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Influence of Diet and Dietary Crude Protein Content on the Reproductive Performance of the Snail *Archachatina marginata* (Swainson, 1821)

Nygblé Angèle Epse Piba Sika [a], Kouassi Jerome N'dri [a*] and N'dri Saint Clair Amani [b]

[a] Laboratory of Animal Biology and Cytology, UFR Natural Sciences, Nangui Abrogoua University of Abidjan, Côte d'Ivoire.
[b] Department of Animal Biology, UFR Biological Sciences, Péléféro Gon Coulibaly Universiy of Korogho, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The pressure to collect snails due to the high demand for their meat has encouraged snail farming. However, snail farming faces a number of problems, including the formulation of a feed that meets the nutritional requirements of snails. The aim of this study was to evaluate the influence of plant-based diets and three concentrated diets, and then to determine the protein level to be incorporated into a concentrated feed capable of positively influencing the reproductive performance of the *Archachatina m*arginata snail. The study was conducted on 225 Arch. marginata juveniles fed two plant-based diets (R1 and R2) and 3 concentrated meal diets (R3, R4

**Corresponding author: E-mail: jeromekasb@yahoo.fr, ndrikjer.sn@univ-na.ci;*

and R5) with different protein contents (10.5%, 14% and 17.5%) for two years in experimental rearing. The R1 diet consists of *Lactuca sativa, Carica papaya*, *Brassica oleracea*, *Cecropia peltata*, *Laportea aestuans* and *Phaulopsis falcisepala*. The R2 diet was obtained by adding *Leucena leucocephala* leaves to R1. The first eggs were laid at 12 months on the R1 and R2 green forage diets and at 10 months on the R3 concentrate diet. Snails fed concentrated diets R4 and R5 laid their eggs at 9 months. The number of eggs laid per clutch and the average number of eggs per clutch in the animals fed the concentrated diets were statistically higher than those obtained with the vegetable diets (between 0.34 and 0.45 clutches and between 6.10 and 7.46 eggs respectively). The concentrated diets significantly improved the reproductive performance of *Arch. marginata* compared with the vegetable diets. In addition, reproductive performance was optimal on the R5 diet with the highest protein incorporation rate.

Keywords: Archachatina marginata; diet; protein content; reproduction.

1. INTRODUCTION

Giant African snails are becoming increasingly rare in Côte d'Ivoire's forests as a result of the pressure exerted on collecting them. These animals are a high-quality delicacy due to their high protein content, several trace elements such as iron, magnesium, phosphate and calcium, and their low lipid content. These snails are also consumed for their beneficial effects on human health. According to [1,2] African giant snails are consumed or used to treat various diseases such as haemorrhage, anaemia, hypertension, pain, constipation, cough, measles and tuberculosis. They are also used to treat various gynaecological conditions [3]. The scarcity of these animals has led to their breeding, also known as purchase-breeding. However, several factors, such as feed, are still limiting this type of farming. The aim of this study was to determine the nature of the diet and the level of crude protein to be incorporated into the concentrated feed in order to optimize reproductive performance.

2. METHODS

2.1 Biological Material

The animals used in this study are Achatinidae molluscs belonging to the genus Archachatina. The species studied is *Archachatina marginata* (Swainson, 1821). The snails chosen for this study were born at the Nangui Abrogoua University's experimental farm from parents themselves born at the said farm. *Arch. marginata* is an essentially terrestrial species with two variants: one with black pigmented flesh and the other with white pigmented flesh. People prefer to eat those with dark-coloured flesh, believing it to be tastier than white flesh [4].

2.2 Experimental Site

This study was carried out at the experimental farm of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). This center includes a building where breeding takes place under shelter and an open-air experimentation area. The mean monthly temperature and relative humidity in the barn were 26.7 ± 1.4°C and 82.6 ± 1.4%, respectively. The photoperiod was 12 hours light and 12 hours dark. The snails were reared in plastic tanks 0.66 m long, 0.6 m wide and 0.2 m high, giving a base area of about 0.4 m^2 and a volume of 0. 08 m^3. These enclosures are equipped with a mosquito net type cover constituting an anti-leakage device. Their bottom is covered with soil to a height of 4 cm thick.

