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Biochemical study of some metabolic indicators of fish *Mastacembelus mastacembelus* (Banks & Solander, 1794) and its parasite tapeworm *Senga mastacembeli*

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Abstract

This study is the first global attempt to evaluate the influence of the parasitic tapeworm *Senga mastacembeli* on its fish host, *Mastacembelus mastacembelus*, using tissue biomolecules to bridge the informational void regarding this fish. Forty-six alive and mature fish were gathered from a fish market in Mosul, Iraq. We removed and extracted tapeworms from the fish's intestine. Also, an extract of the liver and intestinal tissues of both infected and uninfected fish was made for estimation of proteins, carbohydrates, aspartate transaminase, alanine transaminase, alkaline phosphatase, and lactate dehydrogenase activities using colorimetric techniques. The findings at $p \leq 0.05$ showed that the intestine of infected fish had concentrations of total proteins of 178.6 and carbohydrates of 122.1 mg/g wet weight that were significantly lower than those of uninfected fish, which had concentrations of 232.2 and 183.3 mg/g wet weight, respectively. On the other hand, the protein and carbohydrate concentrations of the tapeworm tissues were 106.9 and 94.5 mg/g wet weight, respectively, which were significantly lower than those of infected and uninfected fish. Regarding liver enzyme activities, the infected fish's alanine transaminase and alkaline phosphatase, measuring 83.3 and 63.0 IU/L, respectively, were significantly lower than the uninfected fish's 109.1 and 125.0 IU/L, while the infected fish's lactate dehydrogenase activity was higher at 223.6 IU/L compared to the uninfected fish's 198.4 IU/L. However, the aspartate transaminase activity of infected and uninfected fish did not differ significantly. Furthermore, the most sensitive ratio to parasite infection was the aspartate transaminase/alkaline phosphatase, which was followed by the aspartate transaminase/alanine transaminase and lactate dehydrogenase/aspartate transaminase ratios. The study concludes that the studied biomolecules can be used as reliable biomarkers of the severity of fish infection with parasitic tapeworms.

Keywords: Aquatic animals, Biomolecules, Parasitic biochemistry, Parasitic diseases, Tissue enzymes

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Introduction

The Mesopotamian spiny eel, *Mastacembelus mastacembelus*, is a member of the Mastacembelidae family, characterized by its elongated body. It inhabits freshwater environments in the Tigris and Euphrates rivers and surrounding regions in the Middle East. As a carnivore, this species plays a crucial ecological role in controlling the populations of smaller aquatic organisms, such as fish and crustaceans. Additionally, its sediment-burrowing behavior contributes to improving water quality and habitat diversity for other species, providing insights into ecological dynamics and helping maintain ecosystem balance (Alit et al., 2023). Economically, the spiny eel is a valuable source of protein; reports indicate that spiny eel meat contains a high calorie content, reaching up to 303 calories per 100 grams (Jahan et al., 2020). However, like other aquatic animals, Spiny eel is susceptible to parasitic infections. One such parasite is the tapeworm *Senga mastacembeli*, which commonly infects *M. mastacembelus*. This parasite has been reported in Iraq with a high prevalence of up to 73.77% (Bilal et al., 2017). As an endoparasite, *S. mastacembeli* settles in the alimentary canal of its host and obtains its nutrients through its highly specialized tegument and potentially alters the host's nutritional status (Banerjee et al., 2017).

Parasites are responsible for approximately 70% of fish diseases, and about half of them are due to the tapeworms, significantly impacting host biochemistry and physiology by slowing growth, reproduction, flesh quality, and survival; therefore, the severity of tapeworm infections is often assessed by analyzing biomolecules related to the infection (Oliveira et al., 2024). Numerous investigations have confirmed that tapeworm infections alter protein and carbohydrate quantities in fish tissues. For example, fish *Clarias batrachus* is parasitized by tapeworm *Lytocestus* sp. (Jawale, 2023), *Barbus grypus* is parasitized by *Khawia armeniaca* (Al-Niaeemi & Dawood, 2021), and *Siluris glanis* is parasitized by *Postgangesia armata* (Al-Niaeemi et al., 2019). Protein contents of the tapeworms *Lytocestus* sp. (Jawale, 2023) and *P. armata* (Al-Niaeemi et al., 2019) were 44.0 and 9.26 mg/g of wet weight, respectively.

