



## Comparative Calcium Carbonate Profiling of Avian Eggshells in Benue State, Nigeria: Analytical Benchmarking and a Circular Biogenic-Calcium Valorization Platform

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**Abstract:** Eggshell is a non-edible by-product with little saleable value and biologically active compounds. Calcium carbonate is the major component of an eggshell and its analysis can be carried out volumetrically by characteristic reaction of carbonate compounds with acids. The eggshells of five different birds (local hen, hybrid hen, turkey, pigeon and duck) were collected domestically and from poultry farms in Gboko local government area of Benue State after hatching. The eggshells sample were thoroughly washed with distilled water to remove any adhering membranes and organic matter, Air-dried, crushed and ground into fine powder stored in clean, airtight containers for further analysis. The  $\text{CaCO}_3$  contents were determined volumetrically using back titration. The concentration of  $\text{CaCO}_3$  in the eggshell followed the sequence hybrid hen > domestic duck > pigeon > turkey > local hen with percentage composition of  $\text{CaCO}_3$  as  $96.34 \% \pm 0.17$ ,  $95.97 \% \pm 0.69$ ,  $91.38 \% \pm 0.79$ ,  $87.45 \% \pm 0.45$  and  $80.54 \% \pm 0.07$  respectively. The weight of  $\text{CaCO}_3$  in the eggshells were 0.42 g, 0.54 g, 0.47 g, 0.50 g and 0.36 g for local hen, hybrid hen, turkey, pigeon and domestic duck respectively. The result indicates a significant difference associated with the species identity, district and their interaction. This indicates that both intrinsic biological factors (such as nesting strategy and species-specific physiology) and extrinsic regional factors (such as calcium availability and habitat conditions) influence eggshell composition in birds.

**Keywords:** eggshell, calcium carbonate, titrimetric, birds, percentage composition

### 1.0 Introduction

Calcium carbonate is a natural occurring chemical substance with the chemical formula  $\text{CaCO}_3$ . It is commonly found in rocks as calcite and aragonite. It also constitutes the main components of pearls, shells of marine organisms such as crabs, snails, and eggs shells. Calcium carbonate can also be extracted by mining or quarrying (Suraj, et al., 2020).

### 1.1 Sources of calcium carbonate

#### Geological sources

Calcium carbonate accounts for more than 4 % of Earth's crust and is found in various forms across the globe. Limestone, chalk and marble are the most common natural sources of  $\text{CaCO}_3$ . These types of rock are formed through the sedimentation of shells and coral over a period of millions of years. Irrespective of its form, calcium carbonate is an extremely versatile material that can be used for a variety of practical purposes. Research has also explored the use of calcium carbonate in carbon capture, sustainable manufacturing, and environmental remediation applications. Figure 1 shows the Calcium Carbonate or rocks.



**Figure 1: Calcium Carbonate rocks (marble and Limestone) (Moslih, et al., 2018)**

### ***Biological sources***

The principal biological sources of calcium carbonate are the eggshells, seashells and snail shells. The shells of Oyster have recently been recognition as a source of dietary calcium, although are also a practical industrial source. Green vegetables such as kale and broccoli are known to contain dietarily significant amounts of calcium carbonate but are not considered as industrial sources of calcium carbonate (Suraj, et al., 2020). Figure 2 shows the chicken eggshells used in the study.



**Figure 2: Chicken Eggshell**

## **1.2 Birds**

Birds are referred to as a group of endothermic vertebrates, characterized by toothless beaked jaws, feathers, laying of hard-shelled eggs, high metabolic rate, a four chambered heart, and a strong and light weight skeleton. The chicken (*Gallus gallus domesticus*) is a type of domesticated fowl, a sub species of the red jungle fowl. It is one of the most common and wide spread domestic animals, with a total population of more than 19 billion as of 2011. There are more chickens than any other bird or domesticated fowl. Humans keep chickens primarily as a source of food and more rarely, as pets (Nimisha, et al., 2020). Chickens and turkeys are ground-dwelling birds from the order *Galliformes*. The genus and species name for chickens is *Gallus gallus domesticus* while turkey is *Meleagris gallopavo*. Ducks are waterfowl from the order *Anseriformes*. The genus and species name for the Pekin duck is *Anas platyrhynchos*, while for the *Muscovy duck* the genus and species name is *Carinia moschata*. Chickens were domesticated approximately 4000 years ago, while Pekin ducks were domesticated approximately 2000 years ago. However, turkeys and Muscovy ducks were domesticated within the last 1000 years and some believe maybe even more recently (Wendy, 2005).

