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Evaluation of the Phenotypic Characters in *Archachatina marginata* Hatchlings Fed Leaves of *Phyllanthus amarus* (Schonn. and Thonn.)

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Abstract

A breeding investigation was carried out to evaluate the effect of *Phyllanthus amarus* (Schonn. and Thonn.) on the phenotypic characters of hatchlings of Giant African Land Snails (GALS), *Achachatina marginata.* The phenotypic traits of one hundred (100) hatchlings investigated were live (body) weight (BW), shell mouth length (SML), shell mouth width (SMW), shell length (SL), shell width (SW) and Feed Intake (FI). The snails were randomly assigned to treatment groups A(10%), B(20%), C (30%) inclusion of *Phyllanthus amarus* powdered leaves into the normal chow of the hatchlings and D (control), with twenty-five (25) snails in each group. 5 weeks after commencement of treatment, the live (body) weights (BW) and shell length (SL) of the snails showed significant differences ($p<0.05$), while observed differences in the other phenotypic traits were not significant ($p>0.05$) The feed intake was highly significant ($p<0.01$) in all the groups. From the results obtained, *P. amarus* leaf meal was well tolerated by the hatchlings and is recommended to snail breeders, as a feed supplement for *A. marginata* hatchlings bred for commercial purposes.

Keywords: *Phyllanthus amarus*, *Achachatina marginata*, Hatchlings, Phenotypic characters, GALS, Heliculture, feed supplement.

Contents

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1. Introduction

The use of herbs as feed supplements is not new in Africa. It is an age long practice that is gradually being accepted for live stock improvement world over [1]. The shortage of good quality feeds needed to sustain livestock growth, especially during the dry season has been a major challenge to the industry in the developing countries. Thus crop residues, agro-industrial by- products and non conventional feed resources which abound during the dry season are being evaluated to access their nutritive potential to support livestock productivity. Several factors have been generally identified as limiting to the utilization or high incorporation of non conventional feedstuffs in livestock feed. These include low protein content, high fibre, amino acid imbalance and presence of anti-nutritional factors [2-4]. Herbs like *P. amarus* (Schum and Thonn) have attained the status of a miracle plant because of its broad spectrum applicability as a cure for several ailments including jaundice, dysentery, diabetes, thyphoid, asthma, hepatitis and fevers ([5]; [6]; [7]). Several crude infusions, concoctions and decoctions of the herb are being consumed for their medicinal value here in Nigeria [8]. *Phyllanthus amarus* (Plate 1) is a small erect tropical herb that grows to a height of 10-60cm. It is an annual plant which is widespread throughout the tropics and subtropics. The plant is a common tropical weed that grows very well in moist, shady and sunny places [9]. *A. marginata* (Plate 2) commonly known as the Giant African land Snail (GALS) is native to West Africa – Cameroon through Democratic Republic of Congo [10]. GALS are considered as omnivorous animals, based on their habit. They are known to feed on decaying materials such as dead plants and animals carcasses, thus, they are termed good end converters (Scavengers) [11]. They also feed on their own wastes (Coprophagia) and eat up their dead or weak mates under certain conditions (canibalism). However, the growth obtained through feeding young *Archachatina marginata* on plant food material supplemented with compounded feed was significantly better than that obtained through feeding it only plant food materials, Ejidike, et al. [12]. The growth of snails like other animals differs with respect to what they are fed. There is also a strong and positive relationship between nutrient of the feed and growth of snail Okonkwo, et al. [13]. Adu, et al. [14] pointed out the need for research studies on the use of compounded ration for snails in order to solve the problem of scarcity of fruits, tuber and leaves during the dry season. Some of the plant materials which they feed on include tubers, avocado pear, guava, oil palm, ripe pawpaw, ripe plantain, pineapple, orange, mango, and bread fruit. Other materials include, plant leaves such as pawpaw, sweet potato, cocoyam, fluted pumpkin and household wastes like yam peel, cassava, bread, remnant food without table salt, rotten plantain [15]. The growth rate in GALS, as micro livestock, has generated great interest among Nigerian researchers as farmers need feed formulas and supplements for raising snails to market weight in less time [16]. One possible source of cheap protein is the leaf meal of some tropical legume plants. Leaf meals do not only provide protein source but also some essential vitamins such as vitamins A and C, minerals and oxycarotenoids [11]. *Phyllanthus amarus* has been reported to be well tolerated in broiler diet, hence the necessity to investigate the effect(s) of the herb, *Phyllanthus amarus* on the phenotypic characters in *A. marginata* hatchlings in order to present it as an affordable, readily available growth enhancing dietary supplement for heliculturists, if the effects are positive.