Parasitic infections can also induce changes in enzyme activities in hosts. Estimations of enzyme activity in tapeworms and fish are reported by various authors (Al-Niaeemi et al., 2020; Frolova et al., 2023). Enzymes play crucial roles in metabolic pathways, including proteins and carbohydrates, providing basic molecules and energy demands, and enzyme activity is a good indicator of tissue injury (Shukla, 2024). Alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) are commonly used as indicators of liver and tissue health (Anuprasanna et al., 2024). Transaminases and LDH represent metabolic links between carbohydrate and protein biochemical pathways through gluconeogenesis (Gupta & Sharma, 2023). ALT interchanges alanine - pyruvate in the Cahill cycle and regenerates glucose in an aerobic condition. LDH transforms lactate into pyruvate through the Cori cycle and regenerates glucose in an anaerobic condition. For aerobic glycolysis, AST is more important and facilitates the transfer of NADH from the cytoplasm to the mitochondria through the malate-aspartate shuttle (Holeček, 2024). The ratios of these enzymes provide valuable insights into the extent of tissue damage and metabolic stress caused by parasitic infections, including the AST/ALT ratio (De Ritis ratio) in fish *Totoaba macdonaldi* (Trejo-Escamilla et al., 2021).

Given the environmental and economic importance of the present spiny eel fish and the impact of *S. mastacembeli* infections, there is a need for comprehensive investigations on the biochemical effects of such infections. To our knowledge, global research has primarily focused on blood biomolecules, leaving a knowledge gap regarding the role of tissue enzymes as metabolic biomarkers for assessing the pathophysiological consequences of *S. mastacembeli* infections. This study could serve as a first report that aims to address this gap by exploring the role of tissue enzymes as biomarkers, which could have implications for nutrition, health, and economics.

Materials and methods

Specimen collection

Forty-six alive and mature *M. mastacembelus* fish were gathered in an isothermal box containing crushed ice from Mosul's fish market in Iraq and transferred to the Biology Department Laboratory. The period of collecting was May to June 2024. The fish's length ranged from 52 to 63 cm, with an average of 56.4 cm, and their weight ranged from 315 to 485 g, with an average of 391.3 g. The fish were taxonomized according to Dwivedi & De (2024). Twenty-one (45.7%) of the fish were uninfected with any parasite, and twenty-five (54.3%) were infected, resulting in a 1:1.19 uninfected/infected ratio. The tapeworm *S. mastacemblei* was identified according to Bilal et al. (2017). Nine of the total fish were found to be only infected with *S. mastacemblei*, whereas sixteen were found to be infected with other parasites,

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sometimes including *S. mastacemblei*, and these fish were ignored from the study. Fish immersed in crushed ice were dissected immediately at Mosul University's Biology Department Science College research lab. After the tools and dissection area had been cleaned, blunt-tipped scissors were used to make a tiny incision in front of the anus and extending along the fish's ventral midline to the middle of the lower jaw, then a cut from the tiny incision in front of the anus upward, across the fish's body, and towards the head was done to remove the flap of skin covering the abdominal cavity. The intestine and liver were then carefully cut out, and no unusual signs or indications were observed in their morphology during the dissection, being careful to clean and rinse the tools between each step. The fish's small intestine was the source of the adult tapeworm *S. mastacemblei*. After being collected, the tapeworms were placed in a Petri dish, cleaned five times using a pH 7.4 phosphate-buffered saline (PBS; Invitrogen, Waltham, MA , USA), and inspected using a dissecting microscope (Hamilton, Ontario, Canada). Each tapeworm had a predetermined wet weight. Then frozen at a temperature of –20°C in a deep freezer (Haier, Pune, India). The tapeworms were dehydrated, clarified, and mounted with dibutyl phthalate polystyrene xylene (Sinopic, Beijing, China) in order to prepare them for taxonomication. Additionally, the small intestine and liver of ten uninfected and nine infected *M. mastacembelus* fish were chopped, scrubbed, and rinsed with PBS pH 7.8. Each sample's fresh weight was then estimated, and it was frozen at –20°C for the ensuing biochemical analyses.

