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# DETERMINATION OF TOTAL CONCENTRATION OF PROTEINS AND CARBOHYDRATES IN TAPEWORM POSTGANGESIA ARMATA AND INTESTINES OF INFECTED AND NON-INFECTED FISH HOST SILURUS GLANIS

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## ABSTRACT

The study investigated the concentration of some biochemical parameters in the tissues of the *Postgangesia armata* and intestine of their final host (Fish) *Silurus glanis*. The study showed significant increases($P \leq 0.05$) in protein, carbohydrate concentrations in intestine of non-infected fish compared with infected one, while The concentration of proteins and carbohydrates in tapeworm tissues were (9.26 ± 0.05) and (5.96 ± 0.01) mg / g of wet weight respectively and these were less than those in infected host (15.13 ± 0.08, 11.75 ± 0.05) mg / g of wet weight. Electrophoresis technique(SDS-PAGE) showed two different proteins, the first is approximately 37,000 Dalton, and the second is 52,000 Dalton.

**KEYWORDS:** Protein, Carbohydrate, Electrophoresis, Cestoda, Silurus Glanis.

## INTRODUCTION

Molecules with high molecular weights are the basic components of living cells and called macromolecules. They are proteins, carbohydrates, fats and nucleic acids. Biochemical molecules have different chemical structures and functions. They are energy-rich sources, synthetic components and carriers of genetic traits (Rao and Suryalakshmi, 2011). Proteins are the most abundant organic molecules in cells and make up about 50% of dry body weight. Proteins enter into a number of basic functions in all tissues; they have more structural and

supportive roles, an energy source, and participate in synthesis of a number of vital compounds such as enzymes, hormones, antigens and antibodies. Some proteins contain sugars, fats, or metal group such as iron in the hemoglobin (Harvey and Ferrier, 2011).

Total protein contents in parasite tissues range between 20-80% of dry weight. The proteins of tissue contain two main groups, soluble and insoluble. Soluble proteins include enzymes, hormones and antigens, while insoluble proteins are associated with cellular membrane and membranous structures within the cell and they have structural and supportive functions such as collagen, keratin-like proteins, and sclerotin (Chappell, 1980). Carbohydrates are the main source of energy in the body and represent about 90% of the total energy that body needs. Carbohydrates are very important. They carry out synthetic and physiological functions and are part of important compounds of the cells such as mucoproteins, glucuronic acid, and heparin (Deb, 2011).

Carbohydrates in protozoa and parasitic worms form a major source of energy. Interoparasits degrade carbohydrate an aerobically to obtain energy regardless of the amount of oxygen available in its environment. Consequently, a large number of parasites store polysaccharides for production of adenosine triphosphate ATP (Daoud and Abdel-Karim, 1989).

Intestinal parasites settle in the intestines of the host and feed on its food, and metabolic processes in the parasites depend on the type and quantity of food in the intestines of the host, as parasites use this food for growth and development, and this participates in available bimolecular that are found in parasite bodies, Hence, a number of studies have been conducted to investigate the molecular content and its place in the tissues and organs of the parasites as well as to determine their concentrations Based on this, the knowledge needs more detections about biological content of parasites, and these results increase the information of total knowledge in this regard. Hence, this study aimed to know the amount of food (biomolecules) that is consumed by the tapeworm From the intestine of its host as well as a comparative study of proteins, carbohydrates between infected and non-infected intestine of fish.

## MATERIALS AND METHODS

Collection of samples 72 samples of European fish *Silurus glanis* were collected from Tigris river in Rashidiya area in the north of Mosul in Iraq. The fresh fish were brought to the laboratory to search for internal parasites, especially the tape worm. A longitudinal incision

was made from the exit area to the anchored area and the small intestine was placed in a petri dish containing the saline solution of fish 0.65%, then intestine was opened and its contents were examined for the tapeworm. The tapeworm samples and intestine of their infected and non infected final host (fish) were cleaned using a small brush and washed with saline and distilled water several times, then they were placed individually in small bottles containing saline solution and frozen at (-20)°C. Preparation of the extract. The samples were lifted from the freezer and each sample was placed on the filter paper to remove excess water. The method of Pappas (1988) was adopted to prepare the extracts of worm, infected and non-infected intestine with a concentration of 100 mg /3ml of buffer solution (2ml of 0.05 M Triss-HCL buffer, pH7.8 and 1 ml of 1mM ethylene Diamine Tetracetic Acid). The worm and samples of infected and non-infected intestines were crushed alone using the Homogenizer, which is placed in a beaker containing ice to prevent high temperature and tissue damage, then complete the crushing using ultrasonic 4000 Pulse / sec for 30 seconds. This process was repeated four times with a 15-second interval to maintain a low temperature of the extract. The extract was separated by centrifuge 10,000 rpm for 20 minutes at 4 °C. Then sediment was neglected and used the supernatant to study the biological parameters.

