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The influence of starvation on the calcium content in the shell and hemolymph of *Achatina fulica* Bowdich, 1822

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Abstract. The influence of different periods of starvation (1, 2, 3 and 4 weeks) on the calcium content in the hemolymph and deposits in the shell of *Achatina fulica* Bowdich, 1822 were analyzed. Throughout the starvation periods studied, the content of this ion in the hemolymph did not vary significantly when compared to control snails, evidencing the existence of a homeostatic mechanism to calcemia. But, after starvation the content of calcium in the shell was reduced ranging 38.50 % lower in comparison to control group which reached 330.90 ppm of $CaCO_3$/g of shell. The results were discussed.

Keywords: *Achatina fulica*, homeostasis, acid-base equilibrium, inorganic metabolism

Introduction

In the past years the studies emphasized the importance of abiotic factors as limiting factors to snails' adaptation to environment and their spreading on it. The physiology of this group of animals has been studied and some authors described changes in their metabolism, occurring when they are exposed to stress conditions.

Pinheiro & Amato (1995), Brandolini & Amato (1996) and Moreira *et al*. (2003) related that changes in inorganic metabolism of snails submitted to stress conditions, as, parasitism and starvation, leading to other alterations to minimize the damages to stressed animals.

Under stress conditions, as starvation (Pinheiro, 1996) and parasitism by larval trematodes (Pinheiro & Amato, 1994; Azevedo *et al.,* 1997), the carbohydrates deposits in digestive gland, cephalopedal mass and albumen gland of *Bradybaena similaris* (Fèrussac, 1821), were reduced beyond to 80%. In spite of this severe depletion of carbohydrates stores, mainly in response to starvation, the snail has a long survival period. Probably, the reduction in the main stores of energetic substrates (carbohydrates) causes the utilization of other substrates as alternative energy source, as proteins.

Stanislawsky & Becker (1980) observed that the total proteins and free aminoacids contents in the hemolymph of *Biomphalaria glabrata* Say, 1817 starved was hughly reduced, suggesting that these substances are used in alternative pathways to glicemia maintenance in this snail. In *Lymnaea truncatula*, the starvation causes an increase of lisossomal enzymes activity, followed by the fast reduction on carbohydrates deposits and cellular damages, by autolysis, in digestive gland cells (Moore & Halton, 1973).

Under starvation conditions the metabolic rates of protein and lipids is increased, leading to the formation of high amounts of nitrogenous products of degradation and free fatty acids, respectively, in the hemolymph of *Achatina fulica* Bowdich, 1822 with alterations on pH of internal medium of the snail (SOUZA *et al.,* 2000).

Calcium is an essential metal in the biology of mollusks and one of the main inorganic compounds of the shell. This ion has been described as an important factor that limits the distribution and the adaptation of adult snails, and on their ovipository activity and eggs and embryo development. (THOMAS *et al*., 1974; NDUKU & HARRISON, 1976; APPLETON, 1978; DAWIES & ERASMUS, 1984). Calcium is also involved in the acid-base equilibrium of snails being stored in the shell as calcium carbonate ($CaCO_3$), participating on the bicarbonate buffer formation (SMINIA *et al.,* 1977; DE WITH & SMINIA, 1980).

The snail *A. fulica* is widely distributed throughout Brazilian territory, considered as a plague to horticulture, partially because of its feeding habits nonspecific. Beyond this, this snail is intermediate host of helminthes of veterinary importance, as *Angiostrongylus cantonensis* Morera & Céspedes, 1971 (Nematoda, Angiostrongylidae) (THIENGO, 1995).

In this study the alterations on calcium contents in the hemolymph and shell of *A. fulica* starved were analyzed.

MATERIAL AND METHODS

SNAILS COLLECTION AND MAINTENANCE

Specimens of *A. fulica* were collected manually in the early morning from residential gardens in Seropédica, RJ, Brazil (22°46'59"S-43°40'45"W, 33m height). The snails were maintained under laboratory conditions for three weeks before the beginning of experimental procedures. They were maintained in plastic boxes (50 x 30 x 15 cm) with a layer of 3cm of earth at the bottom. The snails were fed with fresh lettuce leaves *ad libitum.*

Two groups were formed: Control (C1, C2, C3 and C4), that were fed with described above throughout experimental period, and starved (J1, J2, J3 and J4), and each sub group was constituted by 10 snails per box.

Dissection and hemolymph and shell collection

After 1, 2, 3 and 4 weeks of starvation the snails were dissected, the hemolymph collected through cardiac punction and stored in microtubes at -10°C. After that, the shell was removed, washed in tap water and dried in at room temperature.

CALCIUM DETERMINATION

The total calcium content in the hemolymph of *A. fulica* was determined according to TRINDER (1969) (Doles®) and the results were expressed in mg of calcium/dl of hemolymph. The calcium content in the shell was determined according to SOIDO *et al.* (2009) and the results expressed as ppm of $CaCO_3$/g of ash.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation and submitted to Tukey test for mean comparison (α=5%).

