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Replacing dietary vitamin-mineral premix with leaf meal composites sustained the bone characteristics of broiler chickens

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Abstract

Effects of replacing dietary vitamin-mineral premix (VmP) with leaf meal composite (LMC) on bone characteristics of broiler chickens were undertaken in this study. Four leaves (Celosia argentea, Telfairia occidentalis, Moringa oleifera, Vernonia amygdalina) were air-dried to constant weight, individually milled to powder and combined in equal proportions to become LMC sample. Diets containing 0.000, 0.125, 0.250, and 0.375% levels of commercial VmP were formulated and the LMC was incorporated to replace dietary VmP at 1.5, 3.0 and 4.5% of the diets. In an experiment lasting six weeks, one-day old Arbor Acres broiler chicks (n=936) were randomly allotted to 13 dietary treatments. Each treatment was replicated eight times; each replicate comprised nine chicks. The experiment was a 3×4 +1 *augmented factorial arrangement in a completely randomised design. At day 36, blood (3 mL) was sampled and analysed for serum calcium and phosphorus. Eight birds per treatment were sacrificed and the left tibiae were harvested for bone morphometric parameters as well as mineral composition determination. Bone robusticity index of 5.61in broiler chickens on control was significantly higher ($p<0.05$) than in other treatments. Sera calcium range of 13.21to 14.49 mg/dL and phosphorus of 3.67 to 4.29 mg/dL differed significantly ($p<0.05$) with dietary VmP and LMC. Effect of interaction of dietary CLM and VmP inclusion levels on tibia morphometric parameters, bone calcium and phosphorous compositions differed significantly ($p<0.05$). Serum phosphorus correlated positively with serum calcium ($r=0.868$, $p<0.01$) and dietary phosphorus ($r=0.698$, $p<0.01$). Dietary phosphorus however, highly correlated negatively with bone calcium ($r=0.408$, $p<0.01$) and serum calcium ($r=0.607$, $p<0.01$). Replacements of VmP with CLM and their combinations sustained bone morphometry as well as calcium and phosphorous utilization in broiler chickens.*

Keywords: Vitamin-mineral premix, composite leaf meal, broiler chickens, bone morphometry, tibia bone

Le remplacement du prémélange alimentaire de vitamines et de minéraux par des composites de farine de feuilles a maintenu les caractéristiques osseuses des poulets à griller

Résumé

Les effets du remplacement du prémélange alimentaire de vitamines et de minéraux (PVM) par un composite de farine de feuilles (CFF) sur les caractéristiques osseuses des poulets à

griller ont été étudiés dans cette étude. Quatre feuilles (Celosia argentea, Telfairia occidentalis, Moringa oleifera, Vernonia amygdalina) ont été séchées à l'air jusqu'à poids constant, broyées individuellement en poudre et combinées en proportions égales pour devenir un échantillon CFF. Des régimes contenant 0,000, 0,125, 0,250 et 0,375 % de VmP commercial ont été formulés et le CFF a été incorporé pour remplacer le PVM alimentaire à 1,5, 3,0 et 4,5 % des régimes. Dans une expérience d'une durée de six semaines, des poussins de chair Arbor Acres âgés d'un jour (n = 936) ont été répartis au hasard dans 13 traitements diététiques. Chaque traitement a été répété huit fois ; chaque répétition comprenait neuf poussins. L'expérience était un arrangement factoriel augmenté 3 × 4 + 1 dans une conception complètement aléatoire. Au jour 36, du sang (3 ml) a été prélevé et analysé pour le calcium et le phosphore sériques. Huit oiseaux par traitement ont été sacrifiés et les tibias gauches ont été récoltés pour les paramètres morphométriques osseux ainsi que la détermination de la composition minérale. L'indice de robustesse osseuse de 5,61 chez les poulets de chair témoins était significativement plus élevé ($p < 0,05$) que dans les autres traitements. La plage de calcium sérique de 13,21 à 14,49 mg/dL et de phosphore de 3,67 à 4,29 mg/dL différait significativement ($p<0,05$) avec les PVM alimentaires et les CFF. L'effet de l'interaction des niveaux d'inclusion de CLM et de PVM alimentaires sur les paramètres morphométriques du tibia, les compositions osseuses en calcium et en phosphore différaient significativement ($p <0,05$). Le phosphore sérique était corrélé positivement avec le calcium sérique ($r=0,868$, $p<0,01$) et le phosphore alimentaire ($r=0,698$, $p<0,01$). Cependant, le phosphore alimentaire était fortement corrélé négativement avec le calcium osseux ($r=0,408$, $p<0,01$) et le calcium sérique ($r=0,607$, $p<0,01$). Les remplacements de PVM par CLM et leurs combinaisons ont soutenu la morphométrie osseuse ainsi que l'utilisation du calcium et du phosphore chez les poulets à griller.

