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ORIGINAL RESEARCH ARTICLE

Morpho-Anatomical Changes, Calcium and Glycolipid Deposition in the Gut of *Archachatina marginata* During Starvation

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ABSTRACT

The gut morpho-anatomy and physiology are related to the nature of diet and feeding pattern. The morpho-anatomical parameters, calcium and glycolipid deposition in the gut were determined in 0, 4 and 8 weeks starved giant African land snails, Archachatina marginata. A total of Forty-five (45) apparently healthy and matured snails (Archachatina marginata) with weight range of 150 – 250 g, laid out in completely randomized design, with 3 treatments having 3 replicates (5 snails per replicate) were utilised in this experiment. The organs of study for morpho-anatomical and histological dynamics were stomach and intestine. They were observed for calcium and glycolipid deposition using the Von Kossa (VK) and the Periodic Acid Schiff (PAS) stain respectively over the varied lengths of starvation. Data from morpho-anatomical parameters such as (list of indices) were subjected to Analysis of Variance (ANOVA) using the Genstat package (12th edition) and means were separated using Duncan Multiple Range Test, where significant differences ($p<0.05$) existed. In morpho-anatomical parameters, intestines increase in length at the 4th weeks of starvation, while stomach decrease in weight from 0 – 8 weeks of starvation. Presence of calcium deposit in the intestinal tissue and in the stomach tissue was not seen at 0 week of starvation which represent the non-starved period. Deposition of calcium progressively increases over the varied starvation lengths of 4th weeks and 8th weeks in the mucosa, submucosa and muscularis externa of the stomach and intestinal tissue. The intestinal and stomach deposition of glycolipid was however consistent over the starvation lengths of 0 week, 4th weeks and 8th weeks. Starvation of snails during the breeding season did not lower the presence of calcium and glycolipids in intestinal and stomach tissues.

Keywords: *Feed deprivation, histochemical, morpho-anatomical, digestive, glycolipids*

INTRODUCTION

Giant African land snails (*Archachatina marginata*) are invertebrates that have a soft body and a covering of hard shell. *A. marginata* are the second largest snail and most popular species of snail kept and reared in Nigeria (Okon *et al.*, 2012; Akanni and Akinnusi, 2013). Certain prevailing condition can initiate starvation or fasting in the snail, and this could impact on its biological performance.

Starvation is the biological condition when animals are unable to feed due to an extrinsic limitation on their food supply, however fasting means that the animals cannot eat due to intrinsic factor, while food is available (Ahmed *et al.,* 2016). This is characterized by inactivity and a lowered metabolic rate (Miller, 2007). When this naturally occurs. It is referred to as aestivation which is a programmed fasting in GALS (Omoyakhi, 2007). Meanwhile, inability to eat either in fasting and/or starvation could affect morpho-anatomical parameters, calcium and glycolipid deposition of the digestive tract in animals.

Both intracellular and extracellular mechanisms of calcification are found in land gastropods. The deposition of the body shell is one of the more investigated processes of extracellular calcification and a great wealth of details has been exposed about shell structure, formation and regeneration (Fournie and Chetail, 1984). In land gastropods intracellular calcification occurs in calcium cells located in the digestive gland and in several parts of the connective tissue.
Calcium cells have been described in all species of land gastropods whose digestive glands have been investigated (Fournie and Chetail, 1982). It seems most likely that their existence is a general feature in Stylommatophora. These calcium cells belong to a particular type of epithelial cells lying between the digestive and excretory cells of the acinar epithelium of the gland. Also,
lipids are an integral part of molluskan tissues. However, almost all data included in molluskan lipid study concern the entire organism and only scanty reports on the anatomical distribution of total lipids are available (Rakshit *et al*., 1997; Misra *et al*., 2002). Meanwhile, many mollusks store complex carbohydrates in the form of glycogen, in the mantle and adductor muscle while lipids are accumulated in the digestive gland (Napolitano and Ackman, 1992).
The need for calcium which is major component of the shell formation and glycolipid which form the basis for energy requirement in snails have been ascertained in active snails. Little is known on the availability of calcium and glycolipid in snails during starvation. The present study aimed at obtaining comprehensive knowledge on calcium and glycolipid distribution in tissues of the gut: stomach and intestine of the giant African land snail, *Archachatina marginata* over varied lengths of starvation.

