East African Scholars Journal of Agriculture and Life Sciences
Abbreviated Key Title: East African Scholars J Agri Life Sci
ISSN 2617-4472 (Print) | ISSN 2617-7277 (Online) |
Published By East African Scholars Publisher, Kenya
Volume-2 | Issue-11 | Nov-2019 |

OPEN ACCESS

Research Article

Study of Sexual Maturation in Limicolaria Fiammea Snail (Müller, 1774), Subjected To Two Types of Diet

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Abstract: A total of 200 spats were submitted to two types of diets. One hundred of them were fed on a mixture of *Carica papaya* (Caricaceae) leaves, *Manihot esculenta* cassava while the other 100 were fed on feed concentrate.Every month, 20 snails from each batch are taken to the laboratory and to be observed under electron microscope, the various histological sections made in individuals ootestis underwent disinfection and coloring practices. The objective is to determine the sexual maturity age for *Limicolaria flammea* histologically.After three months, snails fed on concentrate were able to reach this age, while those fed on mixture of leaves and shell reached sexual maturity from six months of age. Note that this sexual maturity is marked by the presence of spermatozoa in the lumen of tubules and the mature oocytes**.**

Keywords: *Limicolaria flammea* , sexual maturity, ootestis, oocyte, spermatozoa.

INTRODUCTION

The breeding of snails is experiencing an increasing growth in sub-Saharan Africa. Species of giant African snails such as *Achatina and Archachatina* types are the most preferred bush meat in West-Africa. In Ivory Coast, researches related with a cheating culture have been focused on some species: *Achatina achatina (*Otchoumou, 2003a), *Archachatina ventricosa* (Kouassi, 2007a) *Achatina fulica (*Otchoumou, 2004a), and now *Archachatina marginata*, a species which is more frequently consume in Ivory Coast (Kouassi, 2008). These researches proved that the biological performances of these animals were strongly related to diet (Adou, 2011). The concentrated diet has indeed strongly influenced their growth and sexual maturity. Moreover, (Agongnikpo, 2010), (Odjo, 1992) and then (Akinnusi, 2002) have shown that the leaves of certain plants were among the most common green feeds consumed by snails (*A. achatina*, *A. fulica*, and *A. marginata)* and helped them get better biological performances.

Some many research on many parameters including: relative weight growth, feeding behavior and sexual maturity age. As for *Limicolaria flammea*, it remains poorly known in Yvory coast.

THE GENERAL OBJECTIVE OF THIS WORK IS TO CONTRIBUTE TO A STRONG PRODUCTION OF LIMICOLARIA SNAILS GENUS. THE SPECIFIC OBJECTIVES ARE THEREFORE AIM TO:

- investigate the influence of feed on reproduction by determining the age of first sexual maturity of snails according to each dietary ration (concentrate and mixture of plant leaves)
- study the influence of each diet on the relative weight growth of the snails.

MATERIAL AND METHODS

Study Area

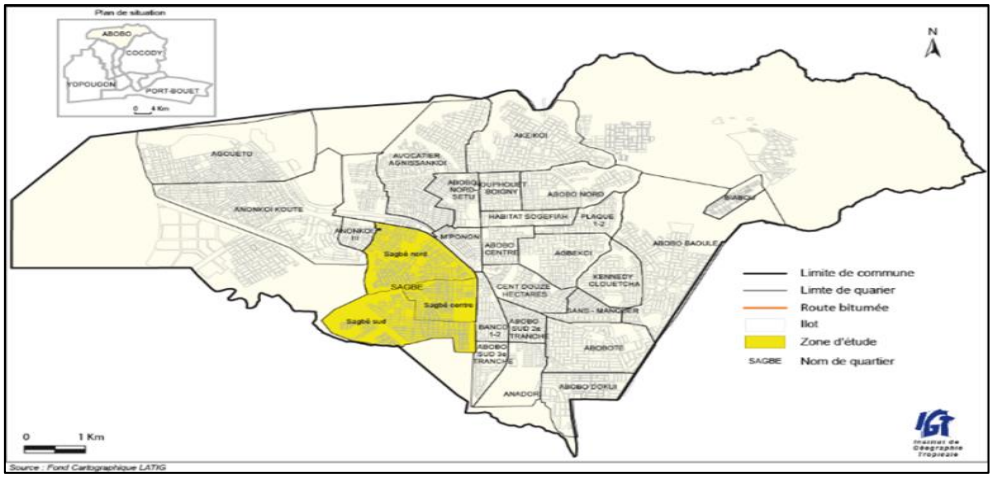
The study took place in the area of Abidjan (Ivory Coast) precisely in the district of Abobo. This work began in early June and ended in November 2015.

