

Effect of *Acacia angustissima* Leaf Meal on the Physiology of Broiler Intestines

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Abstract

The effect of *A. angustissima* leaf meal based diets on intestinal physiology and dressed carcass weight was investigated on 150 day old chicks. Three diets, 0, 5 and 10% *A. angustissima* were used in a three phase feeding programme. At days 14, 28 and 42, two birds from each replicate were slaughtered, dressed and weighed. The weights and lengths of the duodenum, jejunum and colon were measured. Approximately 1 cm specimen was taken from each organ, fixed in formalin and stained for histological analysis. Using a light microscopy, the digestive and absorptive properties of the tissues were assessed. Inclusion of *A. angustissima* leaf meal increased intestinal wall thickness, epithelial thickness, and villus height of the duodenum ($P < 0.05$). There was no effect on the proportional weight and length of the jejunum, jejunum villi height and villi width ($P > 0.05$) but jejunum wall thickness decreased with increasing levels of the leaf meal ($P < 0.05$). Leaf meal inclusion resulted in an increase in weight, intestinal wall and mucosal thickness of the colon ($P < 0.05$) and a decline in sub-mucosal fold height and haustra coli width of the colon ($P < 0.05$). Dressed weight was the same across diets at two weeks ($P > 0.05$). At four and six weeks, broilers on the control and 5% diet had superior dressed weights to the 10% fed broilers ($P < 0.05$). It was concluded that for intestinal physiological adaptation that will not compromise weight gain in broilers, up to 5% *A. angustissima* could be included in broiler diets.

Keywords: *Acacia angustissima* leaf meal, crude fiber, digestive, duodenum, jejunum, morphology

1. Introduction

Poultry production has a major role to play in the economy of developing countries (Shaikh & Zala, 2011; Oladokun & Jonhson, 2012; Sambo et al., 2015) but the cost of feed is thwarting production (Casartelli, Filardi, Junqueira, Laurentiz, & Duarte, 2006; Moreki, 2011; Ja'afar-Furo & Gabdo, 2010; Ntuli & Oladele, 2013). Increasingly the deficit of traditional ingredients such as soyabean (Martens, Temann, Bendelle, Peters, & Lascano, 2012; Mutayoba, Dierenfed, Mercedes, Frances, & Knight, 2011), increasing trends of severe droughts (Sheffield & Wood, 2010) and competition for feed resources between humans and chickens (Ncube, Hamudikuwanda, & Banda, 2012a) continue to be blamed for high cost of poultry feeds. This has come to the attention of scientist, thus the search for alternative protein poultry ingredients and especially those from drought tolerant plants also not attracting competition between people and animals.

Among the ingredients of interest is leaf meal from *Acacia angustissima*, a tropical legume multiple-purpose tree of the Mimosaceae family (Dzowela, 1994). The tree is adaptable to drought conditions (Dzowela, 1994) and produces up to 12.4 t Ha⁻¹ of biomass (Preece & Brook, 1999). A number of scientists have reported reasonable crude protein levels from the leaf meal ranging from 19%-26.5% (Rubanza, Shem, Bakengesa, Ichinohe, & Fujihara, 2007; Odenyo et al., 2003; McSweeney et al., 2005; Mukandiwa, Mugabe, Halimani, & Hamudikuwanda, 2010; Ncube et al., 2012a; Gusha, Ngongoni, & Halimani, 2013), indicative of its potential use as a protein source. Its potential in broiler diets has already been established (Ncube et al., 2012a; Ncube, Hamudikuwanda, & Saidi, 2012b; Ncube, Saidi, & Halimani, 2015) but research supporting its use in broiler diets is still at its infancy. Most of the information available relate to its chemical composition (Reed et al., 2001; Odenyo et al., 2003; McSweeney et al., 2005; Mukandiwa et al., 2010; Gusha et al., 2013), appropriateness of processing techniques and harvesting stage for broiler feeding (Ncube et al., 2015) and effect on growth performance (Ncube et al., 2012a, 2012b).

