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SCIENTIFIC ARTICLE

Effect of feed protein on reproduction, proximate composition, and hemolymph metabolite profile of snail (*Achatina achatina*)

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ABSTRACT

The study was conducted at the Teaching and Research Farm of the Faculty of Agriculture and Veterinary Medicine of the University of Buea, Cameroon, to assess the impact of protein on the reproduction, proximate composition, and hemolymph metabolite profile of *Achatina achatina* snails. Ninety newly hatched snails were divided into three treatments of five animals each, with six replicates. Each treatment group was allocated to one of the experimental diets with different protein levels (T1: 20%, T2: 22%, and T3: 24%). Results revealed that snails fed a diet containing 22% protein (T2) exhibited the earliest age of maturity onset and the highest number of spawns per treatment (7.00 ± 0.00). Conversely, within the same treatment group, snails had the lowest number of eggs per clutch (4.83 ± 0.38). The weight, length, and diameter of the eggs were influenced by the protein level in the diet, with the significantly highest values recorded in snails receiving 20% protein (T1). Animals fed the lowest protein level diet (T1) exhibited reduced values across various parameters, including the fertilization rate (33.00 ± 0.00), incubation period (26.25 ± 0.00), rate of newly hatched snails (75.00 ± 0.00), and newly hatched weight per laying (0.69 ± 0.00). In contrast, those fed a diet containing 22% protein (T2) showed higher values for these parameters, along with the lowest protein value in the hemolymph. Additionally, a correlation was

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observed between the protein level in the diet and a decrease in total cholesterol in the hemolymph. Notably, values such as live weight, shell weight, soft tissue, total meat, viscera, shell-meat ratio, and pedal mass exhibited an increase with 22% protein (T2) content in the diet. In conclusion, incorporating a protein level similar to T2 in the diet of adult snails is recommended for commercial purposes.

Keywords: *Achatina achatina*, hemolymph, protein, proximate composition, reproduction.

RESUMEN

Efecto de la proteína del alimento sobre la reproducción, la composición proximal y el perfil de metabolitos de la hemolinfa del caracol (*Achatina achatina*). El estudio se llevó a cabo en la Granja de Enseñanza e Investigación de la Facultad de Agricultura y Medicina Veterinaria de la Universidad de Buea, Camerún, para evaluar el impacto de la proteína en la reproducción, composición proximal y perfil de metabolitos de hemolinfa de los caracoles *Achatina achatina*. Noventa caracoles recién eclosionados fueron divididos en tres tratamientos de cinco animales cada uno, con seis réplicas. Cada grupo de tratamiento fue asignado a una de las dietas experimentales con diferentes niveles de proteína (T1: 20%, T2: 22%, y T3: 24%). Los resultados revelaron que los caracoles alimentados con una dieta que contenía un 22% de proteína (T2) alcanzaron la madurez más temprana y tuvieron el mayor número de desoves por tratamiento (7.00 ± 0.00). Por el contrario, dentro del mismo grupo de tratamiento, los caracoles presentaron el menor número de huevos por puesta (4.83 ± 0.38). El peso, longitud y diámetro de los huevos fueron influenciados por el nivel de proteína en la dieta, con los valores más altos significativamente registrados en los caracoles que recibieron un 20% de proteína (T1). Los animales alimentados con la dieta con el nivel de proteína más bajo (T1) mostraron valores reducidos en varios parámetros, incluyendo la tasa de fertilización (33.00 ± 0.00), período de incubación (26.25 ± 0.00), tasa de caracoles recién eclosionados (75.00 ± 0.00) y peso de caracoles recién eclosionados por puesta (0.69 ± 0.00). En contraste, aquellos alimentados con una dieta que contenía un 22% de proteína (T2) mostraron valores más altos para estos parámetros, junto con el valor más bajo de proteína en la hemolinfa. Además, se observó una correlación entre el nivel de proteína en la dieta y una disminución en el colesterol total en la hemolinfa. Vale la pena

destacar que los valores como peso vivo, peso de la concha, tejido blando, carne total, vísceras, relación carne-concha y masa del pedal exhibieron un aumento con un contenido de proteína del 22% (T2) en la dieta. En conclusión, se recomienda incorporar un nivel de proteína similar al de T2 en la dieta de caracoles adultos para fines comerciales.

