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Effect of Different Sources of Phosphorus on Bone Mineral Composition and Their Bioavailability Test In Broiler Chickens

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Abstracts
This study was conducted to investigate the utilization of rock phosphate as an alternative phosphorus source to bone meal and dicalcium phosphate in broiler diet. Rock phosphate is rich in calcium and phosphorus, but its utilization has been limited due to its fluorine contents. A total of 250 Sayed broiler chicks were purchased at day old and brooded. After brooding, 210 very active and healthy chicks were allotted at random to 3 dietary treatments, each with 5 replicate pens such that 14 birds were assigned to each replicate and a total of 70 birds made up a treatment. Data collected were analyzed using SPSS statistical software. Results indicated that, different sources of phosphorus had no significant (P>0.05) effects on bone characteristics of broiler finisher chickens (56 days of age) across treatments. However, the bioavailability of P in this study indicated significant difference (P<0.05) among dietary treatments. Bone ash and rock phosphate had a better bioavailability of phosphorus than dicalcium phosphate. Results further showed that at 2.5 Kg/100 Kg diet, rock phosphate can be utilized without adverse effect on bone characteristics and its bioavailability. This also means that diet containing rock phosphate can compete favourably with bone ash which serve as a control and therefore safe cost for broiler chickens production. It is recommended that, rock phosphate can be utilized without adverse effect on bone characteristics and bioavailability for broiler chickens production.
Key words: *Bioavailability, Dicalcium phosphate, Broiler chicken, Raw rock phosphate.*

Introduction

Bone meal according to Tion *et al*. (2012) has become unattractive for animal diets in developed countries due to fear of disease transfer from animal to animal via feed. Tion *et al*. (2012) also stated that when consumers of monogastric animal meat become aware of the inherent danger that may arise from eating meat of animals that are fed bone meal containing diets, they may shy away from such meat consumption as is the case of cholesterol stigma in fatty meats and eggs. Therefore, alternative sources of phosphorus should be found aside from bone meal. Dicalcium phosphate and other reagent grade phosphates (Monocalcium phosphate and deflourinated phosphate) are used in developed economies to supply the phosphorus requirement of animals but dicalcium phosphate is an imported resource which is very costly and will make the production cost via feed to increase in a country where an average Nigerian is unable to afford meat due to high cost (Tumova *et al*., 2004). Rock phosphate occurs in different parts of this country and is mined for fertilizer manufacture. Fears about the use of rock phosphate as a source of phosphorus in animal diets especially poultry is based on its inherent content of fluorine which is toxic to poultry at levels over 40 mg per day (Godoy and Chicco, 2001). Advanced countries have the technology to process the raw rock phosphate to remove the fluorine content. Such rock phosphate is called deflourinated or soft rock phosphate. In this country, we have not acquired that technology and the utilization of raw rock phosphate in animal diets is not conventional. It is possible that varying quantities of fluorine can occur at different sites or deposits.

The objective of this study is to evaluate the utilization of rock phosphate can as an alternative phosphorus source to bone meal and dicalcium phosphate in broiler diets and its effects on blood biochemical characteristics and bioavailability of broiler finisher chickens.

Materials and Methods

Location and Site of Study

The experiment was carried out at Divisional Veterinary Complex, in North Bank, Makurdi, Benue State in one of the poultry houses. Makurdi is located between latitude 7.68 ^{0} North and Longitude 8.62 ^{0} East, the flood plain between 106 m to 113 m above sea level. The area is warm with a minimum temperature range of 17.3 ^{0}C to 24.5 ^{0}C and maximum temperature range of 29.8 ^{0}C to 35.6 ^{0}C. During the dry hot season between February and March, temperature may reach 35 ^{0}C to 40 ^{0}C, and rainfall is between 1500 mm to 1800 mm (Wikipedia, 2013).

Sampling and Data Collection Procedures

The raw rock phosphate (which is the test material) was sourced from Federal Superphosphate Fertilizer Company, Kaduna. The company disclosed that, the product was procured from Sokoto State. Dicalcium phosphate was purchased from Ibadan. Bone meal and other ingredients like maize, soya bean cake, oyster shell, methionine, lysine, and iodized salt were bought from Wadata market in Makurdi, Benue State. The chemical composition of rock phosphate was analysed at the National Geoscience Research Laboratories Centre, Kaduna using Energy Dispersive X-ray fluorescence (EDXRF) Spectrometer of model 'Minipal 4' and gravimetric methods. The samples were pulverized using auger pulverizing machine (Planetary Micro Mill Pulverisette 7). The ground samples were ensured to pass 150 micro mesh sieves. This was to ensure homogeneity of the samples. A gravimetric method was used to determine moisture content. It is done by heating 1g of the powdered sample in a cleaned weighed crucible at 1000^{o}C. After which the crucible and the content were weighed to get the difference in weight before and after heating.

