Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without *Moringa oleifera* leaf meal

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Abstract

The intestinal morphology and pH of digesta of broiler chickens at 35 days old were studied. Birds were reared on these dietary treatments (T). T1, positive control, contained 668 g salinomycin and 500 g zinc bacitracin per kg of feed. Treatments, T2, T3 and T4, contained graded levels of *Moringa oleifera* leaf meal (MOLM) per kg of feed, namely starter (1, 3, 5 g), grower (3, 9, 15 g) and finisher (5, 15, 25 g). T5 was a negative control (without supplementation). Birds were provided feed and water ad libitum. Birds in T3 had the highest proventriculus digesta pH, and T5 birds the lowest. Birds that were supplemented with MOLM – and surprisingly those from the negative control – had significantly higher caecal digesta pH, while T1 had the highest ileal digesta pH. Duodenal villous length was longest in T2, and shortest in T4. Jejunal villous length was longest in T3 and shortest in T2 birds. T3 and T4 jejunal villi were widest, with T1 birds having the shortest. Ileal villous was longest in T2 and T5 birds, while T3 birds had the shortest. Duodenal surface area for absorption was larger in T2 and T5, and smaller in T4, while T3 had the largest ileal surface area, and T1 the smallest. The jejunal surface area was largest in T3 (53.2) and T4 (50.7), and smallest in T1 (25.0). The current results reveal a regulatory effect of MOLM on the gastrointestinal tract, which could be attributed to the coarseness of the diets, thus raising the pH and resulting in thicker digesta viscosity, which is a clear sign of a healthy gut.

Keywords: Digestive physiology, intestinal morphology, plant additives, poultry
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Introduction

Perceptions of consumers on raw and cooked meat quality have created significant interest in increasing the understanding of digestive physiology and the dynamics of the gut microflora (Dibner & Richards, 2005). Physiological studies reveal that a functional gastrointestinal tract (GIT) is vital for the digestion and absorption of nutrients required for the bird's maintenance and growth (Mateos *et al*., 2002; Baurhoo *et al*., 2009). Gut integrity, its absorptive capacity and subsequent bird performance are improved by access to feed and water immediately post hatch, accompanied by intake of exogenous feed (Uni *et al*., 1998). Without a healthy gut, the best feed can be formulated, but does not ascertain optimal broiler performance (Yegani & Korver, 2008). The surface area of the intestinal lining (mucosa) is greatly expanded owing to finger-like projections called villi, which in the avian gut exist throughout the length of the small and large intestines, steadily decreasing in height along the way (Hoerr, 2001).

At hatch, the anatomy and functional capacity of the digestive system of chicks are immature, with morphological and physiological changes of the GIT occurring only during the post-hatch period, including increases in the surface area for digestion and absorption (Panda *et al*., 2006). The early development of the GIT, together with supply organs such as the liver and heart, is of high priority in supplying nutrients to the rest of the body and the frame on which muscles accumulate (Zuidhof *et al*., 2006). The action of supply organs in the upper digestive tract of poultry is crucial to degrading feed to a form that is accessible to intestinal digestion since birds do not have teeth to facilitate mechanical breakdown of feed at ingestion (Rodgers *et al*., 2012).

In monogastric animals, dietary fibre content is the main substrate for bacterial fermentation because it cannot be hydrolysed by endogenous enzymes (Montagne *et al*., 2003). Qualitative and quantitative microbial colonization begin soon after birth, and develop gradually with age, rendering the maternal

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intestinal flora and surroundings as the main bacterial proliferation sources to the new-born digestive system (Fortun-Lamothe & Boullier, 2007; Steiner & Wegleitner, 2007). At this stage, this is a crucial process for all growing animals. However, in commercially bred poultry the process of intestinal microflora development is delayed because of the lack of natural contact between chicks and mother hens (Applegate *et al*., 2010). Consequently, the digestive tract becomes burdened with distinguishing between nutrients and non-pathogenic microorganisms from harmful microorganisms while ensuring good nutrient absorption, but excluding digestive microorganisms, harmful and non-harmful (Fortun-Lamothe & Boullier, 2007; Leaphart & Tepas III, 2007).