Palabras clave: *Achatina achatina*, hemomolinfa, proteína, composición proximal, reproducción.

INTRODUCTION

The African land snail (*Achatina achatina*) is a gastropod mollusc belonging to the Order *Stylommatophora*, whose flesh is widely consumed in Africa (Fagbuaro et al., 2006). Snail has a protein content of 88.37% compared to pigs' (82.42%) and beef's meat (92.75%), and is low in fat (1.64%), saturated fatty acids (28.71%) and cholesterol (20.28 mg/100 g) (Tchowan et al., 2018). These same authors reported that the flesh is also rich in calcium (185.70 mg/100 g), phosphorus (61.24 mg/100 g), and iron (45-50 mg/100 g) as well as in amino acids such as lysine, leucine, isoleucine, and phenylalanine (Ademolu et al., 2004; Imevbore, 1990; Stiévenart and Hardouin, 1990).

Their soft and tasty flesh represents an animal protein source (Aboua, 1990; Adeyeye, 1996) and revenue for many households in Africa (Kouassi et al., 2007). The increased pressure to collect snails due to the growing demand for consumption and the destruction of their biotope by man for agricultural purposes (cultivation of oil palm, pineapple, plantain, and others) constitutes a threat to the survival and sustainability of the species. However, the development of achatiniculture is very important because it will reduce collection pressure, the seasonal deficit, the preservation of its resources, and meet consumption needs. To improve the level of productivity of this species, various farming techniques for African land snails have been developed, such as the determination of nutritional needs (Tchowan et al., 2022).

Among these nutrients, crude protein stands out because an in-depth study on the minimum level of this element for adequate diet formulation has not yet been conducted (Tchowan et al., 2018). Therefore, proteins catalyze the reactions of synthesis and degradation necessary for cell metabolism; they provide a structural role within the cytoskeleton or tissues. Additionally, certain

proteins act as molecular motors that enable motility, condition Deoxyribonucleic Acid (DNA), and regulate gene expression, energy metabolism, or the transmission of cellular signals (Tchowan et al., 2022).

The main objective of this study was therefore to contribute to the productivity of *Achatina achatina*, and more specifically to evaluate the effect of the protein on the reproductive characteristics, the carcass, the bromatological and mineral composition of the flesh, and the hemolymph metabolite profile of the African land snail (*Achatina achatina*).

MATERIALS AND METHODS

Site of the study

The study was conducted at the Teaching and Research Farm of the Faculty of Agriculture and Veterinary Medicine, University of Buea, Southwest region of Cameroon. It is located at an average altitude of 2482 m above sea level (a.s.l.), the mean annual rainfall is 3000 mm, the relative humidity is 90%, and the average annual temperature is 25 °C.

Animal and accommodation

Snails of one-month-old, bred at the snailery of the Teaching and Research Farm of the Faculty of Agriculture and Veterinary Medicine weighing between 1 and 1.5 g with a shell length between 15.5-23.85 mm and a shell diameter between 12.60-16.85 mm were used.

Snails were placed in circular pens (3 mm wall thickness) of 30 cm in diameter and 20 cm deep at the density of 5 snails/0.01413 m^2, each equipped with a feeder and drinker of 5 cm diameter. The bottom of each pen consisted of 5 cm of loose soil substrate. Pens were covered with mosquito-type netting (1 mm mesh) constituting an anti-leak device, then placed in a cinder block building (4 m long by 3.5 m wide and 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).

Conduct of the trial and collection of data

Ninety 1-month-old snails were divided into three treatments of thirty animals each. The animals were fed ad libitum; the experimental feeds contained 20%, 22%, and 24% protein, with fresh pawpaw (*Carica papaya*) leaves as the basal diet (Table 1). The pens were cleared daily of leftover feed and feces to avoid the development of possible pathogens, and the loose soil of each treatment was watered every day for 24 months. The snails were weighed at the beginning of the experiment and every week using a Sartorius brand balance with 0.05 g precision.