Extract preparation of worms and fish intestine and liver

Ten grams of wet weight of worms were suspended in 100 ml of 0.05 M Tris-HCl buffer pH 7.8 (Medicago AB, Uppsala, Sweden), and this was done for intestine or liver tissues of each uninfected and infected fish. The suspensions were then homogenized in a grinder (Harvest Hi-Tech, Coimbatore, India).

To disrupt cell membranes, an ultrasonic disintegrator (Crest Ultrasonics, Ewing Township, NJ, USA) was used at 12 kHz for 30 s in an ice bath. The suspensions were subjected to four sonication cycles. The cooled ultracentrifuge (Beckman Coulter, Brea, CA, USA) was used to do ultracentrifugation at 15,000 g for 30 min. Biochemical investigations were conducted using the supernatant fraction (Al-Naftachi, 2006).

Total protein determination

The concentration of total proteins in the extracts from the worms and fish's gut and liver was determined by the colorimetric method according to Lowry et al. (1951). Wavelength 750 nm was used to measure absorbance using a spectrophotometer (Shimadzu, Kyoto, Japan). Based on the standard curve of protein, the protein content was estimated.

Total carbohydrate determination

The concentration of total carbohydrates in the extracts from the worms and fish guts was determined according to the Gottschalk technique (Gottschalk, 1985). Wavelength 488 nm was used to measure absorbance. Based on the carbohydrate standard curve, the content of carbohydrates was estimated.

Enzyme activity determinations

AST, ALT, LDH, and ALP were estimated in tapeworm tissue *Senga mastacemblei* and liver extract of the uninfected and infected fish *M. mastacembelus* by using specific analysis kits (Biolabo, Maizy, France) as follows: Activity of the ALT assay was estimated utilizing the Reitman and Frankel method (Reitman & Frankel, 1957). The ALT enzyme converts alanine to pyruvate, then pyruvate reacts with the indicator 2, 4-dinitrophenyl hydrazine (2, 4 DNPH) to generate a reddish-brown complex of 2, 4 DNPH , which is absorbed with a spectrophotometer at 505 nm in alkaline solution. ALT activity was estimated using the standard curve as a guide. Activity of the AST assay was estimated utilizing the Reitman and Frankel method (Reitman & Frankel, 1957). The AST enzyme converts aspartate to oxaloacetate, then oxalate reacts with the indicator 2, 4 DNPH to generate a reddish-brown complex of 2, 4 DNPH, which is absorbed with a spectrophotometer at 505 nm in alkaline solution. AST activity was estimated using the standard curve as a guide. Activity of the LDH assay was estimated utilizing the Morgenstern et al. approach (Morgenstern et al., 1965). LDH converts pyruvate into lactate when the coenzyme NADH is present. The determination of LDH activity depends on the decrease in absorbance of NADH due to the conversion of it to NAD^+. Absorption was measured at 340 nm, and LDH activity was estimated using the standard curve as a guide.

Activity of the ALP assay was estimated according to the Kind and King method (Kind & King, 1954). ALP converts phenylphosphate to phenol and phosphate. Determination of ALP activity depends on the reaction of free phenol with 4-amino-antipyrine to form a red complex in alkaline solution. Absorption was measured at 510 nm, and ALP activity was estimated using the following equation.

ALP activity (IU/L) = [(sample absorbance – specimen

blank absorbance) / standard absorbance] × 141.8.

Statistical analysis

The SPSS Statistics program version 23.0 was used to analyze the data. The mean values of tapeworm tissue, uninfected and infected fish liver, and intestine were compared statistically using ANOVA and Duncan's test. A mean ± SD is presented for every data point. At $p \leq 0.05$, all differences were considered significant.

Results

The total protein and carbohydrate levels in the intestinal tissue of infected fish, *M. mastacembelus*, were significantly lower than that of uninfected ones ($p \leq 0.05$). Also, the concentration of proteins in the infected fish's liver tissue was significantly lower than that of uninfected ones ($p \leq 0.05$). In addition, we analyzed the major biochemical constituents of the tapeworm *S. mastacembeli*; a significant decrease was revealed in the protein and carbohydrate levels in the tissues of the tapeworm compared with those in the intestine and liver tissues of the uninfected and infected fish. In addition, all studied specimens contain more proteins than carbohydrates. As well as the carbohydrate-to-protein ratio of the tapeworm tissues was higher than those in the uninfected and infected fish (Table 1).

