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Effects of aqueous *moringa oleifera* leaf extracts on gut morphology and pH of hubbard broiler chickens

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Abstract

This study was conducted to determine the effect of varying aqueous Moringa oleifera leaf extracts (AMOLE) on the gut morphology and pH of Hubbard broiler chickens. A total of 240 Hubbard broiler chickens were randomly allocated into six treatments with four replicates in a completely randomized design. The treatments were positive control (AMOLE), negative control (AMOLE), $AMOLE_{60}$ (60mL of the extracts per litre), $AMOLE_{90}$ (90mL of the extracts per litre), $AMOLE_{120}$ (120mL of the extracts per litre) and $AMOLE_{150}$ (150mL of the extracts per litre). Data on gut morphology weight and length and their pH were measured and analysed by one way analysis of variance. The results showed that dressed, crop, large intestine, kidney and lung weights of birds were influenced ($P<0.05$) by the treatments. Birds in the control treatments (94.67 %, 94.93 %) had the higher ($P<0.05$) dressing percentage. Birds on the $AMOLE_{60}$ had the higher ($P<0.05$) crop and large intestine weights (4.47 and0.23 %, respectively). The birds on the $AMOLE_{90}$ treatment had the largest kidney weight of 0.33 %. Absolute crop and caecum length were influenced byaqueous Moringa oleifera leaf extracts while the relative caecum length was the only relative organ length that was influenced by aqueous Moringa oleifera leaf extracts treatment.The pH of the internal organs showed that the gizzards, duodenum, jejunum, ileum, caecum, kidney and lung were influenced ($P<0.05$) by the treatments. Birds on positive control treatment had higher pH values in most of the parameters measured. Birds on the $AMOLE_{120}$ had the lowest ($P<0.05$) duodenum, jejunum and lung pH. This study revealed AMOLE had influence on the gut morphology and pH of broiler chickens. Therefore, it can be concluded that the substitution of antibiotics as a growth promoter with aqueous Moringa oleifera leaf extracts up to 120 ml improved the gut morphology and pH of the intestinal segmentsin broiler chickens. Thus, is recommended that farmers, poultry producers and nutritionists to administer between 60 and 120 mL/litre of AMOLE in the drinking water of broiler chickens to nourish them for good gut morphology and optimum pH of their internal organs.

Keywords: Aqueous, morphology, hubbard, *Moringa oleifera*

Introduction

Research on meat production globally indicates poultry as the fastest growing livestock sector especially in developing countries (Chang, 2007). Poultry was strategic to address animal protein shortage in human feeding because of its incomparable competence in transforming nutrient to high quality animal protein (Oluyemi and Roberts, 2000 and Isika, *et al*., 2006).

Following the in-feed antibiotic growth

promoters (AGP) ban within the European Union in 2006, consumer perceptions have shifted to the quality and safety of animal products making feed manufacturing exponentially complex in terms of accountability and traceability of feeds and their component ingredients (Leeson, 2008). Prior to the ban, gut integrity of poultry was mainly dependent on AGPs to control intestinal pathogens (Wallace *et al.*, 2010). However, because of the rising concerns on the extensive loss in poultry due to gastro-intestinal complaints and implementation of the laws to use of harmful synthetic drug or antibiotics in products for human consumption an alternative disease control resources to enhance gut health and to reduce the use of AGPs is in high demand (Mirzaei-Aghsaghali, 2012). Interest and some useful research on various natural growth promoters (NGPs) such as phytobiotics (essential oils, powders, extracts and phytochemicals), probiotics, prebiotics, synbiotics, organic acid, clay minerals, egg yolk antibodies, exogenous enzymes, recombinant enzymes, nucleotides, polyunsaturated fatty acids and miscellaneous compounds has increased the impetus for revisiting to look for new, useful additives that can enhance gut health and productivity of birds; one of such NGPs is the miracle tree called *Moringa oleifera*. *Moringa oleifera (Moringaceae)* is a highly valued plant, distributed in many countries of the tropics and subtropics. It is one such plant with an impressive range of medicinal uses, including growth promotion, anti-microbial and antioxidant effects (Makkar and Becker 1997; Moyo, et al., 2011 and Mbikay, 2012). The nutritional profile of dried M. oleifera leaves has shown high levels of lipids and amino acids important in poultry productivity (Makkar and Becker, 1997). *This p*lant that has been studied for many years for use by human is now being investigated for its fast growth, higher nutritional value, and utilization as livestock fodder crop (Noumanet *et al.*, 2013). Incorporation of this herb and its products in livestock feeds and water to stimulate the effective use of feed nutrient may result in more rapid gain, higher production and better feed efficiency. This is because it contains active substances that can improve digestion and metabolism and possess bacterial and immuno stimulant activities (Ghazalah and Ali, 2008). There are limited study on the effect of this plant extract on the gut morphology and internal organ pH. Thus, this study determined the influence of varying concentration of aqueous *Moringa oleifera* leaf extracts on the gut morphology and internal organs pH of Hubbard broiler chickens.