In the fourteenth month of the experiment, the substrate from each pen was stirred by hand daily to collect the eggs. Eggs were incubated (4 cm deep in the soil, at the depth of the spawning) until hatching. The early and late embryonic mortality was determined according to the method proposed by Dafem et al. (2008), the unhatched eggs were broken and the state of development of the embryos was observed. In the twenty-fourth month of the experiment, all the animals were sacrificed. Hemolymph was collected by cardiac puncture (Naresh et al., 2013) to assess the total protein and cholesterol in the hemolymph. Live weight, shell weight, soft tissue, total meat, viscera, shell-meat ratio, pedal mass, gonad, and relative weight were also evaluated. The gonadosomatic index was also determined by dividing the organ´s weight by its live weight.

The proximate composition and the mineral content of the pedal mass of all the animals in each treatment were evaluated in the Laboratories of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang.

Dry and organic matter, protein, lipid, and ash were determined according to the method proposed by Cunniff and Washington (1997). The snails´ flesh was analyzed with three repetitions for each sample. The bromatological snails´ flesh analysis was about the estimation of the whole nitrogen through the Kjeldahl method, crude fiber, and fat (AOAC, 1990). 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 at 550 °C for 24 hours.

The minerals were analyzed from solutions obtained by dry ashing the samples at 550°C and

dissolving the ash in standard flasks with distilled, de-ionized water containing a few drops of concentrated hydrochloric acid. Phosphorus was determined colorimetrically with KH_2PO_4 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 using an atomic absorption spectrophotometer (Pauwels et al., 1992).

Experimental feed

The diets were made weekly; the composition and the nutritional values are presented in Table 1 and Table 2.

Table 1. Ingredients of experimental diet.

Ingredients (%)	Treatments		
	T1	T2	T3
Corn flour	30.00	27.00	22.20
Cassava flour	24.00	22.50	20.00
Soybean flour	06.00	5.10	5.00
Peanut meal	27.00	33.00	38.50
Palm kernel meal	3.00	2.00	3.00
Fish meal	2.00	2.10	2.00
Shell	1.42	1.42	1.42
Bone meal	2.83	2.83	2.83
Palm oil	3.50	3.80	4.80
Vitamin premix 2%	0.25	0.25	0.25
Total	100.00	100.00	100.00

Table 2. Nutritional values of experimental diet.

Calculated Nutritional Values	Treatments		
	T1	T2	T3
Crude protein (%)	20.09	22.04	24.00
Metabolizable-energy (kcal/kg)	3000.50	3000.01	3000.85
Energy-protein ratio	149.35	136.11	125.03
Fat (%)	8.49	9.34	10.75
Calcium (%)	1.82	1.83	1.83
Phosphorus (%)	0.81	0.84	0.86
Phosphocalcic ratio (%)	0.44	0.45	0.46
Lysine (%)	0.85	0.91	0.99
Methionine (%)	0.27	0.29	0.31

Studied parameters

Reproductive parameters and characteristics:

- Average number of eggs per clutch
- Number of clutches per treatment
- Egg weight (g)
- Egg length (mm)
- Egg diameter (mm)
- Gonad (ovotestis) weight
- Age of sexual onset = time taken (months) to reach maturity
- Incubation period = time taken (days) for eggs to hatch
- Fertilization rate = (number of embryonated eggs / number of eggs laid) x 100 (Dafem et al., 2008).
- Average newly hatched rate = (mean number of newly hatched / mean number of hatched eggs) x 100 (Dafem et al., 2008).
- Early embryonic mortality rate = (number of eggs with a dead embryo without shell/number of eggs laid) x 100 (Dafem et al., 2008).

- Late embryonic mortality rate = (number of eggs with a dead embryo with shell/number of eggs laid) x 100 (Dafem et al., 2008).

- Adult mortality rate = (number of adult snails that died during the breeding period/total number of adult snails) × 100 (Dafem et al., 2008).

- Gonadosomatic index (GSI) = weight of gonad/life weight of animal (Dafem et al., 2008).

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the means. When the differences were significant, Duncan's test, conducted at the 5% level, was utilized to separate them. Statistical analysis was performed using SPSS 20.0 software (Meyers et al., 2013).

RESULTS

Effect of feed protein on reproduction

Feed protein effect on reproduction is summarized in Table 3. It is observed that the protein level in the diet significantly affected the reproductive characteristics. Thus, a lower result for the age of onset of sexual maturity, number of eggs per clutch, egg weight, egg length, egg diameter, incubation period, new hatch rate per laying, and newly hatched weight per laying were obtained in snails receiving 22% protein (T2) in the diet. On the other hand, the highest values were recorded in animals receiving 20% (T1) of protein in the diet, except for the fertilization rate.

