

Original Research Article

Response of Broilers Fed with Diets Supplemented with *Moringa oleifera* Leaf Meal and Raw and Cooked Seed Meal Vis-à-vis Synthetic Antibiotic

ABSTRACT

The effects of *Moringa oleifera* leaf meal and raw and cooked seed meal as a replacement for synthetic antibiotic (tetracycline) on carcass characteristics, heart and liver histology of broiler chickens were assessed in a 7-week feeding trial.

The design of the study was a completely randomised design. There were 5 treatments: negative control (D1), positive control (D2), treatments 3, 4 and 5 (D3, D4 and D5) contained 0.25 g/kg of feed of raw air-dried *Moringa oleifera* leaf meal, 0.25 g/kg of feed of raw air-dried and cooked air-dried *Moringa oleifera* seed meal respectively. Each treatment had four replicates of 8 birds per replicate.

The results of the study indicated no significant difference across the treatments for most of the carcass characteristics. Birds on D1 recorded the lowest mean values for heart (6.94 g), spleen (1.56 g), wings (121.00 g) and drumstick (154.24 g). Birds on D4 compared well with those on D2 in terms of drumstick, spleen, heart and wings. No visible lesions were observed in the heart histology of the experimental birds. Liver photomicrograph of birds on D1 indicated mild dissociation of hepatic cords, those on D2 showing closely-packed hepatic plates. Those on D3 showed a few foci of mild random single-cell hepatocellular necrosis. Those on D4 showing no visible lesions while those on D5 showed severe diffuse fatty change of hepatocytes with a few normal hepatocytes.

Birds on raw *Moringa oleifera* seed meal compared well with those on synthetic antibiotics, however, the liver histology of those on synthetic antibiotics indicated potential danger of liver damage.

Keywords: Antibiotics, Broilers, Feeding Trial, *Moringa oleifera*, Toxicity

1. INTRODUCTION

Food safety and security are a global concern; hence, we all need to ensure that our need for animal protein is met. The poultry industry remains an important food supplier in the world. Chicken meat represents an important source of animal proteins [1]. Aside from feed cost, disease outbreak is another important factor mitigating against poultry production and productivity in Sub-Saharan Africa. Outbreaks usually result in great economic losses as a result of the accompanied morbidity, mortality, destruction of the intestinal epithelium which

18 consequently results in reduced body weight gain (BWG), feed conversion ratios (FCR) and
19 reduced egg production [2, 3, 4]. Therefore, the existence and control of disease outbreak in
20 chickens remain a threat to animal food safety and security in sub-Saharan Africa which call
21 for urgent attention.

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23 Several approaches have been used to control disease outbreak in poultry flocks such as
24 improved animal husbandry, higher hygiene standards, the use of drugs and vaccination.
25 However, the spread of the disease is heightened by the development of resistance to some
26 of the drugs by the causative protozoan parasites and the escalating cost of drug.
27 Consumers' request for poultry products that are free from residual drugs is another factor
28 limiting the use of chemotherapeutic agents or antibiotics [5].

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30 Wild plants for reasons of cost, availability and accessibility have been exploited in Africa in
31 the past in order to bring solution to numerous health challenges of man. *Moringa oleifera* is
32 one of the numerous plants being exploited. *Moringa oleifera* is believed to contain an
33 antibacterial agent 4(α -L-rhamnosyloxy) benzyl isothiocyanate, which is a plant synthesised
34 derivative of benzyl isothiocyanates [6]. Anticoccidial property of *Moringa oleifera* extracts
35 had also been reported [7]. It then becomes necessary to test the efficacy of *Moringa*
36 *oleifera* leaf and differently seed meal as a potential replacement for synthetic antibiotics in
37 broiler production in order to assess their comparative effects and toxicity.

38 2. MATERIAL AND METHODS

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40 The feeding trial was carried out at the Poultry Unit of Teaching and Research Farm of
41 Landmark University, Nigeria. There were 5 treatments: negative control (D1) which
42 contained neither *Moringa oleifera* leaf or seed meal; positive control (D2), treated with
43 0.25% tetracycline in water; treatments 3, 4 and 5 (D3, D4 and D5) contained 0.25 g/kg of
44 feed of raw air-dried *Moringa oleifera* leaf meal, 0.25 g/kg of feed of raw air-dried and
45 cooked air-dried *Moringa oleifera* seed meal respectively. Each treatment had four replicates
46 of 8 birds per replicate. Fresh feeds and clean, cool water were supplied to the birds *ad*
47 *libitum* throughout the period of the study. The study lasted for 7 weeks. Only birds on D2