Mots-clés : Prémélange vitamino-minéral, farine de feuilles composite, poulets de chair, morphométrie osseuse, os du tibia

Introduction

Leg weakness, lameness and other bone abnormalities associated with several metabolic disorders are major problems in rapidly growing broiler chickens which lead to production losses (Julian, 2005; Dibner *et al*., 2007). It also affects the welfare of birds and food hazards such as presence of bone fragments in meat products and meat discolouration (Rath *et al.,* 2000). The causes of bone abnormalities in broiler chickens are usually complex. Although it has an ample genetic component, it is highly correlated with growth rate (Mignon-Grasteau *et al*., 2016). Nutrition, however, can also be a causative factor of bone disorder (Bradbury *et al*., 2014). This is because vitamins and minerals are usually the main nutritional factor crucial to bone health and proper skeletal development (Świątkiewicz and Arczewska-Wlosek, 2012). Calcium and phosphorous makeup over 70% of the total elements in the body. The relative content of calcium and phosphorous in the bone is nearly constant even when there is a dietary deficiency of only one of these elements (McDowell, 1992). Vitamins and minerals are incorporated into poultry diets in form of vitamin-mineral premix (VmP). Although needed relatively in small quantity (DSM, 2019), they are highly essential nutrients in poultry diets and at the same time, one of the livestock feed ingredient that contributes in large extent to the high cost of feed (Abudabos *et al*., 2013; DSM, 2019). Reports showed that leaves of plants such as *Vernonia amygdalina, Moringa oleifera, Occinum*

spp, Celocia argentea, Manihot spp, Telferia occidentalis, Chromoleana odorata amongst others are rich in reasonable quantity of vitamins, minerals, amino acids and antioxidants (Aro *et al.,* 2009; Ogbe and John, 2011; Adegbenro *et al.,* 2012a). Adegbenro *et al.*(2012b) reported that leaves of plants could adequately replace conventional VmP in livestock diets either singly or as a composite mixture and helps to lower dependence of farmers on imported VmP, reduce cost of production and consumer's concern on the toxicity of synthetic origin of the premixes (Botsoglou *et al.,* 2002). Report on leaf meal composite as replacement for the conventional VmP in broiler production is scanty. Recent studies on suitable replacement of VmP with LMC in broiler production (Adegbenro *et al*., 2012a, b; Ogunwole and Mosuro, 2020) and in rat diets (Mosuro and Ogunwole, 2020) have been documented. The present research was therefore aimed at assessing the bone characteristics of broiler chickens fed dietary LMC in place of VmP.

Materials and methods

One-day old Arbor Acres broiler chicks (n=936) purchased from Farm Support Hatchery, Ibadan were randomly allotted to thirteen treatments, each replicated eight times. A replicate comprised nine chicks each. Experimental diets and clean water were offered *ad libitum* to the respective chickens. Air dried leaves of *Moringa oleifera (drumstick tree), Celosia argentea (African spinach)*, *Vernonia amygdalina (Bitter leaf),* and *Telfaria occidentalis (Fluted pumpkin) were* mixed in equal proportion into a leaf meal composite (LMC) as test material. Detailed procedure of the test LMC production had been documented (Mosuro, 2018; Mosuro and Ogunwole, 2019).

In a (3x4) +1 augmented factorial arrangement (three levels of test LMC sample: 1.5, 3.0, and 4.5% and four levels of commercial VmP: 0.000, 0.125, 0.250, and 0.375%) The thirteen diets were altogether formulated as highlighted below:

Diet 1 = 0.000% VmP + 1.5%LMC
Diet 2 = 0.000% VmP + 3.0% LMC
Diet 3 = 0.000% VmP + 4.5% LMC
Diet 4 = 0.125% VmP + 1.5% LMC
Diet 5 = 0.125% VmP + 3.0% LMC
Diet 6 = 0.125% VmP + 4.5% LMC
Diet 7 = 0.250% VmP + 1.5% LMC
Diet 8 = 0.250% VmP + 3.0% LMC
Diet 9 = 0.250% VmP + 4.5% LMC
Diet 10 = 0.375% VmP + 1.5% LMC
Diet 11= 0.375% VmP + 3.0% LMC
Diet 12 = 0.000% VmP + 4.5% LMC

The thirteenth diet (Diet 13) had only 0.25% VmP incorporated (control).

