



## Effects of Feeding Different Rations on Growth and Some Innate Immune Parameters in The Haemolymph of African Giant Land Snail (*Archachatina marginata*)

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### ABSTRACT

Nutritional status is recognized as a major factor that modulates immune responses, although interactions between specific nutrients and some immune responses are poorly understood. This study evaluated the effects of different plant feeds on protein coagulation and Phenoloxidase activities in the haemolymph of *Archachatina marginata*. Eighty grower of giant land snails, *Archachatina marginata* were grouped into treatments A, B, C and D based on feeding with standard feed, Carica papaya, Tridax procumbens, and Asclepsia carlifonia respectively. The Weight gain and growth parameters of experimental snails were monitored and measured for 4 weeks. The activities of some innate parameters after four weeks were determined through Phenoloxidase activity and protein coagulation in fractions of snails' haemolymph. The result showed that weight gain of plant fed snails is significantly ( $p < 0.05$ ) higher ranging from 7.11-26.41 g, compared to the standard (1.77 g). Similarly, the growth performance of plant fed snails, (4.20-14.92 cm) were high compared to the standard (3.05-12.77 cm). The protein coagulation in different fractions increased significantly, (0.03-0.32) compare to the standard (0.02-0.06). Likewise, significant increase in Phenoloxidase activity was observed in snails fed plants (0.02-0.08) compared to the standard (0.02). Results of this study suggests that the nutritional compositions of the feeds fed affects both the growth and the innate immune response of *A. marginata*.

**Keywords:** Growth, Parameters, *Archachatina marginata*, and nutrition,

### INTRODUCTION

*Archachatina marginata* (*A. marginata*) belongs to a group of animals (snail) called mollusks which are known to be naturally available (Yoloye, 1986). The species (black snails) is commonly found in the Southern part of Nigeria with tropical weather and vegetation conditions usually found in forest of the derived Guinea Savannah in West Africa (Odaibo, 1997). *A. marginata* consists of numerous extremely, close-set, criss-cross lines, making the surface of the periostracum looking as if it is pressed with a very finely woven cloth. It is used as food and medicine all over African (Monny, 1994). The meat is tasty, tender and highly nutritious, with fine texture, which make it generally accepted in many African countries (Agbogidi and Okonta, 2011). Studies have revealed that the meat of *A. marginata* is rich in iron and good quality protein with high levels of lysine, leucine, isoleucine and phenylalanine (Agbogidi et al, 2008). It is also reported in several studies that the meat of *A. marginata* is rich in Potassium (K), essential amino acids, vitamin C, B complex, but low in fat (Agbogidi

et al., 2008, Cobbinah, 2008, Baba and Adoleke, 2006). Traditionally, ortho-calcium phosphate extracted from the shell of snails was reported to cure kidney diseases, tuberculosis, anaemia, diabetics and asthma (Ademolu et al., 2006).

Generally, snails survive on most food taken by man and can also be fed on compounded and supplemented ration but predominantly, snails eat vegetables and can utilize a number of feeds for growth. Haemolymph is the fluid present in invertebrates meant purposely for circulation. It helps to remove waste materials, transport nutrients and clean the system of pathogens and debris, a process that is responsible for the innate immunity of the snail (Ogunsanmi et al., 2003). Innate immune system is the first line of inducible host defense against bacteria, fungi,

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and viral pathogens (Haebe *et al.*, 2004). The system is essential for the survival of snails because they lack immunoglobulin, but have developed means of detecting and responding to microbial attack (Begun *et al.*, 2000). Haemolymph coagulation, melanin formation, and lectin-mediated complement activation are the prominent immune responses in snails (Salawu *et al.*, 2011).

Phenoloxidase (PO) is another key component of the invertebrates' immune system that plays an active role in the sustainability and survival of snails. It is an important enzyme responsible for the activation of melanogenesis in invertebrates, which could be used as a tool against several pathogens (Cerenius and Soderha II, 2004). Its main role in melanogenesis is to convert phenols to quinones, which could be polymerized to form melanin (Cerenius and Soderha II, 2004). PO in invertebrates undergo activation and inhibition, which involve different cell types (hemocytes, plasmatocyt, and crystal cells). However, the innate immune systems in the Haemolymph of *A. marginata* subjected to different feedings have not been studied.

Therefore, this study was designed to examine the effects of different plant feeds on the growth of *A. marginata* as well as Phenoloxidase activity and protein coagulation in its haemolymph.

