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The photoperiod may modulate the carbohydrate metabolism of *Bradybaena similaris* (Férussac, 1821) (Mollusca, Bradybaenidae)[1]

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Abstract. The snail *Bradybaena similaris* is a pulmonate gastropod with great medical and economic importance. In this study, the effects of different photoperiods (0, 6, 12, 18 and 24 hours of photophase) on glycogen deposits in digestive gland (DG) and cephalopedal mass (CM) of *B. similaris* were analyzed. The higher content of glycogen in the cephalopedal mass was observed in the snails exposed to a photoperiod composed by 12 hours of photophase, ranging 0.39mg of glucose/g of tissue, wet weight. And the lowest value was that obtained from snails maintained under six hours of light, 0.01mg of glucose/g of tissue, wet weight. In digestive gland the maximum and the minimum values were observed to the snails maintained under 24 and 0 hours of light, respectively. The 12 hours photoperiod resulted in a better rate of synthesis/mobilization of glycogen in the CM of *B. similaris*. So, the photoperiod that resulted higher content of glycogen in the DG was that composed by 24 hrs of light.

Key words: glycogen, comparative physiology, mollusc, land snail.

Resumo: O fotoperído pode modular o metabolismo de carboidratos em *Bradybaena similaris* (Férussac, 1821) (Mollusca, Bradybaenidae). O molusco *Bradybaena similaris* é um gastrópode pulmonado com grande importância médica e econômica. Neste estudo, o efeito de diferentes fotoperíodos (0, 6, 12, 18 e 24 horas de fotofase) foi analisado nos depósitos de glicogênio na glândula digestiva (DG) e massa cefalopodeal (CM) de *B. similaris.* O maior conteúdo de glicogênio na massa cefalopodeal foi observado em moluscos expostos a um fotoperíodo composto por 12 horas de fotofase, variando de 0,39mg de glicose/g de tecido, peso úmido. O menor valor foi obtido de moluscos mantidos sob seis horas de luz, 0,01mg de glicose/g de tecido, peso úmido. Na glândula digestiva, os maiores e menores valores foram obtidos em moluscos mantidos sob 24 e 0 horas de luz, respectivamente. O fotoperíodo de 12 horas resultou em uma melhor taxa de síntese/mobilização de glicogênio no CM de *B. similaris.* Assim, o fotoperído que resultou em uma maior concentração de glicogênio na DG foi o composto por 24 horas de luz.

Palavras-chave: glicogênio, fisiologia comparada, molusco terrestre.

INTRODUCTION

The snail *Bradybaena similaris* (Férussac, 1821) is a pulmonate gastropod, probably came from Asia (THOMÉ *et al.,* 1996). This mollusc exhibits a great medical and veterinary importance because it may be used as an intermediate host by many parasites, as the nematode *Angiostrongylus costaricensis* (Morera & Céspedes, 1971) (TIENGO, 1995), and the digenetic trematodes *Eurytrema coelomaticum* (Giard et Billet, 1892) Looss, 1907 (PASCHOAL, 1991) and *Postharmostomum gallinum* Witenberg, 1923 (AMATO & BEZERRA, 1989).

Beyond this, the wide distribution of *B. similaris* (ARAÚJO, 1989) plus to the fact that this snail is a plague to the agriculture due to its varied food

requirements bring the need of study aspects of its biology, as the response to physiological stress.

The carbohydrates are the source of energy to the snails (JOOSSE, 1988), and, in the snails, they are stored as glycogen and galactogen, being the first one the main source of general energy, and the second one is restricted to the albumen gland and it serves only to the reproductive process (GERAERTS, 1992). The content of the glycogen are deeply influenced by the physiological conditions of the snails, as food availability, presence of parasite, abiotic alterations (BECKER, 1980, LIVINGSTONE & ZWAAN, 1983). The mobilization of glycogen reserves occurs when the glucose content in the hemolymph is reduced, under stress conditions, as starvation (PINHEIRO, 1996) and parasitism by larval trematodes (PINHEIRO & AMATO, 1994, AZEVEDO *et al*., 1997) these deposits are highly reduced in *B. similaris.* The photoperiod is related to the exposition of an organism to the light, specially in relation to the effects on reproduction, development and growth.

