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ORIGINAL RESEARCH ARTICLE

Gastrointestinal Microbial Assays of *Archachatina marginata* Under Varied Starvation Length

Oyedeji, A.O, *Omoyakhi, J.M. and Okhale, O.E

Department of Animal Science, Faculty of Agriculture,
University of Benin, Benin City, Nigeria

ABSTRACT

The nature of microbes in the gastrointestinal tract suggest the feeding habit of an animal. In this experiment, the gastrointestinal microbial assays were determined in giant African land snails, Archachatina marginata. The experiment was conducted using a total of forty-five (45) apparently healthy and matured snails (Archachatina marginata) with weight range of 150-250, laid out in completely randomized design, with 3 treatments having 3 replicates (5 snails per replicate). The treatment consisted of 0 week, 4 weeks and 8 weeks of starvation. The content of the gut (crop, stomach, intestine) were subjected to serial dilution technique and pour plating for microbial culture of bacteria and fungi using nutrient agar (NA) and potato dextrose agar (PDA) respectively as culture media. Bacteria were counted off the petri dish to determine the colony forming unit (CFU) which 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. Colony characterization and staining technique using lactophenol cotton blue was employed for bacteria and fungi identifications respectively. A progressive decline in CFU was observed from the 0 – 8 weeks of starvation. The CFU was highest in the intestine at 0, 4 and 8 weeks of starvation. The diversity of microbes also varies at starvation lengths. Staphylococcus spp and Enterobacter spp were encountered in the intestine throughout the starvation length. Lactobacillus spp occurred in all examined organs at 0 weeks (non starved snails). Escherichia coli was present in the stomach at 4 weeks of starvation. Aspergillus nudilans and Aspergillus niger were present in the stomach at 0 and 4 weeks of starvation while Aspergillus flavus was cultured in the stomach in nonstarved snails. Present study helps to understand complexity of bacteria and fungi community in snail gut when they are fed and deprived of feed for 0, 4 and 8 weeks.

Keywords: Starvation *Archachatina marginata*, Microbes, Gastrointestine

INTRODUCTION

Snail is a common name applied to most of the members of the molluscan class among the Gastropoda that have coiled shells in the adult stage. 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 kept and reared in Nigeria (Okon *et al.*, 2012; Akanni and Akinnusi, 2013). However, food and feeding condition could alter the microbial organisms inhabiting various organs of any biological specie, including the giant snail.

A microbial organism often called microbes, are microscopic living organisms, which may be single-celled, or multicellular (Madigan and Martinko, 2006). Microorganisms are very diverse and include all bacterial, archaea, most protozoa, some species of fungi and algae. Gastrointestinal microbiota is a complex community of microorganisms that live in the digestive tracts of humans and other animals, including insects. Snails contain a lot of organisms in its gut, and a variety of enzymes can be extracted and produced from it (Oyeleke *et al.,* 2012). The dependence of snails on microbial activity within their gut would explain their extraordinary efficiency in plant fibre digestion up to 60 – 80 percent (Oyeleke *et al.,* 2012). Animal guts would show morpho-anatomical and physiological adaptation to diet and this is especially obvious in primary consumers. Based on studies on chitinases of *Helix pomatia*, Charrier and Brune (2003) reported that , the digestion of plant components in the gut of all phytophagous snails could be attributed to their intestinal microbiota. Starvation and hibernation triggered a fall in bacterial counts, but a great diversity of bacteria survived in the snail gut during

these periods (Watkins and Simkiss, 1990; Charrier *et al.,* 2006). Studies has helped to understand the complexity of bacterial community in snail (*Cornu aspersum*) gut and their role in digestion of cellulose, pectin, lignin, containing plant constituents (Sayyad and Shaik, 2015). The aim of this study was to investigate the dynamic of the gut microbes of the snail *Archachatina marginata* during the different starvation lengths.

MATERIALS AND METHODS

Location and experimental animals

The research work was carried out at the University Benin Teaching and Research Farm, University of Benin, Edo State, Nigeria. 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. Benin City is located within the tropical rain forest vegetation zone of Southern Nigeria. It is located within longitude 5°E and 6°42'E and latitude 5°45 and 7°34'N of the equator (FAAN, 2018). It is bounded on the north by Kogi State, to the east by Anambra State, south by Delta State and west by Ondo State. The climate of Edo is humid. The climate of Edo State is tropical. It has two distinct climatic seasons the rainy and the dry seasons. The rainy season is between April and October. Average rainfall is between 150cm in the far north of the state to 250cm in the south. The average temperature ranges between 25°C in the rainy season and 28°C in the dry season. Generally, the climate is humid tropical in the southern areas of the state and sub-humid in the north

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 in this study by withdrawal of feed and water. This study was carried out between the months of March to May.