Not much has been established regarding its effect on the physiology of gastrointestinal organs. Ncube et al. (2012b) noted a gross increase in the proportional weight of the intestines with increasing levels of the leaf meal. However the study did not go further to check the actual physiological changes causing intestinal weight increases. Such investigations are important because the gastrointestinal tract is the primary surface of contact with digesta (Koutsos & Arias, 2006). Its physiological state will determine the digestive and absorptive capacity of the intestine, and consequently growth performance of broilers (Roberts et al., 2005; Haoyu, Ivarsson, Lundh, & Lindberg, 2013). Thus, it is important to investigate the effect of the leaf meal on the digestive and absorptive properties of intestines. Such information would be helpful in determining inclusion levels that support the development of the gastrointestinal tract without interfering with live weight gain. It was the objective of this study therefore to assess the physiological adaptation of the broiler intestines to increasing levels of *A. angustissima* leaf meal and how these may influence carcass weight. The study hypothesized that broiler intestines are able to adjust to increasing levels of *A. angustissima* leaf meal in their diets without a compromise on carcass weights.

2. Materials and Method

Acacia angustissima leaves were harvested at mid-maturity, air dried (Ncube et al., 2015) and ground through a 1 mm sieve. Dry matter, crude protein, crude fiber and ash were determined using AOAC (1990) methods and condensed tannins were determined using the Butanol-HCL method (Porter, Hrstich, & Chan, 1986) (Table 1). Three iso-nitrogenous and iso-energetic diets were formulated for a three phase feeding programme at 0%, 5% and 10% leaf meal inclusion (Table 2). Formulation was done using Spesfeed Express Formulation programme from Capital Foods, Harare, Zimbabwe, targeting, the National Research Council recommended nutrient levels for broilers (NRC, 1994). The diets were analysed for dry matter, crude protein, crude fiber, crude ash, P and Ca (AOAC, 1990), condensed tannins (Porter et al., 1986).

A total of 150 day old unsexed Cobb 500 chicks with an average weight of 41.7 ± 1.560 g, were randomly allocated to 15 cages of 1 m width \times 1 m length, with 10 birds per cage. The cages were randomly allocated to the three diets, replicated five times in a completely randomized design. The cages were located in a temperature controlled room. The starter, grower and finisher diets were fed from week 1 to 2, week 3 to 4 and week 5 to 6, respectively. Feed and water were provided *ad libitum* throughout the trial. All other growing conditions were constant across treatments. All procedures in this experiment followed guidelines by the Zimbabwe Scientific Animal Act, 1963, subsection 2 of section 4, License Number L624.

Table 1. Chemical composition of *Acacia angustissima* leaf meal

Chemical component	Percentage (%)
Dry matter	90.00
Crude Ash	4.77
Crude protein	23.40
Crude fibre	13.00
Calcium	0.94
Phosphorus	0.17
Condensed tannins	1.06