48 were given the synthetic antibiotic throughout the period of the study. The proximate
49 composition of the *Moringa oleifera* leaves and experimental diets were carried out using the
50 method of the AOAC [8]. Gross experimental composition and determined chemical
51 compositions of the experimental diets are presented in Tables 1 and 2 respectively.
52 At the end of experimental period, 2 birds from each replicate were randomly picked up and
53 sacrificed. The birds were taken off feed 12 hours before slaughter. The organs and carcass
54 in each group were separated into different parts and weighed: leg, shank, wing, thigh,
55 breast, and drumstick. The organs: liver, heart, gizzard and spleen were also weighed using
56 the sensitive scale.

57

58 Hearts and Livers of experimental birds were harvested, preserved in 10% buffered formalin
59 solution and analysed for histological studies. Histologic sections were prepared from the
60 livers and were fixed in bouin's solution (mixture of 75ml of saturated picric acid, 25ml of
61 40% formaldehyde and 5ml of glacial acetic acid) for 12 hours. This was embedded in
62 paraffin using conventional method and cut into 5nm thick sections and stained using
63 hematoxylin- eosin dye and finally mounted in diphenyl xylene [9]. The sections were then
64 observed under the microscope for histopathological changes in liver architecture and their
65 photomicrographs were taken.

66 Data obtained were analysed within the framework of general linear models of analysis using
67 Statistical Analysis System [10], while significant means were separated using Duncan's
68 multiple range test of same software.

69 **3. RESULTS AND DISCUSSION**

70 Table 3 shows the carcass characteristics of experimental birds, while figures 1 and 2 show
71 the photomicrograph of hearts and livers of experimental animals. The results of the study
72 indicated no significant difference across the treatments for most of the carcass
73 characteristics. Birds on D1 recorded the lowest mean values for heart (6.94 g), spleen (1.56
74 g), wings (121.00 g) and drumstick (154.24 g). Birds on D4 compared well with those on D2
75 in terms of drumstick, spleen, heart and wings. No visible lesions were observed in the heart
76 histology of the experimental birds. Liver photomicrograph of birds on D1 indicated mild
77 dissociation of hepatic cords, those on D2 showing closely-packed hepatic plates. Those on
78 D3 showed a few foci of mild random single-cell hepatocellular necrosis. Those on D4
79 showing no visible lesions while those on D5 showed severe diffuse fatty change of
80 hepatocytes with a few normal hepatocytes.

81 Growth performance characteristics of the birds treated with synthetic antibiotics (D2)
82 (1.57kg/bird) and those fed with diet supplemented with raw air-dried *Moringa oleifera* (D4)
83 (1.58 kg/bird) had significantly ($P=0.05$) higher body weight gain compared with those fed

84 negative control (1.45 kg/bird). Body weight gain of birds in D5 (1.51 kg/bird) and D3 (1.45
85 kg/bird) was statistically similar to those of negative control (1.45 kg/bird). Feed intake was
86 higher for those fed D2 (2.09 kg/bird), D4 (2.16 kg/bird) and D5 (2.17 kg/bird) than the
87 negative control (1.90 kg/bird). Feed intake by birds on D3 (raw moringa leaf meal) (2.01
88 kg/bird) was statistically similar to that of negative control (1.90 kg/bird), as recorded in
89 another paper. Ologhobo et al [11] earlier reported that *Moringa oleifera* leaf meal did not
90 result in significance difference among treatments for most of the carcass parameters of
91 broilers measured. The result of the findings by Nuhu et al [12] earlier showed that there
92 were no significant differences among treatments for carcass characteristic for weaner
93 rabbits fed *Moringa oleifera* leaf meal. Ayssiwede et al [13] also reported that there were no
94 significant differences for carcass parameters across treatments except for lung and spleen
95 weights which were significantly increased in birds fed 8% and 16% *Moringa oleifera* leaf
96 meal. The authors reported that inclusion of Moringa leaf meal had no adverse effect on
97 dressing carcass, liver weight, and all organs weight ratios. Also, Zanu et al [14] found out
98 that none of the parameters measured for carcass characteristics in birds fed diets
99 containing *Moringa oleifera* leaf meal was affected significantly.

