

Growth performance and gut health of broilers fed *Moringa oleifera* and *Azadirachta indica* as an alternative to antibiotics

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Abstract

High usage of antibiotics has led to an increase in bacteria developing antibiotic resistance. A resistant strain of human pathogenic bacteria has been found in the non-curative usage of antibiotics for animal production. *Moringa oleifera* and *Azadirachta indica* are phytobiotics which have been proven to be natural, with no residual effect and are thought to be ideal for animal production. An experiment was conducted to determine the growth performance and gut health of broilers fed *Moringa oleifera* and neem *Azadirachta indica* as an alternative to antibiotics. Two hundred and ten day-old broiler chicks were randomly allotted to seven dietary treatment groups of three replicates each. Each replicate had 10 birds. The experimental design was Completely Randomized Design (CRD). The diets were T1- (positive control) diet with antibiotics (oxytetracycline); T2- diet with 600g moringa leaf meal (MLM)/100kg feed; T3- diet with 600g neem leaf meal (NLM)/100kg feed; T4- diet with 50%(300g) MLM and 50% (300g) NLM/100kg feed; T5- diet with 75%(450g) MLM and 25% (150g) NLM/100kg feed; T6- diet with 25%(150g) MLM and 75% (450g) NLM/100kg feed; T7-(negative control) diet without any additive. Routine management and vaccination procedures were followed while fresh feed and water were supplied ad libitum. The experiment lasted for eight weeks. The feed intake, weight gain and feed conversion ratio was determined, also the villus height and the crypt depth was measured. There was no significant difference observed in the weight gain of the birds, also no significant difference was observed in the feed intake. Treatment 3 (diet with 600g neem leaf meal (NLM)/100kg feed) had the lowest feed conversion ratio which was not significantly different from other treatments. Birds on diets supplemented with moringa and neem had an increased villus height and crypt depth, though not significantly different to other diets. The combination of plant extracts at different inclusions improved the villus height and crypt depth of broiler birds. The study showed that moringa and neem had antibiotic effect on growth performance and gut health of broilers.

Keywords: Broilers, Moringa leaf meal, Neem leaf meal, crypt depth, villus height

Performances de croissance et santé intestinale des poulets à griller nourris avec *Moringaoleifera* et *Azadirachta indica* comme alternative aux antibiotiques



Résumé

L'utilisation élevée d'antibiotiques a entraîné une augmentation du nombre de bactéries développant une résistance aux antibiotiques. Une souche résistante de bactéries pathogènes humaines a été trouvée dans l'utilisation non curative d'antibiotiques pour la production animale. *Moringa oleifera* et *Azadirachta indica* sont des phytobiotiques qui se sont avérés naturels, sans effet résiduel et qui sont considérés comme idéaux pour la production animale. Une expérience a été menée pour déterminer les performances de croissance et la santé intestinale des poulets nourris au *Moringa oleifera* et au neem *Azadirachta indica* comme alternative aux antibiotiques. Deux cent dix poussins à griller âgés d'un jour ont été répartis au hasard en sept groupes de traitement alimentaire de trois

répétitions chacun. Chaque réplique avait 10 oiseaux. Le plan expérimental était un plan complètement randomisé (PCR). Les régimes étaient un régime T1 (témoin positif) avec des antibiotiques (oxytétracycline); T2 - régime avec 600 g de farine de feuilles de moringa (FFM)/100 kg d'aliment ; T3- régime avec 600 g de farine de feuilles de neem (FFN)/100 kg d'aliment ; T4- régime avec 50 % (300 g) de FFM et 50 % (300 g) de FFN/100 kg d'aliment ; T5- régime avec 75 % (450 g) de FFM et 25 % (150 g) de FFN/100 kg d'aliment ; T6- régime avec 25 % (150 g) de FFM et 75 % (450 g) de FFN/100 kg d'aliment ; Régime T7-(témoin négatif) sans aucun additif. Les procédures de gestion et de vaccination de routine ont été suivies tandis que des aliments frais et de l'eau ont été fournis à volonté. L'expérience a duré huit semaines. La prise alimentaire, le gain de poids et le taux de conversion alimentaire ont été déterminés, ainsi que la hauteur des villosités et la profondeur de la crypte ont été mesurées. Il n'y avait pas de différence significative observée dans le gain de poids des oiseaux, pas plus qu'aucune différence significative n'a été observée dans la prise alimentaire. Le traitement 3 (régime avec 600 g de farine de feuilles de neem (FFN)/100 kg d'aliments) avait le taux de conversion alimentaire le plus bas qui n'était pas significativement différent des autres traitements. Les oiseaux recevant des régimes complétés par du moringa et du neem avaient une hauteur de villosité et une profondeur de crypte accrues, mais pas significativement différentes des autres régimes. La combinaison d'extraits de plantes à différentes inclusions a amélioré la hauteur des villosités et la profondeur de la crypte des poulets à griller. L'étude a montré que le moringa et le neem avaient un effet antibiotique sur les performances de croissance et la santé intestinale des poulets à griller.

