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In vitro ANTIMICROBIAL ACTIVITY, MINERAL AND VITAMIN COMPOSITIONS OF NERA BLACK EGG CUTICLE

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ABSTRACT

Avian egg cuticle serves as a barrier to movement of particles, water and microorganisms into the egg. It is speculated to be rich in nutrients with antimicrobial characteristics *in ovo* but, not clear if these characteristics will be exhibited *in vitro.* The study was aimed at evaluating the antimicrobial activity, mineral and vitamin compositions of Nera Black egg cuticle. Fresh maize cobs were cooked, left overnight and 30 eggs were boiled in the broth for 10 minutes, again left overnight with the eggs completely submerged in the broth. The boiled eggs were carefully removed, rubbed gently with fingers and scraped slightly with knife to harvest the cuticle. Samples of the cuticle were air-dried, processed for mineral, vitamin and microbial sensitivity determination following standard procedures. The results indicated that Nera Black egg cuticle contained calcium (123.7 mg/100 g), phosphorus (313.6 mg/100 g), iron (17.7 mg/100 g), sodium (112.6 mg/100 g), potassium (335.5 mg/100 g) and magnesium (26.4 mg/100 g). Also, zinc (1.12 mg/100 g), copper (0.25 mg/100 g), selenium (0.006 mg/100 g) and manganese (0.205 mg/100 g) were detected in the egg cuticle. More importantly, vitamins A (828.64 µg/100 g), B1 (0.515 mg/100 g), B2 (0.016 mg/100 g), B3 (0.77 mg/100 g), B6 (0.53 mg/100 g), C (1.68 mg/100 g) and E (7.31 µg/100 g) were found in the egg cuticle. *Listeria monocytogenes, Enterobacteria, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were resistant to the egg cuticle *in vitro.* So, avian egg cuticle maybe added to livestock feed to improve the nutritional quality but, may not be suitable as an antimicrobial agent. Nevertheless, in depth investigations on antimicrobial potency of crude and extracted components of avian egg cuticle is pertinent to consolidate earlier reports.

Keywords: Avian egg cuticle; eggshell pigmentation; microbial resistance; Nera Black; poultry feed.

INTRODUCTION

Poultry eggshell encloses the egg content thereby protecting the integrity. Although, the eggshell has pores that allow gaseous exchange between the egg and the surroundings, it serves as a barrier to mechanical impact, dehydration and microbial contamination. The eggshell layers are believed to contain basically mammillary and spongy matrixes, deposited in a unique arrangement during egg formation in the segmented complex avian oviduct. Kodali et al. [1] stated that eggshell was composed of three main layers with multifaceted morphological structures (i.e. cysteine-abundant proteinaceous shell membrane, mineralized hard composite layer and an outer non-mineralized cuticle. The egg cuticle is believed to be deposited on the eggshell surface in the uterus at about 2 hours before oviposition [2].

Eggs of many poultry species have cuticle and the composition was speculated to be similar to the organic matrixes of the

eggshell, even though there are controversies about where the egg cuticle is formed in poultry species. Recently, Wilson et al. [3] clearly shown that egg cuticle is secreted and deposited at the shell gland pouch of the uterus and not the vagina as widely speculated. Also, there are diverse opinions that there is a relationship between cuticle formation and eggshell pigmentation. For instance, Wilson et al. [3] reported no direct correlation between deposition of pigment and cuticle in avian eggs. It was stressed that though there was obvious reliance of one event on the other during eggshell pigmentation and cuticle secretion on the eggshell. There was no connection between the genes controlling each trait and that the deposition of pigment was not dependent on the presence of an intact cuticle. Whereas, Samiullah and Roberts [4] reported that about 80% of the avian eggshell pigments were found in the outer calcified layers with only about 20% in the cuticle. Earlier, Lang and Wells [5] stated that eggshell pigmentation was commonly associated with the eggshell cuticle.

In any case, the essence of avian egg cuticle secretion on the eggshell is to limit movement of particles, water and microorganisms into the egg via the shell pores hence, Board and Tranter [6] stated that egg cuticle functions as a physical barrier against microbial attack and contamination of the egg. This phenomenon makes avian egg's natural defence mechanism important, due to possible trans-shell contamination, whereby microorganisms penetrate the eggshell and contaminate the egg content [7-9]. Avian egg cuticle has been reported to contain about 90% proteins, 4% polysaccharides, 3% lipids, calcium carbonate, magnesium, phosphate and porphyrin pigments [8,10-12]. These chemical compositions were reported to have played vital roles in limiting microbial contamination and preserving the egg quality [10,13,14].