100
101 It has been observed that antibiotics are a common cause of drug-induced liver injury [15].
102 Although the frequency of serious antibiotic-induced hepatotoxicity is low compared with the
103 amounts prescribed each year—population-based estimates suggest that it occurs in <5 per
104 100000 population [16]. Antibiotic-induced hepatotoxicity is usually asymptomatic, transient
105 and associated with only mild hepatic impairment [17]. Asha et al [18] earlier reported the
106 effect of tetracycline at two doses of 50 and 200 mg kg⁻¹ daily on pancreatic and liver
107 tissue function for 14 and 21 days in adult male albino rats. The authors found out that free
108 radical levels and reduced glutathione were within the normal range at the 50 mg kg⁻¹ level
109 in the liver and significant changes were observed at 200 mg kg⁻¹. The antioxidant status
110 was unaffected in liver after treatment with tetracycline at the 50 mg kg⁻¹ level and a
111 significant decrease was observed at the higher dose. The results revealed that the safe
112 nature of tetracycline with respect to the liver at the lower dose tested, whereas, both the
113 higher and lower doses seem to have detrimental effect on the pancreas.

114 *Moringa oleifera* leaf extract did not produce histologically observable deleterious effects on
115 the liver elements but however produced extensive histological disruptions of the
116 reproductive organs- testis and epididymis, thus acting as an anti-fertility agent. Nilanjan et
117 al [20] noted that *Moringa oleifera* leaf extract prevented and cured high fat diet-induced liver
118 damage, and observed normal hepatic architecture in mice exposed to *Moringa oleifera* leaf
119 extract.

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Table 1. Gross composition of experimental diet fed to broiler chickens

Ingredients (%)	Starter	Finisher
Maize	54.00	66.00
Soya bean	31.00	20.00
Fish meal (72% CP)	3.00	2.00
Palm oil	1.50	0.00
Wheat offal	6.00	8.00
Bone meal	2.00	2.00
Oyster shell	1.00	1.00
Salt	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Premix	0.25	0.25

ME= metabolisable energy; Premix contains: vit A (10000000IU); vit D3 (3000000IU); vit E (30000IU); vit K (2300mg); vit B2 (5000mg); vit C (2000mg); niacin (30000mg); panthotenic acid (8000mg); vit B6 (3000mg); vit B12 (16mg); folic acid (800mg); biotin (600mg); choline chloride (200000mg); cobalt (200mg); copper (600mg); iodine (25000mg); iron (20000mg); manganese (85000mg); selenium (120mg); zinc (50000mg); antioxidant (1250mg). D1 = negative control, D2 = 0.25% of synthetic antibiotics (oxytetracycline) in water, D3 = 0.25g of raw *Moringa oleifera* leaf meal/kg feed, D4 = 0.25g of raw *Moringa oleifera* seed meal/kg feed, D5 = 0.25g of cooked *Moringa oleifera* seed meal/kg feed

Table 2. Determined chemical composition of experimental diets

(finisher phase)

Nutrients (%)	D1	D2	D3	D4	D5
Moisture	10.56	10.59	10.75	10.76	10.70
Crude protein	19.66	18.35	18.80	18.88	18.73
Crude fibre	2.50	2.37	2.44	2.36	2.44
Fat	5.54	4.88	5.47	5.46	5.70
Ash	10.69	8.92	10.26	10.01	10.34
Calcium	0.62	0.38	0.66	0.68	0.65
Phosphorus	0.78	0.74	0.75	0.75	0.76
Starch	37.21	39.28	37.89	37.93	38.00
Total sugar	3.42	3.57	3.45	3.44	3.54

136 D1 = negative control, D2 = 0.25% of synthetic antibiotics (oxytetracycline) in water, D3 = 0.25g of raw
 137 *Moringa oleifera* leaf meal/kg feed, D4 = 0.25g of raw *Moringa oleifera* seed meal/kg feed, D5 = 0.25g
 138 of cooked *Moringa oleifera* seed meal/kg feed
 139

140 **Table 3. Carcass characteristics of broilers fed diets supplemented with *Moringa***
 141 ***oleifera* leaf and seed meal**
 142