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjals/

Article History
Received: 25.10.2019
Accepted: 06.11.2019
Published: 15.11.2019

DOI: 10.36349/easjals.2019.v02i11.003



ANIMALS

The animals used in this work are Molluscs, Gastropods, Pulmonae. They belong to the order of Stylommatophores, the super family of Achatinaceae, the family of Achatinidae, the genus Achatina and the limicolaria fiammea species

THE BREEDING ENCLOSURES

The breeding snail enclosures consist of cemented rectangular pits. The height extends to 0.50 m for snail enclosures reserved for seed and 0.75 m for snail enclosures reserved for breeding stock.

STUDY MATERIAL FOR THE HISTOLOGY OF OVOTESTIS

The Material Used For The Histological Study Of Snail Ovotestis Consists Of:

- a magnifying glass to facilitate the visualization of ovotestis.
- Rubber boxes marked with a number identifying the organ taken;
- 5% formalin to include organ fragments
- 70 °, 95 ° and 100 ° alcohol, paraffin and toluene were used for the inclusion of fragments;
- cassettes for isolating the organ;
- a microtome for cutting blocks made up of gonad fragments;
- tap water, for rinsing sections;
- hemalum and eosin for the coloring of the sections made;
- some glue (kitt), slides and microglass
- a photonic microscope for the observation of assembled slides.

METHODS

This study of the growth and reproduction performance of Limicolaria flammea required a total of 200 snails and 100 of which were fed on mixtures of papaya leaves, cassava and the other 100 are fed on a feed based on flour concentrate. They were watered with a watering can and regularly cleaned. The average ambient temperature of the breeding building was 25.5 ± 2.2 ° C and the relative humidity of the air was 91.7%. Regular watering of the entire room is done twice a week to create a moist microclimate conducive to the activity of snails.

This snail feed made is based on corn, cottonseed meal, soybeans, remolding soft wheat, bicalcium phosphate, vitamins, calcium carbonate, salt and trace elements (Aboua, 1990) **(Table I).**

Table I: Percentage composition (g/100g) of flour concentrate diet

Corn	Soybean Meal	Soja bean	Dicalcium phosphate	Vitamin	Calcium carbonate	NaCl	Trace elements	Remolding soft wheat
10	16	16	4	0.5	38	0.4	0.10	15

Table II: Characteristics (in% MS) of the various ingredients of compound feed

Gross Energy cal/g	Nitrogenous material total	Calcium total	Phosphate total	Fat	Amidon	Free sugar	Free cellulose	Ash	Total
2785	17,48	12,02	1,2	,71	12,56	03,10	4,76	33,43	100

The characteristics of the various ingredients of this feed are indicated in Table II.

Both types of feed are distributed ad libitum. The feed refusals are recovered from snail enclosures two days later.

The forms of the cereals flour and the cotton meal obtained are mixed with the other constitutive products of the feed concentrate. Here the cereal grains are less than or equal to 1 mm in size (Figure 1).

Figure 1: Flour form of feed concentrate

The feed concentrate in pellet form is obtained with the flour concentrate feed. This feed is hydrated at 10 cm3 of water / kg of feed. After mixing, the feed is spread on flat and smooth plastic container. The pellets are then cut out and served to the snails (Figure 2).

Figure 2 : Pellet form of concentrate feed

HISTOLOGY OF GONADS (OVOTESTIS)

➢ **Realization Of Histological Sections**

The first forty snails of which twenty per diet are dissected for the purpose of collecting the ovotestis of each individual. In fact, the ovotestis is formed of big size lobules and various forms in which the gametes maturation takes place. It is visible in the digestive gland; the functional unit of ovotestis is acinus. This organ (ovotestis) is treated according to standard histology techniques.

