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Dietary effects growth and shell calcium incorporation of *Bulimulus tenuissimus* (Stylommatophora : Bulimulidae) in laboratory conditions

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Resumo. A alimentação exerce grande influência no desenvolvimento dos moluscos terrestres. Desse modo, objetivou-se com esse estudo verificar a influência de diferentes dietas sobre o crescimento e o ganho de massa corporal de *B. tenuissimus*. Para tal, grupos de moluscos foram alimentados com seis itens diferentes (batata, chuchu, pepino, cenoura, maçã e ração), os quais foram oferecidos separadamente ou combinados por um período de 210 dias. Observaram-se os efeitos da alimentação nos dois parâmetros avaliados. Os resultados obtidos indicam que a combinação de itens alimentares favoreceu o crescimento, incorporação de cálcio na concha e o ganho de massa corporal nessa espécie, provavelmente, devido ao aumento na disponibilidade de nutrientes.

Palavras-chave: nutrição. desenvolvimento. moluscos terrestres.

Abstract. Diet influences the development of terrestrial snails. The objective of this study was to verify the influence of different diets on growth, body mass gain and content of calcium in the shell of *Bulimulus tenuissimus* (d' Orbgny). For this purpose, groups of snails were fed with six different items (potatoes, chayote, cucumber, carrot, apple and feed), which were offered separately or combined, for a period of 210 days. We observed the effects of feeding in both available parameters. The results indicate that the combination of food items favored the growth, body mass and calcium incorporation in this species, probably due to increased availability of nutrients.

Keywords: nutrition, development, land snail.

Introduction

Pulmonate terrestrial are known for their economic and medical-veterinary importance. However, the literature concerning this group emphasizes aspects of systematic and geographical distribution, and studies of the biology of snails are still incipient (Almeida & Bessa, 2000), especially for native species.

Lack of knowledge about patterns of development and reproduction of terrestrial snails complicates the management and conservation of these animals in nature, as well as their control while native species and/or transmitting parasite, respectively (D'Ávila *et al.,* 2004, Simone, 2006).

Bulimulus tenuissimus (d' Orbigny, 1835) is a native species, widely distributed in Brazil (Lange-de-Morretes, 1949; Simone, 2006), which has potential in the transmission of helminthes.

There are few studies on the biology of *B. tenuissimus* (Silva *et al*., 2008; 2009; 2010), and little is known about the effects of diet on the life cycle of this

species (Meireles *et al.,* 2008; 2010), and the influence of energy content, nutritional values, structure and texture of food on the growth and reproductive success of these animals (Thomas *et al.,* 1983; Mcshane *et al*., 1994; Foster *et al*., 1999).

One of the problems encountered in the breeding of snails in laboratories is the formulation of diets that aim to meet the nutritional needs of these animals, as well as growth, homeostasis, and hormone and enzyme activities (Ademolu *et al*., 2004). Thus, the formulation of artificial diets becomes necessary for the development of work on snails in different studies.

The objective of this study was to compare the effect of different diets on growth, body mass gain and calcium incorporation by *B. tenuissimus* during 210 days of observation.

Matherial and Methods

Newly hatched animals were obtained in the post-graduation laboratory of Biological Sciences - Animal Behavior and Biology, Juiz de Fora Federal University (UFJF) (21°45'13"-21°46'13"S, 43°21'19"-43°22'15"W) - in Juiz de Fora, Minas Gerais State, Brazil.

The snails were kept in plastic terrariums (14.0 cm in diameter and 9.0 cm deep), sealed with cotton and elastic tissue for a period of 210 days. Humus sterilized (120°C/1 hour) was used as substrate, and dampened with tap water at one-day-intervals (Bessa & Araújo, 1995). The work was conducted under laboratory conditions of photoperiod, temperature and relative humidity (Meireles *et al.,* 2008).

The animals used in this work were deposited in the malacology collection of Prof. Maury Pinto de Oliveira Museum, UFJF (registration number 8364/8365/8366).