**Measurement of Biochemical Parameters**

**1. Proteins:** Proteins were measured according to the method (Lowry *et al*., 1951).

**2. Carbohydrates:** Carbohydrates were measured according to the method (Herbert *et al*., 1971).

Total protein separation with SDS Protein separation by sodium dodcylsulphate polyacrylamide gel electrophoresis technique (SDS-PAGE): Protein was separated by Laemli method (1970).

**Statistical Analysis**

T-test was applied to find the difference in mean values between the infected and non-infected fish populations at the probability level (0.05) after ANOVA test of proteins, carbohydrates concentration (Alrawi, 2000).

**RESULTS AND DISCUSSION**

**Biochemical Parameters:** Biochemical studies were carried out for the tapeworm *Postgangesia armata* and its final host, European fish *Silurus glanis*. The results were as follow.

**1- Determination of total protein concentration**

Table (1) shows the total concentration of biomolecules (proteins, carbohydrates) in the intestines of non-infected and infected fish with *Postgangesia armata*, as well as in *Postgangesia armata* itself as shown in Table (2). The highest concentration of total protein was found in intestines of non-infected fish (25.35 ± 0.36) mg / g wet weight and the lowest concentration was found in the tapeworm (9.26 ± 0.05) mg / g wet weight, while the concentration in the intestines of infected fish was (15.13 ± 0.08) mg / g of wet weight. Statistical analysis showed a significant difference ($P \leq 0.05$) in protein concentration between the intestines of Infected and non-infected fish. These results agree with the findings of (Bhure *et al*, 2011; Nanware *et. al*., 2012; Sonune, 2012) in terms of the presence of protein concentration in the non infected host compared to the infected host, and also agree with (Jawale *et al*., 2011; Bhure *et at*.,2011; Nanware *et al*., 2012; Sonune, 2012; Hassan *et al*., 2016) in terms of the presence of the protein at a lower concentration in worm tissue compared to its infected host. The high concentration of protein in the non infected host (fish) may be due to the European fish are carnivorous fish and live close to the bottom of the river. Other fish such as *Cyprinidae* is a major food for it, followed by crustacea, insects, frogs, birds, annelida and nematoda (Czarnecki *et al*., 2003). Nutrition in these fish varies depending on their age and size, where their young's, less than one year, feed on phytoplankton, while ages (4-2 year) feed on aquatic animal species, particularly fish (Copp *et al*., 2009). Bora and Cül (2004) showed the food of European fish consists of Diptera insects 60.5%, Odonata insects22.8%, and Gammarus0.34%. Orlova and Popova (1987) pointed out that fish are the best source of animal protein because they contain the essential amino acids and quantities of free amino acids. They also pointed out that the food of European catfish consists of vertebrates such as fish and frogs in large ages.

Dörücü (2000) explained that the main source of energy in fish is proteins and fats. Unlike mammalians, the source of energy is carbohydrates and fats. This may be due to food fish contains high protein. Fish metabolism is adaptive to this type of food and fish have the ability to remove nitrogenous waste rapidly and continuously, as well as the activity of Lysosomal enzymes is faster in fish compared to mammalians.

The reason of low protein concentration in the tapeworm compared to its infected host is dependence of the tapeworm on the final host in food because it does not has a digestive system, where it lives in the intestine and absorb nutrients from intestinal cavity of the host

but diffusion or active transport across body cutaneous (Sonune, 2012). The reason of low protein concentration is that tapeworms need pre-formed amino acids to protein synthesis. Worms secrete Protease, which decompose host proteins outside worms, producing amino acids that are absorbed by worms for protein synthesis. This process is necessary for growth (Hassan *et al.*, 2016).

