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# Biochemical composition of eggshell and egg yolk of giant african land snail (*archachatina marginata*) during gestation period

Joseph, M. E.; Kehinde, O. A.; Adewumi B. I.; Adeyinka A. A.; Victoria, O.

Federal University of Agriculture, Abeokuta. Nigeria.

Aditional Keywords

Eggshell.
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Metabolites.
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## SUMMARY

The eggshell is a structure that is highly specialized in minerals, which protects the contents within the egg, provide nourishment to the embryo, and shield it from microbial attacks by serving as an immune system. Twenty (20) matured A. marginata snails weighing from 125.75g to 134.85g were used to produce the eggs for this study. The results revealed that at lay the average weight of snail egg ranges from 1.62 to 2.02 g (mean of 1.82 g). The egg lengths and widths ranges from 12.60 - 17.20 mm (mean of 14.90 mm) and 9.40 to 12.2 mm (mean of 10.80 mm) respectively. The shape of the eggs were spherical and yellowish in colour, which slightly fade away with time. A reduction in average weight of A. marginata egg from 1.83g to 0.73g was observed as days progressed. The metabolites (glucose, protein, cholesterol and triglycerides) and minerals (Mg2+, Ca2+, P and Fe) of the eggshell and egg yolk during gestation period was also determined. There was an increase in the metabolites and the minerals from day 1 to day 14, with a sharp drop on day 21. The values in metabolite and mineral compositions are highest on day 14. The quality of the eggshell and yolk can be associated with the biochemical composition, which can affect the survival of the organism. This study therefore inferred that the metabolite and minerals observed are required for proper development and growth of the embryo.

Palabras Clave

Cáscara de huevo.
Yema.
Archachatina marginata.
Metabolitos.
Minerales.

**Composición bioquímica de la cáscara y la yema de huevo del caracol terrestre gigante africano (Archachatina marginata) durante el período de gestación**

## RESUMEN

La cáscara del huevo es una estructura altamente especializada en minerales, que protege el contenido del huevo, nutre al embrión y lo protege de los ataques microbianos al servir como sistema inmunológico. Veinte caracoles A. marginata maduros con un peso de 125,75 g a 134,85 g se utilizaron para producir los huevos para este estudio. Los resultados revelaron que en la puesta el peso promedio del huevo de caracol oscila entre 1,62 y 2,02 g (media de 1,82 g). Las longitudes y anchuras de los huevos oscilan entre 12,60 y 17,20 mm (media de 14,90 mm) y entre 9,40 y 12,2 mm (media de 10,80 mm) respectivamente. La forma de los huevos era esférica y de color amarillento, que se desvanece ligeramente con el tiempo. Se observó una reducción en el peso promedio del huevo de A. marginata de 1,83 g a 0,73 g a medida que avanzaban los días. También se determinaron los metabolitos (glucosa, proteína, colesterol y triglicéridos) y minerales (Mg2+, Ca2+, P y Fe) de la cáscara y la yema de huevo durante el período de gestación. Hubo un aumento de los metabolitos y los minerales desde el día 1 hasta el día 14, con una fuerte caída el día 21. Los valores en las composiciones de metabolitos y minerales son más altos en el día 14. La calidad de la cáscara y la yema de huevo puede estar asociada con la composición bioquímica, lo que puede afectar la supervivencia del organismo. Por lo tanto, este estudio infiere que los metabolitos y minerales observados son necesarios para el correcto desarrollo y crecimiento del embrión.

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egbebijoseph1@gmail.com

## INTRODUCTION

Snails lay eggs after copulation with variation in weight and number of eggs laid among species. Clutch size in *Archachatina marginata* can range from 2 to 12 with large size eggs (Akinnusi, 1998). Eggs consist of eggshell, yolk and albumen. The eggs of *A. marginata* can be spherical, translucent and yellowish in color (Okon and Ibom, 2012) or spherical and cream yellow in color (Ogogo, 2004) or chalky white (Raut and Barker, 2002). The weight of the eggs of giant African land snail ranges from 1.54 - 2.45 g with the capability of laying 4 -18 eggs in 1- 2 minutes (Okon *et al.*, 2013).

Everything required by an embryo for safe development through to hatching in harsh environmental conditions are contained within the eggshell, which is a complex bio-mineral that superposes functionality, mechanical stiffness, and aesthetic appearance (Inta *et al.*, 2021). The eggshell of giant African land snails is a

highly specialized mineralized structure, comprised of an inner and outer membranes and composed mainly of proteins and glycoproteins, and minerals mainly of the calcite polymorph of $CaCO_3$ (Chien, *et al.,* 2008; Kristl *et al.,* 2019), containing 84.33% $CaCO_3$ content (Inta *et al.* 2021). Many internal and external factors including oviposition, age, and genotype as well as housing system, nutrition, microclimate, etc. affects the quality of eggshell (Ketta and Tumova, 2016).

