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Biochemical Changes in Muscles and Liver in Relation to Helminth Infection of Koshar Fish, *Epinephelus summana* in Jeddah, Saudi Arabia

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Abstract: Koshar fish, *Epinephelus summana* are characterized by their economic importance and widespread in the Red Sea. The effect of infection by some helminthes (trematodes, cestodes and nematodes) infecting fish liver, intestine and stomach on some organic constituents such as total protein and carbohydrates in muscles and fish liver was examined. A total number of 102 *E. summana* were collected randomly from the Red Sea, Jeddah coast during the period of March to September 2014. Helminthss have been detected in the liver, intestine and stomach of the infected fish. Total protein and carbohydrates in muscles and liver have been quantified using spectrophotometer. The obtained results showed that the total muscle protein of fish and total carbohydrates of both muscles and liver decreased significantly in infected fish. Intestinal trematodes have higher effect on the muscles protein rather than stomach trematodes whereas; intestinal nematodes have a low effect on muscles protein than stomach nematodes. This decrease may be due to the ability weakness of the intestinal absorption due to the consumption of digested food by the parasite.

Key words: Koshar fish · *Epinephelus* · Helminthes · Intestine · Liver · Stomach · Muscles · Protein · Carbohydrate

INTRODUCTION

Fish contain carbohydrates, vitamin A and D, iron, calcium and other mineral salts. The nutrients in fish are more than those found in eggs and poultry meat [1]. Protein is an essential nutrient for both maintenance and growth of fish. At maintenance level, fish requires protein for replacement of damaged tissues and proteinaceous product such as intestinal epithelial cells, enzymes and hormones [2]. Among Red Sea fishes, Koshar fish, *Epinephelus summana* is considered the most abundant and widespread. *Epinephelus* species belongs to the Family Serranidae and Subfamily Epinephelinae. The nutritive value of fish may be degraded through the activities of helminth parasites and their cysts as well as lowering the quality of the fish meat due to a fall in the absorbed amino acids which is essential in protein synthesis. Infection with helminth parasites can lead to severe changes in protein content and may result in host mortalities in commercial fish farming. Helminthic infection may affect protein content in fish muscle and glycogen in liver. The muscle protein in the fish host, *Saurida tumbi* and glycogen in fish muscle and liver has decreased in the case of infection with adult of cestode, *Penetrocephalus ganapatii* [3-5].

The helminth infection in some cases may cause increase in the total muscle protein. The percentage of protein in the muscle of prawn was high in infected fish. Protein and free amino acid metabolism in *Catla catla* and *Labeo rohita* due to the acanthocephalan, *Pallisentis nagpurensis* infection was higher in parasitized [6, 7].

The present study have dealt with the effect of helminthes invasion, trematodes, nematodes and larval cestodes on biochemical constituents of the Koshar fish, *Epinephelus summana*, such studies are poorly discussed in Saudi Arabia.

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MATERIALS AND METHODS

Fish under Study: A total number of 102 fish were collected randomly from local fish market, the Red Sea, Jeddah coast, Saudi Arabia, during the period from March to September 2014. Fish were transferred to the laboratory by using small containers containing seawater with aeration. Fresh fish were dissected and examined microscopically for endoparasites by using a dissecting microscope. Helminthes were fixed in alcohol-formalin-acetic acid (AFA) for two hours, washed several times in distilled water, stained with borax carmine, dehydratedin ascending grades of alcohol, cleared in clove oil and mounted in Canada balsam.

Biochemical Analysis: Sodium phosphate buffer 1 M (pH 7) was prepared by mixing 390 ml of 1 M NaH_2PO_4 (monobasic) and 610 ml of 1 M Na2HPO_4 (dibasic) stock solutions, one gram of muscles and liver tissues were blended well by blender and were put in separate tubes. About 10 ml of phosphate buffer (pH 7) was added into each tube. Samples were centrifuged at 5,000 X G. for 15 minute at room temperature and then supernatant was collected. Protein concentration was determined according to dye binding method by Bradford [8] with bovine serum albumin (BSA) as a standard. Total soluble carbohydrates were determined by the method of Dubois *et al*. [9].

The analysis of data was assessed using One-way ANOVA analysis to compare the biochemical analysis between infected and non-infected fish. All statistical analysis were performed using SPSS software program (version 16).

RESULTS

Table 1 shows that there was significantly decrease in muscle total protein and carbohydrates in both muscle and liver when cestodes infect the liver. Nematodes inhabiting the fish liver have caused significant decrease in muscles total protein and total carbohydrates either in muscles or liver. Nematodes have a greater effect on muscles protein compared to cestodes but the latter had great effect on total carbohydrates in muscle tissues.

