# Effect of calcium sources and particle sizes on performance characteristics, age at first egg and blood parameters of egg type chicken

Akpiruo, C. E.
*Department of Animal Nutrition,*
*Federal University of Agriculture, Abeokuta, Nigeria.*
**Corresponding author:** chrispiruo2014@gmail.com; +2348029757385

**Abstract**

*The importance of Calcium to animals in general and egg type chicken in particular has been of interest to researchers for a long time. This interest in calcium varies from the search for the optimal sources of calcium for egg type chicken in their feed, a search for the optimal or ideal calcium in their feed, as well as the influence of calcium (positive and negative) on egg production and metabolism. This study was conducted to evaluate the effect of using different calcium sources (eggshell, limestone and oyster shell) and particle sizes (<1mm, 1-2mm and 2-3.8mm) in the diets of 540, one day old pullet chicks (Nera Black) up to pre - laying phase in a 3 x 3 factorial design consisting of nine dietary treatments. There were 60 birds per treatment replicated thrice with 20 birds each. Three isocaloric and isonitrogenious diets with three calcium particles sizes were formulated for each of the calcium sources totaling nine diets. The experiment comprised of starter (2-9 weeks), grower (10-17 weeks) phase and pre- laying (18-22 weeks) respectively. The levels of oyster shell, limestone and egg shell were adjusted so that the level of calcium in the chick and grower mashes was 1%. Growers mash was also used for pre-laying phase. Data were collected on growth performance, serum calcium, alkaline phosphatase and age at first egg. Diets containing eggshell of particle size <1mm produced birds with the highest (p<0.05) final live weight (523.64g) at the starter phase and also had the best overall live weight (1476.62g) at 17 weeks. Diets that contained eggshell of particle size 1-2mm recorded the highest (p<0.05) value for serum calcium (8.82mg/dl) and alkaline phosphatase (53.55u/l). Birds in this group were the first to start laying. It can be concluded that rapid improvement in growth could be achieved by the inclusion of eggshell of particle size (<1mm) in the diets as the main calcium source and eggshell of particle size (1-2mm) for older pullet to enhance early egg production.*

**Keywords**: Calcium, particle size, egg type chicken, blood parameters, age at first egg

## Introduction

Calcium plays a vital role in pullet chick's metabolic processes. Calcium is found in blood of chickens, as well as other animals, in three forms: bound to plasma proteins, bound to inorganic compounds and freely dissociated or ionized (Ahammad and Mahammud, 2005). The physiologically active component is ionized calcium, which has been firmly established to have a major role in many biochemical processes, including nerve conduction, muscle contraction, blood clotting and hormonal regulation of bone metabolism (Cohn and Hamilton, 1976). Consequently, the regulation of blood ionized calcium ($Ca^{2+}$) within a narrow range is of great importance since this blood calcium fraction affects the functions of the aforementioned biological processes. Calcium is usually supplied as calcium carbonate from limestone in poultry rations or from other sources, such as marine shells in diets for chicks (McNaughton *et al.,* 1974; Gerry, 1980) or in hens (Roland, 1986). The calcium sources differ in their origin (animal or mineral deposit) and their particle size; as a consequence, their physiochemical characteristics are different (Mongin and Sauveur, 1977; Ehtesham and Chowdhury, 2002) observed that poultry producers are always interested in high production at minimum expenditure on nutrients so as to economize their feeding practices. Limestone and oystershell are the sources of calcium commonly used in chicken feed. Limestone is obtained from soil deposit while oyster shell is a marine product. Eggshell is a waste material from domestic sources such as hatcheries, poultry farms, egg product factories, homes, and restaurants. Many studies have looked for ways to use the eggshell waste, for example, by using eggshell powder as a stabilizing material for improving soil properties (Amu *et al*., 2005), as a source of Ca for piglets (Schaafsma and Beelen, 1999), and as a source of Ca in human nutrition (Schaafsmaand Pakan, 1999; Schaafsma*et al.,* 2000). In addition, extruded eggshell, including membranes, can be used as a Ca source in layer diets without adverse effects on egg production (Froning and Bergquist, 1990). Tadtiyanant *et al*. (1993) demonstrated no detrimental effects on egg weight, egg production, feed conversion, and egg specific gravity in laying hens fed diets containing extruded eggshell meal as a source of Ca. These researchers reported that high temperature-short time extrusion was an alternative method for converting poultry industry residues into high-quality poultry feedstuffs. Drying the eggshell product at a high temperature is critical, to decrease the potential for contamination by pathogens.