2.3 Experimentation

Two hundred and twenty-five (225) *Archachatina marginata* snails aged approximately one week were randomly divided into five (5) batches. Each batch was fed one of five diets, two of which consisted of green fodder (R1 and R2) and three of which consisted of concentrated meal (R3; R4 and R5). These animals were distributed at a rate of 15 individuals per rearing tank and therefore three times for each diet. The experiment therefore required the use of 15 breeding chambers, i.e. 45 snails tested per type of food. The R1 green fodders plant diet consists of leaves of Lactuca sativa (Asteraceae), Carica papaya (Caricacea), Brassica oleracea (Brassicaceae), Cecropia peltata (Moracae), Laportea aestuans (Urticaceae) and Phaulopsis

Table 1. Composition of diets based on green fodder

Components (g)	R1	R2
Carica papaya	16,68	14,28
Lactuca sativa	16,68	14,28
Brassica oleracea	16,68	14,28
Cecropia peltata	16,68	14,28
Phaulopsis falcisepala	16,68	14,28
Laportea aestuans	16,68	14,28
leucena leucocephala	00	14,28
Total (g)	**100**	**100**

Table 2. Composition of diets based on concentrated flours

Components (g)	R3	R4	R5
Maize	15,3	15,3	15,3
Soybean meal	16	16	16
Soybeans	5	10	20
Soft wheat	15	15	15
Di-calcium phosphate	4	4	4
Vitamin complex	0,5	0,5	0,5
Calcium carbonate	28,7	28,7	28,7
Salt	0,4	0,4	0,4
Trace elements	0,1	0,1	0,1
Agar agar	15	10	0
Total (g)	**100**	**100**	**100**

falcisepala (Acanthaceae). The R2 diet, in addition to the leaves used for the R1 diet, is supplemented with leaves of Leucena leucocephala (Fabaceae-Mimosoideae), a plant relatively rich in protein (Table 1).

As for diets based on concentrated flours, they are formulated by varying their content of soy flour, a source of protein (Table 2).

To avoid the simultaneous variation of the rate of other nutrients following the decrease in the rate of the protein source in the R3 and R4 diets, powdered agar-agar is incorporated into them. Thus, to diets R3 and R4, 15% and 10% of this compound are added respectively in order to adjust their percentage composition to 100%. Thus, the proportions of seed soy flour in the R3, R4 and R5 diets are 5%, 10% and 20% respectively. This made it possible to obtain diets with variable protein contents (10.5% for R3; 14% for R4 and 17.5% for R5). Food is weighed before being served to them every two days. At the end of the two days, the food refusals are weighed and the feeders are properly washed before being reused. For each food served, a control of 100 g is placed under the same experimental conditions in tanks containing no animals. Weighing these control foods at the same time as the food refusals makes it possible to make weight corrections due to desiccation for plant diets of green fodder and to hydration for concentrated flour diets. The breeding substrates are watered daily morning and evening with tap water at the rate of 0.30 litre/substrate/watering, in order to maintain a relatively constant humidity. The substrates are regularly cleared of food refusals, dead animals and faeces to avoid the development of possible pathogens. The dead animals are inventoried by diet and replaced by animals of the same age and substantially the same weight, reared under the same experimental conditions in order to maintain the initial densities. The snails are weighed every two weeks.

Snails are fed the same diets until sexual maturity. During laying periods, the substrates are regularly inspected to detect possible eggs. The prospection of the substrates is practiced manually to avoid breaking the eggs. Eggs found simultaneously in the same location (buried or on the surface of rearing litter) are considered to constitute a clutch. They are removed with a plastic spoon, brood counted and weighed. The large and small diameters of these eggs are measured to the nearest millimeter. The eggs of each clutch are incubated separately, protected from light. The

incubation substrates are lightly watered once a week in order to maintain a relatively constant humidity. After 9 days, the setter trays are regularly inspected to detect hatchlings and remove them from the trays to prevent them from attacking unhatched eggs. The average of the egg incubation times for each clutch is considered the incubation period for that laying. Hatchlings are counted and hatch rates determined. Thus, the reproductive performances of the animals, by diet, are estimated from the age of first laying, the number of broods per snail, the frequency of the broods, the number of eggs per brood, the weight, the large and small egg diameters and their incubation times. The data collected allowed to estimate the total number of layind per snail and the hatching rate according.

2.4 Data Analysis

A correlation analysis was performed to estimate the degree of relationship between reproductive parameters and protein content of the diet. The correlation coefficient measures the level of joint variation of two variable measures. Also, the mean values of the reproduction parameters were compared by an analysis of variance (ANOVA) respectively according to Tukey's HSD test. These various statistical treatments were carried out using the software STATISTICA version 7.1

