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Appraisal of changes in the Glycogen Contents of Helminth Infected Fishes and Its Host Tissue of *Mastacembelus armatus*

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ABSTRACT

Helminth infections affected the amount of glycogen in the intestine, muscles and liver of different fish species. Metabolic alterations in hosts can result from interactions with helminths. By triggering their internal defense system and mending tissue damage, the host uses their reserves to maintain important bodily functions while also providing the parasites with the energy they need to grow. Our objectives were to assess the glycogen levels in the host tissue that had been infected with the helminth parasite in an experimental setting. Following an experimental trematode infection, it was shown that the muscles of immature cyprinid fish had more glycogen. The same held true for those who had trematode infections naturally.

These alterations could be attributed to fish swimming less frequently because of muscle damage brought on by parasites. After 30 days of trematode maintenance, the fish's spontaneous motor activity significantly decreased, which may be a contributing factor to the buildup of glycogen in the infected fishes' muscles. When the fish were infected with the cestodes, glycogen levels decreased concurrently. The intestine of an infected fish has less glycogen, which indicates that the intestine isn't functioning properly. As a result, the activity of the primary digesting enzymes (proteinases and glycosidases) in the gut is reduced. As a result, trematode and cestode infections have varied effects on the amount of glycogen in fish tissues and may lead to various symptoms.

Keywords: Glycogen Contents Infected Fishes, *Mastacembelus armatus*

Introduction

Carbohydrates form the chief energy source in fish and parasitic cestodes. The Helminths parasites utilize the food from the intestinal gut of host. The metabolism depends on the feeding habits and the rich nourishment available in the gut of the host. The parasites use this nourishment for their normal development and growth. A major part of energy source utilized by the parasite is from Carbohydrates, the percentage and location of Carbohydrates in the host, where the environment is rich for nourishment normal development and reproduction

of the parasite is accounted in the host diet. The host carbohydrate also has an effect of growth; worms grow better in a host, feed on protein free diet containing carbohydrates.

Glucose is a very important energy source for many helminths in habiting the gut of vertebrates. It is generally believed that helminths absorb glucose against a concentration gradient and use their endogenous carbohydrates as an energy source only when it is unobtainable from outside. Similarly, glycogen in most of the cestodes provides a significant reserve store of energy, particularly in forms which are parasitic in animals, and which exist in environments of low oxygen tension.

Literature provided reveals that the carbohydrate plays more important role in cestodes, than in most other parasitic worms which are characteristics by different growth patterns. These carbohydrates are utilized exogenously, their mechanism of uptake is not known, but the evidence indicating that this takes place by active mechanism undoubtedly is entangled in carbohydrate transport of helminths.

It has been known for more than a century by Bernard 1859, that parasitic worm contains polysaccharides; Weinland in his classical work (Weinland 1901) illustrated that the metabolism of intestinal worms is characterized by the Fermentation of Carbohydrates. Following the work of these and other pioneers who studied some phase of Carbohydrate relationship of the parasites (Von Brand 1970), it has very soon become obvious that many endoparasites have a pronounced carbohydrates metabolism.

Sufficient literature is present for parasitic worms in relation to distribution of carbohydrates. The quantitative values found in previous and of many the recent literature e.g. Weinland 1901, Von Brand 1934, Salisbury and Anderson 1939, Daugherty and Taylor, 1956, Good Child, 1962 and others have been obtained by rather unspecific chemical method, there often given higher values than those obtained by means of an enzymatic procedure (Glucose oxidize). The use of various analytical procedures may explain for example the widely differing glucose values reported by Fairbairn (1950), For *Moniezia expansa* reliable quantitative or semiquantitative data has also been obtained by means of paper chromatography.

The glycogen content of cestodes depends to some extent on the stage in the life cycle in few cestodes developmental history changes the growth and parasite is rapid at the first 18-24 hours and then slows down even if the concentration is high as it was in the early phase. It has been observed that the same in *Hymenlepis diminuta* increases from 15% of the dry substance in 5 to 7 days old worms to 37% of the dry substance in 13 to 16 days old specimens. It has also been observed that the uptake of glucose is very much more effective when CO_2 is present in the surrounding than when it is absent.