Details of the basal experimental starter and finisher diets have been documented (Ogunwole and Mosuro, 2020) and are shown in Table 1. The broiler chickens were allocated to these diets in a completely randomised design and were fed experimental starter diets and the finisher each for three weeks *ad libitum.*

At day 36 of the experiment, eight broiler chickens with weight closest to the average weight of each treatment (one chick per replicate) were selected and sacrificed. Blood sample (precisely 3 mL) was collected from the jugular veins of the chickens and the serum harvested for calcium and phosphorus analyses (AOAC, 2000). The left drumstick of the same birds was also removed and immersed in boiling water (100 ˚C) for 10 minutes before defleshing. The bones were air-dried at room temperature for 24 hours for bone characteristics determination. The length and the weight of the tibiae were taken and the weight/length index was estimated by dividing the tibia weight by its length. The robusticity index was calculated using the equation of Reisenfeld (1972). The tibia

Table 1: Gross composition of basal experimental starter and finisher diets (control diet)

Ingredients	Level of Inclusion (%) starter	Level of Inclusion (%) finisher
Maize	50.00	58.00
Soybean Cake	38.00	30.00
Soya Oil	2.00	2.00
Wheat Offal	7.24	7.24
Salt	0.25	0.25
Oyster shell	0.50	0.50
Di-calcium Phosphate	1.50	1.50
*Vitamin-mineral Premix	0.25	0.25
Methionine	0.15	0.15
Lysine	0.05	0.05
Coccidiostat (Lasalocid)	0.06	0.06
Total	100.00	100.00
Calculated values:		
Crude Protein (%)	22.89	19.65
Metabolic Energy (Kcal/Kg)	3038.39	2915.39
Calcium %	1.00	1.18
Available Phosphorous %	0.56	0.58

'*1kg of Premix contains: Vitamin A 10,000,000IU; Vitamin D3 -2,000,000IU; Vitamin E-20,000IU; Vitamin K-2,250mg; Thiamine B1 -1,750mg; Riboflavin B2 -5,000mg; Pyridoxine B6 -2,750mg; Niacin -27,500mg; Pantothenic acid- 7,500mg; Biotin-50mg; Choline chloride-400g; Antioxidant- 125g; Magnesium-80g; Zinc-50mg; Iron-20g; Copper-5g; Iodine-1.2g; Selenium-200mg; Cobalt-200mg

bones were oven dried at 105 ˚C for 24 hours and ashed in a muffle furnace at 600 ˚C for three hours for percentage ash determination.

Precisely, 0.5g of the bone ash was dissolved in 5mL perchloric and nitric acid at the ratio of 2:1. The solution was heated at 105 ˚C until a light yellowish colouration was obtained. The digest was marked up with distilled water and analysed for bone calcium and phosphorus using atomic absorption spectrophotometer (AOAC, 2000).

Statistical analysis

Data were subjected to descriptive statistics, Pearson correlations and analysis of variance (SAS, 2002). Means were separated using orthogonal contrast option of the same software at $\alpha_{0.05.}$

Results

The main effect of CLM on tibia morphometric parameters of broiler chicken fed varying CLM inclusion levels is shown in Table 2. Varied dietary inclusion levels of CLM had no significant effect ($p>0.05$) on all the tibia morphometric parameters of broiler chickens. The main effect of varying dietary VmP on tibia morphometric parameters of broiler chickens is shown in Table 3. All assessed tibia morphometric parameters (tibia weight, tibia length and weight length index) except the robusticity index were similar ($p>0.05$). Robusticity index of tibia bone of broiler chickens on control (5.61) though similar ($p>0.05$) to those on 0.000% (5.49), 0.125% (5.51) and 0.375% (5.49) was significantly higher ($P<0.05$) than those on 0.250% dietary VmP (5.34).

Table 2: Main effect of dietary vitamin-mineral premix replacement with composite leaf meal on the tibia morphometric parameters of broiler chickens

	Percentage Inclusion					
Parameters	0	1.5	3.0	4.5	P value	SEM
Tibia weight (g)	3.23	3.19	3.22	3.19	1.00	0.009
Tibia length (cm)	8.25	7.91	8.10	8.15	0.29	0.008
Robusticity index	5.61	5.39	5.43	5.54	0.18	0.005
Weight /length index (mg/mm)	38.92	40.22	40.40	39.15	0.84	0.089

SEM = standard error of means

Table 3: Main effect of vitamin-mineral premix on tibia morphometric parameters of broiler chickens

	Percentage Inclusion						
Parameters	Control	0	0.125	0.25	0.375	P value	SEM
Tibia weight (g)	3.23	3.2	3.02	3.31	3.28	0.17	0.009
Tibia length (cm)	8.25	8.20	7.96	7.94	8.10	0.08	0.008
Robusticity index	5.61^{a}	5.49^{ab}	5.51^{ab}	5.34^{b}	5.49^{ab}	0.48	0.005
Weight/length index (mg/mm)	38.92	40.39	37.97	40.56	40.17	0.36	0.089

$^{a,b,:}$ means with different superscript within the same row differs significantly (P<0.05)
SEM = standard error of means