Table 3. Effects of feed protein on the characteristics of reproduction.

Characteristics of reproduction	Treatments		
	T1 (n = 30)	T2 (n = 30)	T3 (n = 30)
Age of sexual onset (months)	21.50 ± 0.00^{a}	16.00 ± 0.00^{b}	19.50 ± 0.00^{a}
Number of spawns per treatment	1.00 ± 0.00^{a}	7.00 ± 0.00^{b}	4.00 ± 0.00^{c}
Number of eggs per clutch	6.00 ± 0.00^{a}	4.83 ± 0.38^{b}	5.10 ± 0.71^{b}
Egg weight (g)	1.00 ± 0.00^{a}	0.69 ± 0.48^{a}	0.82 ± 0.40^{a}
Egg length (mm)	14.67 ± 0.51^{a}	13.73 ± 0.76^{b}	14.15 ± 0.80^{ab}
Egg diameter (mm)	11.67 ± 0.51^{a}	10.50 ± 0.80^{b}	10.92 ± 0.49^{b}
Fertilization rate (%)	33.00 ± 0.00^{a}	75.00 ± 0.00^{b}	91.75± 0.00^{b}
Incubation period (days)	27.25 ± 0.00^{a}	26.25 ± 0.00^{a}	27.00 ± 0.00^{a}
Newly hatch rate per laying (%)	100.00 ± 0.00^{a}	75.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Newly hatch weight per laying (%)	1.00 ± 0.00^{a}	0.69 ± 0.48^{a}	0.82 ± 0.40^{a}
Early embryos mortality	67.00 ± 0.00^{b}	26.60 ± 0.00^{a}	12.50 ± 0.00^{a}
Late embryos mortality	0.00 ± 0.00^{a}	13.20± 0.00^{a}	8.25 ± 0.00^{a}
Adults' mortality	13.63 ± 0.00^{a}	18.75 ± 0.00^{a}	20.00 ± 0.00^{a}

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

Also, the lowest values of early embryo mortality were obtained in the treatment receiving the highest protein content T3 (24 %) in the diet; whereas a significantly higher number of adult mortalities ($p < 0.05$) was recorded in the same treatment compared to the other treatments, which were otherwise comparable.

Effects of feed protein on the gonadosomatic index

The effect of feed protein on the gonadosomatic index is illustrated in Figure 1. This index showed an increase in the amount of protein in the diet, but no significant difference ($p > 0.05$) was observed among the treatments.

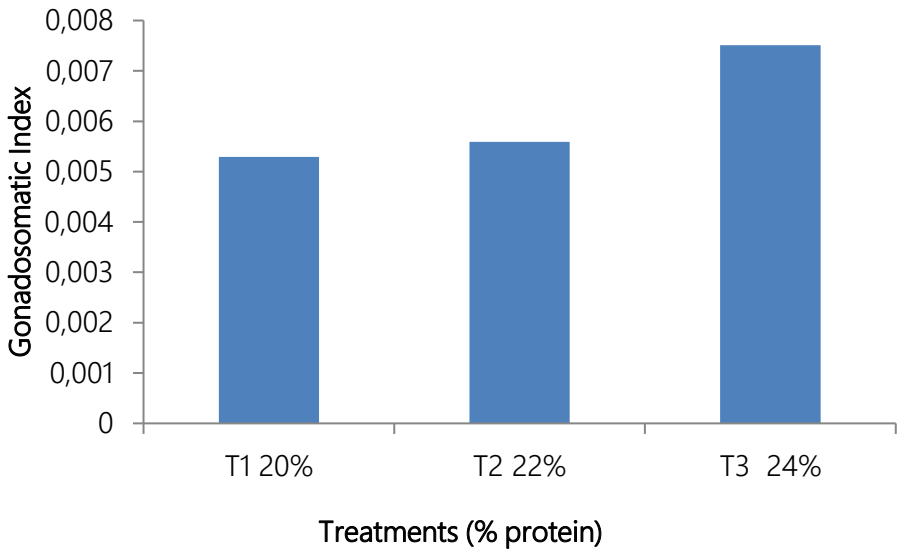

Figure 1. Effects of feed protein on the gonadosomatic index.