Microbiological Test

Snails were sacrificed at 0 week starvation (0S), 4th weeks starvation (4S), 8th weeks starvation (8S) and the gut cut open, contents of the crop, stomach and intestine were immediately collected into sterilized test-tubes that were appropriately labeled. Equal volume of peptone water was added to each test-tube to make the stock solution for bacterial population determination. Nutrient agar and potato dextrose agar were used as the medium of bacteria and fungi culture respectively. Disposable petri dishes were used to culture the bacteria in the incubator at 37 ºC and total viable bacterial colony count was determined after 24 hours. The bacterial isolates were characterized based on colonial morphology, cultural characteristics and biochemical tests as described by Oyeleke and Manga (2008). For fungi, small portion of the mycelia growth were carefully picked with the aid of a pair of sterile inoculating needles and placed in a drop of lactophenol cotton blue on a microscope slide and covered with a cover slip. The slides were examined under the microscope, first with (x4) and then with (x10), (x20), objective lens for morphological examination described by Oyeleke and Manga (2008).

RESULTS AND DISCUSSION

Bacterial colony forming unit (CFU) of *Archachatina marginata* under varied starvation length

It is assumed that the different region of the gastrointestinal tract which include crop, stomach and intestine are highly specialized compartments which could have distinct independent role to play in digestion. The result from the experiment shows that the gut of the *A. marginata* harbours bacteria (Table 1). Although not statistically significant, the total bacterial colony forming unit (CFU), representing the total viable bacterial population distribution across the gut was highest in the intestine followed by the stomach and the crop at 0 week of starvation. Quantitative real-time polymerase chain reaction (qPCR) analysis indicated significant variation in bacteria load in different gastrointestinal tract region of active and non-active snails (Parwar *et al.,* 2012). It was noted by this author that the gastrointestinal (GI) tract of invasive land snail *Achatina fulica* is known to harbor metabolically active bacterial communities and this is abundant across the gastrointestinal tract at 11 %, 16 % and 22 % sequences in respective crop, stomach and intestine. This finding corresponds with the dynamic recorded at 4 and 8 weeks of starvation with the highest CFU of bacteria recorded in the intestine in the current study.

The gastrointestinal tract colony forming units (CFU) were in their highest at 0 week of starvation when compared with 4 and 8 weeks of starvation as a significant drop in bacterial load was noticed. This result also corroborate with the findings of Parwar *et al*. (2012) recording a decrease in the bacterial load in the intestine, rectum, crop, stomach and oesophagus when snails (*Archatina fulica*) entered aestivation. Feed limitation is a physiological challenge being faced by some animals that may alter their gut microbiota as regard to microbial diversity and relative abundances (Kohl *et al.,* 2012).

Table 1: Bacterial colony forming unit (CFU) of *Archachatina marginata* under varied starvation length

	Starvation Length (Weeks)			±SEM
	0	4	8	
Crop (x10^{6}CFU/mL)	20.12^a	11.12^b	1.88^c	2.21
Stomach (x10^{6}CFU/mL)	35.00^a	12.71^b	1.86^b	5.31
Intestine (x10^{6}CFU/mL)	77.00^a	23.50^b	4.12^b	8.46

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

Isolate of Bacteria in Gut (Crop, Stomach and Intestine) of *A. Marginata*

The result from the isolate of bacteria in gut (crop, stomach and intestine) of *A. marginata* (Table 2, 3 and 4) shows that the highest speciesof bacteria at 0 week of starvation when compared with the 4 and 8 weeks of starvation respectively. The abundance of bacterial diversity at 0 week of starvation may be as a result of the important role they play in digestion of plant fibre and other materials from their feed which was absent at 4 and 8 weeks of starvation thereby reducing the gut microbial content concentration. Boulange *et al*. (2016) stated that gut ecosystem alteration could trigger a wide range of physiological disorders, low-grade inflammation inclusively, and excess lipid accumulation and metabolic disorders. *Staphylococcus spp* and *Enterobacter spp* isolate were widely distributed in the intestine across the varied starvation length of 0, 4 and 8 weeks while *Acinebacter spp* isolate was only recorded at 0 week of starvation. Distribution of *Escherichia coli* was however restricted to the stomach of the snails at 4th weeks of starvation. The *Lactobacillus spp* isolate were thoroughly distributed across the different regions of the gastrointestinal tract which are the crop, stomach and the intestine at 0 week of starvation while an isolate was recorded in the intestine at the 4th weeks of starvation. The wide range distribution of *Lactobacillus spp* throughout the gastrointestinal tract at 0 week of starvation might be symptomatic of the feeding habit of snails (Parwar *et al.,* 2012) because they are crucial for the breakdown of cellulose in plants feed. However, their availability seems altered during starvation period in this experiment. Another apparent feature of the bacterial communities is the distribution of *Streptomyces spp* in the stomach of *A. marginata* at 0 and 4 weeks of starvation.