Table 2. Ingredient and chemical composition of the diets

Ingredient(kg)	Starter Diets			Grower Diets			Finisher Diets		
	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2	Control	Diet 1	Diet 2
Soya Meal, 46% CP	30.00	25.00	20.00	18.7	13.70	8.70	18.60	13.60	8.60
Meat and Bone Meal, 45% CP	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Sorghum meal, 9% CP	10.00	0.00	10.00	0.00	9.90	10.00	0.00	0.00	0.00
Acacia leaf meal, 23% CP	0.00	5.00	10.00	0.00	5.00	10.00	0.00	5.00	10.00
Blood meal 80% CP	0.00	0.00	0.00	0.00	2.00	3.00	1.20	1.80	3.00
Sunflower cake	2.50	1.30	0.00	1.70	1.50	2.10	0.00	0.00	0.00
L. Threonine	0.06	0.06	0.03	0.05	0.00	0.45	0.00	0.00	0.00
Soya oil	0.00	0.00	0.00	0.00	1.60	3.00	0.00	1.30	2.40
Wheat bran	0.00	0.00	2.10	0.00	0.00	0.00	0.00	0.00	0.00
Soya oil	0.00	0.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00
Maize meal, 8% CP	48.60	56.90	44.00	68.1	55.00	51.40	73.00	70.40	67.50
Fish meal 65% CP	1.20	4.90	5.00	4.6	4.60	5.00	0.10	1.00	2.00
DL Methionine	0.30	0.29	0.79	0.19	0.16	0.11	0.15	0.15	0.07
Lysine HCL	0.26	0.22	0.28	0.21	0.14	0.12	0.00	0.00	0.00
Monocacium phosphate	0.50	0.30	0.30	0.2	0.30	0.30	0.16	0.15	0.07
Limestone	0.88	0.43	0.40	0.65	0.50	0.27	0.74	0.55	0.36
Salt	0.40	0.30	0.30	0.3	0.30	0.25	0.35	0.35	0.30
Broiler Premix ¹²³	0.30	0.30	0.30	0.3	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100	100	100	100
Chemical composition									
Crude protein (g/kg)	226.00	226.13	225.28	199.90	199.74	200.12	175.00	174.94	174.93
ME (MJ/kg)	12.50	12.46	12.39	13.09	13.07	13.08	13.20	13.21	13.18
EE (g/kg)	36.80	39.04	51.94	41.64	55.17	67.71	39.19	51.27	61.45
CF (g/kg)	41.50	40.15	49.98	34.38	39.88	46.84	31.90	37.88	43.86
Ca (g/kg)	9.98	9.52	9.88	9.22	5.59	5.60	4.93	8.63	8.74
P (g/kg)	7.08	7.10	7.04	6.53	6.61	6.58	6.00	6.02	6.08
Condensed tannins (%)	0.004	0.059	0.076	0.0036	0.056	0.083	0.0043	0.055	0.077

Note. ¹ Composition: 9.9 u.i vitamin A, 1.95 u.i vitamin D₃, 30 u.i vitamin E, 2.9 g Vitamin K₃, 2 g Vitamin B₁, 7.5 g Vitamin B₂, 30 g Vitamin PP Niacin, 12.1 g Vitamin B₅, 3 g Vitamin B₆, 1 g vitamin B₉ Folic Acid, 150 mg Vitamin B₇/Biotin, 20 mg Vitamin B₁₂, 300 g Choline, 60 g Iron, 10 g Copper, 100 g Manganese, 100 g Zinc, 1 g, Iodine, 0.5 g Cobalt, 300 mg Selenium;

² Composition: 8 u.i vitamin A, 2 u.i vitamin D, 25 u.i vitamin E, 2 g Vitamin K₃, 1.75 g Vitamin B₁, 6 g Vitamin B₂, 25 g Vitamin PP Niacin, 10 g Vitamin B₅, 2 g Vitamin B₆, 1 g vitamin B₉ Folic Acid, 100 mg Vitamin B₇/Biotin, 15 mg Vitamin B₁₂, 250 g Choline, 50 g Iron, 8 g Copper, 80 g Manganese, 80 g Zinc, 1 g, Iodine, 0.5 g Cobalt, 250 mg Selenium;

³ Composition: 6 u.i vitamin A, 1.5 u.i vitamin D₃, 20 u.i vitamin E, 1.5 g Vitamin K₃, 1.5 g Vitamin B₁, 5 g Vitamin B₂, 25 g Vitamin PP Niacin, 8 g Vitamin B₅, 1.5 g Vitamin B₆, 0.6 g vitamin B₉ Folic Acid, 80 mg Vitamin B₇/Biotin, 15 mg Vitamin B₁₂, 200 g Choline, 40 g Iron, 6 g Copper, 80 g Manganese, 60 g Zinc, 1 g, Iodine, 0.25 g Cobalt, 200 mg Selenium.

