

DYNAMICS OF PARASITE POPULATION, AND ITS HISTOPATHOLOGICAL AND HISTOPHYSIOLOGICAL EFFECTS IN THE STOMACH OF A FRESHWATER FISH

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ABSTRACT

The caryophyllaeid cestode *Lytocestoides fossilis* infects the freshwater catfish *Heteropneustes fossilis*. The study was conducted for two consecutive years (2004-06) to record the biostatistical data of the parasite. The incidence of infection, intensity of infection, density of infection and index of infection of the parasite has been recorded. The infection was more during June to September, moderate during February to May and low during October to January. The parasite brought about severe histopathological changes in the stomach of infected fish. The changes observed in the stomach of fish included structural damage of the villi, inflammation, and fibrosis associated with hyperplasia and metaplasia. The hypertrophy of mucous layer led to vacuolation and necrosis. Histochemical changes were noticed with enhanced carbohydrate, protein and lipid contents. The enhanced substrate content in the infected organ might be due to the disfunctioning of the digestive tract, which results in the accumulation of various metabolites. Mucus secretion was triggered as a protective interaction against parasitic invasion. The parasitic infection affects the general metabolic state of the host and as the result, the fish becomes sluggish and moribund.

Keywords: *Heteropneustes fossilis*, *Lytocestoides fossilis*, histopathology, histochemistry

INTRODUCTION

Lytocestoides fossilis is a caryophyllaeid cestode parasite of freshwater fishes. The freshwater teleost *Heteropneustes fossilis* was found to be infected with this parasite. The parasite inhabits the stomach and intestine. The occurrence of cestodes, particularly caryophyllaeids, was reported in the piscine hosts by Moghe (1925, 1931),

Mackiewicz *et al.*, (1972), Satpute and Agarwal (1974a, b), Hayunga (1979), Kadav and Agarwal (1982, 1983), Kanth and Srivastava (1984), Chakravarthy and Tandon (1989), and Chakravarthy and Veena (1989). In the present work, a study has been made on the parasitic density, percentage of infection and seasonal variation in the infection of *L. fossilis* in *H. fossilis*. An attempt has been made to

visualize the histopathological and histochemical changes that occur in the fish due to the parasitic infection.

Cestodes have been found to infect freshwater fishes and cause pathological effects. Authors who have studied the caryophyllaeid cestode infection, especially in fishes, are Mackiewicz *et al.* (1972), Ramadevi (1973), Hayunga (1979), Kadav and Agarwal (1982, 1983), Chakravarthy and Tandon (1989), Rahman (1989), and Zaman and Seng (1990). Others who have contributed to the knowledge on the histopathological changes that occur in fishes due to cestode infection include Satpute and Agarwal (1974a), Amin (1990), Hasnain (1992), Lyngdoh (1995), Hiware and Garad (2002), Reddy and Benarjee (2006), and Benarjee and Reddy (2006).

MATERIAL AND METHODS

For the present study, *H. fossilis* was procured from freshwater bodies such as rivers, tanks, lakes and ponds located in Warangal district of Andhra Pradesh. To identify the infection and also to collect the parasites, the fish were dissected and various visceral organs such as digestive tract and liver were examined carefully after placing them in petri dishes containing normal saline. The parasites collected from the infected fish were enumerated and permanent slides prepared by preserving the parasites in 10% formaldehyde for 48 hours. These were later stained with alum carmine (Pearse, 1968; Bancroft, 1975). The parasites were identified after Yamaguti (1959).