Small fragments of about a few grams (0.2 g to 0.4 g) in the area of the visceral mass where the diffuse gonads are found are then taken and fixed in 5%

formalin contained in labeled boxes for 3 to 5 days. The time for the sampling and numbers are marked on the different boxes containing the organ taken.

PERFORMING HISTOLOGICAL SECTIONS REQUIRES SEVERAL STEPS:

❖ **Fixation of sampling**

IT HAS THREE STAGES:

- Performing of recuttings of the sample in small fragments;
- Placing of the sections into cassettes and identification of cassettes;
- Placing of cassettes in 5% formalin for at least 24 hours.

❖ **DEHYDRATION**

This step involves getting rid of the tissue or the organ from water it contains. It occurs in a dehydrating or circulating automaton (Histokinette, 2000). This device consists of 12 trays and the cassettes last 02h in each tray. First of all, the cassettes are placed in the basket of the automaton and the passage from tray to tray is done as follows:

- Tray 1: 5% formalin (uses to rinse les samples)
- Tray 2: water (allows to continue fixation)
- Tray 3: 80° alcohol
- Trays 4 and 5: 95° alcohol
- Trays 6; 7 and 8: absolute alcohol
- The trays 3 to 8 allow the degreasing and dehydration of the sample.
- The trays 9 and 10: They continue the degreasing and the dehydration and then take the place of the alcohol in the sample. They will also facilitate the entry of paraffin into the sample.
- The tray 11 and 12: They make it possible to harden the sample, to coat the tissue in block form and to prepare it for the histological section.

❖ **Casting of the blocs**

It consists of putting the sample into a mold and make the paraffin flow through the mold. It takes place in a device named Histocentre having two parts:

- A hot part: Containing liquid paraffin with a melting temperature of 58 to 62 ° C. It is in this part that the blocks are molded and the paraffin poured above.
- A cold part: It is a cooling plate. It is used for the quick cooling of molds. It has a temperature of -20 ° C.

❖ **CREATION OF HISTOLOGICAL SECTION**

Histological sections are made using a microtome. Each cassette is mounted on the device (apparatus) to scrape the paraffin in order to reach the organ which has been coated therein. The scraping is done at 35µm. When the organ is reached, the actual sections are made at 04µm.

❖ **CHANGEOVER TO THE WATER BATH**

The sections made are put in the water bath (water at 40 ° C + 1ml of albumin). The water bath facilitates the spreading of the sections in order to choose the best ones.

❖ **MOUNTING ON MICROSCOPE SLIDE AND OVEN DRYING**

The best sections are put on the microscope slides previously identified. These slides are put in the oven for 24 hours in order to properly secure the section that is mounted.

➢ **COLORING OF SLIDES**

It is done for about 01h. The slide will go into 12 trays and its duration in each tray is 05 minutes maximum.

- Trays 1 and 2: Toluene
- Tray 3: Absolute alcohol
- Tray 4: 95 ° alcohol
- Tray 5: Hemalum: The hemalum allows the coloring of the nuclei (blue to purple).
- Tray 6: Hydrochloric acid: It fixes the hemalum.
- Tray 7: Saturated Lithium: It neutralizes the action of hydrochloric acid in order to avoid it

INTERACTION WITH FUTURE SOLUTIONS

- Tray 8: Eosin: color the cytoplasm and its organelles (pink to red).
- Tray 9: 95 ° alcohol
- Tray 10: Absolute alcohol

THE PASSAGE OF THE SLIDES IN INCREASING ALCOHOL CONCENTRATIONS ALLOWS THE DEHYDRATION OF THE SECTIONS AND ALSO THEIR RINSING.

- Trays 11 and 12: Toluene: It makes it possible to lighten the slides.

NB: From tray 4 to 9, the slides are quickly rinsed with water before being put back into the next tray.

❖ **ASSEMBLY OF MICROGLASS AND MICROSCOPIC OBSERVATION**

This assembly consists of placing a drop of glue (Eukitt) on a micro cover glass. To do this, the slides are removed from the last medium (toluene) and are quickly covered by a micro glass. The preparation is turned over while avoiding to include air bubbles between the slides and the microglass. The whole thing is left in the ambient air to allow the fixation of the microglass on the slides. The slides are therefore ready for microscopic observation, for reading and interpretation.

