Effect of Infection With *Fasciola Gigantica* on Protein, Lipid Content and Some Enzymes of the Intermediate Host Snail *Lymnea natalensis*

F.A. Bakry, F.M. Ragab, S. El-Bardicy and A.T. Sharaf, El-Din

*Environmental Research and Medical Malacology Department,*
*Theodor Bilharz Research Institute, Cairo, Egypt.*

**TOTAL PROTEIN** and lipid contents were estimated in hemolymph of *Lymnea natalensis* snails infected with *Fasciola gigantica*. There was significant reduction (p<0.05) in total protein content while the lipid content in infected snails showed a significant elevation (p<0.01). Also, there was a significant increase in the activity of hexokinase (HK) by 48.9% and glucose phosphate isomerase (GPI) by 40% and a decrease in lactate dehydrogenase (LDH) by 53.7% in infected snails when compared to control snail group.

**Keywords:** Enzymes, *Fasciola gigantica*, Lipid, *Lymnea natalensis*.

Fascioliasis, the disease caused by infection with either *Fasciola hepatica* or *Fasciola gigantica* has a cosmopolitan distribution and is prevalent in sheep-raising countries. It is an increasingly important parasitic disease of man in the Mediterranean countries (El-Shazly *et al.*, 1991). Parasitic helminths have different stages in their life cycle and apart from the definitive host, most parasites need a specific intermediate host to complete their complex life cycle. Between two successive parasitic stages, free-living stages exist that migrate from one host to the next. Variations in external conditions force the parasite to adapt its energy requirement. Parasites obtain their nutrient and many of their structural elements from the host for growth and energy generation (Tielens *et al.*, 1994). By invading a host, the parasite gains an ecological niche and relies on the host as its food resource and living space (Becker, 1980). It is generally believed that energy metabolism of the intramolluscan parasitic stages is
anaerobic (Tielens et al., 1992). The generation of ATP by the parasite is less sufficient and this is reflected in a much higher rate of carbohydrate utilization by the parasite which is very sensitive to changing ATP level (Kurelec, 1975). The aim of the present work is to evaluate the protein and lipid contents and the activities certain enzymes (lactate dehydrogenase, hexokinase and glucose phosphate isomerase) in hemolymph of the snails, *Lymnaea natalensis* infected with *Fasciola gigantica*.

**Material and Methods**

Adult *F. gigantica* worms were obtained from condemned livers of cattle and sheep. The infected liver was cut across and squeezed to recover flukes and eggs from the open ends of the biliary ducts. Adult worms were incubated at 37°C for two hr. Large number of eggs were collected from adult worms. The remaining eggs in the worms are obtained by dissecting worms under microscope. The eggs were put in numbers of Petri-dishes containing dechlorinated tap water (DTW) at 26°C for about 11 days.

A laboratory culture of *L. natalensis* was started with snails from irrigation canals in Giza Governorate in October, 2000. Placing each 10 adult snails in a plastic aquarium containing 1 liter of DTW and it was provided with pieces of thim plastic sheets. The snails were fed on blue-green algae and dried lettuce leaves. Egg masses were isolated by cutting the plastic sheet around each egg mass. Egg masses were kept in Petri-dishes containing DTW for approximately 14 days at 20°C. A few days after hatching the juvenile snails were transferred to another Petri-dish containing blue-green algae. During the first two weeks, about 10 newly hatched snails were kept in each Petri dish and reached infection size (3-5 mm in shell height) 1 to 2 weeks after hatching.

Snails exposed to *F. gigantica* miracidia and maintained in the same type of Petri dishes, but the number of snails not exceed 15. Each snail was exposed to three to five miracidia following standard procedures (Malck, 1980 and Moens, 1991). Metacercariae were collected by placing cercariae-shedding snails individually in a petri dish (3cm) and 5 ml DTW with a sheet of plastic. Shedding of cercariae was allowed for 72 hr and no food was provided Metacercariae were found mainly just beneath the water surface.

Biochemical analysis

Preparation of hemolymph and tissue homogenates

Hemolymph samples were collected by removing a small portion of the shell and inserting a capillary tube into the heart. Hemolymph was pooled from 10 snails collected in a 1.5 ml vial tube and kept in ice-bath. For preparation of tissue extracts of both exposed and unexposed snails, one gram of soft tissues from each snail groups, was homogenized in 5 ml ice cold bidistilled pH 7.5. A glass homogenizer was used and the homogenate was centrifuged for 15 min. at 3000 r.p.m. The supernant was used.

All physiological parameters determined in this study were analyzed spectrophotometrically by using reagent kits purchased from BioMerieux Company, France. Total protein and Lipid contents were determined according to Lowry et al. (1951) and Knight et al. (1972), respectively.