At week 2, 4 and 6, two chicks from each replicate were slaughtered and dressed. Dressed weight was measured. The duodenum, jejunum, and colon were removed and weighed. Intestinal length was measured. The weight and lengths of organs were expressed as proportions of hot dressed weight. Of each intestinal segment 1 cm was cut, intestinal contents were flushed with distilled water and fixed in 10% saline formalin. The fixed fragments were stained on slides using the procedure described by W. Bacha and L. M. Bacha (2000). Using a Leitz MD5 light microscope fitted with an eye piece graticle, the functional capacity of the duodenum, jejunum and colon was assessed as follows:

At x4 magnification, 10 observations were recorded for each of the following duodenum and jejunum morphometric parameters: intestinal wall thickness, epithelial thickness, villus height and villus width.

Measurements for each parameter were averaged into one value per bird. On the colon, ten points along the intestines were chosen to measure intestinal wall thickness, mucosal layer thickness, semi-luna fold height and width and haustra coli width at x4 magnification and mean values calculated. To determine the effect of diet on intestinal morphology and dressed weight, ANOVA was computed using the General Linear Model Procedure of SAS (2000) version 9.3. Comparison of means was done using Tukey's test.

3. Results

Inclusion of leaf meal resulted in an increase in thickness of the duodenum wall ($P < 0.05$). The 5% leaf meal fed birds exhibited the thickest duodenum wall which was not different from the 10% fed birds (Table 3). Duodenum villi height increased significantly with increasing leaf meal levels ($P < 0.05$) (Table 3). Birds fed the control diet recorded the shortest villi height while those fed the 10% diet exhibited the longest villus. The average width of duodenum villus was the same across treatments ($P > 0.05$). Duodenum epithelium thickness increased significantly with addition of leaf meal in the diet ($P < 0.05$) (Table 3).

Table 3. Effect of increasing levels of *A. angustissima* leaf meal of duodenal parameters

Duodenum Parameter	Control	5% A.A	10% A.A	SE
Proportionate weight (%)	1.29	1.41	1.39	0.048
Proportionate length (%)	40.13 ^a	43.26 ^a	48.60 ^b	1.748
Wall thickness (mm)	0.21 ^a	0.34 ^b	0.33 ^b	0.011
Villi height(mm)	0.88 ^a	0.98 ^{ab}	1.11 ^b	0.036
Villi width (mm)	0.11	0.11	0.11	0.003
Epithelial thickness(mm)	1.17 ^a	1.22 ^{ab}	1.32 ^b	0.040

Note. ^{abc} Within a row, means without a common superscript differ ($P < 0.05$).

Treatment did not affect the proportional weight and length of the jejunum, jejunum villi height and villi width ($P > 0.05$) but affected jejunum wall thickness ($P < 0.05$) (Table 4). The jejunum wall thickness decreased with increasing levels of leaf meal in the diet ($P < 0.05$) (Table 4). The thickest wall was from the control fed birds while the 10% leaf meal fed birds exhibited the thinnest jejunum wall.

Table 4. Effect of increasing levels of *A. angustissima* on jejunum parameters

Jejunum Parameter	Control	5% A.A	10% A.A	SE
Proportionate weight (%)	3.040	3.071	4.462	0.6433
Proportionate length (%)	88.100	86.470	93.060	3.1391
Wall thickness (mm)	0.340 ^a	0.301 ^b	0.259 ^b	0.0140
Villi height (mm)	0.550	0.572	0.597	0.0203
Villi Width (mm)	0.110	0.111	0.102	0.0032
Epithelium thickness (mm)	0.800	0.823	0.846	0.1975

Note. ^{abc} Within a row, means without a common superscript differ ($P < 0.05$), A.A = *A. angustissima*.

