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Research Article

Evaluation of the Reproductive Performance, Body Proportions, Nutritional Value, and Biochemical Parameters of *Achatina achatina*

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To assess the effect of energy levels on the reproductive performance of African Land Snails, ninety young snails of one month of age, weighing between 1 and 1.5 g, of shell length between 15.5 and 23.85 mm and shell diameter between 12.60 and 16.85 mm and free from wounds or shell defects were divided into 3 groups of 5 snails each and 6 replicates in cages equipped with feeders and drinking troughs. Each treatment was randomly assignedto one of the experimental feeds with variable energy levels (2600, 2800, and 3000 kcal/kg) in addition to pawpaw leaves as a staple feed. The cultured substrates were watered daily, and the animals were monitored for a period of 24 months. The results showed that the shortest ages of onset of maturity as well as the highest number of spawns per treatment were observed in snails receiving 2600 kcal/kg of energy in the feed compared to other treatments. The highest egg morphometric and fertility characteristics were found in animals fed the highest energy level of the feed. The highest protein level in the hemolymph was observed in snails given 3000 kcal/kg energy of the feed while the lowest cholesterol level was observed in animals receiving the same treatment. The highest total meat and gonad weight were observed in animals receiving 2800 kcal/kg energy in the feed compared to other treatments. The levels of dry and organic matter, protein, and fat in the ash increased with the energy level of the diet. Animals that received 2800 kcal/kg energy in the diet recorded a significantly lower calcium level in the flesh compared to the other two treatments. On the other hand, the percentage of phosphorus increased significantly with the energy level of the diet. In conclusion, the 2600 kcal/kg energy level can be retained in the feed of adult snails.

1. Introduction

Most of the conventional animal protein sources such as beef, goat, pork, and poultry products have become too expensive for the average citizen. These major sources are decreasing at an alarming rate due to persistent drought disease, high cost of feed, and primitive husbandry techniques [1]. To provide a cheaper source of protein for human consumption, there is a need for an intensive system of micro livestock species such as snails, rabbits, and cane rats [2]. Snails are invertebrate, shell bearing animals that are inactive during the day, but very active at night and at dusk. Snail meat tastes good and it is considered a delicacy in some cultures. Snail meat is particularly rich in protein [3]. Imevbore and Ademosun [4] indicated that snail meat has a protein content of 88.37% (on a dry weight basis), low total fat (1.64%), saturated fatty acids (28.71%), and cholesterol (20.28 mg/100 g) (fresh sample). Snail meat is also rich in calcium, phosphorous, and iron with values of 185.70 mg/100 g, 61.24 mg/100 g, and 45–50 mg/kg, respectively, for dry samples [5] as well as in such amino acids as lysine and leucine. The availability of giant land snails in the world is decreasing gradually through indiscriminate hunting and deforestation, with the latter leading to the destruction of the snail's natural habitat [5]. Thus, the development of snail farming is of great importance because it makes it possible to reduce the collection pressure and the seasonal deficit, fight against the depletion of natural stocks of achatines, achieve the preservation of its resources, and meet the needs. One of the important elements in breeding is nutrition because it ensures good growth of individuals, optimal reproduction, and low mortality [6]. In order to improve the level of productivity of this species, it is necessary to develop techniques for breeding Giant African Snails, more specifically to determine nutritional needs. Among these nutrients, one can cite the energy of the diet because no in-depth study has yet been carried out on the minimum level of this element for an adequate food formulation. The general objective of the study was to contribute to the preservation and enhancement of snails and more specifically to assess the effect of the energy level on the parameters of the following:

(i) Reproduction (morphometric characteristics of eggs and fertility)
(ii) Growth (weight, weight gain, and morphometric characteristics of the shell)

2. Materials and Methods

2.1. Period, Area, and Site of the Study. This study was carried out between September 2015 and December 2017 at the snail farm of the University of Buea, South-West region (LN: 4°12′–4° 25′ and LE: 9°19 - 9° 20′), at an altitude of 870–4095 m). The prevailing climate is equatorial, characterized by a short dry season (mid-November to mid-March) and a long rainy season (mid-March to mid-November). Rainfall ranges from 2,000 to 4,000 mm per year and relative humidity ranges from 85 to 95%. The annual average temperatures oscillate between 20 and 29°C.

2.2. Animal Material, Housing, and Experimental Diet

2.2.1. Animal Equipment and Housing. A total of 90 one-month-old snails (*Achatina achatina*) spat, bred at the Snailery of the University of Buea, weighing between 1 and 1.5 g, of shell length and diameter of between 15.5 and 23.85 mm and 12.60 and 16.85 mm, respectively, free of injury or breakage, were used.

The spats were placed in perforated plastic cages (3 mm side) 30 cm in diameter and 20 cm deep (density of 5 snails/0.01413 m^2), each equipped with a plastic feeder and a drinker of 5 cm in diameter. The bottom of each cage consisted of 5 cm thick of loose soil substrate previously disinfected with virunet, two weeks before the animals were introduced. Virunet is a broad spectrum disinfectant that kills all types of bacteria, viruses, and fungi. Virunet is packed in powder form and only mixed with water during disinfection. For this study 0.1 g of virunet was mixed with 0.5 L of water and spread on the substrates using a sprayer (MATABI brand). The cages were covered with mosquito-type netting (1 mm mesh) constituting an antileak device and then placed in a cinder block building (4 m long by 3.5 m wide with the floor made of cement) covered with a metal sheet at room temperature (25°C) and natural lighting (12 hours of light and 12 hours of darkness).

