Effect of Larval Trematode Infection on the Nutritional Value of *Nerita Orbignyana* Marine Snails

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ABSTRACT

Snails are rich in high-quality proteins and low fat, but little is known about the nutritional value of edible snails that can meet the world's nutritional needs. Parasitic infection of larval trematodes to these snails affects their nutritional value. Studies in Saudi Arabia on parasitic infections in marine snails are rare. The current study aimed to screen the collected snails for the prevalence of larval trematode infections and biochemical changes in proteins and carbohydrates for one whole year using parasitological methods for 132 marine snails belonging to *Nerita orbignyana*. Snails were collected randomly and monthly from November 2018 to December 2019 from the Red Sea Obhor bay, Jeddah city, Saudi Arabia. The number of proteins and carbohydrates in the Digestive Gland Gonad complex (DGG), hemolymph, and Snail Conditioned Water (SCW) of non-infected and infected snails was estimated using an Elisa DSX best 2000. The snails were found infected with two types of cercariae, Trichobelharzia and Ascorhytis charadriformis cercariae. The infection prevalence was highest in the snail length of 19-20 mm with a percentage of 42%. The study showed that there is a significant decrease in the concentration of proteins and carbohydrates in DGG, hemolymph, and SCW in infected snails. The nutritive value of the snails is affected by infection through the decrease of protein and carbohydrates in infected snails. Further qualitative studies are needed.

Key Words: *Nerita orbignyana, Carbohydrate, Protein, Cercaria, Red sea*

eIJPPR 2021; 11(2):62-68

HOW TO CITE THIS ARTICLE: Turkstani AO, Hassan A, Rabah SO. Effect of Larval Trematode Infection on the Nutritional Value of *Nerita Orbignyana* Marine Snails. Int J Pharm Phytopharmacol Res. 2021;11(2):62-8. https://doi.org/10.51847/D2CHtkWEbS

INTRODUCTION

Molluscan meat has a high nutritional value as it contains essential amino acids, proteins vitamins, and minerals [1]. Snails are rich in high-quality proteins and low fat and little is known about the nutritional value of edible snails which can meet the nutritional needs of humans [2-6]. These nutrients are valuable for growth, the brain, and the nervous system and also, vital for proper body functions. The quality of seafood protein types is much healtier than that of poultry and meat thus play a major role in human nutrition [7-9]. Moreover, snail secretions and mucus contain a great diversity of natural constituents, which have beneficial and therapeutic properties for human skin, such as allantoin and glycolic acid [10]. In the crude fiber contents of the meat powders, the different protein levels will make them suitable as complementary foods. This powder can be applied in food product formulation and development, which will increase the consumption of these cheap and available protein sources [9].

Gastropods are a very important group of molluscs that have interesting host-parasite relationships. Most molluscan hosts are infected with several parasites [11]. Accordingly, several pathological changes are induced inside the body of the gastropod hosts, ranging from extremely too severe minor alterations. Trematode parasites have a complex life cycle, where they develop into adult flukes in the bile ducts of infected mammals, which pass immature eggs in feces. After several weeks, the cycle starts from eggs hatching, producing a parasite form known as the miracidium, sporocyst, redia, cercaria,

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Received: 23 February 2021; **Revised:** 19 April 2021; **Accepted:** 22 April 2021

metacercaria and ending with adults [12-14]. Snails need food sources to complete their life and growing, therefore, the infection affects these sources like proteins, carbohydrates, lipids, and others. Proteins are the building blocks for the body and the most abundant nutrient in the body. They are used to repair and build damaged tissues and to fight infections and are a good source of energy. They provide fiber and other health-promoting nutrients. The protein will carry out its specific functions when the body receives enough quantities of proteins, fats, and carbohydrates. Carbohydrates consist of simple and complex carbohydrates. Many carbohydrates also supply fiber which is a type of complex carbohydrate found in food that comes from many different sources. Carbohydrates provide the body with the energy it needs and are the main source of many vitamins and minerals. Much attention has been apid to the biochemical characteristics of molluscan-parasite interactions ın recent years. Several studies have been made on the protein and carbohydrates of gastropods parasitized by larval trematodes [15].

