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Comparative study on calcium content in egg shells of different birds

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Abstract
This study was designed to evaluate the calcium carbonate in different birds' eggshell because it has a potential application in the development of a new option of pharmaceutical excipient. Egg shells are the rich source of mineral salts, mainly calcium carbonate, which is probably the best natural source of calcium and it is about 90% absorbable. The composition of the egg shell is approximately 98.2, 0.9, 0.9% Calcium carbonate, Magnesium and Phosphorous (phosphate) respectively. Discarded eggshells are often used as a plant fertilizer and are effective liming sources. This is because eggshells contain calcium that raises, or neutralizes, the pH level in surface layer of acidic soils. In the present study, the highest Calcium content was determined in the eggshell of lapwing (96.46%) and hybrid hen (95.28%), this was followed by duck and Pigeon and least in the Swift eggshell (68.06%). Chicken eggshells can be used as an alternative soil stabilizer like lime since they have the same chemical composition. The multiple uses of eggshells in nutrition and medicine and can be utilized for various purposes that minimize their effect on environmental pollution.

Keywords: eggshell, calcium, weight and percentage, birds

Introduction
Calcium is a vital component of a healthy diet and a mineral necessary for life, particularly in cell physiology where movement of the calcium ion in and out of the cytoplasm functions as a signal for many cellular processes. The amount of calcium in the body depends on the amount of calcium you get in your food. Eggshells are waste materials from hatcheries, homes and fast food industries (Bain, 1997; Nys, 2004,) [3, 9] 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 (Amu *et al.*, 2005) [2].
Eggshell is non-edible by-product with little saleable value but they may contain biologically active compounds (Nakano *et al*., 2003) [8]. Calcium is the major component in an eggshell. There is also a small amount of magnesium in the eggshell and trace amounts of other micro elements. Eggshell calcium is the best natural source of calcium and it is about 90% absorbable, than limestone or coral sources. The whole medium eggshell makes about one teaspoon of powder (Nakano *et al.*, 2003; Kingori, 2011) [8, 7]. Birds lay hard-shelled eggs, most birds' eggs are incubated in nests and are often highly conspicuous, so their colour serves as camouflage. However, the shells are porous enough to allow oxygen to enter the egg and carbon dioxide to leave. 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 (Adeyeye, 2009) [1].
The composition of the egg shell is approximately 98.2, 0.9, 0.9% Calcium carbonate, Magnesium and Phosphorous (phosphate) respectively. One whole medium sized eggshell makes about one teaspoon of powder, which yields about 750-800 mgs of elemental calcium (Nys, 2004) [9]. The composition of an eggshell is very similar to that of our bones and teeth. It is recommended that people with osteoporosis take 400-500 mg calcium per day to supplement dietary sources. In laying hens in the late production phase, eggshell powder has been found to increase egg production and improve the quality of shells (Kingori, 2011) [7].
In wild before eggshell formation begins, the egg content has to be almost completed. That makes sense! Female great tits were found to deposit 60% of the calcium in the shell in less than 8 hours, prior to laying. Whereby, the fat and protein deposited in the egg is extended over a 4 day period. A general feature of many wild birds is to lay down the calcium needed to lay the night before laying (Bee, 2011) [4]. Chemical analyses showed that, eggshell is composed of about 97% calcium carbonate, and most of the research on shell quality concentrated on this fact (Hunton, 2005) [6].
However, eggshell is a valuable source material or additive for numerous branches of the industry, such as, among others fodder and fertilizer producers, paper manufacturers. Numerous types of collagens, hyaluronic acid, or amino acids of interest may be extracted from the egg shell membrane and purified for numerous uses (e.g. dietary supplements, cosmetics), which makes the separated eggshell membrane a highly valuable material. This way the egg producers could avoid the cost of waste disposal, moreover they can make extra profit by selling their side product (Gaonkar and Chakraborty, 2016) [5].