The relationship between plasma alkaline phosphatase activity and the performance traits of chickens has been studied in several laboratories. Singh *et al*. (1983) found that the alkaline phosphatase activities were higher in pullets selected for higher production. However, the data obtained by (Gootwine and Brody, 1979) failed to show a relationship between alkaline phosphatase activity and production traits. Age and body weight at sexual maturity are important economic traits, which effect on potentiality of egg production. The level of several blood constituents is quite different in female birds when various reproductive states are compared (Bacon *et al*., 1980). The study of such variation in blood constituents in chickens could be associated with growth and egg production. The respective association may explain the physiological basis concerned with performance of both productive and reproductive traits. Numerous investigations were conducted to relate chicken performance with some physical and chemical parameters of blood (Mady, 1990, and El-Bogdady *et al*., 1993). However, the findings of these investigators demonstrated some conflict results in this respect. The objective of this study is to determine the ideal calcium particle size in feed of pullet chicks and establish the relationship between age at first egg and blood parameters of pullets.

## Materials and methods

### *Location of experimental station*

The research was carried out at a poultry farm in Oke-Ata Housing Estate, Abeokuta, Ogun State.

### *Experimental birds and management*

#### *Phase I – Starter phase (2-9 weeks)*

A total of 540-day old pullet chicks of commercial strain (Nera Black) were obtained from a reputable hatchery. The brooding house and brooding equipment were thoroughly washed and disinfected before the arrival of the chicks. The chicks were housed and brooded in a deep litter floor poultry unit. The dimension of the poultry unit was 10.97m by 3.65m. Chicks were brooded together for the first seven days (to allow for physiological adjustment) during which they were fed with commercial feed. Thereafter, they were individually weighed, grouped according to their weight ranges before they were distributed equally at random among the nine experimental diets with three replicates per treatment. Diets were presented in mash form with the three sources of Ca (Oyster shell, limestone and eggshell) ground and separated into three particle sizes (<1mm, 1-2mm and >2-3.8mm) using sieves with the various pore sizes. The experimental birds were fed adlibitum from the 14$^{th}$ day to the end of the experiment. Chick starter was fed from the 2$^{nd}$ to 9$^{th}$ week.

***Phase II – Grower phase (10-17 weeks)***

Growers mash was fed adlibitum to the birds from the 10$^{th}$ – 17$^{th}$ week. Diets were also presented in mash form with the various calcium sources and particle sizes that were used for the chick phase. The overall performance was included in the research to get a holistic view of bird's performance distributed among the nine experimental diets. Overall performance was obtained by adding values for various parameters in the starter and grower phases.

***Phase 111- Pre-laying phase (18-22 weeks)***

Grower mash was also fed to birds at this stage until the first egg dropped.

***Preparation of experimental diets***

A total of nine experimental diets were prepared. The compositions of the diets are as indicated in Tables 1 and 2. The Ca contents of oyster shell, limestone and brown egg shell were determined from literature and laboratory analysis to assess the levels to be included in the diets to contribute equal amounts of Ca in the diets. The eggshell was heated for 30 minutes to kill any salmonella and other pathogenic organism that may be present in it. The levels of oyster shell, limestone and egg shell were adjusted so that the level of Ca in diets 1-9 was 1%. Other ingredients were kept constant in all the diets. All diets were formulated to be Isonitrogenous and Isocaloric. Bone meal was kept constant to get constant level of Calcium (Ca) and Phosphorous (P) ratio in all the diets.

The phytate phosphorus was determined using the method outlined by A.O.A.C 1990. Thereafter the value was deducted from the value of the total phosphorus to get the value of the NPP (non phytate phosphorus).

