AHC -21

Assessment of Antimicrobial Properties of Neem (*Azadirachta indica*) and Moringa (*Moringa oleifera*) Leaf Meal in Broiler Chicken Production

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Abstract
This study was designed to determine the effects of different levels of Moringa leaf meal (MLM) and Neem leaf meal ((NLM) as substitutes to antibiotics on broiler production. Two hundred and ten-day-old broiler chicks were randomly allotted to seven dietary treatments of three replicates (10 birds /replicate). The treatments were T1 (25g of oxytetracycline/4kg feed), T2 (600g MLM/100 kg feed), T3 (600 g NLM/100 kg feed), T4 (300g MLM + 300g NLM /100kg feed), T5 (450 g MLM + 150g NLM/100 kg feed), T6 (150g MLM + 450g NLM/100kg feed) and T7 (control).Antibacterial activities of MLM and NLM extracts were determined *in-vitro*, using disc diffusion method. Data were analyzed using analysis of variance. A total of 188*species*of bacteria were isolated and identified with *Escherichia coli* being the most frequently (29.3%) isolated while *Clostridium* species were the least (0.5%) of the isolates. Total bacterial counts were highest (3.75×10^3 Log cfu/g) in T7 and lowest (3.29×10^3 Log cfu/g) in T3. The highest (3.9±0.35 mm) zone of inhibition was recorded in T3, while T1 recorded the lowest (1.40±0.10mm) zone of inhibition. In conclusion, T3 (600g/100kg feed) of neem leaf meal in broiler diet produced the highest antibacterial effects.

Keywords: Antibacterial activity, *Moringa oleifera, Azadirachta indica*, broiler chicken,

Introduction

Antibiotics are naturally occurring synthetic or semi-synthetic compounds with antimicrobial activities. The high usage of antibiotics has led to an increased risk of bacteria developing antibiotic resistance. In 1997, the World Health Organization (WHO) recommended the end of non-therapeutic use of antibiotics in animal husbandry because resistant strains of human pathogens have been identified. This has compelled researchers to explore the use of other non-therapeutic alternatives like enzymes, probiotics, prebiotic, immune stimulants, organic acids and phytobiotics as feed additives in animal production. Compared to antibiotics, phytobiotics have proven to be naturally less toxic, residue-free and are thought to be ideal feed additives in food animal production. Ogbe and Affiku (2012) reported that *Moringa oleifera* extract has antibacterial properties while Elangovan *et al.* (2000) opined that *Azadirachta indica* contains compounds which have antimicrobial, antihelminthic, and antifungal activities.

This study was to determine the bacterial counts and bacterial growth inhibition using a methanolic extract of M*. oleifera*, A*. indica,* and Oxytetracycline on bacteria isolated from faecal and organ samples of broiler birds.

Materials and Methods

Fresh leaves from Neem *(Azadirachta indica)* and Moringa *(Moringa oleifera)* trees were harvested from a cultivated farmland. The leaves were washed thoroughly under a running tap water to remove dust and other foreign particles,air dried for five days and later ground into powder. Leaf extracts were prepared using methanol. Ground leaves of neem and moringa were mixed with the experimental diets at varying inclusion levels of 150g, 300g, 450g, and 600g/100kg of feed respectively.Two hundred and ten (210) day-old Arbor Acres broiler chicks were allotted to seven dietary treatments (T) of three replicates containing 10 birds each in a completely randomized design. The dietary treatments includes;T1 (25g of oxytetracycline/100kg feed), T2 (600g MLM/100 kg feed), T3 (600 g NLM/100 kg feed), T4 (300g MLM + 300g NLM /100kg feed), T5 (450g MLM + 150g NLM/100 kg feed), T6 (150g MLM + 450g NLM/100kg feed) and T7 (no additive).

A total of forty-eight (faecal and organs) samples were analyzed for microbial quality as described by FAO (2007).Two birds per replicate were selected at random for the collection of faecal and organ (Heart, Kidney, Liver, and Lungs) samples. The faecal samples were collected at day 28 (starter phase) and day 56 (finisher phase), while organ samples were collected at day 56. Pure isolates of the resulting growth were identified using morphological and biochemical methods as described by Jolt *et al.*(1994). Bacteria sensitivitytest was carried out

to determine the zones of inhibition by using two-fold serial dilution method (Chandrasekaran and Venkatesalu, 2004). Small paper discs containing antibacterial medication of Neem and Moringa extracts, and Oxytetracycline, respectively, were placed on the surface of the growth media.

All data were subjected to analysis of variance, using General Linear Model (SAS, 1999), and the means were separated using the Duncan Multiple Range Test.

Results and Discussion

Tables 1and 2 show the bacterial isolates of faecal and organ samples. *E. coli* occurredmost out of a total of 188 speciesof bacteria identified. Of the total bacteria count observed, *E. coli, Staphylococcus* species, and *Salmonella* species, accounted for 29.3%, 24%, and 12% respectively. The least encountered bacterium was *Clotsridium* species(0.5%) while *Bacteroides* species accounted for approximately 1% (Table 1). There was no significant ($p>0.05$) difference in the mean total bacterial counts across the treatment diets (Table 2). The Mean total bacterial count from the faecal and organ samples was highest (3.57±0.55 Log CFU/g) in T7, closely followed by T1 (3.49±0.35 Log CFU/g), but lowest (3.29±1.30 Log CFU/g) in T3. Neem leaf extract (T3) alone exhibited the highest (3.57±0.35mm) zone of inhibition, but lowest (1.40±0.10mm) zone of inhibition in T1 as depicted (Figure 1). From this findings, the bacterial pathogens isolated were similar with the microorganisms reported (Oladele-Bukola *et al*., 2017; Fasanmi *et al*., 2010) from meat scrapings and beef visceral. The highest percentage occurrence of *E. coli* in this study also corroborated the findings of Fasanmi *et al*. (2010) that *E. coli* is a major meat contaminant. However, the range of values (3.29±1.30 (T3) and 3.57±0.55 Log CFU/g (T7) of the mean total microbial counts in this study exceeded the standard limit (1.0 Log CFU/g) of values by FAO/WHO for water and food products. The highest antibacterial activity of Moringa and Neem leaf meal extract against the major bacterial isolates compared to oxytetracycline observed in this study was also similar with the antibacterial activity of ethanolic extracts of some selected medicinal flora reported (Nair *et al.,* 2005). This implied that *M. oleifera* and *A. indica* leaves contain bio-component whose antibacterial potentials are highly comparable to the conventional antibiotic (oxytetracycline) against all Gram-negative and Gram-positive bacteria tested.