In Table 4, the effects of interaction of varying dietary inclusion levels of CLM and VmP on tibia morphometric parameters of broiler chickens are shown. Effects of the different dietary combinations of CLM and VmP on tibia morphometric indices (weight, length, robusticity index and weight length index) differed significantly ($p<0.05$). Broiler chickens on diet 12 recorded bone weight of 2.78g which was significantly of lower ($p<0.05$) weight than 3.75g in chicks on diet 10. Effect of the different combinations of VmP and LMC were similar on bone length (cm) of broiler chickens on diets 2 (8.25), 3 (8.44), 8 (8.09), 9 (8.33), 10 (8.39) and 11(8.11) which all had significantly higher ($p<0.05$) bone length than those on diet 7 (7.40). Bone robusticity index was lowest for broilers on diet 7 (5.18) and highest for those on diet 13 (5.61). Bones of broiler chickens on diets 12 (37.75) had a lower weight length index compared with those on diet 10 (44.44). The main effect of feeding varying levels of dietary CLM on bone mineral content of broiler chickens is shown in Table 5. Effect of treatment on broiler chicken tibia ash content was similar ($p>0.05$). However, bone calcium and phosphorous compositions differed significantly ($p<0.05$) with dietary inclusions of CLM. Bone calcium was higher in broilers on 0% CLM (11.82%) than those on 3.0% (10.65) but similar to those on 1.5% (11.07) and 4.5% (10.96). Similarly, bone phosphorous was significantly higher ($p<0.05$) in broiler chickens on 0% (1.37) CLM and least in those on 4.5% (1.26). The main effect of varying dietary VmP on bone mineral composition of broiler chickens is shown in Table 6. There was no significant effect ($p>0.05$) of treatment on both the bone ash and calcium contents. However, phosphorous was significantly higher ($p<0.05$) in broiler chickens on control (1.37) and 0.375% (1.38) than 0 (1.22).

Table 4: Effect of interaction of varying dietary composite leaf meal levels and vitamin-mineral premix on the tibia morphometric parameters of broiler chickens

	Vitamin-mineral premix (%)	Leaf meal (%)	Weight(g)	Length (mm)	Robusticity index	Weight/length index (mg/mm)
Diet 1	0.000	1.5	3.00^{ab}	7.91^{ab}	5.50^{ab}	37.98^{ab}
Diet 2		3.0	303^{ab}	8.25^{a}	5.43^{ab}	42.68^{ab}
Diet 3		4.5	3.58^{ab}	8.44^{a}	5.54^{ab}	42.31^{ab}
Diet 4	0.125	1.5	3.08^{ab}	7.92^{ab}	5.47^{ab}	38.90^{ab}
Diet 5		3.0	2.95^{ab}	7.95^{ab}	5.55^{ab}	37.06^{ab}
Diet 6		4.5	3.03^{ab}	8.02^{ab}	5.52^{ab}	37.95^{ab}
Diet 7	0.250	1.5	2.93^{ab}	7.40^{b}	5.18^{b}	39.57^{ab}
Diet 8		3.0	3.60^{ab}	8.09^{a}	5.28^{ab}	41.36^{ab}
Diet 9		4.5	3.40^{ab}	8.33^{a}	5.56^{ab}	40.76^{ab}
Diet 10	0.375	1.5	3.75^{a}	8.39^{a}	5.42^{ab}	44.44^{a}
Diet 11		3.0	3.30^{ab}	8.11^{a}	5.46^{ab}	40.49^{ab}
Diet 12		4.5	2.78^{b}	7.81^{ab}	5.58^{ab}	35.57^{b}
Diet 13	0.250	0.0	3.73^{ab}	8.25^{a}	5.61^{a}	38.92^{ab}
	P value		0.07	0.07	0.65	0.21
	SEM		0.009	0.008	0.005	0.089

$^{a,b,:}$ *means with different superscript within the same row differs significantly (P<0.05)*
SEM = standard error of means

Table 5: Main effect of dietary composite leaf meal on the bone mineral content of broiler chickens

	Percentage Inclusion					
Parameters (%)	0.0	1.5	3.0	4.5	SEM	P values
Ash	38.91	36.10	37.33	38.81	0.25	0.91
Calcium	11.82 ^{a}	11.07^{ab}	10.65 ^{b}	10.96^{ab}	0.023	0.27
Phosphorus	1.37^{a}	1.28^{bc}	1.34^{ab}	1.26^{c}	0.002	0.014

$^{a,b,c,:}$ *means with different superscript within the same row differs significantly (P<0.05)*
SEM = standard error of means.

