

Effect of Diet and Crude Dietary Protein Content on Reproductive Performance of The Snail *Achatina fulica* (Bowdich, 1720)

Sika, Nygblé A.

Laboratory of Animal Biology and Cytology, UFR Natural Sciences
Nangui Abrogoua University of Abidjan, Côte d'Ivoire

Amani, N'dri S. C.

Departement Animal Biology, UFR Biological Sciences,
Péléféro Gon Coulibaly University of Korogho, Côte d'Ivoire

N'dri, Kouassi J.

Laboratory of Animal Biology and Cytology, UFR Natural Sciences
Nangui Abrogoua University of Abidjan, Côte d'Ivoire

ABSTRACT

The effect of diet and protein content on reproductive parameters was studied of 225 juveniles of *Achatina fulica*. For this, these snails were subjected to two green fodders (R1 and R2) and three concentrated flours diets (R3, R4 and R5) with different protein contents (10.5%; 14% and 17.5%), for two years in the experimental farm of Nangui Abrogoua University. The R1 diet includes *Lactuca sativa* (Asteraceae), *Carica papaya* (Caricaceae), *Brassica oleracea* (Brassicaceae), *Cecropia peltata* (Moraceae), *Laportea aestuans* (Urticaceae), and *Phaulopsis 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 relatively protein-rich plant. The animals submitted to the diets consisting of green fodder began to lay from the seventh month, whereas with the concentrated diets (R3, R4 and R5) the snails laid eggs from three months, i.e. with a reduction in the age of first lay by four months. Concentrated diets provide higher average monthly laying numbers (between 0.41 and 0.57) as well as a higher number of eggs per laying (between 215 and 233 eggs) compared to R1 and R2. The highest protein R5 diet is the one that provides the best reproductive performance. Additionally, the strong positive correlation ($r=0.99$) between diet protein content and laying and egg number per laying indicates that the protein content of the diet significantly improves the reproductive performance of *A. fulica*.

Keywords: *Achatina fulica*, diet, protein content, reproduction

INTRODUCTION

In most countries of sub-Saharan Africa, an important proportion of animal protein for human consumption has so far come from wild animals. Several animals, among which snails feature prominently, are threatened with extinction due to poaching. In Côte d'Ivoire for example, a study by Kouassi *et al.* 2008 on the markets of the economic capital (Abidjan) revealed that for the only year 2008, more than 1700 tons of snails were marketed. Indeed, snails have been

widely consumed and have even been part of dietary habits since prehistoric times (Murphy, 2001). If the meat of these animals is so much appreciated and increasingly consumed by the Ivorian populations, it is partly thanks to its scent and flavor (Kouassi *et al.*, 2007). In addition, this meat contains important nutritional qualities. Indeed, it is an important source of protein, amino acids (lysine, phenylalanine and leucine), energy and mineral matter (Fagbuaro *et al.*, 2006; Diomandé *et al.*, 2008; Otchoumou *et al.*, 2010) and very poor in lipids. It is rightly recommended in dietetics in low-fat diets (Engmann *et al.*, 2013). The species *Achatina achatina* and *Archachatina ventricosa*, which were once the most consumed species, are becoming increasingly rare, forcing populations to introduce new species such as *Achatina fulica* into their eating habits. In order to have snail meat continuously available, several works have been initiated for the development of breeding of these animals. In this sense, various food formulations have been tested to substitute plant foods. These concentrated feeds must contain optimal proportions of certain elements such as proteins to improve zootechnical performance. The importance of this study lies in the fact that it provides an answer on the effect of diet and the influence of protein content on the reproductive performance of the snail *Achatina fulica*.

METHOD

Biological Material

The animals used for this study are Achatinidae molluscs belonging to *Achatina fulica* (Bowdich, 1720) species with light brown pigmented flesh. These snails are, with *Achatina achatina* and *Archachatina ventricosa*, the most consumed snails in Côte d'Ivoire. The snails chosen for this study were born at the Nangui Abrogoua University's experimental farm from parents themselves born at the said farm. The choice of this species is justified by the fact that *Achatina fulica* is the most prolific species of the *Achatina* genus (Kouassi *et al.*, 2010; Otchoumou *et al.*, 2003). In addition, *A. fulica*, formerly little consumed in Côte d'Ivoire, is increasingly consumed today. Regarding biological performance, *A. fulica* can reach 250 g at the adult stage. It is a species with a short reproductive cycle. Its duration of sexual maturity in rearing environments can be reduced up to 3 months of age (Otchoumou *et al.*, 2003).

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 $26.7 \pm 1.4^{\circ}\text{C}$ and $82.6 \pm 1.4\%$, 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 m² and a volume of 0.08 m³. 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.