Materials and methods

Study location

This study was carried out at Abeezainab Integrated Farms along Minna-Bida road, Niger state, Nigeria. Minna is located between latitude 9°37` North and longitude 6°33`Eeast. It is located in the Southern Guinea savanna zone of North Central Nigeria. The mean monthly minimum and maximum temperatures are 38°C and 42°C respectively. The mean annual rainfall is between 1200mm – 1300mm while the mean monthly relative humidity is 65 % (Niger State Agricultural Development Project, 2009). The experiment lasted for seven weeks.

Source of the test ingredient and preparation of the extracts

Moringa oleifera leaves were purchased from the Central (Kure) Market in Minna. The leaves were air-dried for five days and ground into fine particles using a simple hammer mill, 60g of the ground particles was then soaked in 1 litre of boiled drinking

water for 24hours and this was done daily. The preparation was then filtered using a muslin cloth to separate the debris and filtrate; the extracts were then placed in a clean container and diluted using water (volume/volume) into 60 mL/1000 mL, 90 mL/1000 mL, 120 mL/1000 mL and 150 mL/1000 mL water for the respective treatments. This procedure was carried out daily and the filtrate served to the experimental birds in their drinking water.

Source of the experimental birds, experimental design and experimental diets

A total number of 240 day old Hubbard broiler chicks were purchased from Bnot Harel Hatchery from 1 J.K close, Oluyole Extension Ring road Ibadan, Oyo State, Nigeria. The birds were randomly allocated into six treatments level of aqueous *Moringa oleifera* leaf extract (AMOLE) in a completely randomized design experimental model. Each of the treatment had four replicates with ten birds per replicate. The birds were acclimatized for one week and after this the birds were given the experimental treatments. Treatment 1 was the positive control of which ordinary water was given while Treatment 2 were the negative control and was given water and antibiotics; Treatment 3, 4, 5 and 6 were given 60 ml, 90 ml, 120 ml and 150 ml per litre of aqueous *Moringa oleifera* leaf extracts (Table 1), respectively.

Broiler management

Twenty four pen units, with an area of a onesquare meter each that can accommodate twenty broilers were constructed at Abeezainab Farms Broiler Production Unit. The walls and floors of the pens were disinfected with Germicide (Izal) after washing with detergent and water. Litter materials made of old newspapers were used for the first one week of chicks' life.

Table 1: Aqueous Moringa oleifera Leaf extracts inclusion levels

Treatments	Inclusion level
$AMOLE_{0+}$	Positive control(Ordinary water)
$AMOLE_{0-}$	Negative control(1.25g/L)
$AMOLE_{60}$	60 mL/l
$AMOLE_{90}$	90 mL/l
$AMOLE_{120}$	120 mL/l
$AMOLE_{150}$	150 mL/l

Clean and disinfected feeders and drinkers were set in a place accessible to the birds. Each pen unit was properly labelled for easy identification of each treatment group. In addition, a traditional charcoal pot was placed at the strategic area of each pen unit. The distance of the charcoal pot was adjusted based on the response of chicks, weather condition and feather growth. The charcoal pot was removed during the third week when feathers are fully grown. In terms of feeding, a super starter feed containing a crude protein of 26.60 % and metabolizable energy of 559.13kcal/100 g of was given during the first two weeks and the starter feed during the third and fourth week. Finisher feed containing crude protein of 24.85 % and metabolizable energy of 585.68kcal/100 gwas given during fifth week of age till the sixth week. Feeds were given *ad libitum* and shifting from one form of feeds to another was done gradually to avoid digestive disorder. During feeding, a predetermined amount of

feed as well as the leftover was weighed and recorded. After the first one week, old newspaper used as the litter material were removed and replaced with wood shavings. Medications and proper vaccinations were given to the birds based on the recommendations of the Nigerian Veterinary Medical Association (NVMA) as recommended for this region.