## METHODOLOGY

### The study area

The research was carried out in the Department of Biochemistry laboratory, Ibrahim Badamasi Babangida University, Lapai, Niger State of Nigeria. Lapai Local Government is located in the Southern part of Niger State, and in the Southern Guinea Savanna zone. It is situated between latitude 9° N, 5° E of the equator.

### Materials

Endotoxin, Biuret reagent, pyrogallol, sodium acetate buffer, glucose, hydrogen peroxide, ethylenediaminetetra-acetic acid all of which are of analytical grade were purchased from Sigma Co. (St. Louis, Missouri USA) except endotoxin that was obtained from Dr Salawu Musa of the Department of Biochemistry, University of Ilorin, Kwara state, Nigeria. Fresh leaves of *Carica papaya*, *Tridax procumbens*, and *Asclepsia carlifonia* were daily obtained from IBB University Farm and identified in the

Department of Biological Sciences IBB University, Lapai, Nigeria while the standard feed used was bought from Mikky animal feeds in Ilorin, Kwara state.

### Animals Husbandry Conditions

The procedure for animal maintenance and use were performed in accordance with OECD Guideline of 2006. Eighty (80) pieces of *Archachatina marginata* of six (6) months old were obtained from a local snail farm (Mikky Snail farm), Ilorin, Kwara State, Nigeria and housed at the animal house of Biochemistry Department, IBB University, Lapai, Niger State. The animals were acclimatized by feeding with standard feed for 2 weeks before they were divided into four (4) groups (A, B, C, and D) of 20 snails each based on feeds offered. Group A, B, C, and D were fed daily with standard feed, leaves (0.4 g) of *C. papaya*, *T. procumbens*, and *A. carlifonia*, respectively, for 4 weeks.

### Methods

Haemolymph extracts were obtained in accordance with apical cracking method described by Ogunsanmi *et al.* (2003). Haemolymph plasma, and fractions; haemocytes lysate (HL), haemocytes lysate supernatant (HLS), and haemocytes lysate debris (HLD) were prepared and stored frozen in accordance with the method of Salawu *et al.* (2011). The methods of Salawu *et al.* (2011) were used to determine the average protein coagulation and Phenoloxidase activities in haemolymph of snails fed with different rations for 4 weeks. The average weekly growth performances; shell length (SL), mouth length (ML), mouth radius (MR) were determined using thread and ruler by subtracting the initial length from final length and weight gain by subtracting the initial weight from final weight after 4 weeks of experiment.

## RESULTS

### Weight gain

The average weight gain of snails subjected to different feeds were expressed in gram, as shown in Table 1. Results show a significant difference ( $p < 0.05$ ) in weight gain of snails fed with different plants compared to those fed with standard feed ( $1.77 \pm 0.13$ ). Snails fed with *C. papaya* had the highest weight gain ( $26.41 \pm 0.24$ ), followed by those fed with *T. procumbens* ( $10.53 \pm 3.02$ ) whereas those fed *A.*

*carlifonia* had the lowest weight gain of  $7.11 \pm 2.66$  (Table 1).

Table 1. Average weight gain in the snails fed with different plant rations at the end of 4 weeks

Feed	IW (g)	FW (g)	WG (g)
Standard	$171.63 \pm 3.98$	$173.40 \pm 40.11$	$1.77 \pm 1.13^a$
<i>C. papaya</i>	$168.26 \pm 2.75$	$194.67 \pm 39.32$	$26.41 \pm 0.24^b$
<i>T. procumbens</i>	$165.78 \pm 1.14$	$176.31 \pm 42.06$	$10.53 \pm 3.08^c$
<i>A. carlifonia</i>	$162.13 \pm 3.78$	$169.24 \pm 51.64$	$7.11 \pm 2.66^d$

Values are Mean  $\pm$  SD of 3 determinations. The values with same superscript (a or b) are not significantly different ( $p < 0.05$ ) from the standard. IW: Initial Weight, FW; Final Weight, WG; Weight Gain

### Growth Parameters

The results of the average physical growth parameters of the snails fed different rations were expressed in centimeter as shown in Table 2. The result shows significant differences ( $p < 0.05$ ) in growth parameters measured in snails fed different plant rations and those fed with standard feed. Those fed with *C. papaya* had the highest growth of  $14.92 \pm 0.67$  cm (SL),  $7.33 \pm 0.59$  cm (ML),  $6.93 \pm 0.56$  cm (MW), and

$4.36 \pm 0.55$  cm (MR) as presented in Table 2. However, the snails fed with *T. procumbens* and *A. carlifonia* had their growth parameters significantly different ( $p < 0.05$ ) with  $14.72 \pm 1.34$  and  $14.74 \pm 0.92$  cm (SL),  $7.10 \pm 0.88$  and  $7.14 \pm 0.75$  cm (ML),  $6.33 \pm 0.51$  and  $6.27 \pm 0.83$  cm (MW), and  $4.20 \pm 0.52$  and  $4.34 \pm 0.54$  cm, respectively, compared with snails fed with standard ( $12.77 \pm 1.12$  cm (SL),  $5.80 \pm 0.81$  cm (ML),  $6.03 \pm 0.82$  cm (MW), and  $3.05 \pm 0.23$  cm (MR) as shown in Table 2.