## **1.3 Eggshell**

Eggshell is a non-edible by-product with little saleable value and biologically active compounds. Eggshells are known to contain calcium, magnesium and trace amount of other microelements. Calcium carbonate is the major component of an eggshell and its analysis can be carried out volumetrically by characteristic reaction of carbonate compounds with acids (Mittal, et al., 2016). Eggshell also contain small amount of magnesium and trace amounts of other micro elements. Eggshell calcium is described as the best natural source of calcium and it is about 90 % absorbable than limestone or coral sources (Bonke and Dhiren, 2021). Birds lay hard-shelled eggs which are

incubated in nests and are often highly conspicuous and their colour serving as camouflage. However, the shells porosity allows inflow of oxygen and outflow of carbon dioxide. The shell and the albumen (egg white) protect and cushion the developing embryo. The albumen is also a source of protein and water for the embryo (Nasruddeen and Usman (2013)). Eggshells are agricultural waste and can cause environmental pollution if not properly disposed. Similarly, they can be used in a wide variety of applications. There is increasing interest in eggshells, which are used as adsorbents in the removal of heavy metals and dyes through adsorption.

Shwetha, et al., 2018 compared the calcium contents in the eggshells of different bird and reported its composition as approximately 98.2, 0.9, and 0.9 % calcium carbonate, magnesium and phosphorous (phosphate) respectively, while Chavan and Chondekar, 2025 reported the percentage composition of  $\text{CaCO}_3$  in Red-wattled lapwing as (95.18 %), domestic hen (94.93 %), Baya weaver (93.20 %) and Spotted munia (91.50 %). Nimisha, et al., 2020 reported a relatively low values of calcium carbonate. In their study, chicken variety Aseel (Porukozhi) eggshell recorded 92.90 %  $\text{CaCO}_3$  while Silky's chicken egg shell recorded 86.75 %  $\text{CaCO}_3$ . In duck the highest calcium carbonate concentration was 91 % recorded in the eggshell of Plain duck whereas Patha Tharavu eggshell contained 75 %  $\text{CaCO}_3$ .