Recently, a number of additives derived from plants have been reported to contain aromatic properties that affect gut microflora, nutrient digestibility, intestinal morphology and meat quality of poultry, as with antibiotic growth promoters (Cross *et al*., 2007). *Moringa oleifera* Lam (Moringaceae) is documented to have an impressive range of medicinal uses, including growth promotion, and antimicrobial and antioxidant effects (Mbikay, 2012). For instance, cinnamic and caffeic acids, which are common representatives of a wide group of phenylpropane-derived compounds that have high oxidation properties (Cowan, 1999), are found in *M. oleifera* leaves. The nutritional profile of its fresh and dried leaves is reported to contain high levels of lipids and essential amino acids that are important in poultry productivity (Moyo *et al*., 2012) and selenium, a constituent of the cytosolic enzyme, glutathione peroxidase. Glutathione peroxidase is one of the most important antioxidants in the body and of significant importance for the digestive tract, gastrointestinal mucosa and the liver (Bengmark, 1998). Thus, further exploration of such morphological changes in gastrointestinal tissues, which are supposedly caused by plant feed additives, could provide further information on possible benefits to the digestive tract (Windisch *et al*., 2008; Issa *et al*, 2012). The objective of this experiment was to determine the effects of dietary additive supplementation of *M. oleifera* leaf meal (MOLM) on digestive organ size, intestinal morphology and digesta pH of broiler chickens.

Materials & Methods

Fresh, green and undamaged *M. oleifera* leaves were air-dried during the day without exposure to direct sunlight, and being constantly turned over to avert fungal growth. After five days of drying, the leaves were ground to a fine powder to pass through a 0.15-mm sieve. The leaf meal was tightly packaged in polythene plastic bags, sealed and kept at room temperature until required.

The feeding programme consisted of starter (0 to 21 d), grower (22 to 28 d) and finisher (29 to 35 d) basal diets (Tables 1, 2 and 3), which were formulated to meet the birds' dietary nutrient requirements (NRC, 1994). Each basal feed was split into five treatment (T) groups, which were prepared as follows. T_1 was the positive control with 668 g salinomycin/ton and 500 g zinc bacitracin/ton. Graded levels of MOLM were mixed with other ingredients in T_2 (1, 3 and 5 g/kg), T_3 (3, 9 and 15 g/kg) and T_4 (5, 15 and 25 g/kg) in the starter, grower and finisher diets, respectively. T_5 was a negative control with no supplementation. All diets from starter to finisher were pelleted. Proximate analysis was performed on all experimental diets and on MOLM samples according to the methods of the Association of Official Analytical Chemists (AOAC, 2000). The techniques described by Van Soest *et al*. (1991) were used to determine the concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF).

A total of 2400 day-old unsexed Cobb 500 broiler chicks were purchased from a commercial hatchery, weighed and randomly allocated to 30 floor pens containing fresh wood shavings to the depth of 10 cm in an environmentally controlled house. The experiment was a completely randomized design divided into five dietary treatments with six replicate groups of 80 birds per pen (5 diets × 6 replicates). House temperature was set and maintained at 34 ºC during the first week, then reduced by 3 ºC per week until 22 ºC was reached. It was then maintained at this level until the end of the experiment. Birds were vaccinated for Marek's disease and Infectious bursal disease at the hatchery, but vaccines were not administered during rearing. Feed and fresh water were offered ad libitum throughout the 35-day rearing period. Bodyweights (BW) and feed intake (FI) per pen were recorded weekly, and feed per gain was calculated accordingly. Mortality was recorded daily (pen, date, BW and cause). Care and management of birds were in accordance with principles of animal care in experimentation (NRC, 1985). The experiment was also subjected to an assessment for its ethical acceptability and approved by the Ethics Committees of the University of Fort Hare (Animal Ethics No.: NKU01-1SWAP01).

At 35 days old, 12 birds were randomly selected per treatment, two per replicate, and fasted for six hours, with water offered ad libitum. Bodyweight was recorded per bird before they were electrically stunned at 70 volts and slaughtered by cervical dislocation. Birds were exsanguinated by a unilateral neck cut that severed the right carotid artery and jugular vein. After bleeding, scalding, plucking and washing, the feet, head and neck were removed. Carcasses were then eviscerated manually, and the GIT was excised. The small intestine was separated into duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum

(from the pancreatic loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the caecal junction).