Experimentation

Two hundred and twenty-five (225) approximately 3-day-old *Achatina fulica* snails were randomly allocated after five (5) diets, including two green fodder compounds (R1 and R2) and three flour concentrates (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* (Caricaceae),

Brassica oleracea (Brassicaceae), *Cecropia peltata* (Moraceae), *Laportea aestuans* (Urticaceae) and *Phaulopsis 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).

Table 1 : Composition of diets based on green fodder

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

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

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

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 laying per snail and the hatching rate according.

Datas 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

RESULTS

Egg-Laying Characteristics

The number of broods per snail is higher during rainy seasons and lower during dry periods, regardless of the protein content of the diet (Figure 1). During reproduction periods, egg-laying peaks are recorded during June's month. In addition, the average number of broods per snail increases with age. Thus, the numbers of laying recorded during the first year are lower than those obtained during the second year. The first laying of snails submitted to plant diets R1 and R2 takes place at the age of 7 months (Table 3). On the other hand, those subjected to the R3 concentrated diet lay eggs at 5 months when, those subjected to the R4 and R5 diets lay eggs at 4 months. Thus, the age at first laying of *A. fulica* can be reduced with the crude protein content of their diet. Animals fed concentrated diets have better reproductive performance than those fed vegetable diets. Both, fodder plant diets show the same effects on snail egg-laying characteristics. Statistical analysis reveals no significant difference in Tukey's HSD test at $P < 0.05$, between the incubation period and the hatching rate of eggs collected from snails subjected to vegetable diets of green forage (R1 and R2). The average monthly number of

broods per broodstock in animals subjected to concentrated diets varies between 0.41 and 0.57 with an average number of eggs per brood varying between 215 and 233. The number of broods observed per snail subjected to R5 is higher than that recorded among those subject to R3. Statistical analysis indicates that the R4 diet has an intermediate effect compared to these two concentrated diets on laying performance of snails. There was no significant difference in Tukey's HSD test at $P < 0.05$ between the concentrated diets for the mean number of eggs per snail, which varied between 215 and 233.

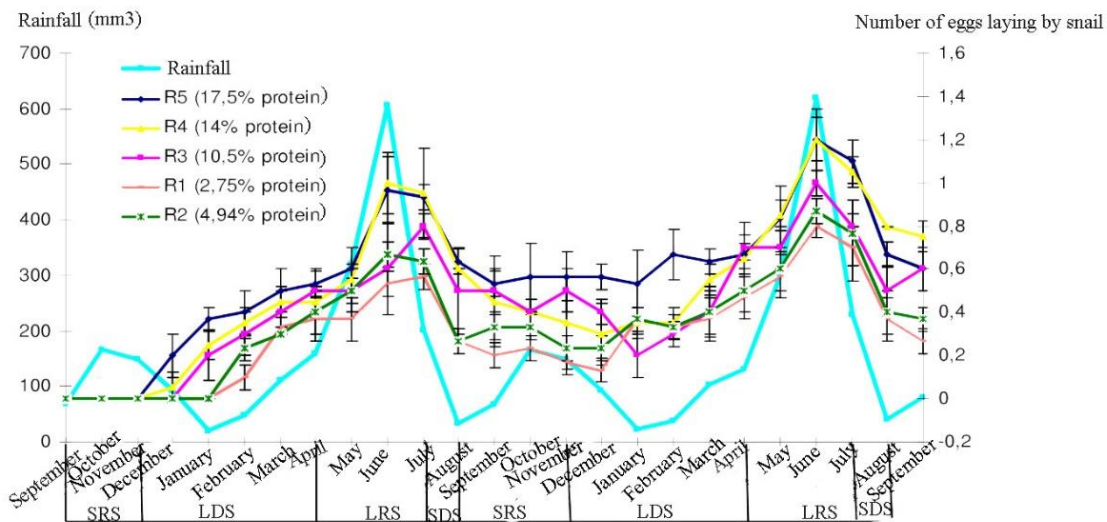


Figure 1 : Evolution of laying according to regimes and seasons, SDS: small dry season ; LDS : long dry season ; SRS : small rainy season ; LRS : long rainy season

