2: Diet with 600 g of MLM/100 kg of feed; T3: Diet with 600 g of NLM/100 kg of feed; T4: Diet with 50% (300 g) MLM and 50% (300 g) NLM in 100 kg of feed; T5: Diet with 75% (450 g) MLM and 25% (150 g) NLM in 100 kg of feed; T6: Diet with 25% (150 g) MLM and 75% (450 g) NLM in 100 kg of feed; T7: Negative control was diet without any additive.

Successful broiler production is measured by the final weight gained by the birds among other things. Eventual broiler weight is a function of the composition and quality of feed taken by the birds (24).

The treatment group where the highest feed intake and weight gain were recorded was fed 600 g MLM/100 kg feed. The high intake of feed and weight gain by the broilers in this group compared to other groups were maintained from starter to the finisher phase of the study. It has been reported that moringa diets are well tolerated and accepted by broilers (11). High intake of feed with moringa

additives in broilers were also reported by Djouhou et al. (25), the report however, stated that addition of higher quantity of moringa in broiler feed may reduce its intake because of the saponins in moringa which decreases the palatability of the feed (11, 25).

The final weight of broilers fed with diets containing MLM and NLM additives were all higher than those fed with diets without any additive and those containing oxytetracycline. The group of broilers fed with 40 g oxytetracycline/100 kg feed diet recorded the lowest feed consumption and weight gain in comparison to other experimental diet groups.

This may be attributed to the presence of calcium, magnesium, sodium, potassium, copper, iron, zinc, manganese, e-tocopherol, β carotene, ascorbic acid and other bioactive components in moringa and neem (25, 26). Increase in weight gain in broilers fed with diets having moringa and neem additives have been reported by other researchers (27, 28).

If the least record of mortality recorded in broilers in the experimental diet groups with moringa and neem is compared with the highest mortality recorded in the treatment group without any additive and that with oxytetracycline, it will suggest moringa and neem helped reduce death among broilers in this group. Reduced mortality in groups to which MLM and NLM were fed were also reported by Alabi *et al.*, Paul *et al.* and Djouhou *et al* (25, 29, 30).

That diet groups with MLM and NLM had the lowest bacterial load in the study suggests moringa and neem may have properties that helped reduce faecal bacteria count.

The low bacteria count recorded in MLM and NLM fed groups this study and the lower mortality recorded in broilers in these diet groups may have been enhanced by moringa and neem. Moringa and neem have been reported to contain bioactive compounds with diverse pharmacological properties. These compounds include methanol, chloroform, ethyl acetate and ethanol which are responsible for the antimicrobial properties of moringa and neem (15, 31). Other researchers like Onu and Aniebo, Djouhou *et al* and Ansari *et al.* (25, 28, 32) have also reported low mortality and bacterial load in broilers experimentally fed moringa and neem leaf meals.

The day old broilers' low glucose level in this study is in agreement with the submission of the research that reported hypoglycaemia in chicks that were just hatched (33). Hypoglycaemia could lead to nervous related symptoms and mortality. Hypoglycaemia in day old chicks has been attributed to low body

fat after hatching (34). Low level monocytes in broilers at 4 weeks may lead to reduction in the level of immunity and consequently decrease in their ability to fight diseases. Tijani *et al.* (35) however did not report any significant difference in the value of monocytes among broilers fed moringa leaf meals in their study. Although numerical increase in PCV, Hb, and RBC were recorded in moringa treatment groups, all values were within normal range. The general haematological values that were within normal range after 8 weeks of feeding broilers in all the treatment groups MLM and NLM suggests moringa and neem may not contain components that constitute major toxicological threat to the health of the birds. This is in accordance with the report of Fuglier (36) and Djouhou *et al.* (25). Low Haemoglobin and glucose could lead to impaired oxygen blood circulation and hypoglycaemia respectively while low AST could imply absence of abnormal liver function.

