Quantification of avian eggshells as calcium zones for developing embryos in local (Kerala, India) breeds of chicken, *Gallus gallus domesticus*

Siny G. Benjamin*1, Kurian Mathew Abraham2, Nandini Narayana Pillai Jayaprabha3, Saji K. John4

1Department of Zoology, All Saints College Chackai, Thiruvananthapuram, India
2Department of Aquatic Biology and Fisheries, University of Kerala Kariavattom, Thiruvananthapuram, India
3Department of Zoology, University College Palayam, Thiruvananthapuram, India
4Department of Chemistry, St. Alberts College, Ernakulam, India

**ARTICLE INFO**

Article history:
Received on: January 15, 2021
Accepted on: April 07, 2021
Available online: July 10, 2021

**Key words:**
Calcium, ontogeny, incubation, naked neck, broiler, Vanaraja, Gramapriya

**ABSTRACT**

The growth and production of any animal husbandry including poultry depends on early development, growth, feeding, environmental, and other factors. Early and late embryonic developments form a significant parameter in poultry production, during which human or technological intervention is practically impossible. Similarly, embryonic development is governed by very many internal factors, among which egg calcium utilization is of prime importance. An experiment was set up to investigate the egg calcium utilization of different poultry breeds during its embryonic development. Four breeds of locally (Kerala, India) available chickens (*Gallus gallus domesticus*), Broiler, Naked neck, Vanaraja, and Gramapriya breeds, were selected and embryonic egg utilization was assessed by comparing calcium content of eggshells before and after hatching and the results showed that layer breeds, Vanaraja, utilizes maximum (0.20 g) and significantly (*p* < 0.01) higher quantity eggshell calcium for embryonic development, followed by Naked neck breed (0.16 g); the least used variety was Broiler breed (0.09 g). Eggshell calcium utilization in different breeds is discussed in relation to its ontogenic breed properties like broiler and layer, i.e., production of meat and eggs, respectively, as the literature on ontogenic calcium utilization of breeds is meager.

**1. INTRODUCTION**

Poultry is perhaps one of the most easily available and preferred protein sources for human consumption, which is constantly in demand worldwide, and forms a trade industry across continents. The rapid growth in the demand for livestock products, especially from developing countries known as the ‘livestock revolution’, is being met, at least in part, by the rapid expansion in the production of poultry meat [1]. Poultry production enhancement at every level, from its embryonic development to its grow out stages, has been an endless effort at its academic and industrial research, of which embryonic development warrants utmost importance as early growth plays a pivotal role in later growth and production. The development of avian embryos or oviparous embryos depends on the nutrients in egg yolk and calcium primarily utilized from the shell, especially during the later stage of development [2,3]. The nutrient intake by developing embryos from yolk, eggshells, or other parts attracted the attention of evolutionists as the ontogenic development has influenced the phenotypic characteristics [3], medical researchers [4], and developmental study researchers [5–7].

Eggshell consistency is one of the most important factors affecting the poultry industry; it affects egg production and hatchability in an economic sense [8]. The components of eggshell are a matrix of protein fibers and columns of calcium carbonate in the proportion 1:50 [9]. The acid produced by the cells of the chorioallantoic membrane facilitates the dissolution of minerals from the shell [10]. The eggshell supplies 98.2% of calcium to the developing embryo along with magnesium (0.9%) and phosphorus (0.9%) [9], in addition to copper, zinc, and iron [10]. Hence, eggshell

*Corresponding Author
Siny G Benjamin, Department of Zoology, All Saints’ College Chackai, Thiruvananthapuram, Kerala. E-mail: sinyben@gmail.com

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is an important source of calcium for embryonic development and different species and/or races uses eggshells differently during embryonic development and grows differently [6]. The hatchability and effective completion of embryonic development depend on quality characteristics of eggshell membranes [11]. Multiple factors affect the eggshell strength, such as diet [12,13], genotype [14,15], egg shape [16], flock age [17], and housing system [18,19].

The study aims to astutely analyze and establish the crucial role of calcium content in the development and sustenance of the embryo. The primary objective is to arrive at an accurate estimate of post-hatch embryonic egg utilization by exhaustive study and subsequent analysis. Moreover, the breed difference in egg calcium utilization warrants the utmost importance as evident from its breed properties in the production of meat and eggs. Layer breeds and broiler breeds may differ in embryonic development as layers may utilize more calcium during its ontogeny [2,3] and requires more calcium content in food for brooders in general and layer brooders in particular [6]. Four locally available varieties of chicken, Broiler, Naked neck, Vanaraja, and Gramapriya breeds were selected for the present study. The objective of the study is to find the eggshell calcium utilization through absorption by the developing embryo and to analyze the importance of calcium in eggshells. The ontogenic characteristics can be promoted to make the layer breeds more productive practically by incorporating adequate calcium content in the feeding regimen. It was pertinent to assert and establish the suitability of the specimens selected for the study and the researchers took ample measures to ensure that stringent standards were followed so as not to compromise the viability of the findings.

