# EFFECTS OF GENOTYPE, DIETARY PROTEIN AND ENERGY ON THE REPRODUCTIVE AND GROWTH TRAITS OF PARENTS AND F<sub>1</sub> HATCHLINGS OF Achatina achatina (L) SNAILS IN NIGERIA

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## Abstract

Ninety (90) adult breeder snails, forty-five (45) each of the black-skinned (BS) and white-skinned (WS) ectotypes were fed on a mixed feeding regime of pawpaw leaves (Carica papaya) supplemented with formulated diets containing different protein and energy levels to evaluate the reproductive and growth performance traits of these snails and their  $F_1$  offspring respectively. The weights of the parent snails ranged from 58.25 g to 60.75 g. Three (3) mating groups or genotypes namely BS x BS, WS x WS and BS x WS consisted the five (5) treatment groups, corresponding to the different protein and energy level diets. Each group was replicated three (3) times with two (2) snails to a replicate in the randomized complete block design (RCBD). Traits measured on the parent breeder snails included clutch size, incubation period (days), percent hatchability and weight of hatchling at day-old (g), while final body weight (g) and feed intake (g) were measured on the  $F_1$  hatchlings. Body weight gain (g), daily growth rate (g/day) and feed efficiency of the  $F_1$  hatchlings were estimated from the two traits earlier measured. Results of the study showed that significantly differences (P < 0.05) existed in reproductive traits (clutch size, egg weight and percent hatchability) and growth performance traits (final weights, increment in shell length, shell width, aperture length and aperture width). The purebred BS genotype is the best genotype in terms of clutch size among the three genotypes studied. Prospective snail farmers are encouraged to select BS genotype of A. achatina for rearing under good environment with 23 % protein and 2995 Kcal/kgME for faster reproductive and growth traits, and better return on investment.

Key words: Genotypes, snails, traits, protein, energy, Nigeria

#### **INTRODUCTION**

The performance of any animal is dependent upon the inherent genetic make-up and the environment in which it is raised. Thus Cole (1972) and Fredeen (1972) noted that variation observed within a population is the product of both genetic and environmental factors. Within the different genetic make-up, the different breeds perform differently within the same environment. Unlike the environmental factors, the genetic make-up of the animal is permanent and cannot be modified in subsequent generations except through breeding and selection. Such genetic factors include breed effects, genotype effects, maternal effects, sire effects and effects of systems of breeding etc (Okon, 2008). Breed type has a marked effect on performance and productivity of snails than all other factors considered. This is attributed to physiological adaptability to the environment and genetic variations among and within breeds.

Snails belong to a group of invertebrate called Mollusk (Odunaya and Akinyemi, 2008). They are one of the most compact groups of animals which show a wide diversity (Odunaiya, 1995). In Nigeria, there are different breeds of snails and they vary in size, colour, adaptability and performance (Amusan and Omidiji, 1998, Okon *et al.*, 2012b). These according to the authors include *Archachatina marginata*, *Achatina achatina*, *Achatina fulica*, *Limicolaria species* and *Thapsia species*. According to Nisbert (1974) and Jennifer (1975) *Achatina achatina* is the largest land gastropod recorded in the Guiness Book of records. In Nigeria, it is the second most popular breed of snail kept.

According to Ani *et al.* (2009), one of the problems facing the rearing of snails is formulating a balanced/standard diet that will meet their nutrient requirements. Protein level of the snails' diet is very necessary because of its immerse role in animals' well being which includes growth, maintenance, hormonal and enzymatic activities (Adomola *et al.*, 2004). Thus Ani *et al.* (2009) opined that the use of balanced/standard diets in the feeding of snails would help to increase their growth rates and meat quality. But Ebenebe *et al.* (2001) opined that information on the rates of growth for *A. marginata*, *A. achatina* and *A. fulica* snails are scare in the literature. This study was therefore intended to report the effects of genotype, dietary protein and energy levels on reproductive and growth traits of the *Achatina achatina* snail commonly reared in Nigeria.