Lactate dehydrogenase (LDH) was assayed according to the method of Cabaud and Wroblewski (1958) in which pyruvate is reduced by incubation with the enzyme in the presence of the coenzyme nicotinamide adenine dinucleotides (reduced NADH).

Hexokinase (HK): was assayed according to the method of Uyeda and Racker (1965) in which glucose-6-phosphate formed by the hexokinase reaction is measured by adding glucose-6-phosphate dehydrogenase and NADP and following NADPH formation. Isomerization of glucose-6-phosphate to fructose-6-phosphate was determined colorimetrically with resorcinol (King, 1974).

Results

The infection rate was 68% and the mean cercarial production was 524.5 ± 45.8 metaceracriae from L. natalensis infected with F. gigantica (Table 1). Duration of cercarial shedding was 19 ± 8.3 days.

There was a significant reduction (p<0.05) in total protein content of L. natalensis snails infected with F. gigantica when compared with their respective control group (Table, 2). Lipid content in snails infected with F. gigantica showed a significant elevation (p<0.01) in comparison with the corresponding control groups.
There was a significant increase in the activity of HK (48.9%) and GPI (40%) and a decrease in LDH (53.7%) in infected snails when compared to control snail group (Table 3).

**TABLE 1. Infection rate and cercarial production from Lymnaea natalensis infected with Fasciola gigantica.**

<table>
<thead>
<tr>
<th>Infection rate (%)</th>
<th>Prepatent period (Day)</th>
<th>Longevity of infected snail (Day)</th>
<th>Duration of cercarial shedding (Day)</th>
<th>Mean number of metacercariae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>68</td>
<td>37.5 ± 1.7</td>
<td>56.5 ± 7.5</td>
<td>19.0 ± 8.3</td>
<td>524.5 ± 45.8</td>
</tr>
</tbody>
</table>

**TABLE 2. Total protein and lipid contents in hemolymph of Lymnaea natalensis infected with Fasciola gigantica**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total protein (mg/ml)</th>
<th>Total lipids (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control snails</td>
<td>25.4 ± 2.8</td>
<td>18.7 ± 3.7</td>
</tr>
<tr>
<td>Infected snails</td>
<td>17.5 ± 3.8**</td>
<td>22.6 ± 4.2*</td>
</tr>
<tr>
<td>% change</td>
<td>-31.1%</td>
<td>+20.8%</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01

**TABLE 3. Levels of lactate dehydrogenase (LDH), hexokinase (HK) and glucose phosphate isomerase (GPI) in the tissues of Lymnaea natalensis infected with Fasciola gigantica.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enzyme activity (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH</td>
</tr>
<tr>
<td>Control snails</td>
<td>48.6 ± 8.6</td>
</tr>
<tr>
<td>Infected snails</td>
<td>22.5 ± 9.8**</td>
</tr>
<tr>
<td>% change</td>
<td>-53.7%</td>
</tr>
</tbody>
</table>

**p<0.01, *** p<0.001

Discussion

Fascioliasis is an important parasitic disease affecting sheep, goats, cattle and buffaloes. It causes a serious economic loss worldwide. Infection with *Fasciola* also has public health significance, causing human fascioliasis.

The present results showed that the protein concentration was significantly reduced in infected snails than that of control snails with percentage reduction 31.1%. This may be due to the presence of parasites which reproduce heavily in a short time, extracting much nutritive substances from their snail host. This finding agrees with many investigators working on different snail-parasite combinations who reported that infection of snails with trematodes caused an obvious changes in the protein metabolism (Lee and Cheng, 1972; Gress & Cheng, 1973 and Sharaf El-Din & El-Sayed, 2001). Ishak et al. (1970) observed an obvious decrease in the metabolic activities of trematode-infected snails, *Lymnaea stagnalis* and *Biomphalaria alexandrina*. Stanislawski and Becker (1979) detected hypo-proteinaemia in infected *Biomphalaria* snails with *Schistosoma mansoni*. Lee and Cheng (1972) found a decrease of total protein in hemolymph of infected *B. glabrata* and attributed this reduction partially to the hydrolysis of snail hemoglobin and uptake of heme by schistosome sporocysts and partially to hemorrhage caused by emergence of cercariae. El-Sheikh and Nagi (1991) stated that the reduction in the protein content in infected *B. glabrata* and *Bulinus truncatus* could be due to the proteolysis of tissue protein external to the parasite which then absorbed as micromolecules by developing parasite. Also, Sharaf El-Din & El-Sayed (2001) reported that infection significantly reduces protein levels in hemolymph of *B. alexandrina* snails infected with either *S. mansoni* or *Echinostoma liei*. This was attributable, in part, to utilization of amino acids by the parasites. The disturbance of the snails’ protein and enzymes could be attributed also to the action of penetrating miracidia which could interfere with the internal control mechanisms of the snails. In this respect, Bayne and Locker (1987) reported that growing and proliferating trematode larvae extract nutrients from, and expel wastes into, the host's haemolymph. Thus, pronounced metabolic and physiological alterations occurred in infected snails.

