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STOMACH ADAPTATIONS OF BROILERS FED *Acacia angustissima* LEAF MEAL BASED DIETS

Sharai NCUBE[1], Tinyiko Edward HALIMANI[1], Musavengana Tapera TIVAPASI[2], Solomon DHLIWAYO[2], Edward Venancio-Imbayarwo CHIKOSI[1] and Petronella Tapiwa SAIDI[1]

[1]*University of Zimbabwe, Faculty of Agriculture, Department of Animal Science, Box MP167, Mt Pleasant, Harare, Zimbabwe*
[2]*University of Zimbabwe, Faculty of Veterinary, Department of Clinical Veterinary Studies, Box MP167, Mt Pleasant, Harare, Zimbabwe*

Email: sharaincube7@gmail.com

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ABSTRACT: The study determined effect of *Acacia angustissima* leaf meal on the stomach physiology of broilers. 150 day old chicks were randomly allocated to 0%, 5% and 10% *A. angustissima* leaf meal based diets for six weeks with five replicates per treatment. At weeks 2, 4 and 6, two birds from each replicate were slaughtered, dressed and weighed. The weights of the proventriculus and gizzard were measured. Approximately 1 cm specimen was taken from each organ, fixed in formalin and stained for histological analysis using a light microscopy. Proventriculus muscle layer thickness, gland diameter and secretory layer thickness decreased with increasing leaf meal levels (P<0.05). *A. angustissima* leaf meal had no effect on the physiology of the gizzard during the starter phase (P>0.05). Continued use of the of *A. angustissima* leaf meal in the grower and finisher diets resulted in increased weight and muscle thickness of the gizzard (P<0.05). It was concluded that *A. angustissima* leaf meal reduced the physiological capacity of the proventriculus to secrete digestive juices and enhanced the physiological capacity of the gizzard for mechanical digestion.

Keywords: *Acacia angustissima*, Broilers, Gizzard, Grinding capacity, Proventriculus

INTRODUCTION

The increased demand of poultry meat has directed breeding focus towards a fast growing broiler with increased feed efficiency and greater final weights (Olanrewaju et al., 2006; Petracci and Cavani, 2012). To support the fast growth in broilers, soybean and maize have been the most conventionally used protein and energy sources (Ochetim, 1993; Onuh et al., 2010). However the increasing cost and scarcity of these conventional ingredients has been reported to affect broiler production in most developing countries (Rao et al., 2005; Khattak et al., 2006; Anaeto and Adighibe, 2011; Gadzirayi et al., 2012; Diara and Devi, 2015), prompting use of alternative ingredients.

In line with this drive, Ncube et al. (2012ab) assessed the potential of *A. angustissima* leaves as a broiler protein ingredient. Based on their findings, inclusion of *A. angustissima* at 5-10% in diets can support growth of broiler. However, findings also show that inclusion of 5 to 10 % of the leaf meal can result in 14.28 to 17.86 % increase in the weight of the gizzard (Ncube et al., 2012c), possibly implying some physiological changes on the organ. It's important to check if increase in size of the gizzard is of a pathological nature or simple adaptations to leaf meal presence. It was the objective of this study to determine effect of graded levels of *A. angustissima* leaf meal based diets on the physiology of the proventriculus and gizzard.

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MATERIALS AND METHOD

A. angustissima leaves were harvested at mid-maturity stage of growth, air dried and ground through a 1mm sieve. Dry matter, crude protein, crude fiber, ash (AOAC, 1990), condensed tannins (Porter et al., 1986), soluble and insoluble fibres (Parsaie et al., 2006) were determined (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). One hundred and fifty day old unsexed Cobb 500 broiler chicks were randomly allocated to 15 groups with 10 birds per group. The groups were randomly allocated to the three diets in five replicates. 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.

At weeks 2, 4 and 6, ten birds per treatment were slaughtered and dressed. The proventriculus and gizzard were removed and weighed. The weight of the organs was expressed as proportions of hot dressed weight. For purposes of histopathology, approximately, 1 cm of the proventriculus and gizzard were cut and fixed in 10% saline formalin. The fixed fragments were stained on slides (Bacha and Bacha, 2000).

