Journal of Agriculture, Food, Environment and Animal Sciences
Tarım, Gıda, Çevre ve Hayvancılık Bilimleri Dergisi
http://www.jafeas.com, ISSN: 2757-5659

J. Agric. Food, Environ. Anim. Sci.
6(1): 101-112, 2025

Replacing Soybean Meal with *Acacia Angustissima* in Broiler Diets Strengthens Birds Skeletal Frame

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Research Article

Article History:
Received:03 September 2024
Accepted:14 January2025
Published online: 01 June 2025

***Keywords*:**
Alternative Feeds
Bird Welfare
Broiler Bone Strength
Leaf-Meal
Leg Problems

ABSTRACT

A robust skeletal system is vital for optimum health and vitality in broiler chickens and consequently the profitability of meat-type bird enterprises. Intense selection of meat-type commercial birds has resulted in rapidly growing birds called broilers whose skeletal frames, unfortunately, buckle under heavy weights. This study aimed to determine the effect of incorporating *Acacia angustissima* leaf meal at 0%, 5%, and 10% inclusion rates on tibia bone physical characteristics (length, weight, strength) and mineral (ash, calcium, and phosphorus) content. One hundred and fifty day-old Cobb 500 broiler chicks weighing 41.7 ± 1.56g were randomly allocated to 15 battery cages with each cage housing 10 chicks. Experimental diets were randomly assigned to cages. Birds were reared on a three-phase feeding system constituting starter, grower, and finisher diets. Water and feed were provided *ad libitum* for 42 days. Voluntary feed intake was recorded weekly. At day 42, two birds per replicate were randomly selected, weighed, and humanely slaughtered. The tibia bone was collected for physical and mineral content analyses. ANOVA revealed that treatment did not influence feed intake in birds (P=0.0625), but at 10% leaf meal, live body weight was depressed. Including *A. angustissima* leaf-meal at 5 and 10% strengthened (P<0.05) broiler tibia bones and increased (P<0.05) their calcium content. It was concluded that incorporating *A. angustissima* leaf meal at 5 % in broiler diets increased broiler bone strength without any adverse effects on the performance of broiler chickens.

To Cite : Hodzi P, Mutibvu T, Mbiriri DT., 2025. Replacing Soybean Meal with Acacia *Angustissima* in Broiler Diets Strengthens Birds' Skeletal Frame. Agriculture, Food, Environment and Animal Sciences, 6(1): 101-112.

INTRODUCTION

Genetic improvement has resulted in meat-type birds that rapidly put on muscle but also has some consequences that burden the skeletal frame (Çapar Akyüz et al, 2020; Hartcher et al., 2020; Avicola et al., 2022; Jiang et al., 2024). This condition is called non-infectious skeletal disorder. Rapid weight gain on underdeveloped, weak skeletal tissues results in bone deformations (Çapar Akyüz et al., 2020; Ahmad et al., 2022). Affected birds experience difficulties in walking and standing and, therefore, cannot feed effectively leading to a decline in body weight (Świątkiewicz and Arczewska-Wlosek, 2012). Such birds may also produce a low-quality carcass due to bruising through direct contact with the ground and in worst cases starve, leading to increased mortalities or culling. Bone deformities and lameness in broilers, apart from infringing on animal welfare, thus result in huge economic losses globally (Greger, 2011; Tainika et al., 2023; Jiang et al., 2024). Interventions come mainly in the form of dietary mineral supplements. These supplements, however, add a significant cost to the feed and have also recently been singled-out as environmental hazards as they may find their way into the environment due to varying bioavailability profiles.

Studies incorporating leaf-meals, as fairly affordable protein sources, in commercial birds' diets have focused on growth performance ignoring potential benefits related to skeletal development since leaf meals are known to be rich in minerals (Melesse et al., 2011; Olabode et al., 2023; Omoikhoje et al., 2024; Faisal et al., 2024). Previous studies (Ncube et al., 2012abc) reported comparable performance of broilers fed a commercial diet and those fed a diet replacing soymeal with up to 10% A. angustissima leaf-meal. This study was designed to determine the effect of similar inclusion levels on birds' bone physical and mineral content characteristics.