The study was approved by the Ethical Committee of the Department of Animal Science of the University of Dschang (ECDAS-UD_S 20/03/2017/UD_S/FASA/DSAES) and was in conformity with the internationally accepted standard ethical guidelines for Laboratory animal use and care as described in the European community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

2.2.2. Experimental Diet. Three experimental isoprotein diets were made every week from local products and by-products to prevent mold. The composition and bromatological characteristics of the said diets are summarized in

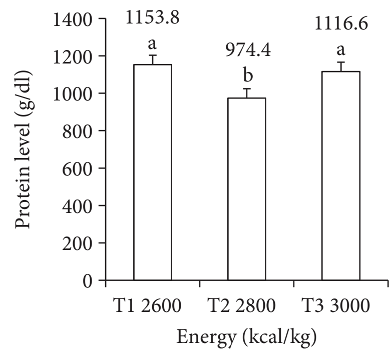

Figure 1: Effects of the energy level in the diet on the total protein level in the hemolymph of the Giant African Snail. a, b: histograms assigned to the same letter do not differ significantly ($p > 0.05$).

Table 1: Percentage composition and bromatological values of isoprotein experimental diets.

	Treatments (kcal/kg)		
Ingredients and nutritional values	T_1 2600	T_2 2800	T_3 3000
Ingredients			
Corn flour	20.10	21.9	22.20
Cassava flour	18.6	22.05	20.00
Soybean flour	2.3	4.20	5.00
Peanut meal	37.50	38.50	38.50
Palm kernel meal	13.90	4.80	3.00
Fish meal	2.00	2.05	2.00
Shell	1.42	1.42	1.42
Bone meal	2.83	2.83	2.83
Palm oil	1.10	2.00	4.80
Vitamin premix 2%	0.25	0.25	0.25
Total (kg)	100.00	100.00	100.00
Calculated nutritional values			
Crude protein	24.00	24.00	24.00
Metabolisable energy (kcal/kg)	2600.36	2800.18	3000.85
Energy-protein ratio	118.19	127.28	136.40
Fat	7.07	8.06	10.75
Calcium	1.85	1.84	1.83
Phosphorous	0.91	0.88	0.86
Phosphocalcic ratio	0.49	0.47	0.46
Lysine	0.96	0.98	0.99
Methionine	0.32	0.31	0.31

Table 1. The feed was stored in labeled bags and classified according to the treatments in a hermetically sealed plastic bucket. All the diets were prepared and stored at room temperature.

2.3. Conduct of the Experiment and Data Collection. A total of 90 one-month-old snail spats (*Achatina achatina*) were randomly assigned to 3 batches (T_1-T_3) of 5 comparable snails (weight and size). Three batches (T_1-T_3) in six replicates were randomly assigned to one of the experimental isoprotein diets (ad libitum) previously described, corresponding to 2600, 2800, and 3000 kcal/kg of energy. Each of the batches received fresh pawpaw leaves (20 g) as a staple diet previously prepreened for 24 hours. Diet from each batch was distributed every day and left over were collected. Every day, the rearing substrates were watered (0.5 L/substrate) and the animals were monitored until the age of onset of maturity (14 months).

In each treatment, every morning, the substrate from each rearing cage was stirred entirely and minutely to collect the eggs to determine the laying and morphometric characteristics of the eggs during 10 months' periods. The eggs were then placed 4 cm deep (average spawning depth of spawners) in loose soil substrates (5 cm thick in plastic jars) until hatching to assess the characteristics of fertility. The unhatched eggs were broken and the state of embryonic development [7] was observed to determine the rate of early embryonic mortality (number of eggs with a dead embryo without a shell) and late embryonic mortality (number of eggs having a dead embryo with shell). At the end of the experiment, each animal of each treatment was sacrificed; the hemolymph was removed by cardiac puncture according to the method proposed by [8] to determine the total protein and cholesterol. Shell, soft tissue, pedals mass, total meat, and gonads were collected for evaluation of carcass characteristics and relative weight [9]. The samples of the pedal mass were transported to the Laboratories of Animal Nutrition and Feeding and of Soil Sciences of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, to determine, respectively, the bromatological characteristics and the mineral content of the carcass.

2.4. Parameters and Characteristics Studied

2.4.1. Reproductive Parameters and Characteristics

(1) Spawning characteristics: the characteristics of reproduction considered were

- (i) Age of onset of maturity
- (ii) Average number of eggs per clutch
- (iii) Number of spawns per treatment

(2) Morphometric characteristics of eggs were

- (i) Weight (g)
- (ii) Length (mm)
- (iii) Diameter (mm)

(3) Characteristics of fertility were

- (i) Weight of the gonad (ovotestis)
- (ii) Incubation period = time taken (days) for the eggs to hatch
- (iii) Fertilization rate = (number of embryonated eggs/number of eggs laid) × 100 [7]
- (iv) Hatch rate = (number of eggs hatched/number of eggs laid) × 100
- (v) Average spat rate per laying = (average number of spat/average number of hatched eggs) × 100
- (vi) Average spat weight = spat weight at hatching

(4) Embryonic and reproductive mortality rates were

- (i) Early embryonic mortality rate = (number of eggs with a dead embryo without shell/number of eggs laid) × 100
- (ii) Late embryonic mortality rate = number of eggs with a dead embryo on shell/number of eggs laid) × 100
- (iii) Adult mortality rate (number of adult snails that died during the breeding period/total number of adult snails) × 100 [7]

