Influence of the Nutritional History of Mature Giant African Land Snails (*Archachatina marginata*) fed at graded protein levels in the supplemental diet on the subsequent reproductive performance of their F1 offspring.

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Abstract

Background: This study was aimed at investigating the influence of the nutritional history of the parents (Archachatina marginata) snails on the subsequent reproductive performance of their F1 offspring fed different levels of protein in the supplemental diets.

Materials and Methods: This study was carried out between April 2020 to April 2021 at the Helvy Farms-WVED Cameroon located at Station-Bamenda. Data on subsequent reproductive performance were collected for 16weeks (January-April 2021). 150 sexually mature Archachatina marginata snails weighing between 68 and 78g, full shelled free of any form of injury previously under a growth trial on the same farm were followed up for subsequent reproductive performance. These snails were randomly allotted to four treatments of different protein levels (16%CP, 18%CP, 20%CP, and 22%CP) and a control (natural plant feed) replicated three times with 10 snails each per replicate. The age at maturity, numbers of egg clutches, clutch size, egg weight, duration of incubation and snailets weight were noted. The egg breadth and length were measured with a vernier caliper (0.02mm accuracy).

Results: The results showed that snails fed supplemental diets reached sexual maturity 70-81days earlier than snails fed just the basal diet. Snails fed 22% CP were significantly higher (P<0.05) in the mean number of clutches (5.88 ± 0.13), mean clutch size (17.67 ± 0.12), and mean egg weight ($1003.02\pm9.3mg$). For the mean egg length and breadth, all eggs from snails fed the supplemental diet were significantly higher (P<0.05) than those fed the control diet. Snail eggs from snails fed 22%CP hatched at 23days of the incubation period. Percentage survivability of F1 snailets was also significantly higher (P<0.05) in snailets from parents fed 22%CP and snailets weight was also significantly higher in snails from parents fed 22%CP. There was an improvement in reproduction with the snails fed the supplemental diet and especially with those fed 22%CP.

Conclusion: We can therefore recommend that 22%CP be included in the diet of Archachatina marginata breeding snails for better reproductive performance.

Keywords: Nutritional history, reproductive performance, snailets, Archachatina marginata, dietary protein level, survivability.

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I. INTRODUCTION

Snails have a high rate of productivity and prolificacy; with ten breeding snails, about 400,000 snails can be produced at the end of the first year of reproduction [1]. Similarly, Akinnusi,[2] reported that a snail farm initiated with just 50 snails has the capacity of producing about 5000 snails in 3 months. Snails growth, survival, reproduction, and development just like in other animals, largely depends on the quality of feed [2-7]. Apart from the quality of the feed, the nutritional history and management of the snail may have far-reaching consequences on their subsequent productivity [3, 4, 8]. It could also have an effect on the subsequent reproductive performance of their F1 offspring.

Furthermore, a rational understanding of the factors underpinning improved productivity requires a clear knowledge of how to manipulate positively reproductive parameters (age at maturity, egg characteristics, duration of incubation, and weight of snailets). This knowledge will permit an informed choice on strategies that could be adopted for the proper feeding of such species. Such feeding could be tailored to meet the snail's specific nutritional requirement for reproduction [3, 4, 9-11]. For example, such a strategy will permit them to attain physical and sexual maturity within a shorter time.

The present study was designed to investigate the influence of the nutritional history of mature snails fed at graded protein levels in the supplemental diet on the subsequent reproductive performance of their F1 offspring.

II. MATERIALS AND METHODS

Study Period and Site This study was carried out from January to April 2020 (4months) under experimental conditions at the snailery unit of Helvy farms upskill Research at Station Bamenda of the Western Highlands of Cameroon situated on latitude 5o57'5.98" N and longitude 10o10'10o04" E, at an altitude of 1,258 m above sea level [12]. It is characterized by a tropical climate consisting of two distinct seasons; a wet season from March to October and a dry season from November to February. The mean annual rainfall is about 2,145 mm and a mean annual temperature of 21.5 °C with the highest temperatures recorded in February (27oC) and the lowest temperatures recorded in July and August (18°C). The average relative humidity stands at about 75%, with February recording the lowest mean relative humidity and July being the most humid month [13].