***Chemical analysis***

The percentage compositions of Ca and P in the feeds were determined using the procedure described by A.O.A.C (1990).

***Performance characteristics***

The birds were weighed on a weekly basis on weighing scale. Each replicate was given the same amount of feed daily and the feed residue was also weighed daily to determine feed consumed. Mortality was recorded on weekly basis. Feed conversion ratio was calculated.

Feed conversion ratio =

$$\frac{\text{Feed Consumed (g)}}{\text{Weight gain (g)}}$$

***Collection of blood samples***

At the end of the experiment, three birds per treatment (making a total of 18) were selected and blood was collected from the jugular vein by the use of a syringe into EDTA bottle for Ca, and alkaline phosphatase analysis (A.O.A.C 1990).

***Statistical analysis***

The data obtained in these studies were subjected to Analysis of Variance (ANOVA) using 3x3 factorial arrangement using SAS (2000). Significant means were separated using Duncan's Multiple Range Test. (Duncan, 1955). All analysis was carried out using Minitab Analytical Computer Package (Minitab Inc. 1989).

***Statistical model***

$$Y_{ijk} = \mu + T_i + L_j + (TL)_{ij} + e_{ijk}$$

Where

$Y_{ijk}$= observed value of dependent

**Table 1: Composition of experiment diets (2 – 9 Weeks) (Starter Phase)**

| | Calcium Sources | | |
|---|---|---|---|
| | Limestone | Oystershell | Eggshell |
| Ingredients | 1 | 2 | 3 |
| Maize | 50.00 | 50.00 | 50.00 |
| Soyabean Meal | 10.00 | 10.00 | 10.00 |
| Groundnut cake | 20.00 | 20.00 | 20.00 |
| Wheat offal | 15.00 | 15.00 | 15.00 |
| Fish Meal | 1.70 | 1.70 | 1.70 |
| Bone Meal | 1.50 | 1.50 | 1.50 |
| Limestone | 1.00 | 1.00 | 1.00 |
| Oyster shell | 0.00 | 1.00 | 0.00 |
| Egg Shell | 0.00 | 0.00 | 1.00 |
| Lysine | 0.15 | 0.15 | 0.15 |
| Methionine | 0.15 | 0.15 | 0.15 |
| Salt | 0.25 | 0.25 | 0.25 |
| *Premix | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 |
| **Calculated composition** | | | |
| Metabolizable Energy (kcal/kg) | 2844 | 2844 | 2844 |
| Protein | 22.70 | 22.70 | 22.70 |
| Crude Fibre (%) | 4.00 | 4.00 | 4.00 |
| Ether Extract (%) | 4.10 | 4.10 | 4.10 |
| *TP (%) | 0.70 | 0.70 | 0.70 |
| *PP (%) | 0.20 | 0.20 | 0.20 |
| *NPP (%) | 0.50 | 0.50 | 0.50 |
| Calcium (%) | 1.00 | 1.00 | 1.00 |

TP – Total Phosphorus

PP – Phylate phosphorus

NPP – Non phylate phosphorus

variable

$\mu$ = population mean

$T_i$ = the effects of sources of Calcium (í=1-3)

$L_j$ = the effects of the particle sizes of Calcium (j=1-3)

$(TL)_{ij}$ = the interactive effect of Calcium sources and particle sizes.

$e_{ijk}$ = random residual errors

## Results and discussion

Table 3 shows the mean values for the performance of pullet chicks fed the various experimental diets at starter phase. Birds fed diets containing eggshell of particle size(<1mm) recorded the highest value for final live weight(523.64g), weight gain(458.45g) and daily weight gain(7.75g). Birds in this group also recorded the least value for feed conversion ratio (4.70). This is closely followed by birds fed diets containing limestone of particle size(<1mm) with final live weight(497.31g), weight gain(432.13g), daily weight gain(7.33g) and feed conversion ratio (5.02). These findings are in agreement with the report of Guninote *et al.* (1991), who observed that pulverized limestone (< 0.15mm particles) improved calcium retention, 4- week body weight and feed conversion compared to medium (6 to 1.18mm) and coarse particle (> 1.18mm) sizes of calcium sources. Also, (Mc Naughton, 1981) reported that 20 to 60 United States Bureau of Standards (USBS) particle-sized $CaC0_3$ when fed to 1 to 21-