Table 1 Analysed *Moringa oleifera* leaf meal composition on as fed basis

Analysed nutrient composition	
Metabolizable energy (MJ/kg)	11.4
Crude protein (g/kg)	267.6
Crude fibre (g/kg)	157.2
Ether extract (g/kg)	56.3
Moisture (g/kg)	78.3
Ash (g/kg)	108.1
Acid detergent fibre (g/kg)	137.9
Neutral detergent fibre (g/kg)	200.8
Analysed mineral composition	
Phosphorus (g/kg)	3.2
Potassium (g/kg)	24.3
Calcium (g/kg)	28.1
Magnesium (g/kg)	10.1
Sodium (g/kg)	8.0
Iron (mg/kg)	192.0
Copper (mg/kg)	5.7
Zinc (mg/kg)	23.8
Manganese (mg/kg)	86.8
Boron (mg/kg)	33.1
Aluminium (mg/kg)	160.0

Table 2 Dietary inclusion levels of *Moringa oleifera* leaf meal in g/kg

Phase feeding regime	Dietary treatments		
	T2	T3	T4
Starter (0 to 21 d)	1	3	5
Grower (22 to 27 d)	3	9	15
Finisher (28 to 35 d)	5	15	25

The pH measurements were performed by inserting the pH meter probe into the distal sections of the proventriculus, duodenum, jejunum, ileum and caecum. The contents of the duodenum, jejunum, ileum and caeca were collected, then the intestinal segments were flushed with distilled water and stored in airtight containers with formalin until required for histology measurements. The liver, gizzard, heart, spleen and bursa were weighed individually, and expressed as percentages of live bodyweight.

For intestinal morphological examinations, three cross-sections measuring 1 mm to 2 mm thick for each segment were prepared and enclosed in tissue cassettes. The tissues were fixed in 10% neutral buffered formalin over 24 h. For each intestinal segment, a 2-µm section was placed onto a glass slide and stained with alcian blue and hematoxylin-eosin. Slides were viewed on the Motic stereo microscope (SMZ-168 series), and visual measurements were taken of the villous height or length (µm: distance from apex of

villus to the junction of the villus and crypt) and villous width (µm: distance from the junction to the basement membrane of the epithelial cell at the bottom of the crypt). For each parameter, six replicate measurements were taken per treatment, and the average of these values was used in statistical analysis. The surface area for absorption was calculated as: (intestinal portion width ÷ 2 × intestinal portion height).

The data on final performance, digestive organ size, intestinal morphology (villous height, width and surface area) and pH were analysed with the analysis of variance (ANOVA) of SPSS 20 (2011). Differences between treatment means were tested according to Duncan's multiple range test of SPSS. Data were presented as the least square means with standard errors. The model used was $Y_{ij} = \mu + \alpha_i + e_{ij}$, where Y_{ij} = response variable, μ = the common mean, α_i = the effect of dietary treatment (T1, T2, T3, T4 and T5) and e_{ij} = the random error. Significant means value differences were evaluated by Duncan's multiple range test. Differences were considered significant at $P < 0.05$.

Table 3 Composition of basal starter, grower and finisher diets on as-fed basis

Item	Phase feeding regime		
	Starter (0 to 21 d)	**Grower (22 to 27 d)**	**Finisher (28 to 35 d)**
Feed ingredients (g/kg)			
Maize	610.3	664.4	660.4
Soya oilcake	311.5	249.1	253.5
Fishmeal	25.4	33.3	40.0
Sunflower oil	20.0	25.0	16.9
Limestone	14.8	14.0	13.6
Monocalcium phosphate	8.6	7.1	7.6
Methionine	3.1	1.7	2.4
Salt	3.3	2.9	3.1
*Vitamin + mineral premix	1.2	1.2	1.2
Sodium bicarbonate	0.9		
Choline chloride	0.8	0.5	0.2
Lysine	0.2	0.6	0.8
Threonine	0.1	0.2	0.3
Analysed composition (g/kg)			
Crude protein	246.2	206.6	200.6
Crude fibre	30.9	14.1	32.8
Ether extract	50.0	54.7	45.2
Calcium	11.2	10.1	9.8
Available phosphorus	7.7	7.8	6.8
Calculated composition			
Metabolizable energy (MJ/kg)	14.1	14.4	13.9