3.1-RESULTS

3.1.1-Histology of Snail Ovotestis Fed on Concentrate And Mixture of Pawpaw And Cassava Leaves.

Stage 1: Beginning of Gonad Maturation (Early Maturation of Gonads)

This first stage is characterized by the presence of large-size ovotestis in individuals fed on concentrate after two months of breeding (F1) whereas these organs only appear during the third month in individuals which have consumed a mixture of leaves (Fa). Only the digestive gland is observable during the first month in individuals fed on a mixture of leaves.

STAGE 2: MATURATION OF GONADS

Spermatogonia and spermatocytes are visible on the periphery of acini but spermatogonia appears smaller than the spermatocytes characterized by the presence of a small nucleolus. These cells (spermatogonia) begin their appearance in the snails ovotestis fed on concentrate from three months (F2); this stage is acquired in individuals consuming the mixture of leaves from the fourth month (Fb).

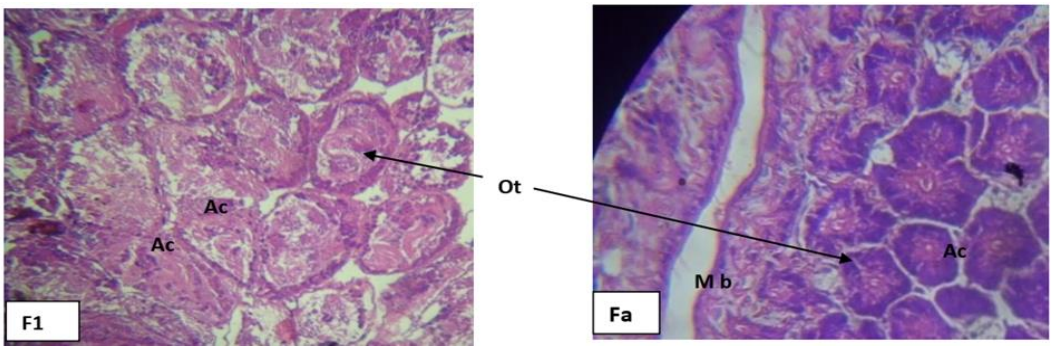
STAGE 3: ADVANCED MATURATION OF GONADS

This third stage of evolution is characterized by the transformation of spermatocytes into spermatids and then into spermatozoa and the appearance of ovogonies at the periphery of the acini. This stage is observed in individuals fed on concentrate, four months of age (F3); we observe all these transformations in the snail ovotestis fed on a mixture of leaves aged 5 months (Fc).

STAGE 4: SEXUAL CELLS MATURITY

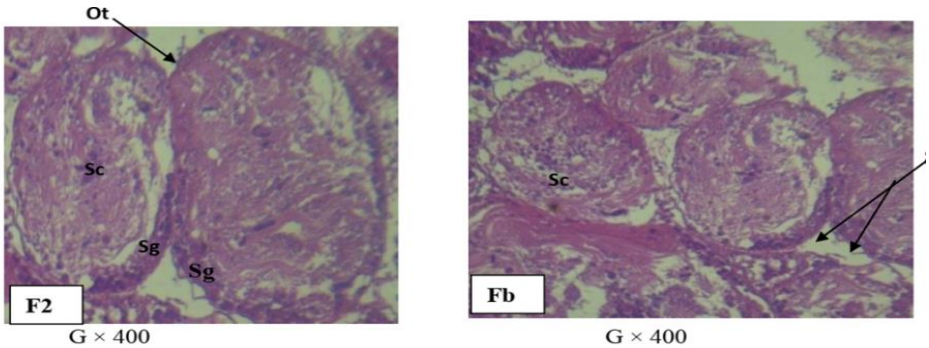
This last stage is characterized by a morphological change in the snail ovotestis with transformation of numerous ovogonies generally located at the periphery of the acini in vetyllogenic oocytes **I**, in vetyllogenic oocytes **II** or mature oocytes, noting however the presence of spermatozoa in the central lumen of the tubules. The vitellogenic oocytes **I** have a wide cytoplasm and are well-endowed with yolk; the nuclear membrane is barely noticeable.