Intestinal trematodes cause an insignificant decrease in the level of muscles protein and significant decrease in total carbohydrates of liver while muscles total carbohydrates were significantly higher in infected fish than non-infected one. With respect to intestinal nematodes, total carbohydrate in fish muscles were significantly low whereas, increase was observed significantly in muscles total protein and insignificantly in liver total carbohydrates of infected fish (Table 2).

Table 1: Concentration of total muscle protein and total muscle and liver carbohydrates (mg/ml) in *Epinephelus summana* infected with liver helminthes

	Total protein in muscle	Total carbohydrate in muscle	Total carbohydrate in liver
Control	0.476 ± 0.108	0.612 ± 0.099	2.057 ± 0.299
Cestodes	0.443 ± 0.079	0.181 ± 0.081	0.551 ± 0.066
P value	0.036*	0.014*	0.019*
Nematodes	0.383 ± 0.089	0.504 ± 0.148	0.460 ± 0.102
P value	0.004*	0.053*	0.027*

* Significant P ≤0.05

Table 2: Concentration of total muscle protein and tolal mucle and liver carbohydrates (mg/ml) in *Epinephelus summana* infected with intestinal helminthes

	Total protein in muscles	Total carbohydrate in muscles	Total carbohydrate in liver
Control	0.476 ± 0.108	0.612 ± 0.099	2.057 ± 0.299
Trematodes	0.459 ± 0.047	0.754 ± 0.243	0.383 ± 0.099
P value	0.071	0.020*	0.000*
Nematodes	0.545 ± 0.100	0.203 ± 0.095	2.861 ± 0.615
P value	0.032*	0.003*	0.27
Trematode and Nematodes	0.491 ± 0.029	0.464 ± 0.300	0.472 ± 0.029
P value	0.026*	0.015*	0.000*

*Significant P ≤0.05

Table 3: Concentration of total muscle protein and total muscle and liver carbohydrates (mg/ml) in *Epinephelus summana* infected with stomach helminthes

	Total protein in muscle	Total carbohydrate in liver	Total carbohydrate in muscle
Control	0.476 ± 0.108	0.612 ± 0. 099	2.057 ± 0.299
Trematodes	0.465 ± 0.017	0.495 ± 0.115	1.657 ± 0.359
P- value	0.004*	0.014*	0.012*
Nematodes	0.503 ± 0.012	0.323 ± 0.171	2.93 ± 1.248
P value	0.032*	0.002*	0.031*

*Significant P ≤0.05

Table 3 shows the significant decline in the level of all protein along with total carbohydrates in the case of fish infected with stomach trematodes. Nematodes infecting kosher stomach cause a significant decline in muscles total carbohydrates only whereas, muscles protein and liver carbohydrate were significantly higher than those in non-infected fish. Based on the above, the effect of stomach trematodes was found more than that of nematodes on muscles protein and total carbohydrates of liver. Nematodes infecting stomach had more effect on muscles total carbohydrates. The results were similar to that obtained from biochemical analysis of intestinal helminths. Intestinal trematodes have higher effect on the muscles protein rather than stomach trematodes whereas; intestinal nematodes have a low effect on muscles protein than stomach nematodes.

DISCUSSION

Nematodes have more effect on muscles protein compared to cestodes but the latter had more effect on total carbohydrates of muscles. Through our findings, it is noticed that the cestode larval cyst affect the concentration of total carbohydrates in the fish liver. This may be due to the large size of larvae which press the liver and may cause partial damage to the liver cells. As well as their nutritional needs make them compete with the host's needs [10, 11]. The nutritive value of fish may be degraded through the activities of helminth parasites and their cysts [4].

Depletion of glycogen in fish infected with cysts is the result of stress since the worm obtains nourishment through cyst wall from the host liver [12-15]. The actual reason beyond the protein and glycogen decrease of the fish infected with the encysted cestode larvae is the obstruction of the bile duct as a result of the presence of a large number of parasites which lead to defect in the digestion because the bile salts do not arrive to intestine [16].

It is clear from the present results that there is a great effect for the nematode larvae on the biochemical contents of the fish. There is a proportional relation between the increase in intensity of infection and the decrease in the level of these contents. These results are in accordance with Ali [15] and Pirus *et al*. [17] who observed a great defect in the infected Gadus fish liver compared with the non-infected ones. Also, Pirus *et al*. [17] pointed out the pathological effect of the adult and larval nematode infections on the blood protein as well as the liver glycogen at different infection intensities. Many studies have focused this effect of the larval nematode *Contracaecum* sp. on the biochemical contents of Al Hamri fish in helminths of stomach [10].

The current results showed that the effect of intestinal trematodes was restricted on muscles protein and total carbohydrate of liver whereas; the intestinal nematodes affect the carbohydrates in muscles only. The double impact of both trematode and nematodes infecting intestine led to decline in total carbohydrates either in muscle or liver compared to non-infected fish, although muscles protein was higher in infected fish. The decrease in protein content may contribute to the effect of gut parasites which feed on gut contents of host fish; this result is the same as that recorded by Habiballah *et al*. [18].