The study was conducted for two consecutive years, *i.e.*, beginning with

July 2004 and ending with June 2006. The following equations were adopted to calculate various biostatistical parameters of the parasite (Reddy *et al.*, 2006):

- a) Incidence of infection (x_1) = $\frac{\text{No. of hosts infected (b)}}{\text{No. of hosts examined (a)}} \times 100$
- b) Intensity of infection (x_2) = $\frac{\text{No. of parasites collected (c)}}{\text{No. of hosts infected (b)}}$
- c) Density of infection (y_1) = $\frac{\text{No. of parasites collected (c)}}{\text{No. of hosts examined (a)}}$
- d) Index of infection (y_2) = $\frac{\text{No. of hosts infected (b)} \times \text{No. of parasites collected (c)}}{\text{No. of hosts examined (a)}^2}$

The stomachs of the infected and uninfected fish were isolated and preserved in Bouin's, Susa, Carnoy and Zenker's fluids for the histopathological and histochemical studies. A battery of histochemical tests were applied (Pearse, 1968; Bancroft, 1975) on the microtome-cut sections of stomach, both infected and uninfected, to realize the histoarchitectural and histochemical changes that occurred in the tissues of the stomachs of the fish due to *L. fossilis* infection.

RESULTS AND DISCUSSION

L. fossilis is a caryophyllaeid tape worm infecting the stomach and intestine of freshwater catfish, *H. fossilis*. In the Indian subcontinent, the dominant hosts for this parasite are siluriform and cypriniform fishes (Schaperclaus, 1954; Amlacher, 1961). Caryophyllaeid tape worms are found in the majority of cyprinid and silurid fishes, especially in the tropics and subtropics (Mackiewicz *et al.*, 1972; Bauer *et al.*, 1973; Satpute and Agarwal, 1974a; Hayunga, 1979; Ahmad and Sanallah, 1979; Bose and Sinha, 1981, 1983; Kadav and Agarwal, 1983;

Fig. 1: Incidence of infection of *Lytocestoides fossilis* in *Heteropneustes fossilis*