The vitellogenic oocytes **II** or mature oocytes are large in size. The peri-nuclear cytoplasm is dense with a clear space between the nucleolus (germinal vesicle) and the nuclear membrane. All these aspects were always visualized during the fourth month of the study of the snails ovotestis fed on concentrate (F4 and F5) and during the sixth month in snails fed on a mixture of leaves. (Fd and Fe).



Stage 1: Beginning of maturation.

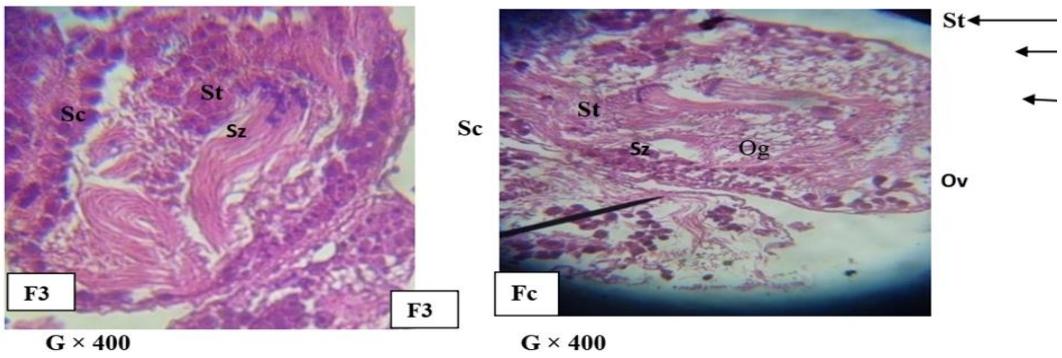
F1: 2 months of age; observation of ovotestis (ot) containing several acini (Ac) in snails fed on concentrate.
Fa: 3 months of age; appearance of ovotestis (ot) containing small size acini (Ac) in individuals fed on a mixture of plant leaves.



Stage 2: Maturation of gonads

F2: 3 months of age; development of the ovotestis and appearance of several spermatogonia **(Sg)** in the basement membrane (**mb**), and some spermatocytes **(sc)** in the lumen of the acini of snails fed on concentrate.

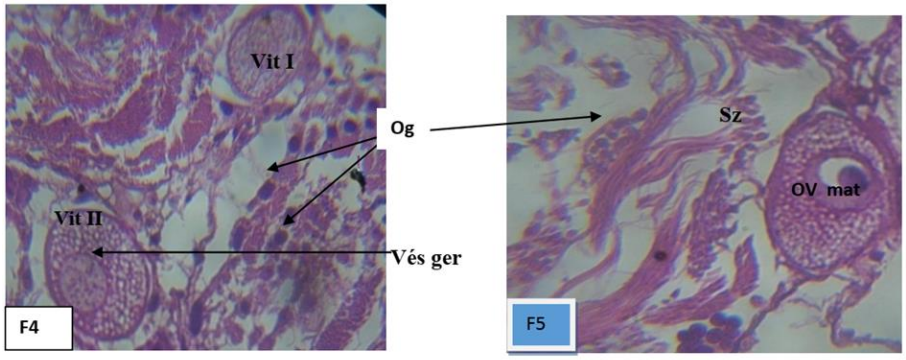
Fb: 4 months of age; development of the ovotestis (**ot**) ; appearance of the spermatogonia **(sg)** in the basement membrane and some spermatocytes **(sc)** in the lumen of the acini of snails fed on a mixture of leaves.



Stage 3: Advanced maturation

F3: 4 months of age: large amplification in ovotestis showing both ovogonia (og) in the basement membrane (mb), spermatids (St) and spermatozoa (Spz) in individuals fed on concentrate.

Fc: 5 months of age; evolution of spermatogonia (Sg) into spermatocytes (Sc), in spermatids (St) and into spermatozoa (Sz) and appearance of some ovogonies in the tunica of individuals consuming a mixture of leaves.



