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Research Article

Analysis of Calcium content and surface profile study of Chicken (*Gallus gallus domesticus*) egg shell

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ABSTRACT

Avian egg shell is a hard mineralized structure that covers the egg contents and provides protection to the growing embryo .The surface of the egg shell of chicken examined for the presence of proteins ,amylase and pores on it revealed the presence of proteins as scattered clusters as well the presence of amylase and the pores of different sizes . The ninhydrin test conducted produced Rheumann purple colour characteristic to protein content on the shell surface in scattered clusters but not as a continuous sheet and a slight modification in photography technique exhibited pore profile with a clarity .Iodine test gave the clue for the presence of amylase that may play a role in the digestion of starch on the egg shell surface, the starch could form a base for the growth of microbes but its digestion by amylase inhibits the microbial growth .The calcium content of the egg shell has been analysed and its utility has been discussed with a suggestion .The ninhydrin staining added a new dimension to egg shell proteins study. In this study of egg shell surface most simple technique used has revealed surface organization with a clarity distinct from the earlier works.

Key words: Shell pores, surface proteins, ninhydrin staining and iodine test.

INTRODUCTION

Avian egg shell is highly organized and mineralized structure essential for protection of the growing embryo and propagation of bird species. The egg shell protects the contents and embryo from microbes and physical environment. Regulates exchange of gases as well provides calcium for the embryonic growth. All avian egg shells have a common mineral content Calcium Carbonate in the form of calcites. The egg shell does not have any type of cells in it. The shell thickness, whole size, shell pores vary from species to species. All these properties are possible because shell is a ceramic material displaying texture gradient.

The shell also has many types of pigment markings in different species of birds, referred to as maculation's. Which serve different functions as reviewed in detail by GoloMaurer et al[1,2], these markings are due to pigments like Porphyrin and Biliviridin derived from haem. In addition to these, there are pores undoubtedly present in all the types egg species of varying sizes that could be viewed by staining. While observing the shell, addition to pores we found several dot like stained regions on the chicken egg shell. The egg shell color pigment was generally white. In this study we discuss the appearance of colored dots on dented like surface of the egg shell of chicken, as well the total Calcium content in this egg shell.

MATERIAL AND METHOD

The chicken eggs were procured from reliable sources were posed to analysis. Egg shells were separated from the egg content by breaking. The broken shells surface were stained using Giemsa stain, and also using Ninhydrin and Starch indicator. The results obtained by staining procedure were

photographed. The calcium content of the egg shell was determined by standard titometric method. The procedure followed in this study are well established and well practiced [26].
Giemsa staining- 2% of Giemsa stain diluted in water was taken, the surface of the egg stained by dipping and allowed to dry to observe dots and pores.

Ninhydrin Test- about 10 ml of 2% Ninhydrin solution was taken in a petridish, warmed and the outer surface of the egg shell was dipped in the Ninhydrin solution and allowed to dry for the observation of dots and pores.

Iodine Test: Starch – 0.5% solution warmed, the outer surface of the egg was dipped and treated with iodine initially allowed for 2-3 minutes, and later the same shell surface was observed to see the reaction and photographs were taken.

Titrimetry: The egg was boiled and allowed to cool. The egg shell was taken off and the attached membrane was removed. The egg shell dried and allowed to cool, later ground to fine powder. 0.2 gms of this powder transferred to a conical flask. 25 ml of Hydrochloric Acid solution and 5ml of Ethanol were added. This mixture was boiled for 15 minutes and cooled. A few drops of Phenopthale in indicator added to this as an indicator and titrated against NaOH solution until a pink color appeared. The titration was repeated to get concordant value, then the calculations were done to find out percentage of calcium.
The Giemsa stained shell was photographed under Stereomicroscope, the Ninhydrin and Iodine treated shells were photographed using mobile phone camera (13pixel) attached with a macro lens, exposed keeping against the light source obtained superior picture than stereo micrographs.