Experiment 1 - Palatability

This experiment was carried out to verify which food items promoted the furthest development of the animals. For this purpose, 360 newly-hatched animals were divided into six groups, each group with 30 individuals each (with two replicates). Each group received one of the following diets for 210 days: Potato Group → potato *Solanum tuberosum* Linné, 1758 (Solanaceae); Cucumber Group →Cucumber *Cucumis sativus* Linné, 1758 (Cucurbitaceae); Carrot Group → carrot *Daucus carota* Linné, 1758 (Apiaceae); Chayote Group → chayote *Sechium edule* Swartz, 1800 (Cucurbitaceae); Apple Group → apple *Malus domestica* Borkhausen, 1793 (Rosaceae) and Ration Group → feed ration for bird growing, enriched with calcium carbonate (Bessa & Araújo, 1996).

One gram of each food, weighed in precision balance, was given to the animals (BOSCH SAE 200) and renewed at one-day-intervals.

Experiment 2 - Selecting Food

To verify what type of diet gave the best development for these animals, 120 newly hatched snails were separated into two groups of 30 individuals each (with two replicates). Animals of each group were fed with the following diets for 210 days: Natural Diet (ND) → potato, cucumber, carrot, apple and chayote, and Combined Diet (CD) → vegetable (potato, cucumber, carrot, apple and chayote) and ration enriched with calcium carbonate, Ration (Ra) → ration enriched with calcium carbonate. For groups of snails it was also offered 1 g of each food, weighed in precision balance, and renewed at one-day-intervals. Table I shows the amount of calcium and water in the food offered to the animals during the study.

Table 1 - Percentage of calcium (mg) and water (g) in 100g of food given to snails (USDA Nutrient Database for Standard Reference).

Food (100 g)	Calcium (g)	Water (g)
Potato	30.0	83.0
Cucumber	16.0	95.0
Carrot	33.0	88.0
Chayotte	17.0	95.0
Apple	6.0	86.0
Ration	33.0	0.0

Dietary Effects on Growth and Body Mass of Bulimulus tenuissimus

In order to evaluate the growth of the individuals, measurements of shell length (cm) of snails were made at 15-day-intervals from the first day of the experiment using a caliper Kanon (Mardened Stainless 1/28 in 1/20mm). The snails were weighed on precision balance, in order to observe the body mass (g) gain.

Calcium Incorporation

To verify calcium incorporation, three shells of each group (Natural Diet, Ration and Combination Diet) were analyzed according to Pinheiro & Amato (1995).

Statistical Analysis

The data were analyzed by the program BioEstat 4.0, using the Kruskal-Wallis and Mann-Whitney ($p < 0.05$) to compare the data found in the different treatments, as well as the length of the shell, body mass and calcium incorporation.

Results

During the experiment the maximum temperature ranged from 22 to 29° C, and the minimum temperature, from 18 to 25° C; and relative humidity between 79-92%. There was no significant difference between the replicates of each treatment ($p>0.5$), the results were analyzed as a single group.

Experiment I – palatability

Growth and Body Mass of Bulimulus tenuissimus

Diet exerted effects on growth and body mass of *Bulimulus tenuissimus* and the ration was the most favorable item to the development of snails. The average length of the shell of snails fed with ration was significantly higher than the other treatments ($H=26.42$; $p=0.0001$). The average size of individuals at the end of the experiment was 3.50 mm ± 0.10 for the Apple Group, 6.6 mm ± 0.33 for the Carrot Group, 7mm ± 0.14 for the Potato Group; 8.1mm ± 0.21 for the Chayote Group, 8.7 ± 0.15 for the Cucumber Group and 18.0 ± 0.26 for the Ration Group (Figure 1).

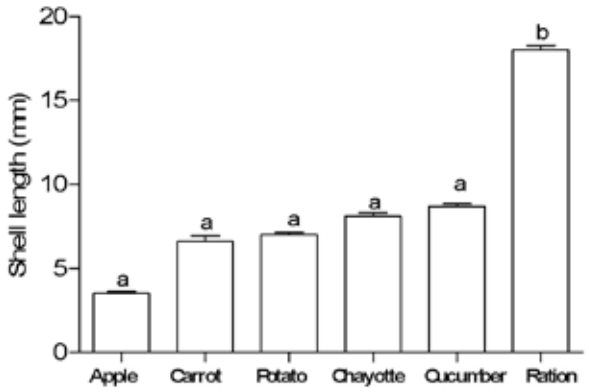

Figure 1: Shell length (mm) of *Bulimulus tenuissimus* - Groups: Potato, Cucumber, Apple, Carrot, Chayotte and Ration in the end of the experiment.
* Different letters indicate significantly different results (Kruskal-Wallis, $p < 0.05$).

