



Physical and Chemical Characteristics of Limestone for use in Layer Feeds in Kenya

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ABSTRACT

An adequate amount of calcium (Ca) is required by laying hens for eggshell formation and other physiological functions. There are many common sources of Ca. The choice and use of each source depends on availability, cost and risk factors present. In Kenya, limestone is the main Ca source used in layers' feeds and is obtained from two main areas, Ukunda in the Kenya South coast (UKC) and Athi River (AR) near Nairobi. There is inadequate information on the physical, chemical and nutritional attributes of these limestone sources. The objective of this study was to determine the physical and chemical attributes of limestone from the two sources. Limestone from two sources was purchased from a raw material vendor in Nairobi and *in vitro* solubility, acid insoluble ash (AIA) and mineral content were determined. *In vitro* solubility of UKC and AR limestone was 63.4 and 39.43%, respectively. AR limestone had a high content of AIA at 23.17% as compared to 3.39% for UKC limestone explaining the difference in solubility. The particle size in limestone was classified into coarse (1-2mm), medium (0.5mm) and fine (0.125-0.5 mm). The proportion of particles in UKC and AR in that order was: coarse 22.6% and 9.9%, medium 17.4, and 28.6% and fine 29.2% and 41.9%. Silt (<0.063mm) formed 17.8 and 10.0% of UKC and AR limestone, respectively. Ca content in UKC limestone was 420 g/kg while in AR it was 340 g/kg. Cr, CU and Zn were found in small concentrations ranging from 0.3 to 8.6 µg/kg in UKC and AR limestone. Fe concentration was 42.6 and 53.6 µg/kg in UKC and AR respectively. Aluminium was relatively high at 1073 and 302 µg/kg for UKC and AR, respectively. Based on Ca and AIA content, solubility, and particle size UKC limestone is a superior Ca source than AR.

Key words: Concentration, Limestone, Particle size, Source, Solubility,

INTRODUCTION

Minerals play vital roles in the physiological functions of an animal, which include structural, homeostasis maintenance, ionic equilibrium, osmotic pressure and enzyme systems. Calcium (Ca) is the most abundant mineral in the body, most of which is found in the skeleton (Jiang, *et al.*, (2013). Laying hens require a high calcium content in their diets compared to other classes of poultry (3.3%) for bone and eggshell formation as well as other physiological functions (NRC,1994). The chicken eggshell is almost 100% calcium carbonate (Rodriguez, 2013). Calcium required for eggshell formation is obtained from the diet and medullary bone reserves. A good dietary supplemental Ca source is therefore required. Limestone, fish meal, bone meal, meat and bone meal and di-calcium phosphate are some of the common calcium sources,

whose use depends on availability, cost and any risk factor present. Limestone and oyster shell which provide calcium in the form of CaCO₃ are widely used in many countries for this purpose. Limestone is cheaper than oyster shell and has been in use for more than 100 years (Saunders-Blades *et al.*, 2009). Limestone, a naturally occurring sedimentary rock, is high in CaCO₃ and/or MgCO₃ and some small amounts of trace minerals. It is extracted from quarries and underground mines, with varying physical and chemical attributes. These include solubility, particle size and Mg content (Ketta and Tùmová, 2016). Pure limestone can be classified into different categories based on CaCO₃ and MgCO₃ content. Thus calcitic limestone contains 95-100% CaCO₃ and 5-0% MgCO₃. On the other hand, dolomitic limestone contains 54-59% CaCO₃ and 41-46% MgCO₃ (<https://dnr.mo.gov/geology/docs>)

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mineralChem.limestone).