MATERIALS AND METHODS

Location and Experimental Animals

The research was carried out at the University of Benin Teaching and Research Farm, University of Benin, Edo State, Nigeria. Benin City is within the tropical rain forest vegetation zone of Southern Nigeria, lying between longitude 5ºE and 6º42'E and latitude 5º45 and 7º34'N of the equator (FAAN, 2018). The climate of Edo is humid. On the north, Edo is bounded by Kogi State, to the east by Anambra State, south by Delta State and west by Ondo State. The histological procedures and microscopic examination of the tissues were carried out at the University of Benin Teaching Hospital and Animal Science Laboratory, University of Benin, Benin City respectively.

Experimental Design and Induction of Starvation

The experiment was conducted using 45 apparently healthy snails, laid out in a completely randomized design, consisting of three (3) treatments, replicated three times with 5 snails per replicate. Snails were induced to starve under prevailing atmospheric conditions by withdrawal of feed and water. Snails in the control group were fed and watered thoughout the experiment while snails in groups 4 and 8 were subjected to a period of starvation for 4 and 8 weeks respectively. This study was carried out between the months of March to May.

Tissue collection and dissection procedures

Snail shells were cleaned thoroughly to remove the adhering water and dissected to remove the stomach and intestine according to the procedures outlined by Segun (1975). The morho-anatomical parameters, which are weight, length, volume and density, were then measured appropriately. The weight was measured using an electronic scale, volume by water displaced method and density calculated using the weight and volume measured.

Histochemical examination

Dissection was by procedures described by Segun (1975). Pieces of organs were promptly and adequately fixed chemically as soon as the organs were detached using 10 % neutral buffered formolseline (Junqueira and Carneiro, 2003). With the aid of an automatic tissue processor (Zeiss Axiostop 40), the tissues were dehydrated and eventually cleared in two changes of xylene at room temperature. Further processing of the tissue followed the sequence of impregnation, embedding (using Leica EG1116), and sectioning (with the aid of a microtome, Leica RM 2235). After this, sections were stained using Von Kossa and Periodic Acid Schiff (often abbreviated VK and PAS respectively) procedures to demonstrate respective calcium and glycolipid deposition (Burkitt *et al.,* 1999). This step was followed by mounting and labeling. The processed slides were examined with the aid of light microscope using a low power magnification (X20). The examined slides were photomicrographed (Celestron Penta View) and presented as such.

RESULTS

Morpho-anatomical and Histochemistry of Starved Snails

Table 1 and 2 shows the morpho-anatomical parameters of the stomach and intestine of *A. marginata* under varied starvation period. There were significant variations in some morpho-anatomical parameters in both stomach and intestine. There was decrease in weight of the stomach from week 0 to week 4, after which the weight stabilised. However, but no significant changes occurred in length. The result on intestinal weight (Table 2) showed no significant changes from 0 to 8 weeks of starvation, but difference was seen in length with value at 4th week of starvation being higher than either of week 0 and week 8. .

Figure 1 and 2 shows the photomicrograph of the stomach and intestine of *A. marginata* over the varied starvation periods. There was no trace of Calcium deposit in 0 week of starvation. However, at 4th week of starvation, calcium deposit which is represented by black stain on the microgragh were noticed in the mucosa, submucosa and muscularis externa of stomach and intestine, represented by the dark coloured stain. At 8 week with Alizarin red, a higher calcium deposit was noticed. Figure 3 and 4 shows the photomicrograph of the stomach and intestine of *A. marginata* over starvation periods. The purple stain noticed represent the consistent presence of glycolipid in the tissue of gut section examined throughout the extended starvation length. It was noticed acrose the 0, 4 and 8 weeks of starvation periods.

Table 1: Morpho-anatomical parameters of the stomach under varied starvation length of *Archachatina marginata*