Table 6: Main effect of varying dietary vitamin-mineral premix on bone mineral composition of broiler chickens

		Percentage Inclusion					
Parameters (%)	Control	0.000	0.125	0.250	0.375	SEM	P values
Ash	38.91	35.45	37.16	38.32	38.73	0.25	0.97
Calcium	11.82	10.86	10.84	10.69	11.17	0.023	0.22
Phosphorus	1.37^{a}	1.22^{c}	1.31^{ab}	1.25^{bc}	1.38^{a}	0.002	0.007

$^{a,b,c,:}$ *means with different Superscript within the same row differs significantly (P<0.05)*
SEM = standard error of means.

The effect of interaction of varying dietary VmP and CLM on broiler chickens bone mineral composition is shown in Table 7. The effect of interactions on bone ash (%) was not significantly different (p>0.05). However, the bone calcium and phosphorous compositions varied significantly (p<0.05) with the varying combinations of LMC and VmP. The main effect of replacing dietary VmP with LMC on broiler chickens' serum calcium and phosphorous are shown in Table 8. Serum calcium and phosphorous were similar (p>0.05) in respective broiler chickens fed 0.0 (14.25 and 4.19), 1.5 (14.07 and 4.12) and 4.5% (14.17 and 4.17) LMC but significantly higher (p<0.05) than in those fed 3.0% (13.50 and 3.84) dietary LMC.

Table 7: Effect of interaction of varying dietary vitamin-mineral premix and composite leaf meal on broiler chickens bone mineral composition

Diets ↓	Vitamin-mineral premix (%)	Leaf meal (%)	Ash (%)	Calcium (%)	Phosphorus (%)
1	0.000	1.5	33.29	11.54^{ab}	1.20^{e}
2		3.0	38.90	10.36^{ab}	1.25^{bcde}
3		4.5	34.17	10.68^{ab}	1.23^{de}
4	0.125	1.5	34.53	11.60^{ab}	1.26^{bcde}
5		3.0	35.86	10.66^{ab}	1.39^{ab}
6		4.5	41.08	10.25^{b}	1.28^{bcde}
7	0.250	1.5	41.66	10.86^{ab}	1.29^{bcde}
8		3.0	37.70	10.40^{ab}	1.23^{e}
9		4.5	35.60	10.82^{ab}	1.24^{cde}
10	0.375	1.5	34.91	10.24^{b}	1.36^{bcd}
11		3.0	30.88	11.20^{ab}	1.50^{a}
12		4.5	44.41	12.07^{a}	1.28^{bcde}
13	0.250	0.0	38.91	11.82^{ab}	1.37^{bc}
	P value		0.93	0.23	0.001
	SEM		0.25	0.023	0.002

$^{a,b,c,d,e:}$ means with different superscript within the same row differs significantly (P<0.05)
SEM = standard error of means.

Table 8: Main effect of replacing dietary vitamin mineral premix with leaf meal composite on broiler chickens' serum calcium and phosphorous composition

	Percentage Inclusion					
Parameters (mg/dL)	0	1.5	3.0	4.5	SEM	P values
Calcium	14.28^{a}	14.07^{a}	13.50^{b}	14.17^{a}	0.0089	0.004
Phosphorus	4.19^{a}	4.12^{a}	3.84^{b}	4.17^{a}	0.0045	0.006

$^{a,b,c,:}$ means with different superscript within the same row differs significantly (P<0.05)
SEM = standard error of means

In Table 9, the main effect of varying dietary VmP on broiler chickens' serum calcium and phosphorous composition (mg/dL) are shown. Serum calcium was significantly higher ($p<0.05$) in chickens on control diets (14.28) compared with those on 0.250% (13.60). Similarly, serum phosphorous was significantly higher ($p<0.05$) in chicks on control (4.19) than in those on 0.25% (3.90)VmP.

In Table 10, the correlation coefficients among bone, feed as well as serum calcium and phosphorous are shown. Serum phosphorus correlated positively with serum calcium ($r=0.868$, $p<0.01$) and dietary phosphorus ($r=0.698$, $p<0.01$). Dietary phosphorus however, correlated highly negatively with bone calcium ($r=0.408$, $p<0.01$) and serum calcium ($r=0.607$, $p<0.01$).

Table 9: Main effect of varying dietary vitamin-mineral premix on serum mineral content of broiler chickens

		Percentage Inclusion					
Parameters (mg/dL)	Control	0.000	0.125	0.250	0.375	SEM	P values
Calcium	14.28^{a}	14.02^{ab}	13.97^{ab}	13.60^{b}	14.07^{ab}	0.0014	0.004
Phosphorus	4.19^{a}	4.06^{ab}	4.09^{ab}	3.90^{b}	4.13^{ab}	0.023	0.006

$^{a,b,:}$ means with different superscript within the same row differs significantly (P<0.05)
SEM = standard error of means.