Table 3 : Reproductive parameters according to diets

Variables	R1	R2	R3	R4	R5
Age of first laying (months)	07 ±2,02 ^a	07±1,50 ^a	05±2,30 ^b	04±1,52 ^c	04±1,15 ^c
Average monthly number of laying/snail	0,30 ±0,12 ^c	0,33±0,14 ^c	0,41±0,15 ^b	0,48±0,22 ^{ab}	0,57±0,19 ^a
Average number of eggs/ laying	200±52,00 ^b	202±44 ^b	215±63 ^a	221±71,00 ^a	233±55 ^a
Average egg weight (g)	0,17±0,02 ^d	0,18±0,03 ^d	0,19±0,05 ^c	0,21±0,08 ^b	0,23±0,06 ^a
Large diameter of an egg (mm)	04,29±0,07 ^d	04,27±0,04 ^d	05,37±0,17 ^c	05,43±0,13 ^b	05,64±0,16 ^a
Small diameter of an egg (mm)	03,35±0,54 ^d	03,41±0,36 ^d	04,31±0,10 ^c	04,35±0,08 ^b	04,45±0,11 ^a
Mean incubation time (d)	16,59±1,97 ^b	16,26±1,52 ^b	14,47±2,09 ^a	15,19±1,78 ^a	14,79±1,92 ^a
Average hatching rate (%)	66,00±1,47 ^b	68,12±2,1 ^b	85,38±2,09 ^a	85,67±3,50 ^a	85,84±2,16 ^a

Characteristics of Eggs

The statistical analysis shows that the eggs laid by the animals subjected R5 (17.5% protein) have a higher average weight, which is 0.23 g. The large diameters and the small diameters of

the eggs laid by R5 are the largest. They measure 5.64 mm and 4.45 mm respectively. Also, the eggs laid by the animals subjected to the R3 diet are smaller (5.37 mm for the large diameter and 4.31 mm for the small diameter) and of less weight (0.19 g) than those laid by the animals subjected to the R4 concentrated diet, with a large diameter of 5.43 mm; a small diameter of 4.35 mm and an average weight of 0.21 g. No significant difference in Tukey's HSD test at $P < 0.05$, is observed between the concentrated diets on the one hand and on the other hand, between the vegetable diets concerning the incubation times and the hatching rates. Hatchability is higher with concentrate diets than with green fodder diets. The correlation analysis between the protein and mineral content of the diet and the reproductive parameters (age at first laying, number of laying and eggs per laying) is summarized in Table 4. The first laying is negatively correlated with the crude protein content of the diet. This explains a reduction in the time of appearance of sexual maturity in snails depending on the protein content of the diet. Moreover, a very strong positive correlation was recorded between the protein content of the diet and the number of laying and eggs per laying ($r = 0.99$). Also, these parameters (number of laying and eggs per laying) are very strongly and positively correlated with mineral content ($r = 0.89$ and 0.90). This reflects an improvement in snail egg-laying performance with the mineral content of the diet.

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

	% protein	% mineral materials	Age of first laying	Number of laying	Number of eggs/laying
% protein	1				
% mineral materials	0,94	1			
Age of first laying	-0,97	-0,99	1		
Number of laying	0,99	0,89	-0,94	1	
Number of eggs/laying	0,99	0,90	-0,94	0,99	1

DISCUSSION

Analysis of the results of this study showed that the reproductive performance of *A. fulica* are influenced by diet. Indeed, the reproductive performance of animals subjected to concentrated diets remains much better compared to those of animals subjected to green fodder. Thus, the age of first laying with the R1 vegetable diet without *Leucaena* is 7 months, that with the R5 concentrated diet is 4 months, ie a difference of 3 months. Also, the number of monthly layings made by a snail subjected to the R1 diet is 0.3 while that of a snail subjected to the R5 diet is 0.57. This corresponds to a monthly egg laying difference of 0.27. This means that after one year, the R5 diet makes it possible to gain about 4 broods more per individual than with the R1 vegetable diet. This difference between the reproductive performance of snails subjected to green fodders and those subjected to concentrated diets could be explained, according to Otchoumou *et al.* (2005) by the level of dietary calcium present in the diet. Indeed, the snail uses dietary calcium for the calcification of these eggs (Bonnet *et al.*, 1990). On the other hand, the results of this study showed that calcium, although necessary, is not the only element responsible for the reproductive performance observed in animals subjected to concentrated diets. Increasing the protein content of concentrated diets resulted in improved reproductive performance in animals. Thus, with a 7% difference in protein between the R3 concentrated

diet and the R5 diet, the age at first lay was reduced by approximately one month. This also allowed a monthly gain of 0.16 laying per individual.

Concerning the average age at the first laying, the results showed that it is 7 ± 2.02 months for R1 and 7 ± 1.5 months for R2 in animals fed green fodders diets. This age falls within the age range of sexual maturity presented by Waitkuwait (1987); Upatham et al. (1988) and Zongo et al. (1990). The R5 concentrated diet containing 17.5% protein reduces sexual maturity time limit until 4 months. Also, the ages of first laying of the animals subjected to the different diets concentrated in this study are late in relation to the period of sexual maturity. This may be due to the high dietary protein content of the diet. However, Otchoumou *et al.* (2005) showed by observation of histological sections of ovotestis from snails of the same species fed with concentrated diets that this time until sexual maturity obtained is 2.5 months.

