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What Separated the Cuticle from the Avian Egg Shell is Unknown: Could it be the Yellow Maize Broth or Heat?

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Authors' contributions

This work was carried out by both authors. Author IKO presented the photographs, managed the literature searches and prepared the manuscript. Author ICO cooked the maize, eggs and observed the possibility of harvesting avian egg cuticle in cooked yellow maize broth. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Avian egg internal components are sources of nutrients for the developing embryo while the external components especially the cuticle, have physical and biological defence mechanisms to protect the embryo against microbial attack. Consequently, the cuticle could be harvested and used in the production of pharmaceuticals for the treatment and prevention of chronic and infectious diseases as well as feed supplement in human and livestock nutrition. Unfortunately, little is known about the possibility of harvesting and utilizing avian egg cuticle hence, the present serendipitous discovery may serve as a guide to commercial production of avian egg cuticle.
Methodology: Six freshly laid eggs of Black Nera where cooked in an attempt to warm six broken yellow maize cobs and were left in the broth overnight.

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Results: It was observed that the cuticles on all the eggs surfaces not submerged in the broth were removed and with gentle rubbing on the surfaces submerged in the broth, more cuticles were harvested. Although, the egg cuticle yield per egg was not determined, it was seemingly indicative that avian egg cuticle could be harvested commercially.
Conclusion: Black Nera egg cuticles could be harvested following cooking of freshly laid eggs left in a broth containing yellow maize overnight. The cooked yellow maize broth was observed to be misty, indicating composition of several substances suspected to be the cause of the egg cuticle's removal. Although, there are apparent prospects of harvested avian egg cuticle that could be explored, some limitations to its optimal utilization may be contemplated. Hence, research activities geared towards avian egg cuticle nutritional profiles determination, pharmacological trial, composition of cooked yellow maize broth and ways to harvest avian egg cuticles should be painstakingly conducted.

Keywords: Avian egg; Black Nera; harvested cuticle; yellow maize.

1. INTRODUCTION

Avian eggs are gradually formed within the oviduct over a period of time ranging from 24 to 27 hours with an average of approximately 26 hours. Egg formation begins with the release of yolk from the left ovary which is swept into the oviduct by the pressure created by the fimbriae of the infundibulum. At the infundibulum region of the oviduct, chalaza is secreted on the yolk that stays for a ¼ hour and then migrates to the magnum which is the longest portion of the oviduct where albumen is secreted around the yolk and spends 3 hours before migrating to the isthmus. At the isthmus, shell membrane is secreted to enclose the yolk and albumen within 1¼ hour and then moves to the shell gland region, where calciferous shell is secreted to enclose the formed egg. Thereafter, cuticle is secreted on the formed shelled egg and it takes about18 to 22 hours at the shell gland before moving to the vagina where the formed egg stays for a minute for mucous secretion to ease laying [1]. Egg cuticle is formed as a thin membrane to cover the entire eggshell at about 1½ to 4 hours before lay. It is made from sticky fluid which is reflective in nature hence it is referred to as "egg shine". Gasses can penetrate the egg cuticle thus dissolves with time (about 96 hours after lay) due to carbon dioxide activity. This will result in its disappearance thereby compromising the defensive mechanisms to prevent microbial invasion [2,3].

According to [4], all eggs are initially white but as shell formation progresses, the epithelial cells lining the surface, synthesize and accumulate pigments. The kind of pigment deposited on the eggshell within the oviduct depends on the breed. White Leghorn is known for its white eggs, Rhode Island Red lays brown eggs whereas Ameraucana lays blue eggs but no particular breed is associated with green eggs. Within a breed, shades of the basic colours are possible hence light brown or tinted and chocolate eggshell colours were reported amongst others [5,6]. White and brown colours with their associated shades of colour are common but blue and green eggs are not and are produced by many different avian species [7]. Protoporphyrin-IX was reported [8] to be the most abundant pigment among others like biliverdin-IX and its zinc chelate derivatives. Eggshell becomes green when blue palisade (i.e. the outer shell layer) is coated with brown pigment. Avian layers are either recessive (o+/o+) wild type oocyan, heterozygous (O/o+) or homozygous (O/O). While brown and white eggshell layers are recessive (o+/o+), blue and green eggshell layers are heterozygous (O/o+) or homozygous (O/O) [9,10].