*Supplied per kg of feed: 60 mg iron; 40 mg manganese; 4 mg copper; 70 mg zinc; 0.8 mg iodine; 0.3 mg selenium; 9000 IU vitamin A; 2000 IU vitamin D; 15 IU of vitamin E; 2 mg vitamin K; 1 mg of vitamin B_{12}; 0.30 mg biotin; 250 mg choline chloride; 0.75 mg folic acid; 20 mg niacin; 5.3 mg pantothenic acid; 7.5 mg pyridoxine; 7.5 mg riboflavin and 2.1 mg thiamine.

Results and Discussion

The effects of dietary treatments (T) on final BW, FI, average daily gain (ADG) and feed conversion ratio or feed : gain (FCR) at 35 d old are shown in Table 4. There were no differences in BW, FI, ADG and FCR from 29 d to 35 d old, although in earlier results by Nkukwana *et al*. (2014) on the production

performance of birds in this study BW, FI, ADG and FCR were highest (P <0.05) in birds fed T3 and T4, respectively. Although not significant, there seemed to be a trend towards increased BW and ADG in T3 birds.

According to Rodgers *et al*. (2012), birds given first access to fibre-rich diets take time to adapt to the feed. However, this changes once the gizzard has developed properly, allowing birds to take full advantage of coarse feeds, thereby increasing their energy utilization efficiency. Moreover, bioactive compounds in certain plant additives can increase the secretion of digestive fluids and improve the immune system of broilers (Steiner & Wegleitner, 2007). It is estimated that for every gram of feed ingested, the chicken gut secretes about 2 g of water, which facilitates digestion and absorption (Hoerr, 2001). These findings may explain the situation with birds that were fed T3 and T4 diets, respectively.

Table 4 shows the effect of diets supplemented with or without MOLM on digestive organ size. Neither antibiotics nor MOLM had an effect on the size of digestive organs, except for the bursa of Fabricius, which was significantly larger in T4 birds (P <0.05). Birds do not have teeth to facilitate the mechanical breakdown of feed at ingestion. Hence, the actions of supply organs in the upper digestive tract of poultry are crucial to degrading feed to a form that is accessible to intestinal digestion (Rodgers *et al*., 2012). The early development of the GIT, together with supply organs such as the liver and heart, is of high priority in supplying nutrients to the rest of the body and developing the frame on which muscles accumulate (Zuidhof *et al*., 2006).

Table 4 Effect of diets supplemented with or without *Moringa oleifera* leaf meal (MOLM) on final bodyweight, average daily gain, feed intake, feed conversion ratio (feed : gain) and digestive organ weight of broilers chickens at 35 days old

Experimental period (d) and parameter	Dietary treatments					SEM	*P*-value
	T1	T2	T3	T4	T5		
Performance parameters							
Bodyweight (g)	2119	2147	2236	2174	2177	14.0	0.094
ADG (g)	60.6	61.3	63.9	62.1	62.2	0.4	0.094
Feed intake (g)	3230	3160	3219	3147	3109	18.9	0.218
Feed : gain (g : g)	1.53^{b}	1.47^{ab}	1.44^{a}	1.45^{a}	1.43^{a}	0.01	0.053
Organ size, % of bodyweight							
Liver	2.2	2.2	2.2	2.1	2.4		NS
Gizzard	1.4	1.2	1.3	1.3	1.3		NS
Heart	0.4	0.5	0.5	0.5	0.5		NS
Spleen	0.10	0.09	0.10	0.11	0.11		NS
Bursa of Fabricius	0.18^{ab}	0.14^{b}	0.19^{ab}	0.23^{a}	0.19^{ab}		0.014

$^{a–c}$ Means within the same row that do not share a common superscript are significantly different (P <0.05); n = 6.
T_1, positive control, 668 mg salinomycin, 500 mg zinc bacitracin; T_2, T_3 and T_4 contained graded levels of MOLM at 1%, 3% and 5% of DM intake, respectively, and T_5: a negative control diet with no supplementation.
ADG: average daily gain.