Experimental Design and Birds Management

The birds were randomly allotted to different diets in a Completely Randomized Design.

A total of 250 Sayed broiler chicks were purchased from Wadata market, in Makurdi, Benue State and brooded using conventional brooding method as outlined by Dafwang and Ogundipe (1987). Birds were brooded on deep litter floor using a commercial broiler starter diet (Vital Feed) for the first one week. Then, 210 very active and healthy chicks were selected for the experiment. Chicks were allotted at random to 3 dietary treatments, each with 5 replicate pens such that 14 birds were assigned to each replicate and a total of 70 birds made up a treatment.

Experimental Diets

Three experimental diets were formulated and mixed manually on the farm. Bone ash (a conventional phosphorus source used in this country), Rock phosphate (the test material) and Dicalcium phosphate 18 % P (a conventional phosphorus source used in developed economies) accounted for the three dietary treatments. Oyster shell was used to balance the calcium deficiency in three dietary treatments. Dietary treatments were formulated to be essentially isocaloric and isonitrogenous at each phase of the study. Diets were chemically analysed to find out if they conform to the calculated formulation. The experimental diets are presented in Table 1.

Table 1: Proximate Analysis of formulated Broiler Starter and Finisher Diets used in the experiments `

Nutrients (%)	Bone Ash T_1	Rock Phosphate T_2	Dicalcium Phosphate T_3	SEM
		Starter Diets		
Crude Protein (CP)	23.05	23.21	23.38	0.31
Crude Fibre (CF)	4.60	5.10	4.80	0.15
Ether Extract (EE)	2.90	3.30	2.80	0.15
Nitrogen Free Extract	60.35	57.80	60.30	0.84
Ash	3.80	4.00	3.90	0.06
Calcium	1.49	1.46	1.10	0.13
Phosphorus	0.70	0.70	0.75	0.02
		Finisher Diets		
Crude Protein (CP)	20.25	20.25	20.25	0.00
Crude Fibre (CF)	5.35	5.45	5.30	0.04
Ether Extract (EE)	3.30	3.60	3.80	0.09
Nitrogen - Free Extract	60.48	59.99	60.25	0.14
Ash	4.12	4.21	4.40	0.08
Calcium	1.46	1.44	1.12	0.11
Phosphorus	0.66	0.66	0.70	0.01

SEM=Standard Error of Means

Parameter Measured

Bone Mineral Determination

Tibia bones were soaked in warm water containing a few drops of 2% sodium hydroxide solution and were allowed to simmer. This facilitated easy removal of residual flesh from bones. Bones were dried at 105^0C overnight in an oven, cooled the following morning in a desiccator and weighed. Whole tibias were defatted by soaking in diethyl ether contained in flat bottom flasks large enough to accommodate whole tibia. The flasks were sealed with re-enforced polythene sheet and kept tight by means of elastic rubber band. The ether was changed each time the 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 60 hours. Tion and Njoku, (2009) reported the process to be about 72 hours. The bones were air dried, weighed and ashed at 600^0C for 24 hours in a Muffle Furnace, cooled in a desiccator and weighed. The ash was then saved for determination of calcium and phosphorus contents.

The Calcium content of bone ash was determined by Atomic Absorption Spectrophotometer Model 290 B, following wet digestion using perchloric acid, nitric acid and sulphuric acid (Perkin-Elmercorp, 1968). The principle was accomplished by weighing 3 g of ground bone ash into a 125 ml Erlermeyer Flask which has been washed with acid and distilled water. Then 4 ml of perchloric acid, 25 ml of concentrated NHO_3 and 2 ml of concentrated H_2SO_4 were added. The excess solution from the Ca determination was used in determination of P bone ash using the Vanado-Molybdate (yellow flame) method as outlined by Kaankuka (1990). Vanado - Molybdate reagent was prepared by firstly dissolving 20 g of NH_4 – Molybdate $\{(NH_4)_6\ Mo\ _7O_{24}\ 4H_2O\}$ in 200 ml hot water and cooled. Then dissolving separately 1 g of NH4 – metavanadate in 120 ml hot water, cooled and 140 ml concentrated HNO_3 was added under a fume hood.