3. RESULT S

3.1 Egg-laying Characteristics

The number of eggs laid on concentrated meal diets remained higher than on green forage diets (Figure 1). Animals fed both green forage diets had approximately the same number of eggs laid throughout the laying period. The number of eggs laid by animals on the R5 concentrated diet was higher than that obtained by animals on the R4 diet. On the other hand, the number of eggs laid on the R4 concentrated diet was higher than on the R3 concentrated diet. Analysis of the curves showed that the number of eggs laid, whatever the diet and dietary protein level, was greater during the rainy seasons than during the dry seasons. The number of eggs laid by snails also increases with the age of the breeder. Thus, the number of eggs laid is lower in the first year of reproduction than in the second. The age of first oviposition was earlier in animals fed concentrated diets than in those fed green fodder (Table 3). The results also show that the age of first egg laying decreases with the protein content of the feed. The first eggs were laid at 12 months for animals fed the R1 and R2 green forage diets, i.e., 2 months after the first eggs were laid for animals fed the R3 concentrate diet (10 months). Snails on concentrated diets R4 and R5 laid their eggs at 9 months, i.e. 3 months before those on vegetable diets. The average number of clutches recorded per breeder and the number of eggs per clutch for animals on concentrated diets R3, R4 and R5 varied between 0.34 and 0.45 clutches and between 6.10 and 7.46 eggs respectively. There was no significant difference in these parameters between diets R3 and R4 using Tukey's HSD test at $P < 0.05$. Animals on the R1 and R2 green forage diets had statistically significantly lower average numbers of eggs laid (0.21 for R1 and 0.23 for R2) and eggs per laying (5.40 for R1 and 5.36 for R2) than snails on the R3 and R4 concentrate diets. The number of laying per snail and the number of eggs per snail improved with the protein content of the diet.

3.2 Characteristics of Eggs

The eggs of animals fed the R1 and R2 green forage diets had lower average weights and larger and smaller egg diameters than those of snails fed the various concentrated diets. Analysis of the results shows that the physical characteristics of the eggs (weight, large and small egg diameters) improve with the protein content of the diet. In fact, the eggs from animals fed the R5 diet had the highest weights and sizes. The eggs harvested from animals fed the concentrated diets had similar average incubation times (29.27 d for R3, 29.06 d for R4 and 29.03 d for R5) and were shorter than those of snails fed the green forage diets R1 (30.36 d) and R2 (30.12 d). Hatching rates were better on the concentrate diets than on the green forage diets. The protein content of the diet was positively correlated with the number of clutches laid per breeder ($r = 0.99$) and the number of eggs per clutch ($r = 0.98$) (Table 4. On the other hand, it was negatively correlated with age at first-laying ($r = -0.97$). The mineral content was also negatively correlated with age at first-laying ($r = - 0.99$) and positively correlated with the number of laying per snail ($r = 0.94$) and the number of eggs per laying ($r = 0.88$).

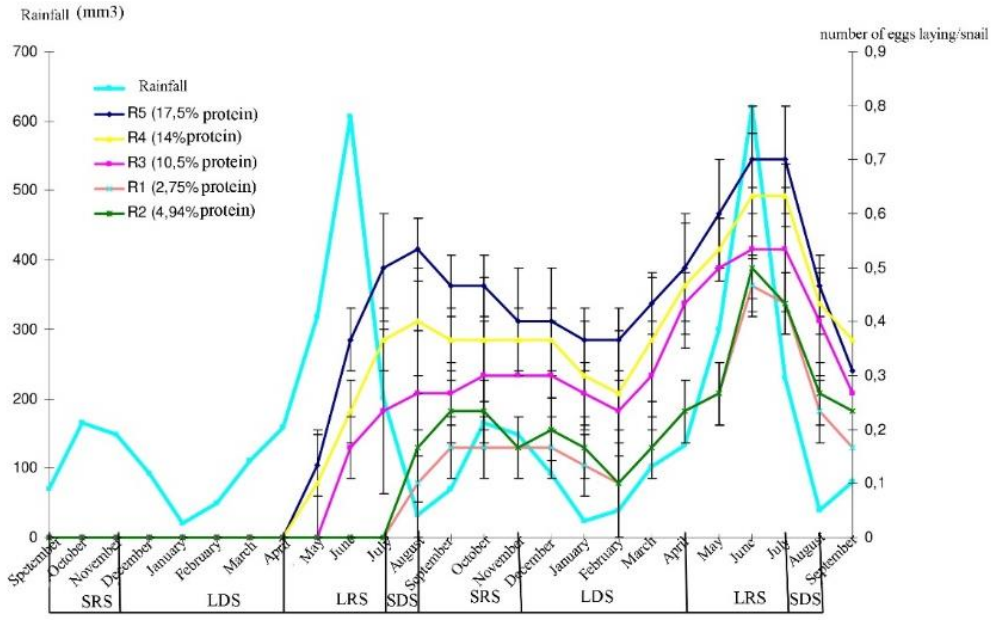

Fig. 1. Evolution of laying according to regimes and seasons

SDS: small dry season; LDS: long dry season; SRS: small rainy season; LRS: long rainy season

