

EFFECTS OF *Moringa oleifera* ROOT EXTRACT ON THE
PERFORMANCE AND SERUM BIOCHEMISTRY OF
Escherichia coli CHALLENGED BROILER CHICKS

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Abstract: The antibacterial and phytochemical effects of aqueous extracts of *Moringa oleifera* roots on *E. coli* challenged broiler chicks were investigated. Ninety one-day old broiler chicks were orally inoculated with *E. coli* at 1.23×10^8 CFU/ml and then divided into six treatment dose levels: 5g/l, 10g/l, and 15g/l *Moringa* root extract (MRE), positive control, negative control and a standard (commercial antibiotics). The extract showed no significant difference ($P > 0.05$) in performance, carcass and nutrient retention indices of birds compared to the controls. However, at 10g/l dose level, serum parameters including cholesterol and uric acid were higher ($P < 0.05$) at 118.9Mmol/l and 4.07Mmol/l respectively, but lower in total protein (4.40g/l, $P < 0.05$). Birds fed 15g/l dosage had lower ($P < 0.05$) serum cholesterol level (77.503Mmol/l) and lower ($P < 0.05$) mortality (1%) compared to other treatments (2.2–3.3%) and the negative control (5.5%). The findings of this study suggest that the active ingredients from *Moringa oleifera* roots could significantly assist in combating endogenous pathogenic activities.

Key words: *Moringa oleifera*, phytochemical, antibiotics, *Escherichia coli*, broiler chick.

Introduction

Disease incidence and management remain a major challenge in the poultry industry accounting for higher annual economic losses. Some of the important pathogens plaguing the sector include *Escherichia coli*, *Salmonella* spp, mycoplasma, infectious laryngotracheitis virus, and fowl pox virus (Bouzoubaa et al., 1992). Several studies have found the prevalence of most pathogens, particularly *E. coli* resistant to ciprofloxacin and erythromycin in

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chickens with colibacillosis (Moniri and Dastehgoli, 2007; Panlilio, 1992). Suggestions have thus been put forward to reduce this problem and these include controlling the use of antibiotics, understanding the genetic mechanisms of resistance, and continuing studies to develop new drugs, either synthetic or natural (Gislene et al., 2000; Cordell, 2000). About 80% of individuals of the rural population use traditional medicine, which has compounds derived from medicinal plants (WHO, 2004), while recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. These can then be developed into medicines or chemically modified for medical use (Heinrich et al., 2004). Plant based antimicrobials represent a vast untapped source of medicines with enormous therapeutic source potential (Cowan, 1999) and in recent years, there has been an increased awareness of the potential that natural plant compounds have in the prevention and treatment of poultry diseases (Chen et al., 2003; Guoet et al., 2003). *Moringa oleifera* is highly esteemed by people in the tropics and sub-tropics for the many ways it is used nutritionally by people and medicinally by a local herbalist. *Moringa oleifera* has pharmacologically important chemical compounds such as carbohydrates, saponins, cardiac glycosides, terpenes, steroids, flavonoids and alkaloids having many known therapeutic values (Ahmad et al., 2006; Ambali and Furo, 2012). Molecules of herbs are said to be safe and they would overcome the resistance produced by the pathogens since they exist in more than one molecule in the protoplasm of plant cell (Prakash, 2006). This study therefore seeks to provide answers to the use of aqueous extracts of *Moringa* roots as an alternative to synthetic antibiotics for poultry.

Material and Methods

Origin and Management of Experimental Birds

The experiment was carried out at the Teaching and Research Farm, University of Ilorin. Ilorin is located at latitude 08 29°N and longitude 004 35°E. An annual temperature range is 22–34°C and annual precipitation is 80–12mm (World Climate, 2013). 90 chickens one-day old were obtained from a commercial farm (Zarm Farms Nig. Ltd.), Ilorin, Nigeria and were raised in metabolic cages for eight weeks. Diets were formulated to meet standard requirements according to NRC (1994) as shown in Table 1. The birds were vaccinated and pre-medicated using Neomycin, Embazine-Forte as anticoccidiostat and multivitamin supplements, while other routine management practices were strictly adhered to.

Table 1. Composition of experimental diet (g/100g DM).