Different limestones sources vary in their calcium (Ca) content (Aila *et al.*, 2012 ; Chambers *et al.*, 2017). The two main sources of limestone in Kenya are Ukunda in the Kenyan coast (UKC) and Athi River (AR) near Nairobi. In 2016, an amount of 0.287 million metric tons of poultry feed were manufactured in Kenya with an estimated use of 21 and 26 tons of UKC and AR limestone, respectively. The physical characteristics of limestone influence its utilization. Thus absorption of mineral is influenced by its solubility and the level of intake (Zhang *et al.*, 2017). Particle size is another factor influencing utilization of limestone. The bigger the particles size the more the retention of limestone in the gizzard (Pelicia *et al.*, 2009), which is associated with more availability of Ca during eggshell formation period. The retention time is however dependent on the source of the Ca, particles size, and the *in vivo* solubility (Zhang *et al.*, 2017 and Pizzolante *et al.*, 2009). Although large amounts of this limestone are used, there is no information on chemical attributes and nutritional value of these limestone sources. The objective of this study was to determine the physical and chemical characteristics of two sources of limestone used in layer feeds in Kenya.

MATERIALS AND METHODS

From two sources, Ukunda in coast region of Kenya (UKC) and Athi River (AR), was used in this study. Both types of limestone were purchased from a feed ingredient raw material vendor in Nairobi and analysed for *in vitro* solubility, acid insoluble ash (AIA) and mineral content. The *in vitro* solubility test was done with the view of estimating the *in vivo* solubility in the gizzard. Approximately 2.0 g of each of the two limestone samples were weighed in duplicates into 500 ml glass beakers. To each beaker 200 ml of 0.2N HCl was added. The beakers were then heated in a water bath at 42°C for 10 minutes under slight agitation. The reaction was stopped by adding 200 ml of de-ionized water into each beaker. The contents were then filtered

through a Whatman 42 ashless filter paper. The residue was dried in an oven at 60°C for 20 h and weighed. Percentages *in-vitro* solubility was calculated by weight difference (Zhang *et al.*, 2017). Briefly, 5.0 g of the limestone sample was boiled in 100 ml 4M hydrochloric acid (HCl) for 30 minutes. The contents were filtered through a Whatman No 42 ashless filter paper and residue washed severally using hot distilled water to remove HCl. The filter paper together with the residue was transferred into a tared silica dish and ashed at 600°C for 6 h. The acid insoluble ash was then determined by weight difference and expressed as a percentage of the sample used.

Particle size was determined using the methods described by Chakraborty (2016). This involved using a vibrator with seven ABNT (Brazilian Association of Technical Standards) sieves, which had mesh ranging in sizes from 2 mm to 0.063 mm. The sieves were stack together from the largest sieve size at the top to the smallest at the bottom. Below the sieves was the collector. One kilogram of the limestone sample was weighed and placed into the first sieve (2 mm mesh sieve). The vibrator was rotated for 30 minutes at a speed of 1450 rpm. The amount of limestone remaining on each sieve was weighed and recorded. Percentage particle size was determined using the formula

% Particle size in respective sieve =

$$\frac{\text{Particle weight (g)}}{1000 \text{ g}} \times 100$$

A preliminary investigation to determine the minerals present in the samples was done using an X-Ray Fluorescence (XRF) method. This was done at the Geology and Mines Department, Ministry of Natural Resources in Nairobi. It involved use of an X-ray fluorescence (XRF) spectrometer to determine qualitative and quantitative elemental composition of each sample. This analysis showed that Ca, Zn, Fe, K, Mg, Al, Co, Cu, Cr, Mn, Na and P were present in limestone samples. The individual concentration of these minerals was determined using AOAC (1990) procedures, 0.5 g

Table 1. *In vitro* solubility and AIA of limestone from two sources in Kenya

Limestone source	<i>In vitro</i> solubility (%)	Acid insoluble ash (%)
UKC	63.04±0.34	3.39±0.36
AR	29.43±0.23	23.17±0.16

of sample was weighed into a 100 ml glass beaker and 15 ml of a digestion mixture, which consisted of three parts 70% concentrated per-chloric acid and one part 70% concentrated nitric acid, was added. The glass beakers were then placed on a sand bath set at a temperature of 100°C and allowed to boil for 3 to 4 hrs until the solution became colourless. They were allowed to cool and 20 ml of distilled water was then added into each beaker. The contents were then filtered through an ashless Whatman filter paper No 42 into a 100 ml volumetric flask, after which it was topped up to the mark using distilled water.