MATERIAL and METHOD

Experimental Site

The rearing of chickens was done in the Bioassay Laboratory at the University of Zimbabwe, Department of Livestock Sciences. Tibia bone parameters were assessed at the same institution and department in the Animal Physiology Laboratory. The study site is located in Harare (1,500 meters above sea level), the capital city of Zimbabwe. Harare experiences a cool period from May to August and a hot season from October to March. According to Mushore et al. (2017), the average temperature of the hottest month, October, is 28.4 °C (82.4 °F, while the coldest month, July, is 14.5 °C (58.2 °F). The city receives a mean annual rainfall of 850 mm.

Animal Ethics

All procedures were conducted in accordance with the Bioassay Laboratory at the University of Zimbabwe, Department of Livestock Sciences Act 1963, approved by institutional ethical review committees and conducted under the authority of the Project number (16/2025).

Processing and Nutrient profiling of Acacia angustissima

The fresh green leaves of A. angustissima used in this research were harvested within the University environment. A. angustissima leaves were harvested at the mid-maturity stage, shade-dried as recommended by Ncube et al. (2015), and milled through a 1mm sieve. Dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), ash calcium, and phosphorus were determined according to the Association of Official Analytical Chemists (AOAC) (1990). Condensed tannins were determined using the Butanol-HCL method as described by Porter et al. (1986) (Table 1).

Table 1. Chemical composition of *Acacia angustissima* used to replace soybean-meal in broiler diets

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

Experimental Diets

Three iso-nitrogenous and iso-energetic starter, grower and finisher diets were formulated. The diets were: the basal diet (control, 0% A. angustissima); the diet with 5% A. angustissima leaf-meal inclusion; and the diet with 10% A. angustissima leaf-meal inclusion. Other feed ingredients were incorporated into the feed during formulation to meet the nutritional requirements for broiler birds at each growth stage. The ingredient and nutrient composition of the experimental broiler diets is presented in Table 2.

Flock Management

One hundred and fifty unsexed day-old Cobb 500 broiler chicks weighing 41.7 ± 1.56g were obtained from Charles Stewart Day Old Chicks Pvt Ltd, a reputable commercial poultry breeder, and reared in the Bioassay Laboratory at the University of Zimbabwe. This research facility has appropriate biosecurity measures to prevent pathogens. The chicks were kept in groups of 10 in temperature-controlled pens (suiting the optimum recommended temperature per age group) with access to clean water. A completely randomized design (CRD) was used to assign the chicks to their respective cages. Experimental diets were randomly assigned to cages. Birds were reared on a three-phase feeding system constituting starter, grower and finisher diets. The starter, grower and finisher diets were fed from weeks 1 to 2, 3 to 4 and 5 to 6, respectively. Feed and water were provided ad libitum and standard management practices, including proper lighting, ventilation, and sanitation, were followed to ensure the health and welfare of the birds. The birds were also closely monitored for any signs of illness or adverse effects, and immediate veterinary care was provided if necessary.

Table 2. Ingredient and chemical composition of broiler starter, grower and finisher diets

Items	**Starter Diets**			**Grower Diets**			**Finisher Diets**		
Ingredient(Kg)	Control	5%*AA**	10%*AA*	Control	5%*AA*	10%*AA*	Control	5%*AA*	10%*AA*
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	-	10.00	-	9.90	10.00	-	-	-
Acacia Angustissima Leaf Meal	-	5.00	10.00	-	5.00	10.00	-	5.00	10.00
Blood Meal	-	-	-	-	2.00	3.00	1.20	1.80	3.00
Sunflower Cake	2.50	1.30	-	1.70	1.50	2.1.0	-	-	-
L. Threonine	0.06	0.06	0.03	0.05	-	0.45	-	-	-
Soya Oil	-	-	-	-	1.60	3.00	-	1.30	2.40
Wheat Bran	-	-	2.10	-	-	-	-	-	-
Soya Oil	-	-	1.50	-	-	-	-	-	-
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	-	-	-
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 (g/kg)									
Crude Protein	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
Ether Extract	36.80	39.04	51.94	41.64	55.17	67.71	39.19	51.27	61.45
Crude Fiber	41.50	40.15	49.98	34.38	39.88	46.84	31.90	37.88	43.86
Calcium)	9.98	9.52	9.88	9.22	5.59	5.60	4.93	8.63	8.74
Phosphorus	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