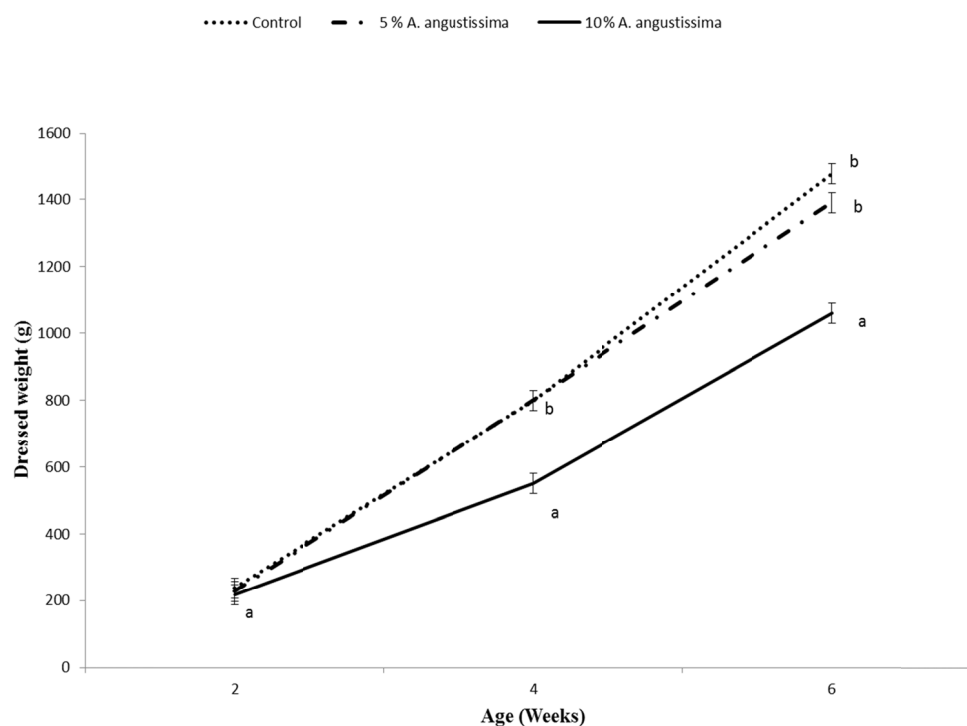
Weight of the colon increased by 51.51% and 36% on addition of 5% and 10% leaf meal, respectively ($P < 0.05$) (Table 5). The intestinal wall and mucosal thickness of the colon increased by 52.80 and 20.31% on inclusion of the 5 and 10% leaf meal, respectively ($P < 0.05$) (Table 5). The increases at 10% inclusion of the leaf meal were only numerical and not different from the control fed birds ($P > 0.05$). A significant decline in sub-mucosal fold height and haustra coli width was noted at 10% leaf meal inclusion ($P < 0.05$) (Table 5).

Table 5. The physiological response of the colons to graded levels of *A. angustissima* leaf meal

Colon Parameter	Control	5% A.A	10% A.A	SE
Proportionate weight (%)	0.330 ^a	0.500 ^b	0.450 ^b	0.0324
Proportionate length (%)	11.670	12.530	12.930	0.6281
Wall thickness (mm)	0.536 ^a	0.819 ^b	0.571 ^a	0.0310
Mucosal layer thickness (mm)	0.396 ^a	0.599 ^b	0.475 ^a	0.0274
Sub-mucosal fold width (mm)	0.174	0.207	0.159	0.0180
Sub-mucosal fold height (mm)	0.479 ^a	0.405 ^{ab}	0.336 ^b	0.0303
Haustra coli width (mm)	1.094 ^a	0.871 ^{ab}	0.789 ^b	0.0891

Note. ^{abc} Within a row, means without a common superscript differ ($P < 0.05$).

The effect of treatment on dressed weight depended on the age of the bird ($P < 0.05$) (Figure 1). No differences among treatments were noted at two weeks of age ($P > 0.05$). At four and six weeks of age, dressed weights from the control and 5% fed broilers outperformed the 10% fed birds.