Experimental Design and Diet

150 sexually active F1 breeding *Archachatina marginata* snails weighing between 68 and 78g, full shelled, free of any form of injury were used for the study. The F1 breeding offspring were constituted of snailets hatched from sexually mature snails fed graded levels of supplementary diets corresponding to the following protein levels 16, 18, 20 and 22 % respectively. The snailets emanating from parents in these treatments were maintained on the same graded dietary protein levels as their parents to investigate the influence of the nutritional history of the parents on the subsequent reproductive performance of their F1 offspring. A comparative analysis was also done between the reproductive parameters of the parents and that of their F1 offspring to assess if there was an improved performance in the offspring when compared with the parents. The resulting snailets were monitored for their reproductive performance before comparisons with previous data from their parents.10 F1 snailets each of similar weights ranges were randomly distributed to 15 pens. There were four (4) treatments corresponding to the supplemental diets (T1, T2, T3, and T4) had 3 replicates (R1, R2, and R3) of 10 snailets per replicate.

Ingredients Proportion of ingredients (%) *T1 (16 %) T2 (18 %) T3 (20 %) T4 (22 %) Corn flour 55.00 55.00 55.00 55.00 Sovbean cake 168 18.9 20.722.8 Palm kernel cake 1.01 3.75 2.65 2.75 Fish meal 4.35 3.35 1.09 1.65 Limestone (CaCO3) 20.0 20.0 20.0 20.0 Iodizedsalt 0.1 0.1 0.1 0.1 Total 100 100 100 100 **Calculated** Composition Crude Protein (%DM) 16.03 18.01 20.04 22.05 Calcium 8.12 8.14 8.12 8.13 2500.01 2500.05 2500.05 ME (Kcal/Kg DM) 2500.03 Fat 3.04 3.03 3.04 3.02

Table 1: Feed formula and calculated composition of supplemental diets

*T= Treatment

		Composition	
Samples			
	Protein	Fat	Ash
Water Leaf	2.03	1.14	4.18
Pawpaw Leaf	2.65	2.01	4.52
Cabbage	1.2	1.1	4.56
Lettuce	1.6	1.1	3.98
Pawpaw Fruit	0.7	0.4	4.21
Watermelon	0.65	1.03	3.34
Ripe Banana	1.2	1.12	2.04
Mean protein content	14		

Table 2: Proximate composition of the basal diet (T0-% Dry Matter Basis)

Animal Management of F1 Snails

The methodology used in snail management, egg collection, and incubation was as described by Agbelusi and Adeparusi, [14], Okon*et al.*[15] and Okon*et al.*, [7]. 150 sexually active F1 Archachatina marginata snails weighing between 68 and 78g, full shelled, free of any form of injury were used for the study. The Archachatina marginata snails were managed intensively in built-up surface trench pens of $1m\times0.5m\times0.5m$ in dimension under a shade made of aluminum roofing sheets in an open space to enable cross-ventilation. To prevent potential attacks on the snails from soil organisms, the soil introduced into the pens was heat-treated using boiled water and allowed for 24hrs before the introduction of the snails. Also, dry banana leaves (*Musa paradisiaca*) were heat-treated by steeping into boiled water to kill ants and other insects before placing in the pens to serve for covering, moisture absorber, and food. Heat treatment of the soil in the pens was also repeated every 3months necessarily to eliminate any potential pests and microbes that could be a nuisance to snail growth and development. Shallow feeding and drinking trays were placed in all pens to prevent the drowning of snails.

150 F1 sexually active snails were randomly allotted to treatments following a completely randomized design into 5 treatment groups with 30 snails of homogenous weight each per treatment. Each pen of T1 T2 T3 and T4 received 30g of supplemental diets containing different levels of protein (16%, 18%, 20%, and 22%) +30g of the basal plant diet while the snails in the Control (T0) pen received only the basal plant diet (60g) daily. The basal plant diet was weighed before feeding and it was made of pawpaw leaves, pawpaw fruits, watermelon, ripe bananas and plantains, cabbage, lettuce, and waterleaf used throughout the experiment. Water was provided ad libitum, snails were fed each day between 4-5 pm following their nocturnal feeding habits. The snail pens were watered daily to maintain the required humidity level for snail reproduction.