For pathological and toxicology (Li et al., 2009), biomarker development (Zhong et al., 2022), and disease diagnosis and classification (Ong et al., 2009), gas chromatography–mass spectrometry (GC-MS) is a helpful technique. GC-MS-based techniques were used to analyze the tissue metabolome of fish intestine to get additional insight into the energy metabolism of the host.

In view of the importance of carbohydrates in helminths any difference in their carbohydrate metabolism and that of their hosts might be useful exploited in helminth control. This work was undertaken with the goal to investigate the changes in carbohydrate metabolism due to infection by helminth parasite by evaluating the glycogen concentration in healthy as well as diseased host following infection.

Material And Methods

Infective parasite forms

Some freshwater fish hosts, *Mastacembelus armatus* and their intestines (Three Hundred Sixty intestines) were brought, and these intestines were dissected for the collection of parasites. Three Hundred Thirty-Six intestines were heavily infected with Helminth parasites. The identical parasites are sorted, few of them, Cestodes and Trematodes were fixed in 4% formalin and Nematodes were fixed in glycerol for identification. The taxonomic observation turns then to a species of the genus *Senga indicus* Sp. Nov., *Senga vishnupurensis* Sp.Nov., *Ptychobothrium armatii* Sp.Nov., *Polyoncobothrium follicularis* Sp. Nov., *Isoparorchis hypselobagri* (Billet, 1898), *Allocredium intestinalis* Sp.Nov., *Camallanus armatusae* Sp.Nov. and *Procamallanus mastacembali* Sp. Nov.

Biochemical analysis

Small pieces of infected, non-infected intestine and parasites viz. *Senga indicus* Sp. Nov., *Senga vishnupurensis* Sp.Nov., *Ptychobothrium armatii* Sp.Nov., *Polyoncobothrium follicularis* Sp. Nov., *Isoparorchis hypselobagri* (Billet, 1898), *Allocredium intestinalis* Sp.Nov., *Camallanus armatusae* Sp.Nov. and *Procamallanus mastacembali* Sp. Nov., were collected and washed thoroughly in distilled water and the Glycogen content was determined by the method of Kemp et.al.1954.

The amounts of Glycogen in the worms were calculated by the Formula:

$$\text{Percentage of Glycogen} = \frac{100 \times U}{1.11 \times S}$$

Where,

U = O.D. of Unknown solution.
S = O.D. of the 100 mg of Glucose standard.
1.11 = Conversion factor of glucose to Glycogen.
S = 2

Preparation of Intestine Extract

Intestinal tissue extracts were processed according to the instructions described earlier (Li et al., 2009). The intestinal tissue was homogenized with 1 ml of methanol at 4°C for three minutes using a Blender. After adding and thoroughly mixing 0.5 ml of Milli-Q water, 0.3 ml of the homogenate was transferred to another tube and divided with 0.2 ml of chloroform. Proteins were extracted from the samples by centrifuging the 300 µl top aqueous layer through a 10-kDa cutoff filter for 15 minutes at 4°C. Glycogen in intestinal tissue extracts were derivatized before GC-MS analysis.

Intestines extract analysis by GC-MS

The intesinal sample was examined in a sequence as described earlier following derivatization process. In N,O-bis(trimethylsilyl) trifluoroacetamide was used to derivatize the dried sample together with 1% trimethylchlorosilane. Then, the sample was heated at 70°C for 60 minutes to create trimethylsilyl derivatives Thermo Finnigan Trace GC 2000 equipped with a Polaris Q mass detector and an Xcalibur software system for the GC-MS study. A 30-m ×0.25-mm (internal diameter) ×0.25-μm (film thickness) DB-5-fused silica capillary column was filled with one microliter of derivatized material.

