Calcium and Phosphorus Retention and Bone Characteristics of Broiler Chickens Fed β - Carotene Bio-Fortified Cassava (*Manihot esculenta* Crantz) Grits Based Diets

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Abstract

Effect of replacing yellow maize with grits from β-carotene bio-fortified whole cassava roots on calcium and phosphorus retention and bone characteristics of broiler chickens was studied in a 42-day experiment. In a completely randomized design, 120 one-day old Arbor Acre broiler chicks were allotted to four dietary treatments each replicated thrice with 10 birds per replicate. Diet 1(control) was the yellow maize based diet in which maize was replaced with cassava grits prepared from -carotene bio-fortified whole cassava roots (TMS 01/1371) at 25 (diet 2), 50 (diet 3) and 75% (diet 4). On day 21 and 42 respectively, two birds were randomly chosen from each replicate for Ca and P retention study. Blood samples were also collected for serum biochemical analysis while the left and right tibiae were harvested at day 42 for bone morphometric parameters and mineral composition determination. There were no significant differences (P>0.05) in calcium retention levels, serum biochemical indices, bone physical characteristics and mineral content across treatments. However, serum alkaline phosphatase (U/L) of finisher birds fed diet 3 (61.83) was significantly lower (P<0.05) compared with 65.17, 66.50 and 74.00 obtained from birds on diets 1, 2 and 4, respectively. Correlation of dietary phosphorus with feed intake and phosphorus retention were positive and highly significant (r = 0.962, P<0.01). Regression of dietary phosphorus and phosphorus retention in finisher chickens fed test diets was linear, positive and highly significant (P<0.01), with the equation: $y = 210.26x – 18.82$; (R^2 = 0.95). In conclusion, calcium and phosphorus retention by broiler chickens were not altered by dietary maize replacement with -carotene bio-fortified cassava grit in this experiment.

Keywords: β-carotene bio-fortified cassava roots, Broiler chickens, Calcium and phosphorus retention, Cassava grits based diets, Dietary minerals.

Introduction

Modern broiler chickens are often associated with problem of skeletal deformities ensuing from genetic faults and nutrition imbalances (Julian, 1998) which, ultimately, affects the process of bone mineralisation in them. Some of the key factors regulating this process are hormones and other systemic factors, local factors (such as cytokines and growth factors), bone cells and bone matrix proteins (Ali, 1992; Kini, 2012). Vitamin A as retinoic acid, acts as hormone which regulates the formation of long and short bones (Blomhoff and Blomhoff, 2006; Daryl, 2009) and consequently, its mineralisation. Retinoic acid is synthesised *de novo* from the oxidation of retinol or directly from provitamin A (β-carotene) (Blomhoff and Blomhoff, 2006). It operates by receptor-mediated repression which is both necessary and sufficient for chondroblast differentiation (Hoffman, 2003), cartilage development and skeletogenesis (Conaway *et al*., 2009; Lind *et al*., 2011) and is one of the key regulators of bone formation and development in animals (Kirimoto *et al*., 2005). Retinoic acid suppresses cell growth (i.e. osteoclastogenesis), cartilage nodule formation, accumulation of proteoglycan, alkaline phosphatase (ALP) activity and mineralisation in a dose-dependent and spatio-temporal manner (Kirimoto *et al.,* 2005). Excessive intake of vitamin A leads to accumulation beyond the capacity of binding proteins, and therefore, causes tissue damage. It also affects calcium homeostasis thereby causing the thickening of long bones, hypocalcaemia, and calcinosis (David and Peter, 2009).

Avitaminosis A related diseases constitute one of the main food security problems facing Nigeria and the Sub-Saharan Africa (Maziya-Dixon *et al.* 2006). This was attributed to inadequate essential micronutrients (such as vitamin A, Fe, and Zn) in cassava mostly cultivated and consumed by the populace (Morante *et al*., 2010). Recently developed β-carotene rich cassava grit has, however, been identified as potential panacea to these problems (Global Panel, 2015). Since, skeletal deformities in fast growing broiler chickens have been attributed to nutritional imbalances among other factors. It therefore becomes necessary, to also document the effect vitamin A intake on mineral utilisation and development of bones in fast growing broiler chickens when fed diets rich in provitamin A.