The snails were allowed to breed naturally following the method described by Ibom, [16] and Ibom *et al.*[17]. This enabled follow-up and data collection of the following reproduction performance parameters; age at maturity, number of egg clutches, number of eggs per clutch, the weight of an egg, egg length, egg breadth, duration of incubation, hatchability, survivability, and weight of snailets (F1).

Trial Management

The age at maturity for these F1 snails was monitored and recorded from the time snails approached each other to start the courtship process. This process consisted of a series of movements that lasted for about 2-12hours that could either end or not in mating. To find a partner, they rely on their sense of smell and touch and also recognize chemicals in the air that indicate receptivity of some other sexually active snails nearby. Interactions increased with the help of their tentacles, they touched each other. Others move in circles and could bite the area of the genital pore. In the final stage of courtship and just before mating, some snails use a unique weapon: "Love darts" which is a structure of calcium or chitin only found in sexually mature snails. With these observations, the age at first egg-lay was used to estimate the average age at maturity for the F1 snails.

The eggs were collected in clutches daily at between 6 and 7 am, egg weights were measured immediately using a KA8-AM digital LCD electronic scale balance of 0.01g to 1000g sensitivity. The number of egg clutches, number of eggs per clutch were counted and recorded, while egg lengths and breadths were measured with a Focket carbon steel vernier caliper of 0.02mm accuracy. After collection, all the eggs for incubation were sorted against cracks and morphological deformities.

The eggs were grouped in incubation batches of ten (10) and each incubation batch was tied in a mesh net and randomly placed in an incubation medium to a depth of 3 to 5 cm and covered sparingly with the incubation substrate which was a moist mixture of sawdust/soil. Incubation temperatures and humidity were measured using a DURAC® PLUS TM Pocket mercury-in-glass thermometer and a ROBU-IN 3-way soil probe respectively. This procedure continued until a total of six hundred (600)

eggs in four batches were incubated in the incubation boxes lined with impermeable plastic film and filled with moist soil/sawdust mixture at the same temperatures of 25-28oC, relative humidity of 70-80%. These boxes were watered twice every week to maintain the humidity level for the proper development of snail eggs.

Eggs in all incubation boxes were checked every 3 to 4 days by simply pulling out the tied mosquito nets and viewing for any possible snailets. After 45 days unhatched eggs were broken to reveal the content. At hatching, the weights of the snailets and incubation duration were also recorded. Hatchability and survivability were calculated thereafter and expressed as a percentage.

Data Collection

Data collected for reproductive performance lasted 16weeks (January to April 2021) and included; age at maturity and egg characteristics (weight, length, breadth), number of egg clutches, clutch size, incubation duration, and weight of snailets. The eggs were observed and checked for snailets and turned every five (5) days from day fifteen (15) of incubation. The hatchlings that emerged were removed, counted, and transferred to growing pens to prevent them from consuming the other eggs still in incubation [17]. Unhatched eggs were left in incubation for up to 45 days [14], after which the eggs were gently cracked to reveal their contents.

The number of egg clutches, numbers of eggs per clutch were counted, and incubation duration was recorded as the number of days eggs took to hatch from the date of incubation. The numbers of eggs hatched were counted as snailets that emerged from the eggs. The snailets were weighed to determine their weight at hatching. Hatchability and survivability were also calculated. The reproductive parameters were calculated using the following formulae as described by Ibom *et al.* [17] and Oyeagu *et al.* [18].

Measurement of the Subsequent reproductive performance characteristics of F1 offspring:

Age at maturity was noted as the age at which the first "love darts" (calcareous substance) appear around the anus of the snail, followed by first egg lay.

Egg weight and weight of snailets were measured using KA8-AM digital LCD electronic scale balance of 0.01sensitivity and a Focket Carbon Steel Vernier caliper of 0.02sensitivity were used to measure egg breadth and egg length respectively.

Number of clutches and clutch size (counted and recorded)

- Eggs were counted clutch by clutch to obtain the total number of clutches and also the total number of eggs per clutch

Duration of incubation (DI)

- Interval between incubation and hatching and the various means calculated.

