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Solubility and bioavailability studies of limestone sources with layers.

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Abstract

Six limestone sources, oystershell, bone meal and dicalcium phosphate were tested for solubility and bioavailability in vitro and in vivo. In the in vitro solubility, 1, 0.5 and 0.25 gram of each source was reacted with 100, 50, 25mls of 12N HCl respectively for 30 minutes. The in vivo bioavailability utilized fasted laying chickens, which were force fed 3.0g calcium from each source. Faecal collection lasted for 48 hours. Calcium was determined by atomic absorption spectrophotometer. Results of in vitro solubility test indicated high solubility rate for dicalcium phosphate (78.9%). Other sources produced low solubility values below 40% except for bone meal. Limestone sources were similar to oystershell. The in vivo bioavailability values of local sources (limestone, oystershell and bone meal) were significantly (P < 0.05) higher (87% - 92%) than the imported dicalcium phosphate (84.0%). Low solubility of local sources resulted in longer retention time in the GIT, which caused the "metering out" effect that produced high bioavailability mean values observed in this study. Oystershell is universally accepted as a good calcium source for layers. The tested limestone samples were similar to oystershell. It was concluded that any of the sources tested can be used in areas of close proximity without fear of adverse effect.

Key words: *in vitro* solubility, *in vivo* bioavailability, calcium sources, layers.

Introduction

The solubility rate of a calcium source is an important factor in determining the availability of calcium from the source. Since the "metering out" hypothesis of Scott *et al* (1971) and Kim, (1971) which attempted to explain why calcium from oystershell improved eggshell quality over that of limestone, other experiments have been conducted which indicated that the difference in the solubility rate of oystershell and limestone was due to particle size of source and not source *per se*, (Meyer *et al*, 1973, and Roland, *et al*, 1972). Savage (1982) indicated that the pH of the solution in which solubility is determined is an important factor apart from particle sizes of the source. He pointed out that some limestone sources may be of small particle sizes (pulverized form) and will have minimal time to react with the HCl of the gastric juice to cause the calcium ion release for absorption in the small intestine. Kuhl and Sullivan (1977) compared the solubility rate of large particle size oystershell and limestone both *in vitro* and *in vivo*. They reported similar *in vitro* solubility values between oystershell and limestone, but *in vivo* retention time significantly favoured limestone

Studies have been conducted to evaluate calcium availability from different calcium sources through bioavailability trial. The meal feeding method has been utilized by many investigators who have reported promising results. Force-feeding of poultry is another method that is said to increase precision. Kim *et al* 1985)[a] force-fed Japanese quails to compare True available calcium with apparent available calcium, using six calcium supplements. They reported that True available calcium values were slightly higher than the apparent available calcium. Particle sizes of calcium source have been reported (Lindsay, 1982) to have an effect on calcium availability especially for laying hens. Kim *et al* (1985)[b] in another trial, studied the level and method of calcium inputs, age and particle size on True available calcium value in breeding Japanese quails. They reported that the True available calcium values of limestone and oystershell were highest (74.67%) on large particle size (40 – 80) mesh and lowest (68.2% - 61.01%) for small particle size (10 30) mesh.

There is little information in the literature on the solubility and availability for poultry of calcium from limestone deposits that occur in different parts of Nigeria and other sources (already tested in temperate climate) which availability may be influenced by hot tropical environment. The objective of this study was to assess the limestone solubility and availability of calcium from the different calcium sources for laying chickens.

Materials and Methods

Solubility Test:

Six limestone deposits {obtained from cement factories of Ashaka (A), Calabar (B), Jakura (C), Sokoto (D), Ukpila (E) and Yandev (F)}.and oystershell, bone meal and dicalcium phosphate, all in pulverized forms, were tested for their solubility rates using the modified method of Roland and Rabon (1985). One gram of each calcium source was placed in a clean 250ml beaker. 100ml, 12N HCl was added to each sample. The mixture was allowed to react for exactly 30 minutes. (Roland and Rabon 1985, allowed the mixture to react for exactly 15 minutes). The extension of time was due to the fact that, at the end of 15 minutes time interval, the reaction was still progressing actively. After 30 minutes, the mixture was immediately passed through a previously weighed filter paper held over a plastic funnel in a 500ml flask. The soluble fraction was filtered completely by the continuous flushing with deionized water. The insoluble fraction remaining on the filter paper was dried at 100°C for 30 minutes, cooled in a desiccator and weighed. The amount of source solubilized was determined by the difference and recorded. The same procedure was repeated using 0.5 gram of each source to react with 50ml, 12N HCl and again 0.25 gram of each source was made to react with 25ml, 12N HCl. The percentage solubility of each source was calculated from the equation:-