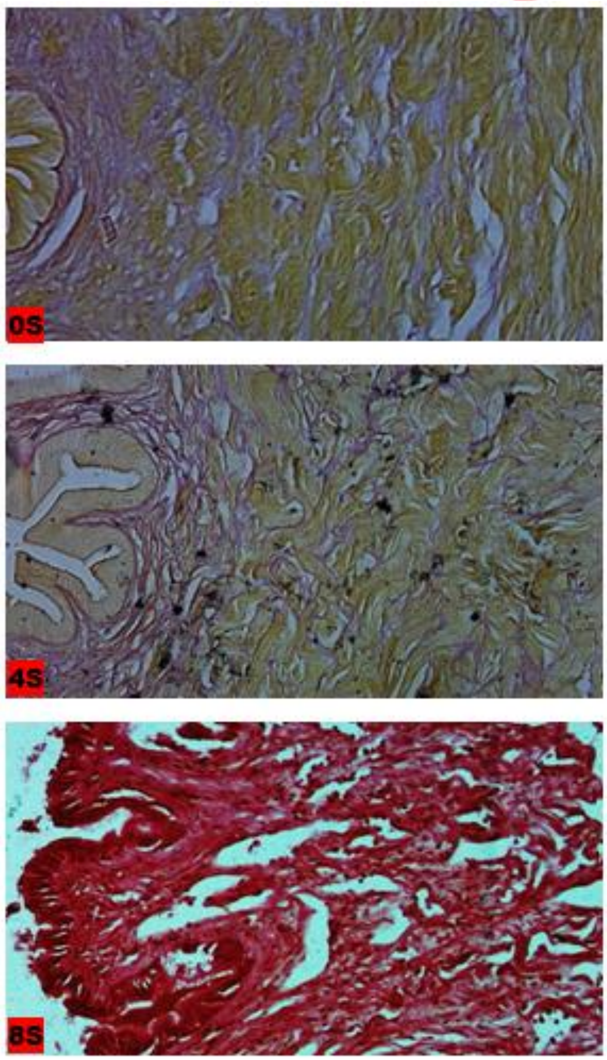
	Starvation Length (Weeks)			±SEM
	0	4	8	
Stomach length (cm)	1.85	1.50	1.75	0.14
Stomach weight (g)	1.90^{a}	1.35^{b}	1.35^{b}	0.10
Stomach volume (mL)	3.97^{a}	0.97^{c}	1.97^{b}	0.03
Stomach density (g/mL)	0.48^{c}	1.35^{a}	0.68^{b}	0.03

^{abc} means in the same row with different superscripts are significantly different (P<0.05)
SEM = Standard Error of Mean

Table 2: Morpho-anatomical parameters of the intestine under varied starvation length of *Archachatina marginata*

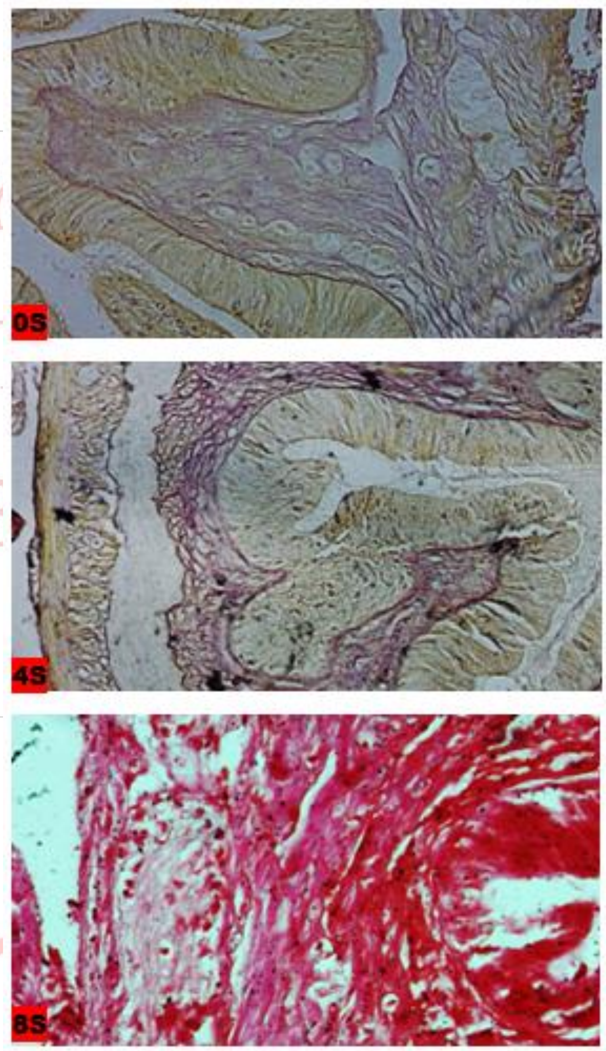
	Starvation Length (Weeks)			±SEM
	0	4	8	
Intestine length (cm)	2.85^{b}	3.80^{a}	2.45^{b}	0.24
Intestine weight (g)	0.30	0.30	0.20	0.03
Intestine volume (mL)	1.00^{b}	0.30^{a}	1.00^{b}	0.03
Intestine density (g/mL)	0.30^{b}	1.08^{a}	0.20^{b}	0.13

^{ab} means in the same row with different superscripts are significantly different ($P<0.05$)
SEM = Standard Error of Mean