According to VAN ELK & JOOSSE (1981), in *Lymnaea stagnalis* (Linnaeus, 1758) the photoperiod exerths an influence on the catalitic activity of the enzyme UDP-galactose 4-epimerase, which converts the UDP-glucose in UDP-galactose, an initial step to the polimerization of galactose. The epimerase activity was reduced when the snails were exposed to short days conditions and increased under long days. The study showed that the photoperiod has an effect on the ovipository activity and the increase in the lenght of the day results in an increase of this activity. So, the UDP-galactose 4-epimerase from the albumen gland reflects the presence of a mechanism of adaptation in the snails to a long day photoperiod.

Factors associated to temperature and photoperiod influence the neuroendocrine control of ovulation and egg laying in *Helix aspersa* (Müller, 1774). In *Helix pomatia* (Linnaeus, 1758), the photoperiod influences the olfactory estimulation (VOSS *et al*., 2002). Effects of the photoperiod on the hormonal behaviour and the oviposition in *L. stagnalis* is knew (DOGTEROM *et al*., 1983).

In spite of the existence of studies about the influence of photoperiod on reproduction of different snails species, there is not information about the influence of this abiotic factor on the glycogen deposits of the molluscs. So, the purpose of this study is to analyze the influence of different photoperiods on the glycogen reserves in digestive gland (DG) and cephalopedal mass (CM) of *B. similaris*.

MATERIAL AND METHODS

Snails collection and maintenance

Specimens of *B. similaris* were manually collected from plants of gardens located at BR465, km 9, Seropédica, RJ, Brazil. The snails were oberved through their transparent shell to investigate the presence of larval trematodes, as metacercariae of *P. gallinum* and samples of snails randomly chosen were dissected to verify the larval stages of trematodes in their soft tissues. The snails free of infection were maintained under laboratory conditions (20 e 25ºC), in glass vivaria with earth at the bottom, moistened with tap water in alternate days (LEAHY, 1984). The snails were fed with fresh lettuce leaves *ad libitum*.

Establishment of photoperiods

The snails with 10mm of shell diameter were divided in groups of 20 specimens, and each group was exposed to a different photoperiod (0, 6, 12, 18 and 24 hours of photophase). The light exposition was made through three lamps (100W). The exposition to different photoperiods was made by a week. The experiments were made in duplicates, using two groups of 20 specimens to each photoperiod analyzed.

Biochemical analysis

After a week the snails were dissected early morning (at about 8 hours). The shells of the living snails were removed with tweezers and the tissues of DG and CM were separated, pooled according to the photoperiod, weighed and stored at -10ºC until their utilization.

The glycogen extraction was made according to PINHEIRO & GOMES (1994) and its determination was made by the 3.5 dinitrosalicylic acid (3.5 DNS)

(SUMNER, 1924), being the results expressed as mg of glucose/g of tissue, wet weight. The sprectrophotometrical analysis were made in triplicates.

The chromatographic analysis of the polysaccharides extracted was made according to VILLELA *et al*. (1973) and PINHEIRO & GOMES (1994).

Statystical analysis

The results were expressed as mean ± standard deviation and the Tukey-Kramer's test was used to compare the mean values. The polynomial regression was applied to verify the relation between the carbohydrates contents and the different photoperiods (α=5%) (GraphPad InStat, GraphPad Prism, Prism Inc.).

