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Assessing calcium availability from limestone sources through bone and blood status of chickens

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Abstract

Calcium (Ca) availability from limestone (procured from different cement factories) was assessed through bone and blood status of chickens using the completely randomized design. Five hundred and twenty five (525) straight run broiler chicks that were 28 days of age (trial 1), four hundred and twenty (420) broiler finishers that were 56 days of age (trial 2), and 210 layers that had been laying for six months (trial 3) were used in the study. Six limestone dietary treatments and a control accounted for the seven diets that were assessed. Twelve birds per treatment and 4 per replicate that had their weights close to the mean of the pen were selected in the broiler trials (trials 1 and 2) and three birds per pen and nine per diet were selected in the layer trial (trial 3) for bone and blood samples analysis. Bone samples were analysed for bone weight, bone length, ash weight, percentage ash, Ca and phosphorus contents of ash and blood samples were analyzed for plasma Ca and alkaline phosphatase activity. Results showed similar influence of sources on bone weight, bone length, ash weight and percentage ash. Ca and P contents of ash were significantly (P<0.05) affected by limestone sources but generally, limestone sources produced mean values that were equal to, or better than the control diet in the starter phase (trial 1). In the finisher phase (trial 2), only Ca content of ashy varied significantly where Jakura source produced the least mean value. The layer trial (trial 3) also produced mean values that were significant (P<0.05) for Ca content of ash with the Sokoto source producing the least mean value. Plasma Ca and alkaline phosphatase enzyme activity attained published values for chickens. It was concluded based on the information from this study that the Ca from tested limestone sources was generally available for chicken production.

Key words: *Limestone sources, Calcium sources, bone and blood parameters.*

Introduction

Calcium (Ca) is required by the chicken in greater amount than any of the other minerals. Cromwell (1982) suggested that approximately 99% of Ca is located in the skeleton where it complexes with phosphates to give rigidity to bones. The remaining <1% is widely distributed throughout the organs and tissues with relatively large amounts found in blood. Simkis (1967) reported that the blood cells are almost devoid of Ca but the serum and plasma contain 9-12mg/dl in most species when not in reproductive activity. The

skeletal system is preferentially sacrificed to maintain serum calcium level vital to the life of the animal and the effect of Ca deficiency or imbalance may first be shown as skeletal aberrations. Peo (1976), suggested that ash weight and ash % together are better criteria for assessing bone mineral changes than either alone. Hurwitz (1973) reported that plasma calcium increase from around 10mg percent (typical of non laying hens) to over 20mg percent (typical of laying hens). Scott *et al* (1971) and Kin (1971) reported that oystershell increased blood plasma Ca level over that of limestone.

Alkaline phosphate (orthophosphoric – momonester – phosphorylase) enzyme activity of plasma increases during growth and at the onset of lay when there is greater demand for Ca for bone calcification and shell formation in the laying hens. Garlich *et al* (1984) reported that alkaline phosphatase level in serum of laying hens was higher than that of cocks, and was lower in adult hen than young chickens. Reichman and Connor (1977) reported that, as the level of dietary Ca increased, plasma Ca also increased significantly in laying hens with the resultant decrease in alkaline phosphatase level. They concluded that phosphatase level was a suitable indicator for Ca status of the animal. The objective of the study was to use the bone and blood Ca status of chickens in assessing the availability of Ca from the various limestone sources acquired for poultry rations.