## Egg Composition

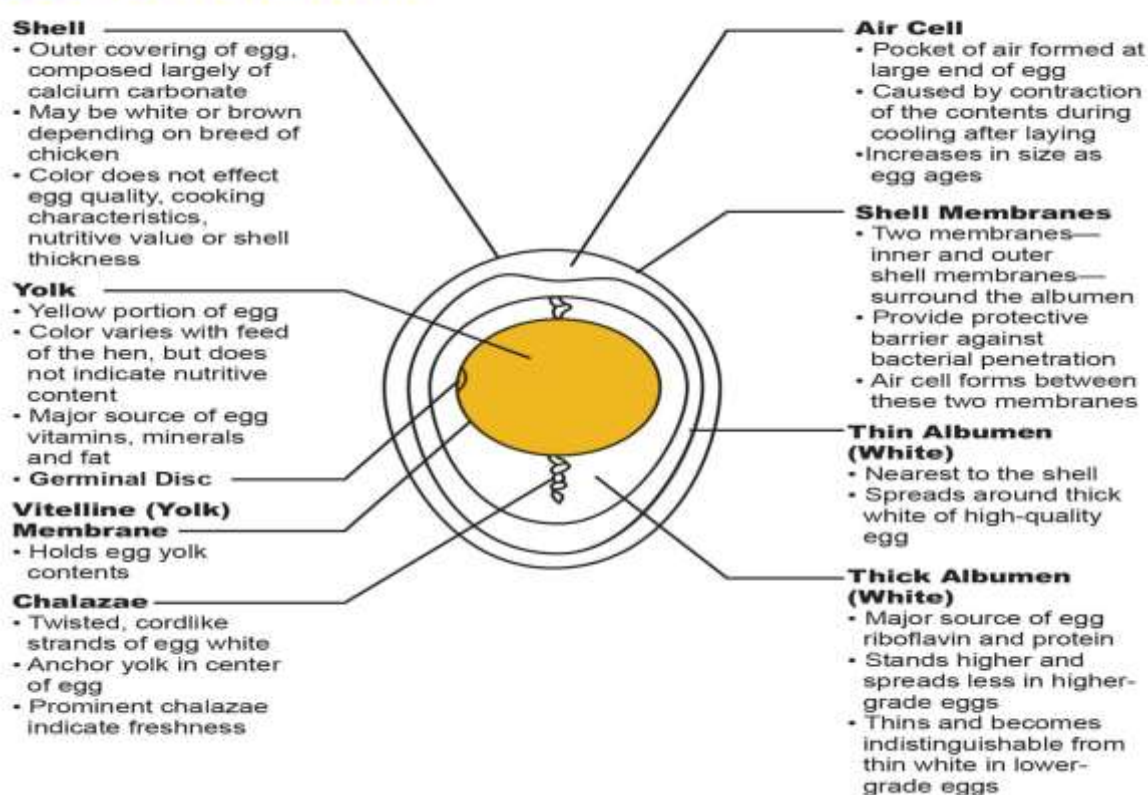


Figure 3: structure of chicken egg showing its composition

Eggshells are the perfect complex bio-mineral superposing functionality, mechanical stiffness, and aesthetic appearance and contains all the necessary requirement by an embryo for safe development. The chicken eggshell is composed mainly of calcium carbonate (95 % w/w), hydroxyapatite crystals (1 % w/w) in the outermost calcified layer of the shell, organic matrix (2 % w/w of the palisade layer), pigments as colorants (0.15-1200 nmol/g) and more than 10 % (v/v) void cavities constituting about 0.4 % (w/w) of water adsorbed (Ostertag, et al., 2019). The surface of the inner shell is composed of two membranes followed and interstitial calcite columns known as mammillary layer that is a regular array of cones, each with a core of concentration of organic matter. The eggshell of the European pond turtle is composed of calcium carbonate in aragonite form, while eggshells of other reptiles and birds contain calcium carbonate in calcite form. When compared to chicken eggs, the ostrich eggs weigh approximately 1.5 kg and 97 % of the ostrich shell is of mineral origin allocated among calcium carbonate (97.4 %), magnesium phosphate (1.9 %), and tricalcium phosphate, TCP (0.7 %). Also, the shell of quail eggs consists of 90 % calcium carbonate with other essential minerals such as copper, iron, potassium, zinc, sodium, calcium, magnesium, sulfur, phosphorus etc. In addition, quail egg weighs about 10 g with variation in eggshell colour from white to blue and green which

have brown or reddish-brown patterned areas on a light background. Similarly, the weight of giant African land snail eggs ranges between 1.54 g to 2.45 g and they are capable of laying 4-18 eggs in 1-2 minutes (Ferreira, et al., 2016). Eggshells are waste materials from hatcheries, homes and fast-food industries (Hanaa and Tajjanah (2026) and can be readily collected in plenty. Eggshell waste disposal contributes to environmental pollution. Challenges associated with disposal of eggshells include cost, availability of disposal sites, odour, flies and abrasiveness (McClelland, et al., 2021). Therefore, the present study focuses on evaluating the percentage content of calcium carbonate content of the eggshells from five different species of birds: local and hybrid hens, turkey, duck and local pigeon using back titration

## 2.0 Materials and Methods

### 2.1 Eggshell sample collection

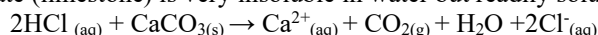
Eggs of local hen and turkey were bought from poultry farms and their shells were collected accordingly, while the shells of hybrid hen, duck and pigeon were collected locally after hatching birds get rid of these shells by dropping them out of the nest (especially the pigeon). These fallen eggshells were collected manually and only naturally broken, hatched, or abandoned shells were used to minimize disturbance to nesting birds and to comply with wildlife conservation ethics. Samples were carefully labelled with species name, collection date. All samples were stored in sterile containers and handled with care to avoid contamination before analysis.