2.4.2. Biochemical Characteristics of the Hemolymph

(1) Total cholesterol level in hemolymph was obtained by the colorimetric method, CHOD-POD enzymatic, according to the instructions of the SGM Italia commercial kit (Ref: 10028–4 × 100 ml). In presence of cholesterol esterase, cholesterol esters are hydrolysed and the total cholesterol is measured by oxidising with cholesterol oxidase to form hydrogen peroxidase. Hydrogen peroxidase reacts with phenol and 4-aminoantipyrine to form a red quinoneimine dye. The intensity of the solution colour is directly proportional to the level of total cholesterol (TC) present in the sample. The sample or standard were pipetted in the tubes. The mixture of each tube was well vortexed, incubated for 10 minutes at 20–25°C, and the absorbance of standard/sample was read against the reagent blank at 500 nm within 60 minutes' interval.

(2) Total protein level in hemolymph was determined by the Biuret colorimetric method, according to the instructions of the CHRONOLAB commercial kit (Ref: 101–0240). Peptides containing three or more amino acid residues will form a coloured chelate complex with cupric ions in an alkaline environment containing sodium potassium tartrate. A similar coloured chelate complex forms with the organic compound biuret (NH_2–CO–NH–CO–NH_2) and the cupric ion. The reaction in which a coloured chelation complex (violet) is formed with peptide bonds in the presence of an alkaline cupric sulfate solution became known as the biuret reaction. The different reagents were added in several times. After mixing all the products, these tubes were shaken and incubated for 10 min at 37°C. The colours were

stable for 30 min, and the optical densities were read with a spectrophotometer at 550 nm against blank.

2.4.3. Growth Characteristics. The characteristics of growth considered were as follows:

(i) Carcass characteristics are as follows:
Shell meat ratio (SMR) is as follows:

$$\text{SMR} = \frac{\text{shell weight}}{\text{total meat weight}}. \quad (1)$$

(ii) Relative weight of organs (RWO) is as follows:

$$\text{RWO} = \frac{\text{weight of the organ considered}}{\text{live weight}}. \quad (2)$$

2.4.4. Bromatological Characteristics of the Carcass

(i) The quantities of dry and organic matter, protein, lipid, and ash were determined according to the method proposed by AOAC [10]. Snails flesh was analyzed and the analysis was repeated three times for each sample. The bromatological analysis of snail flesh (*Achatina achatina*) was about the estimation of the whole nitrogen by way of the Kjeldhal method and the crude fibre and fat by the methods of AOAC 1990. The level of dry matter was estimated on a fraction of the sample which had been dried in the hot room. The ashes were determined after incineration of the dry material under 550°C for 24 hours.

(ii) Rate of minerals in the ash was determined according to the method proposed by [11]. The minerals were analyzed from solutions obtained by dry-ashing the samples at 550°C and dissolving the ash in standard flasks with distilled, deionized water containing a few drops of concentrated hydrochloric acid. Phosphorus was determined colorimetrically with KH2PO4 as a standard. Sodium and potassium were determined using a flame photometer, using NaCl and KCl to prepare the standards. All other minerals were determined by means of an atomic absorption spectrophotometer.

2.5. Statistical Analyses. One-way analysis of variance (ANOVA) was used to compare the means and when the differences were significant, Duncan's test was used to separate them at the 5% level. The Pearson correlation coefficient was determined to establish the correlations between the different characteristics. SPSS 20.0 software was used for statistical analyses.

3. Results

3.1. Effects of Energy Level on the Reproductive Characteristics and Death Rate in the Giant African Snail. The influence of the energy level on the morphometric characteristics of eggs and laying and the characteristics of fertility as well as the mortality rate are summarized in Tables 2–5. The following is shown.

3.1.1. Spawning Characteristic. The influence of energy level on spawning characteristics is summarized in Table 2. The age of onset and the number of eggs per lay increased significantly with the energy level in the diet.

It can be noticed that the lowest values were recorded in snails receiving the lowest energy level (T_1 2600 kcal/kg) and the highest in those receiving the highest energy level (T_3 3000 kcal/kg). Conversely, the number of spawns per treatment decreased with the energy level in the diet.

3.1.2. Morphometric Characteristics of Egg. The effect of energy level on the morphometric characteristics of the eggs is shown in Table 3. It follows that, except for the weight of the eggs, the characteristics were significantly affected by the energy level of the diet. The weight of the eggs increased with the energy level in the diet. The highest value was obtained in snails receiving the high energy level (T_3 3000 kcal/kg), followed by that of the T_2 treatment (2800 kcal/kg), while the lowest value was recorded with the lowest energy level (T_1 2600 kcal/kg). However, no significant difference was observed between the treatments.

Significantly higher egg length and diameter values were obtained in snails receiving 3000 kcal/kg energy in the diet compared to the other two treatments (T_1 2600 kcal/kg and T_2 2800 kcal/kg).

3.1.3. Characteristics of Fertility. It appears from Table 4 summarizing the effect of energy level on the characteristics of fertility that the fertilization rate, incubation period, and hatching rate were not significantly influenced by the energy level in the diet. The fertilization and hatching rate increased with the energy level in the diet. The highest values were recorded in snails receiving the highest level (T_3 3000 kcal/kg), followed by that of T_2 treatment (2800 kcal/kg). The lowest values were obtained in those receiving the lowest energy level (T_1 2600 kcal/kg). However, no significant difference was observed between the treatments.