Table 10: Correla tion coefficients among bone, feed and serum calcium and phosphorous compositions of broiler chickens fed varying inclusion levels of composite leaf meal and vitamin-mineral premix

Parameters	Bone calcium	Feed calcium	Serum calcium	Bone phosphorous	Feed phosphorous	Serum phosphorous
Bone calcium	1.000					
Feed calcium	0.065	1.000				
Serum calcium	0.257	-0.293	1.000			
Bone phosphorous	0.077	-0.048	-0.089	1.000		
Feed Phosphorous	-0.408**	0.057	-0.607**	0.009	1.000	
Serum phosphorous	0.269	-0.064	0.868**	-0.068	0.698**	1.000

***Correlation was significant at the 0.01 level (2-tailed)*

Discussion

Replacement of supplemental VmP with LMC in the diets did not have any major effect on the assessed bone morphometric parameters of broiler chickens in this study as shown in Tables 2 and 3. Thus, either dietary VmP or LMC or combinations would equally influence bone morphometric parameters in similar manners. However, bone robusticity index of the experimental broiler chickens reduced from 5.61 to 5.34 due to the main effect of increased inclusion of dietary VmP in Table 3. Skeletal robusticity refers to the strength of a skeletal element relative to some mechanically relevant measure of body size and is generally considered a reflection of the magnitude of mechanical loads that are habitually incurred by that element as the organism interacts with its environment (CARTA, 2019). A low robusticity index is an indication of strong bone structure (Safaeikatouli *et al*., 2012). Results showed that supplemental VmP at 0.25% of the diet was most favourable for bone robusticity in this study. In Table 4, effects of the different combination levels of VmP and LMP on tibia morphometry are shown. The tibia weight of 3.73g and weight/length index of 38.92 mg/mm in the bones of broiler chickens on Diet 13 was lower than 7.59g and 77.22 mg/mm reported by Mutus *et al.* (2006). The bone strength was maintained with the varying combinations of CLM and VmP, but the bones were less dense as a result of lower weight/length index. Minerals are major cofactors of all metabolic reactions and are implicated in all processes involved in bone formation and growth. The main structural macro minerals: calcium and phosphorus are abundant and are stored in the bone for mobilisation when metabolic needs arise (Cheeke and Dierenfeld, 2010). The bone ash is entirely comprised of minerals like Ca, P, Mg, Na, K, Cl, F and traces of other elements (McDowell, 1992). Tibia ash range of between 33.29 and 44.41% was lower than 47.98 and 49.66% reported by Nkukwana *et al.* (2014). Also, the tibia ash, calcium and phosphorus of the bones of broilers on different diets were lower than 43.2% ash, 17.1% calcium and 8.2% phosphorus reported by Shafey and McDonald (1990) and 22.52% tibia calcium and 10.06% phosphorus documented by Mutus *et al.* (2006). The LMC components were predominantly plant materials which are known to contain nutrients including vitamins and minerals which are preponderantly not bioavailable to monogastric omnivore stocks like poultry (Baker and Stein, 2013).

The tibia calcium of 10.00-12.07% obtained in this study was also similar to the 10.00-14.76% tibia calcium reported by Moravej *et al.* (2013). Vitamin D is a regulator of calcium and phosphorus metabolism and plays an important role in controlling calcium and phosphorus homoeostasis through direct action of the hormone on the bones, intestine and kidney

(Pike *et al.,* 2007). The variations of bone calcium and phosphorus among treatments were not analogous to the mineral composition in the feed (William *et al*., 2000). However, the feed phosphorus correlated negatively with bone calcium and also has low correlation (0.9%) with the bone phosphorus. The serum calcium concentration recorded were above the 8.85 mg/dL reported by Silva *et al.* (2007) and 12.71 mg/dL (Bozkurt *et al.,* 2012) with corresponding low serum phosphorus when compared to 6.21 mg/dL and 6.25 mg/dL, respectively reported by the same authors. Report of Moravej *et al*. (2012) showed no significant effect of dietary VmP replacements on bone parameters of broiler chickens. The findings were premised among others, on the fact that vitamins particularly, fat soluble vitamins which were mostly implicated in bone metabolism (Cheeke and Dierenfeld, 2010) usually, exceed two or three times of the recommended requirement in poultry diets. Fat-soluble vitamins are stored momentarily in the liver and fatty tissue in sufficient quantities to meet requirements for some time. Also, broiler chickens housed in the deep litter like in the present study, were exposed to their faecal droppings which were inadvertently consumed to obviate or mitigate the likely deficiency of vitamin and or minerals consequent on dietary inadequacy as earlier opined (Baker, 1995; Maiorka *et al*., 2002 Alahyari-Shahrasb *et al*., 2011; Alahyari-Shahrasb *et al*., 2012). Such practice may have prevented the expected outcome of the different combinations or replacement observed in this study. Therefore, observation may be different for broiler chickens kept solely in the battery during brooding and rearing. Thus, study of this nature should be carried out on broiler chickens kept in cage.