$$\% \text{ Solubility} = \frac{\text{Weight of source solubilized}}{\text{Weight of source}} \times \frac{100}{1}$$

Bioavailability Test:

Sixty hens already in lay were assigned at random to nine calcium sources and a "calcium deficient" diet accounted for the tenth treatment. The calcium deficient diet was formulated to be adequate in all necessary nutrients for layers except calcium. Birds were paired at random in battery cages such that each source had three replicates of two birds per replicate. The layer ration previously fed was replaced with that of calcium deficient diet and was fed for 48 hours. The period was to get the hens into negative calcium balance such that they will utilize maximally the calcium from the sources. Kim *et* *al* (1985)[a] fed the calcium devoid diet for 24 hours. Birds were force-fed a calculated amount (3 grams) of calcium from each source by delivering the source in the crop. A copper pipe (25cm long and 1.25cm diameter) to which a plastic funnel was fixed facilitated this delivery. The pipe was kept from blocking by means of a piece of wire longer than the delivery pipe and was bent at the distal end to prevent injury to the crops. Particles from source, possibly adhering to the sides of the delivery pipe were washed into the crop with water. Thereafter, birds were returned to their cages to continue to feed on calcium deficient diet.

Calcium sources tested comprised of limestone from six deposits A, B, C, D, E and F, oystershell, bone meal, and dicalcium phosphate. Feed and water were provided *ad libitum*. Faecal output from each treatment group was collected for 48 hours, dried, first in open air and at 105°C in the oven over night, cooled in a desiccator and saved for calcium determination. Sibbald (1982) noted that extension of excreta collection period to 48 hours improved both accuracy and precision. Faecal output of the calcium deficient diet was analysed for endogenous metabolic faecal and urinary calcium. Faecal output of calcium sources was determined for un-utilized calcium from each source. Faecal calcium determination was carried out using atomic absorption spectrophotometer following wet digestion as outlined by Perkin-Elmer (1968). Determined faecal calcium from calcium deficient diet was used as a "correction factor" for faecal calcium from the sources.

Summarized data was analysed statistically using the completely randomized design procedure and mean separation was carried out using the Duncan's New Multiple Range Test as described by Steel and Torrie (1980).

Results and Discussion

Solubility Test (in vitro)

Result of this test is presented in Table 1. Result shows highly significant differences between

Table 1: The *In Vitro* Solubility rate (%) of some calcium sources.

Calcium Sources	% Solubility Rate
Limestone Sources	
Ashaka A	39.9^{c}
Calabar B	37.5^{d}
Jakura C	38.2^{cd}
Sokoto D	38.8^{cd}
Ukpila E	35.5^{e}
Yandev F	38.4^{cd}
Oystershell	38.2^{cd}
Bone Meal	44.0^{b}
Dicalcium Phosphate	78.9^{a}
SEM	0.49

^{a,b,c,d} :Means with different superscripts differ ($P < 0.05$)

SEM = Standard Error of Means

mean values. Dicalcium phosphate produced the highest solubility value (78.9%), which was significantly ($P < 0.01$) higher than all the other sources tested. Bone meal (43.98%) was significantly ($P < 0.01$) superior to the remaining calcium sources. All the limestone sources except E (Ukpila) produced solubility values comparable to oystershell. Limestone sourced from Ukpila "E" that produced low solubility value appeared more granular to the feel even in its pulverized form. This is believed to be the reason for the low solubility value. The percentage solubility values for limestone sources used in this study differ from those reported by Roland and Rabon (1985), although the same methodology (save for time allowed for reaction to take place) was used. The differences in result findings are blamed on time of reaction. Savage (1982) suggested that the period of time feed (calcium source) stays in the proventriculus and gizzard is extremely variable. This ranged from a few minutes to about half an hour depending on many factors, one of which is surface area which is due to porosity and particle size of source.

