Efficacy of tertiary egg membranes as calcium zones for embryonic development in poultry breeds

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Research

Keywords: Egg Shell utilization, Gallus gallus, Broiler, Vanaraja, Gramapriya Chicken

Posted Date: April 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-20972/v1

Abstract

Background Growth and production of any animal husbandry including poultry depends on early development, growth, feeding, environmental and other factors. Early and later embryonic development forms a significant parameter in poultry production, during which human or technological intervention is practically impossible.

Results 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 at its embryonic development. Four breeds of locally available chicken (Gallus gallus domesticus), Broiler, Common domestic variety (Nadan), Vanaraja and Gramapriya breeds were selected and embryonic egg utilization was assessed by comparing calcium content of egg shell before and after hatching and found out that Vanaraja breed utilizes maximum (0.20gm) and significantly ($P < 0.01$) higher quantity egg shell calcium for embryonic development followed by Local breed (0.16gm) and least used variety was Broiler breed (0.09gm).

Conclusions The study highlighted the criticality of the composition of the specific mineral in the calcium zones for embryonic utilization underlining the importance of its only source through food.

Background

Poultry is perhaps one of the most easily available preferred protein source for human consumption. It is also a food source that is constantly in demand. Poultry production enhancement at every level from its embryonic development to 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 depend on the nutrients in egg yolk and calcium primarily utilized from the shell [1]. The components of egg shell are a matrix of protein fibres and columns of calcium carbonate in the proportion 1:50 [2]. The acid produced by the cells of the chorioallantoic membrane facilitate the dissolution of minerals from the shell [3]. The egg shell supply 98.2% of calcium to the developing embryo along with magnesium (0.9%) and phosphorus (0.9%) [2] in addition to copper, zinc and iron [4]. Hence egg shell is an important source of calcium for embryonic development and different species and/or races uses egg shell differently during embryonic development and grows differently. The study aims to astutely analyse 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. Four locally available varieties of chicken Broiler, Common domestic variety, Vanaraja and Gramapriya breeds have been taken up for the present study. 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

Methods

Hatchery certified, disease free and fertilization confirmed eggs of different breeds of hen (*Gallus gallus domesticus*; Phasianidae, Galliformes) were obtained from different government and private hatcheries in and around Thiruvananthapuram, Kerala. Two to three days old fertilized eggs (n = 5) of selected breeds, *viz*, Broiler breed, Local breed, Vanaraja breed and Giriraja breed were placed in laboratory incubator at the same time. They were incubated for the stipulated time of twenty one days and they were rotated twice daily in constant temperature of 35^0C.

Permanganometric method [5, 6] was adopted for the estimation of calcium (gm/gm shell) in pre and post hatching egg shells of all breeds. The difference between pre- and post incubation and/or hatching calcium content was regarded as utilization during embryonic development and was subjected to parametric two way repeated measure ANOVA [7] test for comparison for breeds and pre- and post incubation calcium content. The methods were scrupulously adhered to and the analysis was done with utmost precision to facilitate accuracy of data.

Results

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 is done in the egg shells during pre incubatory phase and post incubatory phase after thorough cleaning of shells to remove the visible remnants. About one hundred egg shells were used in the study. The pre incubatory calcium content is recorded to be high in Gramapriya breed when compared to other three varieties selected for the study.

Fig 1 Here

Fig 2 Here

Post incubatory egg shell calcium analysis also showed increased quantity in Gramapriya in comparison with Broiler, Native/Local and Vanaraja. 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 egg shells of each hen breed under consideration differ each other significantly. Since there was no standard or control egg shell, 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 (gm/gm shell) content of pre- and post incubation of four different breeds with comparison with repeated measure ANOVA.

Table 1 Here

Figure 3 Here

Pre-incubation calcium content was registered maximum in Gramapriya breed followed by Vanaraja, Local and least in Broiler breed (Fig 1). The post-incubation egg shell calcium content also registered the same pattern, with maximum in Gramapriya breed (Fig 2). But shell calcium utilization (Fig.3) assessed in terms of difference between pre- and post incubation shell calcium content was maximum in Vanaraja breed followed by local breed, gramapriya breed and least 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

Discussion

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 egg shell 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 assist in the deposition of calcium during mineralization process [4]. The peculiarity in the structure and composition of avian eggshell help protect the egg against damage and microbial contamination, desiccation, regulation of gas, water exchange for the growing embryo and provides calcium for embryogenesis [2]. The amount of calcium in egg shell varies with the species and diet patterns of hen and habitat preference [8].