Cuticle and eggshell colours were highly correlated and have been speculated to influence egg consumer's preference. For instance, it was stated [4] that the British preferred brown-shelled eggs whereas, the Americans and Spanish preferred white-shelled eggs. In Nigeria however, the cuticle and eggshell colours do not play significant role in the choice of egg to be consumed. Series of studies have been conducted on eggshell pigmentations [11,12,13], eggshell structure [14,15,16], eggshell thickness [17] and eggshell properties [18]. More importantly, the role of egg cuticle [19,20] and eggshell antimicrobial defence mechanisms [21,22,23] have been investigated.

Use of reagents such as ethylene diamine tetra acetic acid (EDTA) to remove cuticle were suggested to enhance hatchability [24] and protoporphyrin-IX determination [25]. Yet, little is

known about how to harvest the cuticle from avian eggshell. Therefore, the present serendipitous discovery may serve as a guide, to commercial production of cuticle for possible use in the production of pharmaceuticals, treatment and prevention of chronic and infectious diseases in humans and livestock as suggested by [26]. More essentially, harvested avian egg cuticle could be utilized industrially in the synthesis of degradable materials, microbial culture media, paints and nutrients supplements amongst other things.

2. MATERIALS AND METHODS

2.1 Brief Climatic Description of the Study Site

The observation was made in the month of June 2016, in a kitchen on Buzzuzzu street, off Kwandere road, College of Agriculture Students' Village, Lafia, situated on latitude 08°35ᴵN, longitude 08°34ᴵE with an altitude of 181m above sea level. The environmental temperature ranges from 32 to 35°C with relative humidity of 58 to 63%, average day light of 9 to 12 hours and mean rainfall of 1,400mm per annum [27]. The vegetation consists of different species of trees, shrubs, leguminous browse plants and grasses with fairly undulated terrain.

2.2 Experimental Procedure

Some fresh and succulent yellow maize were bought from a road side vendor with the sheaths intact. On getting home, the sheaths were removed and six fresh cobs were broken into two parts and cooked with salt on a kerosene stove. Immediately after cooking, some of the cobs were eaten and the remainders were being warmed morning and evening daily for 4 days. On day 3, freshly laid brown eggs of Black Nera layers at 24 weeks of age, were bought from the sales outlet of the Demonstration and Teaching Farm, Nasarawa State University, Keffi, Shabu-Lafia Campus, Lafia. In an effort to conserve kerosene, six of the freshly laid eggs were placed in the pot containing cooked yellow maize and broth during warming (on day 3) in order to cook the eggs. Thereafter, 3 of the eggs were eaten and the remainders were left overnight in the pot containing some of the yellow maize cobs and broth. On day 4 in an attempt to warm the remaining maize cobs and the eggs, it was astonishing to observe that the cuticles on all the eggs surfaces not submerged in the broth were removed and with gentle rubbing on the surfaces submerged in the broth, more cuticles were harvested per egg with ease. In that surprising mode, snap shots of the shocking observation were taken with a digital camera (Cyber-Shot DSC-W330 Sony®).

3. RESULTS

"Fig. 1" shows yellow maize, Black Nera eggs and broth in a cooking pot. It was observed that the cuticles on the eggs were removed, suggesting the possibility of harvesting it commercially. The broth was observed to be misty, signifying its compositions of several substances and the yellow maize appeared moderately cooked and fresh.

Cooked yellow maize and Black Nera eggs in a broth as well as harvested cuticle are presented in "Fig. 2". Removed cuticles were observed only on the parts of eggs not submerged in the broth.