A 5% diphenyl-95% dimethylpolysiloxane cross-linked stationary phase was used to chemically bond this (J&W Scientific). The temperature of the ion source was 200°C, and the injector was 230°C. Start the oven temperature programme at 80°C for 5 minutes. Increase the temperature by 5°C/min to 300°C and hold it for 1 minute. A carrier gas of 1 ml/min was employed: helium. In the electron impact mode (70 eV), the mass spectrometer was in operation. Total ion currents (TICs) were acquired in the full scan mode, with a scan period of 0.58 s, between 50 and 650 m/z. By comparing the retention index and MS spectra with those from commercially available reference substances, peaks of GC-MS were identified.

Histochemical analysis

The collected worms were kept on blotting paper to remove excess water. The material transferred to a previously weighed watch glass and weighed on a sensitive balance. The wet weight of the tissue is taken and kept in a oven at 50^{o}c to 60˚c. for twenty four hours to make the material dry. The dry weight of the material was taken and prepared as a powder. This powder weighed 122.10mgs on a sensitive balance and was homogenized in a mortar pestle, added 5ml of 5% TCA and transferred in a centrifuge tube. This material is digested in boiling water for 15 minutes at 2000 RPM.

1ml of supernatant was taken in a test tube, added 3ml of sulphuric acid and boiled for 5 minutes, the mixture should shake well, then immediately cooled and readings were taken in Erma's Colorimeter at 530mu filter.

Statistical analysis

The biochemical parameters were expressed as mean ± standard derivation and analyzed to one-way ANOVA.

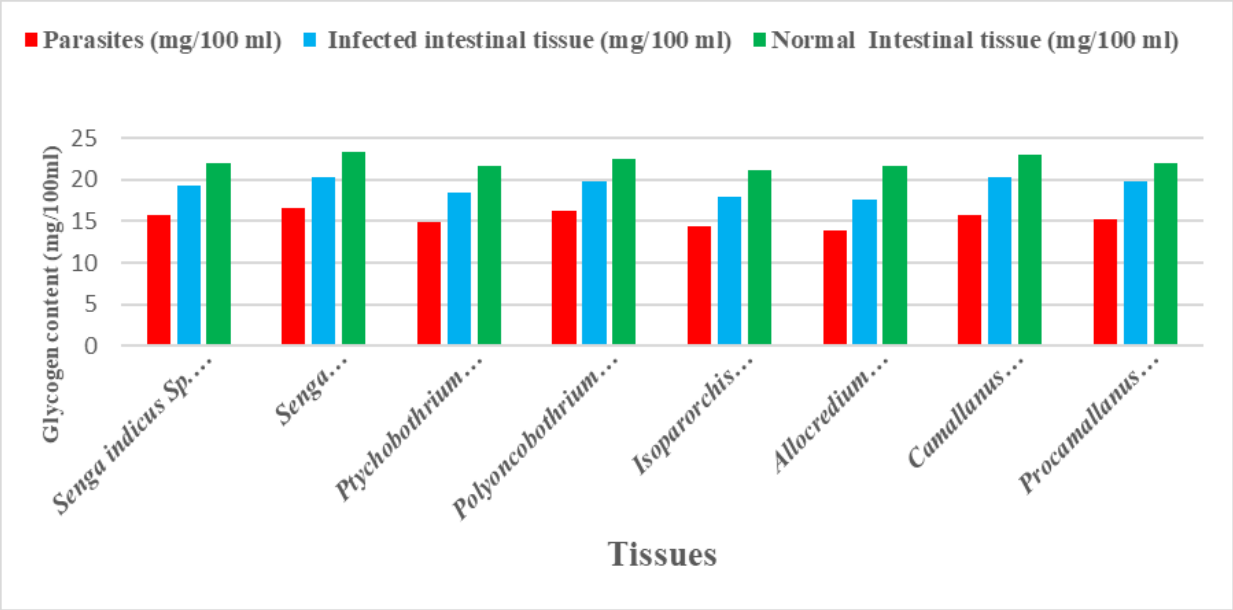
Results and Discussion

Changes in the pattern of carbohydrate metabolism were observed in all infected samples. The glucose concentration in tissue decreased significantly in the groups composed of infected host compared to the control.