The growth rate of the Ration Group was significantly higher, being of 0.08 mm per day (H=14.51; p=0.01). For all groups there was a variation in the length and the final growth rates, which were 0.009 mm per day for the Apple Group, 0.03 mm per day for the Carrot, Potato and Chayote Groups, and 0.02 mm per day for the Cucumber Group.

There was difference between the body mass gain of individuals of *B. tenuissimus* by the end of the experiment (H=33.19; p=0.0001) (Figure 2).

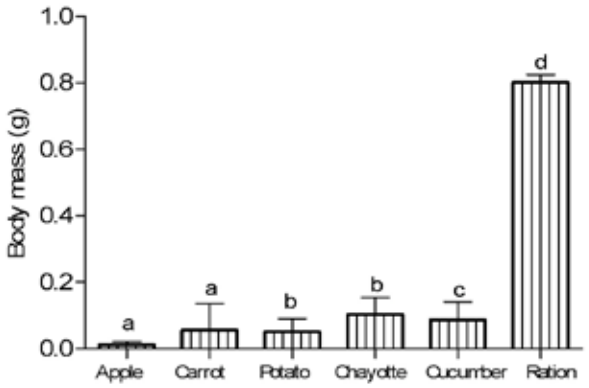

Figure 2: Gain of body mass (g) of *Bulimulus tenuissimus* Groups: Potato, Cucumber, Apple, Carrot, Chayotte and Ration in the end of the experiment.
* Different letters indicate significantly different results (Kruskal-Wallis, p < 0.05).

Experiment 2 - Selecting Food

Growth and Body Mass of Bulimulus tenuissimus

The animals of the Combined Diet Group (CD) had significant greater length of shell than the Natural Diet (ND) Group (t=25.80; p=0.001) and Ration Group (t=37.50; p<0,001). The average size of individuals at the end of the experiment was 11.6 mm ± 0.263 for the ND Group, 15.1mm ± 0.25 and 19.8 ± 0.150 for the CD Group (Figure 3).

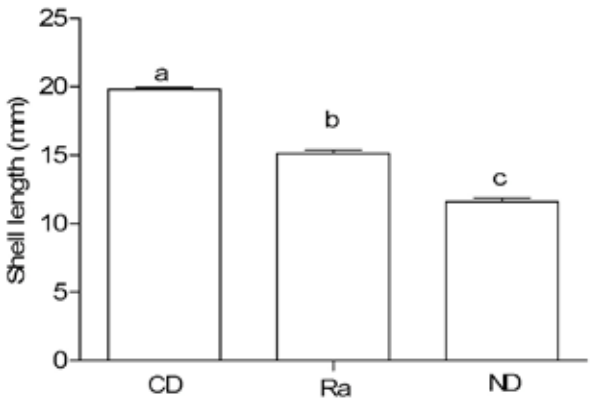

Figure 3: Shell length (mm) of *Bulimulus tenuissimus* of the groups Natural Diet (ND) and Combined Diet (DC), in the end of the experiment.
* Different letters indicate significantly different results (Kruskal-Wallis, p < 0.05).

There was variation in growth rates, and the snails of the CD Group showed greater growth than the ND Group (Z=12.58; p=0.005). The growth rate of the ND and CD Groups were 0.05 mm per day and 0.09 mm per day, respectively.

Snails from CD group obtained higher weight than others (ND: t=33,63; p=0.0004 and Ra: t=38,5; p<0.0001). The average body mass of individuals at the end of the experiment showed in Figure 4.

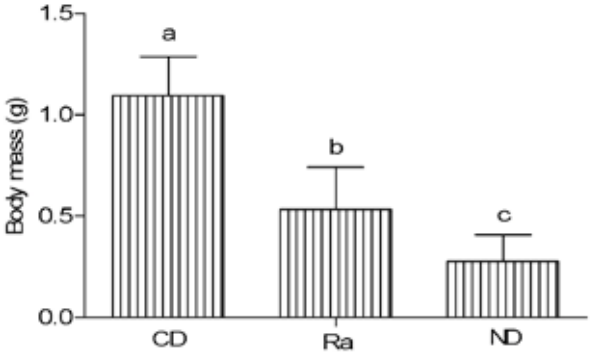

Figure 4: Gain of body mass (g) of *Bulimulus tenuissimus* of the groups Natural Diet (ND) and Combined Diet (CD) in the end of the experiment
* Different letters indicate significantly different results (Kruskal-Wallis, p < 0.05).