STOMACH

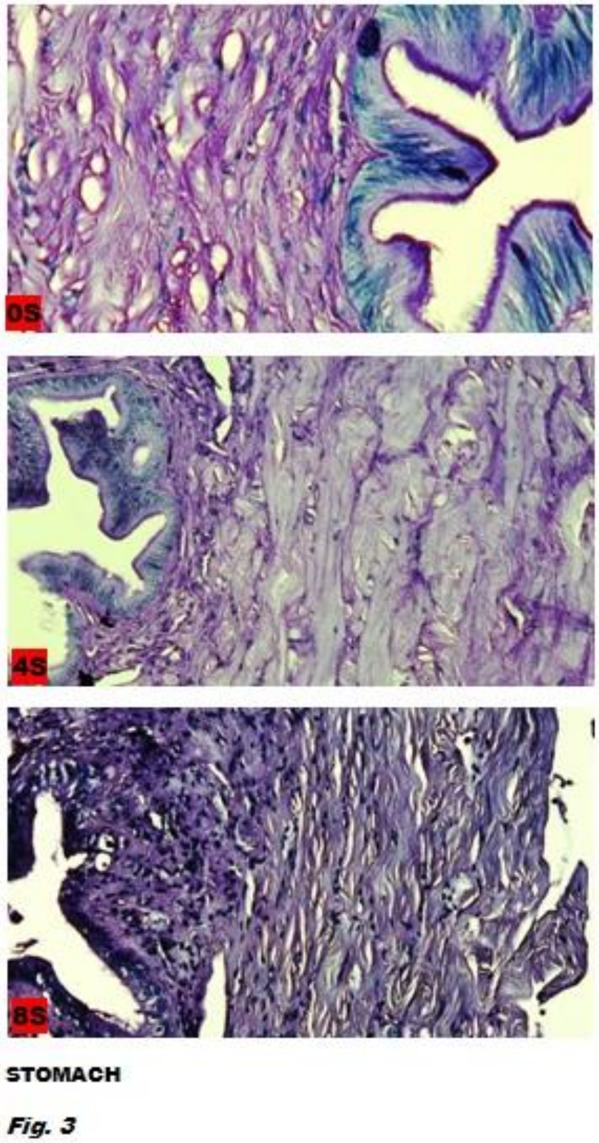
Fig. 1



INTESTINE

Fig. 2

Figure 1 and 2 shows the transverse photomicrograph histological dynamic of the Stomach and Intestine stained with Von Kossa (0 and 4S) and Alizarin Red (8S) viewed at X10 magnification of *A. marginata* over the varied starvation length.0S= 0 week of starvation, 4S=fourth week of starvation, 8S=eight week of starvation.