Stage 4: stage of sexual maturity

F4 and F5: 4 months of age; figure showing the evolution of ovogonies (Og) into vitellogenic oocytes I (vit I), vitellogenic oocytes **II (vit II)** and in mature oocytes **(mat ov)** with the presence of spermatozoa **(sz)** in the lumen of the tubule of snails fed on feed concentrate.

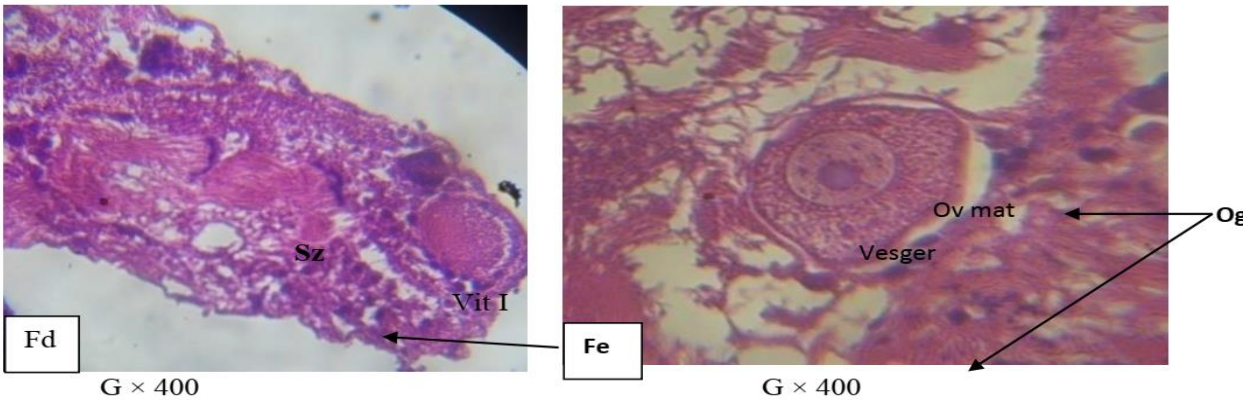


Figure 3: Differents stages of the evolution of snails ovotestis fed on two types of feed ration

Fd and Fe: 6 months of age: figure showing the evolution of ovogonies **(Og)** into vitellogenic oocytes **I (vit I)**, in vitellogenic oocytes (vit**II**) or mature oocytes **(mat Ov)** with presence of spermatozoa **(Sz)** in the lumen of tubule in individuals fed on a mixture of leaves.

DISCUSSION

Whether they were fed on plant leaves or with concentrate, the subjects were subjected to a very good density: the temperature, the relative humidity of the air and the density of 100 snails / m² favored a better growth at *Limicolaria flammea*. It can also be noted that the various foods offered to the subjects were served in quantity and were of good quality.

Indeed, it has been shown that in captivity, the disruption of growth and reproduction at high densities is due to the reduction in the quantity and quality of the food available to snails (N'da, 2004).

Snails subject to different diets show different behavior. The analysis of the different histological sections reveals the necessity of feeding the snails with the concentrate; indeed, this type of ration has a better effect on gametic maturation (spermatogenesis and oogenesis) than the diet based on a mixture of plant leaves.

Snails that consumed concentrate pellets reached sexual maturity after four months and six months for those fed a mixture of papaya leaves and cassava. This aspect was evidenced by the visualization of mature spermatozoa and oocytes at the end of the indicated periods.

Of the two diets proposed, only the ration with concentrate has the highest Ca2 +, K + and Na + levels. In addition, it contains relatively high proportions of nitrogen, fat, total sugars and cellulose. All this could be at the origin of the early acquisition of sexual maturity after four months in individuals who consumed this type of food during this study.

As for *Achatina fulica*, which is widespread in East Africa, Asia and Oceania, this species completes its life cycle in 5.5 months because the sexual maturity of this small species of giant African snails occurs. sometimes from the age of 5 months when the snail weighs 32 grams and measures 6 centimeters in shell length (Upatham et *al*., 1988).

On the other hand, the results obtained by Karamoko (2009) reveal that snails lay after 5 months of captivity and subjected to densities of 100 snails / m2 when fed with flour concentrate. This could further justify the early sexual maturity (4 months) observed in individuals who also consumed the same type of food (Stievenart, 1996).