2. Materials and Methods

GALS hatchlings (Plate 2) used for this research were obtained from a farm in Odukpani Local Government Area of Cross River State, Nigeria. A total number of one hundred (100) hatchlings of *Achachatina marginata* breed of snails were obtained from Odukpani L.G.A. The snails were housed in a wooden vivaria measuring 30cm in length and 13cm in width. The wooden vivaria were properly perforated for easy flow of air in and out of the boxes. The boxes were also filled to 7cm depth with loamy soil exposed to get rid of harmful soil microorganism, moistened regularly to keep favorable humidity for snail growth. The formulated feed given to the hatchlings comprised of Soybeans meal, bone meal, maize powder and vitamin premix during the acclimatization period of two (2) weeks. Fresh feed that was free from mould was given to the hatchlings every day, as left over feed was removed from the feeding trough. They also had access to clean drinking water in water troughs. Feaces was scooped out of the box everyday to prevent microbial infestation and corprophagia. Fresh leaves of *Phyllanthus amarus* (Plate 1) were collected from the botanical garden and authenticated by the curator in the botanical garden, Cross River University of Technology, Calabar. The fresh leaves were thoroughly washed in distilled water and air-dried for 3 days after which they were pulverized into fine powder with an electric blender (Huawai, 787). In a complete Random design (CRD), the snails were randomly assigned to treatment groups, A, B, C and D with twenty five (25) snails in each group. Group D was the control and group A, B and C were treatment groups with 10%, 20% and 30% dietary inclusion of powdered leaves of *Phyllanthus amarus* respectively, into the normal chow of the hatchlings. Initial live (Body) weights (BW) of hatchlings were measured using a digital weighing balance (Zenox, UK) on commencement and subsequently, the body weights were taken every week until the end of the experiment. Shell length (SL) was measured from the apex to the mouth with a measuring tape on a weekly basis. The Shell Mouth Length (SML) was also measured weekly using meter rule.The Shell Mouth Width (SMW) was measured weekly using verniercallipers. The shell widths (SW) were measured weekly using a measuring tape. The total feed consumed by each group of hatchlings was measured daily using a sensitive weighing balance. This is because, feed intake will also determine observable effects on the phenotypic traits to be measured. The data that was obtained from this study was used to compute the descriptive statistics for the *A. marginata* hatchlings. Analysis of variance (ANOVA) test was used to compare the means, that were separated using least significant difference (LSD) at 5% probability level. All analysis was calculated using the SPSS ver.18 statistical package.

3. Results and Discussions

3.1. Effect of *Phyllantus amarus* on Live (Body) Weights of Hatchlings

Result showing the effect on the live (body) weights of *A. marginata* hatchlings fed different doses of *Phyllanthus amarus* is presented on Table 1 and on Fig. 1. Snails in the control group had the highest mean live (body) weight (7.93 ± 0.62) followed by snails fed with 10% inclusion of the test material (7.06 ± 0.45) while the

least body weight was recorded for snails fed with 30% inclusion of the test substance (6.25 ± 0.31). On Table 2, descriptive statistics show the live (body) weights ranges for each treatment group. For groups A, B and C, the weights ranged from 3.00g-16.00g, 2.00g – 14.70g and 3.00g – 9.00g respectively over the treatment period of 5 weeks. ANOVA results (Table 3) show that there were significant differences ($p<0.05$) in the live (body) weights of snails in the different treatment groups. The *P. amarus* leaf meal fed to *A. marginata* hatchlings did not have any adverse or depressing effects on their body weights. The minimum weight ranges (Table 2) agree with reports of [17] that the mean body weight of hatchlings is 2.1g. 10% inclusion of the herb yielded the highest LBW recorded (Fig. 1) unlike the depressing effects of cassava leaf meal in broiler diet recorded by Mantilla, et al. [18] a. Ojelade, et al. [19] also recorded weight loss among snails fed with fresh and dried water leaf meal. Results in this study agree with results recorded by Omole, et al. [20] who observed better performance among snails fed various types of poultry mash with paw-paw leaves inclusion. The rich array of nutrients in the herb, maybe implicated in the observed effect of the leaf meal on the body weights of treated snails.