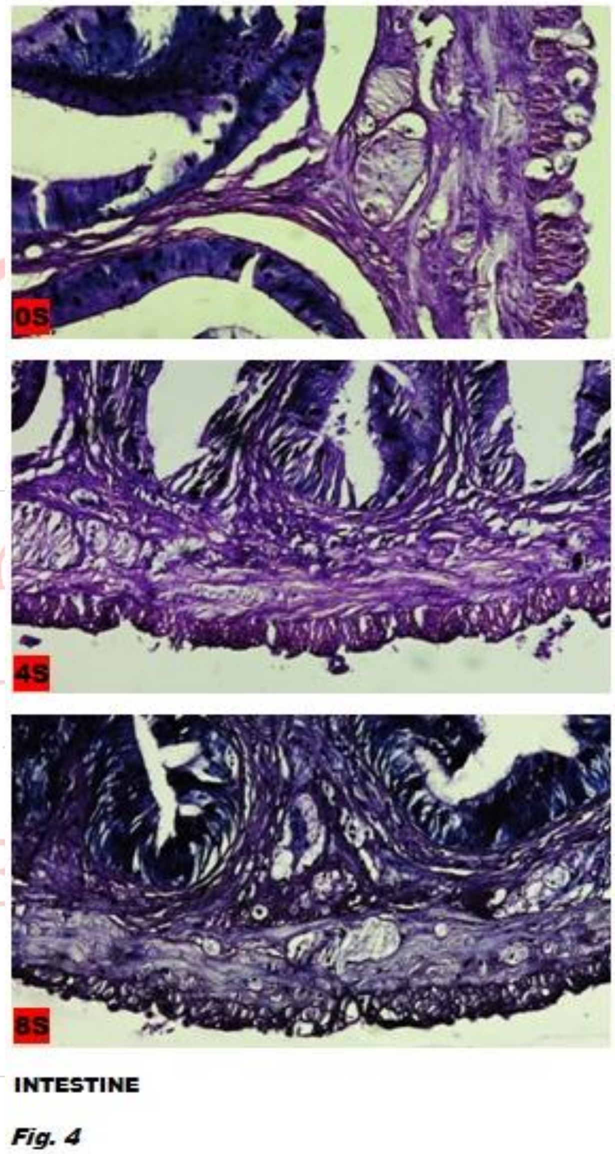


Figure 3 and 4 shows the transverse photomicrograph histological dynamic of the Stomach and Intestine stained with Periodic Acid-Schiff (PAS) viewed at X10 magnification of *A. marginata* over the varied starvation length.0S= 0 week of starvation, 4S=fourth week of starvation, 8S=eight week of starvation

DISCUSSION AND CONCLUSION

There are reports suggesting that animal guts show morpho-anatomical and a physiological adaptation to the diet (Charrier and Brune, 2003). Starvation did not alter the length of the stomach but the weight decreased between 0 and 4 weeks of starvation. The intestinal length was however highest at 4th week of starvation while no changes was recorded in weight. The progressive decrease recorded in the stomach could be as result of tissue atrophy as the stored energy in the tissue is been used by the snails. To corroborate with findings in the study as regard the changes that were not recorded, Makovicky *et al*. (2014) stated that the length of the small intestine which is a component of the gut recorded no change following intermittent starvation in rabbit which may have different physiological adaptation.

Achatina fulica (*Archachatina marginata* inclusive) are herbivorous pulmunate gastropod. The calcium, glycogen and lipid classes in these snails are probably exogenous in nature (RakshitAbrol *et al.*, 2014). Therefore deprivation of feed could result in the lowering of these basic nutrients in snails. Starvation experienced during aestivation could make snails to make a preparatory need for these nutrients. According to previous research work, lipid and glycogen metabolism provides energy during aestivation in gastropod *Pilaglobosa* (Mitra and Sur, 1989) which is also a period of feed deprivation experienced during starvation.

Biosynthesis of lipids in active mollusks has been established, but in aestivating snails information is scanty. Studies on metabolic adaptation in aestivating *Achatina achatina* revealed that glycogen degradation and lipid mobilization takes place in foot, digestive gland and heart muscle at the beginning of aestivation in short-spell forced starved and aestivating snails.. Such mechanism also takes place in other pulmonate (Van der Horst, 1974).Figure 1 and 2 shows the stomach and intestine respectively of *A. marginata* stained Von kossa to outline the calcium deposition in the tissues. The trace of calcium deposit as depicted by dark dotted stain on the photomicrograph was minimal at 0 week (non-starved) of starvation in the stomach and the same was observed in the intestine. This phenomenon could be as a result of the immediate utilization of the exogenous calcium gotten from feed. This however changed at 4 weeks of starvation with gradual calcium deposit seen at the mucosa, sub mucosa and muscularis externa layers of the organs. This could be associated with the need for calcium for embryonic egg shell formation as the research coincided with the breeding season.

At 8 weeks of starvation, there was an upsurge in calcium deposit in both stomach and intestinal tissues. The increase deposition of Calcium in the tissue can also be as a result of the need for calcium in egg formation as this experiment falls into the breeding period of the hot wet and cold wet season. This increase in calcium deposition corresponds with the findings of Omoyakhi and Okhale (2015) who recorded Calcium deposit in the tissue during the period of aestivation in *Archchatina marginata*.

Glucose and lipid are regarded as an integral part of molluscan tissue. Many molluscan stored complex carbohydrates as glycogen, in the mantle and adductor muscle and lipid are accumulated in the digestive organs (Napolitano and Ackman, 1992). Figure 3 and 4 represent the photomicrograph of the stomach and intestine, showing the deposition of glycolipid in the tissues. The deposition of the glycolipid which is represented by the purple stain on the tissue is clearly visible throughout the starvation length, represented by the 0, 4 and 8 weeks. Glycolipids were evenly distributed in the stomach and intestinal tissue throughout the starvation lengths of 0, 4 and 8 weeks.

From this research, it can therefore be deduced that starvation selectively alters the various section of the gut and led to a greater deposition of calcium in the gastrointestinal tract, while glycolipids deposition were constant throughout the starvation. It can therefore be said that starvation did alter the tissue glycolipids of *A. marginata*.

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