Conclusion

The gross indices of broiler chickens' bones were not appreciably altered by the varying inclusion levels and combinations of composite leaf meal and vitamin-mineral premix. In addition, the calcium and phosphorus utilisation were not affected by the varying dietary levels of CLM and VmP. However, similar study should be undertaken for broiler chickens in cages.

References

Abudabos, A. M., Samara, E. M., Hussein, E. O. S., Al-Ghadi, M. Q. and Al-Atiyat, R. M. 2013. Imapct of stocking density on the performance and welfare of broiler chickens. *Italian Journal of Animal Science* 12(1):e11.

Adegbenro, M., Ayeni, A. O., Agbede, J. O. Onibi, G. E. and Aletor, V. A. 2012a. Performanceand cost evaluation of some leaf meals as composite premix in broiler starter diets. *31st Biennial Conference of Ghana Animal Science Association.* Book of Abstracts, pp 2.

Adegbenro, M., Ayeni, A. O., Olowoyeye, J. C., Bankole, O. M., Agbede, J. O., Onibi, G. E. andAletor, V. A. 2012b. Leaf composites mix as alternative premix to commercial premix in broiler finisher diets. *31st Biennal Science association*. Book of Abstracts.

Alahyari-Shahrasb, M., Moravej, H., Shivazad, M. and Gerami, A. 2011. Study of possible reduction or withdrawal of vitamin premix during finisher period in floor and battery cage broiler raising systems. *African Journal Biotechnology*, 33: 6337

Alahyari-Shahrasb, M., Moravej, H., and Shivazad, M. 2012. Effect of different levels of vitamin premix

during finisher period on broiler performance and immunocompetence in battery cage and floor systems, *Cuban Journal of Agricultural Science*, 46 (3).

AOAC. 2000. Association of Official Analytical Chemists. *Official Methods of Analysis 16th ed. Association of Analytical Chemists,* Washington DC.

Aro, S. O., Osho, I. B., Aletor, V. A. and Tewe, O. O. 2009. *Chromolaena Odorata* in livestock nutrition. *Journal of Medicinal Plants Research.* 3(15): 1253-1257.

Baker, D. H. 1995. Vitamin Bioavailability; Bioavailability of Nutrients for Animals; Amino Acids, Minerals, and Vitamins. Eds. C. B. Ammerman, D. H. Baker and A. J. Lewis. Academic Press, San Diego, USA, page 399-423 *http://www.apnet.com*

Baker, D. H. and Stein, H. H. 2013. Bioavailability of minerals and vitamins in feedstuffs. Pages 341-365 in Sustainable Swine Nutrition. Chiba, L. I., ed. John Wiley and Sons Inc.,

Ames, I. A., Botsoglou, N. A., Christaki, E., Fletouris, D.I., Florou-Paneri, P., and Spais, A. B. 2002. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. *Meat Science,* 62,259-265.

Bozkurt, M., Kucukyilmaz,K.,Cath, A.U.,Cmar, M., Abuk, M. and Bintas, E. 2012. Effects of boron supplementation to diets deficient in calcium and phosphorus on performance with some serum, bone and fecal characteristics of broiler chickens. *Asian-Australian Journal of Animal Science,* Vol. 25 (2): 248-255.

Bradbury, E. J., S. J. Wilkinson, G. M. Cronin, P. C. Thomson, M. R. Bedford and Cowieson, A. J.2014. Nutritional geometry of calcium and phosphorus nutrition in broiler chicks. Growthperformance, skeletal health and intake arrays *Animal,* 8:7, pp 1071–1079 The Animal Consortium 2014doi:10.1017/S175173111400103

CARTA. 2019. Centre for Academic Research and Training in Anthropogeny. The Matrix ofComparative Anthropogeny (MOCA)Skeletal Robusticity: https://carta.anthropogeny.org/moca/ topics/skeletal-robusticity

Dibner, J.J,, Richards, J.D.,Kitchell, M.L. and Quiroz, M. A. 2007. Metabolic challenges andearly bone development. *Journal of Applied Poultry Research.* 16:126-137.

DSM. 2019. DSM in Animal Nutrition and Health; Vitamin Nutrition is Dynamic and Cost Effective.
https://www.dsm.com/markets/anh/en_US/Compendium/vitamin_basics/vitamin_nutrition_is_dynamic_and_cost_effective.html

Cheeke, P. R., and Dierenfeld, E. S. 2010. Comparative Animal Nutrition and Metabolism. CABI Publishing, Wallingford.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry - A Review. *Veterinary Journal*, 169 (3): 350-69 *doi: 1016/j.tvjl.2004.04.015*

Maiorka, A., Laurentiz, A. C., Santin, E., Araujo, L. F. and Macari, M. 2002. Dietary vitamin or mineral

mix removal during the finisher period on broiler chicken performance. *Journal o f Applied Poultry Research,* 11: 121-126.