RESULTS AND DISCUSSION

The snails maintained under 0, 6 and 24 hours of photophase presented lower content of glycogen in CM than those observed to snails maintained under 12 and 18 hours of light (Tab.1). The highest content of glycogen was observed to the snails exposed to 12 hours of light, ranging 0.39mg of glucose/g of tissue, wet weight, and this value was significantly different than those obtained in other photoperiods. The polynomial regression analysis did not revealed a positive relation between the photoperiod and the glycogen concentration in the CM tissue in *B. similaris* (r^2=0.67) (Fig. 1).

The deposits of glycogen in muscular tissue of snails are resulting from the balance between the food intake and the locomotory activity during the period of observation. This relation is evidenced when the snails were maintained under extreme photoperiods (0 and 24 hours of light). The snails are most active in the nocturnal period (JUNQUEIRA *et al.*, 2003), due to the lowest temperature and increasing of the moisture at this time. So, the molluscs exposed to 0 hours of light presented an increased physical activity, moving itself most frequently, increasing the probability of the snails found the food supplies. But the group of animals exposed to 24 hours of light, showed a reduced activity, begining an estivation process and this situation limited the movements of the snails and consequently reduced the food meeting.

Table 1. Glycogen content in the cephalopedal mass (CM) and in the digestive gland (DG) of *Bradybaena similaris*, as mg of glucose/g of tissue, wet weight, maintained under different photoperiods for a week.

Photophase (hours)*	N	Glycogen content (mg of glucose/g of tissue, wet weight) X ± SD	
		CM	DG
0	3	$0.0752 \pm 0.0169^{a,d}$	0.0792 ± 0.0171^{a}
6	3	0.0111 ± 0.0074^{a}	0.4164 ± 0.0747^{b}
12	3	0.3878 ± 0.0601^{c}	0.3246 ± 0.0173^{b}
18	3	$0.1449 \pm 0.0060^{b,d}$	0.3246 ± 0.0364^{b}
24	3	0.0523 ± 0.0201^{a}	0.5751 ± 0.0329^{c}

* Two groups of 20 snails were used in each photoperiod analyzed.
X ± SD = mean ± standard deviation. N = number of biochemical determinations. a, b, c, d = means with significant difference among them (α = 5%)

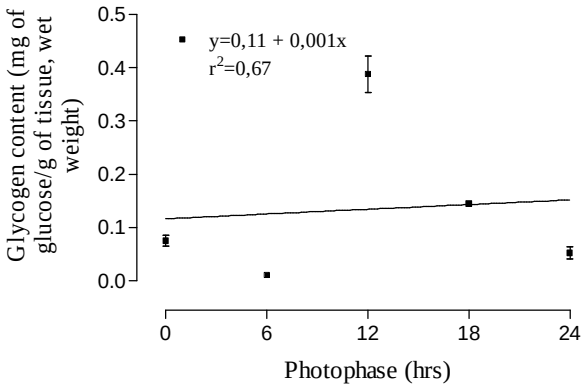

Figure 1. Relation between glycogen content, expressed as mg of glucose/g of tissue, wet weight, in the cephalopedal mass (CM) of *Bradybaena similaris* maintained under different photoperiods for a week. Two groups of 20 snails were used in each photoperiod analyzed.

In *L stagnalis* there are photoregulated enzymes, as UDP-galactose 4-epimerase, that catalyzes the conversion of UDP-glucose to UDP-galactose, being the catalytic activity of this enzyme increased when the day lenght is made greater and the galactogen content raises in response to the increasing of the catalytic activity of this enzyme (VAN ELK & JOOSSE, 1981). This was observed in *B. similaris* that presented higher concentrations of galactogen at photoperiods composed by 18 and 24 hours of light (GARCIA & PINHEIRO, 2005).

Thus, the lowest values of glycogen content in CM tissue presented by the snails maintained under six hours of light, may be related to the fact of these animals were most active, resulting in a waste of energy and reducing of glycogen deposits in this site. In the present study the food consumption was not quantified, but, although the snails presented a higher locomotory activity, we may infer that the food intake was not great enough to compensate the energetic waste of the major locomotion of them. Besides this, the fact of these snails were maintained in the dark 18 hours, take to a high rate of galactogen production, constituting another factor that justify the lower values to the glycogen content once that the galactogen is produced from the glucose that is converted to galactose by the UDP-galactose 4-epimerase.