RESULTS

The titrometric analysis calcium content of the chicken egg shell yielded reliable results with slight or negligible difference between the titre values .The egg shell calcium content analyzed was about 42% to 44.80% in poultry chicken *Gallus gallus domesticus. (table.1)*
The unstained and stained shell surface revealed the presence of pores and dots of variable sizes (Fig 1-a) and giemsa stained egg shell revealed the presence of pores and dots of variable size as scattered in the form round blue structure and blue small dots those were different from pores in size and shape (fig 1-b).where as the non stained shell surface the pores appeared as circular depressions but the dots were comparatively small dent like structures scattered on the shell surface .
The Ninhydrin stained egg shell surface (Fig 2) revealed two types of pores, small and large scattered on the surface viewed with brightly illuminated background. But numerous dotted structure were positively stained bearing colour characteristic to Ninhydrin reaction, indicating these depressions are store house of proteins that react with Ninhydrin, but pores with intact shell membrane did not show positive reaction with Ninhydrin indicating the absence of nihydrin positive proteins on the shell membrane.
The Iodine test done for the shell (Fig 3 a & b), revealed that the shell surface reacted grade wise to begin with, the starch solution smeared and Iodine treated egg shell surface was bluish, after two minutes the same appeared bearing only dilute Iodine color, indicated the surface may be having Amylase enzyme.

DISCUSSION

Egg shell is a semipermeable structure formed of calcium carbonate encloses the pores of the shell and it has a coating referred to bloom or cuticle to keep out bacteria. A typical hen's egg shell contains about 6500 pores with the greatest concentration of pores at blunt end of the shell over the air cell or chamber[3]. The egg shell on inside is supported by shell membrane that separates the albumen and the shell proper. The stony shell membrane is partly formed by Keratin. The shell contains different size pores that could be measured by using different techniques, such as mercury porosimetry[4]. Reduction in pore area has adaptive significance. The pore size reduces with the increase in the altitude to avoid excessive dehydration[5]. The egg cuticle has compositional gradation with its outer part rich in proteins and inner part rich in sulphated polysaccharides and phosphates, and changes in this content composition depends on age of the bird and freshness of the egg. The cuticle that coats the outer egg shell surface plugs the entry to the shell pores[6]. This cuticle composed of Protein 90% mainly glycoproteins, polysaccharides (4%), and lipids (3.5%) rich in phosphorous [7]. cuticle limits movement of particles, water and bacteria through its pore[8]. Eggs with damaged cuticle are more susceptible for bacterial infection [9], thus act as natural barriers. Egg shell waste is used as solid catalyst for bio diesel production[10], for cellulase enzyme production by using Neurospora[11], for production of bio manure[12], used as template for synthesis of Nano rod arrays on carbon fibre for energy storage [13] for harvesting micro algae [14],what makes all these possible may be

the shell surface content and shell architecture ,the protein clusters detected through ninhydrin staining may throw a light on the functional patterns of these by future analysis .

Infection is the main source of mortality for avian embryos but eggs themselves provide a net work of anti microbial defenses. The egg shell cuticle is composed of nanometere sized calcite spheres. It is experimentally proved that the modified egg shells were significantly hydrophobic and better at anti microbial activity as examined in compost nesting birds .Although egg shell presents a considerable barrier to microbial passage it is permeated by thousands of pores[15], this quality of egg shell is of great interest that how even with shell permeability microbial infection is avoided.

Thus egg shell quality and integrity are of high significance to the egg contents and to the market. Glycosylation of the cuticle is highly essential because it increases the mechanical property of the cuticle, lower glycosylation leads to lower quality glycoprotein which increases microbial penetration [16].It is observed by Nyset,al[17] that changes in the uterine fluid constituents with the stages of egg calcification affect the morphology of calcites, egg shell structure and mechanical properties related to control of eggshell fabric .Addition to these there are many factors that affect the thickness of the egg shell such as estrogen and carbonic anhydrase[18].

To examine the topography of the egg shell we used different technique that yielded some topographic picture in different profiles. The Giemsa stained profile yielded large blue circles representing the pores of variable sizes filled with giemsa and blue dotted structure that represented protein. But Ninhydrin staining mechanism applied to study the shell surface yielded a very clear picture of pores and chromatophores. Marie. p et al [19] have used quantitative proteomics to study stages of egg shell mineralization in different stages of shell development and found that 216 types of shell matrix proteins found at four different stagesof development and a few of these proteins were characterized by bio informatics that included surface proteins too. The egg shell cuticle formed mainly of glycoproteins reacted positively with Ninhydrin in the form of purple dots scattered all over the surface of the shell. The pores of varying size appeared clear white boundaries when photographed against the light. The shell membrane supported the pores below remained unreacted to Ninhydrin detects the nature of protein composition of the shell membrane. This staining procedure is suitable for differentiating the protein type dispersal on the egg shell surface. Ninhydrin reagent is majorly used in detection of amino acids and proteins in liquid forms that gives chromogenic reactions at P^H 5.6. The Ninhydrin reacts with primary amino group to produce a characteristic purple color referred to Rheumann purple. This reagent also reacts with imines, indole ring, sulfhydryl group, amino groups and cyanide ions to produce different chromatophores of many interests indicated either one or many of these characters were to be there in the egg shell surface proteins reffered in this study [20].