The meat qualities of broilers from the seven treatment groups implied that the meat colour, appearance, flavour, texture, taste and overall acceptability of the broiler meat from broilers produced from rations containing MLM and NLM can favourably compare with those fed with conventional feeds. Although, numerically meat quality parameters for the group fed with NLM were found to be slightly lower. This is in line with Bonsu *et al.* (32) that reported unsalted cooked meat from chickens fed on 2.5% neem-based diets to be mildly bitter. The high rate of inclusion of neem at 2.5% might have resulted in the deposition of azadiractin in muscle fibres resulting in mild bitter taste.

Conclusion and Applications

1. Feeding broilers with MLM and NLM (singly or jointly) produced higher final weight compared to those fed with conventional feed and feed that

- contained oxytetracycline.
2. Low mortality may be directly related to bacteria count which could be enhanced by the addition of MLM and NLM to broiler feed in demonstrated amounts.
 3. Moringa leaf meal and NLM did not give rise to toxicological and histopathological side effect as evident in the blood parameters of broilers in this study which were generally within normal range.
 4. Meat colour appearance, flavour, texture and overall acceptability of broiler meat produced using MLM and NLM can compare with those produced with conventional feed.
 5. The use of MLM and NLM in broiler feed did not produce notable side effect in broilers, rather helped improve weight gain, reduce mortality, bacteria load, maintain haematological parameters and produce broiler meat that could compare with those produced using conventional feed.

References

1. Food and Agricultural Organization (FAO) (2020). Animal production. Available at: <http://www.fao.org/animal-production/en/>. Accessed on 14th, March, 2020.
2. Augère-Granier, M. (2019). The EU poultry meat and egg sector; Main features, challenges and prospects, in-depth analysis. Available at: [https://www.europarl.europa.eu/RegData/etudes/IDAN/2019/644195/EPRS_IDA\(2019\)644195_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/IDAN/2019/644195/EPRS_IDA(2019)644195_EN.pdf). Accessed on 14th March, 2020.
3. Masud, A.A., Rousham, E.K., Islam, M.A., Alam, M-U., Rahman, M., Mamun, A.A., Sarker, S., Asaduzzaman, M. and Unicomb, L. (2020). Drivers of Antibiotic Use in Poultry Production in Bangladesh: Dependencies and Dynamics of a Patron-Client Relationship. *Frontiers in Veterinary Science*, 7:78. doi: 10.3389/fvets.2020.00078.
4. Phillips, I., Caswell, M., Cox, T., de Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R. and Waddell, J. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy*, 53: 28 – 52. doi:10.1093/jac/dkg483.
5. Lillehoj, H., Liu, Y., Calsamiglia, S., Fernandez-Miyakawa, M. E., Chi F., Cravens R. L., Oh S. and Gay C. G. (2018). Phytochemicals as antibiotic alternatives to promote growth and enhance host health. *Veterinary Research*, 49, 76. <https://doi.org/10.1186/s13567-018-0562-6>.
6. Reimensperger, A. (2011). Ways to replace antibiotic growth promoters. *Biotronic Fact Sheet*. Available at: <https://thepoultrysite.com/articles/ways-to-replace-antibiotic-growth-promoters>. Accessed 18th March, 2020.
7. Xu, J., Sangthong, R., McNeil, E., McNeil, E., Tang, R. and Chongsuvivatwong, V. (2020). Antibiotic use in chicken farms in northwestern China. *Antimicrobial Resistance and Infectious Control* 9, 10. <https://doi.org/10.1186/s13756-019-0672-6>.
8. Agyare, C., Boamah, V. E., Zumbi, C. N. and Osei, F. B. (2018). Antibiotic Use in Poultry Production and Its Effects on Bacterial Resistance. In book: *Antimicrobial resistance - a global threat*. Ed. Yashwant Kumar. Pub: InTech Open Science. DOI: 10.5772/intechopen.79371. Available from: <https://www.intechopen.com/books/antimicrobial-resistance-a-global-threat/antibiotic-use-in-poultry-production-and-its-effects-on-bacterial-resistance>. Accessed 14th March, 2020.
9. Wang, R., Li, D. and Bourne, S. (1998). Can 2000 years of herbal medicine history