Table 2: Bacterial isolates cultured from the crop of *Archachatina marginata* under varied starvation length

Bacteria Isolates	Starvation Length		
	0A	4A	8A
Staphylococcus spp	-	-	-
Acinetobacter spp	-	-	-
Enterobacter spp	-	-	-
Escherichia coli	-	-	-
Lactobacillus spp	+	+	-
Streptomyces spp	+	+	-

A = 0 week of starvation 4A = 4th weeks of starvation 8A = 8th weeks of starvation, + = present (recorded)

Table 3: Bacterial isolates present in the Intestine of *Archachatina marginata* under varied starvation length

Bacteria Isolates	Starvation Length		
	0A	4A	8A
Staphylococcus spp	+	+	+
Acinetobacter spp	+	-	-
Enterobacter spp	+	+	-
Escherichia coli	-	-	+
Lactobacillus spp	+	-	-
Streptomyces spp	-	-	-

A = 0 week of starvation 4A = 4th weeks of starvation 8A = 8th weeks of starvation, + = present (recorded)

Table 4: Bacterial isolates cultured from the Stomach of *Archachatina marginata* under varied starvation length

Bacteria Isolates	Starvation Length		
	0A	4A	8A
Staphylococcus spp	-	-	-
Acinetobacter spp	-	-	-
Enterobacter spp	-	-	-
Escherichia coli	-	+	-
Lactobacillus spp	+	-	-
Streptomyces spp	-	-	-

A = 0 week of starvation 4A = 4th weeks of starvation 8A = 8th weeks of starvation, + = present (recorded)

From Figure 1, different fungi isolates were cultured from the gastrointestinal tract of *A. marginata* over the varied length of starvation. The fungi isolates were more prevalent in the intestine throughout the starvation period, with occurrence of *Cladosporium spp* and *Aspergillus flavus* at 0 week, *Fusarium spp* at the 8 weeks, *Aspergillus nudilans* at the 0, 4 and 8 weeks and *Aspergillus niger* at the 0 and 4 weeks of starvation. Oyeleke *et al*. (2012) successfully isolated *spergillus flavus*, *Fusarium spp* and *Aspergillus niger* from the gut of *Archachatina marginata*. The commercial production of enzymes such as cellulase and protease which are derivatives of *Aspergillus niger* isolated from gut of *A. marginata* can be exploited (Oyeleke *et al.,* 2012). *Trichophyton spp* was however distributed across the gastrointestinal tract (crop, stomach and intestine) of the snails at 8 weeks of starvation.

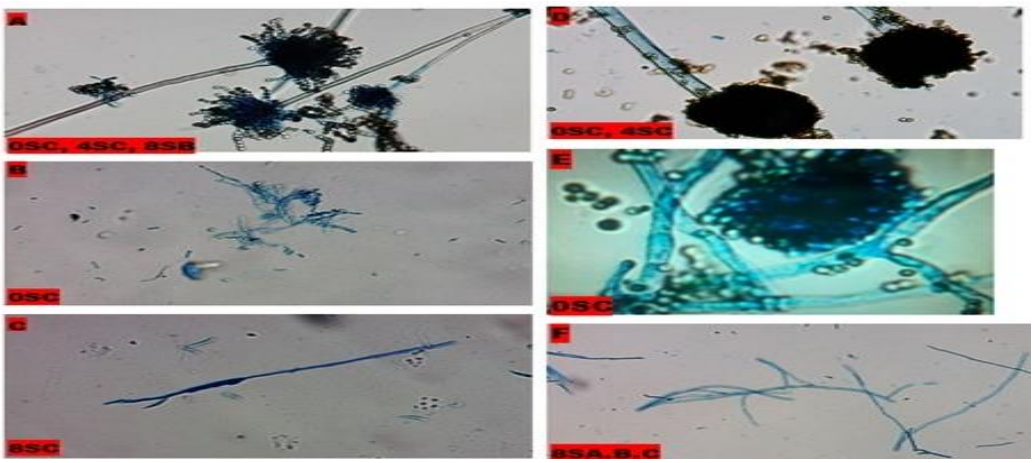

Figure 1 shows the photomicrograph of Fungi isolates across the gut of *A. marginata* stained with Lactophenol cotton blue viewed at X10 (A, B, C, F) and X20 (D, E) magnification. 0SC=0 week of starvation (intestine), 4SC=4th week of starvation (intestine), 8SB=eight week of starvation (stomach), 8SC=8th week of starvation (intestine), 8SA,B,C=eight week of starvation (crop, stomach, intestine), A=*Aspergillus nudilans*, B=*Cladosporium spp*, C=*Fusarium spp*, D=*Aspergillus niger*, E=*Aspergillus flavus*, F=*Trichophyton spp*

CONCLUSION

There is therefore overwhelming evidence suggesting a decrease in total microbial population and diversity (bacteria and fungi) in the gut of *A. marginata* as a result of starvation over an extended period of 8 weeks. These microbes may play major roles in the digestion of food which are basically plant materials. Absence of food materials during starvation drastically reduces the microbes.

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