Nutrient absorption, metabolism, deposition and early as well as late development vary with genetics [9]. Mild variations in the calcium content of egg will be fatal to the embryo during incubation [10]. Nutrient

deficiencies in hen's diet result in the increased mortality of embryos during second week of incubation [11]. Nutrient deficient diets not only affect embryonic development but also egg production by the hen.

The standard mineral content of the egg shell 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 egg shell [12]. The trace deficiencies of minerals will deleteriously affect the viability of the embryos and in some cases may also be fatal [13]. Increased stress conditions eventually affect the mineral content of egg shell [14].

The hen's diet is usually supplemented with energy and nutrients that enhances the egg production while mineral content of the shell is not targeted. The chorioallantoic circulation which is responsible for transporting minerals to embryo from shell is established only after 14 days of incubation. Phosphorus which is a yolk phosvitin origin combines with the calcium in the egg shell during bone mineralization of chick embryos. Mineral loss from the egg shell also result from the increased metabolism with the enzymatic actions in the presence of carbonic acid. The decreased calcium during mineralization with the matrix will make the egg brittle. Diet supplementation by additives should also target the mineral content of egg shell. Providing hens with calcium diets help 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 egg shell utilization thus leads the researcher to formulate the decisive role played by calcium content in embryonic health and later development.

References

1. Johnston PM & Comar CL. Distribution and contribution of calcium from the albumen, yolk and shell to the developing chick embryo. Amr J of Physiol. 1955; 183(3): 365-370
2. Romanoff AL & Romanoff A. The avian egg. New York: John Wiley & Sons; 1949
3. Leeson T, Leeson C. The chorioallantoic of the chick. Light and electron microscopic observations at various times of incubation. J of Anat. 1963;97: 585-595
4. Richards MP, Steele NC. Trace element metabolism in the developing avian embryo: A review. J of Exp Zool. 1987;S1: 39-51
5. Kramer B, Tisdall FF. A simple technique for the determination of calcium and magnesium in small amounts of serum. J of Biol Chem.1921; 475-481
6. Clark EP, Collip JB. A study of Kramer-Tisdall method for determination of calcium with suggested modifications. J of Biol and Chem,1925;63:461-464
7. Zar JH. Biostatistical analysis. Prentice Hall Englewood chiffs, NJ;1984.
8. Shwetha A, Dhananjaya, Shravana Kumara SM, Ananda. Comparative study on calcium content in egg shells of different birds. Int J of Zool Stud.2018;3: 31-33

9. Lillie RJ, Olsen MW, Bird HR. Variation in reproductive response of hens to dietary deficiency. Poul Sci.1951;30: 92-97
10. Wilson HR. Effects of maternal nutrition on hatchability. Poul Sci, 1997;76: 134-143
11. Leeson S, Reinhart BS, Summers JD. Response of White Leghorn and Rhode Island Red breeder hens to dietary deficiencies of synthetic vitamins. Canad J of Ani Scie.1979;59: 569-575
12. Naber EC. The effect of nutrition on the composition of eggs. Poul Sci. 1979; 58: 518-528
13. Savage JE. Trace minerals and avian reproduction. Federation Proceedings. 1968; 27: 927-931
14. Lin YFT, Hsiu-Ling, L, Yi-Chun C. Maternal vitamin E supplementation affects the antioxidant capability and oxidative status of hatching chicks. J of Nutr.2005;135: 2457-2461

Declarations

Ethics approval and consent to participate:

No animals were killed or captured during the study period. Only egg shells were used for analysis of mineral content.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable

Funding

Not applicable

Acknowledgement

First author acknowledge the support and facilities provided by the Manager and Principal, All Saints College, Thiruvananthapuram and Saji John K, St. Albert's College, Ernakulam for the biochemical analysis. We thank the farm supervisors for providing eggs during the experimental analysis.