The proprietary of β-carotene in bio-fortified cassava in alleviating vitamin A shortage is, however, yet to be well established. This is because of the dearth of information on its bioavailability when fed to animals. Earlier study (Ogunwole *et al.*, 2015), on effects of β-carotene bio fortified cassava grits based diet fed to broiler starter chicks revealed no significant effect on liver retinol status, though; there was reported improved broiler performance. Also, replacement of maize with β-carotene bio-fortified cassava up to 75% was documented as an avenue of improving broiler chicken meat shelf life and oxidative stability (Ogunwole *et al*., 2016). The present endeavour was aimed at assessing the effects of feeding β-carotene bio-fortified cassava grits based-diets on the retention of calcium and phosphorus by broiler chickens.

Materials and Methods

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan Nigeria located on latitude $7^0 20^1$ N, longitude $3^0 50^1$ E, and 200 m above sea level in tropical rain forest vegetation zone. The laboratory analyses were carried out at the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental Animals and Management

One-day old Arbor Acre broiler chicks (n=120) were obtained from Amo Farm and Sieberer Hatchery, Awe, Oyo State, Nigeria. The chicks were randomly selected and distributed into four dietary treatments in triplicates of 10 birds per replicate. The birds were raised in a deep litter system with facilities for separate provision of feed and water. Feed and water were offered to birds *ad libitum*. Individual feeds were analysed for calcium and phosphorus to ensure uniform mineral composition in the basal diet. Standard management practice followed was as described (Oluyemi and Roberts, 2000).

Experimental Diets

Four iso-caloric and iso-nitrogenous diets were compounded to contain cassava grits from TMS 01/1371 replacing yellow maize at 25, 50 and 75 % levels, respectively and a yellow maize based diet as the control. TMS 01/1371 is a β-carotene bio-fortified cassava variety with conspicuous yellow root and was processed to grits as documented (Tewe, 2005). Detailed dietary formulation and gross composition of the experimental starter and finisher diets have been published (Ogunwole *et al.*, 2015; 2016) and is shown in Table 1.

Determination of Minerals Apparent Retention in Broiler Chickens

Birds were fed for five days at both the starter and finisher phases (week 3 and 6 respectively) in metabolic cages following 2 days of acclimatization. During the trial, records of daily feed offered and feed left-over were taken. Total voided faecal samples from two birds per replicate were collected, bulked and analysed for faecal calcium and phosphorus following standard procedure (AOAC, 2000). Samples of experimental feeds (including control) at both the starter and finisher phases were analysed to determine dry matter, ash, calcium and phosphorus content using the procedure of AOAC (2000). The percentage faecal calcium and phosphorus were calculated and recorded relative to the ash weight. Apparent retention coefficient of minerals was evaluated by calculating the differences in the calcium and phosphorus content of feed intake and faeces voided (Thomas and Ravindran, 2010).

Serum Biochemical Indices

Blood samples were collected at weeks 3 and 6 from jugular veins of the birds and were allowed to coagulate in non-heparinised tubes. The coagulated blood samples were centrifuged at 3,000 rpm for 15 min. Extracted serum was stored in a freezer at -20 °C and analysed for serum calcium and phosphorus (AOAC, 2000) using commercial colorimetric kits manufactured by (Quimica Clinica Aplicada S.A., Amposta, Spain). Serum alkaline phosphatase (ALP), aspartate amino transferase (AST) by spectrophotometric method (Reitman and Frandel, 1957) and serum creatinine were analysed (Thomas, 1992).

Determination of Bone Characteristics

At week 6, the left and right legs of two birds from each replicates were removed from drumsticks with flesh intact. The drumsticks were labelled and immersed in boiling water (100 °C) for 10 minutes. After cooling to room temperature, the drumsticks were defleshed by hand and the patella was removed. They were then air-dried for 24 hours at room temperature. Prior to breaking, each bone was marked at midpoint, and outside diameters were measured perpendicular and parallel to the direction of the applied force using a digital Vernier calliper with an accuracy of 0.001 cm. After breaking, diameter measurements were made inside and outside the midshaft of the bone, perpendicular and parallel to the direction of the applied force. The thickness of the medial and lateral walls was measured at the midpoint mark using a digital Vernier calliper.