Bioavailability Test

At the end of the finisher phase, 40 birds were assigned at random to three phosphorus source diets and a "phosphorus deficient" diet accounted for the fourth treatment. A phosphorus deficient diet was formulated to be adequate in all necessary nutrients for broiler finisher except phosphorus. Birds were paired at random in battery cages such that each treatment had 5 replicates of two birds per replicate. The broiler rations previously fed were replaced with that of phosphorus deficient diets for 48 hours. The period was to get the birds into negative phosphorus balance such that they will utilize maximally the phosphorus from the difference sources. The birds were force-fed through a process known as dosing where a calculated amount (4g) of phosphorus in each of the material being tested was placed in the mouth of the birds using tea spoon and at the same time forced to drink water using a wash bottle. Thereafter, birds were returned to their cages to continue to feed on phosphorus deficient diet. Feed and water were provided ad libitum. Faecal output from each treatment group was collected for 48 hours by placing polythene leather directly under the cage where the birds were housed. Savage (1982) stated that extension of excreta collection period to 48 hours improved both accuracy and precision for calcium determination. The faeces collected was dried first in an open air and then at 105^0C in an oven over night, cooled in a desiccator. The dried droppings were weighed, pounded and pooled per replicate and treatment. Sample of pounded faeces were taken for determination of un-utilized phosphorus from each source. Faecal phosphorus determination was carried out using calorimetric method following the Vanado-molybdate (yellow flame) as outlined by Kaankuka (1990). The faecal output for the P deficient diet was also analyzed for endogenous metabolic faecal and urinary P which served as a ‘’correction factor’’ for faecal phosphorus sources. Phosphorus sources used for the experiment comprised of bone meal, rock phosphate, and dicalcium phosphate.

Table 2: The Composition of the Phosphorus Deficient Diets of Broiler Finisher Chickens

Ingredients (%)	**Bone Ash T_1**	**Rock Phosphate T_2**	**Dicalcium phosphate T_3**
Maize	57.20	57.20	57.20
Full Fat Soyabean	35.30	35.30	35.30
Rice Offal	1.50	1.50	1.50
Oyster Shell	1.30	1.30	1.30
Methionine	0.20	0.20	0.20
Premix	0.30	0.30	0.30
Salt	0.30	0.30	0.30
Sand	4.00	4.00	4.00
Total	**100**	**100**	**100**
Calculated Nutrient Composition			
CP (%)	20.07	20.07	20.07
ME (Kcal/Kg)	3078	3078	3078
Ca (%)	0.54	0.54	0.54
P (%)	0.24	0.24	0.24
CF (%)	3.65	3.65	3.65

The diet was formulated using the Nutrient Master Plan by Pfizer.
Crude Protein = CP, Metabolizable Energy = ME, Calcium = Ca, Phosphorus = P, Crude Fibre = CF, Flourine = F.
Premix (Optimix Poultry) each 1.25 kg of diet contained the following:
Vits A = 12,000,000 IU. D_3 = 3,000,000 IU. E = 30,000mg. K_3 = 2,500mg. B_1 = 2,000mg. B_2 = 5,000mg. B_6 = 3,500mg. B_{12} = 20mg. Folic acid = 1,000mg. Naicin = 40,000mg. Calpain = 10,000mg. Biotin = 80mg. Minerals: Cobalt = 250mg. Selenium = 250mg. Iodine = 1,200mg. Iron = 40,000mg. Manganese = 70,000mg. Copper = 8,000mg. Zinc = 60,000mg. Choline Chloride = 200,000mg.

Data Analysis

Data collected were subjected to one way Analysis of Variance (ANOVA) according to Statistical Package of Social Science (SPSS) using computer programme (version 20.0 of Window 2007 model) and identified significant difference mean values were separated using the Duncan's Multiple Range Test as outlined by Steel and Torrie (1980).

Results

The effect of different sources of phosphorus inclusion on Bone characteristics of broiler finisher chickens (56 days of age) as expressed in Table 3 indicated that there were no significant differences (P >0.05) across treatments.