Table 1: Results of biochemical test to identify bacterial isolates using general purpose media

Samples	Colour	Gramstain	Oxidase	Catalase	Starch hydrolysis	Motility	Citrate	Glucose	Fructose	Lactose	Probable identity of bacteria isolates	Number and percentage of Occurrence
1	Purple	Cocci	-	-	+	-	-	+	+	+	*Staphylococcus spp*.	45 (24%)
2	Pink	Rod	-	+	-	+	-	+	-	+	*Escherichia. coli*	55 (29%)
3	Pink	Rod	-	+	-	-	+	+	-	+	*Klebsiella spp.*	18 (9%)
4	Pink	Rod	+	+	-	+	+	+	+	-	*Pseudomonas spp.*	7 (4%)
5	Pink	Rod	-	+	+	+	+	+	-	+	*Proteus sp.*	19 (10%)
6	Pink	Rod	-	+	+	+	+	+	-	+	*Enterobacter spp.*	5 (3%)
7	Pink	Rod	-	+	-	+	-	+	-	+	*Salmonella sp.*	22 (12%)
8	Pink	Rod	+	+	+	+	-	+	-	-	*Bacteroides spp.*	2 (1%)
9	Pink	Rod	-	-	-	-	-	+	+	-	*Streptococcus spp.*	3 (2%)
10	Purple	Cocci	-	-	-	-	-	-	-	+	*Clostridium spp.*	1 (0.5%)
11	Pink	Cocci	+	+	-	-	+	+	+	-	*Pasteurellaspp*	11 (6%)
Total												188

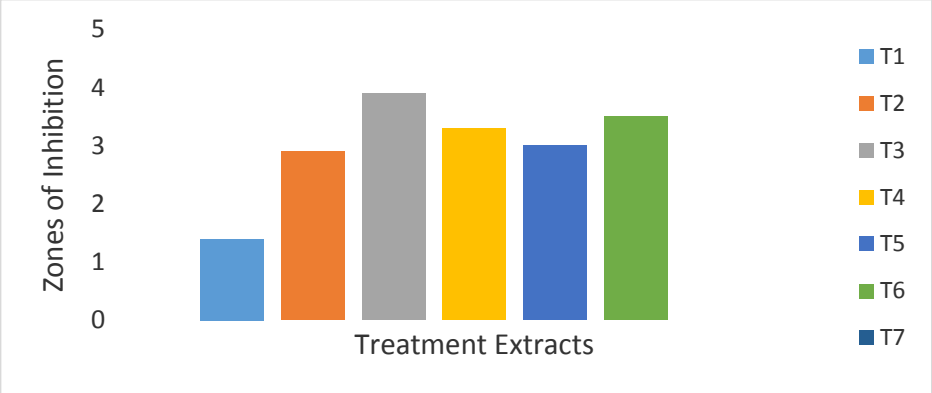

Figure 1: Antibacterial activity of *M. oleifera*, *A. indica*, and Oxytetracycline on bacteria isolates**.**

Table 2: Total bacteria counts (Log CFU/G) of faecal and organ samples of chicken

Period	Sample	T1	T2	T3	T4	T5	T6	T7	P-value
Day 1	Faeces	2.32±0.57	2.32±0.81	2.32±0.19	2.32±0.53	2.31±1.43	2.32±0.32	2.32±0.21	0.49
4thwk	Faeces	3.57±1.21	3.51±0.51	3.51±1.06	3.49±0.81	3.45±0.73	3.46±0.51	3.67±1.05	0.59
8thwk	Faeces	3.54±1.01	3.49±1.10	3.43±1.07	3.46±0.09	3.40±1.11	3.43±1.03	3.61±1.01	0.55
8thwk	Kidney	3.38±1.02	3.36±0.55	3.28±1.00	3.34±0.57	3.32±1.02	3.32±0.17	3.58±1.05	0.35
8thwk	Heart	3.40±0.19	3.32±0.08	3.20±1.01	3.24±1.02	3.23±0.90	3.28±0.07	3.59±0.11	0.47
8thwk	Lungs	3.40±0.12	3.30±0.15	3.28±0.10	3.32±0.12	3.32±0.10	2.33±0.09	3.49±1.05	0.43
8thwk	Liver	2.70±0.51	2.45±0.11	2.10±0.15	2.20±0.10	2.50±0.09	2.10±0.17	3.70±1.01	0.51
Mean Total		3.49±0.35	3.35±0.13	3.29±1.30	3.31±0.10	3.45±1.03	3.41±1.11	3.57±0.55	0.48

Conclusion

Antibacterial activity of *M. oleifera* and *A. indica* leaf meal were remarkable. Diet (T3) containing 600 g of neem leaf has a highest antibacterial effect compared to the conventional antibiotic (Oxytetracycline). The bioactivity of the leaves against both gram-positive and gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant.

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