The carbohydrate levels reduced more than proteins in infected fish (Table 2).

Regarding the enzyme activities in the liver of fish *M. mastacembelus* infested with the tapeworm *S. mastacembeli*. The activities of ALT and ALP of infected fish were significantly lower ($p \leq 0.05$) than those of uninfected ones. Conversely, the liver of infected fish had significantly greater ($p \leq 0.05$) LDH activity than that of uninfected fish. No significant difference was observed in the AST activity in both infected and uninfected fish. Regarding the activities of the four enzymes ALT, AST, ALP, and LDH in the tapeworm tissues, they were significantly lower ($p \leq 0.05$) than those found in the infected and uninfected fish. Moreover, the enzyme ratios AST/ALT, AST/ALP, and LDH/AST were also studied, and they all demonstrated that infected fish had significantly ($p \leq 0.05$) higher ratios (Table 3).

The most affected enzymes were ALP, followed by ALT, then LDH, and finally the AST enzyme is almost unaffected. Regarding the enzymatic ratios, the AST/ALP ratio in infected fish was exceeding the value of the uninfected ones by 1.031; thus, it was the most sensitive ratio to infection with the parasite, followed by the AST/ALT ratio (De Ritis ratio) by 0.339, then the ratio LDH/AST by 0.250 (Table 4).

Table 1. Total biomolecule concentrations in the tissues of fish *Mastacembelus mastacembelus* and tapeworm tissues *Senga mastacembeli*

Biomolecules	Tissues of tapeworm		Uninfected fish		Infected fish	
(mg/g wet weight)	Mean ± SD[1)]	CV (%)	Mean ± SD[1)]	CV (%)	Mean ± SD[1)]	CV (%)
Worm and fish intestinal proteins	106.9 ± 0.92[a]	0.86	232.2 ± 0.91[c]	0.39	178.6 ± 0.91[b]	0.51
Worm and fish intestinal carbohydrates	94.5 ± 1.13[a]	1.20	183.3 ± 3.37[c]	1.84	122.1 ± 2.81[b]	2.30
Worm and fish intestinal carbohydrates / proteins ratio	0.888 ± 0.01[c]	1.35	0.789 ± 0.04[b]	4.69	0. 682 ± 0.03[a]	4.55
Fish liver proteins	-	-	185.2 ± 0.23[c]	0.12	153.1 ± 0.47[b]	0.31

[1)]Each value represents the mean of three replicates ± SD.
[a–c]Letters that differ horizontally signify a significant difference at the probability level ($p \leq 0.05$), according to Duncan's test.
CV, coefficient of variation.

Table 2. The percentage of changing values of biochemical parameters in the infected fish compared to uninfected fish

Biochemical parameters (mg/g wet weight)	Uninfected fish		Infected fish		Changing (%)
	Mean	Parameter (%)	Mean	Parameter (%)	
Intestinal proteins	232.2	100	178.6	76.9	–23.1[1)]
Intestinal carbohydrates	183.3	100	122.1	66.6	–33.4[1)]
Liver proteins	185.2	100	153.1	82.7	–17.3[1)]

Changing (%) refers to changing values of infected fish in contrast to uninfected fish.
[1)]Refers to decrease.

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Table 3. Activity of enzymes and their ratios in the liver of fish *Mastacembelus mastacembelus* and tapeworm tissues *Senga mastacembeli*

Parameters (IU/L)	Tissues of tapeworm		Uninfected fish		Infected fish	
	Mean ± SD[1)]	CV (%)	Mean ± SD[1)]	CV (%)	Mean ± SD[1)]	CV (%)
Alanine transaminase	34.5 ± 0.08[a]	0.23	109.1 ± 2.5[c]	2.29	83.3 ± 1.87[b]	2.24
Aspartate transaminase	43.4 ± 1.02[a]	2.35	142.7 ± 1.8[b]	1.26	137.0 ± 2.41[b]	1.76
Lactate dehydrogenase	95.1 ± 1.9[a]	2.00	198.4 ± 1.01[b]	0.51	223.6 ± 2.01[c]	0.90
Alkaline phosphatase	27.3 ± 2.1[a]	7.69	125.0 ± 1.2[c]	0.96	63.0 ± 0.25[b]	0.40
AST/ALT	1.229 ± 0.016[a]	1.30	1.312 ± 0.023[a]	1.75	1.651 ± 0.033[b]	2.00
AST/ALP	1.593 ± 0.078[b]	4.90	1.144 ± 0.013[a]	1.14	2.175 ± 0.023[c]	1.06
LDH/AST	2.209 ± 0.048[c]	2.17	1.385 ± 0.012[a]	0.87	1.635 ± 0.022[b]	1.35