**Table 2: Composition of experiment diets (10 – 17 Weeks) (Grower Phase)**

| | Calcium Sources | | |
|---|---|---|---|
| | Limestone 4 | Oystershell 5 | Eggshell 6 |
| Ingredients | | | |
| Maize | 40.00 | 40.00 | 40.00 |
| Soyabean Meal | 5.00 | 5.00 | 5.00 |
| Groundnut cake | 5.00 | 5.00 | 5.00 |
| Wheat offal | 26.80 | 26.80 | 26.80 |
| Palm Kernel Cake | 18.50 | 18.50 | 18.50 |
| Fish Meal | 1.50 | 1.50 | 1.50 |
| Bone Meal | 1.50 | 1.50 | 1.50 |
| Limestone | 1.00 | 0.00 | 0.00 |
| Oyster shell | 0.00 | 1.00 | 0.00 |
| Egg Shell | 0.00 | 0.00 | 1.00 |
| Lysine | 0.10 | 0.10 | 0.10 |
| Methionine | 0.10 | 0.10 | 0.10 |
| *Premix | 0.25 | 0.25 | 0.25 |
| Salt | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 |
| **Calculated composition** | | | |
| Metabolizable Energy (kcal/kg) | 2590 | 2590 | 2590 |
| Protein | 17.90 | 18.20 | 18.40 |
| Crude Fibre (%) | 6.10 | 6.00 | 5.90 |
| Ether Extract (%) | 4.30 | 4.30 | 4.30 |
| *TP (%) | 0.63 | 0.60 | 0.60 |
| *PP (%) | 0.28 | 0.20 | 0.32 |
| *NPP (%) | 0.35 | 0.40 | 0.40 |
| Calcium (%) | 1.20 | 1.00 | 1.00 |

TP – Total Phosphorus
PP – Phylate phosphorus
NPP – Non phylate phosphorus

day old broiler chicks produced higher body weight than either USBS 12 to 20 or 100 to 200 particle-sized $CaCO_3$ sources. Guinote *et al.* (1991) and (Mc Naughton ,1981) indicated that calcium utilization by chicks is dependent upon the particle size of the Ca supplement and the most desirable particle size of $CaC0_3$ ranged from a fine to medium particle sizes for producing optimum chick weight and percent tibia ash.

The large particle size (1-2mm) and (>2-3.8mm) recorded the least values for final weight, weight gain and high feed conversion ratio. The transit time for the large particle calcium source to pass through the gastrointestinal tract was longer because most of the particles were stored in the gizzard. This reduced the amount of calcium readily available for muscle formation. This explanation agrees with the report of Guninote *et al.* (1991) who suggested that the calcium retention was lower for broilers fed larger $CaC0_3$ particles because the particles were retained in the gizzard. The calcium source that produced birds with the highest values for final live weight, weight gain and low feed conversion ratio was eggshell. This was followed by limestone while oyster shell had the least values. This is due to the low in vitro solubility of these other sources compared to eggshell.

Significant differences were not recorded for any of the parameters measured in the grower phase. This trend was observed for both particle size and calcium sources. This is in agreement with the findings of Guinotte *et al*. (1991) who observed no effect of calcium source nor particle size on live weight of pullet. Table 4 shows the mean values of the overall growth performance of pullets fed the various experimental diets. Birds fed diets containing eggshell of particle(<1mm) recorded the highest value for final live weight (1476.62g) while birds fed limestone of particle size(1-2mm) had the least value(1361.82g) for the same parameter. The overall performance of the chicks fed the large particle calcium carbonate in the present studies was less than chicks fed smaller particles of calcium carbonate ($CaCO_3$) because of high feed conversion ratio. The lager particles retained in the gizzard may not have provided adequate calcium intake for optimum performance. The chicks fed the larger calcium particles are probably compensating for the poor intake of nutrients in the increased retention time. This finding is similar to the report of Guninote *et al.* (1991) who suggested that the longer transit time needed for coarse particles of $CaCO_3$ to pass through the gastrointestinal tract of broilers because of gizzard retention and the lower solubility of the particles might explain the negative effect of large or coarse particle sizes.