Materials and Methods

Five hundred and twenty five (525) straight run Anak – 180 day old broiler chicks were procured and assigned at random to 21 homogenous pens each of which measured 3.05 x 1.25m^2. An open sided poultry house was used and the sides were covered with polythene sheets to conserve heat during brooding. Experimental diets were formulated to be essentially iso-nitrogenous and iso-caloric and only the six limestone sources and the control diet accounted for the different dietary treatments. Diets were assigned at random to homogenous pens such that each diet had three replicate pens and each pen had 25 chicks. Kerosine stoves and lamps provided heat and light respectively. At the end of the 28 day brooding period, 4 chicks per pen (12 per diet) which had their mean weight values close to the pen average weight were selected for bone and blood sample collection. In the finisher phase, the covered sides of the poultry house were opened for cross-ventilation. 420, Anak – 180 chicks were used. Diets were formulated in a similar manner as in the starter phase except that the protein level was lowered and energy level slightly increased, but the limestone source inclusion rate was maintained. When broiler chickens were 56 days of age, the feeding experiment was terminated and 4 chickens per pen (12 per diet) which had their weights close to the pen average were selected for bone and blood sample collection. In the layer study, 210 layers that were 6 months in lay were used. 3 birds per pen (9 per diet) were selected for bone and blood sample collection.

Feed Preparations

The limestone sources used were chemically analysed for their Ca content. The analysed

samples showed that the sources contained high levels of Ca. This is believed to be as a result of the request of the Researcher who requested for sections of the deposits that were high in calcium carbonate ($CaC0_3$) for this study. The analysed samples showed the following results. Ashaka = 37.6% Ca; Calabar = 38.8% Ca; Jakura = 39.5% Ca; Sokoto 38.21%Ca; Ukpila = 38.8% Ca; Yandev = 38.5% Ca. Iowa Limestone Company (1974), and Spesfeed (2000) adviced that limestone containing 38% Ca (95% Calcium carbonate) is more useful for livestock rations than the limestone containing lower Ca content (36%Ca; 34% Ca; 32% Ca). Bone meal is conventionally used in poultry diets as a source of phosphorus (P), so it was utilized in the control diets where it supplied both P and Ca. Dicalcium phosphate was used as a P source in the tested diets. To incorporate more tested material in the feed, the ratio of Ca: P (1.5:1) was widened beyond recommended ratio, (1.9:1). By this arrangement, Ca contributed by the tested diuets furnished about 1.25 times more Ca than that from the basal ingredients and was believed to fully elicit the effect of Ca on the bone and blood parameters. It was easier to incorporate more test materials in the layer diets where a wider Ca: P ratio (3.5:1) is well tolerated. The compositions of the experimental diets are presented in tables 1, 2, 3. In all trials, feed and water were provided *ad libitum*.

Bone and Blood Sample Collections

Test tubes large enough to accommodate 10mls of blood were marked against each dietary treatment. Few drops (4-5) of anticoagulant

Table 1: The composition of the broiler starter diets

	Control	Limestone sources					
		Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yandev
		%					
Ingredients							
Groundnut cake	44.00	44.00	44.00	44.00	44.00	44.00	44.00
Maize	51.00	49.50	49.70	49.78	49.60	49.75	49.65
Bone meal	4.00	-	-	-	-	-	-
Dical. Phosphate	-	3.40	3.40	3.40	3.40	3.40	3.40
Limestone	-	2.10	1.90	1.82	2.00	1.85	1.95
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.25	0.25	0.20	0.25	0.25	
	100	100	100	100	100	100	100
Determined analysis (DM basis)							
Crude Protein %	24.56	24.15	24.05	23.85	24.21	24.45	24.30
**Energy Kcal/kg ME %	2952	2900	2906	2909	2903	2908	2904
Calcium %	1.53	1.55	1.45	1.45	1.50	1.45	1.48
Phosphorus %	0.95	0.82	0.79	0.75	0.80	0.85	0.77

**Calculated using Pauzenga (1985) formula Kcal/kg ME = (37x%CP+81.8x%EE+35.5x%NFE)

*Premix (Peter Hand) supplied the following additional nutrients per kg of diet:-

Vits. A = 10,000 IU; D_3 = 1920 IU; E = 10 IU; K = 0.02mg; B_2 = 9mg; B6 = 4.00mg; B_{12} = 0.03mg; Nicotinic acid = 50mg; C = 40mg; Calcium D – Panto thenate = 16.02. Minerals: - Choline chloride = 40.02mg; Manganese = 200mg; Fe = 100mg; Zn = 80.02mg; Cu = 4.8mg; Co = 0.41mg; Se = 0.12mg