Using a Leitz MD5 light microscope fitted with an eye piece graticle, ten points of the following parameters were measured and averaged into one value per bird. The proventriculus muscle thickness, secretory layer thickness and diameter, the thickness of the gizzard muscle, gizzard glandular and keratin layer were measured at x4 objective. The number and size of muscle fibers of the gizzard were counted and measured across a 0.2 mm length at x40 objective. All procedures in this experiment followed guidelines by the Zimbabwe Scientific Animal Act, 1963, subsection 2 of section 4, License Number L624. To determine effect of diet on the physiology of the organs, ANOVA was carried out using PROC GLM procedure of SAS version 9.3. Comparison of means was done using Tukey's test.

Table 1 - Chemical composition of *A. angustissima* leaf meal

Chemical Component	Percentage (%)
Dry matter	90.00
Ash	4.77
Crude protein	23.40
Crude fibre	13.00
Calcium	0.94
Phosphorus	0.17
Condensed tannins	1.06
Insoluble Dietary Fibre	9.24
Soluble Dietary Fibre	4.96

Table 2 - Ingredient and chemical composition of 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	30.00	25.00	20.00	18.7	13.70	8.70	18.60	13.60	8.60
Meat and Bone Meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Sorghum meal	10.00	0.00	10.00	0.00	9.90	10.00	0.00	0.00	0.00
Acacia leaf meal	0.00	5.00	10.00	0.00	5.00	10.00	0.00	5.00	10.00
Blood meal	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	2.1.0	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	48.60	56.90	44.00	68.1	55.00	51.40	73.00	70.40	67.50
Fish meal	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
Monocalcium 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

[1]Composition: 9.9u.i vitamin A, 1.95u.i vitamin D_3, 30u.i vitamin E, 2.9g Vitamin K3, 2g Vitamin B1, 7.5g Vitamin B2, 30g Vitamin PP Niacin, 12.1g Vitamin B5, 3g Vitamin B6, 1g vitamin B9 Folic Acid, 150mg Vitamin B7/Biotin, 20mg Vitamin B12, 300g Choline, 60g Iron, 10g Copper, 100g Manganese, 100g Zinc, 1g, Iodine , 0.5g Cobalt, 300mg Selenium. [2]Composition: 8u.i vitamin A, 2u.i vitamin D, 25u.i vitamin E, 2g Vitamin K3, 1.75g Vitamin B1, 6g Vitamin B2, 25g Vitamin PP Niacin, 10g Vitamin B5, 2g Vitamin B6, 1g vitamin B9 Folic Acid, 100mg Vitamin B7/Biotin, 15mg Vitamin B12, 250g Choline, 50g Iron, 8g Copper, 80g Manganese, 80g Zinc, 1g, Iodine , 0.5g Cobalt, 250mg Selenium. [3]Composition: 6u.i vitamin A, 1.5u.i vitamin D_3, 20u.i vitamin E, 1.5g Vitamin K3, 1.5g Vitamin B1, 5g Vitamin B2, 25g Vitamin PP Niacin, 8g Vitamin B5, 1.5g Vitamin B6, 0.6g vitamin B9 Folic Acid, 80mg Vitamin B7/Biotin, 15mg Vitamin B12, 200g Choline, 40g Iron, 6g Copper, 80g Manganese, 60g Zinc, 1g, Iodine , 0.25g Cobalt, 200mg Selenium

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Table 3 - Physiological response of proventriculus parameters to graded levels of *A. angustissima* leaf meal

Parameter	Control	5 % AA	10 % AA	SE
Proventriculus weight (%)	0.846	0.855	0.855	0.0341
Proventriculus muscle (mm)	0.221[a]	0.213[a]	0.169[b]	0.0065
Proventriculus gland diameter (mm)	1.267[a]	1.180[b]	1.094[c]	0.0237
Secretory cell layer thickness (mm)	0.605[a]	0.571[ab]	0.556[b]	0.0135