The total protein concentration of tapeworm in the current study is different from the results of the above studies. The variation in the concentration of the protein by worm type may be due to variation in the stages of maturation of the worms and the formation of eggs (Al-Egaidy, 2011; Al-Kallak,2001) or the difference in the metabolic activity of the organism related to DNA which determines the types of proteins present and therefore their importance in each species as a reaction to the host's tissues to form anti-parasitic substances. This in turn stimulates the parasite to produce immune proteins for the parasite, or the organism's reaction to internal stimuli such as nutrition, enzyme synthesis, and amino acid absorption (Al-Naftachi, 2006).

Also the concentration of proteins between different worms varies according to the type of host and the nature of the food, where protein ratio in the parasite depends on the host's protein-rich substances (Al-Kallak, 2001). Since fish are rich in protein, they account for about 15-24 % of body weight, so parasitic worms benefit from this characteristic and show a high level of protein compared with other types of worms that infect other vertebrates. also there are other factors affecting the amount of protein in fish such as sex, age, species, seasonal and genetic variations (Mohamed *et al.*, 1967) as well as the aquatic environment in which fish live, where the aquatic environment and its aquatic organisms alter the amount and type of foods in host intestines including protein, this reflects on the amount and quality of nutrients absorbed by tapeworms from the intestines of the host (Pallewad *et al.*, 2015).

**2- Determination of total carbohydrate concentration**

The total carbohydrate concentration in tapeworm tissue was (5.96 ± 0.0l) mg / g of wet weight, and in the intestines of infected and non-infected fish was (11.75 ± 0.05, 16.10 ± 0.06) mg / g of wet weight, respectively. A significant difference in the concentration of carbohydrates between the intestines of infected and non-infected fish was observed at the probability level ($P \leq 0.05$), which is consistent with the results of (Bhure *et al*., 2011; Lanka *et al*., 2011; Jadhav *et al*., 2008; Hassan *et al*., 2016; Nanware *et al*., 2014; Pallewad *et al*.,

2015; Sonune, 2012; Jawale *et al*., 2011) in terms of low carbohydrate content in worm tissues compared to their infected and non-infected host.

The total carbohydrate concentration in the tapeworm under study differs from its concentration in the results of (Nanware *et al.*, 2014; Hassan *et al.*, 2016). This variation may be due to the type of tapeworm or host and the nature of its food. Decreasing the amount of carbohydrates In the host's food, it will be reflected in the amount of carbohydrates absorbed by the worms that are necessary for the growth of eggs leading to the abnormal growth of the eggs and thus causing a developmental disability (Cheng, 1986). The variation in total carbohydrate concentration may depend on the host type. Al-Naftachi and Al-Khan (2011) pointed out In their study, that the concentration of carbohydrates in tapeworms varies according to their vertebrate hosts due to the different physiological nature of the digestive system in the vertebrates that cause a difference in the supply of the raw materials of the worm, which in turn affects the nature of the worm's metabolism.

Nanware *et al*. (2014) attributed the difference in glycogen concentration *Moniezia expansa*, parasite in *Caprahircus*, to its concentration in the other tapeworms to the size of the worm and its location inside the host. Nanware and Bhure (2011) also showed a difference in the glycogen amount in segment of tapeworms that are isolated from *Capra hircus*. It was observed that immature segments contained small amounts of glycogen compared to mature and gravid segments. The reason is that mature segments contain many cells absorbs the food from the host. The difference in the amount of glycogen in segments reflects the variation in the rates of metabolism in them, which are related to the anatomical differences and permeability characteristics of segments. Glucose sugar is an important source of energy for many parasitic worms that live inside the intestine of vertebrates. Glucose is absorbed through the sodium channel that found in tegument tape worm. The difference of tissue nature of the tegument in the different worms affects the amount of sugar absorption, which causes a difference in its quantity between them (Starling, 1975).

**Table. 1: Total concentration of biomolecules (proteins, carbohydrates) In the intestine of infected and non-infected fish.**

| Biomolecules | Biochemistry Concentrates (Standard Deviation ± Rectifier) (mg / g wet weigh) | |
|---|---|---|
| | Intestine of infected fish | Intestine of non infected fish |
| Proteins | 15.13±0.08* | 25.35±0.36 |
| Carbohydrates | 11.75 ±0.05* | 16.10 ±0.06 |

*There is a significant difference between the two groups of fish between at (P≤0.05) according to T-test.