The incubation period was comparable for all treatments. In contrast, the significantly highest spat weight and rate per laying were obtained in snails receiving the highest energy level in the diet (T_3 3000 kcal/kg).

3.1.4. Mortality Rate. The influence of the energy level on the mortality rates is summarized in Table 5. It shows that the mortality rate did not change under the effect of the energy level in the diet. The early embryonic mortality rate decreased with the energy level in the diet. The highest value was obtained with the lowest energy level (T_2 2600 kcal/kg) and the lowest with the highest energy level (T_3 3000 kcal/kg). Conversely, the adult mortality rate increased with the energy level in the diet.

Table 2: Effects of the energy level of the diet on characteristics of laying in the Giant African Snail.

Spawning characteristics	Treatments (kcal/kg of energy)		
	T_1 2600 $n = 30$	T_2 2800 $n = 30$	T_3 3000 $n = 30$
Age of onset (months) of maturity	11.50 ± 0.00^a	16.50 ± 0.00^b	24.00 ± 0.00^c
Number of spawns per treatment	14.00 ± 0.00^a	11.00 ± 0.00^b	1.00 ± 0.00^c
Number of eggs per clutch	5.00 ± 0.67^a	5.80 ± 0.90^b	7.00 ± 0.00^c

a, b, c: on the same line, the values assigned to the same letter do not differ significantly ($p > 0.05$). n = number of snails.

Table 3: Effects of energy level in the diet on morphometric characteristics of the eggs.

Morphometric characteristics of eggs	Treatments (kcal/kg of energy)		
	T_1 2600 $n = 30$	T_2 2800 $n = 30$	T_3 3000 $n = 30$
Weight (g)	1.28 ± 0.25^a	1.32 ± 0.25^a	1.33 ± 0.39^a
Length (mm)	15.38 ± 0.97^a	14.63 ± 0.65^b	16.52 ± 0.39^c
Diameter (mm)	11.65 ± 0.55^a	11.73 ± 0.64^a	13.14 ± 0.69^b

a, b, c: on the same line, the values assigned to the same letter do not differ significantly ($p > 0.05$). n = number of snails.

Table 4: Effects of energy level in the diet on characteristics of fertility.

Characteristics of fertility	Treatments (kcal/kg of energy)		
	T_1 2600 $n = 30$	T_2 2800 $n = 30$	T_3 3000 $n = 30$
Fertilization rate (%)	59.35 ± 32.88^a	72.80 ± 27.15^a	85.71 ± 0.00^a
Incubation period (days)	29.42 ± 2.87^a	29.00 ± 4.32^a	29.00 ± 4.35^a
Spat rate per laying (%)	63.64 ± 50.45^a	100.00 ± 0.00^b	100.00 ± 0.00^b
Spat weight (g)	1.29 ± 0.27^a	1.01 ± 0.32^b	1.5 ± 0.00^a
Hatch rate (%)	51.92 ± 39.97^a	69.64 ± 25.65^a	85.71 ± 0.00^a

a, b: on the same line, the values assigned to the same letter do not differ significantly ($p > 0.05$). n = number of snails.

Table 5: Effects of energy level in the diet on the mortality rate.

Mortality rate (%)	Treatments (kcal/kg of energy)		
	T_1 2600 $n = 30$	T_2 2800 $n = 30$	T_3 3000 $n = 30$
Early embryos	24.87 ± 25.29^a	19.52 ± 25.87^a	14.28 ± 0.00^a
Late embryos	10.76 ± 14.79^a	5.66 ± 13.15^a	14.28 ± 0.00^a
Adults	30.00 ± 0.00^a	37.00 ± 0.00^a	37.50 ± 0.00^a

a: on the same line, the values assigned to the same letter do not differ significantly ($p > 0.05$). n = number of snails.

The value of the late embryonic mortality rate was highest in snails receiving T_3 treatment (3000 kcal/kg) and the lowest in snails receiving 2800 kcal/kg energy from the ration. However, no significant difference was observed between the treatments.

3.2. Effects of Energy Level in the Diet on Total Protein and Cholesterol Rate in the Hemolymph of the African Giant Snail. The influence of energy level on the total protein and cholesterol rate in the hemolymph is illustrated in Figures 1 and 2.

3.2.1. Protein Level. The significantly lower total protein level (Figure 1) was recorded in snails receiving 2800 kcal/kg (T_2) energy in the diet compared to the other two treatments, which are otherwise comparable.

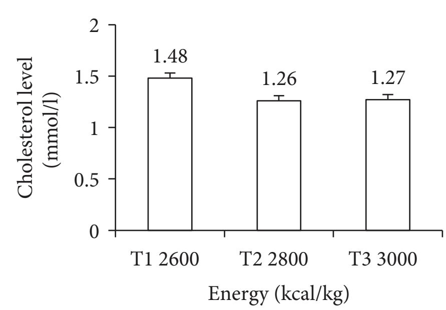

Figure 2: Effects of energy level in the diet on the total cholesterol level in the hemolymph of the Giant African Snail.

3.2.2. Cholesterol Level. The total cholesterol level (Figure 2) decreased with the energy level in the diet. The highest value was recorded in snails receiving 2600 kcal/kg of energy (T1) followed by treatment T_3 (3000 kcal/kg). The lowest value was obtained with 2800 kcal/kg energy level in the diet. However, no significant difference was observed between the treatments.