3.2. Effect of *Phyllanthus amarus* on Shell Length (SL) of Hatchlings

This result is presented on Table 1 and on Fig. 1. As presented, the shell length at 10% inclusion of *P. amarus* into the normal chow was the highest with a mean value of 4.54 ±0.61**.** Shell lengths were the same forboth 20% and 30% inclusion of *P. amarus* while that of the control was 4.48 ± 0.48. Table 2 shows the size ranges for the three treatment groups to be 1.50g – 8.70g, 1.30g – 9.50g and 2.30g-10.50g for groups A, B and C respectively. ANOVA results (Table 3) shows there were significant ($p< 0.05$) differences in the shell length of hatchlings. The 10% leaf meal inclusion had the highest effect on the shell length of treated snails (Fig. 1). This shell length performed even better than the mean control shell length. Snail shell size is a polymorphic phenotypic character dependent on environmental effects [21, 22]. Thus the increase in snail shell length may have been largely due to the *P. amarus* treatment.

3.3. Effect of *Phyllanthus amarus* on Shell Width (SW) of Hatchlings

The result of the effect of *P.amarus* leaf meal on the shell width of snail hatchlings is presented on Table 1 and Fig. 1. Results show that the means for groups A, B, C and D were 5.19±0.88, 5.22±0.82, 5.07±0.76 and 5.25±0.83 respectively. On Table 2, the width ranges observed were 1.70g - 7.60g, 2.20g - 9.20g, 2.00g - 7.60g and 2.00g-8.00g for groups A, B, C and D respectively. ANOVA results (Table 3), show that there was no significant ($p>0.05$) difference in the effects of the leaf meal on the hatchling shell width across the groups. The snail shell width means were not affected significantly by the *P. amarus* leaf meal treatment (Table 1). Observed differences in this phenotypic parameter can be attributed to chance. This could also mean that this phenotypic trait in the snail is genetically determined and is not influenced by the environment. The shell width growth ranges (Table 2) however show ranges that compare favorably with that of the control group.

3.4. Effect of *Phyllanthus amarus* on Mouth Shell Width (MSW) of Hatchlings

The result of the effect of *P. amarus* leaf meal on the shell mouth width of the hatchlings fed *P. amarus* leaf meal is presented on Table 1 and in Fig. 1. Observed means were 1.42±0.10, 1.56±0.15, 1.37±0.16 and 1.53±0.17 for groups A, B, C and D respectively. Table 2 shows the mouth shell width (MSW) ranges across the groups to be 0.90g -2.30g, 0.60g - 3.20g, 0.10 g-3.10g and 0.30g -3.00g for groups A, B, C and D respectively. ANOVA results (Table 3) show that there were no significant differences ($p>0.05$) in the *P.amarus* effect on the MSW of *A. marginata* hatchlings. The shell mouth width of hatchlings fed leaf meal of *Phyllanthus amarus* showed differences that were not significant (Table 1 and 3). The hatchlings fed *P. amarus* leaf meal, performed as well as the control hatchlings (Fig. 1) showing that the leaf meal did not confer any deleterious effects on the snail shell widths. This could also indicate a polygenic influence on this trait,that is not environmentally determined as reported by Okon and Ibom [23].

3.5. Effect of *Phyllanthus amarus* on Mouth Shell Length (MSL) of Hatchlings

The result of the effect of *P. amarus* leaf meal on the mouth shell length of *A. maginata* hatchlings is presented on Table 1 and Fig. 1. From Table 1, mean values observed are 2.26 ± 0.126, 2.19 ± 0.087, 2.26 ± 0.074 and 2.35 ± 0.079 for groups A, B, C and D respectively. Table 2 shows ranges of 1.10 – 7.60, 1.20 – 4.60, 1.30 – 3.50 and 1.20 – 3.60 for 10%, 20%, 30% treatment levels and the control respectively. ANOVA results (Table 3) show there were no significant differences ($p>0.05$) in the effect on MSL of hatchlings. The Shell mouth length (SML) of *A. marginata* hatchlings fed 10% inclusion of *P. amarus* performed better than the control group with the highest mean value of 2.67, (Table 1 and Fig. 1). Statistically, however, the values were not significantly affected by the *P. amarus* leaf meal inclusions (Table 3). Like for the SMW, it may be that this trait is controlled by genes peculiar to this breed of snails as such, the environmental influence on it is insignificant. The SML means observed in this investigation were also smaller than means recorded by Okon and Ibom [23]. These differences may be attributed to the age of the hatchlings at the time of investigations.