RESULTS

Differences in calcium content in the hemolymph and shell between the control group mean (0) and treated group (1, 2, 3 and 4 weeks) were observed (Tab.1). Similar variation was observed when means of control and starved groups were compared, thus reinforcing the idea of some authors, those related the existent of an homeostatic mechanism to maintenance of basal levels of calcium ion in the hemolymph of snails when they are exposed to stress conditions, as starvation.

Table 1. Calcium content in the hemolymph, expressed as mg/dl, and in the shell, expressed as ppm of $CaCO_3$/g of ash, of *Achatina fulica* starved by 4 weeks. X ± SD= mean ± standard deviation; percentual variation in relation to control group; means followed but different letters differ significantly (α=5%).

Time of starvation (Weeks)	Calcium content in the hemolymph (mg/dl) X ± SD	Percent change (%)	Calcium content in the shell (ppm $CaCO_3$/g of ash) X±SD	Percent change (%)
0 (control group)	0.05941 ± 0.00733[a]	0	330.90 ± 14.23[a]	0
1	0.06033 ± 0.002517[a]	+1.54	203.50 ± 6.241[b]	-38.50
2	0.06333 ± 0.009074[a]	+6.59	291.10 ± 27.134[c]	-12.02
3	0.06394 ± 0.002309[a]	+7.62	298.45 ± 8.690[c]	-9.80
4	0.06400 ± 0.005292[a]	+7.72	247.26 ± 14.581[c]	-25.27

When we analyze the calcium content in the shell, a significant difference between the control and starved groups was observed, with maximum and minimum values observed to groups of third (298.45 ± 8.690) and first (203.50 ± 6.241) weeks, respectively. Throughout period analyzed the starved group presented a reduced calcium content in the shell when compared to control group, with highest variation observed at first and the lowest at third weeks of starvation (38.50% and 9.80%), respectively.

Discussion

According to De With & Sminia (1980) and Thompson & Lee (1986), the hemolymph composition is very precisely regulated, being a homeostatic parameter of the internal medium. Lira *et al*. (2000) related that in *Bradybaena similaris* occurred severe alterations in the calcium content in the hemolymph and theses changes are related to carbohydrates (free, as glucose, and stored, as glycogen and galactogen) contents when starved. Under this condition of physiological stress, commonly occur an exponential increase in the organic acids content, due to the higher proteins and, mainly, lipids catabolism, aiming the maintenance of basal levels of glucose, monosaccharide that represents the most important source of energy to snails (Pinheiro & Amato, 1994). This depletion of carbohydrate reserves, combined with simultaneous elevation of protein degradation, pointed to an interrelationship of pathways that act in the glicemic control in these snails. So, in response to this biochemical rearrangement the elevation of acids levels is evidenced (Bezerra *et al*., 1999) establishing alterations in the pH, one of the homeostatic parameters in the internal medium.

In this study, the absence of significant variations in the calcium content in the hemolymph of starved snails, when compared to feed ones, characterizes the existence of a metabolic pathway between the shell and the hemolymph that acts to maintenance of the calcemia. Starved organisms had an increase of the organic acids in the hemolymph in response to lipids and proteins degradation to glicemia maintenance. To the maintenance of acid-base equilibrium there is $CaCO_3$ mobilization from to shell and utilization of the CO_2 in the bicarbonate buffer formation to establish the homeostasis, consequently Ca^{++} ions is also withdrawal from the shell (SMINIA *et al*., 1977; DE WITH & SMINIA, 1980). In this way, the intense mobilization of calcium, as $CaCO_3$, from the shell and its rapid dissociation to the formation of bicarbonate buffer, may, in part, explain the absence of significant variations in the calcium content in the hemolymph between the control (fed) and starved groups. Furthermore, the Ca^{++} is an important second messenger in muscle contraction. So, the maintenance of calcium levels in the hemolymph allow a most intense muscular activity, and consequently, major displacement of the snails, increasing the likelihood of finding food (THOMAS *et al*., 1974).

According to the results presented, it seems clear that the period of greatest physiological stress occurs in the first week of starvation, justified by the largest percentual decreasing of this ion in the shell. This mechanism enables the snail to minimize the deleterious effects induced by starvation. The absence of significant variation between groups from the second week of starvation lead us to suggest a better plasticity of this organism in response to stress, as even withdraw calcium content were significantly lower than the group of first week of starvation, nevertheless the calcium content in the hemolymph changes close the basal levels.

The reduction of calcium contents in the shell of *A. fulica* starved helps to elucidate the presence of a homeostatic mechanism as described above, showing the great importance of this ion in the survival of this species of mollusks.

REFERENCES

APPLETON, C.C. 1978. Review of literature on abiotic factors influencing the distribution and life cycles of bilharziasis intermediate host snails. **Malacological Review 11:** 1-25.

AZEVEDO, V.P.B.DE; PINHEIRO, J.; GOMES, E.M. & CHAGAS, G.M. 1997. Variação do conteúdo de galactogênio da glândula de albúmen de *Bradybaena similaris* em conseqüência da infecção com *Eurytrema coelomaticum*. *In*: **XII Reunião Anual da FESBE, Caxambu, Brasil.** pp. 274.