- help us solve problems in the years 2000? Biotech in the feed Industry: Proceedings of Alltech's 14th Annual Symposium. Kentucky, USA. 273 -291.
10. Yang, Y., Iji, P.A. and Choct, M. (2019). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Science Journal*, 65: 97-114. doi:10.1017/S0043933909000008.
 11. Su, B. and Chen, X. (2020). Current Status and Potential of *Moringa oleifera* Leaf as an Alternative Protein Source for Animal Feeds. *Frontiers in Veterinary Science*, 7:53. doi: 10.3389/fvets.2020.00053.
 12. Alzohairy, M. A. (2016). Therapeutics Role of *Azadirachta indica* (Neem) and their Active Constituents in Diseases Prevention and Treatment. *Evidence-Based Complementary and Alternative Medicine*, 7382506. <https://doi.org/10.1155/2016/7382506>.
 13. Amabye, T. G. and Tadesse, F. M. (2016). Phytochemical and Antibacterial Activity of *Moringa oleifera*. *Journal of Analytical and Pharmaceutical Research*, 2(1): 00011. DOI: 10.15406/ japlr. 2016. 02. 00011.
 14. Gandji, K., Chadare, F. J., Idohou, Salako, R., Assogbadjo, V. K., A. E. and Glèlè Kaka, R. L. (2018). Status and utilisation of *Moringa oleifera* Lam: A Review. *African Crop Science Journal*, 26: 137 – 156.
 15. Alzohairy, M. A. (2016). Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Evidence-Based Complementary and Alternative Medicine*: 7382506. <https://doi.org/10.1155/2016/7382506>.
 16. National Research Council (NRC) (1994). Nutrient requirements of poultry. 9th Revised Edition. *National Academy Press*, Washington DC, USA. <https://doi.org/10.17226/2114>.
 17. Yi, Z., Li, X., Luo, W., Xu, Z., Ji, C., Zhang, Y., Nie, Q., Zhang, D. and Zhang, X. (2018). Feed conversion ratio, residual feed intake and cholecystokinin type A receptor gene polymorphisms are associated with feed intake and average daily gain in a Chinese local chicken population. *Journal of Animal Science and Biotechnology*, 9: 50. <https://doi.org/10.1186/s40104-018-0261-1>.
 18. Turkson, P. K. and Ganyo, E. Y. (2015). Relationship between haemoglobin concentration and packed cell volume in cattle blood samples. *The Onderstepoort Journal of Veterinary Research*, 82(1), 863. <https://doi.org/10.4102/ojvr.v82i1.863>.
 19. Ihedioha, J. I., Okorie-Kanu, C.O. and Ugwu, C. P. (2011). The blood picture and serum biochemistry profile of the African pied crow (*Corvus albus*). *Comparative Clinical Pathology*, 20: 239–250. <https://doi.org/10.1007/s00580-010-0985-6>.
 20. Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) (2003) Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. WHO/CDS/CSR/EPH/2002.15. <http://www.ritm.gov.ph/arsp/Manual%20for%20Lab%20ID,%20AST-cdc,%20who.pdf>. Accessed on 16 July, 2019.
 21. Chengula, A., Lushino, A., Mbise, J., Mzula, A., Mafie, E., Mwega, E., Makundi, I. and Peter, E. (2014). Determination of bacterial load and antibiotic susceptibility testing of bacteria isolated from students' toilets at Sokoine University of Agriculture, Morogoro.