Table 1

Table 1. Analysis of variance (Two Way Repeated Measure ANOVA) of calcium content (gm/gm shell) comparing breed eggs and incubation period.

Breed Eggs	Pre Incubation		Post Incubation	
	Mean	± SD	Mean	± SD
Broiler	0.28	0.008	0.19	0.003
Native	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	2241.397**
Intercept	9.40	1.00	9.401	81669.105**
Incubation	0.17	1.00	0.17	1478.756**
BREED	1.61	3.00	0.538	4669.812**
Incubation × BREED	0.01	3.00	0.0044	38.205**
Total	11.267	31		
Corrected Total	1.809	30		

Figures

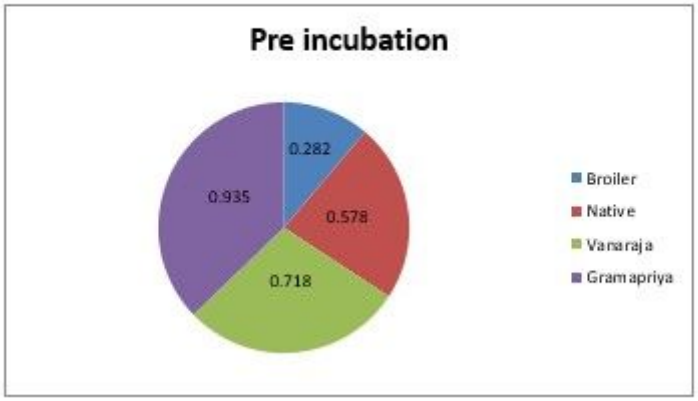

Figure 1

Calcium content (in gms) in different breeds before hatching

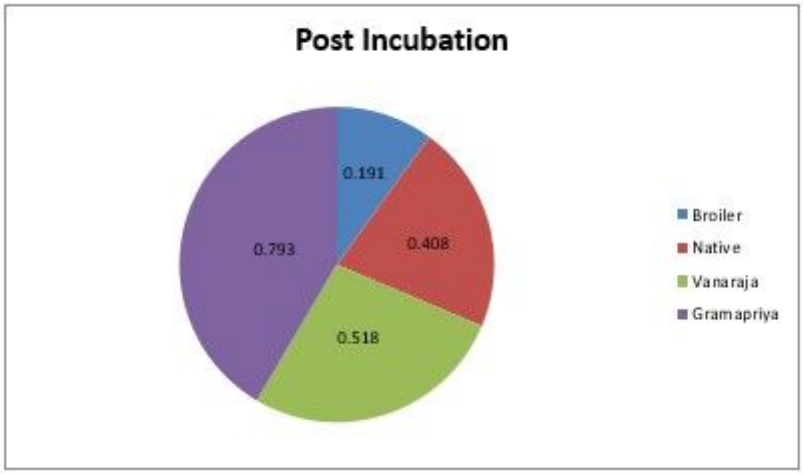

Figure 2

Calcium content (in gms) in different breeds after hatching

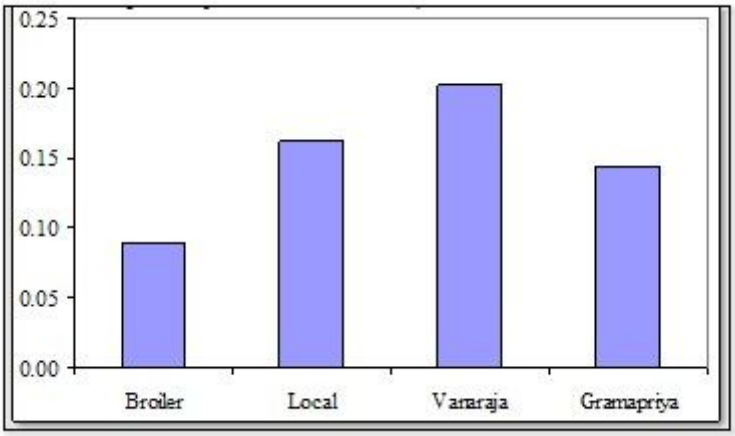

Figure 3

Mean embryonic egg shell calcium (gm/gm shell) utilization (pre- and post incubation difference) in different hen breeds