BEZZERRA, J.C.B.; KEMPER, A. & BECKER, W. 1999. Profile of organic acid concentrations in the digestive gland and hemolymph of *Biomphalaria glabrata* under estivation. **Memórias do Instituto Oswaldo Cruz 94** (6): 779-784.

BRANDOLINI, S.V.P.B. & AMATO, S. B. 1996. *Eurytrema coelomaticum* (Giard et Billet) (Digenea, Dicrocoeliidae) em *Bradybaena similaris* (Gastropoda, Xanthonychidae): alterações nos depósitos de cálcio e na funçào reprodutiva do primeiro hospedeiro intermediário. **Revista Brasileira de Zoologia 13** (2): 411-418.

DAWIES, T.W. & ERASMUS, D.A. 1984. An ultrastructural study of the effect of parasitism by larval *Schistosoma mansoni* on the calcium reserves of host, *Biomphalaria glabrata.* **Cell and Tissue Research 236:** 643-649.

DE WITH, N.D. & SMINIA, T. 1980. The effects of the nutritional state and the external concentration of the ionic composition of the haemolypmh and on calcium cells in the pulmonate freshwater snail *Lymnaea stagnalis*. **Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen 83**: 213-227.

Lira, C.R.S.; Pinheiro, J.; Gomes, E.M. & Chagas, G.M. 2000. Influência do jejum severo sobre o conteúdo de proteínas totais e de amônio na hemolinfa de *Bradybaena similaris* (Gastropoda). **Revista Brasileira de Zoologia 17**: 907-913.

Moore, M.N. & Halton, D.W. 1973. Histochemical changes in the digestive gland of *Lymnaea truncatula* infected with *Fasciola hepatica*. **Zeitschrift fur Parasitenkunde 43**: 1-16.

Moreira, C.S.D.R.; Gomes, E.M.; Chagas, G.M. & Pinheiro, J. 2003. Calcium changes in *Bradybaena similaris* (Férussac, 1821) (Mollusca, Xanthonychidae) under starvation. **Revista Brasileira de Zoociências 5**: 45-54.

Nduku, W.K. & Harrison, A.D. 1976. Calcium as a limiting factor in the biology of *Biomphalaria pfeifferi* (Krauss) (Gastropoda: Planorbidae). **Hydrobiologia 49:** 143-170.

Pinheiro, J. & Amato, J.F.1994. *Eurytrema coelomaticum* (Digenea: Dicrocoeliidae): The effect of infection on carbohydrate contents of this intermediate snail host, *Bradybaena similaris* (Gastropoda, Xanthonychidae). **Memórias do Instituto Oswaldo Cruz 89**: 407-410.

Pinheiro, J. 1996. Influence of starvation on the glycogen and galactogen contents in the snail *Bradybaena similaris* (Férussac, 1821) (Mollusca, Gastropoda). **Brazilian Archives of Biology and Technology 39**: 349-357.

Pinheiro, J. & Amato, S.B. 1995. Calcium determination in the Shell of *Lymnaea columella* (Mollusca, Gastropoda) infected with *Fasciola hepatica* (Platyhelminthes, Digenea). **Brazilian Archives of Biology and Technology 38**: 761-767

Sminia, T.; De with, N.D.; Bos, J.L.; Van Nieuwmegen, M.E.; Witter, M.P. & Wondergem, J. 1997. Structure and function of the calcium cells of freshwater pulmonate snail *Lymnaea stagnalis*. **Netherlands Journal of Zoology 27**: 195-208.

Souza, R.M.; Gomes, E.M.; Chagas, G.M. & Pinheiro, J. 2000. The influence of starvation and *Eurytrema coelomaticum* infection on the nitrogenous products of degradation in the hemolymph of *Bradybaena similaris*. **Brazilian Archives of Biology and Technology 43:** 365-371.

Stanislawski, E. & Becker, W. 1980. Influences of semi-synthetic diets, starvation and infection with *Schistosoma mansoni* (Trematoda) on the metabolismof *Biomphalaria glabrata* (Gastropoda). **Comparative Biochemistry Physiology 63**: 527-533.

Thiengo, S. 1995. Técnicas malacológicas. *In:* ***Tópicos* em Malacologia Médica** (Barbosa, F.S. org.), pp. 255-260, Rio de Janeiro: Ed. Fiocr.

Thomas, J.D.; Benjamin, M.; Lough, A. & Aram, R.H. 1974. The effects of calcium in the external environment on the growth and natality rates of *Biomphalaria glabrata* (Say). **Journal Animal Ecology 43**: 839-860.

Thompson, S.N. & Lee, R.K.W. 1986. Comparison of starvation and infection by *Schistosoma mansoni* on tissue viability and the 31P NMR spectrum of *Biomphalaria glabrata*. **Zeitschrift für Parasitenkunde 72**: 417-421.

Soído, C.; Vasconcellos, M.C.; Diniz, A.G.J. & Pinheiro, J. 2009. An improvemen of calcium determination technique in the shell of mollusks. **Brazilian Archives of Biology and Technology 52:** 93- 98.

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