$$\text{Retention coefficient} = \frac{(Feed\ intake \times Mineral_{diet}) - (Faecal\ output \times Mineral_{excreta})}{(Feed\ intake \times Mineral_{diet})}$$

Medullary canal diameter was calculated by subtracting the thicknesses of the medial and lateral walls from the diameter at the diaphysis (Mutus *et al*., 2006). The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor *et al*., 1991). The tibiotarsal and the robusticity indices were determined using the formulae of Barnet and Nordin (1960) and Reisenfeld (1972), respectively as follows:

$$\text{Tibiotarsal Index} = \frac{\text{Diaphysis diameter} - \text{Medullary canal diameter}}{\text{Diaphysis diameter}} \times 100$$

$$\text{Robusticity Index} = \frac{\text{Bone length}}{\text{Cuberoot of bone weight}}$$

Tibiae Mineral Determination

The tibia bones were oven-dried at 105 °C for 24 h and ashed in a muffle furnace at 600 °C for 4 hours. The percentage ash was determined relative to dry weight of the tibia (i.e. pre-ash tibia multiplied by 100). From each replicate, 0.2 g of bone ash was dissolved in 10 mL of 50 % concentrated hydrochloric acid. Digested samples were filtered and diluted with de-ionized water to the required volume and analyzed for calcium and phosphorus using UV spectrophotometer at wavelengths 540 nm and 650 nm,

Table 1: Gross composition (g/100 gDM) of experimental starter and finisher diets fed to broiler chickens

Replacement levels of TMS 01/1371 cassava grits (%)								
	STARTER				FINISHER			
INGREDIENTS	**0** **1**	**25** **2**	**50** **3**	**75** **4**	**0** **1**	**25** **2**	**50** **3**	**75** **4**
Yellow Maize	50.00	37.50	25.0	12.50	52.00	39.00	26.00	13.00
White Maize	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TMS 01/1371	0.00	12.50	25.00	37.50	0.00	13.00	26.00	39.00
Soyabean meal	35.90	39.00	41.50	43.14	30.90	34.00	36.50	39.90
Wheat offal	8.24	5.14	2.64	1.00	11.34	8.24	5.74	2.34
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Palm oil	2.50	2.50	2.50	2.50	2.40	2.40	2.40	2.40
Dicalcium Phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Table Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Avatec	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Nutrients Composition								
ME (Kcal/kg)	3080.3	3091.8	3098.4	3097.7	3062.4	3082.5	3097.4	3119.9
CP (%)	20.97	20.88	20.64	20.19	19.58	19.42	19.11	19.03
DL Methionine (%)	0.52	0.51	0.50	0.49	0.50	0.50	0.48	0.48
Lysine (%)	1.30	1.33	1.36	1.37	1.19	1.23	1.25	1.29
Av. P (%)	0.52	0.52	0.52	0.51	0.51	0.50	0.50	0.50
Calcium (%)	0.98	1.00	1.03	1.05	0.82	0.85	0.87	0.90

DCP- Dicalcium phosphate; ME- Metabolisable energy; CP- Crude protein; Av P- Available phosphorus

*Each 2.5 kg vitamin/mineral premix contain: vitamin A-10,0000,0000 I.U, vitamin D3 22000000 I.U., vitamin E-10,000 mg, vitamin K3-2,000, Folic Acid-500 mg, Niacin-15,000 mg, Calpan-5000 mg, vitamin B2-5,000 mg, vitamin B12-10 mg, vitamin B1-1500 mg, vitamin B6-1500 mg, Biotin-20mg, antioxidant-125,000mg, selenium-200 mg, iodine-1000 mg, iron-40,000 mg, cobalt 200 mg, manganese-70,000 mg, copper-4000 mg, Zinc-50,000 mg, choline chloride 150,000 mg and yolk colorant.

respectively. Tibiae Ca and P were determined using commercial colorimetric kit (Quimica Clinica Aplicada S.A., Amposta, Spain) as described (Fiske and Subbarow, 1925; Goodwin, 1970).