Ingredients	Starter	Finisher
Maize	55.00	55.50
Groundnut cake	23.00	-
Brewer's dried grain	-	6.77
Corn bran	7.00	-
Soya bean meal	10.00	23.40
Palm kernel cake	-	5.00
Fish meal (72%)	1.23	-
Blood meal	-	3.33
Palm oil	-	3.00
Bone meal	2.00	2.00
Oyster shell	0.50	0.50
DL-Methionine	0.25	0.25
Lysine	0.25	0.25
Salt	0.25	0.25
Vit./Min. premix	0.25	0.25
Total	100.00	100.00

Preparation and Administration of Moringa Root Extract

Fresh *Moringa oleifera* roots were harvested within the Ilorin metropolis, washed, shade-dried and crushed, then soaked in water for seven days (7 d) to reduce its perceived anti-nutritional constituents. The extracts were recovered using a rotary evaporator until slurry was obtained, dried and ground into fine powder. The powdered extract was then administered orally (via drinking water) using a six-treatment design, as described: birds neither inoculated nor treated with Moringa root extract (MRE) were assigned as a positive control, birds inoculated but not treated with either of MRE or commercial antibiotic were assigned as a negative control, birds inoculated but treated with only commercial antibiotics were assigned as the standard. The three remaining treatments utilized 5g/l, 10g/l, and 15g/l of Moringa root extract (MRE) respectively as a phytobiotic for the *E.coli* challenged birds. The treatments were replicated thrice, each comprising five (5) birds in a completely randomized design.

Bacteriology

Strains of *Escherichia coli* were cultured at the Department of Microbiology, University of Ilorin. The plate was placed in an incubator set at 37°C to allow for growth for 24 hours. After the growth of the *E. coli* on the agar plate, samples

were then inoculated into a test tube containing 10ml of nutrient broth that will aid the growth of the bacteria and again placed in an incubator at 37°C. After 24 hours, the solution became cloudy indicating the growth of bacteria. The cloudy solution was then poured into a conical flask containing 200ml of the same broth. The birds were challenged orally with *Escherichia coli* throughout the experiment at the rate of 1.23×10^8 CFU/ml (counted using Serial dilution method). Birds were observed twice daily for 9 days post-challenge for clinical signs, and each individual was assigned daily clinical scores as follows: 0, normal; 0.5, slightly abnormal appearance, slow to move; 1, depression, reluctant to move; 2, inability to stand or reach food or water; and 3, dead. All of the birds were scored daily for 9 days post-challenge. Some birds that received a clinical score of 2 were euthanized by cervical dislocation. Chickens that were found dead or euthanized were necropsied immediately for various pathological lesions indicative of *E. coli* incursion.

Data Collection and Analysis

Proximate analyses of the feed and fecal samples were carried out using the method of AOAC (1990). Performance indices including feed intake, body weight gain, feed conversion ratio (FCR) were also examined. Carcass characteristics of the broilers were collected by fasting and subsequently slaughtering the birds (exsanguinations) and then weighing the primal parts as a percentage of dressed weight. Dressing percentage was calculated as a ratio of dressed weight to live weight. Nutrient retention study was carried out for crude protein (CP), ash, crude fibre (CF) and ether extract (EE) using the total collection method. Blood samples for serological indices were collected through transverse cut of the jugular veins of the birds into Bijou bottles without anticoagulant to allow for blood clotting. The blood samples were spinned in a centrifuge at 200rpm for 10 minutes and the resultant serum was decanted into a well-labelled bottle. The serum biochemical analysis was carried out at the University of Ilorin Teaching Hospital (UITH) for such indices as uric acid, glucose, creatinine, cholesterol and total protein. Data obtained from the experiment were subjected to analysis of variance of the Completely Randomized Design (CRD) according to Steel and Torrie (1980). Significant means were separated using Duncan Multiple Range Test (Duncan, 1955).

Results and Discussion

The effects of *Moringa oleifera* root extract on the performance and carcass characteristics of broilers challenged orally with *Escherichia coli* at 1.23×10^8 CFU/ml are shown in Table 2.

Table 2. Effects of treatment on the performance of the broilers.

Parameters	Positive Control	Negative Control	Commercial Antibiotics	5g/l MRE	10g/l MRE	15g/l MRE	±SEM
Feed Intake (*g/b/d)	83.62	76.73	81.80	82.74	78.72	80.59	0.89
Weight Gain (g/b/d)	35.85	29.99	31.14	35.63	31.96	33.15	1.02
FCR	2.33	2.56	2.63	2.32	2.46	2.43	0.15
Mortality (%)	0.00	5.50	2.20	2.20	3.30	1.00	0.03

*gram/bird/day.

Table 2 shows no effect ($P>0.05$) of MRE treatment on the performance of the birds. The negative control recorded the highest mortality at 5.5%, attesting to the virulence and economic losses associated with the inoculated pathogen. Birds treated with 15g/L MRE showed a relatively better combative capability against the pathogen, and at this level, 1% mortality was recorded compared with birds fed commercial antibiotics (2.20%). In Table 3, it was observed that birds treated with 10g/L MRE had reduced liver weight (38g, $P<0.05$) compared with the positive control, however there were similar ($P>0.05$) differences observed across other treatments. Dressing percentage was also appreciable across treatments, though observable differences were similar ($P>0.05$).