3.3. Effects of Energy Level in the Diet on Carcass Characteristics and Relative Weight of the Giant African Snail

3.3.1. Carcass Characteristics. The influence of energy level on the characteristics of the carcass is shown in Table 6. It shows that the highest values of live weight, total meat, and

Table 6: Characteristics of the carcass of the Giant African Snail in relation to the energy level in the diet.

Carcass characteristics (g)	Treatments (kcal/kg of energy)		
	T_1 2600 $n = 30$	T_2 2800 $n = 30$	T_3 3000 $n = 30$
Live weight	62.60 ± 8.06^a	69.90 ± 19.08^a	67.10 ± 7.93^a
Shell weight	7.30 ± 1.44^a	8.60 ± 3.66^a	6.40 ± 0.54^a
Soft tissue	17.40 ± 2.60^a	17.12 ± 4.95^a	14.50 ± 3.10^a
Pedal mass	8.90 ± 1.63^a	12.10 ± 3.41^a	12.20 ± 2.88^a
Total meat	17.30 ± 2.84^a	22.90 ± 4.99^a	22.10 ± 3.57^a
Viscera	3.40 ± 0.41^a	3.00 ± 0.70^a	3.60 ± 0.54^a
Shell/meat	0.69 ± 0.17^a	0.59 ± 0.30^a	0.44 ± 0.12^a
Gonads	0.29 ± 0.05^a	0.37 ± 0.09^a	0.34 ± 0.08^a

a: on the same line. The values assigned to the same letter do not differ significantly ($p > 0.05$). n = number of snails.

Table 7: Relative weight of organs of the Giant African Snail in relation to the energy level in the diet.

Relative weight of organs (%)	Treatments (kcal/kg of energy)		
	T_1 2600 $n = 30$	T_2 2800 $n = 30$	T_3 3000 $n = 30$
Shell	11.97 ± 3.52^a	13.28 ± 7.01^a	9.65 ± 1.51^a
Soft tissue	28.40 ± 6.82^a	26.53 ± 12.78^a	21.89 ± 5.62^a
Pedal mass	14.52 ± 3.75^a	17.84 ± 5.60^a	18.12 ± 3.40^a
Total meat	28.25 ± 7.03^a	34.13 ± 9.89^a	33.11 ± 5.50^a
Viscera	5.46 ± 0.62^a	4.49 ± 1.46^a	5.45 ± 1.22^a
Gonads	0.47 ± 0.11^a	0.57 ± 0.24^a	0.51 ± 0.10^a

a: on the same line. The values assigned to the same letter do not differ significantly ($p > 0.05$). n = number of snails.

gonads were obtained in snails receiving 2800 kcal/kg of energy from the diet. The lowest values are recorded in those receiving the lowest energy level (T_1 2600 kcal/kg).

The highest values for pedal mass and viscera were obtained in snails receiving the highest energy level (T_3 3000 kcal/kg) compared to other treatments. However, no significant difference ($p > 0.05$) was recorded.

The shell-to-meat ratio decreased with the energy level in the diet. The highest value was recorded in snails receiving the lowest energy level in the diet (T_1 2600 kcal/kg), followed by those in treatment T_2 (2800 kcal/kg). The lowest value was obtained in snails receiving the highest energy level in the diet (3000 kcal/kg). However, no significant difference ($p > 0.05$) was found between the treatments.

3.3.2. Relative Weight of Organs. It appears from Table 7 summarizing the effect of energy level on the relative weight of organs that the highest values of relative shell weight, total meat, and gonad were recorded in snails receiving 2800 kcal/kg (T_2) energy in the diet compared to the other two treatments.

The weight of the soft tissue decreased with the energy level in the diet. Thus, the highest values were recorded in the snails receiving the lowest energy level (T_1 2600 kcal/kg) followed by snails in treatment T_2 (2800 kcal/kg). The lowest value was obtained with the highest energy level (T_3 3000 kcal/kg). The reverse has been observed with regarding the pedal mass. However, no significant difference ($p > 0.05$) was recorded between the treatments regardless of the characteristics considered.

3.4. Influence of Energy Level in the Diet on Bromatological Characteristics of the Flesh of the Giant African Snail. The effects of energy level on the levels of dry and organic matter, the level of protein, lipid, and ash in the snail flesh are illustrated in Figures 3 and 4.

3.4.1. Dry and Organic Matter, Proteins, Lipids, and Ash. The levels of dry (Figure 3(a)) and organic (Figure 3(b)) matter, protein (Figure 3(c)), and lipids (Figure 3(d)) increased with the energy level in the diet. Thus, the highest values were obtained in snails receiving the highest energy level in the diet (T_3 3000 kcal/kg) and the lowest with the lowest level (T_1 2600 kcal/kg).

3.4.2. Ashes. Significantly highest percentage of ash (Figure 4) content was recorded in snails receiving the lowest energy level in the diet (T_1 2600 kcal/kg) followed by treatment T_3 (3000 kcal/kg). The lowest value was obtained in snails receiving 2800 kcal/kg of energy.

3.4.3. Mineral Content in the Ash of the Giant African Snail in Relation to the Energy Level in the Diet. The influence of energy level on the mineral content in the ash is summarized in Table 8. It shows that a significantly lower calcium level was recorded in snails receiving 2800 kcal/kg of energy in the diet compared to the two other treatments (T_1 2600 and T_3 3000 kcal/kg).