Table 1:- Glycogen contents in *Mastacembelus armatus* Normal host intestinal tissue, Infected intestinal tissue and their helminth parasites

sr	Parasite	Glycogen mg/100 ml		
		Parasite	Infected host tissue	Normal host tissue
1	*Senga indicus* Sp. Nov.	15.77	19.37	22.07
2	*Senga vishnupurensis* Sp. Nov.	16.67	20.27	23.42
3	*Ptychobothrium armatii* Sp.Nov.	14.86	18.47	21.62
4	*Polyoncobothrium follicularis* Sp. Nov.	16.22	19.82	22.52
5	*Isoparorchis hypselobagri* (Billet, 1898)	14.41	18.02	21.17
6	*Allocredium intestinalis* Sp. Nov.	13.96	17.57	21.62
7	*Camallanus armatusae* Sp. Nov.	15.71	20.27	22.97
8	*Procamallanus mastacembali* Sp.Nov.	15.32	19.82	22.07
	SE	0.3235	0.3701	0.2655
	CD	1.7105	1.9568	1.4041

Figure 1:- Graph showing glycogen contents in Normal host intestinal tissue, Infected intestinal tissue and their helminth parasites.



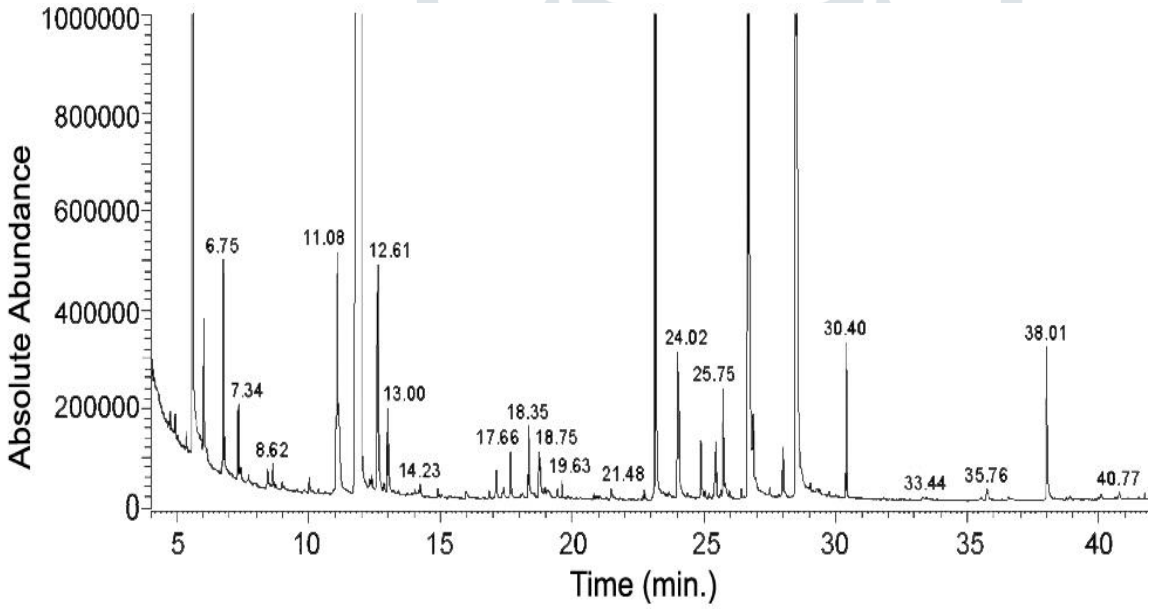
The current study's findings demonstrate that glycogen content varies across parasite, normal, and infected intestines, since helminth parasites possess less glycogen than their host intestines. This was true for all of the worms, as shown in table no.1. Jadhav et al. (2007) reported this type of fluctuation in glycogen content, i.e. the amount of glycogen or carbs present in *Davainea shindei* is lower (15.17 mg/100 ml) than in the host gut (17.56 mg/100 ml).(Figure 1)