Effects of feed protein on the hemolymph metabolite profile

The influence of feed protein on the hemolymph metabolite profile is presented in Table 4. The highest total protein and cholesterol levels were registered in the treatment receiving the reduced protein content in the diet (T1, 20%), while the lowest values were obtained in snails of treatment T2 (22%). However, no significant difference ($p > 0.05$) was observed between the treatments.

Table 4. Influence of feed protein on the hemolymph metabolite profile.

	Treatments		
	T1 (n = 30)	T2 (n = 30)	T3 (n = 30)
Protein (g/dl)	1073.20 ±77.02[a]	977.02 ± 161.24[a]	1066.00 ± 31.17[a]
Cholesterol (mmol/l)	1.59 ± 0.54[a]	1.33 ± 0.60[a]	1.34 ± 0.20[a]

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

Effects of feed protein on carcass characteristics

The influence of feed protein on carcass characteristics is summarized in Table 5. Regardless of the considered carcass characteristics, the values increased with the protein content in the diet, with higher values observed in snails receiving the highest protein content (T3, 24%), and lower values recorded in snails with the lowest protein content (T1, 20%). However, no significant difference (p > 0.05) was observed between the treatments, except for body weight, soft tissue, and viscera.

Table 5. Carcass characteristics according to the protein feed.

Carcass characteristics (g)	Treatments		
	T1 (n= 30)	T2 (n= 30)	T3 (n= 30)
Live weight	58.60 ± 4.96[a]	68.00 ± 12.67[ab]	77.20 ± 12.80[b]
Shell weight	6.10 ± 2.04[a]	7.40 ± 1.88[a]	8.10 ± 2.21[a]
Soft tissue	11.40 ± 1.78[a]	16.30 ± 4.46[b]	18.30 ± 2.86[b]
Pedal mass	9.90 ± 2.04[a]	12.30 ± 3.25[a]	12.10 ± 2.07[a]
Total meat	19.00 ± 3.93[a]	23.90 ± 5.29[a]	25.40 ± 5.59[a]
Viscera	1.90 ± 0.96[a]	4.00 ± 0.93[b]	4.10 ± 1.08[b]
Shell/meat ratio	0.59 ± 0.54[a]	0.48 ± 0.47[a]	0.42 ± 0.54[a]
Gonads	0.31± 0.10[a]	0.38 ± 0.10[a]	0.58 ± 0.68[a]

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

Effects of feed protein on the proximate composition of snail flesh

The influence of feed protein on the proximate composition is presented in Table 6.

Table 6. Effects of feed protein on the proximate composition of snail flesh.

Proximate composition (g/DM)	Treatments		
	T1 (n= 30)	T2 (n= 30)	T3 (n= 30)
Dry matter	91.33[a]	90.33[b]	92.00[a]
Organic matter	80.67[a]	78.33[b]	82.00[a]
Protein	45.00[a]	45.00[a]	48.67[b]
Lipids	3.00[a]	3.00[a]	0.33[b]
Ashes	2.00	3.00	2.00

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

A significantly higher percentage of dry and organic matter was obtained in the snails receiving the diet richest in protein: T3 (24%). The lowest values were recorded among those receiving treatment T2 (22%). Furthermore, the protein content in the flesh was significantly higher in snails receiving the maximum protein level (T3 24%), compared to the two other treatments (T1 20%; T2 22%) which were otherwise comparable. The highest percentage of ash was recorded in snails receiving 22% (T2) protein in the diet compared to treatments T1 and T3.

Effect of feed protein on the mineral content of the snail flesh

The influence of feed protein on the calcium, magnesium, potassium, sodium, phosphorus, and iron content in the carcass of the snail is summarized in Table 7. The percentage of calcium increased significantly with the protein level in the experimental diet. Thus, snails fed the diet with the maximum protein content (T3 24%) exhibited the greatest calcium concentration, while those given the minimum protein level (T1 20%) showed the least calcium concentration. An opposite trend was observed for phosphorus.

Table 7. Effect of feed protein on the mineral content.