2. MATERIALS AND METHODS

Hatchery certified, disease-free, and fertilization-confirmed eggs of different breeds of chicken (Gallus gallus domesticus; Phasianidae, Galliformes) were obtained from different government and private hatcheries in and around Thiruvananthapuram district of Kerala state, India. Two to three-day-old fertilized eggs (n = 10) of selected breeds, viz, Broiler, Naked neck, Vanaraja, and Gramapriya breeds, were placed in a laboratory incubator at the same time. They were incubated for the stipulated time of 21 days and they were rotated twice daily at a constant temperature of 35°C. Pre- and post-incubation eggshells of all local breeds were used for calcium content analysis (Fig. 1). The experiments were triplicated for consistent results.

Permanganometric method [20,21], followed by flame photometric confirmation [22–24] estimation, was used for the estimation of calcium (g/g shell) in pre- and post-hatching eggshells of selected chicken breeds. The difference between pre- and post-incubation and/or hatching calcium content was regarded as utilization during embryonic development and was subjected to a parametric two-way repeated measures analysis of variance (ANOVA) test for comparison of breeds and pre- and post-incubation calcium content [25]. The methods were scrupulously adhered to and the analysis was carried out with utmost precision to facilitate accuracy of data.

3. RESULTS AND DISCUSSION

Avian embryonic development in eggs is governed by many internal factors, among which egg calcium supply is of prime importance. An experiment was set up to investigate the egg

![Figure 1: Eggshells of four breeds of poultry used for the study.](image-url)
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Table 1: ANOVA (two-way, repeated measures ANOVA) of calcium content (g/g shell) comparing breeds and incubation period.

| Breed eggs | Pre-incubation | Post-incubation |
|------------|----------------|-----------------|
|            | Mean ± SD      | Mean ± SD       |
| Broiler    | 0.28 ± 0.008   | 0.19 ± 0.003    |
| Naked neck | 0.58 ± 0.013   | 0.42 ± 0.019    |
| Vanaraja   | 0.72 ± 0.007   | 0.52 ± 0.008    |
| Gramapriya | 0.93 ± 0.012   | 0.79 ± 0.009    |

| Source                  | Type III sum of squares | df | Mean square | F       |
|-------------------------|-------------------------|----|-------------|---------|
| Corrected model         | 1.81                    | 7.00 | 0.258      | 2,241.397** |
| Intercept               | 9.40                    | 1.00 | 9.401      | 81,669.105** |
| Incubation              | 0.17                    | 1.00 | 0.17       | 1,478.756** |
| Breed                   | 1.61                    | 3.00 | 0.538      | 4,669.812**|
| Incubation × Breed      | 0.01                    | 3.00 | 0.0044     | 38.205**  |
| Total                   | 11.267                  | 31   |             |         |

Figure 2: Mean embryonic eggshell calcium (g/g shell) utilization (pre- and post-incubation difference) in different chicken breeds.

Calcium utilization of different poultry breeds at its embryonic development. Four breeds of locally available chickens (*G. domesticus*), Broiler, Naked neck, Vanaraja, and Gramapriya breeds, were selected and embryonic egg utilization was assessed by comparing the calcium content of eggshells before and after hatching and it was found that the Vanaraja (0.20 g) breed utilized the maximum and significantly (*p* < 0.01) higher quantity eggshell calcium for embryonic development, followed by the Naked neck (0.16 g) breed; the least used variety was Broiler (0.09 g) breed (Table 1).

Various factors determine the amount of calcium in shells prior to laying like the time the egg remains in the shell gland (uterus) and the time at which egg is laid. Calcium content analysis was carried out in the eggshells during the pre-incubatory phase and post-incubatory phase after thorough cleaning of shells to remove the visible remnants. About 100 eggshells were used in the study. The pre-incubatory calcium content is recorded to be high in the Gramapriya breed when compared to the other three varieties selected for the study.

Post-incubatory eggshell calcium analysis also showed increased quantity in the Gramapriya breed in comparison to the Broiler, Naked neck, and Vanaraja breeds. All the four breeds of chicken are of significant importance in the poultry industry. The embryonic utilization for development induced the reduced amount of calcium in the post-incubatory phase.

The calcium content of eggshells of each hen breed under consideration differ among each other significantly. Since there was no standard or control eggshells, the calcium utilization during embryonic development can be considered as the difference between pre- and post-incubation calcium content. Table 1 shows the mean calcium (g/g shell) content of pre- and post-incubation of four different breeds in comparison with repeated measures ANOVA.

