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Article

The Impact of Bothriocphalus Achelognathi on the Biochemical Characteristics of Blood and Meat in Infected Fish

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Abstract: The fish samples were collected in the study from the Great Zab River in Eski Kalk area during the period 1/5/ 2023 to 3/8 2023. The number of fish was 108 fish of three different species (Cyprinus carpio, Silurus triostegus, Liza abu). 66 of them were infected with Bothriocphalus achelognathi and 42 of them were not infected. The current study was conducted to know the effect of infection with the parasite Bothriocphalus achelognathi on the chemical characteristics of fish meat such as (protein, fat, moisture) and the biochemical characteristics of blood such as (total protein, cholesterol, glucose). And blood characteristics such as (RBC, WBC, PCV) and compare them with healthy fish. The results showed a significant decrease in protein values in the muscles of infected fish, and healthy liza abu fish recorded the highest increase in muscle protein. A significant decrease (P≥0.05) was also observed in the percentage of fat in the muscles of infected fish, while there were no significant differences in the moisture percentage. As for blood biochemical tests (total protein, cholesterol, glucose). The results showed a significant decrease in the values of total protein in the blood of infected fish except for silurus triostegus fish, where there were no significant differences (P≥0.05). As for cholesterol and glucose, a significant decrease was recorded in all infected fish. The results of blood tests in the fish of the current study showed differences in blood parameters between healthy fish and those infected with the parasite Bothriocphalus achelognathi and it was found that there was a significant increase in the number of white blood cells in all infected fish compared to healthy fish, with a significant decrease in the number of red blood cells in the fish, as well as the ratio of the measurement of the Packed cell volume.

Keywords: Bothriocphalus achelognathi, Fish parasites, Biochemical characteristics, Blood parameters, Fish meat composition

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1. Introduction

Freshwater fish account for about 40% of the total number of fish species and 20% of vertebrate species (Lynch et al., 2016). Fish are important in human life from both nutritional and non-nutritional aspects. Iraq is one of the countries rich in various water bodies, with an estimated area of 1074 thousand hectares, represented by rivers, lakes, streams, ponds, marshes, and other wetlands (Muheisen, 1987). Freshwater fish are widely distributed and easily accessible due to the diversity and widespread presence of their habitats in different natural and artificial environments. This gives them significant importance as a primary food resource and economic value, providing around 61 million jobs worldwide in the freshwater fishing sector and related industries (Song et al., 2018).Fish are recommended as a fundamental part of a healthy diet, as their meat is an important component for essential nutrition and for preventing heart diseases. Their benefits extend to providing a healthy and natural source of energy and high-quality nutrients such as proteins, vitamins (D, A, E, B12) and essential minerals (selenium,

manganese, copper). They are also a source of long-chain fatty acids (Tørris et al., 2018; Gil & Gil, 2015). In recent decades, fish resources have faced significant and multiple problems affecting biodiversity and fish numbers in various environments worldwide, especially freshwater environments (Reid et al., 2013). All living organisms are susceptible to diseases and harmful parasites. Despite fish having high resistance to diseases under good environmental and living conditions, they are still at risk of diseases like other animals (Muheisen, 1983). If these infections are not controlled, they can lead to mass fish deaths or affect their growth. In some cases, they can be a source of infection for humans and other vertebrates that consume fish (Tesfaye et al., 2018). Parasites cause an estimated 80% of diseases affecting freshwater fish in Egypt (El-Tantawy & El-Sherbiny, 2010; Pawluk et al., 2018). This is evident through infections in fish by all major groups of animal parasites such as Protozoa, Myxozoa, Helminthic worms, and Crustacea (Sudhagar et al., 2018). These parasitic infections vary from mild, which involve competition for food or chemical damage from metabolic byproducts and toxins, to severe mechanical damage to the tissue and function of internal organs (Noor El-Din Deen et al., 2015).This study aimed to investigate the impact of the tapeworm Bothriocphalus acheilognathi on the biochemical properties of blood and meat in infected fish and compare these properties with those in healthy fish.