Statistical Analysis

Data were subjected to one-way analysis of variance using the Statistical Analysis Systems package (SAS, 2002), means were separated using Duncan's multiple range test (DMRT) option of the same software at $\alpha_{0.05}$.

Results

Apparent calcium and phosphorus retention of starter broiler chicken

Calcium and phosphorous content of cassava grits based diets fed to broiler chickens at starter and finisher stages are shown in Table 2. There were significant differences (P<0.05) in calcium content of starter diets and phosphorus content of finisher diets. Retention of calcium and phosphorus by starter broiler chicken at week 3 is presented in Table 3. There were no significant differences (P>0.05) in calcium and phosphorus intake, in the droppings and retention across the treatments. Feed intake (g) of birds ranged from 44.88 to 49.24 which was statistically similar (P>0.05) to 48.43 g obtained from birds on diet 1. Birds fed diet 3 had higher (P<0.05) calcium intake (1.01 g) compared with those fed diets 2 and 4 although, statistically similar (P>0.05) to those on diet 1 (0.92 g). Phosphorus intake of birds in TMS group ranged from 0.30 to 0.34g which were significantly different (P<0.05) from 0.39 g for birds on diet 1.

Table 2: Calcium and Phosphorous Composition of Cassava Grits Based Diets Fed to Broiler Chickens at the Starter and Finisher Phases

	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Starter Diet					
Ca (%)	1.90^{ab}	1.86^{ab}	2.25^{a}	1.62^{b}	0.09
P (%)	0.80	0.67	0.75	0.61	0.04
Finisher Diet					
Ca (%)	0.58	0.64	0.68	0.63	0.04
P (%)	0.26^{b}	0.34^{a}	0.24^{b}	0.29^{ab}	0.02

Means with different superscripts within the same row differed significantly (P<0.05).
Ca = calcium. P = phosphorus. SEM = standard error of means.

Table 3: Calcium and Phosphorus Retention of Starter Broiler Chickens Fed TMS 01/1371 Cassava Grits Based diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Daily feed intake (g/bird)	48.43	45.42	44.88	49.24	1.40
Ca intake	0.92^{ab}	0.85^{bc}	1.01^{a}	0.80^{c}	0.04
P intake	0.39^{a}	0.31^{b}	0.34^{b}	0.30^{b}	0.02
Daily Feacal Voided	9.78^{b}	13.89^{a}	13.22^{ab}	10.94^{ab}	0.44
Ca excreted	0.25	0.23	0.21	0.20	0.01
P excreted	0.09	0.09	0.08	0.07	0.00
Ca retention (%)	0.73	0.73	0.79	0.75	0.02
P retention (%)	0.78	0.71	0.75	0.77	0.02

Means with different superscripts within the same row differed significantly (P<0.05).

Apparent Calcium and Phosphorus Retention of Finisher Broiler Chickens Fed TMS 01/1371 Cassava Grit Based Diets

The calcium and phosphorus retention by finisher broiler chickens at week 6 is presented in Table 4. There were no significant differences (P>0.05) in feed intake, voided, and retained calcium as well as phosphorus across the treatments. Calcium intake by birds fed TMS 01/1371 based diets (2, 3, and 4) ranged between 0.97 and 1.05 g/bird/day and were significantly different (P<0.05) from those fed diet 1 with the value of 0.95 g/bird/day. Similarly, phosphorus intake of birds fed diets 2, 3 and 4 were significantly different (P<0.05) from one another and from those on diet 1 (0.42 g/bird/day). Phosphorus retention increased (P<0.05) with increased levels of TMS 01/1371 cassava grits in the diets. Birds fed diet 3 had higher phosphorus retention level of 55 % compared with those on diets 1, 2 and 4 with values of 41, 34 and 39 %, respectively.

Correlations of Dietary Phosphorus with Feed Intake, Mineral Retention and Bone Phosphorus in Finisher Broiler Chickens Fed Graded Levels of TMS 01/1371 Based Diets

The correlation of dietary phosphorus with feed intake, mineral retention (calcium and phosphorus) and bone phosphorus in finisher broiler chickens fed graded levels of TMS 01/1371 based diets at the finisher phase is presented in Table 5. Dietary phosphorus was highly positively correlated with phosphorus retention (r = 0.962, P<0.01), feed intake (r = 0.917, P<0.01); but highly negatively correlated with bone phosphorus (r = 0.953, P<0.01). Similarly, feed intake was highly positively correlated (P<0.01) with calcium retention (r =0.878) and phosphorus retention (r= 0.990). This was the same for the relationships between bone calcium and feacal phosphorus (r =0.936, P<0.01). Phosphorus retention however, was negatively correlated (P<0.01) with bone phosphorus (r = 0.858).