DI= Date of incubation to Date of hatching

% Hatchability =
$$\frac{\text{number of hatched eggs}}{\text{numberofeggsincubated}} \times 100$$

% Survivability= $\frac{\text{Numberoflivesnailets}}{\text{totalsnailetshatched}} \times 100$

Data analysis

Data collected were subjected to a one-way Analysis Of Variance-ANOVA (variable being protein level) following the general linear model (GLM), according to the following statistical model: $X_{ij}^{ij} = n + n^{ij} + n^{ij}$

 $Xij = \mu + \alpha i + eij$

Xij = Observation on the animal having received treatment or ration i

 μ = general mean

 αi = Effect of protein level (i)

eij = residual error caused by animal j having received the treatment or ration i

Separation of significant means was done using Duncan's test at 5% level of significance [19]. Paired T-tests were used to compare the reproductive performance of mature and F1 snails. Statistical Analyses were done using SPSS 18.0.

III. RESULTS

Effect of protein level on age at maturity of F1 snails

The results in Table 3 show that the age at maturity dropped as the protein level increased. There was a remarkable difference of 81days (23%) between snails fed 22% protein level in supplemental diet and those fed solely the basal diet. All the snails fed the supplemental diets reached maturity 70-81days earlier than those fed the basal diet. There were significant ($p \le 0.05$) differences between all snails fed the supplemental diets and the control group.

Treatments	Age at maturity (days)	% reduction age at maturity as compared with values for the basal diet (T0).				
T0 (1.4%)	357.07±0.02 ^a	-				
T1 (16%)	287.01±0.02 ^b	19				
T2 (18%)	284.00 ± 0.03^{b}	26				
T3 (20%)	279.01±0.02 ^c	22				
T4 (22%)	276.02±0.04 [°]	43				

Means with different superscript letters differ significantly (P<0.05). T0=1.4% T1= 16%, T2=18%, T3=20% & T4=22% of dietary protein levels respectively

Effect of protein level on egg characteristics of F1 Snails (A. marginata)

The results (Table 4) show the effect of protein level on egg characteristics of F1 snails. There was an increase in all the egg characteristics as the protein level in the diet increased. The highest in the number of clutches, clutch size, egg weight, egg breadth, and egg length was obtained with snails fed 22% crude protein in the supplemental diet and the lowest obtained from the control group. There were significant differences (P<0.05) between the snails fed different levels of the supplemental diet and those fed solely the basal diet. The snails fed 20% and 22% protein levels were significantly higher (P<0.05) in the number of clutches, clutch size, egg weight, egg breadth, and egg length compared to the snails fed 16%, 18% protein levels, and the control group.

Table 4: Effect of	protein level	on egg	characteristics	of F1	Snails
	Transformer				

	Treatments	ents					
T0(1.4%)	T1 (16%)	T2 (18%)	T3 (20%)	T4 (22%)			
1.69 ± 0.07^{a}	3.85±0.13 ^b	4.38±0.13 ^c	4.96±0.12 ^c	5.88±0.13 ^d			
$9.35{\pm}0.60^a$	$14.48{\pm}0.12^{\text{b}}$	$15.15 \pm 0.13^{\circ}$	16.52±0.11 ^c	$17.67 {\pm} 0.12^{d}$			
$357.77{\pm}0.96^{a}$	$772.15{\scriptstyle\pm1.3^{b}}$	$777.65 {\pm} 0.98^{b}$	$824.83{\pm}8.80^{\circ}$	$1003.02{\pm}9.3^{d}$			
$0.96{\pm}1.20^a$	$1.31{\pm}1.06^{\text{b}}$	$1.60{\pm}1.19^{\circ}$	$1.85{\pm}1.07^{d}$	$1.96{\pm}0.89^{d}$			
$1.38{\pm}0.24^{a}$	$2.01{\pm}0.06^{\text{b}}$	$2.09{\pm}0.11^{b}$	$2.31{\pm}0.07^{\circ}$	$2.42{\pm}0.98^{\circ}$			
	$T0(1.4\%)$ 1.69 ± 0.07^a 9.35 ± 0.60^a 357.77 ± 0.96^a 0.96 ± 1.20^a 1.38 ± 0.24^a	T0(1.4%)T1 (16%) 1.69 ± 0.07^{a} 3.85 ± 0.13^{b} 9.35 ± 0.60^{a} 14.48 ± 0.12^{b} 357.77 ± 0.96^{a} 772.15 ± 1.3^{b} 0.96 ± 1.20^{a} 1.31 ± 1.06^{b} 1.38 ± 0.24^{a} 2.01 ± 0.06^{b}	T0(1.4%) T1 (16%) T2 (18%) 1.69±0.07 ^a 3.85±0.13 ^b 4.38±0.13 ^c 9.35±0.60 ^a 14.48±0.12 ^b 15.15±0.13 ^c 357.77±0.96 ^a 772.15±1.3 ^b 777.65±0.98 ^b 0.96±1.20 ^a 1.31±1.06 ^b 1.60±1.19 ^c 1.38±0.24 ^a 2.01±0.06 ^b 2.09±0.11 ^b	T0(1.4%) T1 (16%) T2 (18%) T3 (20%) 1.69±0.07 ^a 3.85±0.13 ^b 4.38±0.13 ^c 4.96±0.12 ^c 9.35±0.60 ^a 14.48±0.12 ^b 15.15±0.13 ^c 16.52±0.11 ^c 357.77±0.96 ^a 772.15±1.3 ^b 777.65±0.98 ^b 824.83±8.80 ^c 0.96±1.20 ^a 1.31±1.06 ^b 1.60±1.19 ^c 1.85±1.07 ^d 1.38±0.24 ^a 2.01±0.06 ^b 2.09±0.11 ^b 2.31±0.07 ^c			