Data collection

Determination of the gut morphology and pH

At day 49, four chickens from each replicate were randomly selected and were kept off feed for eight hours but water was supplied to satisfactionwhile the birds were weighed before slaughtering. Each bird was weighed and killed by cervical dislocation, then scalded and de-feathered. The gastro intestinal tract (GIT) of each bird was eviscerated immediately and placed in a tray at room temperature and gently uncoiled to avoid tearing or stretching. The pH was measured with a calibrated digital pH meter (Labtech Digital pH metre from USA). The pH values for different segments of the gastro-intestinal tract were measured by inserting a glass electrode directly in the openings made in the organs with digesta. In order to evaluate the organ weight and morphometrics of the chickens at 49 days of age, a tailor measuring tape and scale (Camry premium weighing scale) were used to measure the length and weight of the gizzard, proventiculus, small intestines and large intestines. respectively. The gizzards were weighed after their contents were removed and cleaned. After removal of the contents, the small and large intestines were cut in segments, cleaned, weighed and measured. The lengths of the small intestines were measured from the site where the duodenum emerges from the gizzard and the beginning of the caeca, while the large intestines were the length of the colon and the rectum. The chickens were weighed before slaughter. Relative organ such as heart, spleen, lung, liver weight of the chickens were determined by weighing each internal visceral and their pH were also measured.

Data analysis

Data obtained on absolute and relative gut morphology and pH of the internal organs were pooled and subjected to one way analysis of variance using SAS version 9.3 (SAS, 2015). Where means were significantly different ($P<0.05$), they were separated using the Duncan Multiple Range Test.

Results and discussion

The effect of aqueous *Moringa oleifera* leaf extracts (AMOLE) on the relative gut morphology weight of Hubbard broiler chickens aged 49 days is presented in Table 2. The AMOLE treatments significantly ($P<0.05$) influenced the dressing percentage, crop, large intestine, kidney and lung. The results showed that the dressing percentageof birds in the positive and the negative control had highest value and were significantly higher ($P<0.05$) than birds on $AMOLE_{120}$ treatments. The reason for this is not known as the result did not follow any specific pattern. However, the result is similar to those of Etalem *et al.* (2013) who found that birds on diet containing 0% *Moringa oleifera* leaf meal (MOLM) had higher dressing percentage than those on MOLM diets. Contrast to this result, Ogbe and Affiku (2012) did not find any difference in dressing percentage of broiler chickens administered polyherbal aqueous extract from *Moringa oleifera*, gum Arabic and wild *Ganoderma lucidum*. In another study, David *et* al. (2012) observed that birds fed *Moringa oleifera* powder meal diet had higher dressing percentage than those on the negative control diet which had 0%

flavomycine (antibiotic growth promoter). The relative crop and large intestinesweightsof birds on $AMOLE_{60}$ treatment had the highest value compared to the other treatments. This might mean that at 60 mL per litre of AMOLE these organs were improved.Large crop means larger compartment for feed storage (Birger, 2014) and larger intestine implies more contents can be stored for reabsorption.

The relative kidney weight of birds on $AMOLE_{90}$ treatments had highest value compare to the other treatments. There are limited studies on the effect of *Moringa oleifera* extract on the kidney of broiler chicken. However, on mice and rat studies showed that *Moringa oleifera* extract has no detrimental effect on the kidney (Patwa *et al.*, 2011). Thus, administration of *Moringa oleifera* extract does not have any negative effect on broiler chickens.

The relative lung weight of birds on $AMOLE_{150}$ treatment had the highest value and were significantly higher ($P<0.05$) than the other treatments. This results is similar to those obtained by Odetola *et al.* (2012) who noted that rabbit feed 15 % MOLM had the highest lung weight. The result of bigger lung weight might be as a result of oedema and thickened inter alveolar septa area as reported by Ojo *et al.* (2013).

Table 2: Effect of aqueous Moringa oleifera leaf extracts on relative gut morphology of Hubbard broiler chickens aged 49 days

Parameters	Treatments						SEM
	Control (Positive)	Control (Negative)	$AMOLE_{60}$	$AMOLE_{90}$	$AMOLE_{120}$	$AMOLE_{150}$	
Live weight (g)	2525	2450	2250	2275	2425	2300	66.67
Dressed %	94.67^{a}	94.93^{a}	93.33^{ab}	91.23^{ab}	89.67^{b}	91.20^{ab}	0.712
GIT (%)	7.37	7.11	6.98	7.76	8.14	7.71	0.218
Crop (%)	3.76^{ab}	4.22^{ab}	4.47^{a}	2.66^{b}	3.51^{ab}	2.57^{b}	0.266
Proventiculus (%)	0.26	0.31	0.31	0.26	0.27	0.31	0.011
Gizzard (%)	1.83	1.92	2.35	2.02	1.99	2.26	0.163
Small intestine (%)	1.62	1.66	1.90	1.66	2.20	1.47	0.010
Large intestine (%)	0.12^{b}	0.14^{b}	0.23^{a}	0.14^{b}	0.09^{b}	0.15^{b}	0.241
Heart (%)	0.32	0.38	0.37	0.39	0.41	0.37	0.015
Liver (%)	1.38	1.19	1.26	1.44	1.40	1.33	0.046
Kidney (%)	0.16^{ab}	0.24^{ab}	0.24^{ab}	0.33^{a}	0.27^{ab}	0.11^{b}	0.026
Lung (%)	0.25^{b}	0.24^{b}	0.30^{ab}	0.21^{b}	0.25^{b}	0.32^{a}	0.011
Spleen (%)	0.07	0.06	0.15	0.11	0.09	0.10	0.013