Figure 1. Effect of increasing levels of *A. angustissima* leaf meal on dressed weight at different ages of growth

4. Discussion

The morphological changes in the duodenum and colon indicate increased digestive and absorptive capacity of the intestines. The increase in the length and weight of the intestinal segments in the current study is a physical adaptation to presence of the *A. angustissima* leaf meal. This represent enhanced development of the intestinal segments (Jiang et al., 2012) and thus increased capacity and volume, possibly as a result of greater quantity than quality of feed (Chiou, Lu, Hsu, & Yu, 1996). The increase in intestinal volume was therefore a physiological adaptation to take care of increased feed volumes associated with the leaf meal based formulations. While high fiber content in the leaf meal based formulations imply increased feed volume, it is also associated with a decrease in nutrients density (Shafey, Almufarrij, & Albatshan, 2013) possibly explaining the compromised growth from the 10% fed broilers even after the increase in intestinal capacity.

Increase in the length of the duodenum could also be a result of an increase in gastro-duodenal refluxes as triggered by the high fiber content in leaf meal based diets (Mateos, Jimenez-Monero, Serrano, & Lazaro, 2012;

Martens et al. (2012). In general, increase in fiber component of the diet increases digesta reflux between the gizzard and the duodenum (Sacranie, Svihus, Denstadli, Moen, Iji, & Choct, 2012). Such a response can be explained by the need to prolong the feed to both chemical and mechanical digestion for increased nutrient release. The increases in the colon could be a response to increased bulkiness of the feed due to dietary fiber (Chiou et al., 1996).

Although the adaptation by the duodenum and the colon could represent an increase in capacity of the organs, at 10% inclusion of the leaf meal, growth is negatively affected during the last two phases of feeding. Intestinal adaptation to 10% leaf meal inclusion could support weight gain during the starter phase as noted by the none significant effect on dressed weight. During the grower and finisher phases of feeding, the dressed weight indicated failure of intestinal adaptation to support weight gain at 10% leaf meal inclusion. This due to diversion of nutrients from growth of edible carcass (Iyayi, Ogunsola, & Ijaya, 2005) growth of the gastrointestinal organs. An increase in intestinal capacity is also associated with increasing energy and amino acid requirements for maintenance by the animal (Johnston & Noll, 2003). Thus, the increase in the intestinal weights and length at 10% inclusion of the leaf meal do not translate to good carcass weights. This concurs with findings by Ncube, Hamudikuwanda, and Saidi (2012c) who concluded that continued use of *A. angustissima* leaf meal diet at 10% compromised growth of birds. Shafey et al. (2013) also noted an increase in intestinal capacity after feeding olive leaves and this was also associated with a decline in growth performance of birds as olive leaves increased in broiler diets.

Thickening of the duodenum and colon muscle layer with increasing leaf meal could also be explained by the intense work load or effort by the intestines in propelling digesta along the digestive tract as dietary crude fiber in diets increased. Any thickening of the muscle layer along the digestive tract represents stronger movements during digesta transportation. It is also indicative of a highly activated digestion and absorption function (Al-Tememy, Al-Jaff, Al-Mashhadani, & Hamodi, 2011). Unlike in the duodenum and colon, the jejunum muscle layer decreased in thickness with addition of the leaf meal. It is not clear why the response differed in the jejunum because according to Block, Vahl de Lange, Van de Braak, Henke, and Hessing (2002), when birds are fed diets with poorly enzymatically digestible materials, intestinal walls should get thicker. The different responses could be related to the roles of the intestinal segments in digestion. The duodenum muscle is also involved in the grinding cycle together with the gizzard (Svihus, 2014), and this could easily explain the thickening of the muscle. Presence of fiber in poultry feed increases the intensity of gastro-duodenum refluxes (Mateos et al., 2012). The duodenum area is also the main site for mixing digesta and secretions while the colon is responsible for transportation of digesta to the cloacae (McDonald et al., 2010), thus thickening of the walls of these two intestinal segments could be representative of their functions. While the increase in crude fiber delays passage of feed from the gizzard for further grinding, it is also said to accelerate the passage of feed through the intestines. The involvement of the duodenum in the grinding cycle could better explain its thickening while the decrease in intestinal thickness in the jejunum could possibly be explained by the faster rates of digesta movement along the segment.