In poultry, the presence of dietary fibre increases the rate of digesta passage as well as the physical capacity of the GIT, but may impair gizzard function and reduce nutrient digestibility and broiler performance if finely ground (Jiménez-Moreno *et al*., 2010). The gizzard is responsible for regulating GIT motility, and its poor development is believed to impair nutrient digestibility and broiler growth (González-Alvarado *et a*l., 2008). In the current study, however, there were no differences in gizzard size. These findings are in agreement with the suggestion that broilers that are in good health may not respond to performance-promoting additives when reared under optimal bio-security conditions (Bozkurt *et al*., 2012).

As shown in Table 5, the various segments of the digestive system had significantly different digesta pH levels (P <0.05). Dietary pH for monogastric animals is usually reported to vary between 5.5 and 6.5 (Ao *et al*., 2008), and changes as digesta transit different segments of the GIT. As expected, digesta in the

proventriculus were acidic, pH being lowest (P <0.05) in birds that were fed low MOLM or negative control diets, while birds fed diets with antibiotics and high MOLM levels, respectively, had slightly less acidic digesta. Digesta pH drops gradually as digesta reach the proventriculus or glandular stomach, where hydrochloric acid and pepsinogen are secreted and mixed with digesta through muscular movements in the gizzard (Svihus, 2014). Feed form may also play a role in the digesta pH in the stomach. Svihus (2011) reported that average values in stomach pH for broiler chickens ranged between 3 and 4 for normal pelleted diets.

Table 5 Least square means (± SE) on the effects of diets supplemented with or without *Moringa oleifera* leaf meal on intestinal digesta pH of broiler chicken at 35 d old

Intestinal portion	Dietary treatments					*P*-value
	T1	T2	T3	T4	T5	
Proventriculus	3.7^{ab} ± 0.34	3.3^{ab} ± 0.24	3.9^{a} ± 0.26	3.7^{ab} ± 0.23	3.1^{b} ± 0.24	0.048
Duodenum	5.3^{ab} ± 0.14	5.6^{ab} ± 0.07	5.2^{b} ± 0.14	5.6^{ab} ± 0.11	5.7^{a} ± 0.24	0.047
Jejunum	5.7^{ab} ± 0.09	6.0^{a} ± 0.13	5.7^{ab} ± 0.07	5.9^{ab} ± 0.13	5.5^{b} ± 0.24	0.044
Ileum	6.6^{a} ± 0.12	6.1^{ab} ± 0.21	5.7^{b} ± 0.35	5.9^{ab} ± 0.31	6.3^{ab} ± 0.23	0.021
Caecum	6.1^{b} ± 0.09	6.4^{a} ± 0.10	6.5^{a} ± 0.14	6.6^{a} ± 0.03	6.6^{a} ± 0.06	0.003

$^{a–b}$ Means within the same row that do not share a common superscript are significantly different (P <0.05).
T_1, positive control, 668 mg salinomycin, 500 mg zinc bacitracin; T_2, T_3 and T_4 contained graded levels of MOLM at 1%, 3% and 5% of DM intake, respectively: T_5: negative control diet with no supplement.

The gizzard, which is in close proximity to the stomach, had lower pH levels (Table 5). Besides, the pH of gizzard contents has been shown to decrease by a magnitude of 0.2 to 1.2 units when structural components or fibre materials are added to broiler diets (González-Alvarado *et al*., 2008; Sacranie *et al*., 2012). This may have been the case with birds that were supplemented with higher MOLM levels, as observed in the pH of their stomach digesta. The duodenal pH across treatments was lower than in the jejuna and ileal digesta, particularly in T1 (P <0.05). Morphologically, this might have been expected. Khalaji *et al*. (2011) studied the effects of dried *Artemisia sieberi* leaves on digesta pH in broiler chickens, and reported a significant increase in pH of jejuna contents.