Table 3. Effects of treatment on the carcass characteristics of the broilers g/100g body weight.

Organs and Primal cuts (%)	Positive Control	Negative Control	Commercial Antibiotics	5g/l MRE	10g/l MRE	15g/l MRE
Heart	8.33	7.00	10.33	9.33	9.33	9.33
Wings	140.00 ^{ab}	129.33 ^a	151.67 ^b	153.00 ^b	142.33 ^{ab}	146.33 ^{ab}
Leg	132.33 ^b	89.00 ^a	94.67 ^{ab}	98.67 ^{ab}	85.67 ^a	102.00 ^{ab}
Breast muscle	266.33	221.67	253.00	297.00	249.33	321.00
Neck	105.33 ^b	68.33 ^a	98.33 ^{ab}	98.33 ^{ab}	90.33 ^{ab}	101.33 ^{ab}
Liver	51.67 ^b	31.33 ^{ab}	41.33 ^{ab}	43.00 ^{ab}	38.00 ^a	41.33 ^{ab}
Dressing %	87.0	81.14	84.48	86.14	83.04	84.54

a, b: Means with different superscripts within a row are significant ($p<0.05$). Other indices: Head, Thigh, Breast muscle, Backbone, Gizzard, Dressed weight and Live weight were similar ($p>0.05$) across treatments.

Table 4 shows the percentage nutrient retention of birds fed different treatments. It was observed that the MRE treatments were comparable ($P>0.05$) with commercial antibiotics for the basic nutrients in the diet. Birds challenged with *E. coli*, but not medicated with either MRE or antibiotics (negative control), expectedly showed poor nutrient retention tendencies ($P<0.05$). Specific serum biochemical constituents obtained from the blood assay (Table 5) show higher cholesterol levels (118.9mmol/l, $P<0.05$) in birds treated with 10g/l MRE, and comparing across

treatments, the lowest value was observed for birds fed the 15g/l MRE treatment diet (77.50Mmol/l, $P<0.05$). This trend was consistent for birds on 10g/l MRE treatment, as observed in serum uric acid composition and serum protein which were significantly different ($P<0.05$) compared to other treatments. Creatinine and glucose levels were found to be similar ($P>0.05$) across all treatments.

Table 4. Nutrient retention.

Parameters(%)	Positive Control	Negative Control	Commercial Antibiotics	5g/l MRE	10g/l MRE	15g/l MRE	±SEM
CP	60.58 ^b	48.76 ^a	59.54 ^b	60.33 ^b	58.91 ^b	56.91 ^b	2.52
ASH	58.60 ^b	46.06 ^a	53.80 ^{ab}	57.99 ^b	60.00 ^b	54.93 ^{ab}	1.20
CF	61.95 ^{ab}	54.52 ^a	59.81 ^{ab}	62.78 ^b	61.59 ^{ab}	63.59 ^b	1.34
EE	79.76 ^b	71.42 ^a	82.55 ^b	80.98 ^b	81.62 ^b	78.48 ^b	1.20

a, b: Means with different superscripts within a row are significant ($p<0.05$). Crude protein (CP), ash, crude fibre (CF) and ether extract (EE).

Table 5. Serum biochemistry.

Indices	Positive Control	Negative Control	Commercial Antibiotics	5g/l MRE	10g/l MRE	15g/l MRE	±SEM
Cholesterol (Mmol/l)	106.53 ^{ab}	108.19 ^b	102.66 ^{ab}	108.89 ^b	118.91 ^c	77.503 ^a	4.22
Protein (g/l)	11.82 ^b	9.87 ^{ab}	13.19 ^b	8.26 ^{ab}	4.40 ^a	10.23 ^{ab}	1.59
Creatinine(Mmol/l)	0.26	0.31	0.27	0.33	0.39	0.41	N.S
Glucose (Mmol/l)	7.49 ^b	4.08 ^a	5.08 ^{ab}	5.50 ^{ab}	5.66 ^{ab}	5.54 ^{ab}	0.60
Uric acid (Mmol/l)	0.55 ^a	0.76 ^a	1.37 ^a	1.66 ^{ab}	4.07 ^c	2.69 ^b	0.28

a, b,c: Means with different superscripts within a row are significant ($p < 0.05$).