The major constituents of the yolk are proteins and lipids, which is present mainly in the form of lipoproteins and contains minerals and carbohydrates, most of which are oligosaccharides bound to protein, as well as pigment (Sugino *et al.,* 1997). The biological activities of egg yolk components are the antimicrobial activity, antiadhesive and antioxidant properties, nutrient bioavailability and health and development (Kovacs-Nolan *et al.,* 2005).

Since all the aforementioned biochemical components and activities affects the survival of the organism, it is imperative to investigate the mineral contents (Calcium, Phosphorus, Magnesium, and Iron), metabolite composition and lipid profile of the eggshell and egg yolk of snail.

## MATERIALS AND METHODS

The study was carried out at the Snailery Unit of the Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (7º2′N 3º4′E). The fertile fresh eggs (**figure I**) used in this study were laid by twenty (20) matured giant African land snails *A. marginata* (weight ranges from 125.75g to 134.85g). The snails were purchased from Forestry and Wildlife (FWM) department, FUNAAB, Abeokuta, Ogun State, Nigeria.

The eggs were handpicked and taken to the laboratory. The soils sticking to the eggs were carefully removed. They were also sorted against cracks and morphological defects. Data collected were egg weight (g), egg length (mm) and egg width (mm). A sensitive weighing scale (Mettler PM 11-K) was used to measure egg weight, while Vernier Caliper was used to measure length and width. The egg colour was observed using simple colour chat.

The data of eggs for day 1 was taken and then cracked by hand and the shells were separated from egg white, egg yolk, and the inner membranes. The remaining eggs were then placed in an incubation chamber filled with loamy soil and maintained at moderate soil temperature range from 25˚C to 29˚C, relative humidity range from 70% - 80% for proper development of the snail eggs. The eggs were later removed from the soil at seven (7) days interval, following the procedures for day 1 to study the shell's proximate analysis and the yolk for biochemical analysis.

The minerals (Calcium, Phosphorus, Magnesium, and Iron) composition was determined according to the methods of Association of Official Analytical Chemist A.O.A.C. (2019).

**Figure I**: Eggs of A. marginata at lay (1st day).

Total protein was analysed by Biuret method (Henry *et al.,* 1977), while glucose was determined calorimetrically (Baumniger, 1974). Lipid profile (cholesterol and triglyceride) was assayed by method of Grant (1987).

Data collected from experiments were analyzed using One-way analysis of variance (ANOVA) and separation of significant means was done by Duncan Multiple Range. P-value was set at 0.05.

## RESULTS

The eggs (**figure I**) laid were spherical and yellow in color. The mean egg weight at lay obtained in the study is 1.82 g, range (1.62 - 2.02g) (**table I**). The mean egg weight decreased nonetheless with increase in day (**table II**).

There is a reduction in average weight of *A. marginata* egg from 1.83g to 0.73g as the days progressed. A change in colour was also observed from yellow on day 0 and day 7 to yellow and white on day 14 and finally white on day 28, as the yellow colour has completely faded (**table II**).

*A. marginata* egg yolk shows variation in the concentration of the metabolite content (protein, carbohydrate, cholesterol and triglyceride) (**table III**). There is decrease in the total protein from 5.3g to 3.5g, the

**Table I.** Egg characters of *A. marginata* eggs (Caracteres de los huevos de A. marginata).

| Characters | Mean values/Range |
|---|---|
| Average weight (g) at lay | 1.82 (1.62 - 2.02) |
| Average length (mm) at lay | 14.90 (12.60 - 17.2) |
| Average width (mm) at lay | 10.80 (9.40 - 12.2) |

**Table II.** Morphometric and color variation of *A. marginata* eggs during experimental days ( Variación morfométrica y de color de huevos de A. marginata durante días experimentales).

| Experimental Days | 1 | 7 | 14 | 21 | 28 |
|---|---|---|---|---|---|
| Average weight (g) | 1.82 | 1.83 | 1.66 | 1.14 | 0.73 |
| Colour of eggs | yellow | yellow | Partly yellow and white | Yellow fades and becomes whiter | White |

glucose increases from 4g to 11.4g on day 14 then decrease to 10.7g on day 21. The cholesterol (22.6g, 44.6g, 52.7g, 24.7g) and triglyceride (10.7g, 38.5g, 65.2g, and 42g) also showed similar increase from day 1 to day 14 then a drop on day 21 respectively (**table III**).