Mots-clés : poulets à griller, farine de feuilles de Moringa, farine de feuilles de Neem, profondeur de crypte, hauteur des villosités.

Introduction

Physiological studies have shown that a functional gastrointestinal tract is vital for digestion and absorption of nutrients required for the bird's maintenance and growth (Mateos *et al.*, 2002; Baurhoo *et al.*, 2009). Access to feed and water immediately post-hatch accompanied by intake of exogenous feed stimulates rapid development and growth of the gastrointestinal tract as well as its absorptive capacity; causing improvements in gut integrity and subsequent performance (Uni *et al.*, 1998). Early development of the gastrointestinal tract together with supply organs such as the liver and heart as well as the skeletal system are of high priority in supplying nutrients to the rest of the body and the frame on which muscle accumulates (Zuidhof *et al.*, 2006). Antibiotics are substances that inhibit the growth of microorganisms. They may be synthetic or

naturally occurring substances. The targets of antibiotics are pathogens that inhibit the ability of animals to grow. Antibiotics also keep animals healthy by boosting the immune system to fend off sickness. The use of antibiotics as growth promoters in the poultry industry has been banned in some countries especially in the European Union because of the harmful effects on human health. This was predicated on the development of microbial resistance to these products (William and Losa, 2001 and McCartney, 2002). Consequently herbs, spices and various plant extracts considered to be natural products that consumers would accept have received increased attention as possible alternatives to antibiotic growth promoters following their ban by the European Union in 2006 (Catala-Gregori *et al.*, 2008). Phytogetic feed additives, have been recognised as very promising alternatives as they meet the requirements

of consumers in terms of food safety and solve the problem of bacteria resistance that occurs as a result of using antibiotics as growth promoters (Da Silva Cardoso *et al.*, 2012). Recently, a number of additives derived from plants which were reported to contain aromatic properties that have an impact on gut micro-flora are now being used in poultry diet to enhance the performance of the immune response of birds (Saki *et al.*, 2012). Compared with synthetic antibiotics or inorganic chemicals, these plant-derived products have proven to be natural, less toxic, residue-free and are thought to be ideal feed additives in food animal production (Wang *et al.*, 1998). One of such plants is *Moringa oleifera* commonly known as the drumstick tree (Makker and Becker, 1997). *Moringa oleifera* Lam (*Moringaceae*) is documented to have an impressive range of medicinal uses, including growth promotion, antimicrobial and antioxidant effects (Mbikay, 2012). The nutritional profile of its fresh and dried leaves is reported to contain high levels of lipids and essential amino acids important in poultry productivity (Moyo *et al.*, 2012). Yang *et al.* (2007) evaluated the effect of *Moringa oleifera* on the growth performance, immune function and ileal microflora in broilers. The result showed that dehydrated leaves of *Moringa oleifera*, when given in the diet, revealed significant enhancement of duodenal traits, increased *Lactobacillus* counts in the ileum while reducing *E. coli* and enhancing the immune system of the broilers. Neem (*Azadirachta indica*) belongs to the *Meliaceae* family and is a fast-growing evergreen tree has the potential to provide medicinal and nutritive value to broilers (Schmutterer, 1990). Various parts of the tree have been reported to contain chemicals like *azadirachtin*, *nimbin*, *nimbindin*, *quercetin* among others (Makeri *et al.*, 2007) which have antimicrobial, antihelminthic, antioxidant,

antifungal, insecticidal, antiprotozoal and spermicidal properties. The importance of *Azadirachta indica* has been recognised by the U.S. National Academy of Science which published a report in 1992 titled "Neem: a tree for solving global problems". More than 135 compounds have been isolated from different parts of the tree. They have been divided into isoprenoid and non-isoprenoid compounds (Elangovan *et al.*, 2000). Tamilvanan *et al.* (2017) concluded that immediate post-hatch supplementation of extracts of aloe and neem in the water of Vanaraja chicks enhanced the gut health and development. This study evaluated the effect of replacing synthetic antibiotics with *Moringa oleifera* and *Azadirachta indica* leaf meals on the performance and gut morphology of broiler chickens.