#### MATERIALS AND METHODS

The study was conducted at the Botanical garden of the University of Calabar, Nigeria. The description of the area and climate were as prescribed in Okon *et al.* (2009a, b). Ninety (90) adult breeder snails, forty-five (45) each of the black-skinned and white-skinned ectotypes were used. The weights of the snails ranged from 58.25 g to 60.75 g. The snails were selected based on active appearance and no injury on the foot and/or shell from base population. The selected snails were grouped into five (5) treatment groups. There were three (3) mating groups or genotypes namely black-skinned (BS) x black-skinned (BS), white-skinned (WS) x white-skinned (WS) and Black-skinned (WS), and each mating group or genotype replicated three (3) times with two (2) snails to a replicate in the randomized complete block design (RCBD).

The replicates were managed in wooden cage compartments measuring 0.60 x 0.50 x 0.30 cm<sup>3</sup>. The stocking density of two snails to a cage compartment was to ensure that eggs obtained from them were as a result of the mating between these two snails. The snails were fed on a mixed feeding regime pawpaw leaves (*Carica papaya*) supplemented with formulated diets. The experimental dies were formulated to contain different dietary protein and energy levels. The dietary protein vs energy levels were 23.04 % vs 2995.20 Kcal/kgME (T<sub>1</sub>), 22.42 % vs 2928.38 Kcal/kgME, (T<sub>2</sub>), 21.80 % vs 2838.08 Kcal/kgME, (T<sub>3</sub>), 21.18 % vs 2794.75 Kcal/kgME, (T<sub>4</sub>) and 20.56 % vs 2727.94 Kcal/kgME, (T<sub>5</sub>). Feed and water were given *ad libitum* throughout the study peiod.

Measured parameters for growth traits of  $F_1$  hatchlings or baby snails included mean final body weight (g) and feed intake (g), while body weight gain (g), daily growth rate (g/day) and feed efficiency were estimated from these two parameters earlier measured. The reproductive traits measured from parent snails were clutch size, incubation period (days), percentage hatchability and weight of hatchlings at day-old. Data on reproductive and growth performance traits were subjected to analysis of variance using the GENSTAT (2007) software package. Significant means were separated using Least Significant Difference (Steel and Torrie, 1980).

#### **RESULTS AND DISCUSSION**

The results of reproductive traits of *Achatina achatina* snails are presented on Table 1. Mean clutch size per snail based on genotypes were significantly different (P<0.05) with the purebred BS genotype recording the highest value of 24, followed by the crossbred BS X WS genotype with 19 and the purebred WS genotype having the lowest value of 15. These results though slightly lower, fall within the mean clutch size range per snail of 20 to 28 for purebred BS genotype, 12 to 18 for purebred WS genotype and 12 to 17 for crossbred BS X WS genotype of *Achatina achatina* reported by Okon *et al.* (2012). However, the mean clutch size per snail obtained in this study were quite higher than the mean clutch size range of 4 to 18 for *Achatina achatina* in Omole and Kehinde (2005). The results were also higher than those reported in literature by Ibom *et al.* (2008), Okon *et al.* (2009a,b), Okon *et al.* (2011a,b). These results further revealed that the purebred BS genotype is the best genotype in terms of clutch size among the three genotypes studied.

The variations recorded in mean clutch size in this study may be attributed partly to genotype effects, thus the significant genotype effect on it (Table 1) and partly due to the environmental effects (protein and energy levels) as most of the authors cited either used diets with different protein and energy levels or forages alone. This confirms the views of Akintomide (2004) and Okon *et al.* (2009a,b) that clutch size was dependent on snails' genotypic composition, parity and the composition of the diet used. The incubation periods obtained varied significantly among the three genotypic groups with the purebred WS genotype recording the highest period from 19 to 30 days (mean of 26 days), followed by the crossbred BS X WS genotype, from 17 to 30 days (mean of 24 days) and the purebred BS genotype having the lowest, from 14 to 30 days (21 days). Again the BS genotype had the best mean incubation period of 21 days.