2. Materials and Methods

Fish samples were collected from the local markets in the city of Kirkuk and from the Greater Zab River near the Eski Kalak area, 40 km away from Erbil, during the period from 1/5/ 2023 to 3/8 2023 A total of 108 fish from three different species were collected, of which 66 were infected with B. acheilognathi and 42 were uninfected. The following table (1) shows the number of fish used in the experiment:

Tabel 1. Shows the Number of Fish Used in the Experiment

Fish	N	Infected	Uninfected
Cyprinus carpio	35	20	15
silurus triostegus	35	18	17
liza abu	38	28	10

The fish were transported with a quantity of river water in a large plastic tank to the laboratory. The live fish were then transferred to 50-liter circular plastic containers filled with pre-stored tap water. These fish were left in the containers for 2-3 hours in a relatively stable and dark place for acclimatization according to the laboratory conditions described by(Pasternack and Rahkonen 1998), as bright light affects some blood measurements. After the acclimatization period, the live fish were gently handled with a cloth to avoid stress and were struck on the head to prepare them for blood tests and parasite identification. The internal organs of the fish were examined in a Petri dish containing a saline solution for B. acheilognathi. The digestive tract was cut into sections and each section was opened longitudinally using scissors or a needle and tweezers to search for internal parasites. The body cavity was examined with the naked eye and then with a Kruss light microscope (German-made) with magnification ranging from 16 to 40 times to search for parasites.

Measurement of Blood Parameters

The fish were placed on their side in a dissection dish and the scales were removed using a scraper from the caudal peduncle area. The area was then cleaned of water and mucus with blotting paper. A vertical incision was made in the area between the anal opening and the caudal fin. Pressing on the end of this area caused blood drops to flow from the caudal artery, and the first drops of blood were always discarded. Blood samples

were then taken to perform the following measurements: counting red blood cells, white blood cells, hematocrit

Biochemical Analyses

Blood samples were collected directly using regular glass test tubes. The blood was then centrifuged at 3000 rpm for 15 minutes to separate the serum for testing. The following tests were conducted on the serum: measuring total protein concentration, glucose concentration, and cholesterol concentration in the blood.

Chemical Analyses

Chemical analyses were performed to determine the chemical composition of fish muscles based on the methods outlined in (AOAC 1980(.

Moisture Content in Meat

The moisture content was estimated by placing 5 grams of pure meat in a pre-weighed porcelain crucible. The crucible with the sample was placed in an electric oven at 105°C for 24 hours. The crucibles were then removed, weighed, and the percentage of moisture was calculated by subtracting the dry matter percentage from one hundred (AOAC, 1980)

Estimation of Protein Content in Meat

The protein content was estimated using the Semi-micro Kjeldahl method (AOAC, 1980) for determining protein in fish meat samples.

Estimation of Fat Content

A meat sample weighing 1-5 grams was taken and placed in the organic solvent ether, then in the Soxhlet fat extraction device at a temperature of 70°C for 4 hours. After extraction, the fat was weighed and its percentage was calculated.

Ether Extract (%) = (Weight of beaker after extraction - Weight of empty beaker) / Weight of sample

Statistical Analysis

Data were analyzed using the Completely Randomized Design (CRD) to study the effect of treatments on the studied traits. The significance of differences between treatments was compared using Duncan's Multiple Range Test (Duncan, 1955) with the help of the Statistical Analysis System (SAS, 2005) based on the following model:

$Yij = \mu + Ti + eij$

Where:

- Yij : Observation value for the treatment
- μ : General mean for the studied trait
- T i: Effect of the treatment
- eij : Random error

3. Results and Discussion

Parasites and pathogens are among the most significant problems facing fish in the aquaculture sector, whether in natural waters or fish farms. They cause economic losses directly by killing most of the infected fish and indirectly by sharply reducing the growth rates of surviving infected fish (Daham, 1990 and Al-Khafaji, 1988). The relationship between fish and the parasites and diseases they contract extends to include changes in the chemical composition of the fish (Radhakrishnan et al., 1983). The tapeworm B.acheilognathi is considered one of the most dangerous types of worms and poses a significant threat to freshwater fish populations, especially carp (Heckmann et al., 1987)

Table (2) shows the chemical compositions of the muscles of infected and uninfected fish B.acheilognathi