Means within the same column with different superscript letters differ significantly (P<0.05); T0=1.4% T1= 16%, T2=18%, T3=20% & T4=22% of dietary protein levels respectively

Effect of protein level on duration of incubation of F1 Snails

From the results (Table 5), the duration of incubation of eggs decreased as the protein level in the diet increased. The duration of incubation was shorter by 19days (45%) in snails fed 22% protein level in the supplemental diet when compared with snails fed solely the basal diet. The longest (42 days) incubation duration was observed in eggs from the control group while the shortest (23days) was observed in eggs from snails receiving 22% protein level in the supplemental diet. The number of days of incubation of eggs from snails fed a supplemental diet at 22% protein level was significantly shorter (P<0.05)than for eggs from all the other experimental groups (including the control group).

Treatments	Duration of Incubation (days)	% Reduction in Incubation time			
T0 (1.4%)	$41.92{\pm}2.84^{a}$	-			
T1 (16%)	26.75 ± 0.62^{b}	36			
T2 (18%)	26.13±0.52 ^b	38			
T3 (20%)	$24.92 \pm 0.07^{\circ}$	40			
T4 (22%)	23.00 ± 0.95^{d}	45			

Means with different superscript letters differ significantly (P<0.05). T0=1.4% T1= 16%, T2=18%, T3=20% & T4=22% of dietary protein levels respectively.

1.3.4. Effect of protein levels on hatchability of eggs and survivability of snailets

The results in Table 6show that there was a gradual increase in hatchability of eggs and survivability of snailets as the level of protein supplementation increased in the diet. The eggs from snails fed the diet containing 22% protein showed significantly (p<0.05) higher hatchability and survivability rates compared to all the other treatments. The lowest hatchability (71.32%) and survivability (47.41%) rates were observed in the control group. In comparison to the control group, a 22% protein level in the supplemental diet increased the hatchability of eggs by 17% and doubled the survivability of snailets.

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Treatments	Hatchability (%)	Survivability (%)	
T0(1.4%)	71.32 ± 1.01^{a}	47.41±6.23 ^a	
T1 (16%)	75.53±1.34 ^b	91.75 ± 7.11^{b}	
T2 (18%)	77.34±1.07 ^b	93.61±7.61 ^b	
T3 (20%)	$81.25 \pm 1.18^{\circ}$	$95.43 \pm 8.13^{\circ}$	
T4 (22%)	88.41 ± 1.12^{d}	97.63 ± 9.15^{d}	

Means within the same column with different superscript letters differ significantly (P<0.05). T0=1.4% T1= 16%, T2=18%, T3=20% & T4=22% of dietary protein levels respectively

Effect of protein levels on Snailets weight at hatching

The results in Table 7 show that there was an increase in snailets weights as the protein levels in the supplemental diet increased. The highest (2.16g) snailets weights were recorded in snailets from snails fed 22% protein level in supplemental diet with the lowest (0.98g) from the control group. The snailets from snails fed 22% protein level in supplemental diet weighed 54% more than snails fed solely the basal diet. The snailets weight was significantly (P<0.0) higher in snails fed 22% protein levels and differed from the other treatment levels and the control.