Studies dealing with this type of marine host-parasite systems remain rare in Saudi Arabia. Therefoe, the current study aimed to investigate more information about marine snails. Identify different types of cercariae and their sporocysts or rediae. Also, to know the impact of these larval trematode infections on the levels of proteins and carbohydrates and their profile structure in the hemolymph, digestive gland gonad complex (DGG) and snail conditioned water (SCW).

MATERIALS AND METHODS

Study area

Gulf of Obhor, also known as Obhor, is located about 30 km north of Jeddah in the eastern Red Sea in the west of Saudi Arabia, with the Global Positioning System (GPS) reading of N 21.745469, E 39.130233. Obhor is located south of King Abdullah Economic City and south of Salman Bay **(Figure 1)**.

Figure 1. Snail collection site at Obhor, Jeddah coast, Red sea (Google map)

Collection of snails

Marine snails were collected randomly and monthly from the Gulf of Obhor, the Red Sea, Jeddah coast, Saudi Arabia, during the period from November 2018 to December 2019. Snails were collected by hand. They were placed in glass jars including fresh seawater and were moved to the Parasitology Laboratory, Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah. The snails were identified by shell morphology using identification keys [16, 17]. Shedding and harvesting of cercariare are carried out according to Aboelhadid *et al.* [18] and stained with methylene blue vital stain according to Hassan *et al*. [19].

Digestive gland-gonad (DGG) collection

Each digestive gland-gonad (DGG) of marine snails has been dissected by removing the shells and removing the DGG from the viscera. Care was taken to remove residual intestinal tract material and then stored at −20 °C until analysis [20].

Hemolymph collection

Hemolymph has been obtained by cracking the shell in a Petri dish, tipping the foot, and collecting the hemolymph through cardiac punction and from the edge with a pipet and stored in microtubes and maintained at –20 °C until their utilizations for the biochemical analysis. The samples have been maintained in an ice bath during the dissections [21, 22].

Snail conditioned water (SCW) collection

SCW was collected in a 10-ml glass beaker by placing the snail (10- mm shell diameter) in 1 ml of distilled water for 2 hrs. at room temperature. The water has been filtered through a column of glass wool to get rid of debris, and the filtrate has been collected in a glass vial. SCW used the same day of collection [9].

Determination of protein, carbohydrates, and glucose of the snails

The recommended methods of the Association of Official Analytical Chemists (AOAC) were used to determine the crude protein, ash, crude fat, and moisture content of the dried snail. For the determination of the crude protein content of a 1.0 g sample of dried milled snail, the Kjeldahl method (%N × 6.25) was used [23]**.** Total carbohydrate and glycogen contents of the snails were done using the method of Brockelman and Sithithavorn [24].

Statistical analysis

To assess data analysis, T-Test analysis was used to compare the infection rate capacity between non-infected snails and infected and compare the biochemical changes between non-infected and infected snails. All statistical analysis was performed using the SPSS software program (version 25). Microsoft Office Excel 2010 was used for drawing graphing [25].

RESULTS AND DISCUSSION

A total of 132 snail species *N. orbignyana* **(Figure 2)**, was found infected by two types of cercariae, *Trichobilharzia regenti* (ocellate furcocercus) and *Ascorhytis charadriformis* (xiphidiocercaria) **(Figures 3 and 4)**. The infection prevalence was highest in the snail length of 19-20 mm with the percentage of 42% and lowest in the length of 15-16 (5%). The length of 17-18mm, 21-22mm, 23-24mm and 25-26mm with an infection percent of 19%, 16%, 11.11%, 6.6% respectively. The highest prevalence of cercarial infection was recorded during winter (November, December, January, and February) with the prevalence of 23.4%, while it was lowest during autumn (September, October, and November) with an infection prevalence of 13.7%.

Figure 2. Photomicrograph showing *Nerita aorbignyana* marine snail

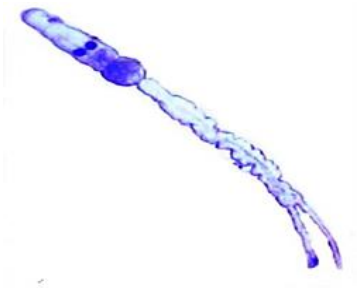

Figure 3. *Trichobelharzia regenti* cercaria collected from *Nerita aorbignyana* snail

Figure 4. *Ascorhytis charadriformis cercaria* collected from*Neritaa orbignyana snail*

