



PROXIMATE COMPOSITION OF THE TIBIA AND FEMUR BONES OF BROILER BREEDERS FED SUPPLEMENTAL LEVELS OF ACETYLSALICYLIC ACID.

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Abstract

Leg deformity is one of the causes of poor breeding soundness of the male breeder cock. Deficiency of the diets in the required nutrients needed for maintaining the integrity of the tibias and femora – the two main bones of the leg saddled with weight bearing function in bipeds is often responsible for this deformity. This problem may be exacerbated in diets with inclusion or supplementation of acetylsalicylic acid (ASA) because of the link between this drug and bone physiology. This experiment was therefore conducted to investigate the effects of supplemental levels of ASA on proximate composition of the tibias and femora of Marshall Broiler breeder cocks. Four standard breeder cocks diets labelled D₁, D₂, D₃ and D₄ were formulated and supplemented with 0.00, 0.05, 0.10 and 0.15% of ASA respectively. These diets were fed to 24 Marshall breeder cockerels throughout the sixteen weeks of the experiment during which the crude protein, moisture, ash, fat and organic matter contents of the tibia and femur bones of the cockerels were determined. Results showed that the moisture content of the tibias decreased while those of the femora increased with increase in the level of ASA supplementation. The crude protein content of both bones was depressed ($P < 0.05$) at 0.15% ASA relative to the control but the bone ash content was lowest at 0.10% ($P < 0.05$) ASA supplementation. The lowest organic matter content for both bones was observed in birds fed the 0.15% ASA supplementation. Supplemental ASA levels decreased bone fat content of the femur while a significant decrease with ASA supplementation relative to the control was only observed at 0.15% level in the tibia bones. In conclusion, ASA supplementation of Marshall Broiler breeder's diets could depress the crude protein, fat and organic matter contents of the tibia and femur beyond 0.10% level.

Keywords: Acetylsalicylic acid, bone, proximate composition, femur, tibia.

Introduction

The breeder cocks are very pivotal to the success of any hatchery operation either as sperm donors in hatchery operations where artificial insemination holds sway or mating partners with hens where natural mating is the order of the day. Breeder cocks are therefore managed in such a way that will ensure their optimum reproductive soundness. Some of the approaches at maintaining the reproductive capability of the breeder male stock are the use of stock from superior breeding lines, conducive housing environment and placing the breeders on a good plain of nutrition that will furnish them the required nutrients needed for their peculiar and unique task. Rekaya *et al.* (2013) suggested that emphasis should now be placed on breeding programmes that will simultaneously address genetic improvement in leg soundness in broilers alongside innovative husbandry practices that will not put the welfare of the birds at risk. This suggestion is borne out of the spate of leg deformity/lameness currently plaguing the modern day broiler birds (Knowles *et al.*, 2008; Shim *et al.*, 2012; Suzer *et al.*, 2017).

The use of acetylsalicylic acid (ASA) is one of the husbandry/management practices directed at solving some of the welfare issues related to heat stress especially under the oppressive tropical environment. This is because this drug is widely known for its anti-pyretic, analgesic and anti-inflammatory roles in both man and his livestock (Weissmann, 1991; Aro *et al.*, 2017a). Its roles in egg production (Aro *et al.*, 2017b), growth (Al-Obaidi, and Al-Shadeedi, 2010) and immuno-modulation (Wu *et al.*, 2015) in domestic chickens have been severally reported. Acetylsalicylic acid has also been reported to play some roles in the physiology of bone, more importantly on its effects in the cylo-oxygenase pathway in the biosynthesis of prostaglandins (Rohleder, 2008). Since ASA is now a recommended drug to combat heat-stressed livestock in the tropics, its risk factors *vis-a-vis* the spate of occurrence and prevalence of leg deformities in the present day broiler breeds of domestic chickens should be examined directly in such an environment. The



strains of weight bearing in birds just like the other bipeds are more on skeletal structure of the legs particularly on the femur, tibia and at the tibio-tarsal joint. This explains why investigations on leg deformities are often directed at these anatomical landmarks and the adjoining musculature and fascia. This experiment was therefore conducted to investigate the effect of supplemental ASA on the proximate composition of the tibia and femur bones of Marshall Broiler breeder cocks.

Materials and Methods:

This research work was carried out at the Teaching and Research Farm (Livestock section) of the Federal University of Technology, Akure, Nigeria. The farm is located in the humid rain forest zone of western Nigeria which is characterized by two rainfall peaks and high humidity during the rainy season. The mean annual rainfall is about 1,500mm and lasts for 9 months usually from March to November every year. The mean annual relative humidity is over 75% and that of temperature is about 27°C.