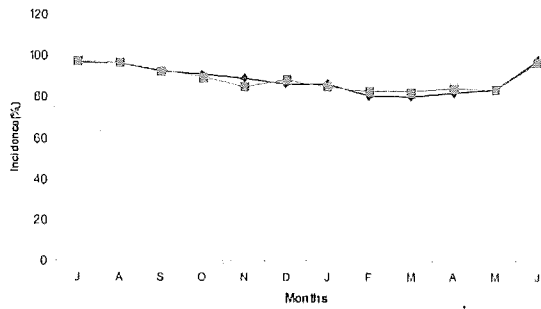


Fig. 2: Intensity of infection of *L. fossilis* in *H. fossilis*

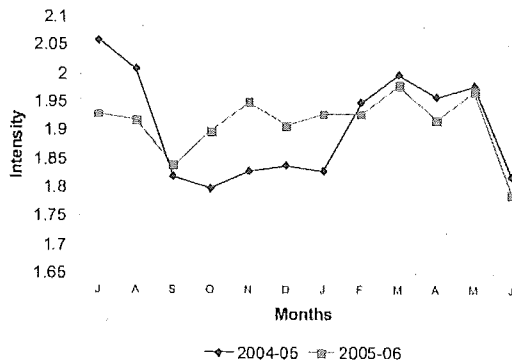


Fig. 3: Density of infection of *L. fossilis* in *H. fossilis*

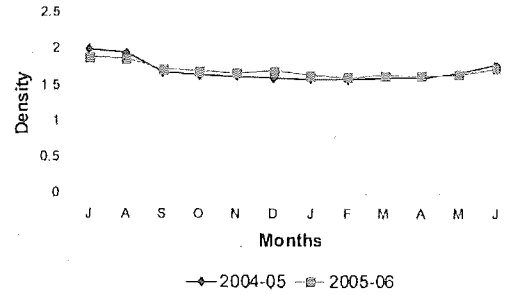


Fig. 4: Index of infection of *L. fossilis* in *H. fossilis*

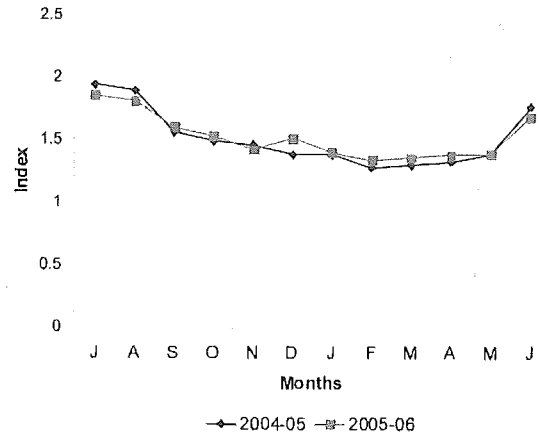


Table 1: Biostatistical indices of *Lytocestoides fossilis* in the population of *Heteropneustes fossilis*

Month	No. of hosts examined (a)	No. of hosts infected (b)	No. of hosts un-infected	No. of parasites collected (c)	Incidence of infection (x1)	Intensity of infection (x2)	Density of infection (y1)	Index of infection (y2)
July 2004	134	130	4	268	97.01	2.06	2.00	1.94
Aug.	136	132	4	265	97.06	2.01	1.95	1.89
Sep.	136	126	10	229	92.64	1.82	1.68	1.56
Oct.	134	122	12	220	91.04	1.80	1.64	1.49
Nov.	134	120	14	219	89.55	1.83	1.63	1.46
Dec.	136	118	18	217	86.76	1.84	1.60	1.38
Jan. 2005	136	118	18	216	86.76	1.83	1.59	1.38
Feb.	136	110	26	215	80.88	1.95	1.58	1.28
Mar.	134	108	26	216	80.60	2.00	1.61	1.30
Apr.	136	112	24	219	82.35	1.96	1.61	1.33
May	136	114	22	226	83.82	1.98	1.66	1.39
June	136	134	2	244	98.53	1.82	1.79	1.77
Total	1624	1444	180	2754	1067.03	22.90	20.35	18.18
July 2005	137	134	3	259	97.81	1.93	1.89	1.85
Aug.	137	133	4	256	97.08	1.92	1.87	1.81
Sep.	134	125	9	230	93.28	1.84	1.72	1.60
Oct.	128	115	13	218	89.84	1.90	1.70	1.53
Nov.	129	110	19	215	85.27	1.95	1.67	1.42
Dec.	126	112	14	214	88.89	1.91	1.70	1.51
Jan. 2006	129	110	19	212	85.27	1.93	1.64	1.40
Feb.	137	114	23	220	83.21	1.93	1.61	1.34
Mar.	134	111	23	220	82.84	1.98	1.64	1.36
Apr.	137	116	11	223	84.67	1.92	1.63	1.38
May	139	117	12	230	84.17	1.97	1.65	1.39
Jun.	140	136	4	243	97.14	1.79	1.74	1.69
Total	1607	1433	154	2740	1069.48	22.97	20.45	18.28

Kanth and Srivastava, 1984; Reddy and Benarjee, 2006). Helminth infections commonly occur in almost all the organs of vertebrates. The parasites make their entry into the fish body and establish at particular sites and initiate the pathological processes. However, the degree of pathogenicity and the extent of damage depend on the intensity of infection.

The observations made on the biostatistical indices of the parasite (Fig. 1-4) revealed that the incidence of infection is more during June, July, March, April and May, whereas the incidence of infection is low during October to January and moderate from February to May. However, the intensity of infection was more during June, July, August and September (monsoon). During monsoon, the fish were more prone for parasitic infection and the density of infection was also higher during this period. During monsoon, the Index of infection was also more. The index of infection (Table 1) was, however, low during October to January (postmonsoon). These findings are in agreement with the observations made earlier by other investigators with respect to helminth parasites found in freshwater fishes, *Channa gachua*, *C. striatus* and *Anabas testudineus* (Satpute and Agarwal, 1974a; Shomorendra *et al.*, 2005; Reddy *et al.*, 2006). Though the water temperature does not have a direct impact (Jha *et al.*, 1992) on parasitic infection, it has some influence on the occurrence of infection in aquatic organisms (Chubb, 1977; Madhavi, 1979). Therefore, the ecological factors may have influence on the intrapopulation