The effect of infection with ocellate furcocercus cercaria and xiphidiocercaria on the concentration of protein, glucose, and glycogen in *Nerita orbignyana* snails was studied. The snails in the range of 19-20mm length during the winter season (about 25ºC) were used in the analysis. There is a significant decrease in protein, glucose, and glycogen in the DGG of *Nerita orbignyana* comparing with non-infected snails **(Table 1)**. It was found that infection with ocellate furcocercus and xiphidiocercaria cause a significant decline in the concentration of protein, glucose, and glycogen of hemolymph **(Table 2)**. *Nerita orbignyana* snails infected with ocellate furcocercus and xiphidiocercaria have a significant decrease in protein and glycogen and insignificant decrease in glucose concentration in SCW than non-infected ones as shown in **(Table 3)**.

Table 1. Concentrations of protein, glucose and glycogen in DGG (mg/g) of *Nerita orbignyana* in infected versus uninfected snails. T-test performed at 95 % confidence level ($p \leq 0.05$)

DGG	Uninfected (mg/g)	Infected with ocellate furcocercus cercaria (mg/g)	p-value	Infected with xiphidiocercaria (mg/g)	p-value
Protein	7.249 ± 1.456 ± 0.515	4.983 ± 0.387 ± 0.158	0.001*	5.060 ± 0.568 ± 0.254	0.006*
glucose	78.375±23.458±8.294	44.500 ±5.320 ±2.172	0.008*	45.800 ± 5.450 ±2.437	0.030*
glycogen	75.62 ±14.392 ±5.088	36.833± 4.834 ±1.973	0.001*	37.400 ± 5.413 ±2.421	0.002*

Table 2. Concentrations of protein, glucose, and glycogen in hemolymph (mg/g) of *Nerita orbignyana* in infected versus uninfected snails. T-test performed at 95 % confidence level (p≤0.05).

heamolymph	Uninfected (mg/g)	Infected with ocellate furcocercus cercaria (mg/g)	p-value	Infected with xiphidiocercaria (mg/g)	p-value
protein	7.200 ± 1.841 ± 0.651	4.507 ± 0.694 ± 0.283	0.003*	4.648 ± 1.018 ± 0.455	0.006*
Glucose	71.125 ±15.085± 5.333	47.000 ± 5.933 ± 2.422	0.008*	49.800 ± 5.263 ± 2.354	0.030*
glycogen	67.125 ± 18.527 ± 6.550	37.000 ± 8.832 ± 3.606	0.020*	38.400 ±10.479 ± 4.686	0.045*

Table 3. Concentrations of protein, glucose, and glycogen in SCW (mg/g) of Nerita orbignyana in infected versus uninfected snails. T-test performed at 95 % confidence level (p≤0.05).

SCW	Uninfected (mg/g)	Infected with ocellate furcocercus cercaria (mg/g)	p-value	Infected with xiphidiocercaria (mg/g)	p-value
protein	6.913 ± 1.053 ± 0.372	4.747 ± 0.411 ± 0.168	0.001*	4.680 ± 0.409 ± 0.183	0.002*
glucose	75.250 ± 24.517 ± 8.668	46.667 ± 4.633 ± 1.892	0.081	48.000 ± 4.743 ± 2.121	0.127
Glycogen	69.500± 24.272 ± 8.582	34.333 ± 7.062 ± 2.883	0.008*	36.800 ± 7.662 ± 3.426	0.030*

In the present study, we investigated the influence of infection with two different types of cercariae (ocellate furcocercus and xiphidiocercariae) on the amount of protein, glucose, and glycogen in the DGG, hemolymph, and SCW of marine snail, *Nerita orbignyana.* This study showed that there is a reduction of protein and carbohydrate levels in infected snails. The study was done in winter where the highest infection prevalence of *N. orbignyana* was observed. The most prevalent infected snail's length was 19-20mm which is used in the study. Earlier studies focused on the snail's length and age and their role in the infection rates and their contents of fats and proteins [26]. The influence of parasitic infection on some marine snails such as Chariya and Brockelman was studied [27] and they investigated the effect of parasitism and stress on heamolymph protein of the african giant snail, *Achatina fulca***.** Sauerlander [28] used the experimental snails infected with trematode larvae and discussed the physiological and pathological changes which play a major role in reproduction, growth, and shell formation. Their results lead to the understanding that when snails are living in a habitat that resembles the natural one, the snails can adapt to the infection through natural selection by which the snail can adjust its food needs and can benefit from the maximum limit of the host supply. The feeding of intramolluscan stages such as sporocysts and rediae which feed on the snail's hepatopancreas cells by ingestion or absorption, cause a reduction in the snail's blood protein and glucose [29]. The result of this competition depends on the intensity of parasitic infection and the ability of the host and its size. A large host such as the terrestrial gastropod, *Achatina fulica* snail has a large amount of hemolymph and a big hepatopancreas in which free and bound amino acids are localized [30]. The reduction of food such as starvation did not cause a decline in total protein since snails can regulate hemolymph protein and adapt to this condition. However, repeated bleeding due to parasitic infection caused a decrease in protein amount. There was a heavy demand on the energy reserves to resynthesize the missing hemolymph volume as well as to carry out shell renewal after this loss.