Nonetheless, the ileum, the last segment of the small intestine, which ends at the ileo-ceco-colic junction, had slightly higher pH levels than the jejunum (Table 5). Ileal pH was highest (P <0.05), which may be due to the regulatory effect of antibiotic growth promoters. The ileum is thought to play a role as a site for water and mineral absorption, although some digestion and absorption of fat, protein and starch may occur. The functionality of the caeca is affected largely by diet, and the caeca enlarge because of an increased amount of fermentable material in the diet (Svihus, 2014), which may explain the rise in pH towards alkalinity. Gastric acidity can be detrimental to some of the microflora that inhabit the hindgut. Thus, maintaining caeca pH is important in promoting gut health (Ricke, 2003).

The effects of dietary supplementation with or without MOLM on the villi height, width and surface area in the duodenum, jejunum and ileum of broiler chickens are shown in Table 6. Birds fed T2 diets had the longest duodenal villous (P = 0.002), while T4 birds had the shortest. The jejunum villous was longest in T3 birds (P <0.001), and shortest in T2. Post hatch, the GIT undergoes morphological changes, resulting in increases in intestinal length, villous height and density, which are accompanied by rises in pancreatic and digestive enzymes activity (Panda *et al*., 2006).

Jamroz *et al*. (2006) studied the effects of maize diets supplemented with plant extracts on broiler chickens. They reported a significant increase in jejuna wall villi. Apparently, rapid increases in villous height and surface area occur at different rates in chick intestinal segments, reaching a plateau at 6 to 8 days in the duodenum and after 10 days in the jejunum and ileum (Uni *et al*., 1996). Long villi are correlated with improved gut health, and an increase in duodenal and jejuna height or length (Baurhoo *et al*., 2009). Thus, birds whose GIT were stimulated earlier in life show better response to growth performance and nutrient utilisation efficiency.

As shown in Table 6, birds that were fed T3 and T4 had the widest jejuna villous (P <0.001). But, as reported previously, narrow or thinner villous width, as observed in T1, does not necessarily mean that they are susceptible to breakage, resulting in contamination of carcasses during processing (Miles *et al*., 2006). In T1 birds, however, the antibiotic effect may not be ruled out in reducing villi properties. Miles *et al*. (2006)

reported the smallest total villous area and shortest villous height and crypt depth in the ileum of chicks that were supplemented with the antibiotic virginiamycin. On the other hand, *M. oleifera* leaves contain glutathione, a conjugate element of glutamate, the most abundant amino acid in blood, which plays a vital role in maintaining mucosal integrity (Rao & Samak, 2012). However, other than their health status, it is difficult to explain why birds on T5 diets had longer and wider intestinal segments than those on T1 diets. Although not significant, T5 birds exhibited a wider ileum even than the T4 birds.

Table 6 Least square means (± SE) on the effects of diets supplemented with or without *Moringa oleifera* leaf meal on small intestine morphology of broiler chicken at 35 d old

Intestinal portion	Dietary treatments					*P*-value
	T1	T2	T3	T4	T5	
Villous height (µm)						
Duodenum	26.4^{a} ± 1.52	28.4^{a} ± 1.54	21.2^{b} ± 1.89	19.9^{b} ± 0.84	26.3^{a} ± 0.46	0.002
Jejunum	18.7^{bc} ± 0.90	16.6^{c} ± 0.72	22.3^{a} ± 0.67	20.7^{ab} ± 1.12	20.4^{ab} ± 0.35	<0.001
Ileum	6.4^{c} ± 0.54	12.1^{a} ± 0.93	7.5^{c} ± 0.42	12.0^{a} ± 0.23	9.9^{b} ± 0.32	<0.0001
Villous width (µm)						
Duodenum	3.8 ± 0.34	4.2 ± 0.12	4.2 ± 0.34	4.2 ± 0.19	4.6 ± 0.29	0.481
Jejunum	2.7^{c} ± 0.25	3.5^{b} ± 0.18	4.8^{a} ± 0.26	4.9^{a} ± 0.18	4.3^{a} ± 0.27	<0.0001
Ileum	3.5^{b} ± 0.39	4.8^{a} ± 0.51	3.1^{b} ± 0.37	3.9^{ab} ± 0.23	4.8^{a} ± 0.35	0.019
Villous surface area (µm)						
Duodenum	51.1^{ab} ± 7.10	59.6^{a} ± 4.16	43.9^{b} ± 4.94	41.8^{b} ± 3.53	59.7^{a} ± 3.64	0.052
Jejunum	25.0^{c} ± 1.64	28.9^{c} ± 2.37	53.2^{a} ± 2.65	50.7^{ab} ± 4.25	43.6^{b} ± 2.54	<0.0001
Ileum	11.0^{b} ± 1.68	29.6^{a} ± 5.01	11.5^{b} ± 1.10	23.3^{a} ± 1.14	23.6^{a} ± 1.49	<0.0001