Table 2: The composition of broiler finisher diets

		Limestone sources					
	Control	Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yandev
		%					
Ingredients							
Groundnut cake	36.00	36.00	36.00	36.00	36.00	36.00	36.00
Maize	59.10	57.35	57.55	57.63	57.45	57.60	57.50
Bone meal	4.00	-	-	-	-	-	-
Dical Phosphate	-	3.65	3.65	3.65	3.65	3.65	3.65
Limestone	-	2.10	1.90	1.82	2.00	1.85	1.95
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
	100	100	100	100	100	100	100
Determined analysis (DM basis)							
Crude Protein %	21.05	20.45	20.50	20.35	20.25	20.48	20.15
**Energy Kcal/kg							
ME %	3024	2964	2970	2973	2967	2972	2968
Calcium %	1.45	1.56	1.49	1.49	1.54	1.49	1.52
Phosphorus %	0.82	0.80	0.81	0.79	0.85	0.78	0.83

**Calculated using Pauzenga (1985) formula Kcal/kg ME = (37x%CP+81.8x%EE+35.5x%NFE)

*Premix (Zoodry) supplied the following additional nutrients per kg of diet -

Vits. A = 10,000 IU; D_3 = 1020 IU; E = 10 IU; K = 0.02mg; B_1 = 9mg; B6 = 4.00 mg; B_{12} = 0.03mg; Nicotinic acid = 50mg; C = 40mg; Calcium D – Panto thenate = 16.02; Minerals - Choline chloride = 40.02mg; Manganese – 200mg; Fe = 100mg; Zn = 80.02; Cu = 4.8mg; Co=0.41mg; Se = 0.12mg.

heparin) were put in each tube. Each bird was bled by severing the brachial vein and blood gushing out was received in each of the marked tubes until each tube was two third (2/3) full. Each tube was sealed with celophone paper and rocked gently to mix the blood and the anticoagulant and the tube was immediately immersed in ice block. Samples were centrifuged within one hour of collection at 750G for 5 minutes (Njoku 1980), and blood plasma on top was aspirated into a sterile plastic tube with the aid of a plastic syringe and all plasma containers were stored in a freezer for analysis.

After blood sample collection, birds were finally sacrificed and tibia excised. Bones were soaked in warm water containing a few drops of 2% sodium hydroxide (NaOH) and allowed to simmer. This facilitated easy removal of residual flesh from bones. Bones were dried at 105°C overnight in an oven, cooled in a dessicator and weighed. Whole tibia was defatted by soaking in diethyl ether contained in flat bottomed flask large enough to accommodate the whole tibia. The flask was sealed with re-enforced polythene sheet and kept tight by means of elastic rubber band. The ether was changed each time the

Table 3: The composition of the layer diets

	Control	Limestone sources					
		Ashaka	Calabar	Jakura	Sokoto	Ukpilo	Yandev
		%					
Feed Ingredients							
Decorticated Cotton seed cake	33.00	33.00	33.00	33.00	33.00	33.00	33.00
Maize	55.00	55.24	55.33	55.68	55.43	55.59	55.49
Bone meal	3.00	-	-	-	-	-	-
Oyster shell	7.74	-	-	-	-	-	-
Dical. Phosphate	-	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	-	7.50	7.41	7.06	7.31	7.15	7.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Methionine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
*Premix	0.65	0.65	0.65	0.65	0.65	0.65	0.65
	100	100	100	100	100	100	100
Determined analysis (DM basis)							
Crude Protein %	17.14	17.88	17.59	17.81	17.19	17.11	17.09
**Energy Kcal/kg ME %	2620	2650	27301	2725	2660	2680	2705
Calcium %	3.65	3.56	3.61	3.79	3.71	3.71	3.52
Phosphorus %	0.66	0.71	0.72	0.68	0.75	0.69	0.80

**Calculated using Pattersya (1985) formula Kcal/kg ME = (37x%CP+81.8x%EE+35.5x%NFE)

*Premix (Peter Hand) supplied the following additional nutrients per kg of Diet -