Treatments	Snailets weight (g)				
T0(1.4%)	0.98±0.08a				
T1 (16%)	1.43±0.21b				
T2 (18%)	1.51±0.26b				
T3 (20%)	$1.94{\pm}0.44c$				
T4 (22%)	2.16±0.05d				

Means with different superscript letters differ significantly (P<0.05). T0=1.4% T1= 16\%, T2=18%, T3=20% & T4=22% of dietary protein levels respectively

Comparative analysis of the reproductive performance of the mature and F1 snails fed supplemental dietary protein

Results of the comparative analysis of the reproductive performance of mature snails fed graded levels of protein and corresponding parameters for their F1 offspring (Table 8), shows a remarkable increase in performance characteristics. There was a significant increase (P<0.05) in performance from the mature snails to their F1 offspring within the respective treatments for the number of clutches (P<0.01), clutch size (P<0.01), weight of an egg (P<0.01), egg breadth (P<0.05) and egg length (P<0.01 from T0-T3 and P<0.05 for T4). However, with the other

characteristics, significant differences (P<0.05) were observed only within certain treatments. This is the case for incubation duration which was only significant for three treatments (T1, T2, and T4), the weight of snailets (T1, T2, and T3), and survivability (T0, T2, and T3). In the case of hatchability, there were highly significant differences (P<0.01) within all the dietary protein supplemented treatments. Meanwhile, there was no significant difference in the characteristics between the mature snails and their F1 offspring in the control treatment fed the basal diet. In

the case of the percentage survivability, there were highly significant differences (P<0.01)observed between the mature females and their offspring for T0, T2 and T3. This was not the case for treatment T1 and T4 where there were no significant differences.

	-			-	-					
Fac	T0 (1.	4%)	T1 (16%)	T	2 (18%)	Т3	(20%)	T4 (2	2%)
characteristics	Mature	Fl	Mature	Fl	Mature	Fl	Mature	F1	Mature	F1
No of clutches	1.56±0.51	1.69±0.07(**)	2.89±0.11	3.85±0.13 (**)	3.04±0.11	4.38±0.13 (**)	3.44±0.12	4.96±0.12 (**)	3.93±0.15	5.88±0.13(**)
Clutch size	9.25±0.13	9.35±0.60(**)	10.63±0.26	14.48±0.12(**)	10.30±0.28	15.15±0.13 (**)	13.07±0.43	16.52±0.19 (**)	13.78±0.49	17.38±0.17(* *)
Weight of an egg (mg)	255.74±2.04	357.77±0.9 6(**)	265.70±2.41	772.65±0.98 (**)	269.48±1.49	777.65±0.98 (**)	277.56±3.29	824.83±8.80 (**)	283.07±3.57	1003.02±9,(* *)
Egg breadth (cm)	0.78±0.27	0.96±1.20(**)	1.13±0.28	1.31±1.06 (**)	1.15±0.26	1.60±1.19 (**)	1.27±0.32	1.85±1.07 (**)	1.32±0.26	1.96±0.89 (**)
Egg length (cm)	1.22±0.27	1.38±0.24(**)	1.81±0.27	2.01±0.06 (**)	2.11±0.26	2.09±0.11 (**)	2.21±0.32	2.31±0.07 (**)	2.37±0.27	2.42±0.98 (*)
Incubation duration (days)	41.97±0.60	41.92±2.84 (ns)	26.97±0.14	26.75±0.62 (*)	26.17±0.13b	26.13±0.52 (ns)	25.57±0.12	24.92±0.07 (**)	24.07±0.14	23.00±0.95 (*)
Weight of snailets(g)	0.95±0.24	0.98±0.08 (ns)	1.36±0.25	1.43±0.21 (**)	1.47±0.33	1.51±0.26 (**)	1.77±0.37	1.94±0.44 (**)	1.86±0.41	2.16±0.05 (ns)
Hatchability (%)	70.61±6.11	71.32±1.01 (ns)	73.85±9.32	75.53±1.34(**)	75.42±6.71	77.34±1.07(**)	79.21±7.38	81.25±1.18 (**)	86.23±6.02	88.41±1.12 (**)
Survivability (%)	43.39±7.21	47.41±6.23 (**)	90.92±9.31	91.75±7.11 (ns)	91.73±8.81	93.61±7.61 (**)	93.64±9.26	95.43±8.13 (**)	96.88±8.75	97.63±9.15 (ns)