density of the parasite; hence, the parasitic population was more during June to September. However, it was observed that 50% of mature and 50% of immature worms were found during December to March. It also agrees with the acanthocephalan infection of lake fishes of Michigan (Amin and Burrow, 1977), Gupta *et al.* (1984) have noticed a similar trend while studying the population dynamics of endohelminths in *Channa punctatus*. A similar trend was noticed earlier with *Lytocestus indicus* in *Clarias batrachus* in this region (Reddy *et al.*, 2006).

Rainfall had an adverse effect on the incidence of infection and low rainfall always favoured an increase of infection level in the piscian host. The temperature and rainfall play significant roles in the dispersion of the infective agents (Rao and Rao, 1996). The frequencies of infection and maturation of the parasites reach the peaks during the spring. It may be presumed that the mature worms are virtually eliminated during the summer and a new immature generation pressed into the host during early autumn. New infections always occur between autumn and spring. The worms were 50% mature and 50% immature during December, 75% mature during January to March and most of the worms get matured during summer. The gravid proglottides may disperse along with the run of water to spread the infection which might probably occur during the monsoon.

The body of *L. fossilis* is elongate, tapering anteriorly and rounded posteriorly. The infestation of this parasite disrupts the basic structural organisation of the stomach of the host. The parasite

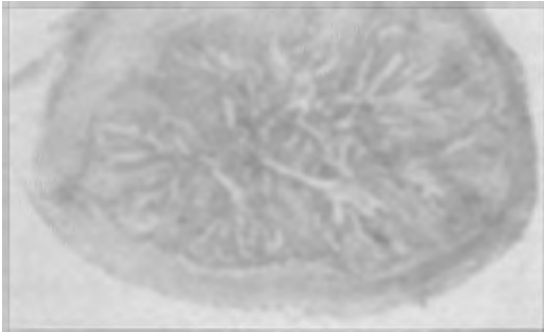


Fig. 5: Transverse section of the stomach of control fish

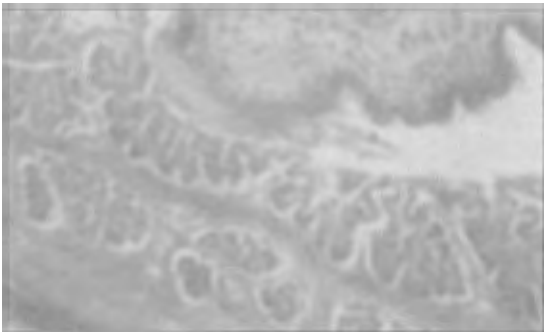


Fig. 6: Transverse section of the stomach of infected fish (shortening of villi)

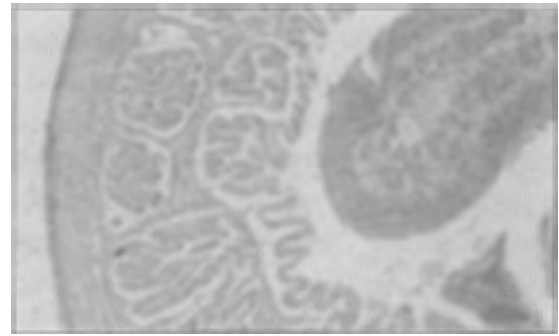


Fig. 7: Transverse section of the stomach of infected fish (damage of mucosa)