Parameters	D1	D2	D3	D4	D5	SEM
Live Weight (kg/bird)	1.58	1.72	1.59	1.70	1.64	0.03
Slaughtered Weight kg/bird)	1.52	1.60	1.47	1.60	1.54	0.02
Defeathered Weight (kg/bird)	1.46	1.62	1.52	1.62	1.55	0.03
Dressed Weight (kg/bird)	1.15	1.23	1.21	1.27	1.23	0.02
Visceral(kg)	0.26	0.27	0.28	0.28	0.24	0.01
Drumstick(g)	154.24 ^b	215.17 ^a	173.82 ^{ab}	175.58 ^{ab}	166.25 ^{ab}	10.25
Liver(g)	51.89	61.93	48.27	51.47	51.65	2.32
Spleen(g)	1.56 ^b	1.82 ^{ab}	1.79 ^{ab}	2.05 ^a	1.66 ^{ab}	0.08
Abdominal fat(g)	25.93	19.75	20.19	23.34	20.36	1.19
Empty gizzard(g)	30.31	31.76	30.69	33.80	32.32	0.62
Heart(g)	6.94 ^b	8.73 ^a	7.63 ^{ab}	7.93 ^{ab}	7.76 ^{ab}	0.29
Wings(g)	121.00 ^b	140.20 ^{ab}	134.95 ^{ab}	145.43 ^a	133.18 ^{ab}	4.09
Legs(g)	65.33	77.09	65.42	73.06	70.94	2.27
Shank length(cm)	7.75	7.50	7.83	7.92	7.58	0.08
Breast(g)	306.33	321.57	308.33	310.13	337.06	5.73
Thigh(g)	180.22	169.25	181.06	192.83	181.20	3.73

143 Means with different superscripts within the same rows are significantly different. P= 0.05.

144 D1 = negative control, D2 = 0.25% of synthetic antibiotics (oxytetracycline) in water, D3 = 0.25g of raw
 145 *Moringa oleifera* leaf meal/kg feed, D4 = 0.25g of raw *Moringa oleifera* seed meal/kg feed, D5 = 0.25g
 146 of cooked *Moringa oleifera* seed meal/kg feed
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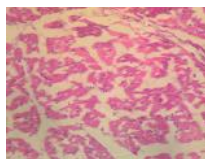


Fig.1a. Heart photomicrograph of birds on D1 showing no visible lesions (NVL) and normal cardiomyocytes

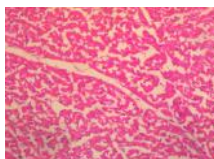


Fig.1b. Heart photomicrograph of birds on D2 showing NVL and normal cardiomyocytes

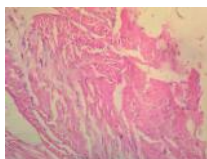


Fig.1c. Heart photomicrograph of birds on D3 showing NVL and normal cardiomyocytes

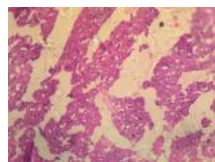


Fig.1d. Heart photomicrograph of birds on D4 showing NVL and normal cardiomyocytes

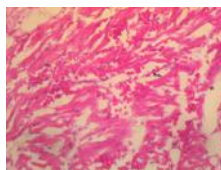


Fig.1e. Heart photomicrograph of birds on D5 showing NVL and normal cardiomyocytes

Mg X 400



Fig.2a. Liver photomicrograph of birds on D1 showing mild dissociation of hepatic cords

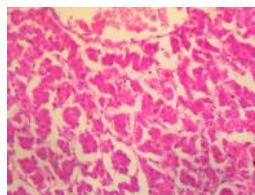


Fig.2b. Liver photomicrograph of birds on D2 showing closely-packed hepatic plates.

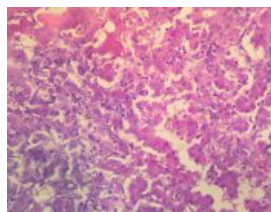


Fig. 2c. Liver photomicrograph of birds on D3 showing a few foci of mild random single-cell hepatocellular necrosis

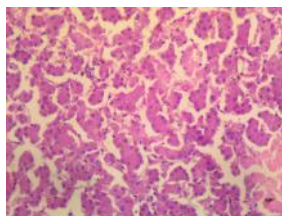


Fig.2d. Liver photomicrograph of birds on D4 showing no visible lesions

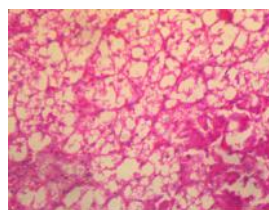


Fig. 2e. Photomicrograph of birds on D5 showing severe diffuse fatty change of hepatocytes with a few normal hepatocytes

Mg x400

4. CONCLUSION

Birds on raw *Moringa oleifera* seed meal compared well with those on synthetic antibiotics, however, the liver histology of those on synthetic antibiotics indicated potential danger of liver damage.

158 **ETHICAL APPROVAL**

159 Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were
160 followed. All experiments have been examined and approved by the appropriate ethics
161 committee.

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