Avian egg antimicrobial and antifungal activities have been propounded. For example, Wellman-Labadie et al. [15] discovered that purified ovocleidin-17 and ansocalcin were bactericidal against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Similarly, Abdou et al. [16] stated that the eggshell membranes contain several bacteriolytic enzymes (lysozyme and N-acetylglucosaminidase) and other membrane components which may alter the thermal resistance of Gram-positive and Gram-negative bacterial pathogens like *Salmonella Enteritidis, Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. Recently, Chen et al. [17] compared precocial and altricial birds' eggshell antibacterial effectivity on *Escherichia coli* and deduced that the *E. coli* penetration rate of altricial birds' eggs was significantly higher than that of precocial birds' eggs.

Yet, there is dearth of information on minerals and vitamins compositions as well as antimicrobial activity of Nera Black egg cuticle. More significantly, it is not clear if *in ovo* characteristics of egg cuticle will be exhibited *in vitro.* Hence, this study was aimed at evaluating the mineral and vitamin compositions as well as antimicrobial activity of Nera Black egg cuticle.

MATERIALS AND METHODS

Climatic Condition of the Study Location

The experiment was conducted at Bafawat Biomedical and Research Laboratory, Lafia, located on latitude 08^{o} 35'N and longitude 08^{o} 33'E in the Guinea savannah zone of north central Nigeria.

Lafia has an altitude of 181m above sea level, temperature ranging from 32 to 35^{o}C, relative humidity between 58 and 63%, average day light of 9 to 12 hours and approximately 1,400 mm rainfall per annum [18].

Harvesting of Nera Black Eggs Cuticle

Six sizable fresh yellow maize cobs were cooked in plenty water and left overnight. The following day, 30 Nera Black eggs were boiled completely submerged in the maize broth for ten minutes and further left overnight. By the following day, the cuticle was harvested by rubbing the eggshell gently with fingers as described by Idahor and Idahor [19] and a slight scraping with a knife (see Plates 1 – 3).

Plate 1. Scraping of eggshell surface

Plate 2. Scraped eggs and harvested cuticle

Plate 3. Harvested cuticle

Determination of Minerals and Vitamins in Nera Black Eggs Cuticle

The harvested cuticle was air-dried, packed properly and sent to Institute of Agricultural Research and Training, Moore Plantation, Ibadan, Nigeria for the determination of vitamins and minerals using standard procedures [20]. In each case, every parameter was determined thrice but the mean value was recorded.

Antimicrobial Potency Test of Nera Black Eggs Cuticle

Slants of known microbes (*Listeria monocytogenes, Enterobacteria, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were obtained from the Department of Microbiology, University of Ibadan and revived on nutrient agar broth according to Bedrani et al. [21] before use. The cuticle samples were ground into finer powder and distilled water was added to it at 2:1 g/v ratio to form a solution. A 2 mL of the cuticle solution was taken with micropipette, smeared on the surface of a tryptic soy agar and streaked with an inoculum of about 1 x 10^{5} cfu/mL of each of the revived microbes and incubated at 37^{o}C overnight according

to Guyot et al. [22]. To ensure sterility during the experiment, the materials used and the bench were sterilized regularly. The plates were carefully assayed macroscopically, for morphological growth which was recorded either as susceptible or resistant.

RESULTS AND DISCUSSION

Detection of Minerals and Vitamins in Nera Black Eggs Cuticle

Mineral and vitamin compositions of Nera Black eggs cuticle are presented in Table 1. It was shown that egg cuticle contains vitamins, macro- and micro-minerals.