Table 5 shows the value for age at first egg, blood calcium and serum alkaline phosphatase.

Birds fed diets containing eggshell of particle size(1-2mm) were the first to lay eggs(20.67weeks) while those fed diets containing limestone of particle size(1-2mm) and eggshell of particle size(<1mm) were the last to start laying (21.67weeks). This can be attributed to the fast transit time of small particles size eggshell through the gastrointestinal tract. Birds fed diets containing limestone of particle size (1-2mm) recorded different value for age at first egg (21.33weeks) when compared to eggshell due to the solubility differences. This assertion is supported by Rabin and Roland (1985) who posited that the solubility of limestone particle of similar size from different sources varies by 62%.

Birds fed diets containing eggshell of particle size (1-2mm) recorded the highest value for serum calcium (8.14mg/dL) and alkaline phosphatase (53.55u/l). This is closely followed by limestone of particle size(1-2mm) with value 6.15mg/dL and 44.15u/l for the same parameter. This result posited that serum calcium and alkaline phosphatase has a positive correlation with age at first egg. The result of this study revealed birds with higher alkaline phosphatase activity started laying earlier than those of lower activity. These results are in agreement with those of Singh *el al.* (1983), El- Attar and Bahieldin (1995) and Kalama *et al.* (2000) who indicated that higher enzyme alkaline phosphatase activity is associated with earlier sexual maturity than those with lower enzyme activity.

**Table 3: Effect of interaction between Ca Sources and Particle sizes on the performance of pullet chicks (2-9 weeks)**

| | **Oyster shell Particle size** | | | **limestone Particle size** | | | **Egg shell Particle size** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | < 1mm | 1-2mm | >2-3.8mm | < 1mm | 1-2mm | >2-3.8mm | < 1mm | 1-2mm | >2-3.8mm | SEM |
| Initial weight (g/bird) | 65.05 | 65.32 | 65.13 | 65.18 | 65.17 | 65.17 | 65.19 | 65.26 | 65.11 | 0.11 |
| Final weight (g/bird) | 475.61$^{d}$ | 490.2$^{bcd}$ | 498.19$^{abcd}$ | 497.31$^{abcd}$ | 478.61$^{dc}$ | 498.68$^{abcd}$ | 523.64$^{a}$ | 515.06$^{ab}$ | 508.29$^{abc}$ | 8.75 |
| Weight gain (g/bird) | 410.56$^{d}$ | 424.93$^{bcd}$ | 433.06$^{abcd}$ | 432.13$^{abcd}$ | 413.44$^{cd}$ | 433.51$^{abcd}$ | 458.45$^{a}$ | 449.79$^{ab}$ | 443.18$^{abc}$ | 8.86 |
| Daily weight gain (g/bird) | 6.99$^{d}$ | 7.22$^{bcd}$ | 7.35$^{abcd}$ | 7.33$^{abcd}$ | 7.04$^{cd}$ | 7.36$^{abcd}$ | 7.75$^{a}$ | 7.62$^{ab}$ | 7.51$^{abc}$ | 0.12 |
| Daily feed intake (g/bird) | 36.71$^{ab}$ | 36.65$^{ab}$ | 37.04$^{a}$ | 36.81$^{ab}$ | 36.96$^{a}$ | 36.89$^{ab}$ | 36.43$^{ab}$ | 36.02$^{b}$ | 36.76$^{ab}$ | 10.21 |
| Total feed intake (g/bird) | 2055.88$^{ab}$ | 2052.35$^{ab}$ | 2074.37$^{a}$ | 2061.12$^{ab}$ | 2069.96$^{a}$ | 2065.98$^{ab}$ | 2039.83$^{ab}$ | 2016.90$^{b}$ | 2058.70$^{ab}$ | 17.10 |
| Feed Conversion ratio | 5.25$^{a}$ | 5.08$^{ab}$ | 5.08$^{abc}$ | 5.02$^{abcd}$ | -5.23$^{a}$ | 5.03$^{abc}$ | 4.70$^{d}$ | 4.73$^{d}$ | 4.90$^{bcd}$ | 1.75 |