Materials and methods
Eggshell Sample
Eggs of local and hybrid hen were bought from the market and their shells were collected. Initially, nests of birds [Swift,

Forest Pigeon, Lap wing] were traced and a constant observation on these nests for a few days, and the birds were identified. After hatching birds get rid of these shells by dropping them out of the nest. These fallen eggshells were collected manually. These collected samples were compared and identified by using resources and based on the morphology of the egg.

Determination of Caco$_3$ in an egg shell

The Major component of eggshell is Calcium Carbonate ($CaCO_3$). This analysis will be done volumetrically by using a characteristic reaction of carbonate compounds, namely their reaction with acids. Calcium Carbonate (limestone) is very insoluble in pure water but will readily dissolve in acid according to the reaction.

$$2HCl\ (aq) + CaCO_3(s) \rightarrow Ca^{2+}(aq) + CO_2(g) + H_2O + 2Cl^-(aq)$$

This reaction cannot be used directly to titrate the $CaCO_3$ because it is very slow when the reaction is close to the endpoint. Instead the determination was achieved by adding on excess of acid to dissolve all of the $CaCO_3$ and then titrating the remaining H_3O^+ with NaOH solution to determine the amount of acid which has not reacted with the calcium carbonate. The difference between amount of the acid (HCl) initially added and the amount left over after the reaction was equal to the amount used by the $CaCO_3$. The reaction used to determine the leftover acid is

$$HCl\ (aq) + NaOH\ (aq) \rightarrow H_2O + Na^+(aq) + Cl^-(aq)$$

Procedure

1. Clean the collected eggshells with water and dry them in an oven.
2. Use a mortar and pestle to grind the eggshells into a powder.
3. Mass out 0.50 grams of the eggshell powder into a 100 ml flask and record the mass of the powder.
4. Add several drops of ethanol to the flask (this helps to the HCl to dissolve the eggshell but is not part of the reaction).
5. Pipette 10.0 ml of 1.0 M HCl into the flask. Then add 2-3 drops of Phenolphthalein.
6. Fill the burette to above the 0.0 ml mark with 0.1 M NaOH. Open the value and allow the NaOH to drain into a small beaker until the solution was at the 0.0 ml mark. The small amount of NaOH in the beaker may be discarded.
7. Titrate the eggshell sample until the solution turns pink and stays for 30 seconds. Read and record the final volume on the burette.
8. Calculate the amount of $CaCO_3$ present in eggshell using obtained values.

Result and discussion

The eggshell is a highly specialized mineralized structure, which provides protection against physical damage and penetration by microorganisms. The egg shell consists of the inner and outer shell membranes, the true shell and the cuticle. The crystalline layer of the shell, which is responsible for its mechanical strength, consists of more than 90% calcium in the form of calcium carbonate. Calcium is absorbed from the feed in the intestine. Provided that sufficient calcium (3.8–4.2%) is present in the feed, the process of calcium uptake, deposition and excretion is regulated by vitamin D3 and its metabolites.

According to our experimental analysis of eggshell samples, the lap wing has the highest value of calcium egg weight (0.4823 g), percentage of $CaCO_3$ was 96.46. The swift has the lowest value of calcium egg weight (0.3403g), percentage of $CaCO_3$ was 68.06 all on the average basis of seven eggs involved in each case.

Present experimental results showed that the Lap wing which has highest $CaCO_3$ level (0.4823 gm, i.e. 96.46%), followed by the Hybrid hen (0.4764 gm, i.e. 95.28%), this is followed by Forest Pigeon (0.4554 gm, i.e. 91%), then Domestic Pigeon (0.4344 gm, i.e. 87%), then Rat snake (0.4034 gm, i.e., 81%), then Local Hen (0.4 gm, i.e. 80%), and the swift eggshell (0.3403 gm, i.e. 68.06%) sample which has the least level of CaCO3.