- Tanzania. Journal of Health, Medicine and Nursing*, 5:1-11.
22. Choi, Y. S., Hwang, K. E., Jeong, T. J., Kim, Y. B., Jeon, K. H., Kim, E. M., Sung, J. M., Kim, H. W., & Kim, C. J. (2016). Comparative Study on the Effects of Boiling, Steaming, Grilling, Microwaving and Superheated Steaming on Quality Characteristics of Marinated Chicken Steak. *Korean Journal for Food Science of Animal Resources*, 36(1), 1–7. <https://doi.org/10.5851/kosfa.2016.36.1.1>.
 23. Mitruka, B. M., Vadehra, D. V., Rawnsley, H. M. (1976). *Animals for medical research: models for the study of human disease*. New York: John Wiley and Sons. Pp 591.
 24. Neves, D. P., Banhazi, T. M. and Nääs, I. A. (2014). Feeding behaviour of broiler chickens: a review on the biomechanical characteristics. *Brazilian Journal of Poultry Science*, 16(2), 01-16. <https://doi.org/10.1590/1516-635x16021-16>.
 25. Djouhou, M. C. F., Nwaga, D., Mafogang, B. and Fokou, E. (2020). Inclusion of Moringa Oleifera Leaf Powder in Broiler Chicken Feed and its Effect on Growth Performance, Biochemical Profile and Metabolism Stimulation: Case Study of the Synthesis of Some Sulphur Compounds. *Biomedical Journal of Science and Technological Research* 28 (4). DOI:10.26717/BJSTR.2020.28.004693.
 26. Jeba Malarab, T. R. J., Antonyswamy, J., Vijayaraghavanc, P., Kim, Y. O., Al-Ghamdie, A. A., Elshikhe, M. S., Hatamlehe, A. A., Al-Dosarye, M. A., Na, S. W., Kim, H. (2020). In-vitro phytochemical and pharmacological bio-efficacy studies on *Azadirachta indica* A. Juss and *Melia azedarach* Linn for anticancer activity. *Saudi Journal of Biological Science*, 27: 682-688.
 27. Anwar, F., Latif, S., Ashraf, M. and Gilani, A. H. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research*, 21: 17-25.
 28. Onu, P. N. and Aniebo, A. O. (2011). Influence of Moringa Oleifera leaf meal on the performance and blood chemistry of starter broilers. *International Journal of Food Agriculture and Veterinary Science*, 1: 38-44. Available at <http://www.cibtech.org/jfav.htm2011>. Accessed 21st July, 2020.
 29. Alabi, O. J., Malik, A. D., Ng'ambi, J. W., Obaje, P. and Ojo, B. K. (2017). Effect of Aqueous Moringa Oleifera (Lam) Leaf Extracts on Growth Performance and Carcass Characteristics of Hubbard Broiler Chicken. *Brazilian Journal of Poultry Science*, 19(2), 273-280. <https://doi.org/10.1590/1806-9061-2016-0373>.
 30. Paul, T. K., Hasan, M. M., Haque, M. A., Talukder, S., Sarker, Y. A., Sikder, M. H., Khan, M, Sakib, M. N. and Kumar, A. (2020). Dietary supplementation of Neem (*Azadirachta indica*) leaf extracts improved growth performance and reduced production cost in broilers, *Veterinary World*, 13(6): 1050-1055.
 31. Wang, L., Chen, X. and Wu. A. (2016). Mini Review on Antimicrobial Activity and Bioactive Compounds of Moringa oleifera. *Medicinal Chemistry (Los Angeles)*, 6: 578-582. doi:10.4172/2161-0444.1000402.
 32. Bonsu, F. R. J., Kanya-Agyemang, J. K., Kivenin, W. K. J. and Zanu, H. K. (2012). Medicinal response of broiler chicken to diet containing neem (*Azadirachta Indica*) leaf meal, haematology and meat sensory analysis. *World Applied Science Journal*, 19(6): 800-805, 2012.
 33. Ansari, J., Khan, S. H., Haq, A. and Yousaf, M. (2012). Effect of the levels of *Azadirachta indica* dried leaf meal as phytogenic feed additive on the growth performance and haemato-biochemical parameters in broiler chicks. *Journal of*

- Applied Animal Research*, 40:4, 336-345, DOI: 10.1080/09712119.2012.692329.
34. McNaughton, J. L., Deaton, J. W. and Reece, F. N. (1978). Effect of Sucrose in the Initial Drinking Water of Broiler Chicks on Mortality and Growth. *Poultry Science*, 57: 985-988.
35. Tijani, L. A., Akanji, A. M., Agbalaya, K. and Onigemo, M. (2015). Haematological and serum biochemical profiles of broiler chickens fed diets containing moringa leaf meals. *Agro-Science*, 14: 7-11.
36. Fuglie, L. J. (1999) The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar, 68. Available at: https://www.academia.edu/19437209/NRC_Poultry_1994_Ninth_revised. Accessed on 17th March, 2020.