GOMOT *et al*. (1989) and GOMOT (1990) reported that the photoperiod composed by long days stimulates the reproduction in *H. aspersa* and reflects an intense synthetic activity in the albumen gland. There must be a compensation of the inhibitory effects of the low temperature by the longest photoperiod. MEDINA *et al*. (1988) also observed that the spermatogenesis in this species of mollusc is accelerated at long days, being the differentiation of spermatocyte II to spermatide most sensible to photoperiod alterations.

The highest content of glycogen in DG was observed to the snails exposed to 24 hours of light (Tab.1), that presented, at mean, 0.57mg of glucose/g of tissue, wet weight. When compared to the values observed to the snails exposed to the other photoperiods, this value was significantly higher and different than other. The lowest value was observed to the snails maintained in the dark (0h photophase), 0.08mg of glucose/g of tissue, wet weight (Tab.1), being significantly different than the results obtained with the other groups of snails. The snails exposed to 6, 12 and 18 hours of light presented the following content of glycogen in the DG 0.42, 0.32 e 0.32 mg of glucose/g of tissue, wet weight, respectively, and there was not significant difference among them.

The polynomial regression analysis revealed a strong positive relation (r^2=0.88) between the glycogen content in the DG and the time of exposition to the light (y=0.16 + 0.015x) (Fig. 2).

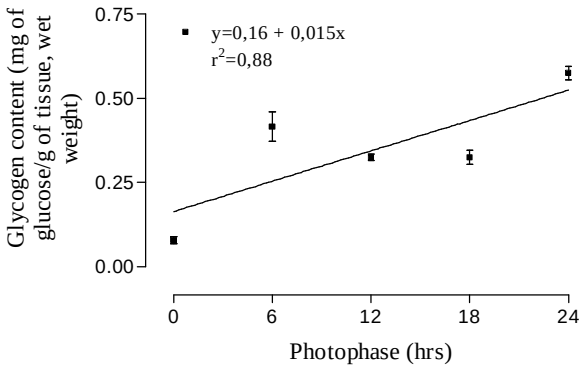

Figure 2. Relation between glycogen content, expressed as mg of glucose/g of tissue, wet weight, in the digestive gland (DG) of *Bradybaena similaris* maintained under different photoperiods for a week. Two groups of 20 snails were used in each photoperiod analyzed.

The snails maintained 24 hours under the light reduced their metabolic activity, moving through by lower distances and staying buried for many hours. These facts take to a reduction on the energetic expenditures of the animal, which is reinforced by the little amount of glycogen found in the CM of the snails maintained under this photoperiod. Also, the glycogen deposits in DG are not extensively used by the animals, being more elevated than the values observed in the other groups. The snails kept under 0 hour of photophase, had higher physical activity, reducing the muscular deposits and from the DG, due the major mobilization rate of this polysaccharide, justifying the results obtained in the present study.

Thus, we can conclude that the photoperiod constituted by 12 hours allow a better relation in the rate of synthesis/mobilization of glycogen in the CM of *B. similaris*, while the photoperiod that resulted in a higher content of glycogen in the DG was that composed by 24h of light. In spite of the great number of studies about the effects of the photoperiod on the behaviour and reproduction of molluscs, there was not clear information about the effects of this abiotic factor on the glycogen reserves in these animals. The knowledgement on the interference of the photoperiod with the glycogen deposits, may be useful in snails control programs and, consequently, of the parasites transmited by them. This is the first study where this relation is investigated using as a

model the snail *B. similaris*, due its medical, veterinary and economic importance, but complementary studies must be made to gave us more information on this subject.

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