Lap wing > Hybrid Hen> forest Pigeon > Domestic Pigeon > Rat Snake> Local hen> Swift

Table 1: Weight and percentage of $CaCO_3$ present in different eggshell samples

Egg samples	Weight of $CaCO_3$ in grams	The % of $CaCO_3$ in Eggshell
Hybrid hen	0.4764	95.28 %
Local hen	0.4	80 %
Domestic Pigeon	0.4344	87 %
Forest Pigeon	0.4554	91 %
Swift	0.3403	68.06 %
Lap wing	0.4823	96.46 %

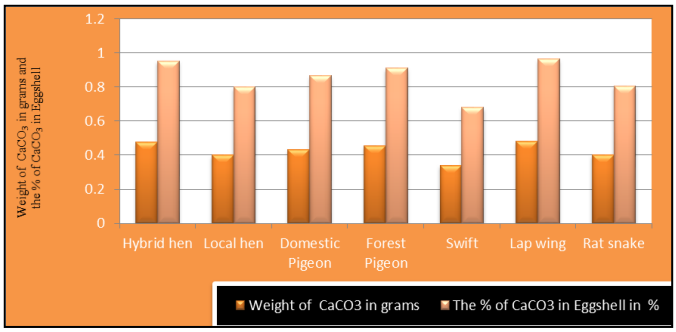

Fig 1: Weight and Percentage of $CaCO_3$ present in eggshell samples.

Increased or decreased calcium level in birds might be depending upon their habitat. In case of domestic birds, they are fed with supplementary feed. This may be the reason for the difference in the calcium content in birds' egg shell. This may increase the quality of egg shell.

Eggshells can be utilized for various purposes that minimize their effect on environmental pollution. Eggshells present healthy, balanced calcium due to its trace amounts of other minerals and is probably the best natural source of calcium (Adeyeye, 2009) [1]. One whole medium sized eggshell makes about one teaspoon of powder, which yields about 750-800 mgs of elemental calcium plus other micro elements. Eggshell powder has been reported to increase bone mineral density in people and animals with osteoporosis (Kingori, 2011) [7]. In laying hens in the late production phase, eggshell powder has

been found to increase egg production and improve the quality of shells. Discarded eggshells are often used as a plant fertilizer and are effective liming sources. This is because eggshells contain calcium that raises, or neutralizes, the pH level of overly acidic soil.

Numerous factors affect the functional quality of the egg shell mostly prior to the egg is laid. The thickness of the shell is determined by the amount of time it spends in the shell gland (uterus) and the rate of calcium deposition during shell formation. If the egg spends a short period in the shell gland, the thickness will be less. Also, the time of the day when the egg is laid determines the thickness of the shell. Many studies showed that saline drinking water, including tap water containing sodium chloride supplied to mature laying hens at concentrations similar to those found in underground bore water, has an adverse effect on eggshell quality while having little effect on feed intake, egg production or egg weight. In contrast some reports indicate that there were no visible shell defects and specific gravity was also not adversely affected.

One of the factors contributing to poorer eggshell quality in hot weather is inadequate feed intake. Eggshell quality is somewhat compromised during summer months. During exposure to warm environmental temperature, the hen reacts by increasing its rate of breathing (panting) in order to cool itself. This causes the lowering of CO_2 in the blood and produces a condition termed "respiratory alkalosis". The pH of the blood becomes alkaline and the availability of calcium for the eggshell is reduced. This disturbance in acid-base balance causes an increase in soft-shelled eggs during summer. Temporary thinning of the egg shell may occur during periods of high ambient temperature (above 25ºC) since feed intake is reduced. The shells quickly regain normal thickness when temperatures are reduced and feed intake increases.

Conclusion

Calcium is vital to breeding birds and is needed in large quantities for egg shell production and for the healthy growth of the new born chicks. Without it young birds can be unhealthy, eggshells can be defective causing clutch desertion as the hatchlings never hatch, deformed or even die. In the hen, calcium deficiency during the breeding period results in poor egg shell formation and even to egg-binding. Giving extra calcium to birds during the breeding and growth season is therefore strongly recommended. Sufficient calcium ensures good egg shell formation and the development of a healthy skeleton. Calcium dietary supplement is administrated for good egg shell formation, for good skeletal development in young birds, during the breeding season to prevent egg-binding and in cases of calcium deficiency or calcium tetany.

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