Ca, Mg, Zn, Cu, Co were analysed for using Atomic absorption spectrophotometer model Varian, spectra AA while Flame emission spectrophotometer was used for estimation of Na and K. Spectrophotometry was used to determine phosphorus at a wavelength of 400 nm.

RESULTS AND DISCUSSION

The *in vitro* solubility and acid insoluble ash content of the two types of limestone are shown in Table 1. The *in vitro* solubility of limestone from UKC was 63.04 ± 0.34% while that from AR was 29.43 ± 0.23 %. Solubility, particle size and amount of acid insoluble ash (AIA) are factors that influence absorbability of minerals (Zhang *et al.*, 2017). Based on the three factors, (solubility, particle size and AIA), UKC is potentially a better source of Ca than the AR limestone. AR had a high content of AIA at 23.17 ± 0.16%

compared to 3.39 ± 0.23 % for UKC limestone. AIA consists of indigestible mineral components, mainly silica (Sales and Janssens, 2003). UKC had a higher proportion of coarse particles than AR limestone. Coarse particles are associated with low digestibility (Zhang *et al.*, 2017). The difference in solubility between UKC and AR limestone can be explained by the amount of AIA in each source.

Data pertaining to particle sizes of the different types of limestone is shown in Table 2. There were 4 different particle sizes, namely coarse medium, fine and silt. This classification was based on recommendations for poultry mineral sources (De Witt *et al.*, 2009). UKC limestone had 2.25 times more coarse particles than the AR. Particle size influences digestion and absorption of limestone (Swiatkiewicz *et al.*, 2015), where digestion is lower for coarse particles than the fine ones. Similar findings have been reported by Zhang *et al.*, (2017) who showed that solubility increased from 30.1% to 58.7 when particle size ranged between 2.75 mm and 0.25 mm, respectively. However, large limestone particles in the diet increase egg shell quality (Tunç and Cufadar, 2014; Zhang *et al.*, 2017). Large particles result in increased retention time in the gizzard resulting in increased Ca bioavailability. Limestone with a high proportion of fine particles (silt) is not desirable because it increases the dustiness of the feed and hence affects feed intake (Pelicia *et al.*, 2009).

The mineral content in the two types of limestone

Table 2. Category of particle size in the limestone samples

Particle description	Size (mm)	UKC limestone %	AR limestone %
Coarse	1-2	22.6±0.15	9.9±0.15
Medium	0.5	17.4±0.15	28.6±0.05
Fine	0.125-0.5	29.2±0.1	41.9±0.15
Silt	<0.063	17.8±0.3	10.0±3.9

Table 3. Concentration of analysed minerals in limestone

Mineral	UKC limestone	AR limestone
Calcium, g/kg	420±0.1	340±0.15
Content in µg/kg		
Aluminium (Al)	1072.5±7.17	302±3.2
Iron (Fe)	42.6±7.9	53.6±2.9
Chromium (Cr)	7.0±1.78	8.6±1.41
Copper (Cu)	3.0±0.01	3.0±0.01
Zinc (Zn)	0.3±0.04	0.0

studied are shown in Table 3. Though the preliminary evaluation using XRF analysis had shown that the limestone samples contained Ca, Mg, Zn, Cu, Co, Mn, Al, Fe, K, Na and Cr, upon analysis using AOAC (1990) procedures, Mg, K, Mn and Co were not detected. The absence of magnesium would lead to the two sources of limestone being classified as calcitic. On other hand, the samples contained Ca, Al, Fe, Cr, Cu and Zn. UKC and AR limestone had high levels of calcium at 420 and 340 g/kg, respectively. A high concentration of calcium is important in that it minimizes the amount of limestone included in the diet thereby reducing dilution effect of this ingredient. The UKC limestone had high levels of aluminium compared to AR limestone. The two samples provide Cr, Cu and Fe but in trace amounts.