The results obtained here were within the reported range of 10 - 31 days for *Achatina achatina* in Hodasi (1979). Mean incubation period range of 20 - 28 days for WS (Albino) *Archachatina marginata* in Okon *et al.* (2009a) and 25.8 days (20 - 30 days) for BS genotype and 22 days (18 - 20 days) for WS genotype of *A. marginata* were almost similar to the results of this study. On the other hand, Okon *et al.* (2010a,b) recorded lower mean incubation periods of 21.59 days (17 - 26 days) for parity 1 and 21.14 days (19 - 23 days) for parity 2 and 24.64 days (20 - 31 days) for parity 1 and 23.75 days (21 - 27 days) for parity the 2 for *A. marginata* respectively. The variations in incubation periods obtained might be attributed to differences in genotypic make-up of the snails, dietary protein and energy levels of the diets used. Besides the prevailing temperature and soil conditions of the study area though not tested may also have affected these results.

There were significant (P<0.05) genotype effect on percent hatchability with the purebred BS genotype recording the highest percentage of 76.28 %, followed by the purebred WS genotype with 72.75 % (Table 1). The crossbred (BS X WS) genotype recorded the lowest percent hatchability of 69.27 %. These values were quite higher than the mean values of 52.45 % (25 – 100 %) for parity 1 and 48.86 % (28.1 – 66.7 %) for parity 2 of  $F_1$  crossbred snails of *A. marginata* in Okon *et al.* (2010b); mean values of 39.78 % (20 – 66.67 %) for parity 1 and 38.33 % (20 – 50 %) for parity 2 for purebred WS (Albino) snails of *A. marginata* in Okon *et al.* (2010a) and mean range values of 60.01 to 61.77 % for three groups of *A. marginata* in Okon *et al.* (2011a). The BS genotype of *A. achatina* in this study had highest percent hatchability of 76.28 % when compared to those cited in literature within the same study area. The reasons for this variation in percent hatchability might be due to the breed or genotype used, size of the eggs, the dietary protein and energy levels of the feed used as well as the soil condition. Egg weight is one of the chief determining factors required for satisfactory hatching of snails, while day old hatchling weight is used as an indicator for hatchling development. Smaller egg size leads to smaller hatched hatchlings and tend to hatch earlier than the standard eggs, while hatching of extra large eggs takes longer period.

On mean weight of hatchlings at day old, there were significant (P<0.05) genotype effects, as the crossbred (BS X WS) genotype recorded the highest mean weight of 0.748 g, followed by 0.718 g for purebred WS genotype and 0.540 g for the purebred BS genotype. Day old hatchling weight is used as an indicator for hatchling development. Here the crossbred (BS X WS) genotype recorded the highest value of 0.748 g, thus may have the fastest growth rate. These values were quite similar to the mean hatchling weight of 0.70 g for parity 1 of purebred WS (Albino) *A. marginata* reported by Okon *et al.* (2010a). However, Okon *et al.* (2011b) recorded higher mean hatchling weights of 0.89 g and 0.83 g for parities 1 and 2 respectively of  $F_1$  crossbred (BS X WS) *A. marginata* in the same study area. The results of mean hatchling weights obtained here suggested that the crossbred (BS X WS) genotype followed or supported the expected superiority heterotic effects from the parents. Besides, the results further confirmed the earlier results that snail genotype with higher clutch size will produce eggs with lower weights which will hatch earlier and consequently lower hatchling weights (Okon *et al.*, 2010a). Table 2 shows the effect of dietary protein and energy levels on reproductive traits of *A. achatina*. Diet 1 (T<sub>1</sub>) with the highest protein and energy levels (23.04 % CP, 2995.20 Kcal/kgME) recorded the highest mean clutch size of 23, while diet 5 (T<sub>5</sub>) with the lowest dietary protein and energy levels (20.56 % CP, 2727.94 Kcal/kgME) recorded the lowest mean clutch size of 15.