Table 4: Calcium and Phosphorus Retention Finisher Broiler Chickens Fed Cassava Grit Based-Diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Daily feed intake (g/bird)	164.62	159.77	154.37	154.91	2.90
Ca intake	0.95^{b}	1.03^{ab}	1.05^{a}	0.97^{ab}	0.04
P intake	0.42^{c}	0.55^{a}	0.38^{d}	0.45^{bc}	0.01
Daily feacal voided	34.02	37.95	39.50	36.05	0.80
Ca excreted	0.56	0.57	0.65	0.64	0.02
P excreted	0.25	0.25	0.25	0.28	0.01
Ca retention (%)	41.00	45.00	39.00	35.00	0.03
P retention (%)	41.00^{b}	55.00^{a}	34.00^{b}	39.00^{b}	0.02

$^{a,b\ d,e,f:}$ means with different superscripts within the same row differed significantly (P<0.05).
Ca = Calcium. P = Phosphorus. DFI- Daily feed intake. DFV-Daily feacal voided. SEM = standard error of means. All parameters measured were in g/bird/day

The regression of dietary phosphorus and phosphorus retention (%) in finisher broiler chickens fed TMS 01/1371 based diets is shown in Figure 1. A unit increase in dietary calcium (g/100 g DM) above 0.24 g/ 100 g produced 2.10 % increase in calcium retention. The strength of association between phosphorus retention (%) and dietary phosphorus (g/100g DM) was positive (R^2 = 0.925). The regression equation obtained was: y = 210.26x - 18.82.

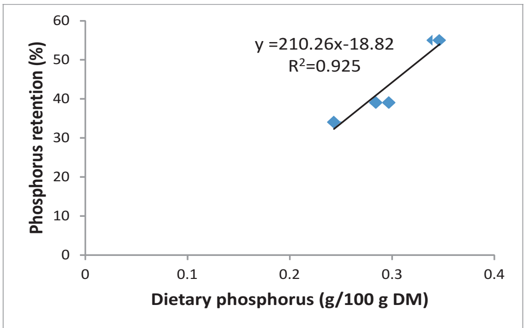

Figure 1: Relationship between dietary phosphorus (g/100g) and apparent phosphorus retention (%) in finishers' broilers chickens fed TMS 01/1371 based diets

Serum Biochemical Indices of Finisher Broiler Chickens Fed Grits Based-Diets

The serum biochemical indices of finisher broiler chickens at day 42 of age are shown in Table 6. The treatments effect was not significant (P>0.05) on sera AST, creatinine, calcium and phosphorus of birds. However, ALP differed significantly (P<0.05). Serum ALP of birds fed diet 1(65.17 U/L) was not significantly different (P>0.05) from those on diets 2 and 4, although, it was higher (P<0.05) compared with the 61.83 U/L obtained from birds on diet 3.

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Table 5: Correlation of Dietary Phosphorus on Feed Intake, Mineral Retention and Bone Phosphorus in Finisher Broiler Chickens Fed Graded Levels of TMS 01/1371 Based Diets

Parameters	Feed intake	P retention	Ca retention	Bone P	Feed P
Feed P	0.971**	0.962**	0.621	-0.953**	1.000
Bone P	-0.797	-0.858**	-0.463	1.000	
Ca retention	0.878**	0.803	1.000		
P retention	0.990**	1.000			
Feed Intake	1.000				

**Correlation is significant at the 0.01 level (2-tailed) Correlation is significant at the 0.05 level (2-tailed). Ca = Calcium, P = Phosphorus