Table 8: Comparison between the reproductive performance of the mature and F1 snails

**P<0.01*p<0.05ns= not significant

IV. DISCUSSION

Age at maturity in F1 snails

The age at maturity obtained in this study was lowest (276 days) in snails fed 22% and highest (357days) in snails fed the basal plant diet. This is an indication that snails fed the supplemental diets were influenced by the nutrients in the diet, especially protein which is a precursor of hormones and in this case, reproductive hormones. The results obtained in this study are comparable with those of Raimi [20] who reported that snails attain sexual maturity at 9-10 months under laboratory conditions.

Egg characteristics

It was observed that the number of clutches ranged from 1.69 in snails fed the basal plant diet to 5.88 in snails fed 22% protein in the supplemental diet, implying many egg clutches from snails fed 22% supplemental diet. The clutch size varied between 9.35 eggs in snails fed the basal plant diet to 17.67 eggs in snails fed 22% protein. These results corroborate with those of Omole and Kehinde[21] whose mean number of clutches and clutch size ranged from 2 - 5 and 4 - 8 eggs, respectively for Achatina achatina and also with Okon *et al.* [22] for *Archachatina marginata* on mixed feeding regime made of natural plant diet and formulated feed. These results are at variance with those of Okon *et al.* [22] who obtained a mean clutch size of 20-28 eggs for the black-skinned purebred cross, 12-18 eggs for white-skinned purebred cross, and 12-20eggs for the black-skinned crossbred cross. These differences in the number of clutches and clutch sizes may be due to the effect of the supplemental diet on the reproductive performance of snails [21]. Protein in a supplemental diet by its amino acid content plays a significant role in egg production in snails.

The egg weights (357mg - 1003.02mg) recorded in this research, however, fall below the range recorded by Okon *et al.*[23] who observed that snail egg weights at laying ranged from 0.54 - 2.45g (mean of 2.00g). Ibom *et al.*[24]had the average egg weights of the black and white-skinned ecotypes of *A. marginata* to be 1.80g and 1.05g, respectively. However, Giant African Land Snail egg weights of 2.3–5.1g with a length of 2.1-3.2cm and breadth of 1.5-2.6cm have been reported [25-27]. The huge variation in the average egg weight ranges could be linked to the species, ages, and nutrition of the Giant African land snail [26].

The egg breadth obtained in this study ranged from0.96cm in snails fed the basal plant diet to 1.96cm in snails fed the supplemental diet at 22% protein level. Meanwhile, the egg length obtained varied between 1.38cm in snails fed the natural plant feed and 2.42cm in snails fed supplemental diet at 22% protein level. These results obtained are similar to those obtained by Ibom *et al.*[24]who observed that egg lengths for black-skinned and white-skinned ecotypes of *Archachatina marginata* were 1.61cm and 1.43cm respectively. Lower egg breadth values of 1.29cm and 1.05 cm for the black-skinned and white-skinned ecotypes of *Archachatina marginata* respectively [16], similar to the mean values obtained in this study have been recorded. Egg length

and breadth observed in this study differ from the egg length range of 13.0-16.0mm (mean of 14.88 mm) and breadths of 9.0-11.0mm (mean of 10.80mm) as reported by Okon *et al.*[23].