Table 3: The Effect of Different Sources of Phosphorus on Bone Characteristics of Broiler Finisher Chickens (56 days of age)

Parameters	Experimental diets			SEM
	Bone Ash T_1	Rock Phosphate T_2	Dicalcium Phosphate T_3	
Undefatted tibia (g)	13.34	13.04	13.50	0.24^{NS}
Fat-free tibia (g)	10.20	9.08	10.18	0.49^{NS}
Tibia ash weight (g)	5.63	5.57	5.60	0.04^{NS}
Tibia ash calcium (%)	31.28	30.96	31.12	0.50^{NS}
Tibia ash phosphorus (%)	16.36	16.20	16.38	0.36^{NS}
Ca:P ratio	1.91:1	1.91:1	1.90:1	-

There was no significant difference (P>0.05) in all parameters measured, SEM=Standard Error of Means, NS=Not Significantly Different (P>0.05)

Bioavailability of Phosphorus

The effect of bioavailability of phosphorus from different phosphorus sources on broiler finisher chickens (56 days of age) is presented in Table 4. The result indicated significant difference (P >0.05) among sources of phosphorus evaluated. Bone meal and rock phosphate were not significant to one another but were both significant to dicalcium phosphate.

Table 4: Effect of Different Sources of Phosphorus Inclusion on Bioavailability of Phosphorus of Broiler Finisher Chickens (56 days old)

Phosphorus Sources	Percent Availability (%)
Bone Meal	86.75^a
Rock Phosphate	87.5^a
Dicalcium Phosphate	81.7^b
SEM	0.70

^{a,b} Means with different superscripts differ significantly (P<0.05)
SEM = Standard Error of Means

Discussion

The Effect of Different Sources of Phosphorus on Bone Parameters of Broiler Finisher

The result of the undefatted tibia for this study did not indicate significant difference (P>0.05) among treatments. The finding in this study fell slightly below the range values of 13.83 – 15.69 g as reported by Tion and Njoku, (2009). Results of Fat - free Tibia in this study did not show

significant difference (P>0.05) among dietary treatments although slightly higher than that of Applegate and Liburn (2002) who reported range values from 7.40 – 9.00 g. Tibia ash from the present study showed no significant difference (P>0.05) among treatments and the findings agreed with the values reported by Tion and Njoku (2009) which ranged from 5.45 – 6.32 g. The tibia calcium indicated absence of significant (P>0.05) effect among dietary treatments. The result of this study agreed with 30.00 – 31.90 % reported by Tion and Njoku (2009).
The result obtained for tibia phosphorus indicated no significant (P>0.05) effect among dietary treatments. The value of this study fell within the range of 15.52 – 16.54 % reported by Tion and Njoku (2009).
The Ca: P ratio of this study showed no significant difference (P>0.05) among dietary treatments which was similar to the reporst of Tion and Njoku (2009) who reported range values of 1.90: 1 to 2.07: 1.

Bioavailability of Phosphorus of broiler Finisher Chickens
The bioavailability of P in this study indicated significant difference (P<0.05) among dietary treatments. The mean values range from 81.7 – 87.5 % P. Rock phosphate had the highest mean value while dicalcium phosphate indicated lowest mean value. Bone meal did not significantly (P>0.05) differ from rock phosphate but significantly (P<0.05) varied from dicalcium phosphate. . Godoy and Chicco (2001) evaluated the bio-availability of phosphorus in Venezuelan rock phosphates, and reported mean value of relative bio-availability of P of six sources on broiler chickens to be between 61.8% and 80.2% P which was lower than the value for this study. Coffey (1994) reported mean value for bioavailability of bone meal to be 78% P for chickens and was lower (86.5% P) than the one recorded in this study. Todd and Angel (2008) reported mean value of 83% apparent utilization of phosphorus from dicalcium phosphate by broiler chicken as determined under deficiency conditions and was lower as compared to the value obtained for this study. The result for this study indicated a better bioavailability of all the sources of phosphorus than those reported. The significant difference (P<0.05) among treatments signified that bone ash and rock phosphate had a better bioavailability of phosphorus than dicalcium phosphate.

Conclusion
The result of this study showed that at 2.5 Kg per 100 Kg diet, rock phosphate can be utilized without adverse effect on growth performance, carcass yield, blood, and bone characteristics. This also means that diet containing rock phosphate compete favourably with bone meal which served as a control and therefore is safe for broiler chickens production.

Recommendation
Effort should be geared towards finding out effect of higher quantities of inclusion in broiler chicken diet and also finding procedures or techniques of removing/reducing fluorine content as the case may be from raw rock phosphate in order to increase its potential feed value for poultry industry.

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