Pre-incubation calcium content was recorded as maximum in Gramapriya breed, followed by Vanaraja, Naked neck, and the least was in Broiler breed. The post-incubation eggshell calcium content also recorded the same pattern, with maximum in the Gramapriya breed. But shell calcium utilization (Fig. 2) assessed in terms of difference between pre- and post-incubation shell calcium content was maximum in the Vanaraja breed, followed by naked neck, Gramapriya breed, and the least was in Broiler breed. The difference in both, different egg breeds and pre- and post-incubation calcium content, was found to be statistically significant (*p* < 0.001) with two-way ANOVA. The findings of the study cogently outline the embryonic utilization of shell calcium in various poultry breeds and reiterate the significance of such a study.

The poultry production industry has often been embroiled in controversy as the unchecked use of hormones and similar food additives raise serious ethical and scientific concerns. Such concerns have to be addressed with renewed urgency and restorative measures adopted to combat further hurdles. Eggshell use for embryonic development by different species as well as breeds with in a species contributes much to early development of quality poultry stock. The deposition of calcium in the eggshell occurs during the movement of egg through the oviduct. The protein matrix in the shell stabilizes the minerals in it, of which...
95%–97% is calcium. The organic matrix assists in the deposition of calcium during mineralization process [20]. The peculiarity in the structure and composition of avian eggshell helps protect the egg against damage and microbial contamination, desiccation, regulation of gas, water exchange for the growing embryo, and provides calcium for embryogenesis [26]. The amount of calcium in eggshell varies with the species and diet patterns of hen and habitat preference [27].

Nutrient absorption, metabolism, deposition, and early and late development vary with genetics [28]. Mild variations in the calcium content of egg will be fatal to the embryo during incubation [29]. Nutrient deficiencies in hen’s diet result in the increased mortality of embryos during the second week of incubation [30]. Nutrient deficient diets not only affect embryonic development but also egg production by the hen. Eggshell quality depends directly on the calcium content of the total feed and particle size of the calcium incorporated in it [31–33] and studies on supplementary diet formulations stresses the importance in various combinations [34].

The standard mineral content of the eggshell is estimated by experiments and this can be effectively utilized for designing proportionate quantities of minerals in the supplement diets for hen. The proportionate quantity of calcium that is utilized during embryonic development is dependent on the amount of calcium that is present in the eggshell [35]. The trace deficiencies of minerals will deleteriously affect the viability of the embryos and in some cases may also be fatal [35]. Increased stress conditions eventually affect the mineral content of eggshell [36], which may affect hatchability as well as further growth of the chick.

The hen’s diet is usually supplemented with energy and nutrients that enhance the egg production, while mineral content of the shell is not targeted. The chorioallantoic circulation which is responsible for transporting minerals to eggshell from shell is established only after 14 days of incubation. Phosphorus which is a yolk phosvitin origin combines with the calcium in the eggshell during bone mineralization of chick embryos [6]. The decreased calcium during mineralization with the matrix will make the egg brittle. Diet supplementation by additives should also target the mineral content of eggshell. Providing hens with calcium diets helps the ingested calcium to stay longer in the gizzard. This helps hen to spare medullary bone reserves. Excessive drawing up on hen’s medullary bone reserves results in the weakening of both hens and eggs. Indeed, a fractured or broken egg that has been fertilized will not lead to chick birth. A close perusal of the diverse factors concerning eggshell utilization thus leads the researcher to formulate the decisive role played by calcium content in embryonic health and later development.

4. SUMMARY AND CONCLUSION

The difference in eggshell calcium utilization by developing embryos of different local (Kerala, India) breeds of poultry showed significantly high calcium absorption in the Vanaraja breed, followed by the Naked neck breed, which are layer varieties, and broiler varieties consumes very little eggshell calcium during its ontogeny, which represented and confirmed the breed properties of chicken. Hence, layer varieties of poultry brooders should be fed with a high content calcium diet for better hatchability and growth cum production characteristics.

5. ACKNOWLEDGMENTS

The first author acknowledges the Manager and Principal, All Saints College, Thiruvananthapuram, for support and facilities. Thanks are due to farm supervisors for providing eggs for the experimental analysis.

6. DISCLAIMER/DISCLOSURE

The authors declare and disclose that this article in its draft format has been uploaded to the pre-print servers (doi: https://doi.org/10.21203/rs.3.rs-20972/v1) and the (draft) pre-prints are available in the site/servers.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

8. DATA AVAILABILITY

All data generated or analyzed during this study are included in this article.

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How to cite this article:
Benjamin SG, Abraham KM, Jayaprabha NNP, John SK. Quantification of avian eggshells as calcium zones for developing embryos in local (Kerala, India) breeds of chicken, Gallus gallus domesticus. J Appl Biol Biotech 2021; 9(04):151–155.