Table 1. Mineral and vitamin compositions of Nera Black eggs cuticle

Parameters	Compositions (mg/100 g)
Macro-minerals	
Calcium	123.7
Phosphorous	313.6
Iron	17.7
Magnesium	26.4
Potassium	335.5
Sodium	112.6
Micro-minerals	
Zinc	1.12
Copper	0.25
Selenium	0.006
Manganese	0.205
Vitamins	
A	828.64 (µg/100 g)
B1	0.515
B2	0.016
B3	0.77
B6	0.53
C	1.68
E	7.31 (µg/100 g)

Calcium was 123.7 mg/100 g, phosphorus (313.6 mg/100 g), iron (17.7 mg /100 g), magnesium (26.4 mg/100 g), potassium (335.5 mg/100 g) and sodium was 112.6 mg/100 g. Also, zinc (1.12 mg/ 100 g), copper (0.25 mg / 100 g), selenium (0.006 mg/100 g) and manganese (0.205 mg/100 g) were detected in the egg cuticle. More so, vitamins A (828.64 µg/100 g), B1 (0.515 mg/100 g), B2 (0.016 mg/100 g), B3 (0.77 mg/100 g), B6 (0.53 mg/100 g), C (1.68 mg/100 g) and E (7.31 µg/100 g) were found in the egg cuticle. The observed macro- and micro-minerals detected in Nera Black egg cuticle, discredited the description of avian egg cuticle as “an outer non-mineralized cuticle” by Kodali et al. [1].

However, these findings corroborated the reports that avian egg cuticle contains proteins, polysaccharides, lipids, calcium carbonate, magnesium, phosphate and porphyrin pigments [8,10]. Macro-minerals such as calcium, phosphorus, iron, magnesium, potassium and sodium as well as micro-minerals like zinc, copper, selenium and manganese were found in the egg cuticle. In addition, vitamins A, B1, B2, B3, B6, C and E were discovered in the eggs cuticle. While the calcium and phosphorus levels were close to 129 mg/100 g and 390 mg/100 g, respectively, sodium value was less than a range of 142 – 166 mg/100 g reported in raw eggs [23]. But the levels of iron, magnesium and potassium were higher than ranges of 1.75 – 2.73 mg/100 g, 5 – 12 mg/100 g and 109 – 163 mg/100 g in that order, recorded in that study. The observed disparities may be due to the egg part analysed and the laboratory protocols adopted in the studies. Similarly, micro-minerals like zinc, copper, selenium and manganese were found in the egg cuticle and the values were within ranges of 1.29 – 2.3 mg/100 g, 0.023 – 0.077 mg/100 g, 0.02 – 0.056 mg/100 g and 0.011 – 0.055 mg/100 g, accordingly, reported in avian

eggs [23]. More significantly, the recorded values were similar to the reports in whole egg and egg parts from different varieties and sources [24,25,26]. The macro- and micro-minerals compositions were similar to what Goran et al. [27] recorded in egg white, yolk and shell from caged and grain fed hens with the highest values in yolk.

Remarkably, vitamins A, B1, B2, B3, B6, C and E were detected in the eggs cuticle and the values were close to what Zang et al. [28] reported in whole eggs. According to FAO [29], vitamins could be categorised as fat-soluble and water-soluble which must be provided in livestock diets except vitamin C. Furthermore, it was expressed that even if vitamin C is not essential in poultry diets, under adverse circumstances such as heat stress, dietary supplementation may be beneficial. It was stressed that the metabolic roles of vitamins were more complex than those of other nutrients and that they are not just body building units and energy sources, they mediate all biochemical pathways in the animals system.

The macro- and micro-minerals levels as well as the vitamins detected in the egg cuticle were within the recommended minimum levels in livestock nutrition [29-32]. Consequently, avian egg cuticle may be used as a supplement in livestock nutrition, to enhance the nutritional quality thereby, supporting the propositions that avian eggshell and egg cuticle may be veritable sources of nutrients in animal nutrition [19,33].

In vitro Antimicrobial Activity of Nera Black Eggs Cuticle

Table 2 shows the susceptibility test of some bacteria strains to Nera Black eggs cuticle.

It was observed that *Listeria monocytogenes, Enterobacteria, Staphilicoccus aureus, Pseudomonas aeruginosa* and *Escherichia coli* grew vigorously on the culture media, suggesting inability of the egg cuticle to suppress microbial growth (see Plates 4 – 8).