CONCLUSION

Dietary protein content influences reproductive parameters of *Achatina fulica*. With the diets made up of green fodder (R1 and R2), the age of first laying is late (7 months) whereas with the concentrated diets (R3, R4 and R5) the age of first laying is reduced at three months. Thus, the age at first laying of snails is reduced with the crude protein content of their diet. Animals fed concentrated diets have better reproductive performance than those fed vegetable diets of green fodders. In addition, concentrated diets result in higher average monthly laying's numbers (between 0.41 and 0.57) as well as a higher number of eggs per laying (between 215 and 233 eggs). Moreover, concentrated diets give eggs better characteristics than diets with green fodder. However, the R5 diet with the highest protein content results in heavier and larger eggs than all other diets. It should also be noted that the nature of the feed (green fodder or concentrate) has no effect on the incubation period and the hatching rate.

References

- Bonnet J.C., Aupinel P. et Vrillon J.L. 1990. L'escargots *Helix aspersa* : biologieélevage. Institut National de la Recherche Agronomique, Editeur, Paris, ISBN: 2-7380-0247-1,124p.
- Diomandé M, Kipré AV, Koussemon M, Kamenan A. 2008. Substitution de la farine de poisson par celle de l'escargot *Achatina fulica* dans l'alimentation des poules pondeuses en Côte d' Ivoire. *Livestock Research for Rural Development*, 20(1): http://www.cipav.org.co/Irrd/Irrd20/1diom2_0002.htm.
- Engmann FN, Afoakwah NA, Darko PO, Sefah W. 2013. Proximate and Mineral Composition of Snail (*Achatina achatina*) Meat; Any Nutritional Justification for Acclaimed Health Benefits? *Journal of Basic and Applied Scientific Research*, 3(4): 8-15.
- Fagbuaro O, Oso JA, Edward JB, Ogunleye RF. 2006. Nutritional status of four species of giant land snails in Nigeria. *Journal of Zhejiang University Science B*, 7(9): 686 - 689
- Kouassi K.D., Otchoumou A. & Gnakri D., 2008. Le commerce des escargots (*Achatina achatina*), une activité lucrative en Côte d'Ivoire. *Livestock Research for Rural Development*, 20 (4). <http://www.Irrd.org/Irrd20/4/koua20058.htm>. Consulté le 22 mars 2023.
- Kouassi K. D., Otchoumou A. & Dosso H. 2007. Effet de l'alimentation sur les performances biologiques chez l'escargot géant africain : *Archachatina ventricosa* (Gould, 1850) en élevage hors-sol. *Livestock Research for Rural Development*, 19 (64). From <http://www.Cipav.org.co/Irrd/Irrd19/5/kona19064>. Consulté le 22 mars 2023.

Kouassi K.D., Karamoko M. & Memel J.D., 2010. Etude comparative de la croissance des principales espèces d'escargots géants comestibles d'Afrique. *Revue du CAMES (Série A)*, 11: 80 – 84.

Murphy B., 2001. Breeding and growing snails commercially in Australia. A report of the rural Industries Research and development Corporation, Barton, A.C.T. at: <http://trove.nla.gov.au/work/32143264>. [Accessed June 2015].

Otchoumou A., N'Da K., Dosso H., & Kouadio D.K., 2005. L'escargot africain *Achatina fulica* (Bowdich, 1820), peste potentielle pour les végétaux des forêts anthropisées de Côte d'Ivoire. *Haliotis* 34: 71-81.

Otchoumou A. Dosso H. & Fantodji A., 2003a. Elevage comparatif d'escargots juvéniles *Achatina achatina* (Linné, 1758), *Achatina fulica* (Bowdich, 1820) et *Archachatina ventricosa* (Gould, 1850): effets de la densité animale sur la croissance, l'ingestion alimentaire et le taux de mortalité cumulée. *Revue Africaine de Santé et de Production Animale*, 1 (2): 146-151

Otchoumou A., Dupont-Nivet M., Ocho Anin, Atchibri L. & Dosso H., 2010.

Body proportions and chemical composition of wild and reared edible snails of Ivory Coast. *Italian journal of food science* 22 (1): 1120-1770.

Upatham E.S., Kruatrachue M. & Baidikul V. 1988. Cultivation of the giant African snail, *Achatina achatina*. *Journal Scientifique Thailandais*, 14: 25 – 40.

Waitkuwait E., 1987. Nutzungsmöglichkeiten der Westafrikanischen Riesenschnecken (Achatinidae), Rapport LACRNA / GTZ. 80 p

Zongo D., Coulibaly M., Diambra O & Adjiri E. 1990. Note sur l'élevage de l'escargot géant africain *Achatina achatina*. *Nature et Faune*, 6: 32 – 44