[abc] Within a row, means without a common superscript differ (*P*<0.05), AA= *Acacia angustissima*
SE= Standard error

Table 4 - Effect of graded levels of *A. angustissima* leaf meal on physiological parameters of the gizzard

Age(Wk)	Gizzard weight (%)			Gizzard muscle thickness(mm)			Keratin Layer(mm)			Myofibril (mm)			Number of Fibers		
	0 % AA	5 % AA	10 % AA	0 % AA	5 % AA	10 % AA	0% AA	5 % AA	10 % AA	0 % AA	5 % AA	10 % AA	0% AA	5 % AA	10 % AA
2	4.420	4.610	4.580	2.880	3.199	3.290	0.269	0.328	0.430	0.064	0.065	0.068	3.194	3.340	3.034
4	2.601[a]	3.472[b]	4.051[b]	3.052[a]	5.098[b]	5.797[b]	0.416[a]	0.577[a]	0.783[b]	0.071[a]	0.121[b]	0.135[c]	3.023[a]	2.013[b]	1.541[b]
6	2.040[a]	2.393[a]	3.062[b]	5.005[a]	8.430[b]	10.095[b]	1.006[a]	0.753[b]	0.346[c]	0.078[a]	0.121[b]	0.141[c]	2.586[a]	1.675[b]	1.485[b]
SE	0.210	0.210	0.210	0.418	0.418	0.418	0.044	0.044	0.044	0.006	0.006	0.006	0.179	0.179	0.179

[abc] Within a row means for a parameter without a common superscript differ (P<0.05), Wk=Week, AA= *Acacia angustissima*, SE= Standard error

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RESULTS

A. angustissima had no effect on proportionate weight of the proventriculus but proventriculus muscle layer thickness, gland diameter and secretory layer thickness decreased with increasing leaf meal levels (P<0.05; Table 3).

The proportionate weight of the gizzard, thickness of gizzard muscle, keratin layer, myofibril thickness and number of myofibrils per unit length was influenced by age of birds. The proportionate weight of the gizzard, thickness of the gizzard muscle layer, keratin layer, fiber muscle and number of fibers (Table 4) was the same across treatments at week two (P>0.05). By week four, an increase in the proportionate weight of the gizzard, thickness of muscle layer, keratin layer, muscle fiber and a decrease in the fiber numbers was noted with increasing level of the leaf meal (P<0.05). By the end of the week 6 the proportionate weight of the gizzard, gizzard muscle thickness, muscle fiber thickness increased with increasing levels of the leaf meal while the keratin layer thickness and number of myofibrils decreased with increasing levels of the leaf meal (P<0.05).

An increase in the level of the leaf meal resulted in significant increases of the gizzard glandular layer thicknesses from 0.731 ± 0.032 mm of the control to 0.842 ± 0.032 mm and 0.762 ± 0.032 mm of the 5% and 10% fed birds respectively (P<0.05; Table 4). Glandular layer for birds on the 5% fed bird increased by 15.18% while those on the 10% diet increased by 4.24%. Gizzard from birds on the 5% diet had the thickest glandular layer, which was not different from the birds on the 10% diet (P>0.05).

DISCUSSION

Reduced muscle layer, gland diameter and thickness of secretory cell layers of the proventriculus at 10% inclusion of the leaf meal could be indicative of reduced capacity to secrete mucus, hydrochloric acid, and pepsinogen in the presence of the leaf meal. As gastrointestinal mass reduces, so does the functional capacity of organs (Starck, 1998). This is usually regulated by presence of food molecules in the organ (Eckert et al., 1988). Presence of the leaf meal, a fibrous ingredient, can reduce feed intake as it can make the diets bulkier.