The unrestricted growth of *Listeria monocytogenes, Enterobacteria, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* on the culture media containing the egg cuticle, evidently demonstrated that the microbes were resistant to the egg cuticle *in vitro*. This observation could be purely due to the nutritional compositions that may support microbial growth, the microbial growth pattern and ability of the microbes to secrete enzymes that can simply destroy the egg cuticle. More so, it could be partly due to egg cuticle concentrations used in the present study. The observed microbial resistance seemingly supports the reports that *Salmonella* and *Pseudomonas* species have the ability to secrete enzymes that degrade egg cuticle [34-36].

Table 2. Susceptibility test of some bacteria strains to Nera Black eggs cuticle

Bacteria	Growth pattern	Response
Listeria monocytogenes	Growth characteristics of the microbe observed	Resistant
Enterobacteria	Growth characteristics of the microbe observed	Resistant
Staphylococcus aureus	Growth characteristics of the microbe observed	Resistant
Pseudomonas aeruginosa	Growth characteristics of the microbe observed	Resistant
Escherichia coli	Growth characteristics of the microbe observed	Resistant

Plate 4. *Listeria monocytogenes* growth

Plate 5. *Enterobacteria* growth

Plate 6. *Pseudomonas aeruginosa* growth

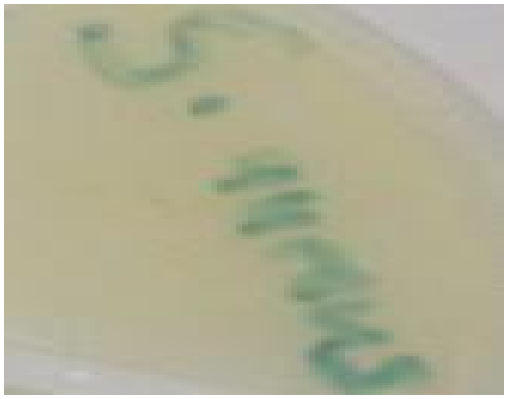

Plate 7. *Staphylococcus aureus* growth

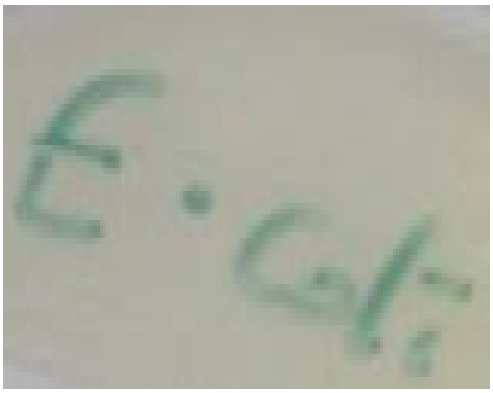

Plate 8. *Escherichia coli* growth

This observation contradicted the reports of Bain et al. [34] and Wellman-Labadie et al. [37] that avian egg cuticle protein, demonstrated activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.* It was stressed that the antimicrobial action of the outer eggshell and cuticle proteins found in a number of avian species, may be the mechanism which enhances avian reproductive success. Furthermore, the present study disagreed with the report that lipophilic extracts of eggshell from three domestic species, played a role in antimicrobial defence [13,34]. The

differences could be largely due to the pure extracts and crude form used in the studies which probably suggested that the reported susceptibility may be due to residual effects of the extraction reagents. Also, it could be partly due to the egg cuticle concentrations administered in the studies. Meanwhile, Irokanulo et al. [38] recorded susceptibility of *Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus flavus, A. fumigatus, Microsporum gypseum and M. audouinii* to quail eggshell powder in the crude state without extraction. Though the antimicrobial properties of the quail eggshell was attributed to ovotransferrin and high calcium content, which were however not determined in that study, it could be largely due to different compositions of the variegated cuticle found on quail egg surface. Therefore, Nera Black egg cuticle may not be suitable as an antimicrobial agent as widely purported.

CONCLUSION

Nera Black egg cuticle composed of calcium, phosphorus, iron, magnesium, potassium and sodium. Also, zinc, copper, selenium, manganese, vitamin A, B1, B2, B3, B6, C and E were found in the egg cuticle. The levels of all these minerals and vitamins were somewhat within the recommended levels in livestock nutrition. Nera Black egg cuticle could be used as a supplement to enhance the nutritional quality of livestock feed. Nera Black egg cuticle does not have antimicrobial activity *in vitro*. Examination of the nutritional quality and antimicrobial activity of crude and extracted avian eggshell components should be conducted.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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