The sodium level was the same for all treatments. In contrast, the percentage of phosphorus increased

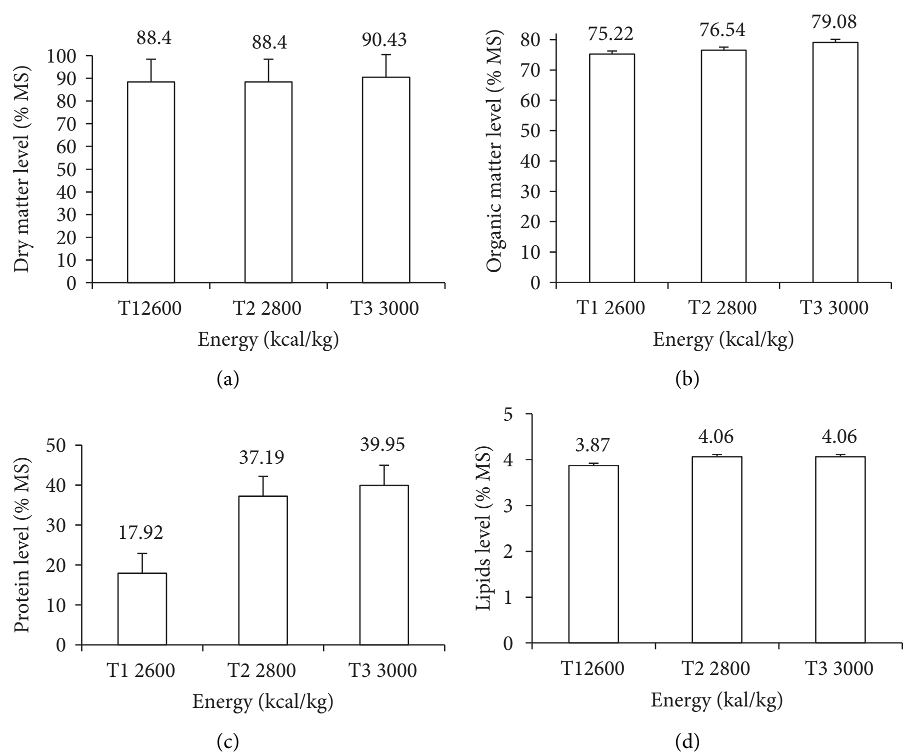

Figure 3: Effects of energy level in the diet on dry matter (a), organic matter (b), proteins (c), and lipids (d) in the flesh of the Giant African Snail.

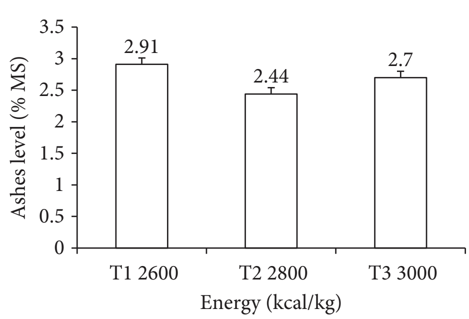

Figure 4: Effects of energy level in the diet on the ash content in snail flesh.

significantly ($p < 0.05$) with the energy level in the diet. The highest values were obtained in snails receiving the highest energy level in the diet (T_3 3000 kcal/kg). The lowest values were recorded in those receiving the lowest energy level (T_1 2600 kcal/kg).

3.5. Correlation between Bromatological Characteristics, Mineral Content in the Carcass of the Giant African Snail, and the Energy Level in the Diet

3.5.1. Correlations between Dry and Organic Matter, Protein, Lipids, Ash, and Energy Level in the Diet. The correlations between dry and organic matter, protein, lipids, and ash in relation to the energy level in the diet are summarized in Table 9. We have the following.

Irrespective of the treatment, a positive and very strong correlation ($p < 0.01$) was generally obtained between the percentage of organic matter and lipids.

When considering the treatments, positive and very strong correlations ($p < 0.01$) were found on the one hand between the levels of organic matter and ash and on the other hand between the levels of lipids and ash in snails receiving the lowest energy level in the diet (T_1 2600 kcal/kg).

The correlation was negative and very strong ($p < 0.01$) between the percentages of dry and organic matter on the one hand and the percentages of dry matter and lipids on the other hand in the treatment that contained 2800 kcal/kg of energy in the diet. In addition, very strong correlations were observed between dry and organic matter, protein, lipids, and ash in snails receiving the highest energy level in the diet (T_3 3000 kcal/kg).

3.5.2. Correlation between the Mineral Contents of the Carcass in Relation to the Energy Level in the Diet. The correlations between the levels of calcium, magnesium, potassium, sodium, phosphorus, and iron in relation to the energy in the diet are presented in Table 10. It emerges that, whatever the treatment, very strong correlations were generally found between the mineral content in the ash.

When taking into account the treatments, negative and strong correlations ($p < 0.01$) were observed between the minerals in snails receiving the lowest level (T_1 2600 kcal/kg) and the highest (T_3 3000 kcal/kg) energy in

Table 8: Mineral content in the ash of the giant African snail in relation to the energy level in the diet.

Minerals (mg/100 g)	Treatments (kcal/kg of energy)		
	T_1 2600 $n = 30$	T_2 2800 $n = 30$	T_3 3000 $n = 30$
Calcium	175.00 ± 1.00^a	75.00 ± 1.00^b	144.50 ± 0.50^c
Magnesium	352.50 ± 0.50^a	838.50 ± 0.50^b	351.00 ± 1.00^c
Potassium	265.50 ± 0.50^a	449.00 ± 1.00^b	387.50 ± 0.50^c
Sodium	21.45 ± 0.15^a	21.45 ± 0.15^a	21.45 ± 0.15^a
Phosphorus	233.00 ± 1.00^a	261.00 ± 1.00^b	459.00 ± 1.00^c
Iron	80.50 ± 0.50^a	81.50 ± 0.50^b	60.50 ± 0.50^c

a: on the same line. The values assigned to the same letter do not differ significantly ($p > 0.05$). n = number of snails.