The increase in proportionate weight and muscle layer thickness of the gizzard with increasing levels of the leaf meal depicts enhanced mechanical digestion by the organ. The need by the gizzard to meet the demand for greater grinding of increased dietary fiber quantities explain the increase in the proportionate weight and muscle layer of the gizzard. Fiber particles are generally harder to grind than other dietary components (Mateos et al., 2012), thus presence has been noted to promote thickening of the gizzard muscle layer (Banfield and Forbes, 2001; Sobayo et al., 2012; Adeyemi et al., 2013) as is also recorded in this study. Similarly to current results, Incharoen (2013) reported increased proportionate weight of the gizzard of broilers fed rice hull based diets and attributed the increase in weight to presence of fibers. Kagya-Agyemang et al. (2007), Borin (2012), Jiang et al. (2012) and Adeyemi et al. (2013) also reported increased proportionate weight of the gizzard on inclusion of *G. sepium*, cassava, Alfalfa and cassava leaf meals respectively. Since the major function of the gizzard is to mechanically break down feed particles through muscle contractions (Sobayo et al., 2012; Adeyemi et al., 2013; Svihus, 2014), the increased proportion of the gizzard relative to body weights is a physiological adaptation to increasing levels of the *A. angustissima* leaf meal.

The increase in the proportionate weight of the gizzard in this study was through thickening of the muscle layer, an indication of increased muscle activity with addition of the leaf meal. According to Akester (1986), Svihus (2011) and Svihus (2014), when the contractile activity of the gizzard increases, the gizzard wall becomes thicker as the organ attempts to increase its digestive efficiency (Waugh et al., 2007). Obun et al. (2008) also says that the development of the gizzard muscle is triggered by the work load imposed on the organ. According to Borin (2012) even minimum increases in crude fiber can stimulate gizzard development. Therefore presence of *A. angustissima* in the diet stimulated gizzard function, resulting in the expressive muscle development observed as thickening of the muscle fiber with increasing levels of the leaf meal in the diet. The increase in the thickness of the gizzard muscle layer was through hypertrophy as seen by the increase in myofibril thickness with an associated decline in muscle numbers. The stimulus to such hypertrophic responses is the contractile activity of the gizzard as the mechanical load on the organ increased (Vaughan and Goldspink, 1979). Such a response would result in an increase in the amount of contractile muscle without an increase in the number of muscle fibers (Paul and Rosenthal, 2002) and is attributed to the increase in the bulkiness of digesta (Starck and Rahmaan, 2003).

The non-significant effect of treatment on all gizzard parameters at the starter phase indicates the ability of all the diets to supply nutrients that could support growth during that growth phase. This could be due the presence of the highly nutritious yolk sac, coupled with the still low nutritional demands by the chicks and the still developing gastrointestinal tract capacity (Nay and Sklan, 2002; Maiorka et al., 2006; Panda et al., 2006). With the residual yolk providing digestible nutrients that are responsible for the development of the GIT (Dibner and Richards, 2004)

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not a lot of grinding is expected from the still developing gizzard. The results indicated that early development of gastro intestinal tract was independent of presence of *A. angustissima* leaf meal in starter diets. The gizzard must also go through a period of development (Maiorka et al., 2006) and can only adapt to fibrous diets when it is fully functionally developed (Nkukwana et al., 2015). As the birds grew older, the increase in nutritional demands can help explain the need for increased gizzard functional capacity.

CONCLUSION

The study concludes that increasing levels of *A. angustissima* leaf meal in broiler diet decreases the proventriculus muscle thickness, proventriculus gland diameter and the secretory layer thickness of the proventriculus. Effect on the gizzard was depended on the age of the broilers. Inclusion of *A. angustissima* in broiler starter diets had no effect on the physiology of the gizzard. In the grower and finisher phases of feeding, increasing levels of *A. angustissima* resulted in increased weight of the gizzard through an increase in muscle layer. The increase in the thickness of the muscle layer was through a hypertrophic response of muscle fibres to presence of *A. angustissima* leaf meal. Therefore inclusion of *A. angustissima* leaf meal in broiler diets reduced physiological capacity of the proventriculus to secrete digestive juices and enhanced physiological capacity of the gizzard for mechanical digestion

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Author's contribution
All authors contributed equally to this work.

Competing interests
The authors declare that they have no competing interests.

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