Duration of incubation

The duration of incubation observed in this study ranged from 23-26days in snails fed the supplemental diet at different protein levels to 42 days in snails fed the basal plant diet. These findings differ from those of Omole and Kehinde [21] who reported an incubation period range of 25-32days and Okon *et al.*, [23] who stated that snails hatched between 28 and 30days with a mean incubation period of 29 days. Also, Ibom *et al.* [17] reported an incubation period of 24-29days. The values obtained in this study are similar to those obtained by Ibom [16] and Okon *et al.* [28] but are quite lower than those reported by Payne and Wilson [29]; Ejidike *et al.* [30] and Ubua [31], whose values rather corroborate with values for the control. The disparity in incubation period may be attributed to variation in genetic factors like breed, strain, age and size of the snail, egg size, and environmental factors like temperature and relative humidity (Okon *et al.*, [23]. Besides, Ibom *et al.*[17] observed that exposure of eggs to fluctuating environmental conditions that differed from their near-constant uterine environment may influence the incubation period. In addition, they noted that incubation conditions such as uptake and loss of moisture, increased transpiration, and water loss resulting from increased heat produced by the developing embryo canal can cause variation in the incubation period.

Hatchability and survivability of snailets

It was observed that egg hatchability obtained in this study for snails fed the supplemental diets and snails fed the basal plant diet ranged from 71.32%-88.41% These findings fall within the range of 67.87%-82% as reported by Ukpong et al. [32] and Agbelusi and Adeparusi, [14] who worked on the effect of different incubation media on hatchability. Also, 0-100% hatchability was reported by several authors [16, 17, 25, 33-35] for purebred white-skinned ecotype of the same snail obtained breed. The values this study agree with those reported in by Adegbaju[36]; Akinnusi[2]; Akintomide[37]; Omole and Kehinde[21], Ibom[16] and Ibomet al. [17]. The results of this study are higher than those of Ogogo[33], Amubode[38], Ibom and Okon, [39]and Okonet al. [28] for Archachatina marginata snails. Ibom, [16] opined that, snail egg's hatchability is highly affected by the prevailing incubation media conditions (temperature, relative humidity, dryness, and water-logging) and whether or not the eggs were fertilized before laying. Snail egg hatchability is greatly impinged by environmental conditions [17].

The findings for survivability obtained in this study for snails fed the supplemental diet agree with the values of 74.67-96.23% obtained by Ibom[16], Ibom *et al.* [17] and Fapohunda *et al.* [40]but higher than the results obtained by Adegbaju [36] who reported a survivability range of >45%. The significant difference in survivability observed between the snails fed the supplemental diets and those fed the basal plant diet implies that feed plays an important role in the survival of snailets. Protein is needed amongst other things in enhancing the immunity and adaptation of animals to their environment.

Snailets weight

It was observed that the snailets of F1 snails fed the supplemental diet containing 22% crude protein level weighed more than those fed the basal plant diet. These results are higher than those obtained by Ogogo[33], Ewa [34], Odido [35], Ibom *et al.* [24], Ibom [16], Ibom and Okon [39], Okon *et al.* [7], Okon *et al.* [28], Okon *et al.* [22] and Ibom *et al.* [17] who reported snailets weights of 0.67- 1.24g for crossbreed Archachatina marginata var. saturalis snails fed chicken feed. Birth weight is a determining parameter for the survival of young animals [41] as seen in Guinea pigs. The weight of the snailets at hatch has a positive impact on the growth and subsequent reproductive performance of the snail.

Comparisons between the reproductive performances of mature Giant African land snails (*Archachatina marginata*) fed at graded protein levels in the supplemental diet and the subsequent reproductive performance of their F1 offspring.

It was observed that the reproductive performance of the F1 snailets fed the supplemental protein diets were higher compared with corresponding mature snail parents fed the same diets. This could be linked to the fact that the previous nutritional history of the snail affects its subsequent reproductive performance [42]. Management of the snails involving regular feeding and watering also improved the subsequent reproductive performance of the F1 snailets fed solely the basal diet.

Introducing a supplemental protein diet to the snails at an early age (at hatch) can greatly boost their growth and subsequent reproduction.

V. CONCLUSION

It can be concluded that the previous nutritional management of snails influences their subsequent reproductive performance. As such, feeding snailets with diets containing 22% crude protein level greatly boosts their subsequent reproductive performance. The minimum age at maturity (276days) was established. The number of egg clutches (5.88), clutch size (17.67 eggs), egg weight (1003.02mg), egg breadth (1.96cm), egg length (2.42cm), duration of incubation (23 days)), hatchability (88%), survivability (97%) and snailets weight (2.16g) generally were improved upon.

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