Minerals (mg/100g)	Treatments		
	T1 (n=30)	T2 (n=30)	T3 (n=30)
Calcium	54.50 ± 0.50[a]	143.00 ± 0.50[b]	164.50 ± 0.50[c]
Magnesium	230.33 ± 0.57[a]	413.67 ± 0.57[b]	230.33 ± 0.57[a]
Potassium	599.67 ± 0.57[a]	691.00 ± 1.00[b]	569.67 ± 0.57[c]
Sodium	21.30 ± 0.30[a]	21.30 ± 0.30[a]	21.30 ± 0.30[a]
Phosphorus	401.67 ± 0.57[a]	401.67 ± 0.57[a]	317.00 ± 1.00[b]
Iron	59.67 ± 0.57[a]	71.67 ± 0.57[b]	60.33 ± 0.57[a]

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

DISCUSSION

This study evaluated the effect of protein levels on the morphometric characteristics of *Achatina achatina* eggs. The results showed that the highest egg weight, length, and diameter values were recorded in snails receiving the lowest protein content in the diet. These results are higher than those of Cobbinah et al. (2008a) in *Achatina achatina* in captivity. The proteins ingested act on the albumin gland of snails (glands responsible for the production of proteins and polysaccharides) in the form of hormones stimulating the synthesis and the accumulation of galactogens and proteins in the yolk constituting the perivitelline layer of eggs. Charniaux-Cotton (1973) showed that, during vitellogenesis in gastropods, protein synthesis is continuous and allows the accumulation of numerous reserves (the yolk), representing 80% of the protein reserves of the cell. It occurs in the cytoplasm of the oocyte as aggregates made up of proteins, lipids, and glycogens. These results could also be justified by the low number of spawning recorded in snails in this treatment because Leclercq et al. (1980) reported that high egg production results in low-weight eggs.

The earliest age of onset maturity (16 months) recorded in snails in treatment T2 (22 %) remains within the range values (14-20 months) obtained by Cobbinah et al. (2008b) but lower than those of Hardouin et al. (1995) in *Achatina achatina* (18-20 months). This would be justified by the fact

that the protein would have acted on the cerebral ganglia of the snail in this treatment by stimulating the production of the egg-laying hormone, which in turn would act on the ovotestis promoting its growth and development, the release of gametes, and the activity of the albuminogenic glands, followed by spawning. Indeed, Morishita et al. (2010) have shown in invertebrates, and particularly in molluscs, that the egg-laying hormone controls the maturation of the gonads and the production of eggs; while the dorsal hormone regulates the cellular differentiation of the accessory female sexual organs, the maturation of the gonads, oocytes, vitellogenesis, and synthesis of galactogens.

The number of spawns increased with the protein level; the highest value (7.00 ± 0.00) was obtained in snails receiving 22% protein in the feed. These results corroborate those of Nyameasem and Borketey-La (2014), who found that any increase in protein level in food intake leads to enhanced egg production. This could be explained by the fact that proteins improve egg production performance in snails (*Achatina achatina*).

The shortest incubation period was recorded in snails that received 22% protein in the diet, but it was longer (26 days) than those of Karamoko et al. (2011) recorded in *L. micolaria flammea* (18.42 ± 5.15). This could be attributed to the small size of the eggs observed in snails under this treatment. Codjia and Noumonvi (2002) reported in giant snails that small eggs with a reduced contact surface exhibit shorter incubation period compared to larger eggs, which have longer ones. Additionally, the incubation period is influenced by factors such as shell calcification, temperature (25-30 °C), and environmental humidity (80-95%) (Cobbinah et al., 2008b).

Snails receiving 20% protein in the feed recorded the highest newly hatched weight. The results are similar to those of Kouassi et al. (2007) in *Archachatina ventricosa*, who demonstrated that the protein level in the diet influences the weight of the eggs. This could be attributed to the high egg weight obtained from the animals in this treatment, as a positive and strong correlation ($p < 0.01$) was found between the weight of the eggs and the newly hatched weight.

The fertilization rate and hatching, both of which increased with the protein level, yielded the highest values in snails consuming the most protein-rich diets. This outcome aligns with the findings of Kouassi et al. (2007) in *Archachatina ventricosa*, indicating an association between

fertilization and fertility rates and dietary protein levels. Similar trends have been observed in other species, as reported by Zougou et al. (2017) in guinea pigs. Miegoue et al. (2016) also noted an increase in fertilization and fertility rates corresponding to higher protein levels. The obtained results' values surpassed those reported by Karamoko et al. (2011) in *Achatina achatina* but were lower than the findings of Dafem et al. (2008) in archachatines (94.65).