The total lipid concentrations in the hemolymph of snails infected with *F. gigantica* were elevated when compared to those of the control groups. This was a result of enhanced lipogenesis secondary to the accumulation of acetyl Co-A.
and glycerophosphate from the anaerobic metabolism of glucose. This interpretation is supported by Plisetskaya and Joosse (1985) who reported that an anaerobiosis was evoked during the exposure of the snails to stressing factors. Therefore, the snail must derive its energy from the anaerobic metabolism. In this respect, Mohamed et al. (2000) reported that haemolymph glucose and tissue glycogen of *Melanoides tuberculata* subjected to different kinds of stress (e.g. starvation, treatment with low concentrations of niclosamide and crude oil) were significantly decreased. Also, Cheng and Snyder (1962) found an increase in fatty acids in *Helisoma trivolvis* parasitised by *Glyphelmins pennsylvaniensis*. Abdel-Kader and Tantawy (2000) reported an increase in the lipid content of *B. alexandrina* snails subjected to the plants, *Agave filifera* and *Agave attenuata*. El-Ansary and Qureshy (1994) reported changes in a number of substances (such as carbohydrates and proteins), in the hemolymph of infected snails, which serve as nutrients for the parasite, while lipids are usually not degraded by the parasite.

The results of the present study showed a significant decrease in LDH activity in the whole tissue extract of *Lymnaea* snails in response to infection with *Fasciola*. This may be due to the release of the enzyme from the tissues as a result of cellular damage caused by the rediae of the parasite. In this respect, Nabih et al. (1990) attributed the degradation of glycogen in *B. alexandrina* infected with *S. mansoni* mainly to dependence on the anaerobic glycolysis because the parasite destroys LD₁, a lactate dehydrogenase isoenzyme associated with the aerobic respiration and stimulates LD₅, the isoenzyme responsible for the maintenance of anaerobic respiration. As a consequence, food depletion of glycogen takes place in order to meet the energy requirements via the anaerobic respiration. Several authors have reported significant decline in LDH activity of tissues of various molluscs in response to some molluscicides (Aboul-Zahab and El-Ansari, 1992).

In the present study a marked elevation of the activity of both HK and GPI enzymes in infected snails. This could be attributed to a highly active glycolytic flux in infected snails. This was supported by El-Ansary (1999) who predicted that glycolysis as an emergency pathway for generating ATP is of critical importance post infection and any disturbance of this metabolic pathway could lead to physiological alteration of the molluscan hosts and render them unsuitable for the development of the parasite. Also several investigators

demonstrated elevation of glycolysis and glycolytic enzyme activities in parasitized hosts. Marshall et al. (1974) reported significant increase of glycolytic enzymes activity including the regulatory enzymes hexokinase and phosphofructokinase in the digestive gland of field collected *Lymnaea saxatilis* infected with *M. simillis*. Also, El-Ansary et al. (2000) demonstrated an increase of glycolytic enzymes in *B. alexandrina* snails parasitized with *S. mansoni*.

References


(Received 14/4/2002; accepted 17/9/2002)
تأثير العدوي بطفلة فاشيولا جيجانتيكا على محتوي كل من البروتين والدهون وبعض الانزيمات للعائل الوسيط ليمنيا ناتالينسيس.

فائز أحمد بكرى، فوزي محمد عبد الرحمن رجب، سامية البرديسي وأحمد طارق شرف الدين
قسم بحوث البيئة والرخويات الطبية - معهد توبور بلعهرس
للابحاث - القاهرة - مصر.

أجرى هذا البحث لدراسة تأثير عدد قوانع ليمنيا ناتالينسيس بمصريا فاشيولا جيجانتيكا على محتوى البروتين والدهون
في هيمولويف المواقع والانزيمات (هيكوسوتينيز و جلوكوز
فوسفات أيزوميريز و لاكتيت ديهيدروجينيز في نسجتها. وقد
وجد أن نسبة عدوى المقاطع 1.87٪ ومتوسط أعداد البيتاسيركاريا
0.42 لكل قوقع. وقد أوضحت النتائج انخفاض ملحوظا في
محتوى البروتين وإرتفاع مهذب في محتوى الدهون في
هيمولويف للمقاطع المقدمة بالمقارنة بالمقاطع الضافية (الخُبر
معروضة للعدوى).، وبيعت الدراسة أن العدوى أدى إلى زيادة
ملحوظة في نشاط كل من انزيم هيكوسوتينيز (0.48٪) و جلوكوز
فوسفات أيزوميريز (0.4٪) وانخفاض معنوي في نشاط انزيم
لاكتيت ديهيدروجينيز (0.7٪) عن المجموعة الضافية.