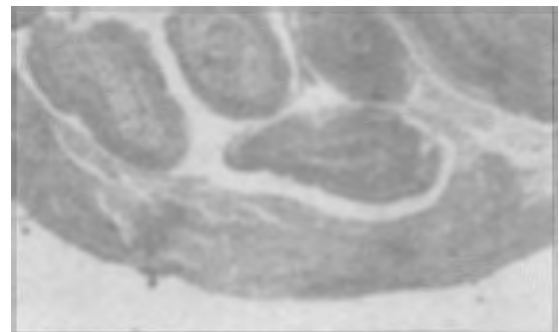


Fig. 8: Transverse section of the stomach of infected fish (degeneration of mucosa and submucosa)

penetrates deep into the stomach walls and reaches up to the muscularis layer. Despite this, there were no nodule formations. However, mechanical displacement and compression of tissue layers, such as mucosa, submucosa and muscularis layer, were noticed in the stomach of fish. The scolex was anchored to the stomach wall. *L. fossilis* brought about severe histopathological changes which include shortening of villi, thickening of muscular layer, destruction of villi, and the damage of both the submucous and mucous layers in the stomach of infected *H. fossilis*. A comparison with the control specimen (Fig. 5) shows that the parasite also caused the dilation of the blood vessels (Fig. 6-8) in the submucous membrane. Satpute and Agarwal (1974a) have observed histopathological changes in the

duodenum of *C. batrachus* due to the infection by a cestode parasite *Djombangia indica*, in which the effects were prominently noticed in the histopathology of the duodenum of infested fish; the muscularis layer becomes thick and the villi shorter. The enormous thickening of the muscularis layer of digestive tract might affect the peristalsis and also general metabolism, and consequently, the fish become sluggish and moribund. However, in contrast to the observations made by Satpute and Agarwal (1974a), in the present investigation, the parasite did not reach the muscularis layer. Therefore, the muscularis layer was intact and the rest of the layers of the intestine were extensively damaged by the parasite.

In the present study, the parasite was found lying in the stomach with its

anterior end pressed firmly into the host tissue and the host tissue surrounding the parasite. Thus, the parasite was found embedded in the stomach wall. Due to the excess pressure exerted by the scolex, at some places, the submucosa of stomach turned hyperplastic. It was observed at some places that a thin mucoid layer is present between the host tissue and the

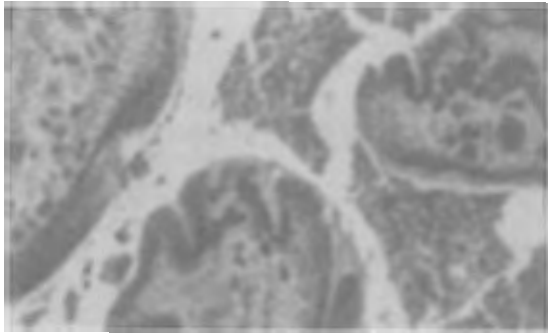


Fig. 9: Transverse section of the stomach of infected fish (further degenerative changes)

scolex. This deep penetration of parasite caused severe damage (Fig. 9). The stomach of *H. fossilis* has undergone drastic histological alterations due to the infection by *L. fossilis*. These histopathological changes include damage of the villi, inflammation, and fibrosis associated with hyperplasia and metaplasia. The epithelial necrosis was clearly noticed. These changes are in agreement with the earlier observations made by Rees (1967), Sircar and Sinha (1980), Kanth and Srivastava (1984), and Hiware and Garad (2002). Rees (1967) had observed inflammatory fibrosis associated with hyperplasia and metaplasia in a cestode infection. Sircar and Sinha (1980) observed degenerative changes that include hyperplasia and hypertrophy of the intestinal villi, vacuolation of submucous layer and proliferative changes which led to the

degeneration of various layers of the intestine. The shallow ulcers as found in the host fish due to *L. indicus* infection as reported earlier by Ahmad and Sanaullah (1979), however, was not noticed in the present study which might occur only after prolonged and chronic infection. This kind of histopathological changes in the host intestine by *L. fossilis* was reported by Kanth and Srivastava (1984). Sircar and Sinha (1980) have also documented these observations with the same parasite in tune with the earlier observations made by Stirewalt (1963). The histopathological changes in the stomach of *H. fossilis* due to helminth infestation and pathogenicity support the earlier reports of Stirewalt (1963), Rees (1967), Ahmad and Sanaullah (1979), Sircar and Sinha (1980), Kanth and Srivastava (1984), and Reddy and Benarjee (2006). The damage of the host tissues may be due to the secretion of lytic enzymes and penetrative damage caused by the parasite while establishing its position in the host.