In the species *Limicolaria flammea* which was the subject of the study; the first ovotestis appeared from the second month for snails fed concentrate. According to Udumsringowsiri et *al*. 1999, this same organ appears in *Achatina fulica* only from two months.

CONCLUSION

The diet has had really an influence not only on the parameters of relative growth but also on the reproductive parameters, namely gametic maturation. In fact, after two months of breeding, the monthly observation of the ovotestis section of the genus *Limicolaria* snails in electron microscopic fed on pellets of feeds concentrate reveals a sexual maturity after four months; however those having consumed a blend of plant leaves reached their sexual maturity after six months.

REFERENCES

1. Otchoumou, A., Dosso, H., & Fantodji, A. (2003a). The edible giant snails: fertility of Achatina achatina (Linné), Achatina fulica (bowdich, 1820) and Archachatina ventricosa (Gould,1850) in humid forest; influence of animal density and photoperiod on fertility inbreeding.Boll. Malacol.39:179-184
2. 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*(5), 16-20.
3. OTCHOUMOU, A., N'DA, K., DOSSO, H., & KOUASSI, D. K. (2003). Inventaire de végétaux sauvages consommés par l'escargot géant africain Archachatina ventricosa (Gould, 1850): préférences alimentaires. *Haliotis*, *33*, 15-22.
4. 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), 2008.
5. Adou, C., Kouassi, D., Karamoko, M., & Octchoumou,A. (2011). L'élevage des escargots comestibles d'Afrique : effets de la qualité du régime et du taux de calcium alimentaires sur les performances de croissance d'Achatina achatina (Linné, 1750). *Rev. CAMES-Série A,12 (1), 6-11, juin.*
6. Agongnikpo, E., Karamoko, M., & Otchoumou, A. (2010). Diet of the giant African land snail Archachatina marginata Swainson, 1821, in Ivory Coast: preferences and estimate of damages. *Revue d'écologie*.
7. Odjo, M. G. (1992). Croissance, développement et composition chimique de l'escargot Achatina achatina (Linné). *Mémoire de DEA, Université Nationale de Côte d'Ivoire (FAST)*.
8. Akinnusi, F. A. O. (2002, March). Comparative Evaluation of fresh fruits leaves and concentrate feed on the growth and reproductive performance of the African land snail (Archachatina marginata). In *Proceedings of the 27 th Annual Conference of the Nigerian Society for Animal Production (NSAP), Federal University of Technology, Akure (FUTA)* (pp. 328-320).

9. Aboua, f. (1990). Chemical composition of Achatina fulica. *Tropicultura, 8: 121-122.*
10. Karamoko, M. (2009). Etude de la biologie, de l'écologie et du comportement d'un escargot terrestre d'intérêt économique, limicolaria fl ammea (Muller, 1774), en milieu d'élevage. *Université Cocody Abidjan, Thèse de doctorat unique, 166.*
11. N'da, k., Otchoumou, A., & Koffi, K. J. C. (2004). Alimentation à base de produits du papayer et maturation ovocytaire chez Achatina fulica (Bowdich, 1820) en Côte d'Ivoire. *Tropicultura, 22 (4),168-172.*
12. Otchoumou, A., N'da, K., & Kouassi, K.D. (2005). L'élevage des escargots géants comestibles d'Afrique: Inventaire de végétaux sauvages consommés par Achatina achatina (Linné 1758) et préférences alimentaires Livest. Res. Rural Dev. *17(3),028.http://www.cipav.org.co/lrrd/lrrd17/3/otch17028.htm*
13. Stievenart, C. (1996). Morphologie coquillière, croissance, reproduction et estivation chez les escargots géants africains: observation au laboratoire sur Archachatina marginata et Achatina fulica. *PhD, Institut de Médecine Tropicale, Prince Léopold, Antwerpen, Belgique, 204 p.*
14. Ngowsiri, U. D. O. M. S. R. I., Sertarugsa, P. R. A. P. E. E., Sobhon, P. R. A. S. E. R. T., Kruatrachue, M., Chavadej, J., & Upatham, E. S. (1989). Development of, and seasonal changes in, the reproductive system of Achatina fulica. *Journal of the Science Society of Thajland*, *15*, 237-249