Table 9: Correlation between dry matter, organic matter, protein, lipids, ash, and energy level in diet.

Treatments (Kcal/kg) and bromatological characteristics (% DM)	Bromatological characteristics (% DM)				
	DM	OM	CP	Lipids	Ashes
T_1 2600 $n = 30$					
DM	1				
OM	−0.32	1			
CP	+0.11	+0.90	1		
Lipids	−0.32	+1.00**	+0.90	1	
Ashes	−0.32	+1.00**	+0.90	+1.00**	1
T_2 2800 $n = 30$					
DM	1				
OM	−1.00**	1			
CP	−0.99	+0.99	1		
Lipids	−1.00**	+1.00**	+0.99	1	
Ashes	−0.96	+0.96	+0.92	+0.96	1
T_3 3000 $n = 30$					
DM	1				
OM	+1.00**	1			
CP	−1.00**	−1.00**	1		
Lipids	+1.00**	+1.00**	−1.00**	1	
Ashes	+1.00**	+1.00**	−1.00**	+1.00**	1

DM: dry matter; OM: organic matter; CP: crude protein. **The correlation is significant at the 0.01 level (two-tailed). n = number of snails.

the diet. In contrast, they were positive and strong ($p < 0.01$) in snails receiving 2800 kcal/kg energy in the diet.

4. Discussion

The effect of energy level on reproduction parameters showed that, considering the morphometric characteristics of eggs (weight, length, and diameter of the eggs), the number of eggs per clutch increased significantly with the energy level in the diet. The highest values were thus recorded in snails receiving the highest level of energy in the diet. Our results are different from those of Nyameasem and Borketey-La [12] in achatines who reported that, for the morphometric characteristics of the eggs, the number of eggs per clutch decreased with increasing energy level in the diet. Similar observations have been made in other species such as laying hens and have shown that, for the morphometric characteristics of the eggs, the number of eggs per clutch increased with increasing concentration of food energy [13, 14]. These results would be justified by an increase in the amount of energy ingested and the energy value of the compound feed received by the snails. Philippe and Juliette [14] found that the oil improves the metabolizable energy (ME) content of food and increases the number and weight of eggs and the total percentage of laying due to the fatty acid profiles it contains. It is responsible for the integrity of the cell membrane and for the synthesis of hormones. It also improves the physical presentation and palatability of compound feeds. Our results could also be explained by the fact that snail flesh contains polyunsaturated fatty acids. An intake of food rich in energy would promote the synthesis of polyunsaturated fatty acids (PUFA) of type $n - 3$ (triglycerides), which are transferred to the oocyte thanks to the very low density lipoproteins (VLDL), which would improve the quality of semen [15] and consequently increase the egg volume [16]. For example, arachidonic acid (which is present in VLDL) stimulates the secretion of progesterone by hen granulosa cells (follicle cells) [17].

The average number of spawns per treatment decreased with the increasing energy level in the diet. The highest number of layings was recorded in snails receiving the lowest energy level (2600 kcal/kg) in the diet. These results corroborate with those of Nyameasem and Borketey-La

Table 10: Correlation between the mineral content of the carcass and the energy level in the diet.

Treatments (Kcal/kg) and mineral content (mg/100 g)	Mineral content (mg/100 g)					
	Ca	Mg	K	Na	P	I
T_1 2600 $n = 30$						
Ca	1					
Mg	+1.00**	1				
K	−1.00**	+1.00**	1			
Na	+1.00**	+1.00**	−1.00**	1		
P	+1.00**	+1.00**	−1.00**	+1.00**	1	
I	+1.00**	+1.00**	−1.00**	+1.00**	+1.00**	1
T_2 2800 $n = 30$						
Ca	1					
Mg	+1.00**	1				
K	+1.00**	+1.00**	1			
Na	+1.00**	+1.00**	+1.00**	1		
P	+1.00**	+1.00**	+1.00**	+1.00**	1	
I	+1.00**	+1.00**	+1.00**	+1.00**	+1.00**	1
T_3 3000 $n = 30$						
Ca	1					
Mg	−1.00**	1				
K	+1.00**	−1.00**	1			
Na	+1.00**	−1.00**	+1.00**	1		
P	+1.00**	−1.00**	+1.00**	+1.00**	1	
I	+1.00**	−1.00**	+1.00**	+1.00**	+1.00**	1

Ca: calcium; Mg: magnesium; K: potassium; Na: sodium; P: phosphorus; I: iron. The correlation is significant at the 0.01 level (two-tailed). n = number of snails.

[12], who found that egg production decreases with the energy level of the diet in achatines. Similar observations were also made in rabbits and revealed that excessive consumption of high-energy food causes an increase in body fat and loss of young rabbits at birth [18]. According to the same authors, there is a competition between the gastrointestinal tract (associated fat) and the reproductive tract for space in the abdominal cavity. In fact, the use of an energy-rich compound feed would have caused the resorption of eggs in snails due to the accumulation of fat in the seminal receptacle and consequently reduced the number of spawns [19]. Our results could also be justified by the fact that the consumption of foods very rich in energy would have decreased the production of functional oocytes in snails by causing a deregulation of the follicular hierarchy. Similar observations have already been reported in broiler chickens [20].