Type of fish	Infection status	% Protein	% fat	%Moisture
Cyprinus carpio	uninfected	A14.1±1.04	A5.30±0.81	71. 1±2.15
	infected	B12.7±0.34	B3.22±0.39	70.5±1.18
silurus triostegus	uninfected	A16.3±0.53	A3.1±0.32	70.2 ±2.30
	infected	B14.8±0.30	B2.1±0.47	70.9 ±1.91
liza abu	uninfected	A17.8±1.09	A4.12±0.73	72.0 ±0.20
	infected	B15.7±1.63	B3.30±0.42	72.21±0.52

*Different letters within a column indicate a significant difference between the coefficients at a probability level of (P≥0.05)

Proteins are among the most essential elements for protection and the construction of living organisms. In fish, proteins are used to protect and build the fish's body, repair damaged tissues from wounds, regenerate epithelial tissues, and synthesize necessary proteins and hormones (Wilson, 1986). Proteins are found in the serum of fish at normal levels under usual conditions, depending on various factors such as diet, fish species, season, sexual maturity, and water temperature (Patriche et al., 2009). Additionally, the levels of globulin and albumin proteins in the blood indirectly reflect the immune and physiological status of fish and other living organisms (Ahmed and Ali, 2013).

In the current study, we observe that the protein content in healthy and infected carp is 14.1% and 12.7%, respectively, with healthy fish having a higher protein content than infected fish. Similarly, for catfish, healthy fish have higher protein content than infected fish at a significance level of (P≥0.05), with averages of 16.3% and 14.8%, respectively. For the rough fish, the protein content for healthy and infected fish is 17.8% and 15.7%, respectively. The primary reason for the reduced protein content in the muscles of most infected fish is that B.acheilognathi feeds on the host fish's intestinal components, preventing the host from benefiting from nutrients to build different body parts. These results align with the findings of (Marcogliese 2008 and Hassan et al. (2015).) Additionally, the parasite B.acheilognathi may indirectly affect muscle tissues by attaching to the intestinal lining, causing inflammation and necrosis, reducing nutrient absorption in the intestines, and consequently affecting muscle growth and development (Perez et al., 2017).

As for fat content, healthy fish outperformed infected fish with the B.acheilognathi parasite across all three types of fish studied.(Rober Britton et al. 2011: and Bingwen Xi and Gutang Wang 2011) explained that the decrease in fat content in infected fish is due to the parasite affecting the fish's metabolism by consuming nutrients, reducing their absorption and metabolism, and diverting the energy that the fish would use for growth and fat accumulation to counter the stress and damage caused by the parasite, thereby reducing body fat content.

Regarding moisture content in the flesh, there were no significant differences at a significance level of (P≥0.05), with slight numerical differences favoring healthy fish. The reason may be that parasitism leads to increased water content and reduced protein and fat levels, as these nutrients are consumed for the parasite's growth and reproduction, lowering the nutritional value of fish muscles (Abbas et al., 2018). Additionally, the increased moisture in the muscles of most infected fish in the current study may be due to a higher rate of infection, as parasites increase inflammation in the fish, as noted by(Natarjan and Balakrishnan 1976).

Table (3) shows the biochemical composition of the blood of fish infected with B.acheilognathi and uninfected fish.

Type of fish	Infection status	Protein(mg/100ml of blood)	cholesterol(mg/100ml of blood)	Glucose(mg/100ml of blood)
Cyprinus carpio	**uninfected**	A 3.06±0.35	A252.56±58.70	A218.12±31.90
	infected	B 2.3. ±0.03	B244.71±71.50	B317.56±14.48
silurus triostegus	**uninfected**	3.54±0.36	A 305.33±63.63	A219.22±44.32
	infected	3.17±0.55	B275.55±31.21	B150.27±29.16
u liza abu	**uninfected**	A3.89±0.43	A316.51±103.90	A288.51±17.25
	infected	B2.31±0.23	B208.66±75.94	B164.88±26.62

*Different letters within a column indicate a significant difference between the coefficients at a probability level of (P≥0.05)

Blood chemistry and physiology are among the most important parameters in evaluating the general physiological state of the body and are also used as indicators of stress (Docan et al., 2012). In clinical chemistry, total blood serum protein is a marker of the health and nutritional status of fish (Yang and Chen, 2003).