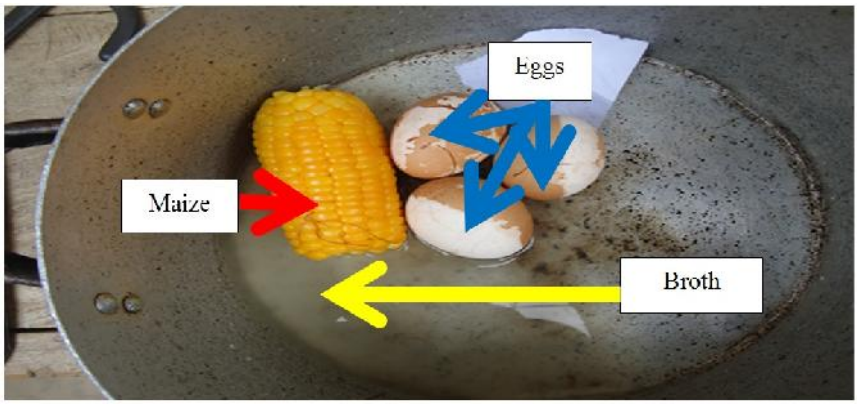

Fig. 1. Cooked Black Nera eggs and yellow maize cob in broth

Given in "Fig. 3" is a cooked Black Nera egg surface indicating where cuticles were removed. It was observed that gentle rubbing on the cooked eggs' surfaces yielded an appreciable quantity of cuticles.

"Fig. 4" provides egg cuticles from Black Nera egg. The seemingly high quantity (though not measured) suggested that it might be feasible to harvest egg cuticles.

4. DISCUSSION

Black Nera eggs cuticles were observed to peel off after cooking in yellow maize broth and left overnight. Although, it was only on the eggs' surfaces outside the broth that the cuticles were seemingly removed, it was observed that gentle rubbing with fingers on the other side submerged in the broth yielded more cuticle though not quantified. This suggested that avian egg cuticle could be harvested commercially. It was observed that the true eggshell colour was revealed to be white after harvesting the cuticle. This corroborated the reports of [4] and [28] that avian egg shell true colour is white. The Black Nera egg cuticle was observed to be a tiny brown membrane which was formed from sticky fluid that covered the whole eggshell and functions as a defensive mechanism to prevent microbial