^{a,b}: Means within rows with different superscripts are significantly different ($p<0.05$)
SEM: Standard Error of Mean
AMOLE: Aqueous *Moringa oleifera* Leaf extracts

The GIT weight, proventiculus, gizzard, small intestine, heart, liver, and spleen weights relative to the live weight were not influenced by the AMOLE treatments.

The effect of aqueous *Moringa oleifera* on the absolute and relative organ length of Hubbard broiler chickens aged 49 days is presented in Table 3. The AMOLE treatments significantly ($P<0.05$) influenced the absolute crop, absolute caecum and relative caecum length. The results showed that the absolute crop length of birds on the positive control treatment had the highest value and were significantly higher ($P<0.05$) than the birds AMOLE and $AMOLE_{150}$ treatments. The crop length seems to be less developed in the AMOLE treated birds than the control particularly those on $AMOLE_{90}$ and $AMOLE_{150}$ treatments. The shorter crop length could be related to the lower dressing percentage of the birds in these treatments. Barash *et*

(1993) reported increased weight of crop when birds were fed one or two times daily than *ad libitum* feeding. The absolute caecum length of birds on $AMOLE_{120}$ had the highest value and were significantly higher than ($P<0.05$) those of birds on $AMOLE_{90}$treatment. Similar to the absolute caecum length, relative caecum length of birds on $AMOLE_{120}$ had the highest value, they were, however, significantly higher ($P<0.05$) than those of birds on control, $AMOLE_{90}$ and $AMOLE_{150}$ treatments. Caecum to a great extend were influenced by diets and the enlarge caecum signifies increased amount of fermentation (Birger, 2014). This might implies that bird on AMOLE treatments had more fermentation going on in their caecum than those on the control treatment. Absolute length of the GIT, proventiculus, gizzard, small intestines and large intestines were not influenced ($P>0.05$) by the treatments (Table 3).

Table 3: Effect of aqueous Moringa oleifera Leaf extracts on absolute (cm) and relative internal organs length (cm) of Hubbard broiler chickens aged 49 days

Parameters	Treatments					SEM
	Control (Positive)	Control (Negative)	$AMOLE_{60}$	$AMOLE_{90}$	$AMOLE_{120}$	$AMOLE_{150}$	
Absolute(cm)							
GIT	208.00	213.00	212.50	203.00	218.00	205.50	3.969
Crop	7.25^{a}	5.50^{ab}	6.50^{ab}	5.00^{b}	6.00^{ab}	5.00^{b}	0.296
Proventiculus	4.75	4.75	4.75	5.00	4.75	4.50	0.097
Gizzard	6.00	6.00	5.75	5.50	6.50	6.25	0.163
Small intestine	162.50	172.50	172.00	160.00	172.50	149.00	3.496
Caecum	17.00^{ab}	18.00^{ab}	18.50^{ab}	15.50^{b}	20.25^{a}	16.25^{ab}	0.567
Large intestine	13.00	11.25	12.75	12.00	12.00	11.50	0.421
Proventiculus	2.28	2.24	2.26	2.49	2.18	2.21	0.076
Gizzard	2.89	2.82	2.73	2.71	2.98	3.07	0.096
Small intestine	78.20	79.75	83.10	79.19	79.12	72.47	1.080
Caecum	8.17^{bc}	8.43^{abc}	8.74^{ab}	7.64^{c}	9.29^{a}	7.90^{bc}	0.180
Large intestine	6.24	5.30	6.03	5.99	5.52	5.62	0.241

^{a,b,c}: Means within rows with different superscripts are significantly different ($p<0.05$)
SEM: Standard Error of Mean
AMOLE: Aqueous *Moringa oleifera* Leaf extracts

The effect of aqueous *Moringa oleifera* on the internal organs pH of Hubbard broiler chickens aged 49 days is presented in Table 4. The AMOLE treatments significantly ($P<0.05$) influenced the gizzard, duodenum, jejunum, ileum, caecum, kidney and lung. The results showed that the pH of the gizzard of birds on the positive controlwere all significantly lower than ($P<0.05$) all the other treatments. Birger (2014) reported that a well-functioning gizzard may reduce survivability of probiotics through an increased retention time and a decreased pH. This could mean that birds on the positive control had a more functional gizzard hence the significant lower pH observed in the treatment.