The *A. marginata* eggshell contains the following mineral; calcium, iron, magnesium and phosphorus (**table IV**). Day 14 compared to other days of the experimentation has the highest value for each mineral concentration with calcium 169.59mg/100 being the highest followed by Phosphorus, 48.63mg/100, then Magnesium 29.66mg/100 and Iron 7.87mg/100 (**table IV)**.

## DISCUSSION

The shape and colour of the eggs obtained in this study was spherical and yellow at lay. This is similar to the observations of Okon *et al.* (2013). The colour gradually fade away with time. The mean egg weight decrease in weight. This is because of the gradual metamorphosing of the egg liquid mass into prospective hatchlings, consequently leading to a decreased egg weight (Ibom, 2009; Okon *et al.*, 2013). It could also be due to transpiration because of inconsistence in environmental temperature as oppose to the very close persistent uterine environment of the egg (Ibom, 2009). The sharp drop or depletion in metabolites can be due to the requirement by the imminent embryo, which according to Kovacs-Nolan *et al*. (2005) are used provide immunity against microbial attacks and nourishment for the embryo. The yolk therefore serves as an immune system that shields the embryo from microbial attacks and albumen contains the nutrients needed to nurture the embryo. The yolk of the egg contains varying concentration of protein, glucose, cholesterol and triglycerides. The total protein and cholesterol decreases with days, which might be due to development of the embryo as it feeds from the contents in the egg. This correlate with the study of Ogunwole *et al*. (2015) that albumin contains protein and cholesterol, which the embryo feeds from during development.

**Table III.** Metabolite Contents of *A. marginata* egg yolk during experimental days ( Contenido de metabolitos de la yema de huevo de A. marginata durante los días experimentales).

| METABOLITE CONTENTS (g/100g) | EXPERIMENTAL DAYS | | | |
|---|---|---|---|---|
| | 1 | 7 | 14 | 21 |
| TOTAL PROTEIN | $5.3^{a}$ | $4.0^{b}$ | $5.3^{a}$ | $3.5^{c}$ |
| GLUCOSE | $4^{d}$ | $7.4^{c}$ | $11.4^{a}$ | $10.7^{b}$ |
| CHOLESTEROL | $22.6^{d}$ | $44.6^{b}$ | $52.7^{a}$ | $24.7^{c}$ |
| TRIGLYCERIDE | $10.7^{d}$ | $38.4^{b}$ | $65.2^{a}$ | $42^{c}$ |

$^{abcd}$Means in the same row having different superscripts are significantly different (P<0.05)

**Table IV.** Mineral Composition of *A. marginata* eggshell during experimental days (Composición mineral de la cáscara de huevo de A. marginata durante días experimentales).

| Mineral Composition (mg/100) | EXPERIMENTAL DAYS | | | |
|---|---|---|---|---|
| | 1 | 7 | 14 | 21 |
| Calcium | $108.28^{d}$ | $154.17^{b}$ | $169.59^{a}$ | $134.9^{c}$ |
| Phosphorus | $38.15^{c}$ | $31.98^{d}$ | $55.29^{a}$ | $48.63^{b}$ |
| Magnesium | $22.61^{c}$ | $14.69^{d}$ | $29.66^{a}$ | $27.23^{b}$ |
| Iron | $2.85^{d}$ | $5.13^{c}$ | $7.87^{a}$ | $5.61^{b}$ |

$^{abcd}$Means in the same row having different superscripts are significantly different (P<0.05)

The eggshell contains calcium, iron, magnesium and phosphorus. Correspondingly, Anthony (2011) documented it that the eggshell is composed of approximately 98.2% Calcium carbonate, 0.9% Magnesium and 0.9% Phosphorus documented it. Calcium and phosphorus are the highest observed minerals, which suggests the durable characteristics of the shell such as strength, resistivity and impermeability. This supports the findings of Inta *et al.*, (2021) that the concentration of calcium carbonate was the highest in eggshell among other component and as documented in the review of Anthony (2011) this constitutes about 90% of the shell component. Calcium is an important mineral for shell production. A lack of calcium according to Ademolu *et al.*, (2013) can cause the formation of thin and transparent egg*shell* as well as a stunted growth and infertility in snails.

## CONCLUSION

The mean egg weight of giant African land snail, *Archachatina marginata* reduces as incubation period increases. The eggs shape are spherical and yellowish in color. The result of this experiment revealed that snail egg (shell and yolk) varies in composition with time because of snail development. This is expected because the biochemical required for the maintenance of various biological systems activities in the egg such as development and the successful survival of the organism decreases. A good quality of the eggshell and yolk is associated with the biochemical composition, which affect the survival of the organism. The metabolite and minerals observed are therefore necessary for proper development and growth of the snail embryo.

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