The results would be justified by the fact that the level of protein received by snails in the compound feed promotes fusion and fertilization of gametes, thereby increasing the rate of hatching. Glabe et al. (2019) corroborate this hypothesis, stating that the consumption of protein-rich food promotes sperm attachment to the egg. According to the same authors, proteins present on the membranes of spermatozoa and ova potentiate the fusion and fertilization of gametes. High hatching rates could also be attributed to the effect of calcium on egg calcification (Bonnet et al., 1990), where determination of the optimal calcium level in the diet could enhance the reproductive characteristics of the snails' eggs.

The increased protein level in the diet resulted in a decreased level of total cholesterol in the hemolymph. The highest value was recorded in snails receiving the lowest protein level in the diet. Collomb and Mayor (2007) reported, on one hand, that soybean is rich in amino acids (such as glycine and arginine), phytosterols (which have structures like cholesterol and may inhibit its absorption), and isoflavones, responsible for decreasing insulin levels in the blood. Lower insulin levels lead to reduced cholesterol production in the liver. On the other hand, amino acids, phytosterols, and isoflavones may also affect the intestinal absorption of cholesterol by inducing a decrease in its presence in the hemolymph (Collomb and Mayor, 2007).

The values of the carcass characteristics also increased with the protein level in the diet; these results are similar to those of Otchoumou et al. (2005). Smith and Adegbola (1982) revealed that the best growth performance and carcass yield are obtained in snails fed on diets containing high protein content. These results are believed to be due to the beneficial effect of the diet containing a high level of protein (24%), necessary for the growth of snails (*Achatina achatina*). The highest relative organ weights are obtained in snails receiving 22% protein in the diet. The values recorded in this study (32.40-35.65%) are higher than those obtained by Otchoumou et al. (2010) in *Achatina fulica* (26.61%). This difference could be related to the compound feed as well as the

species used. This could also be justified by a difference between the parts considered to be consumable (pedal masses and the head) during the various studies (Aman, 2013).

This study also showed that the protein level in snail flesh increased proportionally to the protein content in the diet. This rise might be attributed to the elevated consumption of compound feed in snails receiving a high protein content in their diet, leading to increased intake and absorption of amino acids during digestion. Consequently, this process could stimulate the synthesis of muscle proteins such as collagens, actins, myosins, or cytoskeletal proteins, all of which are stored in the pedal mass of snails. Conversely, the escalation in lipid levels, dependent on the protein level in the diet, could be elucidated by the conversion of amino acids, not utilized for protein synthesis, into pyruvic acids and subsequently into Acetyl Coenzyme A (Acetyl-CoA). These Acetyl-CoA fragments may then condense to form long-chain fatty acids, which would subsequently combine with glycerol to form fats (King, 2002).

The calcium level in the ash increased while the phosphorus level decreased with the rising protein content in the diet. These findings are similar to those of Kerstetter et al. (2005), who reported that increasing the protein level in the diet promotes intestinal absorption of calcium and iron, while decreasing phosphorus. This outcome could be justified not only by the high content of calcium, iron, and phosphorus in the compound feed but also by the presence of vitamins. For instance, vitamin D regulates the intestinal absorption of calcium and phosphorus, while vitamin C promotes iron absorption (Fleet, 2022).

FINAL CONSIDERATIONS

The morphometric characteristics of the eggs (weight, length, and diameter), the number of eggs per clutch, and the highest spat weight were recorded in snails receiving the lowest protein level (T1 20%) of the feed. Conversely, the highest total egg-laying percentage, hatching, and fertilization rate were observed in snails receiving the highest level of protein (T3 24%). Additionally, the total cholesterol level in hemolymph decreased with the protein level in the diet, while carcass characteristics increased with the protein level in the ration. The highest values were obtained in snails receiving the highest protein content (T3 24%). Bromatological characteristics

were influenced by the protein level in the diet: the rates of dry and organic matter increased, while the lipid levels decreased with higher protein content in the ration. Furthermore, the calcium level in the ash increased, while the phosphorus level decreased significantly with the protein level in the diet. Overall, the study suggests that the 22% protein level can be retained in the diet of adult snails. In conclusion, understanding the intricate relationship between dietary protein levels and various physiological parameters can optimize snail husbandry practices for enhanced productivity and health.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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