**AA* - *Acacia angustissima* leaf-meal, [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.

Measurement of Parameters

Voluntary feed intake was recorded weekly. At six weeks old, two birds were randomly selected from each treatment for the determination of slaughter weight and tibia bone parameters. Birds were subjected to a five-hour feed withdrawal period and euthanized by cervical dislocation. The birds were slaughtered by exsanguination and carcasses were scalded in water at 60°C for 63 s. After scalding, carcasses were plucked manually. Five samples of bone per treatment were cleaned by manual removal of flesh and cartilage and stored at -18oC. Tibia bone calcium content was determined using the ethylene diaminetetraacetic acid (EDTA) titration method. Phosphorus and ash contents were determined by the spectrophotometric and proximate analysis procedures, respectively (AOAC, 1990). Tibia bone weight was determined using a digital electronic scale (Jadever JPS-1050, Micro Preciso Calibraton Inc., USA; ± 1 g sensitivity) and the bone length was measured using stainless steel ALPA monoblock Vernier Calipers, Italy, range 0 - 200 mm (accuracy ±0.02 mm). Tibia bone strength was determined as bone hardness using a portable hardness tester (Leeb Hardness Tester (Ver: 2.90, Kairda Group Corporation, Haidian District, Beijing 100096, PR China). The instrument measures the depth of penetration and the force that is required for the penetration. The Leeb hardness tester is equipped with a diamond indenter. Five strikes were made on a single bone on different spots and then averaged to get mean tibia bone hardness. The units obtained were converted from Vickers Hardness (HV) to Newtons using standard conversion tables.

Statistical Analyses

Data were analysed by one-way ANOVA using Graph Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Treatment means were compared using Tukey's multiple comparison tests. The assumptions of Normality, Homogeneity of variance, and Independence were made. Means were tested at a 5% level of significance.

RESULTS and DISCUSSION

Including *A. angustissima* leaf-meal at 5 and 10% strengthened broiler tibia bones. The 10% *A. angustissima* diet, however, resulted in shorter and lighter bones (Figure 1). The current findings on bone strength concur with the higher calcium levels in *A. angustissima*-augmented diets, particularly in the finishing phase – when birds eat the most feed (Table 2). Melesse et al. (2011) incorporated *Moringa stenopetala*, another tropical forage legume, in poultry diets and observed higher calcium levels in diets with Moringa. Similar studies showed that a decrease in calcium content (Moreki *et al*., 2011) and calcium, along with phosphorus (Jamroz et al., 2011) in broiler diets negatively affected the strength and toughness of bones. This is because bones require a specific calcium-to-phosphorus ratio for optimal structure (Veum, 2010). An imbalance of the calcium-to-phosphorus ratio can result in brittleness or deformities, making bones more susceptible to fractures. An imbalance in calcium content alone can significantly upset bone mineralization since about 98-99% of the body's calcium is deposited in the skeleton (Amoroso et al., 2013; Alharbi et al., 2024). The lighter bones observed in the current study could partly explain the lower slaughter weights associated with the 10% *A. angustissima* diet (Table 3).