Table 6: Serum biochemical indices of finisher broiler chickens fed cassava grits based-diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
AST (U/L)	239.00	246.67	222.00	258.83	5.15
ALP (U/L)	65.17[abc]	66.50[abc]	61.83[c]	74.00[a]	1.29
Creatinine (mg/dL)	0.93	1.12	1.07	1.02	0.02
Ca (mg/dL)	39.33	36.00	31.50	34.00	1.01
P (mg/dL)	3.52	3.93	3.63	3.65	0.07

Means with different superscripts within the same row differed significantly (P<0.05).
SEM = standard error of means. Ca = Calcium. P = Phosphorus. AST-Aspartate aminotransferase.
ALP-Alkaline phosphatase

Morphometric Parameters and Mineral Content of Tibia Bone of Finisher Broiler Chickens Fed Cassava Grits Based-Diet

The bone characteristics of broiler chickens at week 6 are presented in Table 7. There were no significant differences (P>0.05) in bone morphometric parameters and mineral (calcium and phosphorus) content of the tibia bones across the treatments. Tibia weight (g), tibia length (mm), tibiotarsal weight to length index, robusticity index, tibiotarsal index, bone ash, tibiae calcium and phosphorus of birds on diet 1 with values of 9.19g, 97.58mm, 93.87mg/mm, 4.67, 36.81, 40.48, 19.95 and 7.74 %, respectively, were similar (P>0.05) to those on diets 2, 3 and 4.

Discussion

Total dietary calcium and phosphorus decreased in diet 1 and across the treatments with age in agreement with specifications in earlier reports (NRC, 1994; Aviagen, 2006).

Average daily feed intake (48.43 g/bird/day) of starter birds fed diet 1 was below 62.04 g/bird/day reported (Ngiki *et al*., 2014). This increased to 164.62 g/bird/day at the end of week 6 which was higher than 124.44 g/bird/day reported (Ngiki *et al*., 2014) and comparable to 167.86 g/bird/day (Okereke, 2012) at day 42. The calcium and phosphorus intake of birds fed diet 1 (0.95 g/bird/day and 0.42 g/bird/day) at the finisher phase compared favourably with calcium intake of

Table 7: Morphometric Parameters and Mineral Content of Tibia Bone of Finishers' Brolier Chickens Fed Cassava Grits Based-Diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Weight (g)	9.19	8.65	10.08	9.60	5.12
Length (mm)	97.58	95.18	97.19	94.83	85.34
Weight/length index (mg/mm)	93.87	90.87	103.70	101.13	2.19
Robusticity index	4.67	4.66	4.50	4.47	0.04
Tibiotarsal index	36.81	37.54	36.60	35.29	0.45
Bone ash, (%)	40.46	39.31	39.99	41.49	0.40
Ca (%)	19.95	18.70	17.37	20.12	0.33
P (%)	7.74	7.28	7.84	7.50	0.22

SEM = standard error of means. Ca = Calcium. P = Phosphorus

0.93 g/bird/day and phosphorus intake of 0.44 g/bird/day reported (Rousseau *et al*., 2012) in broiler chickens fed medium level of calcium. This was similar to the calcium intake (0.92 g/bird/day) of starter birds fed control; the phosphorus intake (0.39 g), however, was lower compared to that reported by Rousseau *et al.* (2012).

Apparent calcium and phosphorus retention levels of 73 and 78 %, respectively by starter broiler chicks fed diet 1 at week 3 conformed to earlier findings (Li *et al*., 2000) that 74 % calcium was retained in broiler chicks fed wild-type corn while the phosphorus retention rose above 67.6 % reported (Li *et al*., 2000). This, however, declined sharply at week 6 to 41 % for calcium and phosphorus retentions, respectively. This agreed with the report (Rousseau *et al*., 2012) that calcium and phosphorus retention coefficient of 46.57 and 46.47 %, respectively in broiler chickens fed 0.57 % calcium and 0.32 % non phytate phosphorus corn-soybean diet.

The drop in retention levels confirmed that young animals absorb more calcium or mineral than mature animals (Horst *et al.,* 1978). Apparent retention of calcium and phosphorus in birds fed test diets also declined with age as stated above. Higher phosphorus retention in finisher birds fed diet 2 with values of 55 % juxtaposed the effect of higher dietary phosphorus (0.39 g) in finisher birds fed diet 2 (Ravindran e*t al.,* 2000 and Sheikhlar *et al.,* 2009).