Materials and methods

Study area

The experiment was conducted at the poultry unit of the Institute of Agricultural Research and Training, Ibadan, Nigeria. Ibadan is in Southwest Nigeria and it lies on the geographical coordinates of 7° 23' 16"N and 3° 53' 47"E.

Processing of neem and moringa leaf meals

Fresh leaves of neem and moringa were harvested and air-dried for 5 days: at which time the leaves were dry and crisp. The dried leaves were then milled using a locally fabricated milling machine and stored in air-tight plastic containers until they were ready to be used.

Experimental diets

The experimental diets are as listed below:

Treatment 1: Positive control [diet with an antibiotic (oxytetracycline)].

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 50% (300g) MLM

and 50% (300g) NLM in 100kg of feed.
Treatment 5: Diet with 75% (450g) MLM and 25% (150g) NLM in 100kg of feed.
Treatment 6: Diet with 25% (150g) MLM and 75% (450g) NLM in 100kg of feed.
Treatment 7: Negative control (diet without any additive).

The diet was a corn/soya diet and was formulated to meet the NRC (1994) nutrient requirements for broiler starters and finishers. Table 1 shows the gross composition of the experimental starter and finisher diet. The diet was formulated to meet the nutritional requirements of broilers as recommended by NRC (1994).

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

Ingredients	Starter	Finisher
Maize	53.00	59.00
Groundnut cake	15.30	13.30
Soyabean meal	18.00	25.00
Wheatbran	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

Intestinal morphology studies

Two birds were randomly picked from each replicate and slaughtered after a period of fasting (12 hours). Samples of ileum and jejunum 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. Five readings each of villus height were taken per specimen and this was done with the aid of a light microscope (Olympus). Villus height was measured from the apical to the basal region which corresponded to the superior portion of the crypts.

Housing and management

Two hundred and ten day-old Arbor Acres

broiler chicks were allotted to seven dietary treatments of threereplicates each. Each replicate had 10 birds. Birds were housed in a well ventilated deep litter pen which was thoroughly cleaned and disinfected with Vinkokill (Chlorophenol [7%]) prior to the arrival of the chicks. The chicks were offered a vitamin/electrolyte solution (vitalyte) upon arrival. Routine administration of vaccines against Infectious Bursal Disease and New Castle Disease were done. Body weights were 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, feed intake and feed conversion ratio (FCR) were obtained by calculation. The experiment lasted 56 days which comprised the starter and finisher phases.

Experimental 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).

Results and discussion

Performance characteristics

Table 2 shows the performance characteristics of birds fed moringa and neem leaves as an alternative to antibiotics. There was significant difference in the final body weight and weight gain of the birds with birds on treatment 2 having significantly higher values than the birds in other treatments. This agrees with the work of KoutElkloub *et al.* (2015) that birds fed on Moringa leaf powder gained significantly higher body weights than birds fed on other diets. This was also in line with the research findings of Anwar *et al.* (2007) who reported that Moringa treated broilers were heavier than those fed a control diet. Aderinola *et al.* (2013) observed that replacing antibiotic growth promoters with herbal supplements of Moringa leaf powder

has beneficial effects on the growth performance of the birds. Birds fed on the combination of both moringa and neem leaves had higher values but not significantly different ($p>0.05$) from the control which was a synthetic antibiotics. Chakravarty and Prasad (1991) reported that boilers fed on diet containing neem (*A. indica*) leaves, had higher body weight gain. This higher body weight gain in broilers consuming neem leaves infusion could be due to its diversified effect on intestinal micro flora, thereby avoiding stressful conditions. There was no significant ($p>0.05$) difference in the feed intake of the birds in the different treatments. However, treatment 2 had the highest value across the treatments. Treatment 3 to Treatment 6 which contains 100%, 50% and 25% of neem respectively showed slight reduction in feed intake. This might be due to mildly bitter taste of neem leaves according to Elangovan *et al.* (2000). Also, Gowda *et al.* (1998), reported significantly lower feed intake when neem leaves were fed to broilers.

Treatment 3 had the lowest feed conversion ratio numerically, although was not significantly different from the other treatments.