These results agreed with Sang-Min and Tim-Jun (2005) views that optimum egg production is obtained at higher protein level. The results further revealed that mean clutch size decreased with decrease in dietary protein and energy levels of the concentrate feed fed to the parent *A. achatina* snails. This also agreed with Okon *et al.* (2011b) views that decreased dietary protein and energy levels of the feed affect the clutch size of breeder snails. However, this variation might as well be due to the breed effects, genotype effects, age and size of the snails and season of breeding among other climatic factors. Mean incubation periods among the different protein and energy levels (Table 2) varied significantly (P<0.05), but still full within the range of 15 – 30 days for diets 1 – 3 (T<sub>1</sub> – T<sub>3</sub>), range of 18 – 30 days for diet 4 (T<sub>4</sub>) and 20 – 30 days for diet 5 (T<sub>5</sub>). Expectedly, the mean incubation period increased with decrease in dietary protein and energy levels of the feed with diet 1 (T<sub>1</sub>) recording the lowest mean incubation period of 22 days while diet 5 (T<sub>5</sub>) recorded the highest mean incubation period of 27 days as in Okon *et al.* (2011b). Besides, Okon and Ibom (2011c) reported that snails of all ages can perform optimally within acceptable dietary protein and energy bench mark levels. However, the higher mean percent hatchability values of 82.87 % (T<sub>1</sub>), 82.27 % (T<sub>2</sub>), 76.40 % (T<sub>3</sub>), 64.45 % (T<sub>4</sub>) and 57.88 % (T<sub>5</sub>) obtained here were quite similar to that reported by Okon *et al.* (2011b) for *A. marginata* fed the same diets within the same study area.

These results also fall within the range earlier reported by Amubode (1994) and Ogogo (2004) for BS genotype of *A. marginata*. This might be attributed to the higher crude protein and energy levels of the diets which could have contributed to a better protein – energy balance. The mean weights of hatchlings obtained for *A. achatina* snails in this study were significantly (P<0.05) different among the dietary protein and energy levels (Table 2). The results revealed a decrease in mean weights of hatchlings with decrease in the dietary protein and energy levels as reported in Okon *et al.* (2011b) for *A. marginata*. These values were quite lower than the range values from  $1.01\pm0.20$  to  $1.03\pm0.13$  g for *A. marginata* in Okon *et al.* (2011a); 0.71 to 1.08 g for parity 1 and 0.67 to 0.98 g for parity 2 of *A. marginata* in Okon *et al.* (2010a) for crossbred BS X WS genotype of *A. marginata*. The differences observed in mean hatchling weights here might be attributed to differences in dietary protein and energy levels of the feed used and genotype effects.

The results of growth performance traits obtained (Table 3) revealed significant (P<0.05) genotypic differences in mean final weights, but non-significant (P>0.05) growth rates among the three different genotypes of snails studied. The purebred BS genotype recorded the fastest growth rate of 0.07 g/d, followed by the crossbred (BS X WS) genotype with 0.06 g/d while the purebred WS genotype grew at a slower rate of 0.05 g/d. Besides genotype effects on the observed differences in mean body weights and weight gains, this could also be attributed to the fact that the hatchlings had enough protein and energy to utilize efficiently for growth (Adegbola and Akinwande, 1981). According to Ebenebe *et al.* (2011), reports on body weights of these snails has remained inconsistent with Omole (2000) reporting hifher mean body weight gains of 0.41 g/d, 0.2 g/d, 0.12 g/d and 0.065 g/d for *A. marginata*, *A. achatina*, *A. fulica* and *Limicolaria* species respectively in six (6) months study period; while Hamzart *et al.* (2005) reported much higher mean daily weight gain of 1.16 g for *A. marginata*; Ebenebe *et al.* (1986) reported growth rates of 0.17 g/d and 0.14 g/d respectively for *A. marginata*. These variations in body weight gains of the snail hatchlings might be attributed to genotype effects, synergistic effect of the levels of protein and energy of the diets (concentrate) fed to the juvenile F<sub>1</sub> snails.