Vits: A = 10,000 I.U., D = 200 I.U. E = 10 I.U. K = 2mg, B_1 = 1.5mg, B_2 = 4mg, B_6 = 15mg, B_{12} = 0.01mg Pantothenic acid = 5mg, Folic acid = 0.5mg, Biotin = 0.02, Minerals - Choline chloride = 0.2mg, Mn = 0.8mg, Zn = 0.05mg, Fe = 0.02mg, Cu = 0.005mg, Se = 0.2mg, Co = 0.2mg

colour was observed to change from clear liquid to varying degrees of yellow until eventually there was apparently no colour change even after 12 hours of soaking. The whole process took about 72 hours. Bones were then air dried, weighed and ashed at 600°C for 24 hours in a muffle furnace, cooled in a dessicator and weighed. The ash was saved for determination of Ca and P contents.

Blood Plasma and Bone Ash Analysis

Blood plasma was analyzed for alkaline phosphatase enzyme by the colorimetric method (King and King 1954), using 0.01M disodium phosphate substrate in a solution of sodium carbonate and sodium bicarbonate buffer and the activity was read with the aid of colorimeter model 252. Plasma Ca was analyzed as outlined by Baginski *et al* (1973) with the aid of a spectrophotometer model PYE UNICAN SP6-300. The Ca content of ash was determined by atomic absorption spectrophometer model 290B, following wet digestion using nitric acid, perchloric acid and sulphuric acid (Perkin-Elmercorp, 1968). The excess solution from the Ca determination was used in determination of P content of bone ash using the Vanadomolybdate (yellow flame) method as outlined by Hesse

(1971). Data collected from the trials were subjected to analysis of variance using the completely randomized design, and identified significant treatment means were separated using Duncan's New Multiple Range Test as outlined by Steel and Torrie (1980) for both procedures

Results and Discussions

Bone development as measured by length and weight of fat free tibia were not significantly P>0.05) different in each phase of broiler growth (Tables 4 and 5). Bone mineral status as measured by ash weight and percent ash were also similar in each phase of growth. Ca status of bone varied significantly (P<0.05) at the two phase of the study. However, in each phase, the tested sources were equal to, or better than the control. Bone lengths responded in a similar manner in each phase of broiler growth. This is an indication of uniformity of growth of birds from different dietary treatments. Njoku (1980), Smith and Kabaija (1985) worked with different Ca sources and levels but reported lack of significant differences in longitudinal tibia growth. Bones were defatted after careful removal of flesh and appendages that were not bone (tendons and ligaments) thus the remaining bone was believed to be composed of mineral matter largely. Therefore, the lack of significant differences among the tested sources and the control was an indication of good bone mineralization. Ash weight and percent ash also showed lack of significant differences (P>0.05) among the sources tested and the control, indicating good mineralization. The percentage Ca composition of the tested sources was increased in the diets while that of P was reduced to create a wider Ca: P ratio (1.9:1). The tested sources then contributed 1.25 times as much Ca as the basal ingredients. Lack of significant difference in ash weight and percent ash is an indication that similar amount of Ca from the

Table 4: The effect of limestone sources on tibia status of broiler starter chicks (28 day's of age)

Sources of limestone	Bone Wt (g)	Bone Length (cm)	Ash wt (g)	%Ash	Ash Ca %	Ash P %	Ca/P ratio
Control (Bone meal)	5.17	6.73	1.90	37.02	30.28	[illegible]	1.84:1
[illegible]	5.16	6.78	1.78	34.59	31.28	[illegible]	1.99:1
Calcite	5.41	6.70	1.96	36.22	31.37	[illegible]	1.96:1
[illegible]	4.90	6.51	1.79	36.53	29.97	[illegible]	2.04:1
[illegible]	5.38	6.66	2.25	37.53	31.01	[illegible]	2.01:1
[illegible]	5.16	6.67	1.91	33.38	30.53	[illegible]	1.92:1
[illegible]	1.72	6.44	1.74	37.02	30.51	[illegible]	1.66:1
SEM	0.81	0.22	0.18	2.44	0.27	0.29	-

a,b,c = means within a column with same or without superscript are not significantly different (P>0.05)