### 2.2 Sample cleaning and preparation

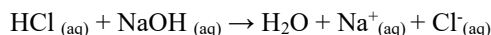
Collected eggshells sample were thoroughly washed with distilled water to remove any adhering membranes and organic matter. Air-drying of the samples was carried out at room temperature for 2-3 days. After drying, the eggshells samples were crushed using a mortar and pestle and further ground into a fine powder to ensure uniformity for analysis. The powdered samples were stored in clean, airtight containers for further analysis.

### 2.3 Determination of Calcium carbonate in Eggshell

Calcium Carbonate (limestone) is very insoluble in water but readily soluble in acid as shown below:



The above reaction cannot be used to titrate against  $\text{CaCO}_3$  directly because it is very slow when the reaction approaches the endpoint. Instead, the desired result was obtained by adding excess HCl acid to dissolve all the  $\text{CaCO}_3$  and then titrate the remaining  $\text{H}_3\text{O}^+$  with NaOH solution to determine the amount of acid which has not reacted with the calcium carbonate (back titration). The difference between amount of the acid (HCl) initially added and the amount left after the reaction was equal to the amount used by the  $\text{CaCO}_3$ . The reaction used to determine the leftover acid is shown below:



#### Procedure for titration

1. The collected eggshells were cleaned with water and dry them in an oven.
2. Mortar and pestle were used to grind the eggshells into a powder.
3. About 0.50 g of the eggshell powder was weighed into a 100 ml flask and the mass of the powder was recorded.
4. Several (2-3) drops of ethanol were added to the content in the flask (this is to enable the HCl to dissolve the eggshell, although it is not part of the reaction).
5. About 10.0 ml of 1.0 M HCl was pipetted into the flask. Then 2-3 drops of Phenolphthalein indicator were added to the solution.
6. The burette was filled to above the 0.0 ml mark with 0.1 M NaOH. The tap of the burette was open to allow the NaOH to drain into a small beaker until the solution was at the 0.0 ml mark.
7. The eggshell sample for each specie was titrated until the solution turns permanent pink seconds. The final volume on the burette was read and record.
8. The amount of  $\text{CaCO}_3$  present in eggshell was calculated using the final burette reading.

## 3.0 Statistical Analysis

All titrations were performed in triplicate for each eggshell sample to ensure the accuracy and reproducibility of the calcium carbonate ( $\text{CaCO}_3$ ) measurements. For each species or sample, the mean and standard deviation (SD) of  $\text{CaCO}_3$  content were calculated based on these replicate titrations.

## 4.0 Results and Discussion

The percentage composition of  $\text{CaCO}_3$  determined the strength of an eggshell and the results for the present study are presented in table 1.

**Table 1: Weight and percentage composition of  $\text{CaCO}_3$  in different eggshell samples**

| Bird species  | Weight of CaCO <sub>3</sub> (g) | Composition of CaCO <sub>3</sub> (%) |
|---------------|---------------------------------|--------------------------------------|
| Local hen     | 0.42±0.01                       | 80.54±0.07                           |
| Hybrid hen    | 0.54±0.04                       | 96.34±0.17                           |
| Turkey        | 0.47±0.04                       | 87.45±0.45                           |
| Pigeon        | 0.50±0.02                       | 91.38±0.79                           |
| Domestic duck | 0.36±0.05                       | 95.97±0.69                           |