A battery of histochemical tests employed on the infected and uninfected tissues of stomach revealed that the stomach layers contain vicinyl hydroxyl groups, weakly acidic sulphate mucosubstances, proteins such as disulphides, sulphhydryls, rich quantities of protein-bound amino groups, glycoproteins, lipids and phospholipids. The sections of stomach when subjected to alcian blue at 1.0 and 2.5 pH, mucous cells were moderately positive indicating the presence of weakly acidic sulphated mucosubstances, hyaluronic acid, sialomucins and sulphated mucosubstances. With alcian blue at pH 1.0, strong alcianophilia was observed by

a deep-blue colour in these cells. But a combined periodic acid Schiff's (PAS) and alcian blue technique exhibited some interesting results. With alcian blue (1.0 pH)/PAS, little quantities of acid mucopolysaccharides were revealed. All these tests gave a negative reaction of serosa, muscularis and submucosa in all the tissues.

In fishes, not much of information is available on the histochemical localization of important chemical substances, but some authors have contributed to the knowledge of histochemical nature of alimentary canal that include Reifel and Travill (1978, 1979), Hirji (1983), Chakravarthy *et al.* (1983), Sinha and Chakravarthy (1984),

and Woodward and Bergeron (1984). Apart from Bucke (1971) about the affinity of the mucous region to alcian blue and PAS, no serious attempts have been made in fishes to localize histochemistry of the muco-substance present in the alimentary canal, in general, and the stomach, in particular. In the present study, an attempt has been made to localize acid and neutral mucopolysaccharides in the stomach of both infected and uninfected fish (Table 2). However, in both the cases, the infected fish gave strong positivity (Fig. 10, 11) which indicates that the parasitic invasion enhances mucus secretion, which is a protective interaction of the host against parasitic infection. Reifel and Travill (1977) reported the complex

Table 2: Histochemical tests for stomach

Test	Result	
	Not infected	Infected
Periodic acid/Schiff (PAS)	++	+++
PAS/Saliva	++	++
Schiff's without oxidation	+	+
Acetylation/PAS	-	-
Deacetylation/PAS	++	++
Alcian blue (pH 1.0)	++	+++
Alcian blue (pH 2.5)	++	+++
Alcian blue (pH 1.0)/PAS	++	+++
Alcian blue (X pH 1.0)/PAS	++	+++
Alcian blue/Aldehyde fuchsin	++	++
Mercuric bromophenol blue	++	+++
Ninhydrin/Schiff's	++	+++
Ferric ferricyanide	++	++
Congo red	+	++
p-DMAB nitrate	-	-
KMnO ₄ /Alcian blue	++	++
Millon's reaction	+	+
Copper pthalocyanin	++	++
Sudan black B	++	+++

+++ Strongly positive; ++ Moderately positive; + Positive; - Negative

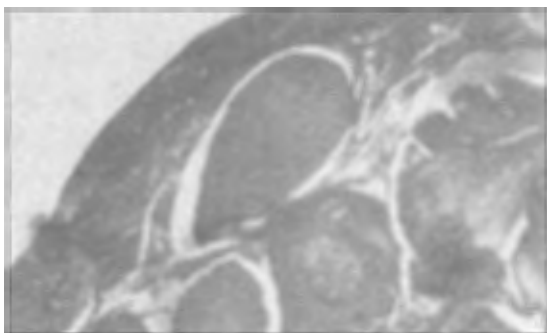


Fig. 10: *Transverse section of the stomach of infected fish indicating the presence of acid mucopolysaccharides*