Egg shell of poultry or domestic chicken has been considered to be an alternate source of calcium for industrial use. The other procedure of experimentation with iodine revealed the presence of amylase on the surface of the egg shell. The shell treated with starch and with the immediate treatment of iodine resulted in bluish color that after 2-3 minutes the blue color disappeared because of total digestion of starch, only iodine color on the shell indicate amylase activity, presence of amylase generally block the accumulation of starch by its digesting activity, otherwise which might help in the growth of fungus and microbes on the surface, thus making the shell surface contents naturally disinfectants. The procedures Ninhydrin staining and starch iodine treatment that we used to analyse egg shell surface will add a new dimension to the technique.

Shells form about 10% of the egg weight, it is formed of calcium carbonate along with other minor elements. The calcium is organized as calcite crystals that form ceramic structure to organized egg shell. On an average White Leg Horn lays about 320 eggs in an year of 60 gms average weight for a total of 19.2 Kg. thus produce 10% of this weight of egg shell having 1.92 Kg weight more than the body weight of hen in a year. Egg shell powder is used in poultry feeds as a substitute for calcium, in India, that works much cheaper than the shell grit and limestone. The effect ,if any, in correlation to body weight of the layers ,egg production ,egg weight ,breaking strength ,the shell thickness had not shown significant differences with the traditional calcium sources as reviewed by Yashothai and Kavithaa[21] but some chemicals like sodium chloride have adverse effect on the eggshell quality when got in through the drinking water[22]. A few workers have experimented with the improving of shell quality and strength by adding herbal extracts in addition to calcium and phosphorus and got positive results regard to improved egg and egg shell quality[23], but these have not examined whether there were changes in the distribution of surface proteins clustered appearance

as recorded by us or were there any changes in pores as the egg shell is formed of three layers containing large number of pores on it for permeability of gases . The utility of the egg shells have been argued and recommended good material for bulk inexpensive, light weight application such as automotive industry, trucks, homes, offices and factories, including as filters in cosmetic industries, feed fertilizers, paper printing ink, pharmaceuticals and starting material for dielectrics and biocatalysts (cross findings): N.Tangbooriboon et.al[24]

The egg shell calcium content was about 42% was almost similar to the findings of N Y Al- awwal[25], it has been discussed that for industrial purposes white leg horn egg shells are more suitable. On an average 368000 tons of poultry chicken eggs are produced each year in India, 10%of this is the proportion of egg shell calcium carbonate, i.e 36800 tons can be utilized for industrial purposes such as cement industries.

CONCLUSION

The egg shell surface has revealed the presence of positive proteins where as shell membrane had no response toninhydrin. The iodine mediated test gave an idea that surface of the shell do contain amylase. The method of photo recording had given a clear picture of shell pores. Nihydrin staining of egg shell opened way for new thinking on egg shell related studies .The egg shell seems to be a promising source of calcium of industrial utility. Organization of pores and proteins have given a new idea for analysis of egg shell topography.

Fig 1a.
Unstained egg shell

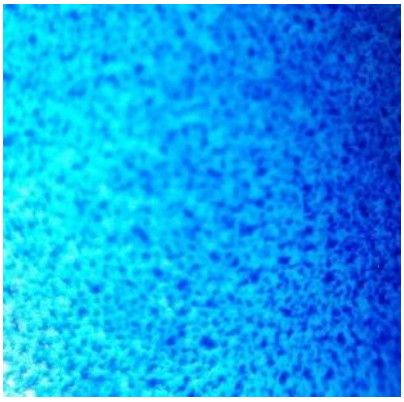

Fig 1b.
Giemsa stained egg shell

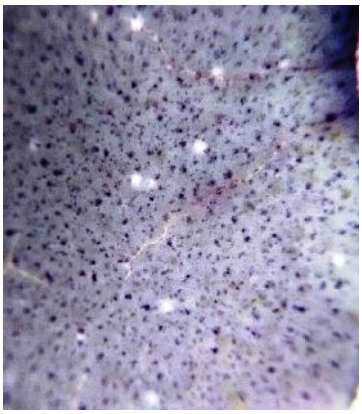

Fig. 2
Ninhydrin stained egg

Fig 3a.
sStarch and Iodine stained shell

Fig 3b.
Starch digested shell surface

Table 1
Calcium content of different egg samples.

Sample no	Percent of Calcium
1	42.01
2	42.75
3	44.02
4	42.06
5	42.80

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