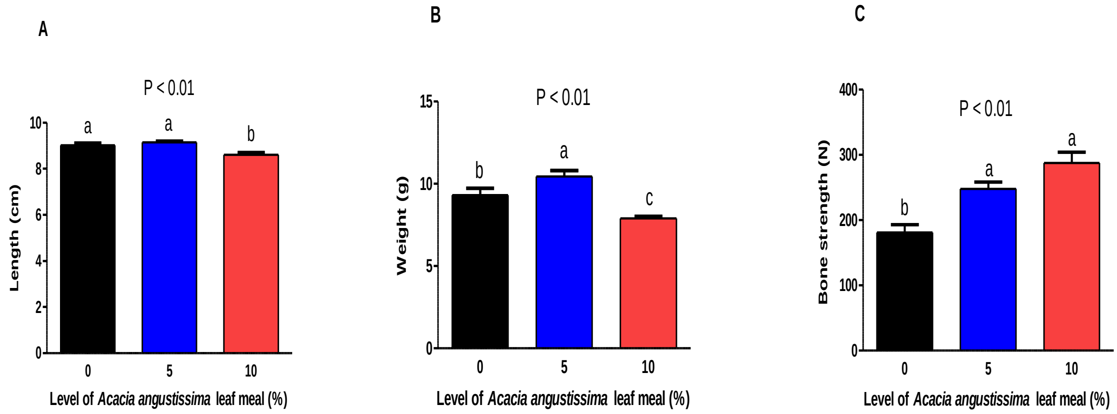
Table 3. Effect of graded levels of *Acacia angustissima* leaf meal (0, 5 and 10%) on the total feed intake (g) and live weight (g) of Cobb 500 broilers

Parameter	Control (0%)	5% *AA**	10% *AA*	*P value*
Total feed intake (g)	3 069±142.7	3 174±208.6	2 921±71.66	0.0625
Slaughter weight (g)	1 772^{a}±121.1	1 773^{a}±116.2	1 402^{b}±36.74	<0.0001

*Acacia angustissima, ^{ab}Means with different superscripts in a row differ significantly (P<0.05)

The 10% *A. angustissima* diet suppressed slaughter live weights (Table 3). Although there was no statistical difference in feed intake, the 10% diet had numerically lower intakes (Table 3) which could partly explain the lower slaughter live weights. The tendency to reduce feed intake as dietary *A. angustissima* content increased is most likely a consequence of increasing levels of tannins. Tannins have always been known to lower feed intake (Kumar *et al*., 1990; Osei *et al*., 1990; Buyse *et al*., 2021; Liu *et al*., 2023; Daghio *et al*., 2024). This is related to their astringency and bitter aftertaste. High levels of tannins in *A. angustissima* leaf meal could have interfered with protein and mineral absorption resulting in reduced slaughter weights of broilers. According to Tapiwa, (2019) tannins bind to proteins forming complexes that are less soluble and harder for digestive enzymes to break down leading to a decrease in the availability of amino acids which are essential for broiler growth and development. Tannins can also bind to minerals such as calcium, phosphorus, and iron, making them less available for absorption as well as disturbing the calcium-to-phosphorous absorption ratio (Tadele, 2015). These minerals are crucial not only for bone development but also for various metabolic processes.

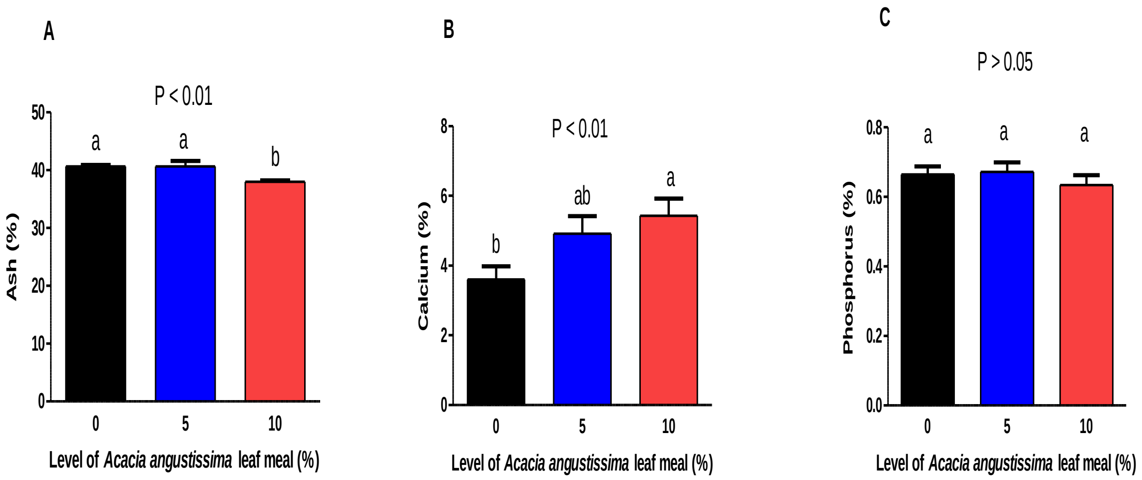
The findings on feed intake are in agreement with trends observed by Ncube et al. (2012b). Interestingly, of the same authors, Ncube et al. (2012c), observed trends contrary to the current findings in a different study. They observed a significant increase in feed intake from day 21 to day 42 (end of the trial) when soybean was replaced by *A. angustissima* leaf meal at the same concentrations as the current study. They gave credible plausible reasons for their observations. We, however, observed that tannin levels were also higher in the current study diets. The different feeding regimes could also help explain the differences in voluntary feed intake.