Table 2. Average increase in the physical growth parameters of snails fed with different plant rations at the end of 4 weeks

Feeds	SL (cm)	ML (cm)	MW (cm)	MR (cm)
Standard	$12.77 \pm 1.12^a$	$5.08 \pm 0.81^a$	$6.03 \pm 0.82^a$	$3.05 \pm 0.22^a$
<i>C. papaya</i>	$14.92 \pm 0.67^b$	$7.23 \pm 0.59^b$	$6.93 \pm 0.56^b$	$4.36 \pm 0.55^b$
<i>T. procumbens</i>	$14.72 \pm 1.34^c$	$7.10 \pm 0.88^c$	$6.33 \pm 0.51^c$	$4.20 \pm 0.52^c$
<i>A. carlifonia</i>	$14.74 \pm 0.92^c$	$7.14 \pm 0.75^c$	$6.27 \pm 0.83^d$	$4.34 \pm 0.52^b$

Values are Mean  $\pm$  SD of 3 determinations. The values with same superscript (a or b) are not significantly different ( $p < 0.05$ ) from the standard. SL: Shell Length, ML; Mouth Length, MW; Mouth Width, MR; Mouth Radius

### Protein coagulation and Phenoloxidase activity in *A. marginata* haemolymph

The results for Phenoloxidase activity in the haemolymph and that of protein coagulation in plasma and other haemolymph fractions of snails are shown in Table 3. The result shows that the average protein coagulations in plasma, HL, HLS, and HLD of snails fed with *C. papaya* are different significantly ( $p < 0.05$ ) with plasma ( $0.12 \pm 0.01$ ), HL ( $0.08 \pm 0.01$ ), HLS ( $0.23 \pm 0.01$ ), and HLD ( $0.32 \pm 0.02$ ) compare to standard with plasma ( $0.042 \pm 0.02$ ), HL ( $0.02 \pm 0.002$ ), HLS ( $0.06 \pm 0.02$ ), and HLD ( $0.15 \pm 0.01$ ) as appeared in Table 3. However,

the plasma ( $0.04 \pm 0.004$  and  $0.05 \pm 0.008$ ) and HL ( $0.06 \pm 0.003$  and  $0.03 \pm 0.02$ ) in snails fed with *T. procumbens* and *A. carlifonia*, respectively, were not significantly different ( $p > 0.05$ ) from standard with plasma ( $0.04 \pm 0.02$ ) and HL ( $0.02 \pm 0.008$ ). Also, the HLS ( $0.13 \pm 0.003$  and  $0.15 \pm 0.03$ ) and HLD ( $0.31 \pm 0.04$  and  $0.24 \pm 0.02$ ) of snails fed with *T. procumbens* and *A. carlifonia* are significantly different ( $p < 0.05$ ) compare to those fed with standard with HLS ( $0.06 \pm 0.02$ ) and HLD ( $0.15 \pm 0.01$ ) as presented in table 3. Likewise, the snails fed with *C. papaya* had their Phenoloxidase activity ( $0.08 \pm 0.005$ )

significantly different ( $p < 0.05$ ) compared to standard ( $0.02 \pm 0.002$ ), while the phenoloxidase activity of snails fed with *T. procumbens*

( $0.02 \pm 0.002$ ) and *A. carlifonia* ( $0.03 \pm 0.002$ ) were not significantly different ( $p > 0.05$ ) from the standard ( $0.02 \pm 0.002$ ) as shown in Table 3.

Table 3. Average protein coagulation and Phenoloxidase activity in haemolymph fractions of *A. marginata* fed with different feeds for 4 weeks.