Stievenant (1992) and Ebenebe et al. (2011) in addition opined that variations in the body weights of snails are related to the hydration stage, stoutness and shell heaviness (i.e. thickness of calcium deposit during the process of shell calcification) as A. marginata has a thicker shell than A. achatina though not measured in this study. The daily feed intake and feed efficiency observed (Table 4) were non-significantly (P>0.05) different among the three genotypic groups. The mean daily feed intake obtained were similar among the three genotypes, although the efficiency of feed utilization of 0.70 obtained in the crossbred (BS X WS) genotype was the best when compared with the purebred BS and purebred WS genotypes. The daily feed intake of 0.16 g obtained for the hatchlings in this study for the three genotypic groups is at variance with the 4.85 g/d reported by Amubode (1994) for A. achatina (L) and 18.74 g/d by Ogogo (1989) for A. marginata. The differences in growth traits (weight gain, feed intake and feed efficiency) studied may be attributed to the genotype effects, protein and energy levels of the diets and the age of the snails, as the growth traits results here are for hatchlings (1 - 4 weeks)of age), while other authors cited worked with either grower or mature snails. Thus, Hodasi (1982) noted that as snails get older, they utilize higher energy food for growth rate. Besides, the report of Sang-Min and Tue-Jun (2005) showed that optimum production was obtained at high dietary protein levels. These results were at variance with that obtained by Hodasi (1979) and Omole et al. (2000) who used diets containing 28 % CP and 2200 Kcal/kgME for mature snails.

Thus, Ani *et al.* (2009) working with grower snails recommended that dietary protein and energy levels of 24 % and 3.2 Mcal/kgME are adequate for the growth of African giant land snails (*A. achatina*) in the humid tropics. The results of morphometric traits (Table 4) were significantly different (P<0.05) among the three (3) genotypic groups. Rates of increment in shell length, shell width, aperture length and aperture width of the  $F_1$  hatchlings for the purebred BS genotype were 0.009 mm/d, 0.006 mm/d, 0.01 mm/d and 0.006 mm/d respectively, while the rates were 0.007 mm/d, 0.004 mm/d, 0.003 mm/d and 0.003 mm/d respectively for the purebred WS genotype. The crossbred (BS X WS) genotype recorded incremental rates of 0.009 mm/d, 0.004 mm/d, 0.008 mm/d and 0.004 mm/d, for the shell length, shell width, aperture length and aperture width respectively for *A. achatina*  $F_1$  hatchlings. These values were for hatchlings at four weeks of age and were quite lower than values reported by Ebenebe *et al.* (2011) for grower snails. This may be attributed to breed differences, genotypic effect, age of the snails as well as the dietary protein and energy levels used in feeding the snails.

In addition, Ebenebe *et al.* (2011) noted that information on the rates of growth for *A. marginata, A. achatina* and *A. fulica* are scare in the literature, thus the results of this study with *A. achatina*  $F_1$  hatchlings (from day old to four weeks) will form an important base-line information for other researchers to work upon.

## CONCLUSION

The results of this study showed that genotype, dietary protein and energy levels have significant effects (P<0.05) on the reproductive and growth traits of *A. achatina* snails studied. However, the purebred BS genotype had the best reproductive and growth traits than the purebred WS and crossbred (BS X WS) genotypes. Prospective farmers could select BS genotype of *A. achatina* and rear under a good environment with 23 % protein and 2995 Kcal/kgME for faster reproductive and growth traits, and better return on investment.

<b>Reproductive Traits</b>	Genotypes			
	Purebred Black- skinned Genotype (BS)	Purebred White- skinned Genotype (WS)	Crossbred Genotype (BS X WS)	
Mean clutch size	24 <sup>a</sup>	15 <sup>c</sup>	17 <sup>b</sup>	
Incubation period (days)	$14 - 30 (22)^{c}$	$19 - 30 (26)^{a}$	$17 - 30(24)^{b}$ $14^{b}$	
Mean no. of hatchling /clutch	17 <sup>a</sup>	$10^{\circ}$	14 <sup>b</sup>	
Percent hatchability	$76.28^{a}$	72.75 <sup>b</sup>	69.27 <sup>c</sup>	
Mean wt. of hatchling at day old (g)	$0.54^{\mathrm{b}}$	$0.718^{a}$	$0.748^{a}$	

#### Table 1: Genotype Effects on Reproductive Traits of Parent Achatina achatina Snails.

<sup>abc</sup>Means along the same column with different superscripts are significantly different (P<0.05).