a, b, c Different superscripts on the bar-graph indicate significant differences between treatments ($P < 0.05$)

Figure 1. Effect of including *Acacia angustissima* leaf-meal up to 10 % in broiler diets on physical characteristics of broiler bones; (A) length (cm), (B) weight (g), and (C) strength (N)

Interestingly, the 10% *A. angustissima* diet had lower ash content than the other dietary treatments (Figure 2). The fact that calcium – one of the chief determinants of bone strength – was significantly higher than the control diet swayed bone strength results in favour of high *A. angustissima* diets. Although we did not observe any confirmed significant differences in bone phosphorus content, it is important to remember that the source of dietary minerals (organic or inorganic) determines the availability of these nutrients with organic sources being associated with greater bioavailability. It is also noteworthy that bone development is a complex sequence of interrelated events (Almeida Paz and Bruno, 2006; Delaisse et al., 2020; Whelan et al., 2023) with two major factors, namely genetic expression of the proteins and dietary nutrient supply being closely linked to and contributing immensely to bone development (Almeida Paz et al., 2008; Bashir et al., 2024). Over and above these factors the interactions among these dietary minerals is also important.



[a, b, c]Different superscripts on the bar-graph indicate significant differences between treatments (P < 0.05)

Figure 2. Effect of including *Acacia angustissima* leaf-meal up to 10% in broiler diets on chemical characteristics of broiler bones; (A) ash, (B) calcium, and (C) phosphorus all expressed as percentages

Ensuring that calcium and phosphorus are provided in adequate concentrations is crucial for broiler skeletal development. Calcium and phosphorus are critical for bone tissue mineralization (Ciosek et al., 2021); hence, their deficiency results in less dense and weaker bones, leading to decreased mechanical strength. Any imbalances in mineral supply, therefore, may have detrimental effects on general bone, hence, skeletal integrity. For instance, in periods of deficiency, excessive bone resorption may occur leading to the weakening of bones. Approximately 98 to 99% of total body calcium and 80 to 85% of phosphorus are located in the bones (Amoroso et al., 2013; Mohamed et al., 2024). Deficiencies in blood lead to the withdrawal of minerals from the bone matrix hence weakening bones among other disorders. The reduced walking ability caused by bone disorders can lead to difficulties in feeding, hence reduced feed intake and a decreased body weight for chickens in production (Świątkiewicz & Arczewska-Wlosek, 2012; Liu et al., 2023). Calcium and phosphorus metabolism is, therefore, complex and is influenced by the intricate balance in other dietary elements. A case in point is the importance of Vitamin D_3 as a crucial factor to calcium and phosphorus absorption and proper skeletal development. It must be emphasized, therefore, that broiler or bird nutrition, in general, must be considered and balanced with these factors in mind.

CONCLUSION and RECOMMENDATIONS

Including *A. angustissima* in broiler diets up to 10% resulted in stronger tibia bones. This is a result of high calcium content in the bones. Feed intake was not affected by incorporating *A. angustissima* leaf-meal. However, at 10%, slaughter live weights were lowered. We recommended that 5% *A. angustissima* leaf me can be included in broiler diets to enhance bone strength without comprising slaughter weight.

ACKNOWLEDGMENT

The authors would like to thank Mr. C Karosi for his cooperation during the broiler feeding trial.

Conflict of Interest

The authors have declared that there are no competing interests.

Authors Contribution

DTM and TM have designed the study and collected the data. PH executed the experiment with the help of DTM, PH wrote the article and critically reviewed by TM.

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