[1)]Each value represents the mean of three replicates ± SD.
[a–c] Letters that differ horizontally signify a significant difference at the probability level ($p \leq 0.05$), according to Duncan's test.
CV, coefficient of variation; ALP , alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; IU, international unit ; LDH, lactate dehydrogenase.

Table 4. The percentage of changing values of liver enzymes and their ratios in the infected fish *Mastacembelus mastacembelus* compared to uninfected ones

Biochemical parameters (IU/L)	Uninfected fish		Infected fish		Changing (%)
	Mean	Parameter (%)	Mean	Parameter (%)	
Liver alanine transaminase (ALT)	109.1	100	83.3	76.4	–23.6[1)]
Liver aspartate transaminase (AST)	142.7	100	137.0	96.0	–4.0[1)]
Liver lactate dehydrogenase (LDH)	198.4	100	223.6	112.7	+12.7[2)]
Liver alkaline phosphatase (ALP)	125.0	100	63.0	50.4	–49.6[1)]
AST/ALT	1.312	100	1.651	125.8	+25.8[2)]
AST/ALP	1.144	100	2.175	190.1	+90.1[2)]
LDH/AST	1.385	100	1.635	118.1	+18.1[2)]

% changing refers to changing values of infected fish in contrast to uninfected fish.
[1)]Refers to decrease.
[2)]Refers to increase.
ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; IU, international unit; LDH, lactate dehydrogenase.

Discussion

This study is the first worldwide report through which we have tried to offer details on evaluating the effect of the parasitic tapeworm *S. mastacembeli* on its fish host *M. mastacembelus* using tissue biomolecular components such as total proteins and carbohydrates, as well as some enzymes like ALT, AST, LDH, and ALP as biomarkers of the fish's overall metabolism.

Our study's findings regarding the total protein and carbohydrate levels in the tissue of the tapeworm *S. mastacembeli* and its host fish *M. mastacembelus* align with several previous reports. Al-Niaeemi et al. (2019) documented lower concentrations of carbohydrates and proteins in the infected fish *Siluris glanis* compared to those uninfected with cestode *P. armata*, also referred to as the lowest concentrations found in the cestode; Nabi et al. (2021) examined the effects of various helminth parasites (*Pomphorhynchus kashmirensis*, *Diplozoon kashmirensis*, and *Adenoscolex oreini*) on transaminases, lipids, and protein in different organs of fish *Schizothorax plagiostomus*.

The depletion of carbohydrates and proteins in the infected host can occur due to the tapeworm's consumption of essential nutrients. Also, this depletion can result from damage to the intestinal mucosa and villi, which are crucial for nutrient absorption (Abdel-Hakeem et al., 2024).

On the other hand, our results contradict those of Hudha et al. (2021), who reported a significant increase in globulin content in parasitized carps and *Schizothorax* fishes. This increase can be attributed to immunological reactions that lead to the release of more globulins as a defensive strategy or adaptation of the host to their parasites (Kumar, 2014).

Regarding the fact that the tissues of tapeworms have lower levels of protein and carbohydrates than the tissues of their

hosts. Our findings are consistent with Jawale (2023), who observed lower levels of protein and glycogen in the intestines of infected and uninfected fish *C. batrachus* compared to the cestode *Lytocestus* sp. Similar results were mentioned by Bhure et al. (2015), showing lower fat, protein, and carbohydrate contents in six Senga species compared to their host fish, *Mastacembelus armatus*. In contrast, Pawar (2020) found that protein and glycogen levels in the parasite tissue *Lytocestus vyasaei* were higher than those of infected and uninfected intestinal tissue of host fish *C. batrachus*, suggesting that tapeworms may compete with the host's nutritional needs or affect the host's macromolecule metabolism (Jeon & Eom, 2024).