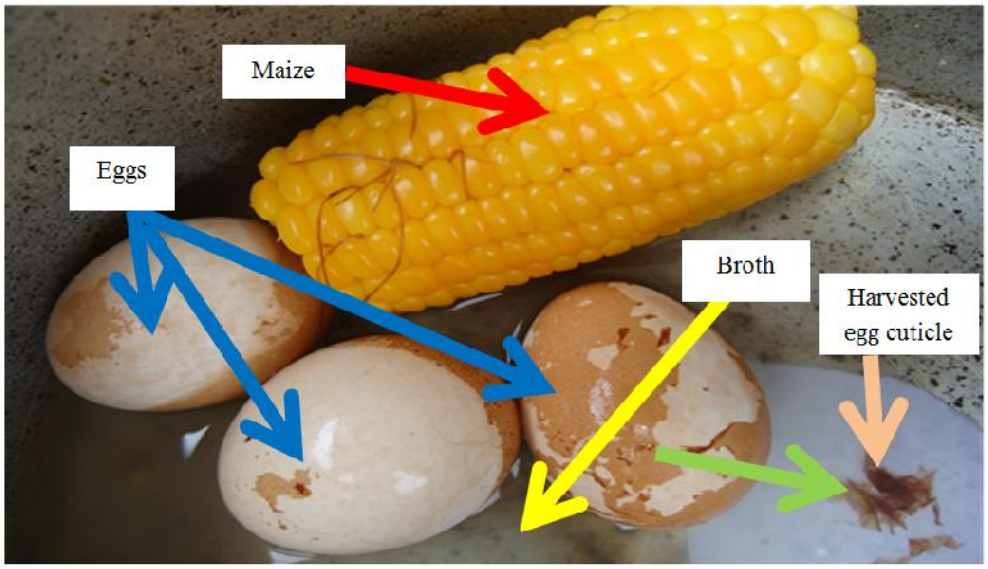

Fig. 2. Cooked Black Nera eggs, yellow maize cob and harvested cuticle

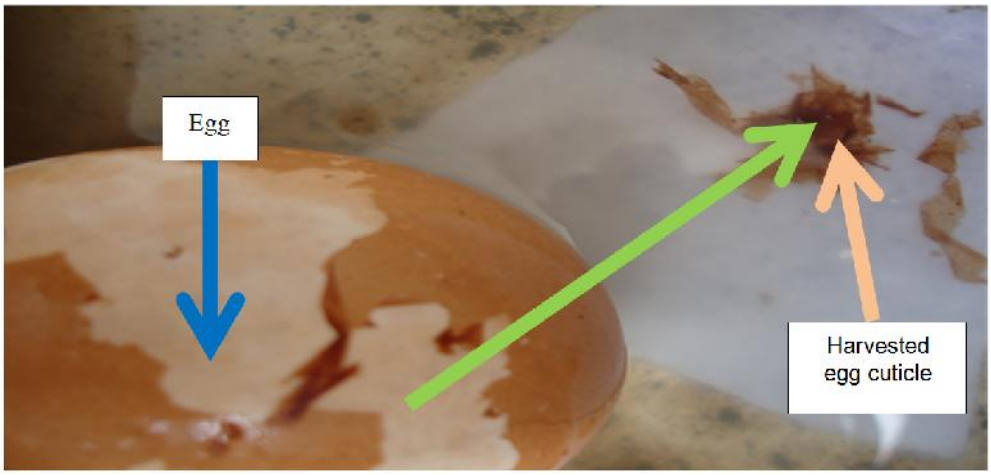

Fig. 3. Cooked Black Nera egg showing removed cuticle

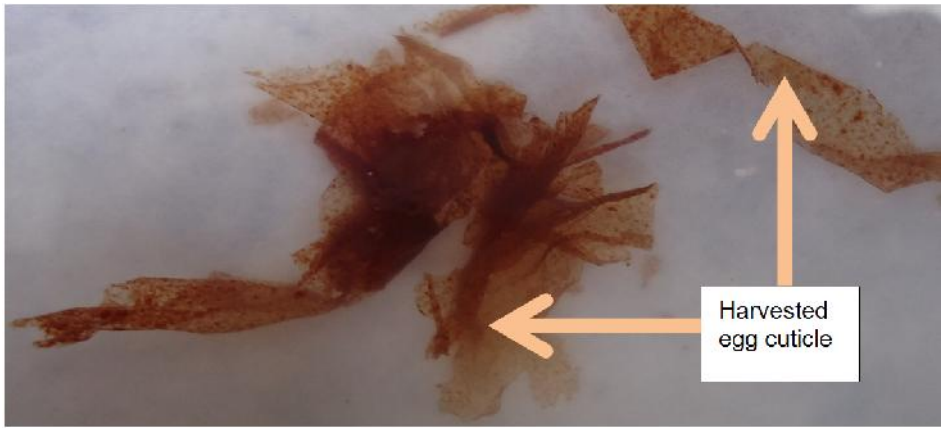

Fig. 4. Harvested Black Nera egg cuticle

invasion [2]. More so, it was reported that eggshell physically obstructed microbial penetration of egg [26]. Since myriad of biological activities with unique applications could be attributable to various egg components especially the cuticle that has antimicrobial, immunomodulatory, anti-neoplastic and inhibitory activities. It could be harvested and used in the prevention and treatment of human and animal diseases. This and other prospects of harvested avian egg cuticle could be highlighted as follows:

4.1 Pharmacological Potentials

Avian egg components have been reported to be highly potent in antimicrobial activities [19]. Eggshell and shell membrane mechanically and biologically constituted barriers against egg penetration by microbes [26]. Although, cuticle functions most effectively within 96 hours after egg lay, it was observed to block the egg pores from bacteria penetration [29]. Unfortunately, it was demonstrated that the cuticle could be damaged by enzymes secreted by *Pseudomonas* and *Salmonella* species [30,31]. According to [32], avian eggshell cuticle modulated the amounts of beneficial verses harmful UV-Light reaching the embryo inside an avian egg. Therefore, harvested avian egg cuticle could be utilized in the production of drugs, ointments or spray to prevent or treat human and livestock diseases.