The results in this study indicated that the muscles and liver protein and total carbohydrates were affected in the case of trematodes inhabiting fish stomach. Nematodes inhabiting stomach have reduced muscles total carbohydrates whilst protein and liver carbohydrate were high in infected fish. Intestinal worms had more effect than stomach worms. These results are in accordance with that mentioned by Ashokan *et al*. [19] where they recorded a reduction in total protein in liver and muscles in the case of helminths either infecting intestine or stomach. The results indicated that intestinal helminthes such as (*Eucreadium*: Trematoda) and (*Camallanus*: Nematoda) are more influential than stomach ones such as (*Fellodistomum*: Trematoda).

Protein and glycogen levels have increased during helminthes infection. The increasing in protein is due to enhanced rate of protein metabolism as a kind of adaptation of host to their parasites [6, 7, 20-27]. Protein in muscles of infected fish will not be affected unless a

severe decrease in energy reserves including glycogen and lipids has occurred [15, 28, 29]. This increase could be attributed to the tissue repair mechanism operating in the host system in order to cope with the parasitic invasion. Amino acids were found to play an important role in meeting the energy demands of an animal by converting them into keto acids which run into citric acid cycle through transamination process. Thus animal might have sought catabolic activities and degraded proteins to amino acids. Parasites have altered the crucial physiological aspects of the host system makes the host to try to face the infection by altering its physiology [7]. Increased muscles protein and liver total carbohydrates in infected fish may be due to that helminthes interfere with the digestion and absorption of digested food causing metabolic disturbances [26]. Fish stress during infection may not cause decline in liver glycogen and carbohydrates in muscles. This may be due to that reserve lipids have transformed to glycogen by gluconeogenesis with glucose consumption in muscles activities [15, 30, 31]. The high hormonal activity of host (insulin) may be the reason for glycogen accumulation in skeletal muscles [27]. In addition, during helminthic infection, some enzymes such as aspartate aminotransferase and alanine aminotransferase which used in the amino acid metabolism as well as linking carbohydrates were increased [32]. These enzymes caused increased pyruvate that may be converted to glycogen through gluconeogenesis and stress led to fish adaptation to infection.

REFERENCES

1. Ghaffar, R.A., 2008. Seasonal variation and histopathology of helminth parasites in the fish *Lutjanus argentimaculatus* (Forsk, 1775) red snapper. Ph D Thesis. Department of Zoology, University of Karachi. Pakistan, pp: 1-14.
2. Hepher, B., 1988. Nutrition of pond fishes. Cambridge University Press, pp: 388.
3. Fraser, C. and A. Mays, 1986. The Merck Veterinary Manual. Merck and Co., Inc. Rahway, NJ, pp: 921-934.
4. Singh, K. and A. Srivastava, 2010. Study on infection intensity and changes in total protein content in tissues of *Channa punctatus* infected with helminth Parasites. Trends in Biosciences, 3: 204-205.
5. Radhakrishnan, S., N.B. Nair and N.K. Balasubramanian, 1983. Adult cestods infection of marine teleost fishes *Saurida tumbil* (Bloch). Actaichthyologicaet piscatorial, 13: 75-94.
6. Dörücü, M., 2000. Changes in the protein and lipid content of muscle, liver and ovaries in relation to *Diphyllobothrium* spp. (Cestoda) infection in powan (*Coregonus lavaretus*) from Loch Lomond, Scotland. Turkish Journal of Zoology, 24: 211-218.
7. Kumar, P.A., 2014. Biochemical effects on protein and free amino acid metabolism in *Catla catla* and *Labeo rohita* due to *Pallisentis nagpurensis* infection. American International Journal of Research in Formal, Applied and Natural Sciences, 6: 82-85.
8. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.
9. Dubois, M., K.A. Gilles, J.K. Hamilton, P. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28: 350-356.
10. Al-Niaeem, K.S., 1999. Histopathological and biochemical effects of some helminthes parasites in three Iraqi freshwater fishes. M.Sc. Thesis, university of Basrah, College of Agriculture, pp: 73 (In Arabic).
11. Roberts, R.J., 2003. Fish Pathology, Bailliere Tindall Books London.
12. Linnik, V.Y. and V.S. Litvyak, 1980. Amino acid composition of the muscle and blood of fish infected with trematode metacercariae. Trudy Belorusskogo Nauchno-Issledovatel'skogo Instituta Eksperimental'noi Veterinarii (Veterinarnaya Nauka--Proizvodstvu), 18: 120-123.
13. Gupta, A.K. and S.M. Agarwal, 1983. Host-parasite relationship in *Channa punctatus* and *Euclinostomum heterostomum*. Current Science, 52: 1141-1143.
14. Gupta, A.K. and S.M. Agarwal, 1984. Host parasite relationships in *Channa punctatus* and *Euclinostomum heterostomum*. III. Transaminases and total proteins and free amino acids. Current Science, 53: 710-711.
15. Ali, A.H., 2001. Pathological effects of helminthes parasitic on some local fishes. M.Sc. Thesis, College of Agriculture, Basrah University (In Arabic).
16. Joshi, B.D., 1979. Biochemical changes in the liver and blood of a freshwater fish, *Rita rita*, infested with a trematode *Opisthorchis pedicellata*. Folia Parasitologica, 26: 143-144.
17. Pirus, R.I., E.E. Basalkevich and Ya. N. Stasyna, 1983. Principal physio-biochemical and histological changes in carp with *Philometroides* infection. Rybnoe Khozyaistvo, Res ikanskii Mezhvedomstvennyi Tematicheskii Sbornik, 36: 64-66.