Positive correlations in dietary phosphorus, apparent phosphorus retention and feed intake in finisher broilers fed TMS 01/1371 based diets suggested that both diets 3 and 4 would also respond in similar trend as diet 2. Negative correlation of dietary phosphorus with bone phosphorus in finisher birds fed TMS 01/1371 based diets could however not be established. Regression of dietary phosphorus on phosphorus retention in finisher chickens fed test diets was positive and highly significant ($p<0.01$) with the linear equation: $y = 210.26x - 18.82$; ($R^2 = 0.95$). The significant differences in the mineral retention were obviously due to the dietary contributions at above 0.24 g/100g dietary phosphorus in TMS 01/1371 grits based diets. Therefore, as the dietary level increases, so would the retention of the minerals.

Serum biochemical indices of experimental birds at week 3 indicated normal levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, sera calcium and phosphorus in birds fed test diets relative to control. This could be attributed to the fairly uniform dietary calcium: phosphorus ratio in all the diets as a small deviation from optimal supply of calcium and phosphorus in diet could engender changes in respective serum levels (Growth and Frey, 1996; Ravindran e*t al.,* 2000).

The ALP are indicative of liver functioning and also the rate of bone mineralization of broiler chickens (Ogunwole *et al.,* 2014), therefore, the higher the value above the upper limit, the higher the abnormality in the bone function (Coles, 1987). Dietary phosphorus, phytate and excessive vitamin A are some of the established factors that can cause upsurge in the level of ALP (Khare, 2000; Philips *et al.,* 2005; Guo *et al.,* 2011). The ALP value of finisher birds fed diet 1 (65.17 U/L) was slightly lower than the 69.0 U/L reported by Bozurt *et al.* (2012). The statistically similar values of ALP in birds fed test diets relative to control indicated that dietary sources made available sufficient minerals for normal functioning of the bone and osteoblasts (Guo *et al.,* 2011; Ogunwole *et al.,* 2014). Higher ALP in birds fed diet 2, however, contradicted earlier reports (Khare, 2000; Philips *et al.,* 2005; Guo *et al.,* 2011).

Different measurements can be used to determine bone mineralization as well as bone structure and health in poultry, including bone ash, breaking strength, bone weight and bone volume (Rao *et al.,* 1993). Also, bone density is evaluated by two indices; the bone ash weight/bone length index, where higher value indicates more density (Monteagudo *et al.,* 1997); and the robusticity index; where lower values indicate stronger bones (Reisenfeld, 1972). The tibia weight (9.19 g) and tibiotarsal weight/length index (93.87 mg/mm) were a little higher in birds fed diet 1 compared with 7.59 g and 77.22 mg/mm reported by (Mutus *et al.,* 2006). Conversely, the tibia length (9.83 cm) and robusticity index (5.01) were almost similar in both experiments. Relatively similar tibiotarsal index values in all experimental birds showed that they all had similar level of mineralization (*Von Hartung and Van Hasselt*, *1988*) and that effect of test diets was not superior to birds fed control diet. The tibia ash, tibia calcium and phosphorus (40.46, 19.95 and 7.74 %, respectively) of birds fed diet 1 compared relatively with 43.2, 17.1 and 8.2 % reported (Shafey *et al.,* 1999), 22.52 % tibia calcium and 10.06 % tibia phosphorus (Mutus *et al.,* 2006), 20.06 % tibia calcium and 8.79 % tibia phosphorus (Adebiyi *et al.,* 2009) and slightly above 33.18 % tibia ash, 11.23 % tibia calcium and 5.71 % tibia phosphorus (Bozkurt *et al.,* 2012).

Conclusion

Calcium and phosphorus retention and bone characteristics of broiler chickens were not altered by replacement of dietary yellow maize with bio-fortified cassava in this experiment. Therefore, β-carotene bio-fortified cassava could successfully replace maize in the diets of broiler chickens without adversely affecting the birds.

Acknowledgements

Authors appreciate the donation of quality day old chicks by Amo Farm and Siebrier Hatcheries, Awe, Oyo State, Nigeria. The entire Staff and Management of IITA, Ibadan are also appreciated for assisting the research with the desired cassava variety.

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