3.5.1. Effect of *Phyllanthus amarus* on Feed Intake (FI) of Hatchlings

On Table 1 results of the feed intake of hatchlings is recorded. Table 1 presents mean values of 2.29 ± 0.215, 1.79 ± 0.176, 2.24 ± 0.221 and 3.30 ± 0.248 for groups A, B, C and D respectively. Table 2 shows value ranges with very high upper boundaries of 0.00-6.00, 0.00-6.00, 0.00-6.00 for the treatment groups and 0.00-9.00 for the control. ANOVA results (Table 3) show highly significant differences ($p<0.01$) between the hatchlings feed intake response to the treatments and the control group. The feed intake values recorded in this study were highly significant($p<0.05$) (Table 3). However, the hatchlings in the control group had the highest mean feed intake value (Fig. 1). This could be attributed to the high feed conversion character of snail hatchlings when compared to other micro-livestock as stated by Ejidike [24]. There could also be a component in the *P. amarus* leaf meal that affected

the palatability of the chow. On a whole, the feed intake will bear heavily on the overall performance of the phenotypic traits as observed by Okonkwo, et al. [13] that the growth of snails like other animals, differs with respect to what they are fed. There is also a strong and positive relationship between nutrient content of the feed and growth rate of snails.

4. Conclusion

Nutrition quality of feeds is critical in micro livestock growth and development. The leaf powder of *Phyllanthus amarus* as a feed inclusion was investigated and has been seen to improve live body weights, shell length and feed intake at 10% inclusion into the normal chow of the hatchlings. *P. amarus* leaf meal, which was well tolerated by the hatchlings and is recommended to snail breeders, as a feed inclusion for *A. marginata* hatchlings bred for commercial purposes.

Plate-1. *Phyllanthusamarus*
Source: Field Work, (2016)

Plate-2. *A. marginata* hatchlings
Source: Field Work,(2016)

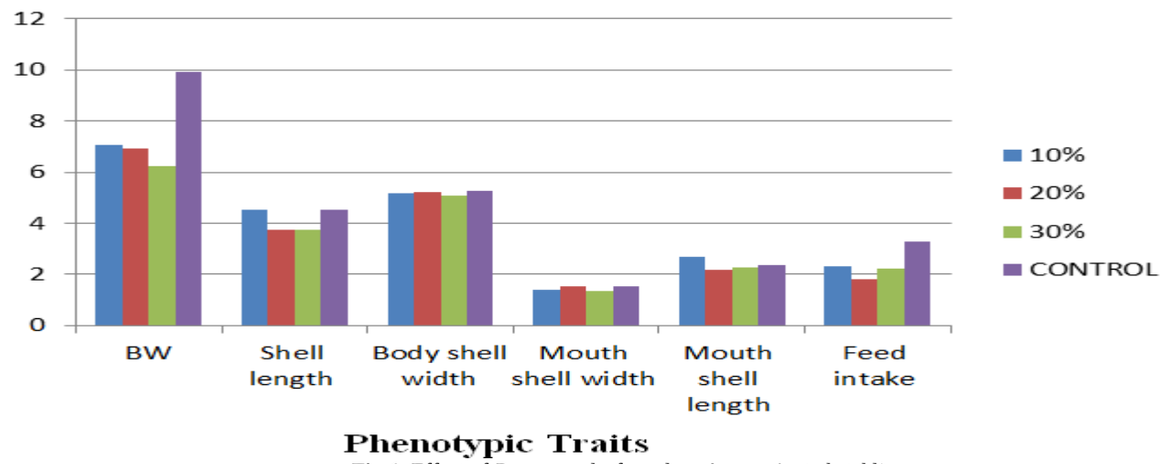

Fig-1. Effect of *P. amarus* leaf meal on *A. marginata* hatchlings
Source: Field Work (2016)