The results show differences in the biochemical properties of the blood of the fish species studied, particularly between infected and non-infected fish. For instance, healthy carp had higher protein levels than infected carp (3.06 and 2.3 mg/100 ml of blood, respectively). The same trend was observed in healthy rough fish (3.89 mg/100 ml) compared to infected rough fish (2.31 mg/100 ml). The reduction in total blood protein in infected fish or the slight variation in its value compared to healthy fish can be attributed to several reasons. One reason is the utilization of protein as an energy source to combat the stress caused by parasitism since proteins and fats are the primary energy sources in fish (Chellappa, 1988). Additionally, the decrease in albumin levels occurs to activate the immune system at the cellular level (Almeida et al., 2011), as fish produce antibodies in response to the presence of parasites (Osmani et al., 2009).

It is well known that total blood proteins are indicators of liver health and function since the liver is responsible for producing important proteins such as albumin present in the blood (Watts et al., 2001). Globulin and albumin are blood proteins, and measuring albumin levels is one of the tests indicating liver efficiency in protein synthesis (Al-Hawari and Azeddin, 1986).

Stress and infection are significant factors that affect total blood protein levels because they reduce the fish's appetite, leading to a decrease in total blood protein (Magnadottir et al., 2010). The reduction in protein levels may also be due to the fish's reduced ability to synthesize or absorb essential proteins due to illness or protein loss during bleeding caused by internal parasites piercing body organs and the digestive tract (Patriche et al., 2011).

From the results mentioned above, it is evident that infection with the parasite B.acheilognathi in three types of fish showed a decrease in cholesterol concentration. This could be because the parasite affects metabolism, thus reducing cholesterol levels (Tanveer et al., 2016). Additionally, Table 3 shows that glucose levels were affected by the parasite, as infected fish had lower blood glucose levels than healthy fish. This reduction is because the parasite B.acheilognathi consumes glucose as a primary energy source, depriving the host fish of nutrients. Moreover, the tapeworm can damage the intestinal lining, reducing the efficiency of nutrient absorption and affecting glucose levels (Wang and xie, 2011; Summer et al., 2012; Perez et al., 2017).

Table (4). Shows The Blood Parameters In Fish Infected With B.Acheilognathi And Uninfected

Type of fish	Infection status	$10^3\times$ WBC cell/mm^3 of blood	$10^6\times$ RBC cell/mm^3 of blood	PCV%
Cyprinus carpio	**Uninfected**	B37.50±3.49	A2.01±2.19	A35.00±1.03
	infected	A45.11±7.15	B3.02±6.18	B31.01±0.41
silurus triostegus	**uninfected**	B22.6±8.11	A3.01±1.40	A32.80±2.10
	infected	A30.3±4.10	B2.90±3.20	B31.0±1.30
liza abu	**uninfected**	B27.90±3.60	A2.01±6.50	A32.01±0.89
	infected	A43.75±2.77	B1.82±4.7	B28.7±2.69

*Different letters within a column indicate a significant difference between the coefficients at a probability level of (P≥0.05)

Hematological values can be indicators of fish health and can be used to monitor their condition when they are sick (Tavare-Dias et al., 2007; Ponsen et al., 2009). This increases the likelihood of diagnosing diseases in fish (Haniffa and Abdul Kader Myden, 2011). In Table 4, it is noted that infected fish (Cyprinus carpio, silurus triostegus , and liza abu) with B. acheilognathi have a higher WBC count compared to healthy fish. An increased white blood cell count is often an indicator of the presence of parasites and bacteria, associated with the body's defensive mechanism against parasitic infections and various diseases. Conversely, RBC and PCV values significantly increase (P≥0.05) in healthy fish and decrease in infected fish. The reduction in red blood cell count in some infected fish may be attributed to the mechanical damage caused by the parasite in the intestines, leading to reduced nutrient absorption (Summer et al., 2012; Ernesto et al., 2011).

4. Conclusion

The study demonstrated that infection with the parasite Bothriocphalus achelognathi significantly affects the biochemical characteristics of both blood and meat in infected fish species, including Cyprinus carpio, Silurus triostegus, and Liza abu. The results showed a decrease in protein and fat content in the muscles of infected fish compared to healthy ones, while moisture content remained relatively unchanged. Additionally, the blood of infected fish exhibited lower levels of total protein, cholesterol, and glucose, alongside significant alterations in hematological parameters such as increased white blood cell counts and decreased red blood cell counts and packed cell volume (PCV). These findings highlight the detrimental impact of parasitic infections on the nutritional quality of fish, which can have significant implications for fish health and the broader aquaculture industry.

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