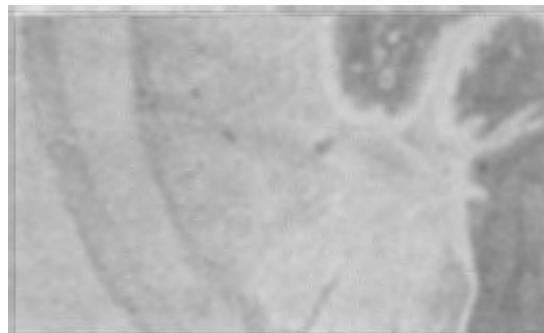


Fig. 12: *Transverse section of the stomach of infected fish indicating the presence of carbohydrates*

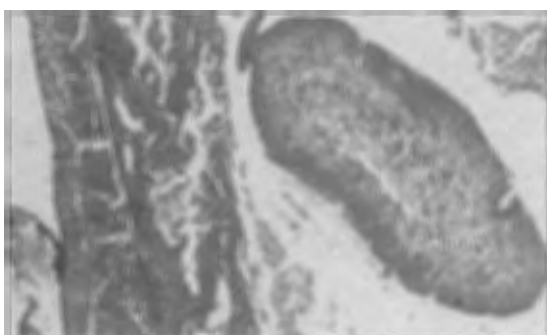


Fig. 11: *Transverse section of the stomach of infected fish indicating the presence of neutral mucopolysaccharides*

morphology and carbohydrate histochemistry in oesophagus and stomach of certain teleost fishes. They demonstrated the presence of sulfomucins, sialomucins and neutral mucosubstances. In the present study, PAS reaction yielded intense positivity in all the four layers of stomach. This reaction has suggested the occurrence of vicinyl hydroxyl groups in these tissues. Further, this reaction indicated the simultaneous occurrence of both acidic and neutral mucopolysaccharides. However, the infected stomach sections showed more positivity to PAS, suggesting intense carbohydrate nature (Fig. 12). The carbohydrates are the major source of energy to the host, although the energy yield is less. When these are consumed in the food material, get

converted into glucose, which enters the blood circulation after absorption. In the present study, the results of the histochemical tests revealed an increase in the amount of carbohydrates and glycogen in the stomach of infected fish. The increase of carbohydrates may be a sort of resistance developed by the host to the pathological condition.

The various layers of stomach responded very strongly to bromophenol blue indicating the occurrence of large quantities of proteins. The glands of the stomach were strongly positive while submucosa, muscularis layer and serous coat were moderately positive. The sections of stomach were positive to Millon's reaction and ferric ferricyanide that suggested the occurrence of tyrosine and sulphhydryl groups of proteins. KMnO_4 /alsian blue was employed which showed the presence of disulphides in the striated border of the stomach, wherein submucosa and muscularis layer have also shown a little quantity of these substances. Similarly, Congo red exhibited a positive reaction showing the occurrence of glycoproteins. On the other hand, tryptophan was found to be absent in all the regions of the stomach as indicated by a negative response to para-

dimethylaminobenzaldehyde (*p*-DMAB) nitrate method, a specific test for the identification of tryptophan-containing proteins.

The infected fish have shown a significant change in the distribution of protein content in the stomach. The bromophenol blue test has confirmed this. The occurrence of the other specific histochemical substances were demonstrated in the stomach by Millon's reaction, *p*-DMAB nitrate, ferric ferricyanide, KMnO₄/alcian blue, ninhydrin/Schiff's and Congo red. The lipid and phospholipid concentrations varied in infected and uninfected stomach which was confirmed by Sudan black B and copper phthalocyanin, respectively. The increase in the lipid content in the infected fish indicates that the parasites hampered the absorption mechanism, which in turn, deprived the host in retaining them. The present study may be an evidence that the lipid content generally increases in the host due to helminth infections.

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