The phytochemical constituents in *Moringa oleifera* aqueous root extract in this study point towards the potential of the extract to have analgesic, anti-inflammatory and adaptogenic effects, which help the host to develop resistance against disease and endurance against stress (Gupta, 1994; Ambali and Furo, 2012). These could be possible as the root extracts contain some antibacterial activities. The flavonoids act as antibiotics by inhibiting its protein synthesis (Hong-Xi and Song, 2001) and although no significant difference ($P>0.05$) was observed in the overall performance of birds fed diets containing Moringa root treatment compared to the control diet, serum parameters however did show some variations in relative responses of birds to the disease condition. Birds that received 10g/l MRE treatment showed a marked increase in serum cholesterol and uric acid levels and lower serum protein composition. It is known that diseases prevent cells from removing cholesterol from the blood or cause the liver to over-produce cholesterol. This can over time lead to plaque deposits on the wall of the arteries and subsequent narrowing of the blood cells making it difficult for the blood to pump and receive an adequate blood supply.

Hypoproteinemia, which was also observed in 10g/l treatment, may be due to decreased cell protein production, or increased protein loss, characteristic of the disease condition. The implication of this is increased tissue degeneration, kidney dysfunction causing hypoalbuminaemia and liver damage. High uric acid is also indicative of kidney dysfunction that may predispose to higher mortality rate (as observed in Table 2). Furthermore, adherence to epithelial cells is a fundamental requirement for colonisation of the respiratory tract by *E. coli*, hence a strong increase in hydrophobicity of the microbial species in the presence of some plant extracts may influence the surface characteristics of microbial cells and thereby affect the virulence properties of the microbes (Kamel, 2001). This may be an important antimicrobial mechanism of some plant extracts. It has also been observed that some plant extracts can influence the growth of commensal gut microflora by facilitating continuous supply of specific substrates for the protective intestinal flora or by minimizing the risk of development of populations in which opportunistic pathogens can thrive (Lanet al., 2005). The protection of the gut environment is now known to play an important role in reducing disease in animals. Several bioactive compounds from plants have been identified as compounds that differentially stimulate favourable bacteria such as *lactobacilli* and *bifidobacteria spp* without promoting the growth of pathogenic species (Guoet et al., 2004; Lanet et al., 2004). Stimulation of these beneficial bacteria by the extracts could have contributed to the balanced gut microflora and might have provided an optimal precondition for effective protection against pathogenic microorganisms and an intact immune system (Piva and Rossi, 1998; Wenk, 2003).

Conclusion

The study validates the use of Moringa roots (aqueous extracts) as an alternative to synthetic antibiotics in combating relevant poultry diseases, particularly those of the *E. coli* origin. Furthermore, extracts administered at 15 g/L dosage are recommendable, since this dose level shows better serological indices compared to other dose levels examined.

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Received: May 5, 2015

Accepted: July 10, 2015

UTICAJI EKSTRAKTA KORENA *Moringa oleifera* NA PROIZVODNE
REZULTATE I BIOHEMIJSKE PARAMETRE KRVI BROJLERA
ZARAŽENIH BAKTERIJOM *Escherichia coli*

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R e z i m e

Antibakterijski i fitohemijski uticaji vodenog ekstrakta korenova *Moringa oleifera* na brojler zaražene bakterijom *E. coli* su ispitivani. Devedeset brojlera uzrasta od jednog dana su oralno inokulisani bakterijom *E. coli* sa $1,23 \times 10^8$ CFU/ml i onda su podeljeni u šest tretmanskih nivoa prema dozama: 5g/l, 10g/l, i 15g/l ekstrakta korena Moringe (MRE), pozitivna kontrolna grupa (pilići bez inokulacije sa *E. coli* i tretiranja sa MRE), negativna kontrolna grupa (pilići inokulisani sa *E. coli* i bez tretiranja sa MRE ili komercijalnih antibiotika) i standardna grupa (komercijalni antibiotici). Ekstrakt nije pokazao značajnu razliku ($P > 0,05$) u pogledu proizvodnih rezultata, trupa i indeksa zadržavanja hranljivih materija kod ptica u poređenju sa kontrolnim grupama. Međutim, pri nivou doze od 10g/l, serumski parametri koji uključuju holesterol i urinsku kiselinu su bili viši ($P < 0,05$), 118,9 Mmol/l odnosno 4,07 Mmol/l, ali niži u ukupnom proteinu (4,40g/l, $P < 0,05$). Ptice koje su hranjenje ekstraktom u dozi od 15g/l su imale niži ($P < 0,05$) nivo serumskog holesterola (77,503 Mmol/l) i nižu ($P < 0,05$) smrtnost (1%) u poređenju sa drugim tretmanima (2,2–3,3%) i negativnom kontrolnom grupom (5,5%). Rezultati ovog istraživanja sugerišu da aktivni sastojci iz korena *Moringa oleifera* mogu značajno da pomognu u borbi protiv endogenih patogenih aktivnosti.

Ključne reči: *Moringa oleifera*, fitohemijski, antibiotici, *Escherichia coli*, brojler.

Primljeno: 5. maja 2015.
Odobreno: 10. jula 2015.

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