Oystershell has been shown to be retained in the gizzard for a longer period of time due to particle size (Scott *et al* 1971, Kim, 1985). This implies that it is less soluble than limestone. The limestone sources used in this study were similar in their solubility rate to that of oystershell. This is believed to be due to particle size effect. All the sources were in pulverized forms. Meyer *et al* (1973), Roland et al (1972) and Kuhl and Sullivan (1977), found no difference in solubility rate between limestone and oystershell that were in the same form. The superior solubility rate of di-calcium phosphate was expected since it is a reagent grade substance with specification standard. Highly soluble sources of calcium will not be advantageous in feeding poultry especially

Table 2: Bioavailibility of calcium (%) from different calcium sources

Calcium Sources	% Availability
Limestone Sources	
Ashaka A	87.0^{bc}
Calabar B	88.6^{b}
Jakura C	92.8^{a}
Sokoto D	90.0^{ab}
Ukpila E	87.3^{bc}
Yandev F	90.0^{ab}
Oystershell	92.6^{a}
Bone Meal	89.6^{ab}
Dicalcium Pphosphate	84.5^{c}
SEM	1.05

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

chickens in lay. Sources are expected to be retained in the gastro-intestinal tract for a longer period of time to enhance steady and continuous release of calcium from the source (metering out) for absorption.

Bioavailability Test (*in vivo*)

Result of bioavailability test is present in Table 2. It shows that generally, all the limestone sources tested except from Ashaka 'A' produced availability values that were similar to those of oystershell and bone meal. Limestone sourced from Jakura 'C' and oystershell produced the highest numerical values, 92.8% and 92.6% respectively but these sources were similar to limestone sourced from Sokoto 'D', Yandev'F' and bone meal (90.0%, 90.0% 89.6%) respectively. Limestone sourced from Ashaka "A" produced mean availability value similar to sources from Calabar(B), sokoto (D), Ukpila (E), Yandev (F) and bone meal (87.0% 90.0%, 87.3%, 90.0% and 89.6%) respectively. Dicalcium phosphate produced the least availability value (84.5%) which was similar to limestone sourced from Ashaka (A) (87.0%), and Ukpila (E) (87.3%) respectively.

Available calcium from dicalcium phosphate reported herein was similar to the values reported by Kim *et al* (1985). They reported values for the true available calcium for breeding Japanese quails to be between 83.58% - 86.12% and apparent available calcium was 83.42% - 86.0%. Available calcium levels from limestone and oystershell are much lower than values obtained in this study. The lower available calcium level from dicalcium phosphate was believed to be due to its reagent grade, which made it more soluble than the other calcium sources. Savage (1982) pointed out that highly soluble sources of calcium may pass rapidly through the gut with minimal time to react with the HCl of the gut to release calcium ions for absorption, and that, once calcium source is in the duodenal loop, the pH is theoretically too high for calcium release, the calcium from that source becomes unavailable.

Calcium sources that are retained in the proventriculus and gizzard for a longer period of time may be more available to poultry. The result obtained in this study with respect to the availability of calcium from oystershell and limestone sources agree with the report of Waldroup *et al* (1964) although the authors employed different criteria for evaluation. Values for limestone sources are in agreement with those values reported by Reid and Wiber (1976) who used similar methodology for evaluating calcium from the different limestone sources.

Conclusion

Dicalcium phosphate is a resource that has similar usage with bone meal, but it is imported and scarce. Routine use is being made of bone meal that is available locally. For egg production, oystershell and limestone are utilized to supply additional calcium required for good quality shells. Oystershell is a proven source of calcium for layer diets the world over. Since all the limestone sources except from Ukpila (E) had similar solubility, and all sources produced high available values, it was concluded that the limestone sources tested in this study can be utilized effectively in layer diets.

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