Table 3. Reproductive parameters according to diets

Variables	R1	R2	R3	R4	R5
Age of first laying (months)	12 ±2,02^{C}	12±2,07^{c}	12±1,02^{b}	09±1,05a	09±1,08^{a}
Average monthly number of laying/snail	0,21 ±0,11^{c}	0,23±0,15^{c}	0,34±0,11^{b}	0,38±0,13^{b}	0,45±0,13^{a}
Average number of eggs/ laying	5,40±1^{c}	5,36±1,16^{c}	6,10±1,12^{b}	6,67±1,51^{b}	7,46±1,79^{a}
Average egg weight (g)	1,42±0,08^{b}	1,45±0,08^{c}	1,46±0,1b^{c}	1,48±0,11^{ba}	1,25±0,09^{a}
Large diameter of an egg (mm)	1,31±0,06^{c}	1,37±0,07^{c}	1,42±0,09^{d}	1,51±0,14^{b}	1,56±0,12^{a}
Small diameter of an egg (mm)	1,14±0,05^{c}	1,15±0,08^{c}	1,17±0,06^{d}	1,19±0,07^{ba}	1,21±0,08^{a}
Mean incubation time (d)	30,35±1,38^{a}	30,12±1,22^{a}	29,27±1,14^{b}	29,06±0,94^{b}	29,03±0,89^{b}
Average hatching rate (%)	67,92±15,52^{b}	69,33±14,33^{b}	78,08±18,89^{a}	82,79±18,78^{a}	84,79±19,46^{a}

Table 4. Correlation between egg-laying performance and protein and mineral content of the diet

	% protéin	% mineral materials	Age of first-laying	Number of laying	Number of eggs/laying
% protéin	1				
% mineral materials	0,94	1			
Age of first-laying	-0,97	-0,99	1		
Number of laying	0,99	0,94	-0,97	1	
Number of eggs/laying	0,98	0,88	-0,93	0,98	1

4. DISCUSSION

Analysis of the results showed that increasing dietary protein content influenced the egg-laying performance of *Arch. marginata*. Animals fed green fodder diets didn't perform well reproductively compared with those fed concentrated diets richer in minerals, energy and protein. This could be explained by the fact that a diet balanced in minerals and proteins improves reproductive performance. In terms of age at first-laying, the results of this study showed that varying the protein content from 10.5% to 17.5% reduced the age at first-laying from 10 to 9 months. This also resulted in an increase in the monthly number of laying per snail from 0.34 to 0.45, followed by an increase in the number of eggs per laying from 6.10 to 7.46. It has been established that the ages of first-egg laying recorded with the different plant and concentrate diets are lower than that presented by Dafem *et al*. [5] for the same species, i.e. 13 months and in *Archachatina ventricosa* in the wild, where the animal feeds almost exclusively on plant food [6]. This difference could be explained by the rearing management (density, feed, humidity, temperature). The highest monthly number of eggs laid recorded with the R5 concentrated diet (0.45) is lower than those obtained by Aman [7] i.e. 0.62, 0.66, 0.82 and 0.95. The difference between the numbers of laying could be explained by the fact that this author enriched the rearing substrate with calcium for snails fed the meal concentrate diet. Similarly, Jess [8] reported that the high level of bioavailable calcium in this snail improved egg-laying performance. Furthermore, our results showed that the best oviposition frequency recorded in this study was also lower than that of *Arch. marginata suturalis* (one oviposition approximately every thirteen and a half days) reported by Stievenart [9]. This could be attributed not only to the genetic difference between the two species of *Archachatina*, but also to a possible age difference between the breeders of the two species concerned. According to Hodasi [10], mature snails have egg counts and laying frequencies that improve with the age of the animal. With regard to meat yield, analysis of the results showed that the quantity of meat produced by animals on concentrated diets was more abundant than that produced on vegetable diets. Similar results were reported by Otchoumou [11].

5. CONCLUSION

The protein content of the diet influences reproductive parameters of *Archachatina marginata*. With diets made up of green forage (R1 and R2), the age of first egg-laying is late (12 months of age) whereas with concentrated diets (R3, R4 and R5) the age of first egg-laying is reduced to 10 months. Thus, the age at which snails lay their first eggs is reduced with the crude protein content of their diet. Animals fed concentrated diets had better reproductive performance than those fed vegetable diets of green fodder. The concentrated diets produced higher average monthly laying numbers and a greater number of eggs per laying. Furthermore, the concentrated diets produced eggs with better characteristics than the green forage-based diets. However, the R5 diet with the highest protein content produces heavier, larger eggs than all the other diets. It should also be noted that the nature of the diet (green feed or concentrate) has no effect on incubation time or hatching rate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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