Little data on carbohydrates and protein concentration in the snail DGG and hemolymph infected by trematode parasites [31] showed that *Lanistes carinatus* snail infection by rediae of gymnocephalus cercariae, sporocysts, and free xiphidiocercaria led to a non-significant increase in total carbohydrates in the snail's hemolymph. Also, the amount of reducing sugar in DGG did not increase significantly whenever the infection was caused by both types of trematode larvae [32]. found both sporocysts and cercariae released excretory-secretory products which hydrolyzed the stored glycogen reserves in the DGG cells of *B. glabrata* to glucose and maltose. Therefore, the glucose level was significantly increased in infected snails in comparison to those uninfected. However, other authors reported that the concentration of haemolymph glucose of *Biomphalaria glabrata* decreased by the infection. [33] showed that *Shistosoma mansoni* absorbed glucose directly from the hemolymph of *Biomphalaia glabrata*. Wagner *et al.* [34] In some cases, there is no reduction in the amount of glucose due to infection [35]. Thompson and Lee reported that although *B. glabrata* snails were infected by *S. mansoni*, they maintained the glucose levels constant in the hemolymph by using the stored carbohydrates [35]. The study showed that infection by sporocysts of xiphidiocercariae caused a significant increase in

hemolymph total proteins. The non-significant increase was recorded whenever the infection was caused by rediae of gymnocephalus cercaria. This alteration in hemolymph protein concentration of infected snails caused by two different types of cercaria may be related to physiological and metabolic damages induced by infection, redirection of the gene expression, immunological response to parasite invasion, and parasite excretory-secretory products [36]. Many authors have focused on the biochemical alterations as a result of infection. Zelck *et al.* [37] found that resistant or susceptible snails for schistosome contained similar quantities of the hemolymph protein. However, Adema *et al.* [38] showed that the freshwater gastropod *B. glabrata* produced hemolymph proteins that bind to and precipitate certain secretory excretory products derived from the cultured intramolluscan sporocyst stages of the parasite, *Echinostoma paraensei*. Similar reactions are absent in the hemolymph of the unexposed snails. This suggested that *B. glabrata* snails respond uniquely to digenean infection by increasing the synthesis of the humoral defense proteins. Regarding the effect of infection on the number of total proteins in *L. carinatus* DGG, was significantly increased whenever the infection was caused by two types of trematode larvae. This may be due to that infection causes stimulation of the activities of sixteen enzymes in the Embden-Meyerhof pathway [39]. In this respect, it was suggested by El-Ansary *et al.* [40]. that the remarkable increase of protein fractions in the tissues of *S. mansoni* parasitized *B. alexandrina* snails could be because of additional proteins in response to this physiological condition and the snail produce.

CONCLUSION

We can conclude that, in the present study, the biochemical changes that resulted in trematode larval infection lead to a decrease in the snail's DGG, hemolymph, and SCW protein and carbohydrates and that the change in the biochemical components of infected snails depends on various factors, such as diet, parasite species, host size, and the environmental conditions to which they are submitted. The nutritive value of the snails is affected by infection through the decrease in the amount of protein and carbohydrates in the infected snails. More qualitative studies are needed.

Acknowledgments: I would like to thank King Abdul Aziz University, the great scientific edifice, the Faculty of Sciences, biological Sciences Department for providing the opportunity to study and to allocate laboratories and private spaces to us.

Conflict of interest: None

Financial support: None

Ethics statement: Informed consent was obtained from all individuals included in this study. The conducted research is not related to human or animal use.

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