The nature of harvested avian egg cuticle could make it suitable for the synthesis of fabric materials especially absorbable medical suture. Since avian egg cuticle was described as a liquid or sticky fluid which dried and became membranous, it could be harvested and processed into syrups and administered as supplements to boost immunity in both humans and livestock alike. For example, [33] discovered uronic acid, sialic acid, nitrogen and an array of amino acids in eggshell and eggshell membranes, while [34] described eggshell membrane as adsorbent. It was reported that *Pseudomonas* and *Salmonella* species have the ability to synthesize enzymes that can damage the integrity of avian egg cuticle [30,31]. Therefore, harvested egg cuticle could be used as substrate for microbial culture media, in diagnostic and confirmatory tests of suspected *Pseudomonas, Salmonella* and other bacteria species.

The nutritional profiles of harvested avian egg cuticle, when determined, may reveal it contains essential nutrients required by humans and livestock to thrive well. The observations of [35] and [36] seemingly indicated that egg cuticles probably contained protein and fat. Thus, harvested avian egg cuticle could be used to supplement human and livestock nutritional requirements. If harvested egg cuticle contains ample concentrations of minerals, vitamins, proteins, carbohydrates, fats and oils, it could be incorporated into livestock feed as an additive to boost essential nutrients intake. Until recently when protein profiles of hatchery egg shell membrane was given [37], it was speculated [31] that little is known about an efficient method of protein and protein-polysaccharide complexes extraction from eggshell membranes.

4.2 Raw Material for the Industry

It has been established that avian egg cuticle has colour that contributes to the overall eggshell

colours [11]. For instance, protoporphyrin-IX, biliverdin-IX and the zinc chelates derivatives concentrations determine the brown, blue, green and their intermediate colours of avian eggshell [13,25,38]. These colours could be extracted from harvested avian egg cuticle and used in industrial production of paints, crayons and other colouring materials.

4.3 Limitations to Utilization of Avian Egg Cuticle

Commercial quantity of harvested avian egg cuticle may not be feasible, thus may not be readily available, accessible and affordable. The nutrients in the avian egg cuticle may be heat labile, resulting in low nutritional value thus, limiting its use as nutritional supplement. Avian egg cuticle may contain toxic factors or allergic compounds that may result in hypersensitivity when used as pharmaceuticals thus, limiting its utilization. The strands may not be long enough to be kneaded into fabrics and may not be easily degraded thus, becomes incompatible in absorbable medical suture synthesis.

The quantity of avian egg cuticle harvested at a given time, may not be enough, thereby resulting in localization in the livestock feed thus, might not be suitable as an additive. The avian egg cuticle may not readily form gel and its antimicrobial tendency may inhibit microbial growth thus, may not be suitable as substrate for growth media. The pigments on the avian egg cuticle may be difficult to extract and separate into basic and intermediate colours. Besides, the quantity of avian egg cuticle may not be enough to be used as a component of paints, crayons or other colourants production.

5. CONCLUSION

Black Nera egg cuticles could be harvested following cooking of freshly laid eggs left in a broth containing fresh yellow maize overnight. The cooked yellow maize broth observed to be misty, indicated its composition of several substances and could be suspected to be the cause of the egg cuticle's removal. The true eggshell colour was revealed to be white, after a gentle rubbing on the eggs' surfaces that were not submerged in the broth signifying possible cuticle harvest. Although, the cuticle yield was not determined, it was apparently indicative that commercial quantity maybe achievable for industrial uses. At the meantime, harvested avian egg cuticle's proficiency, effectiveness and safety in the treatment of diseases as well as feed supplement in humans and livestock, cannot be guaranteed until in-depth researches are conducted exhaustively to elucidate the highlighted prospects. Consequently, prospects of harvested avian egg cuticle could be explored however some limitations to its optimal utilization may be contemplated. Hence, research activities geared towards nutritional profiles determination of avian egg cuticle as well as pharmacological trials should be painstakingly conducted. More significantly, cooked yellow maize broth suspected to be the cause of the egg cuticle's removal should be investigated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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