Table 2: Dietary Protein and Energy Levels on Reproductive Traits of Parent Achatina achat	ina Snails.
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<b>Reproductive Traits</b>	$T_1$	$T_2$	T <sub>3</sub>	$T_4$	<b>T</b> <sub>5</sub>
-	23.04 % CP	22.42 % CP	21.80 % CP	21.18 % CP	20.56 % CP
	2995.20	2928.38	2818.08	2794.75	2727.94
	Kcal/kgME	Kcal/kgME	Kcal/kgME	Kcal/kgME	Kcal/kgME
Mean clutch size	23 <sup>a</sup>	20 <sup>b</sup>	19 <sup>bc</sup>	16 <sup>d</sup>	15 <sup>e</sup>
Incubation period	15–30 (22) <sup>de</sup>	15–30 (23) <sup>cd</sup>	15–30 (24) <sup>c</sup>	18–30 (26) <sup>ab</sup>	20–30 (22) <sup>a</sup>
(days)					
Mean no. of hatchling	$17^{\mathrm{a}}$	$16^{ab}$	$15^{bc}$	11 <sup>d</sup>	$9^{\rm e}$
/clutch					
Percent hatchability	$82.87^{a}$	$82.27^{ab}$	$76.40^{\circ}$	64.45 <sup>d</sup>	57.88 <sup>e</sup>
Mean wt. of hatchling	$0.67^{a}$	$0.65^{\mathrm{b}}$	$0.62^{\circ}$	$0.62^{cd}$	$0.60^{\rm e}$
at day old (g)					

<sup>abcde</sup>Means along the same column with different superscripts are significantly different (P<0.05).

Table 3: Genotype Effects on Gro	wth Traits of F <sub>1</sub> Hatchlings	s of Achatina achatina Snails.
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Growth Traits		Genotypes	
	BS	WS	BS X WS
Mean initial weight (g)	9.93	10.02	12.56
Mean final weight (g)	14.30 <sup>b</sup>	$12.85^{\circ}$	$15.60^{a}$
Daily weight gain (g/d)	$0.07^{\mathrm{a}}$	$0.05^{\mathrm{a}}$	$0.06^{a}$
Mean daily feed intake (g)	$0.16^{a}$	$0.16^{a}$	$0.16^{a}$
Feed efficiency	$0.89^{a}$	$0.78^{\mathrm{a}}$	$0.70^{a}$

<sup>abc</sup>Means along the same column with different superscripts are significantly different (P<0.05).

Morphometric Traits			
•	BS	WS	BS X WS
Mean initial shell length (mm)	3.10	3.49	3.54
Mean final shell length (mm)	3.34 <sup>c</sup>	$3.68^{b}$	$3.80^{a}$
Mean daily shell length increment (mm/d)	0.009	0.007	0.009
Mean initial shell width (mm)	2.10	2.03	2.24
Mean final shell width (mm)	$2.26^{b}$	$2.14^{\circ}$	2.36 <sup>a</sup>
Mean daily shell width increment (mm/d)	0.006	0.004	0.004
Mean initial aperture length (mm)	2.14	2.32	2.39
Mean final aperture length (mm)	$2.45^{\circ}$	$2.47^{b}$	$2.60^{a}$
Mean daily aperture length increment (mm/d)	0.01	0.005	0.008
Mean initial aperture width (mm)	1.62	1.46	1.59
Mean final aperture width (mm)	$1.80^{a}$	1.55 <sup>c</sup>	$1.70^{b}$
Mean daily aperture width increment (mm/d)	0.006	0.003	0.004

#### Table 4: Genotype Effects on Morphometric Growth Traits of F<sub>1</sub> Hatchlings of Achatina achatina Snails.

<sup>abc</sup>Means along the same column with different superscripts are significantly different (P<0.05).

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