Regarding spat weight and rate per lay, fertilization and hatching rate increased with the energy levels of which the lowest values were recorded in snails receiving the lowest energy level (2600 kcal/kg) in the diet. These results are similar to those obtained after assessing the effect of energy level on geese reproduction, which showed that a high energy in the diet decreased the number of spawns and increased fertility [21, 22]. Indeed, a diet rich in energy would have allowed the snails to produce eggs of high weight and size and therefore improve the weight and spat rate. Likewise, the high hatching rate observed could be explained by the high egg weight in the treatment. The correlations found between egg weights on the one hand and incubation time and hatching rate on the other hand seem to show that large eggs hatch earlier with higher hatching rates than the little ones. This observation contradicts that of Codjia and Noumonvi [23] who reported that small-sized eggs hatch earlier than large ones but with a lower hatch rate. The low hatching rate observed with the small eggs is thought to be due to their large surface area of exposure to environmental conditions compared to their volume. The fertilization rate, which was also high in this treatment, would be justified by a better production of ovum and viable sperm.

The spawner mortality rate increased with the energy level, the lowest of which was recorded in snails receiving 2600 kcal/kg energy in the diet. Our results corroborate those of Renouf and Offner [24] who studied the effect of the energy level of food and the distribution period on growth and mortality in rabbits and revealed that the mortality rate increased with the level of energy in the diet.

The increased energy level in the diet resulted in a decrease in the total cholesterol level in the hemolymph. The highest value was recorded in snails receiving the lowest energy level (2600 kcal/kg). This would be justified by the use of soybean flour in our diet. Indeed, Collomb and Mayor [25] reported on the one hand that soybean is rich in amino acids (glycine and arginine), phytosterols (which have structures similar to cholesterol and which would inhibit its absorption), and isoflavones, responsible for the decrease in insulin levels in the blood. When insulin levels are low, the production of cholesterol in the liver decreases. On the other hand, amino acids, phytosterols, and isoflavones would also affect the intestinal absorption of cholesterol by inducing a decrease in it in hemolymph.

The lowest carcass characteristic values were recorded in snails receiving the highest energy level in the diet (3000 kcal/kg). These results are similar to those of Castellini and Battaglini [26] who showed that an increase in fat intake from 2 to 6% increases carcass yield and fat. This could be justified by the high energy-protein ratio observed in snails receiving the highest energy level in the diet. Indeed, when the energy/protein ratio increases in the diet of the snails, the weight gain decreases as well as the values of the characteristics of the carcass. Pla [27] also reported that the distribution of a high energy in the diet tends to improve carcass yield and could be explained in particular by fat deposition.

The levels of dry and organic matter, fat, and protein in the flesh of the snail increased with the energy level in the diet. The lowest value was recorded in snails receiving the lowest energy level (2600 kcal/kg) as well as the lowest energy-protein ratio in the diet. Similar observations have been made by Médale [28] in other species such as fish. According to the same authors, an increase in the energy level in the diet (via the amount of feed distributed or the energy content of the feed) leads practically in all species to an increase in body lipids accompanied by a decrease in water content Corraze and Kaushik [29] and nitrogen discharge. However, there are great disparities between species as to the body compartments in which lipids are stored. Our results could be justified by the presence of fishmeal contained in the diet. In fact, the lipids of the flesh of fish are characterized by their high levels of long-chain polyunsaturated fatty acids (LCPUFA) of the omega-3 series (Tte flesh of snails contains omega-3 fatty acids, which participate in the proper functioning of the cardiovascular system) or $n-3$, in particular eicosapentaenoic acid (C20: 5 $n-3$ or EPA) and docosahexaenoic acid. The intake of energy from food stimulates the synthesis of these fatty acids in snails (C22: 6 $n-3$ or DHA), which are stored in the foot mass in the form of triglycerides, neutral lipids which constitute an easily mobilized form of energy storage. It is the reserve triglycerides that are responsible for almost all of the increase in fat content seen in muscle as a result of increased dietary energy intake.

The level of calcium in the ash decreased while the level of phosphorus increased with the increasing energy level in the diet. Our results are similar to those of Kerstetter et al. [30] who reported that increasing the energy level in the diet decreases the intestinal absorption of calcium and iron, with an increase in phosphorus. Our results would be justified not only by the low content of food composed of calcium, iron, and phosphorus but also of vitamins. For example, vitamin D regulates the intestinal absorption of calcium and phosphorus, while vitamin C promotes the absorption of iron. On the other hand, a diet rich in energy would promote the formation of insoluble soaps involving fatty acids and calcium.

5. Conclusion

At the end of the study on the effect of energy level on the reproductive performance of snail *Achatina achatina*, the main conclusions are as follows.

The lowest age of onset of maturity and the highest number of spawns per treatment were recorded in the treatment receiving 2600 kcal/kg in the diet. The energy 2600 kcal/kg in the diet positively affected the bromatological characteristics of the carcass, the biochemical characteristics of the hemolymph, and rate of minerals in the ash.

We recommend that the 2600 kcal/kg energy level can be retained in the feed of adult snails.

Data Availability

The raw data can be obtained from the first/corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Guy Merlin Tchowan designed the study and carried out the experimental protocol. He wrote the first draft. Jean Paul Toukala participated in data collection. Ferdinand Ngoula analyzed and interpreted the results. Joseph Tchoumboué conceptualized the study and supervised the field. All authors read and approved the final manuscript.

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