McDowell, L. R. 1992. Minerals in animal and human nutrition. Academic Press, San Diego.

Mignon-Grasteau, S., Chantry-Darmon, C., Boscher, M., Sellier, N., Chabault-Dhuit, M., Bihan-Duval, E and Narcy, A. 2016. Genetic determinism of bone and mineral metabolism in m e a t - type chickens: A QTL mapping study**.** *Bone Reports*, 5: 43–50. doi: 10.1016/j.bonr.2016.02.004 PMCID: PMC4926819 PMID: 28326346

Moravej, H., Alahyari-shahrasb, M., K i a n i , A . , B a g h e r i r a d , M.andShivazad, M. 2013. Effects of different levels of vitamin mineral premix in finisher diets on performance, immunocompetence and meat lipid peroxidation of chickens fed on corn-soybean meal. *Veterinary Research Forum,* 4(1):13-18.

Moravej, H., Alahyari, S. M. and Shivazad, M. 2012. Effects of the reduction or withdrawal of the vitamin premix from the diet on chicken. Performance and meat quality. *Brazilian Journal of Poultry Science.* 14(4): 233-304

Mosuro, A. O. 2018. Replacement of dietary vitamin-mineral premix with leaf meal composites in broiler chicken diets. A thesis in the Department of Animal Science, submitted to the Faculty of Agriculture in partial fulfillment for the award of PhD, University of Ibadan, Ibadan, Nigeria. 186pp.

Mosuro, A. O. and Ogunwole, O. A. 2019. Vitamin and provitamin profiles of selected vegetables as affected by storage and different drying methods. *Journal of Agricultural Sciences* Serbia*,* 64 (2): 175-188.

Mosuro, A. O. and Ogunwole, O. A. 2019. Biological response, haematology and serum biochemical indices of weanling Wistar rats as affected by replacement of dietary vitamin-mineral premix with differently processed leaf meal composites. *Tropical Veterinarian*37 (4) 234-255

Mutus, R., Kocabagli, M., Alp, N., Acar, M., Eren, and Gezen, S. S. 2006. The effects of dietary

probiotic supplementation on tibial bone characteristics and strength in broilers. *Poultry Science,* 85: 1621-1625.

Nkukwana, T. T., Muchenje, V., Masika, P. J., Hoffman., L. C., Dzama, K. and Descalzo, A. M. 2014. Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration. *Food Chemistry* 142: 255–261.

Ogbe, A. O. and John, P. A. 2011. Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves harvested at Lafia, Nigeria: Potential benefits in poultry nutrition and health. *Journal of Microbiology, Biotechnology and Food Science,* (3): 296-308.

Ogunwole, O. A. and Mosuro, A. O. 2020. Replacement of dietary vitamin-mineral premix with leaf meal composites improved the growth performance of broiler chicken. *Slovak Journal of Animal Science* Accepted.

Pike, J. W., Zella, L. A., Meyer, M. B.,

Fretz, J. A. and Kim, S. 2007. Molecular actions of 1,25Dihydroxyvitamin D3 on gens involved in calcium homeostasis. *Journal of Bone Minerals Research,* 22(Suppl, 2): V16-V19.

Rath, N. C., Huff, G. R., Huff, W. E. and Balog, J. M. 2000. Factors regulating bone maturity andstrength in poultry. *Poultry Science.* V79: page 1024-1034.

Reisenfield, A. 1972. Metatarsal robusticity in bipedal rats. *America Journal of physical Anthropology*, V40: page 229-234.

Safaeikatouli, M., Boldaji, F., Dastar, B. andHassan, S. 2012. Growth response and tibia bone characteristics in broilers fed diets containing kaolin, bentonite and zeolite.–*Journal of Animal Feed Science* 21: 334-344.

SAS Institute, Inc. 2002. SAS language: Reference (version 6 1st ed.) SAS Institute Inc.,carry North Caroline USA.

Shafey, T. M. and McDonald, M. W. 1990. Effects of dietary calcium: available phosphorus ratio on calcium and phosphorus in performance, phytate P and mineral retention in Japanese quail (*Coturnixcoturnix Japonica*). *International Journal of Poultry Science,* 8(7): 692-695.

Silva, P. R. L., FreitasNeto, O. C., Laurentiz, A. C., Junquiera, O. M. and Fagliari, J. J. 2007. Blood serum components and serum protein test of hybro-PG broilers of different ages. *Brazilian Journal of Poultry Science,* Vol. 9(4): 229-232.

Swiatkiewicz, S. and Arczewska-Wlosek, A. 2012. Bone quality characteristics and performance in broiler chickens fed diets supplemented with organic acids. *Czech Journal of Animal Science,* 57(4): 193-205. DOI: 10.3923/ajava.2013.747.753

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