$^{a–c}$ Means within the same row that do not share a common superscript are significantly different (P <0.05).
T_1: positive control, 668 mg salinomycin, 500 mg zinc bacitracin; T_2, T_3 and T_4 contained graded levels of MOLM at 1%, 3% and 5% of DM intake, respectively, and T_5: a negative control diet with no supplementation.

The jejunum is largely responsible for digestion and absorption of all the major nutrients. Thus, increased villi height may be a consequence of greater need for digestive capacity (Svihus, 2014). The epithelial cells lining the villi facilitate the absorption of fluids and nutrients, secrete electrolytes and fluids, and regenerate and replace damaged cells or those lost to normal attrition (Hoerr, 2001). This may be the reason for the largest jejuna villi surface area (P <0.0001) that was observed in T3 and T4 birds. As reported by Paul *et al*. (2007), the increase of villous height of various small intestine segments may be attributed to the role of the intestinal epithelium as a natural barrier to pathogenic bacteria and toxic substances that are present in the intestinal lumen. Furthermore, *M. oleifera* leaves contain short-chain carbohydrates, glycosides, which are reported to cause major increases in the height of the jejuna villi compared with AGP and AGP-free diets (Baurhoo *et al*., 2009).

The villi greatly expand the surface area of the intestinal lining (mucosa), which in the avian gut exist throughout the length of the small and large intestines, steadily decreasing in height along the way (Hoerr, 2001). This was evident in the current study, as the duodenal surface area for absorption was largest in T2 and T5 birds, and smallest in T4 birds (Table 6). The ileal villous surface area was largest in T3 birds and smallest in T1. The enhanced growth performance reported in Nkukwana *et al*. (2014) in birds that were supplemented with MOLM is directly related to the improved intestinal morphology and villous surface area.

Albeit it is not clear why a similar effect was not obtained in birds that were fed diets containing antibiotics, Baurhoo *et al*. (2009) reported improved gut integrity, as measured by changes in villi height, goblet cell number, and populations of beneficial bacteria, lactobacilli and bifidobacteria in the ceca of chicks that were fed fibre-rich diets. Thus, it is possible that the presence of short-chain monosaccharides in *M. oleifera* leaves was the main component that functioned as a competitive attachment site for pathogenic

bacteria, carrying them out of the gut, rather than binding to the intestine, thus enhancing the growth of beneficial gut microflora. Moreover, the fluid in the upper small intestine plays a protective role, keeping pathogenic bacteria in suspension and washing them downstream, while encouraging the proliferation of growth-enhancing bacteria (Hoerr, 2001).

Conclusion

Findings from this study showed that ground *M. oleifera* leaves can be incorporated in broiler diets as a phytogenic feed additive up to 25 g/kg with significant positive effects on intestinal morphology, digestive organ size and digesta pH. Although the differences in the intestinal morphology and digesta pH of antibiotic and MOLM supplemented birds are difficult to explain, this study supports the hypothesis that under good hygienic conditions, strict biosecurity measures, clean litter, good ventilation and low stocking densities, bird health and productivity may be maintained. As such, there may be no need for antibiotics in the feed, as there would be minimum bacterial proliferation in the chicken gut and the environment. Further studies are necessary to evaluate the effects of ground *M. oleifera* leaves on gut microbial development and profile, ileal nutrient digestibility from 7 d to 35 d old, caecal fermentation and the production of volatile fatty acids.

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