Graff and Allen (1963) determined the glycogen content of male *Moniliformiis dubis*. When expressed as mg glycogen/gm wet weight of tissue, the glycogen level of male worms was more than double that of female worms, at 16.81 (I4.3) in male and 7.87 (I1.76) in female. C.A. Hopkins (1950) recorded the artificial infection of a pigeon with *Schistocephalus* from the corpse of a fish. He estimated the amount of glycogen in the parasite's body to be 11.9% (after 24 hours of infection), 10.8% (after 48 hours), and 10.0%. (after 72 hours). The results

indicates of Standard error and the average deviation of the observed glycogen levels from the expected mean levels within each tissue type. The threshold for determining whether the difference in glycogen levels between any two groups within each tissue type is statistically significant at the 5% significance level (p=0.05). Based on the comparisons, the differences in glycogen levels between the tissue types are all significant, the parasite glycogen against Infected Host Tissue Glycogen was found to be significant, similar parasite glycogen against Normal Host Tissue Glycogen was also found to be Significant and infected Host Tissue Glycogen against Normal Host Tissue Glycogen was also found to be significant. This indicates that there are statistically significant differences in glycogen levels between the different tissue types for each parasite species.

Using these values, comparisons can be made to determine if the glycogen levels differ significantly between the different tissue types (Parasite, Infected Host Tissue, Normal Host Tissue) for each parasite species.

Figure 2 shows a typical GC-MS TIC chromatogram of intestinal tissue metabolites from fish, the number of peaks is quite large. A huge number of distinct peaks were seen when the low-intensity regions were magnified, indicating that the metabolites in the intestine varied significantly. The intestine uses these metabolites—which include fatty acids, carbohydrates, organic acids, amino acids, and lipids—in a variety of metabolic reactions. Their varying presence can shed light on the physiological and pathological impacts within the intestinal system.



This finding suggests that the lung flukes *Haematoloechus complexus* and *H. medioplexus* have much less glycogen than the frog tapeworm *Crepidobothrium saphena*. Nanware et al., (2011) examined the amount of glycogen present in several cestode parasite areas in *Capra hircus*, the juvenile proglottids of both tapeworm species had less glycogen than the mature and gravid proglottids. Because of differences in size and environment, all cestode parasites have varying amounts of glycogen. Habitat is also an essential element in the quantity of glycogen contained in the parasite's body.

According to Bhure et al. (2016), normal intestinal tissue has a higher glycogen content than infected intestinal tissue, while the nematode parasite Camallanus jadhavii (Jadhav and Khadap, 2003) had a lower biochemical

content in Wallago attu (Bleeker, 1857). Because they could draw nourishment from their host, intestinal parasites had high levels of lipid, protein, and glycogen. However, in the current study, there is a significant difference in glycogen content, with the parasite having lower glycogen levels than the infected and normal intestines of its host.

Conclusion

The current study shows that the amount of glycogen in parasite bodies is lower than in infected and normal host intestines. Furthermore, the amount of glycogen contained in all Helminth parasites varies depending on their size and location.

Senga indicus Sp. Nov., *Senga vishnupurensis* Sp. Nov., *Ptychobothrium armatii* Sp. Nov., *Polyoncobothrium follicularis* Sp. Nov., *Isoparorchis hypselobagri* (Billet, 1898), *Allocredium intestinalis* Sp. Nov., *Camallanus armatusae* Sp. Nov. and *Procamallanus mastacembali* Sp. Nov. are quite successful in obtaining a sufficient amount of glycogen from the environment.

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References

Bernard C. (1859) Quoted by Von Brand (1973). In “Biochemistry of Parasites”, Acad .Press, Newyork.

Bhure, D. B., Nanware, S. S., & Kardile, S. P. (2016). Taxonomic and biochemical studies of piscean nematode Camallanus Jadhavii (Jadhav and Khadap, 2013) parasitic in Wallago Attu (Bleeker, 1857). World Scientific News, (34), 98-108.