The duodenum of birds on the positive control, negative control and $AMOLE_{60}$ had higher ($P<0.05$) pH value than those of birds on $AMOLE_{120}$which had similar values with birds on $AMOLE_{90}$ and $AMOLE_{150}$. The jejunal, pH of birds on the positive control had the highest value and it was significantly higher ($P<0.05$) than those of birds on $AMOLE_{120}$ treatment. The chickens on positive control had the highest ileum and caecum pH values and their

values were significantly (P<0.05) higher than those of birds on negative and $AMOLE_{60}$, $AMOLE_{120}$ and $AMOLE_{150}$ treatments.Birds on $AMOLE_{150}$ had the lowest kidney pH value and this value was significantly lower (P<0.05) than those of birds on both negative and positive control treatments. The lung pH values of birds on positive control, negative control and $AMOLE_{60}$ were similar (P>0.05), their values were, however, higher than those of birds on $AMOLE_{120}$, and $AMOLE_{150}$ treatments.

The significant differences observed in the pH of gizzard, duodenum, jejunum, ileum, caecum, kidneyand lung in the present study agree with those of Nkukwana (2012) who reported that the stomach, ileum and caecum pH were influenced at 5 % addition of MOLM and lowest at 10 % addition of MOLM. The variation in the pH of the internal organs could be attributed to the presence of anti-nutritional factors especially tannin which decreased the pH with an increase in tannin concentrations (Muller and Tobin, 1980).

Table 4: Effect of aqueous Moringa oleifera leaf extracts on the internal organs pH of Hubbard broiler chickens aged 49 days

Parameters	Treatments						SEM
	Control (Positive)	Control (Negative)	$AMOLE_{60}$	$AMOLE_{90}$	$AMOLE_{120}$	$AMOLE_{150}$	
Crop	6.28	6.05	6.09	6.74	5.66	5.14	0.208
Proventiculus	5.84	6.27	6.82	6.01	5.84	5.58	0.174
Gizzard	3.76^{b}	7.54^{a}	7.57^{a}	6.97^{a}	6.79^{a}	6.83^{a}	0.400
Duodenum	6.98^{a}	6.86^{a}	6.78^{a}	6.69^{ab}	6.01^{b}	6.44^{ab}	0.112
Jejunum	7.36^{a}	6.84^{ab}	6.68^{ab}	6.76^{ab}	5.79^{b}	6.18^{ab}	0.174
Ileum	7.21^{a}	6.62^{bc}	7.05^{ab}	6.57^{c}	6.65^{bc}	6.33^{c}	0.096
Caecum	7.63^{a}	7.00^{bc}	7.33^{ab}	6.88^{bc}	6.95^{bc}	6.57^{c}	0.109
Kidney	7.21^{a}	7.05^{a}	7.03^{ab}	6.88^{ab}	6.82^{ab}	6.50^{b}	0.079
Liver	6.52	6.52	6.57	6.33	6.30	6.15	0.119
Lung	7.61^{a}	7.68^{a}	7.44^{a}	7.24^{ab}	6.52^{c}	6.84^{bc}	0.131

^{a,b,c}: Means within rows with different superscripts are significantly different (p<0.05)

SEM: Standard Error of Mean

AMOLE: Aqueous *Moringa oleifera* Leaf extracts

Conclusion and recommendations

The results obtained from the present work showed that aqueous *Moringa oleifera* leaf extracts treatments influences gut morphology (dressing percentages and relative weights of crop, large intestines, kidney and lungs; absolute length of crop, caecum) and pH (gizzard, duodenum, jejunum, ileum, caecum, kidney and lung) of the broilers. The inclusion of AMOLE at 150mL had negative effect in lung. Therefore, it can be concluded that the substitution of antibiotics as a growth promoter with aqueous *Moringa oleifera* leaf extracts up to 120 mL improved the morphology of the intestinal segments, tissue accretion and pH in broiler chickens. It is hereby recommended to farmers, poultry producers and nutritionists to administer between 60 ml to 120 ml/litre of AMOLE in the drinking water of broiler chickens to nourish them for good gut morphology and optimum pH of their internal organs.Also, it is recommended that further research should be carried out to investigate other parts (seed, root) of Moringa extracts on performance and gut morphology of chickens.

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