SEM = Standard Error of Mean

Table 5: The effect of limestone sources on tibia status of broiler chickens (56 day of age)

Sources of limestone	Bone Wt (g)	Bone Length (cm)	Ash wt (g)	% Ash	Ash Ca %	Ash P %	Ca: P ratio
Control (Bone meal)	13.52	10.96	5.45	34.19	30.61[a]	16.28	1.90:1
Ashaka	13.69	10.68	6.32	37.53	31.90[b]	16.54	1.93:1
Calabar	15.49	10.68	5.98	38.38	32.28[b]	15.52	2.07:1
Jakura	14.11	10.70	5.67	36.81	29.23[c]	16.23	1.8:1
Sokoto	15.27	10.26	5.36	37.28	31.52[b]	16.54	1.9:1
Ukpila	13.96	10.48	5.96	34.68	30.00[a]	16.28	1.90:1
Yandev	13.73	10.41	5.45	36.49	30.38[a]	16.03	1.90:1
SEM	1.04	0.22	0.33	1.24	0.12	0.43	-

a,b,c,d – means within a column with same or without superscript are not significantly different (P<0.05)

SEM = Standard Error of Mean

tested sources was available for bone mineralization. Tion *et al* (2005) reported that the tested limestone sources have high bioavailability, Cromwell (1982) reported that 99% Ca is stored in bone. Peo (1976) pointed out that ash weight of bones was a better measure of assessing mineral content of bones but concluded that, ash weight and percentage ash should be taken together in assessing mineral status of adult bone. The percentage Ca content of tibia ash varied significantly among sources and the control in all phases including the layer study. However, the tested sources were largely equal to, or better than the control diet. Mean values for the broiler study were similar to the values reported by Hulan *et al* (1985), while the values for layers were similar to those reported by Reynells (1979). In broiler study, the percentage Ca content of ash for the Jakura source was significantly (P<0.05) low among the diets in both phase of the study. This may be an indication of the lower availability of the source (marble). Cromwell (1982) reported that marble had lower availability value for pigs among the supplemental Ca source tested. In the layer study, perhaps, due to longer retention time in the gizzard and the gradual metering out of Ca from source, Jakura source was similar to other diets. The Sokoto source was the least available as measured by percentage Ca in the bone ash of the layer study. This could be explained in the light of its very powdery form which caused rapid passage of feed through the gastrointestinal tract and allowed minimal time for reaction with the hydrochloric acid (HCl) in the gastrointestinal tract for the release of Ca ions for absorption and retention in the bone (Savage, 1982). The percentage P in tibia ash was significantly (P<0.05) different among the various diets in the starter phase of the study only, where Jakura and Sokoto sources produced significantly (P<0.05) lower values. The ratio of Ca: P in the bone (2:1) as reported by Preston *et al* (1977) was essentially attained in this study. P was not one

Table 6: The effect of limestone sources on tibia status of layers.

Sources of limestone	Bone Wt (g)	Bone Length (cm)	Ash wt (g)	% Ash	Ash Ca %	Ash P %
Control (Bone meal)	14.04	10.64	6.40	44.83	36.38[b]	18.87
Ashaka	13.99	10.64	5.84	41.33	36.33[b]	18.52
Calabar	14.30	10.39	7.14	50.32	38.53[a]	18.87
Jakura	16.02	10.94	7.54	46.47	36.42[b]	18.98
Sokoto	14.57	10.47	6.97	47.69	34.79[c]	17.55
Ukpila	14.71	10.32	7.77	51.46	38.42[a]	17.55
Yandev	15.04	11.06	7.05	45.89	38.33[a]	18.33
SEM	0.33	0.28	0.32	0.42	0.17	0.86

a,b,c, – means within a column with same or without superscript are not significantly different (P>0.05).

SEM = Standard Error of Mean

of the minerals tested in this study but was determined because of its close association with Ca in bone tissue.