Table-1. Mean (X ± SEM) effects of different dosages of *P. amarus* on phenotypic Traits of *A. marginata*hatchlings

Parameters	10%	20%	30%	Control
Body weight	b 7.06±0.45	bc 6.93±0.32	c 6.25±0.31	a 7.93±0.62
Shell length	a 4.54±0.61	c 3.76±0.32	c 3.76±0.24	b 4.48 ±0.48
Shell width	a 5.19±0.88	a 5.22±0.82	a 5.07±0.76	a 5.25±0.83
Mouth shell width	a 1.42±0.10	a 1.56±0.15	a 1.37±0.16	a 1.53±0.17
Mouth shell length	b 2.67±0.13	a 2.19±0.16	b 2.26±0.15	a 2.36±0.14
Feed intake	b 2.29 ± 0.215	c 1.79 ± 0.176	b 2.24 ± 0.221	a 3.30 ± 0.248

*mean values with different superscript along the same horizontal line differ Significantly($p<0.0$)from each other

Table-2. Descriptive Statistics for phenotypic traits of GALS hatchlings fed with *P. amarus* leaf meal

Phenotype	Group (%)	N	Mean ± SEM	SD	MIN	MAX
Live (Body) Weight(**BW**)	10	60	7.06 ± 0.278	2.15	3.00	16.00
	20	60	6.93 ± 0.229	1.78	2.00	14.70
	30	60	6.25 ± 0.229	1.78	3.00	9.00
	0	60	7.93 ± 0.441	3.43	2.30	18.20
Shell Length (**SL**)	10	60	4.54 ± 0.260	2.02	1.50	8.70
	20	60	3.76 ± 0.233	1.80	1.30	9.50
	30	60	3.76 ± 0.248	1.92	2.30	10.50
	0	60	4.48 ± 0.266	2.06	2.30	9.50
Shell Width (**SW**)	10	60	5.18 ± 0.262	2.03	1.70	7.60
	20	60	5.22 ± 0.250	1.94	2.20	9.20
	30	60	5.07 ± 0.230	1.79	2.00	7.60
	0	60	5.25 ± 0.250	1.93	2.00	8.00
Shell Mouth Width (**SMW**)	10	60	1.41 ± 0.049	0.38	0.90	2.30
	20	60	1.55 ± 0.061	0.47	0.60	3.20
	30	60	1.37 ± 0.068	0.53	0.10	3.10
	0	60	1.53 ± 0.073	0.56	0.30	3.00
Shell Mouth Length (**SML**)	10	60	2.67 ± 0.126	0.97	1.10	7.60
	20	60	2.19 ± 0.087	0.67	1.20	4.60
	30	60	2.26 ± 0.074	0.57	1.30	3.50
	0	60	2.35 ± 0.079	0.61	1.20	3.60
Feed Intake (**FI**)	10	50	2.29 ± 0.215	1.53	0.00	6.00
	20	50	1.79 ± 0.176	1.25	0.00	6.00
	30	50	2.24 ± 0.221	1.56	0.00	6.00
	0	50	3.30 ± 0.248	1.75	0.00	9.00

Source: Field Work ,(2016)

Table-3. ANOVA Results for mean differences between and within groups of *A. maginata* treated with *P. amarus* leaf meal.

Traits		Sum of Squares	df	Mean Square	F	Sig
LBW	B/W gps	85.676	3			
	Within gps	1338.074	236	28.559	5.037	.002*
	Total	1423.751	239	5.670		
SL	B/W gps	34.153	3	11.384	2.977	.032*
	Within gps	902.387	236	3.824		
	Total	936.540	239			
SW	B/W gps	1.147	3	.382	.103	$.958^{NS}$
	Within gps	874.723	236	3.706		
	Total	875.870	239			
SMW	B/W gps	1.428	3	.476	1.950	$.122^{NS}$
	Within gps	57.640	236	.244		
	Total	59.069	239			
SML	B/W gps	0.822	3	.274	.515	$.672^{NS}$
	Within gps	125.458	236	.532		
	Total	126.279	239			
FI	B/W gps	60.649	3	20.216	8.565	.000**
	Within gps	462.638	196	2.360		
	Total	523.287	199			

Source: Field Work, (2016)

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