The result in figure 4 shows that the percentage of calcium carbonate varied from 96.34 % (hybrid hen) to 80.54 % (local hen) as shown in table 1 while the weight of CaCO<sub>3</sub> in both the hybrid hen and local hen were 0.42 g and 0.54 g respectively. The high value of calcium carbonate in hybrid hen is attributed to hen diet. Similar results have been reported by (Hejdysz, et al., 2024; Nasruddeen, and Usman, 2013). Gautron, et al., 2021, carried out a comparative analysis of calcium carbonate content for selected eggshells of hen, duck and guinea fowl using back titration and their results revealed that Guinea fowl eggshell with brown colour egg was the best sources of calcium compared to duck and hen. The proved that brown eggshells contain more calcium carbonate than white eggshells and the high value was attributed to the difference in hen diet. Hanaa, and Tajjanah, 2026, also reported brown egg to have a higher percentage of calcium (82.25 %) than the white egg (75.82 %).

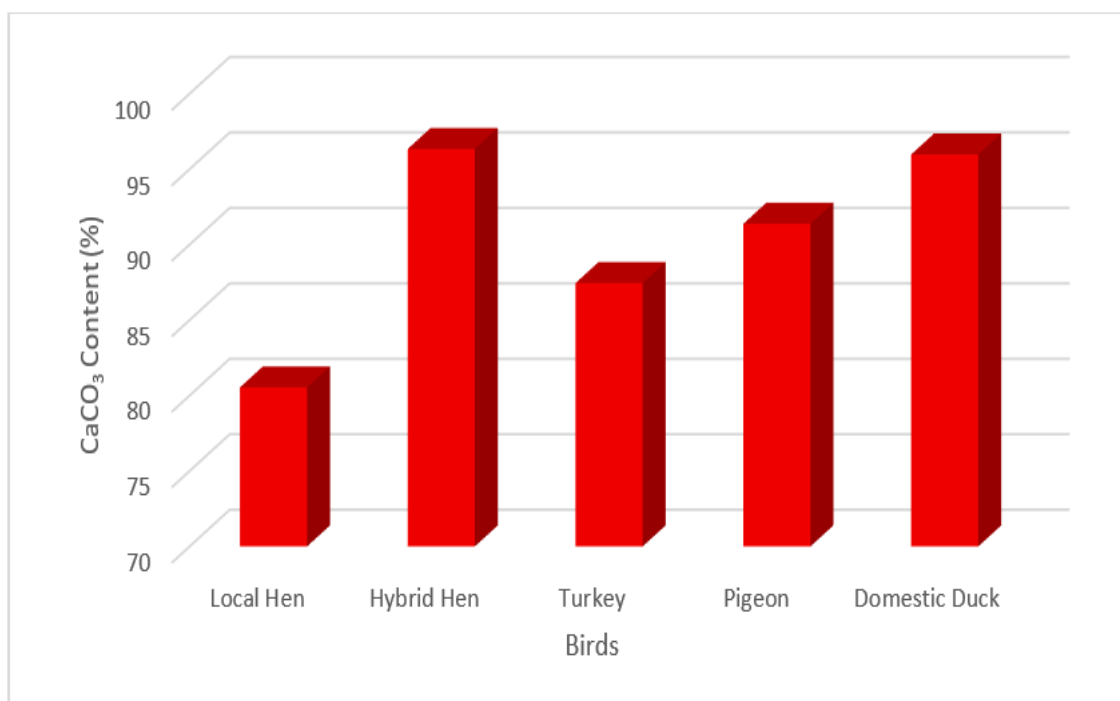
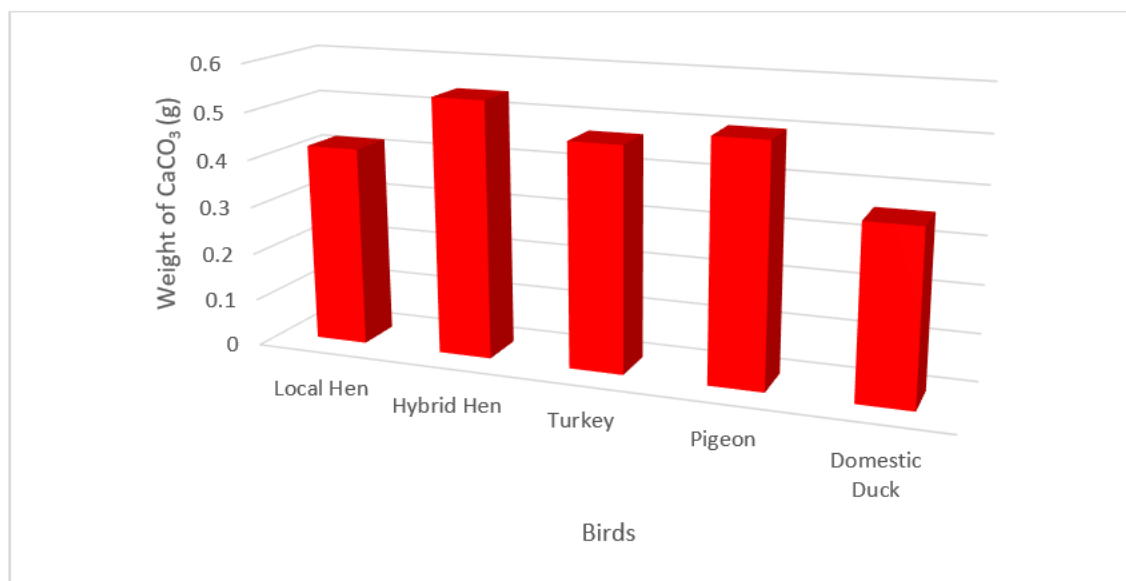