CONCLUSION

The chemical and physical characteristics of limestone obtained from Athi River (AR) and that from the coast (UKC) were assessed. The following conclusions are drawn from this study: (i) the UKC and AR limestone are good sources of calcium, (ii) UKC limestone had higher proportion of coarse particles (1-2 mm) than AR limestone, making it a good source of calcium for eggshell formation, (iii) UKC limestone had a higher solubility than AR one, (iv) Acid insoluble ash was high in AR limestone, reducing its solubility and (v) UKC and AR limestone contained trace amounts of Fe, Cr, Cu and Zn, but UKC limestone had a relatively high content of aluminium (1.07 mg /kg). Both the UKC and AR limestone can be used in poultry feeds. However, based on Ca content, solubility, AIA content, and

particle size UKC limestone is a better feedstuff than AR one.

REFERENCES

- Aila, F.O., Oima, D., Ochieng, I., Ojera, P.B. and Odera, O. 2012. Construction and validation of consumer attitudes on biosecurity principles: A methodological perspective. *Res Hum.Soc. Sci* 2: 240-250.
- AOAC.1990 Official Methods of Analysis, 15th edn, Association of Official Analytical Chemist, Washington DC. Vol. 1, pp. 69-90.
- Chakraborty, A.P. 2016. Chicken eggshell as calcium supplement tablet. *Int. J. Sci. Eng. Manag*, 1:45-49.
- Chambers, J.R., Zaheer, K., Akhtar, H. and Abdel-Aal, E.S.M. 2017. Chicken eggs. In *Egg Innovations and Strategies for Improvements*, Academic Press, pp. 1-9.
- De Witt, F.H., Kuleile, N.P., Van Der Merwe, H.J. and Fair, M.D. 2009. Effect of limestone particle size on egg production and eggshell quality of hens during late production. *S. Afr. J. Anim. Sci.* 39: 37-40.
- Jiang, S., Cui, L., Shi, C., Ke, X., Luo, J. and Hou, J. 2013. Effects of dietary energy and calcium levels on performance, egg shell quality and bone metabolism in hens. *Vet. J.* 198: 252-258.
- Ketta, M., and Tùmová, E. 2016. Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. *Czech. J. Anim. Sci.* 61: 299-309.
- Pelicia, K., Garcia, E., Mori, C., Faitarone, A.B.G., Silva, A.P., Molino, A. B. and Berto, D. A. 2009. Calcium levels and limestone particle size in the diet of commercial layers at the end of the first production cycle. *Brazilian J. Poult. Sci*, 11: 87-94
- Pizzolante, C.C., Saldanha, E.S.P.B., Laganá, C., Kakimoto, S.K. and Togashi, C.K. 2009. Effects of calcium levels and limestone particle size on the egg quality of semi-heavy layers in their second production cycle. *Brazilian J. Poult. Sci*, 11: 79-86.
- Rodriguez, C.M. 2013. Performance of chicken layers as

- affected by calcium supplement. *Int. Sci. Res. J.* 2: 2094-1749.
- Saunders-Blades, J.L. and Korver, D.R. 2014. The effect of maternal vitamin D source on broiler hatching egg quality, hatchability, and progeny bone mineral density and performance. *J. Appl. Poult. Res.* 23: 773-783.
- Swiatkiewicz, S., Arczewska-Wlosek, A., Krawczyk, J., Puchala, M. and Józefiak, D. 2015. Effects on performance and eggshell quality of particle size of calcium *Archiv. Tierzucht.* 58: 301.
- Tunç, A.E., and Cufadar, Y. 2014. Effect of calcium sources and particle size on performance and eggshell quality in laying hens. *Turkish J. Agric. Food Sci. Technol.* 3: 205-209.
- Zhang, B., Caldas, J.V. and Coon, C.N. 2017. Effect of Dietary Calcium Intake and Limestone Solubility on Egg Shell Quality and Bone Parameters for Aged Laying Hens. *Int. J. Poult. Sci.* 16: 132-138.

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