**Table 2: Total concentration of biomolecules (proteins, carbohydrates) In tapeworm tissues.**

| Biomolecules | Concentrating biomolecules (standard deviation ± average) (mg / g wet weigh |
|---|---|
| Proteins | 9.26±0.05 |
| Carbohydrates | 5.96±0.01 |

**Separation of protein content by electrophoresis:** SDS-PAGE was used to separate the protein content of the tapeworm extract using standard proteins with molecular weight ranging from (23000-67000) Dalton.

The electrophoresis of tapeworm tissues showed two bands of protein different in size. The first band has an approximate molecular weight of 37,000 Dalton and it is close to the standard protein (pepsin) with a molecular weight of 36000 Dalton. The second band has an approximate molecular weight of 52,000 Dalton, which lies between the molecular weights 43000 and 67000, i.e. between the two standard proteins egg albumin and bovine serum albumin respectively (Figure 1).

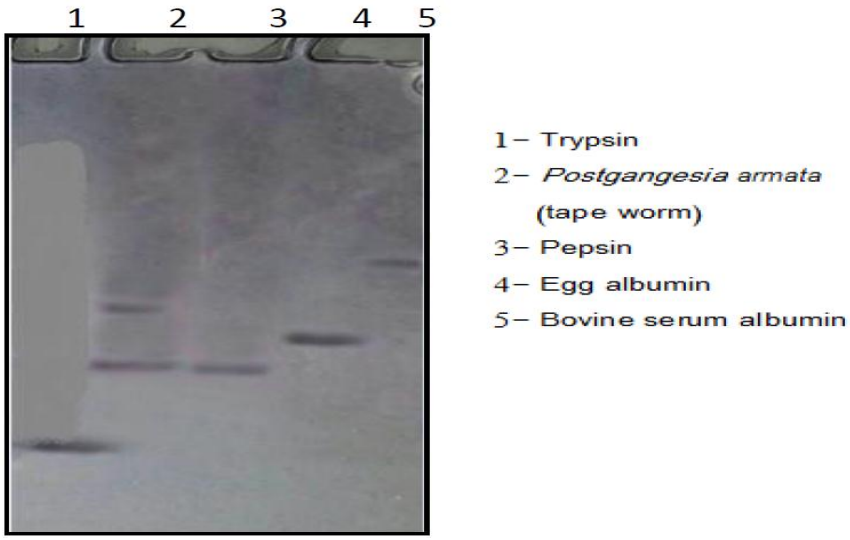


**Figure 1: SPS-PAG protein bundles.**

Studies of the separation of the protein content of tapeworms indicate the difference in protein bands in number and molecular weight. Oaks and Knwoles (1979) showed that the total protein of the *Hymenolepis diminuta* is characterized by several proteins different in

molecular weight, whereas Bursey *et al*., (1980) stated each one of the three species of *Taenia* has protein bands different from each other.

Alkallak (2001) reported the tapeworm *Khawia sp.* has three proteins, while the tapeworm *Proteocephalus sp.* has two protein bands. Al-Naftachi(2006) indicated that extract of parasitic tapeworms in vertebrates has many proteins different in number and size. Al-Mohammed and Youssef (2010) observed sixteen protein bands in the tapeworms *Nematotaenia dispar* parasitic in mammalians.

Al-Mola (2010) recorded a variation in the number and thickness of protein bands of larval stages of *Contracaecum sp.*. The differences were attributed to two reasons. First, there is a significant change in protein quality on cuticle surface after each molt and a change in the amount of proteins after each developmental stage down to the adult worm, and the second may have been attributed to a change in the parasite proteins as a reaction to the host's immune system.

To distinguish worm species and genus, it can be depend on number and size of protein bands in them, electrophoresis one of the techniques utilizes to classify parasitic worms (Sood, 2006), or depend on the differences in amino acids of protein. Smyth and McManus (1989) pointed to the difference in the amount of amino acids in different worms and to the difference in active transport systems of these acids, perhaps the difference is due to the interaction between host food and amount of protein found in the worm (Al-Kallak, 2001) or the difference is due to worm specie, host type and surrounding effects about it (Al-Naftachi, 2006).

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