Table 2: Performance characteristics of Broilers fed Moringa and Neem leaf meals as alternative to antibiotics

Parameters	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	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 ^{ab}	2295.30 ^a	2258.50 ^{ab}	2192.40 ^{ab}	2191.00 ^{ab}	2047.70 ^b	2107.00 ^{ab}	31.13
Weight gain (g)	2122.33 ^{ab}	2256.80 ^a	2220.47	2153.87 ^{ab}	2152.77 ^{ab}	2009.63 ^b	2068.97 ^{ab}	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
Mortality(%)	20.00 ^a	15.00 ^{ab}	5.00 ^c	10.00 ^{ab}	10.00 ^{ab}	10.00 ^{ab}	30.00 ^a	0.11

Source: (Makanjuola *et al* 2020)

^{ab}Mean within the same row with different superscript letters, were different ($P<0.05$)* Level of significance ($P<0.05$)

FCR- Feed conversion ratio

T1: Positive control +oxytetracycline, T2: Diet with 600g MLM/100kg of feed, T3: Diet with 600g of NLM/100kg of feed, T4: Diet with 300g MLM + 300g NLM in 100kg of feed, T5: Diet with 450g MLM and 150gNLM in 100kg of feed, T6: Diet with 150g MLM and 450gNLM in 100kg of fed, T7: Negative control (no additive).

Gut morphology

The villus height and crypt depth of the ileum and jejunum of the small intestine of broilers fed moringa and neem are shown in Table 3. There was no significant difference ($p < 0.05$) in the villus height in T1 to T6 but T7 was significantly different from the rest. The values obtained for the crypt depth also followed the same trend. The result showed that the T1 (oxytetracycline) (positive control) was not significantly different from the other treatments, although numerically, it had the highest value recorded and was followed by T6 (150gMLM+ 450g NLM), T2 (600gMLM), T4 (300MLM+300NLM), T5 (450MLM+150NLM), and then T3 (600gNLM) respectively. All the treatments supplemented with moringa and neem had an increased villus height and crypt depth. This result is in line with Samanya and Yamauchi (2002) that the observed increase in jejunum and ileum villus height at 56 days (finisher phase) of age differed significantly compared to non-supplemented basal diet. Also, Tamilvanam *et al.* (2017) reported that the histomorphometry of the villi were significantly influenced by herbal supplementation. This may confirmed the fact that the additives in the diets increase villus height. It was observed that the T6 (150g MLM and 450 NLM) numerically

had a higher value amongst the different inclusion levels. This agrees with the work of Tamilvanam *et al.* (2017) that neem significantly improved both the height and width of villi on the jejunum of broiler birds. The works of Nkukwana (2015) checked the effect of moringa leaf meal supplementation on the intestinal morphology found out that birds that were supplemented with moringa leaf meal had a longer duodenum, jejunum and ileal villi and also a larger surface area for absorption. The increase in the internal surface area of the intestinal wall makes available a greater surface area for absorption of nutrients (Awad *et al.*, 2008) which will bring about increase in the performance of the birds. The combination of the different plant extract (moringa and neem) at different inclusion levels improved the villus height and crypt depth of the birds. Jamroz *et al.* (2003) stated that the botanicals and powdered extracts have the ability to stimulate the proliferation and growth of absorptive cells (villus and crypt) in the gastrointestinal tract and also enhances the production and activity of the digestive enzymes. It can be concluded from this study that moringa and neem leaf meals showed promising natural antibiotic effect on the growth performance and the gut health of broiler birds.

Table 3: Gut morphology of Broilers fed Moringa and Neem leaf meals as alternative to antibiotics

	T1	T2	T3	T4	T5	T6	T7	SEM
Ileum								
Villus height (µm)	1213.0 ^a	945.9 ^{ab}	980.0 ^{ab}	1001.1 ^{ab}	944.2 ^{ab}	1040.1 ^{ab}	811.4 ^b	41.95
Crypt depth (µm)	260.84 ^a	241.67 ^{ab}	274.19 ^a	269.56 ^a	254.13 ^{ab}	254.06 ^{ab}	229.57 ^b	12.66
Jejunum								
Villus height (µm)	1594.4 ^a	1325.6 ^{ab}	1179.2 ^{ab}	1242.0 ^{ab}	1239.6 ^{ab}	1560.0 ^a	1088.1 ^b	96.97
Crypt depth (µm)	300.65 ^a	258.07 ^b	310.66 ^a	230.29 ^b	323.35 ^a	330.18 ^a	223.73 ^c	16.78

^{ab}Mean within the same row with different superscript letters, were different ($P < 0.05$)* Level of significance ($P < 0.05$)

T1: Positive control +oxytetracycline, T2: Diet with 600g MLM/100kg of feed, T3: Diet with 600g of NLM/100kg of feed, T4: Diet with 300g MLM + 300g NLM in 100kg of feed, T5: Diet with 450g MLM and 150gNLM in 100kg of feed, T6: Diet with 150g MLM and 450gNLM in 100kg of fed, T7: Negative control (no additive).

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