*Means in the same row across each effect with different superscripts differ s ignificantly (P<0.05)*

**Table 4: Effects of interaction between Ca source and particle sizes on overall growth performances of the birds (2-17 weeks)**

| | | Oyster shell Particle size | | | limestone Particle size | | | Egg shell Particle size | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | < 1mm | 1-2mm | >2-3.8mm | < 1mm | 1-2mm | >2-3.8mm | < 1mm | 1-2mm | >2-3.8mm | SEM |
| Initial weight (g/bird) | 65.05 | 65.32 | 65.13 | 65.18 | 65.17 | 65.07 | 65.19 | 65.26 | 65.11 | 0.13 |
| Final weight (g/bird) | $1377.39^{b}$ | $1369.60^{b}$ | $1367.86^{b}$ | $1401.13^{b}$ | $1361.82^{b}$ | $1365.15^{b}$ | $1476.62^{ab}$ | $1475.07^{a}$ | $1388.10^{b}$ | 39.50 |
| Daily weight gain (g/bird) | 11.98 | 11.91 | 11.90 | 12.20 | 11.85 | 11.87 | 12.87 | 12.86 | 12.08 | 0.78 |
| Weight gain (g/bird) | 1312.33 | 1304.28 | 1302.73 | 1335.95 | 1296.65 | 1299.98 | 1411.43 | 1410.40 | 1322.92 | 43.28 |
| Daily feed intake (g/bird) | 55.37 | 55.34 | 55.54 | 55.41 | 55.41 | 55.45 | 55.24 | 55.08 | 55.39 | 1.51 |
| Total feed intake (g/bird) | 6201.05 | 6198.92 | 6220.05 | 6205.97 | 6213.57 | 6210.65 | 6186.36 | 6169.15 | 6203.17 | 22.20 |
| Feed Conversion ratio | 4.62 | 4.65 | 4.70 | 4.57 | 4.68 | 4.68 | 4.31 | 4.30 | 4.59 | 0.34 |

*Means in the same row across each effect with different superscripts differ significantly ($P<0.05$)*

**Table 5: Effects of interaction between Ca sources and particle sizes on serum calcium, alkaline phosphatase and age at first egg in pullets**

| | Oyster shell Particle size | | | limestone Particle size | | | Egg shell Particle size | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | < 1mm | 1-2mm | >2-3.8mm | < 1mm | 1-2mm | >2-3.8mm | < 1mm | 1-2mm | >2-3.8mm | SEM |
| Ca in blood (mg/dl) | 4.28[d] | 5.75[bc] | 6.25[b] | 5.26[bcd] | 6.15[c] | 6.45[b] | 4.86[cd] | 8.14[a] | 6.45[b] | 0.35 |
| Age at first egg | 21.00[b] | 21.33[ab] | 21.00[b] | 21.33[ab] | 21.67[a] | 21.33[ab] | 20.67[bc] | 20.33[c] | 21.00[b] | 0.22 |
| A/P in blood (u/l) | 34.66[d] | 45.12[b] | 39.44[c] | 43.49[bc] | 44.15[bc] | 48.29[b] | 46.54[b] | 53.55[a] | 32.15[d] | 1.14 |

*Means in the same row across each effect with different superscript s differ significantly (P<0.05)*

## Conclusions

This study reveals that there was an increase in the final weight of the chicks when diets containing small particle (<1mm) calcium sources were fed to the chicks. Chicks fed diets containing eggshell as the main calcium source had the highest final live weight, and the best feed conversion ratio at the starter phase. Particle size and calcium source did not influence the growth performance of the chicks at the grower phase. Birds fed the experimental diets containing large/medium size eggshell as calcium source had the highest calcium and serum alkaline phosphatase in the blood. They were also the first to start laying among the experimental birds.

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