Feed	Haemolymph Fractions			Phenoloxidase activity (mmol/min)	
	Plasma (mg/dL)	HL (mg/dL)	HLS (mg/dL)	HLD (mg/dL)	Haemolymph
Standard	$0.04 \pm 0.02^a$	$0.02 \pm 0.008^a$	$0.06 \pm 0.08^a$	$0.16 \pm 0.01^a$	$0.02 \pm 0.002^a$
<i>C. papaya</i>	$0.12 \pm 0.01^b$	$0.08 \pm 0.01^b$	$0.23 \pm 0.01^b$	$0.32 \pm 0.02^b$	$0.08 \pm 0.005^b$
<i>T. procumbens</i>	$0.04 \pm 0.04^a$	$0.06 \pm 0.003^a$	$0.03 \pm 0.003^a$	$0.31 \pm 0.04^c$	$0.02 \pm 0.003^a$
<i>A. carlifonia</i>	$0.05 \pm 0.008^a$	$0.03 \pm 0.02^a$	$0.15 \pm 0.03^c$	$0.24 \pm 0.02^d$	$0.03 \pm 0.002^a$

Values are Mean  $\pm$  SD of 3 determinations. The values with same superscript (a or b) are not significantly different ( $p > 0.05$ ) with standard (control) HL; Haemocyte lysate, HLS; haemocyte lysate Supernatant, HLD; haemocyte lysate debris.

## DISCUSSION

The significant difference ( $p < 0.05$ ) in average weight gains observed in snails fed with different plants may be attributed to the fact that weight gain in animal species is a function of adequate acceptable food at its reach. Ejideke and Adebayo (2014) have reported that weight gain in animals is a function of adequate food. The major factors that favor the physical performance of living organisms is adequate supply of proteins, energy and minerals (Ejideke and Adebayo, 2014). The snails fed with *C. papaya*, which recorded high weight gain could be due to its high nutrient composition than the other plants. Nutrition is one of the major factors that affect the performance of living organisms (Ejideke and Adebayo, 2014). The result of weight gain in this study agrees with the findings of Babalola and Owolabi, 2014, where it was observed that the growth parameters were significantly higher in snails fed with pawpaw leaf compared to those fed with milkweed leaf.

The appreciable increase in the physical growing parameters, which include shell length (SL), mouth width (MW), mouth length (ML), and mouth radius (MR) after 4 weeks of experiment reflected that growth parameters in animals is a function of adequate food and nutrient composition. Ejideke and Adebayo

(2014) demonstrated that for an animal species to survive, increase in size and reproduce in an environment, the adequate supply of foods must be in order. The finding is also in accordance with the report of Babalola and Owolabi (2014). *A. marginata* increase in physical performance is usually concentrated in the body weight, SL, and aperture (Adeparusi and Agbebisi, 1998). The successful increase in the growth parameters of the snails fed with simple weed demonstrates that its rearing could be cost effective and environmentally friendly. Also, the cost of production could be affordable as the feeding materials are cheap and available in the environment.

The significant increase in protein coagulation in *A. marginata* haemolymph plasma and fractions of snails fed with different plants compared to that of standard suggests that the plant feeds may contain some nutrients required for an improved host cellular and immunological functions. The essential minerals and antioxidants are usually utilized by immune system to meet the demand of challenges posed by infectious agents (Spallholz et al., 1990, Sherman, 1990). Previous studies have demonstrated that trace elements are essential in the maturation, activation, and functions of host defense mechanisms (Bendich, 1990, Romero, 2003).

High levels of Phenoloxidase activity observed in *A. marginata* haemolymph of snails fed with different plants compared with standard may indicate that defense mechanisms in snails are sensitive to changes in availability of different chemical components in plant. It has been demonstrated that defense mechanisms in snail are susceptible to changes in environmental factors, such as temperature, salinity, dissolved oxygen, food availability, and presence of pollutants (Matozzo *et al.*, 2005, Monari *et al.*, 2005). Phenoloxidase is a member of tyrosinase group, the main function of which is to oxidize phenols and initiates the activation of melanogenesis in invertebrates (Lee and Sodarholl, 2001). The encapsulation of multicellular pathogens, repairing tissues and defending against other pathogens such as bacteria, fungi, and viral agents are other functions of Phenoloxidase (Bendich, 1990, Romero, 2003).

### Conclusion

The consumption of foods with adequate nutritional properties by animal species, which are known to help combat pathogenic diseases are well documented. In this study, all plants used for feeding gave higher weight gain and growth performance of the snails fed, and enhanced the innate immune parameters in snail haemolymph. However, *C. papaya* looks more promising in enhancing the innate immune system in this animal species. This study has demonstrated that different plants have a significant effect on the growth performance and immune response of *Archachatina marginata* with the snail performing better when fed with pawpaw leaf. Pawpaw leaf is therefore, recommended to snail farmers in the rural areas of Nigeria for optimum growth and improved immune response to pathogenic attack in order to enhance their survival. Pawpaw leaf could also be used as a cheap source of feed to *Archachatina marginata*.

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