The tapeworm *S. mastacembeli* had a greater impact on intestinal carbohydrate than protein levels in the fish's intestine and liver. This result is corroborated by Al-Niaeemi et al. (2019), who found higher protein concentrations than carbohydrates in the intestines of both infected and uninfected *Siluris glanis* fish and tapeworm *Postgangesia armata*. Wahab (2019) noted that the Mastacembelidae family, being carnivorous, may have higher protein concentrations in the tapeworm *S. mastacembeli* due to their preference for predation over herb-based food. However, some studies are quite contrary to our results; for example, Pawar (2020) reported higher glycogen content than protein in the parasite *L. vyasaei* of *C. batrachus*.

In general, it is crucial to consider the type of sample used for enzyme estimation, whether in blood or tissue. An increase in blood enzyme activities may indicate leakage from damaged tissues, leading to decreased tissue enzyme activities. Our findings align with Hudha et al. (2021), who showed elevated serum AST and ALP levels in *Schizothorax* and Carps fishes infected with helminth parasites *Adenoscolex*, *Neoechinorhyncus*, and *Pomphorhyncus* compared to uninfected ones. Similar results were found in serum *Schizothorax plagiostomus* infected either with helminthic parasites *A. oreini* or *Pomphorhynchus kashmirensis* or both of them (Nabi et al., 2021).

Previous studies have shown consistent results in serum enzyme estimation compared to our tissue enzyme findings. Liver necrosis can lead to enzyme leakage into the bloodstream, causing a decrease in tissue levels and increased blood levels (El-Seify et al., 2011). Regarding tissue enzymes, our results differ from those of Vinatha et al. (2013), who demonstrated increased ALT and AST in the muscle of parasitized fish *Channa punctatus* with the *Callodistomum diaphanum* and *Genarchopsis goppo.* These alterations led to the hosts' biochemical adaptations to counteract the parasitic effect. The present infected fish showed higher LDH activity than normal ones, similar to results in the gut and liver of parasitized fish *B. grypus* with tapeworm *K. armeniaca* (Al-Niaeemi & Dawood, 2021). LDH is a crucial enzyme for connecting both glycolysis and the Krebs cycle with alanine and lactate serving as key precursors for gluconeogenesis. However, our infected fish showed increasing LDH activity, and shifting its equilibrium to lactate resulted in less pyruvate, impaired gluconeogenesis, low carbohydrate concentrations, and low ALT activity. In addition, in hypoxic environments (intestinal lumen), tapeworms prioritize anaerobic glycolysis, leading to increased LDH activity and decreased carbohydrate and ALT levels (Bao et al., 2019).

In our study, the most affected enzymes were ALP, followed by ALT, then LDH, and the AST/ALP ratio was the most sensitive ratio to parasite infection. These findings align with those in *Clarius catfish* (Rudneva et al., 2021). The AST/ALT ratio in infected *M. mastacembelus* fish was higher than in uninfected fish, which helps assess organ damage severity (Bao et al., 2019). According to the current report and other research, the biomolecule contents of tapeworms and their hosts vary based on factors such as the type of worm, host, and food consumed, as well as environmental pollution (Kantal et al., 2024; Mustafa et al., 2024).

This study clearly demonstrates that the tapeworm *S. mastacembeli* significantly impacts the biochemical composition of fish *M. mastacembelus*. Further research is necessary to gain a deeper comprehension of the effects of parasites on fish biomolecules. It is recommended to conduct histological investigations on fish organs to confirm damage from parasitic infections.

Conclusion

From this study, it is concluded that parasitic tapeworm reveals a considerable influence on the biomolecule contents of the intestine and liver tissues of the fish host. Notably, the estimations of total carbohydrates and activities of ALT, ALP, and AST/ALP ratio emerge as powerful and crucial biomarkers for evaluating the severity of parasitic tapeworm infections in fish and the adaptive responses within the host-parasite system. Consequently, controlling parasitic infections is an urgent necessity at the health, nutrition, and economic levels.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) of Mosul University, Iraq (UM.VET. -IACUC-2024-001).

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