Calcium Incorporation

The calcium concentration of the shells at the end of the experiment was 677,855mg $CaCO_3$/g for the CD Group, 285,126mg $CaCO_3$/g for the Ration Group and 108,938mg $CaCO_3$/g for the ND Group (H=23,28; p=0.001) (Figure 5).

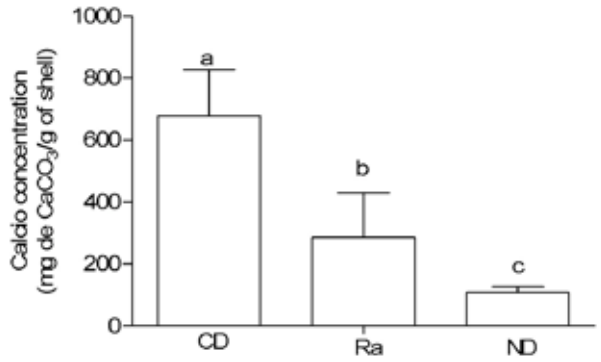

Figure 5: Calcium incorporation ($CaCO_3$/g) in the shells of *Bulimulus tenuissimus* of the groups Combined Diet (CD), Natural Diet (ND) and Ration in the end of the experiment.
* Different letters indicate significantly different results (Kruskal-Wallis, $p < 0.05$).

Discussion

By feeding this species, as well as other animals, acquire energy resources to be allocated for the maintenance of vital processes, somatic growth, reproductive activity and survival (Raut & Panigrahi, 1988; Cichon, 1999; Heller, 2001). This observed in this study through the parameters evaluated: the growth rates, size, body mass and calcium content of the shell in the snails.

The higher growth and body mass gain observed in the Combined Diet (CD) and Ration Groups is probably due to the fact that they have been given food with varied nutritional components. Similarly, the absence of supplementary nourishment explains the lower growth and body mass gain in the others groups.

The quality of the food influenced the rates of growth, mass gain and shell consistence. The nutritional components and digestibility of foods exert influence on the development of these animals (Foster *et al*., 1999). Thus, the use of adequate nutrition is essential for the maintenance of these animals in laboratory.

The results showed that all foods were consumed when offered separately, indicating that a feature of low nutritional quality can be consumed in the absence of other high energy value. However, when they were offered different resources simultaneously, there was clear preference for more nutritious foods. Thus, it was confirmed by this study that consumption of a particular food does not necessarily indicate food preference, but may be related to resource availability.

The artificial diet offered in laboratories provides more amounts of proteins, lipids, vitamins, minerals and carbohydrates that are converted into energy, allowing growth and better reproductive performance (Bessa & Araújo, 1996; Brandolini & Gomes 2002; Meireles *et al,* 2008).

There were no data for the formulation of complete diets for snails (Cuellar, 1986; Rousselet, 1986; Soares *et al*., 1999; Hayashi *et al*., 2000) according to different stages of development. In general, snail breeders have used empirical diets (Hayashi *et al.,* 2000), normally based on wheat bran, soybean and maize or concentrates of poultry food mixed with corn and sources of calcium and phosphorus, independent of the development stage (Rodrigues, 1991; Ferraz, 1999; Soares *et al.,* 1999).

We observed beneficial effects of artificial diets in snails bred in laboratory experiments. The nutritional supplement of artificial diet with vegetables,

protein and calcium carbonate, among others, has proven an advantageous and better development of these animals (Ribas, 1986; Mansur & Machado, 1994; Soares *et al.,* 2002).

It also observed that the physical characteristics (texture) and chemical (water and calcium) were decisive in the choice of food being food softer and more water content and calcium consumed the most. Individuals prefer softer foods and higher water content and calcium. The importance of calcium for terrestrial gastropods is unique in the animal kingdom. Few animals have their ecology so closely linked to one single factor that, directly or indirectly, determines their distribution and response to adverse environmental conditions, ability to aestivation, hibernation and mating, growth rate and reproductive capacity (Beeby &Richmond, 2007).

The combination of various food items provide access to various nutritional components and thereby promote higher growth, body mass gain and calcium incorporation by the snails.

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