Thirty broiler breeder cockerels (Marshall Breed) at fourteen weeks of age were purchased from Obasanjo's Breeders Farms, Lanlate, Oyo State, Nigeria. Prior to sourcing for the birds, the surroundings of the pens were cleaned, the pens were washed and disinfected while the cages were equally disinfected and arranged properly. The cages were labelled according to the treatment diets and their replicates. Four experimental diets with varied levels of acetylsalicylic acid (ASA) were formulated. The first diet was the control with 0.00% of ASA and labelled as D₁ while the remaining three diets were labelled as D₂, D₃ and D₄ with 50g, 100g and 150g of ASA per 100kg of feed respectively. The diets also contained crude protein and energy level of 16.70% CP and 2360 KCalKg⁻¹ (10.50MJkg⁻¹) respectively. Table 1 shows the gross composition of the experimental diets

Table1: Gross composition (g/100g) of the broiler breeder cocks' diets

Ingredients	D ₁	D ₂	D ₃	D ₄
Maize	55.00	55.00	55.00	55.00
Soybean	9.00	9.00	9.00	9.00
Wheat offal	21.00	21.00	21.00	21.00
Groundnut cake	9.00	9.00	9.00	9.00
Fish meal	1.00	1.00	1.00	1.00
Bone meal	2.75	2.75	2.75	2.75
Limestone	1.50	1.50	1.50	1.50
Methionine	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Breeders' Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
ASA	0.00	0.05	0.10	0.15

D₁ = Diet with 0.00% ASA; D₂ = Diet with 0.05% ASA; D₃ = Diet with 0.10% ASA; D₄ = Diet with 0.15% ASA; ASA = Acetylsalicylic acid.

Breeder Premix: Supplied in a kg of the diet; Vit. A, 10,000 IU; Vit. D₃, 2,800 IU, Vit. E, 35,000 IU, Vit. K, 1,900 mg; Vit. B₁₂, 19 mg; Riboflavin, 7,000 mg; Pyridoxine, 3,800 mg; Thiamine, 2,200 mg; Pantothenic acid, 11,000 mg; Nicotinic acid, 45,000 mg; Folic acid, 1,400 mg; Biotin, 113 mg; Cu, 8,000 mg; Manganese, 64,000 mg, Zinc, 40,000 mg; Iron, 32,000 mg; Selenium 160 mg; Iodine, 800 mg; Cobalt, 400 mg; Choline, 475,000 mg; Methionine, 50,000 mg; BHT, 5,000 mg; Spiramycin, 5,000 mg.

The thirty experimental birds were acclimatised for two weeks after which twenty four of them were selected and randomly allotted into the four treatments (diets) which contained six birds each, where each bird served as a replicate of its treatment diet. The feeding trial lasted for 16 weeks. All routine and occasional management practices were strictly adhered to. Adequate health management practices were observed during the period of the trial. All necessary vaccination and medications were administered accordingly.

Proximate analysis of tibia and femur bones



Moisture content of both the tibias and the femora was determined using the oven-drying method while ash content of the bones was determined by ashing representative samples of the bone in a muffle furnace at 600°C until a light grey colour of ash was obtained. Fat and protein contents were determined by AOAC methods (2000) while the organic matter content of the bones was calculated as 100 minus the % ash content of the bones i.e.:

$$\text{Organic matter} = 100\% - \text{Ash}\%$$

Statistical Analysis

All data were subjected to one way analysis of variance in a completely randomised design (CRD) using the SPSS version 22 (SPSS 2016) statistical package. Means were separated using the Duncan Multiple range test of the same statistical package.

Results and Discussion

Table 2 shows the proximate composition of the tibias of the breeder cocks fed dietary supplementation of acetylsalicylic acid (ASA). The moisture content of the tibias decreased with increase in the supplemental levels of ASA. Both the D₃ and D₄ diets differed in the moisture content of the tibia bones in comparison with the control. Hence ASA supplementation at levels above 0.05% or 50g/100kg of the diet could result in reduction in moisture content of the tibia of Marshall Broiler breeder cocks. The crude protein content of the tibia bones was similar in birds fed D₁, D₂ and D₃ diets but all the three differed ($P < 0.05$) from the D₄ diet which recorded the lowest crude protein value. The crude protein content of the bone is mainly derived from the red bone marrow hence 0.10% ASA would favour optimum haematopoiesis in this breed of broiler chickens.