18. Habiballah, S.Y.M., O.F. Idris and M.K. Sabahelkhier, 2011. Morphometric and gross chemical studies of healthy and infected rabbit Fish, (*Siganus rivulatus*) by helminthes parasite in Red Sea Coast, Sudan. Australian Journal of Basic and Applied Sciences, 5: 450-456.
19. Ashokan, K.V., D.S. Mundaganur and Y.D. Mundaganur, 2013. Ecto and Endo parasites in *Labeo rohita*, Major carp (Hamilton) In Krishna River segment in Sangli district. International Journal of Research in Chemistry and Environment, 3: 16-19.
20. Kameswari, M., 1978. Studies on some biochemical and physiological aspects of host parasite relationship in *Rana tigrina* and *Calotes versicolor* with reference to helminth infection, Ph.D. Thesis, Kakatiya University, Warangal, India.
21. Bhonsle, H.R., 1980. Investigation in to some metabolic aspects of *Tanqua tiara*, Linstow, 1897 and its organ of infection. Ph.D. Thesis, Kakatiya University, Warangal, India.
22. Sulochana, T., 1982. Studies on some post helminth infection histochemical and histopathological changes in the intestine of few vertebrates, Ph.D. Thesis, Kakatiya University, Warangal, India.
23. Rao, B.R.H., 1985. On some biochemical aspect of *Lytoces tusindicus* Moghe, 1925 and its adaptation in *Clarius batrachus*, Ph.D. Thesis submitted to Kakatiya University, Warangal. A.P., India.
24. Swamy, S.K.N., 1986. Some biochemical and histochemical aspects of post helminth infection changes in *Rana cyanophlyctics*, Ph.D. Thesis submitted to Kakatiya University, A.P. Warangal, India.
25. Bikshapathi, V., K.S. Reddy and M. Kameswari, 2008. Post helminth infection changes in the protein metabolism of myotomal muscles in *Channa punctatus*. Uttar Pradesh Journal of Zoology, 3: 289-295.
26. Reddy, B.L. and G. Benarjee, 2011. Histochemical studies on *Genarchopsis goppo* from fresh water murrel, *Channa striatus*. Asian Journal of Animal Science, 6: 23-26.
27. Tyutin, A.V. and G.I. Izvekova, 2013. Infection of mollusks and fish by the trematode *Apophallus muehlingi* (Jagerskiold, 1898) and its interrelations with intermediate hosts. Inland Water Biology, 6: 52-56.
28. Mehdi, D.S., 1989. Effect of parasites on the biochemical constituents of fishes *Liza abu*. M.Sc. Thesis, University of Basrah, pp: 98 (In Arabic).
29. Al-Hadithi, I.A.W., A.Y. Khudhair and D.S. Mehdi, 1990. Effect of intensity of Infection with parasites on protein content in *Liza abu* (Heckle). Basrah Journal of Agricultural Sciences, 3: 209-218.
30. Hantoush, A.A., 1998. Seasonal variations in the biochemical constituents for muscles of some freshwater and marine fishes from Shatt Al-Arab River and northwest Arabian Gulf. M.Sc. Thesis, University of Basrah. pp: 93 (In Arabic).
31. Hantoush, A.A., E.A. Abdul-Hassan and H.T. Al-Saad, 2001. Seasonal variations of fatty acids constituents in the muscles of some freshwater and marine fishes from Shatt Al-Arab River and North West Arabian Gulf. *Marina Mesopotamica,* 16: 359-373 (In Arabic).
32. Vinatha, N., V. Bikshapathi and S.R. Babu, 2013. Post helminthes infection changes in AAT and AIAT activity in the myotomal muscles of *Channa punctatus*. Science Research Reporter, 3: 167-170.