The plasma Ca in the two phases of broiler growth, and laying hens (Tables 7 and 8) did not result in significant differences (P>0.05) among the sources tested. Values reported in this study are in agreement with values reported by Simkis (1967) and Hurwitz (1973) who reported 20mg percent for laying hens, Scott *et al* (1971), reported that plasma Ca of laying hens varied from 27 – 28mg/100ml for limestone diets, and that oystershell produced higher plasma Ca values (29.5 – 32.9mg/100ml). Values reported in this study are similar to those of Scott *et al* (1971), where oystershell plasma Ca values were slightly higher. Bone calcification process as measured by the level of plasma alkalin phosphatase activity was high in both phases of broiler growth. The starter phase did not result in significant (P>0.05) differences among the various diets but the finisher phase witnessed significant (P<0.05) variation caused by Calabar and the Jakura sources. The enzyme activity in laying hens also did not show significant (P>0.05) variation among the diets. Apparently, the enzyme activity in the starter phase was higher than the finisher phase and was higher in broilers than in laying hens. This is in agreement with, Garlich *et al* (1984) who reported that alkaline phosphatase activity was higher in young chickens than adult, Paul and Snetsinger (1969), Reichman and Connor (1977) reported that as the level of dietary Ca increased, plasma Ca also increased significantly in laying hens with the resultant decrease in alkaline phoisphtase activity and concluded that alkaline phosphatase was a suitable indicator of Ca status of blood in animals. Roland and Harms (1973) suggested that when dietary Ca is adequate for laying hens, minimal bone resorption take place to meet the demand for shell calcification. This may be due in part to, why the alkaline phosphate activity of blood plasma of layers was low. In this study, Ca supplied by the basal ingredient accounted for only 15% of the required Ca (3.5%) in layer

Table 7: The effect of limestone sources on plasma Ca and alkaline phosphatase enzyme of broiler starter and finisher chicks.

Sources of limestone	Starter phase		Finisher phase	
	Plasma Ca (mg/dl)	Plasma alkaline phosphatase (ui/l)	Plasma Ca (mg/dl)	Plasma alkaline phosphatase (ui/l)
Control (Bone meal)	9.29	1808.60	9.95	1725.3[c]
Ashaka	9.87	1796.30	9.83	1682.7[c]
Calabar	10.25	1952.50	10.12	1617.6[b]
Jakura	9.93	2059.50	10.33	1547.8[b]
Sokoto	9.11	1917.00	10.47	1787.3[c]
Ukpila	9.63	1980.90	10.00	1796.3[c]
Yandev	9.75	1919.90	9.64	1732.4[c]
SEM	0.46	51.07	0.40	46.56

a,b,c=means within a column with same or without superscript are not significantly different (P>0.05)

SEM = Standard Error of Mean

Table 8: ***The effect of limestone sources on plasma Ca and alkaline phosphatase enzyme of laying chickens.***

Sources of limestones	Plasma Ca (mg/dl)	Plasma alkaline phosphatase (ui/l)
Control (Bone meal)	29.53	421.27
Ashaka	28.14	257.97
Calabar	26.63	312.40
Jakura	27.94	325.33
Sokoto	25.73	402.33
Ukpila	26.67	418.90
Yandev	27.07	357.37
SEM	1.53	58.10

Means within a column with same or without superscript are not significantly different (P >0.05)

SEM = Standard Error of Mean

diet. Absence of significant alkaline phosphatase enzyme activity was an indication of good availability of Ca from the sources for shell formation especially when bone and plasma Ca levels attained reported values.

Conclusion

There is indication from the study that Ca from all the tested limestone sources (save for the Jakara source that appeared to be less available for broiler diets) could be used in broiler diets profitability. The Ca from all the tested limestone sources (save for Sokoto source that appered to be less available for layer diets) could be used in laying hen diets profitability

The poultry (chicken) tissues of bone and blood connective tissues can be effectively used in assessing mineral (Ca) availability from Ca sources (limestone) in agreement with Garlich *et al* (1984); Reichman and Connor (1977).

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