Table 2: Proximate composition of tibia bones of Marshall breeder cocks fed dietary supplementation of acetylsalicylic acid

Treatments	% Moisture	% CP	% Ash	% OM	% FAT
D ₁	11.64±0.71 ^a	28.96±0.65 ^{ab}	70.87±3.80 ^{ab}	29.13±3.80 ^b	38.89±2.17 ^{ab}
D ₂	9.89±0.86 ^{ab}	30.67±0.68 ^a	62.28±1.47 ^c	37.72±1.47 ^a	37.67±0.65 ^b
D ₃	8.95±0.71 ^b	30.16±0.61 ^a	67.86±4.91 ^b	32.15±4.91 ^b	39.59±1.19 ^a
D ₄	8.38±0.39 ^b	27.75±0.11 ^b	71.62±0.80 ^a	28.38±0.80 ^b	34.68±1.86 ^c

^{a,ab,b,c} = Means in the same column but with different superscripts are statistically significant ($P < 0.05$). D₁ = 0.00% inclusion level of ASA; D₂ = 0.05% inclusion level of ASA; D₃ = 0.10% inclusion level of ASA; D₄ = 0.15% inclusion level of ASA; CP = Crude protein; OM = Organic matter; ASA = Acetylsalicylic acid.

Data obtained on the ash content of the tibia bones did not follow any particular trend. The values showed that D₁ (70.87±3.80%) was similar ($P > 0.05$) to D₄ (71.62±0.80%) while D₂ (62.28±1.47%) differed significantly to D₃ (67.86±4.91%). This implies that variation observed in these values may not be as a result of direct response to the supplementation of ASA in the diets. Values obtained on bone ash however fell within the 65% average of bone mineral matrix (bone ash) of total bone weight (Vieites *et al.*, 2018). The ASA supplementation in the diets reduced the organic matter component of the tibias at 0.15% in comparison with the control. Figures obtain in the present study however compared with 30% proportion of the organic component in a typical bone (<http://www.c14dating.com/bone.html>). The fat content of the tibia did not follow a particular trend but the general observation was a significant reduction in the fat content of tibia at 0.15% ASA. Reduction in organic matter and fat contents of the bones are pointers to the fact that bone integrity of broiler breeders may be compromised at 0.15% ASA supplementation.

The proximate composition of the femur bones of Marshall Broiler breeder cocks is presented in Table 3.

Table 3: Proximate composition of femur bones of Marshall Breeder cocks fed dietary supplementation of acetylsalicylic acid

Treatments	% Moisture	% CP	% Ash	% OM	% FAT
D ₁	8.84±0.10 ^b	28.22±0.53 ^b	65.82±2.74 ^a	34.18±2.74 ^b	38.58±3.29 ^a
D ₂	9.71±0.33 ^a	28.31±0.64 ^b	66.37±2.95 ^a	33.63±2.95 ^b	29.02±2.02 ^d
D ₃	9.29±0.45 ^{ab}	30.69±1.46 ^a	57.71±5.12 ^b	42.29±5.12 ^a	34.56±0.41 ^b



D ₄	9.97±0.86 ^a	26.55±0.66 ^c	69.15±3.66 ^a	30.85±3.66 ^c	32.55±2.26 ^c
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^{a,ab,b,c} = Means in the same column but with different superscripts are statistically significant ($p < 0.05$). D₁ = 0.00% inclusion level of ASA; D₂ = 0.05% inclusion level of ASA; D₃ = 0.10% inclusion level of ASA; D₄ = 0.15% inclusion level of ASA; CP = Crude protein; OM = Organic matter; ASA = Acetylsalicylic acid.

The moisture content of the femora increased with increase in dietary ASA supplementation—a clear departure from what was observed with the tibias. A different pharmacokinetics of ASA and its metabolism in these bones is thus suggested as the probable cause of this anomaly. The fat content of the femora in the ASA supplemented diets was significantly reduced relative to the control. This could have resulted from a lower moisture content of the femora in the control diet as compared with all the ASA supplemented diets, since water and fat contents in the body are inversely related. The ASA has also been known to reduce triglycerides and cholesterol contents in animal tissues (Alagawany *et al.*, 2017).

Conclusion and Recommendations

Dietary ASA decreased moisture content of tibia but increased that of femur bones. Crude protein, organic matter and fat contents of both the tibia and femur bones were decreased beyond 0.10% ASA supplementation thus suggesting that this level (0.10% ASA) could be optimum for Marshall Broiler breeder cocks.

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