Figure 4: Percentage composition of calcium carbonates in different birds' species



**Figure 5: Weight of calcium carbonate in eggshells of different birds' species**

In figure 5, the percentage composition of CaCO<sub>3</sub> in the eggshells of turkey, pigeon and domestic duck were recorded as 87.45 %±0.45, 91.38 %±0.79 and 95.97 %±0.69 respectively with respective weights of calcium carbonates of 0.47 g±0.04, 0.50 g±0.02 and 0.36 g±0.05. Similar result of calcium carbonate contents in the eggshells of duck was reported by Nimisha, et al., 2020 with average values ranging from 80 %-91 %. Shen and Chen (2003), also reported high concentration of calcium in duck eggshells. Wei Chen, et al., 2015 reported that hen egg shell contains more calcium when compared to duck egg shell. They concluded that dietary calcium deficiency negatively affects eggshell quality and microarchitecture by suppressing shell biomineralization. Shwetha, et al., 2018 also reported similar values for the CaCO<sub>3</sub> contents in the eggshell of domestic and forest pigeon. The value for domestic pigeon was recorded as 87 % while forest pigeon was 91 %.

## 5.0 Conclusion

The result of the analysis reveals significant differences in the composition of calcium carbonate (CaCO<sub>3</sub>) in the eggshells of five different bird species (domestic hen, hybrid he, turkey, pigeon and domestic duck. Among the studies birds, hybrid hen exhibited the highest composition of CaCO<sub>3</sub> (96.34 %±0.17), followed by a similar content of CaCO<sub>3</sub> (95.97 %±0.69) shown by domestic duck and 91.38 %±0.79 was recorded for pigeon. The local hen exhibited the lowest CaCO<sub>3</sub> contents (80.54 %±0.07), while turkey had 87.45 %±0.45 CaCO<sub>3</sub> content. The result indicates a significant difference associated with the species identity, district and their interaction. This indicates that both intrinsic biological factors (such as nesting strategy and species-specific physiology) and extrinsic regional factors (such as calcium availability and habitat conditions) influence eggshell composition in birds. This study also provides important baseline data on eggshell chemistry in birds in Gboko Local Government Area of Benue State and also highlights the usefulness of a simple and cost-effective acid-base back titration method for the comparative studies of eggshell quality.

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## Conflict of Interest

The authors declare no conflict of interest.

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