Because the colon's main motility is through haustra contractions (Sherwood, 2012), significant decreases in haustra width with increasing levels of leaf meal represent the shortening of distance between any two semi-luna folds, thus more folds within a length. Haustra are produced by circular and longitudinal muscle contractions that narrow the lumen and shorten the colon (Alpers & Yamanda, 2009). More folds in the colon would result in increases in the propulsive force, increasing rate of bolus passage along the colon. The increasing fiber content in leaf meal based diets is likely to have stimulated colon motility (Staniforth, Baird, Fowler, & Lister, 1991; Brownlee, Dettmar, Strugala, & Pearson, 2006; Zeng, Lazarova, & Bordonaro, 2014) resulting in increases in the number of colon folds. Such segmental contractions result in increased water absorption and electrolyte exchange (Maykel & Opelka, 2004) and possibly reduced contact time between digesta and intestinal lumen. This is important as it reduces colon mucosal exposure to potential damaging agents of either endogenous or exogenous origin (Brownlee et al., 2006) while allowing the colon to absorb as much water as is possible.

The increase in villi height could have been triggered by the inefficiencies associated with the presence of leaf meal in the diet. Since intestinal tissue possesses the ability to adapt to the nutritional environmental (Mitchell & Moreto, 2006), the increase in villi height could have been an attempt to increase the digestive and absorptive capacity as the inefficiencies associated with presence of the leaf meal increased. Increased villi height suggests an increased surface area for satisfactory digestive enzyme action and higher nutrient absorption (Dibner & Richards, 2004; Watson, 2009; Varastegani & Dahlan, 2014; Shomali et al., 2015). Increase in total epithelial thickness of the intestines is indicative of suboptimal digestion due to the presence of the leaf meal. According to Bedford (2006), the general perception is that increase in size of the gastro-intestinal tract components represents

suboptimal digestion, thus the need for the physiological adaptations. However, such adaptations to match digestion and absorption in normal diets may actually cost the broilers more energy than the benefits. As a result growth may be affected and linked to this is a decline in feed efficiency (Bedford, 2006). This is attributed to the fact that nutrients will first be directed towards maintenance of the chicken's current biological state (Dibner & Richards, 2004; Watson, 2009; Shaw, Gohil, & Basson, 2012) and only after that will any additional nutrients be directed towards growth. Thus, at 10% inclusion of the leaf meal, although the digestive and absorptive capacities of the intestines increased, beyond two weeks of age, carcass weights were negatively affected. The increase in functional capacities during the starter period for birds on the leaf meal based diets did not affect weight gain. However during the grower and finisher phases of growth and possibly due to greater demand for nutrients as the birds grew, only those broiler on the 5% leaf meal diet gained weight as well as the control. Failure of broilers on the 10% leaf meal to attain expected weights could be associated with increase in maintenance requirements of the gastrointestinal tract. This implies that the gastrointestinal maintenance demands for broiler on the 10% leaf meal diets may have cost the broilers more energy than the benefit from the adaptation during the grower and finisher phases of growth.

5. Conclusion

Intestinal adaptations of the leaf meal based diets supported expected carcass weights up to two weeks of age. Beyond two weeks of feeding, physiological adaptations from the 10% leaf meal fed broilers failed to support growth to the expected carcass weights. The results from this study imply that *A. angustissima* leaf meal could be included in broiler diets upto 10% only in starter diets as the intestines can make physiological adjustments without compromising carcass weights. Beyond two weeks of age, the optimum inclusion levels of *A. angustissima* in broiler diets is 5% since at 10% inclusion, intestinal adjustments failed to support optimum growth.

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