C.E. Cannon et al.(1970) Changes in the distribution of *Hymenolepis diminuta* (Cestoda: Cyclophyllidea) within the rat intestine during prepatent development. *Canadian Journal of Zoology(1970).*

Daugherty, J.W. and Taylor, D. (1956) Regional distribution of glycogen in the rat cestode, *Hymenolepis diminuta*. *Expt. Parasitol., 5: 376-390.*

Fairbairn, D. (1950). An improved method for the complete elimination of microorganisms from Ascaris lumbricoides. Journal of Parasitology, 36(6, Sect, 2, Suppl).

Goodchild, D.G. (1962) Carbohydrate content of the tapeworm *H. diminuta* from normal bile less and starved rats.*J. parasit, 47: 401-405.*

Graff, D. and Allen, K. (1963) Glycogen content in Moniliformis dubius (Acanthocephala). *J. parasitol, 49 (2), 204-208.*

Hopkins, C.A., (1950) Studies on cestode metabolism I. Glycogen metabolism in *Schistocephalus solidus* in vivo. *J.Parasit., 36: 384-390.*

Jadhav, B. V,. Shivesh P. Singh, Bhure, D. B. and Padwal, N. D. (2008) Biosystematic studies of *Davainea shindei* n.sp. (Cestoda- Davainidae) Fuhrmann, 1907 from *Gallus gallus domesticus*. *National Acdemy of Science Letter Vol.-31 No.-7&8 pp 245-250.*

Kemp, A. Vankites and Haijnin Gen, A.J.M. (1954) A Colourimetric method for the determination of glycogen in the tissue *Biochem J.56: 646-648.*

Kumar, P. A. (2014). Biochemical effects on protein and free amino acid metabolism in Catla catla and Labeo rohita due to Pallisentis nagpurensis infection. AIJRFANS Am. Int. J. Res. Form. Appl. Nat. Sci, 6, 82-85.

Li, X., Xu, Z., Lu, X., Yang, X., Yin, P., Kong, H., Yu, Y. and Xu, G., (2009.) Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for metabonomics: Biomarker discovery for diabetes mellitus. Analytica Chimica Acta, 633(2),257-262.

Nanware, S. S., & Bhure, D. B. (2011). Studies on glycogen profile of cestodes of Capra hircus. International Multidisciplinary Research Journal, 1(10).

Ong, E. S., Chor, C. F., Zou, L., & Ong, C. N. (2009). A multi-analytical approach for metabolomic profiling of zebrafish (Danio rerio) livers. Molecular BioSystems, 5(3), 288-298.

Ramalingam, K. Vijayalakshmi, V. and Satyaprema, V.A. (2004) Carbohydrate profile in relation to growth and differentiaationn of proglottids in *Avitellina lahorea* (Woodland, 1927), an aoplocephalid cestode *Uttar Pradesh J.Zool 24(3).*

Salisbury and Anderson (1939) Concerning the chemical compostion of Cysticercus fasciolaris *J.Bio. Chem.129: 505-517*

Theron, O., Odlaug, (1955) The quantitative determination of glycogen in some parasites of Amphibia, *The journal of parasitology vol. 41, No.3 pp 258-268.*

Von Brand, T., (1934): Der Stoffwechsel von Ascaris lumbricoides bei Oxybiose und Anoxybiose, Z. Vgl. *Physiol. 23: 220–235.*

Von Brand, T., (1970): Physiology and biochemistry of Trichinella spiralis, in: Trichinosis in Man and Animals (S.E. Gould, ed.), *Charles C. Thomas, Springfield, Illinois, pp. 81–90.*

Weinland E. (1901): Ober Kohlenhydratzersetzung ohne Sauerstolfaufnahme bei Ascaris, einen tierischen Gerungsprozess. *Z. Biol. 42:55-90*

Zhong, L., Liu, H., Zhang, H., Zhang, W., Li, M., Huang, Y., & Yin, L. (2022). High starch in diet leads to disruption of hepatic glycogen metabolism and liver fibrosis in largemouth bass (*Micropterus salmoides*), which is mediated by the PI3K/Akt signaling pathway. Frontiers in Physiology, 13, 880513.