CHAPTER – III
BIOCHEMISTRY
INTRODUCTION

The gastrointestinal tract (GI) is not only an organ for digestion, absorption and excretion, but also it is a residence site to many parasitic organisms. The regulation of helminth population in the hosts GI is a complex process, influenced by host immunological and nutritional status, age and breed of the animal (Von Brand, 1979). Immunological status of the host is very important for helminth infections, because GI is one of the largest immunological organs of the body and it serves as the first line of defense against orally administered antigens (e.g. feed protein or carbohydrates) and intestinal pathogens. Gut associated lymphoid tissues make up about 25% by weight of the gut mucosa and sub mucosa and thus constitute the largest extrathymic sites of lymphocytes (McBurney, 1993). Furthermore, it is very important interaction between helminth infection and nutrition. This interaction can be considered from two inter-related point of views (1) The adverse influence of the helminth infection on the hosts physiology and nutrition (2) The effect of the host nutrition on the helminth population i.e. their establishment, persistence and reproductive capacity (Coop and Holmes, 1996)

The first point of view (the impact of helminth infection on the hosts physiology and nutrition) has been the subject of numerous investigations over the past decade (Stephenson, 1993; Solomons, 1993; Solomons & Scott, 1994; Eduisinghe & Tomkins, 1995; Coop & Holmes, 1996; Knox 2000). The research on the complex interactions among host nutritional status and parasitic infection has mainly focused on the detrimental consequences of parasitic infections on host nutritional status and on
Nutritional deficiencies as a result of intestinal helminth infection have been the subject of several investigations (Hadju, et al., 1996; Lunn & Nothrop-Clewer, 1996). Intestinal helminthes may affect the nutritional status by causing increased nutrient loss, in addition to decreased food intake and nutrient absorption (Edirisinghe & Tomkins, 1995). Detailed investigations of the mechanisms of gastrointestinal dysfunction of the parasitized host have shown that the increased endogenous loss of protein into the gastrointestinal tract is a key feature, partly as a result of leakage of plasma protein but also from increased exfoliation of gut epithelial cells and mucoprotein secretion (Bown et al., 1991).

Curiously, the influence of host nutrition on helminth population (the second type of host-parasite interaction) has received relatively little attention and limited information is available only a few studies have examined the effects of nutrition on the parasitic response in the parasited host, and even fewer have considered the event occurring at the intestinal level, where absorption of nutrients occurs, intestinal parasites reside, and the gastrointestinal associated tissues play role in directing both the local and the more systemic responses. Bundy & Golden (1987) described mechanisms by which host nutrition might influence helminth infection: nutritionally mediated changes in the helminth environment or nutritionally mediated changes in host defence and malnutrition of the parasites. Gastrointestinal helminthes have very specific physico-chemical requirements of their host gut environment, and nutritionally mediated changes might have a direct influence on the parasite population (Crompton & Nesheim, 1976).

Parasitic worms compete for energy reserves with their fish host (Meakin, 1974; Tierney, 1991; Walkey and Meakins, 1970). There is a clear variation among hosts and parasites in the extent of such effects.
Parasitic worms compete for energy reserves with their fish host (Meakin, 1974; Tierney, 1991; Walkey and Meakins, 1970). There is a clear variation amongst hosts and parasites in the extent of such effects. This may be due to differences in the extent to which the parasites compromises nutrient reserves.

The overall aim of the present study was to examine the relationship between cestode infection and nutrient reserves in fresh water fish *Clarias batrachus* (Linnaeus), *Mastacembelus armatus* (Lacepede) and *Wallago attu* (Bloch) in relation to infection with cestode parasite, *Lytocestus teesgaonensis* n.sp, *Circumoncobothrium hemlatae* n.sp and *Gangesis bendsurensis* n.sp.
PROTEIN

Introduction

The main sources of energy reserves in fish are protein, in contrast to mammals in which carbohydrate and lipid are more important. This is perhaps due to the following factors: (1) The diet of fish generally consist of high protein, and the fish metabolism is well adapted to deal with such a diet: (2) Unlike mammals, fish have the ability to eliminate nitrogenous waste rapidly and continuously: (3) Specific activities of lysosomal enzymes which are involved in protein breakdown are greater in fish than in mammals (Chellappa s. 1988).

The relative contributions of protein to energy production in fish depend on number of factors such as the species involved, nutritional state and environmental temperature. In Salmonids, during routine activity, more than 40% of energy production is considered to be due to amino acid catabolism (Cho, C.Y and Kaushik, S.J. 1985). Proteins have many different biological functions, they are ubiquitous in their distribution and there is really no satisfactory scheme of classifying them. The largest groups of proteins are the enzymes, of which nearly 2000 different ones have been described. Proteins are also involved in contractile systems, in Transport, as protective agents, toxins hormones and amino acid reserves and as important structural components. In parasitic helminthes, protein usually constituents between 20 and 40% of the dry weight, but values as high as 70% of the dry weight.

Proteins are absorbed by the parasites by diffusion and transmission. It is naturally available from the host tissue as there is no media to acquire proteins in parasites these protein are naturally available from the host tissue. These worms utilize different degree of protein for producing energy. Literature reveals that the parasites able to adopt
themselves to the parasitic mode of life due to protein metabolism. These parasites excrete amino acids as their end product. For these worm protein are very essential, therefore parasites do not depend on the host’s dietary protein. If host proteins are removed from the diet, even then amino acids occur in the intestinal lumen. When rats were given protein free diet then also *Hymenolepis diminuta* (Phifer, 1960 a, b, c; Fisher, 1965) developed fantastically. Mettrick and Munro (1965) where as low protein diet given to rats, while favoring the migratory phase of *Nipprotrogyulus basiliensis* proved some what unfavourable to the adult worm (Clarke, 1968) but indirect rather than direct influence was probably involved Clarke, (1968).

The essential and non-essential amino acids are required, proteins are also digested at the host parasites interface by the activity of proteolytic enzyme and the cestode tegument secretes these amino acids are absorbed by active transport but some amino acid tries to inhibit the uptake of others. Where as some have no effect. In *Hymenolepis diminuta* there was interference by amino acids if the diet contained an incomplete protein or there was imbalance in dietary amino acids (e.g. Casein or Zein). It may be possible (Mettrick 1971) that inhibition might be due to the tendency of cestode to acquire Carbohydrates (Polyfunctional Carrier System) or changes in the molar ratios of intestinal amino acids. Though other scientist (Good Child and Dennis 1965, Hopkins and Young, 1967) did not get the same to all that due to the parasitic life led by *Hymenolepis diminuta* the composition of the amino acid pool and other parameters is changed (Mettrick, 1971b) this can have an influence on the response of the parasites to an altered host diet. Further studies reveal that the molar ratio of amino acids differ with differing dietary proteins, as well as in various pattern of the gut (Mettrick, 1970) their molar ratios are factors in determining host.
Many workers have gone through the studies of protein metabolism in various cestode parasites. They are amino acid oxidizes in *Hymenolepis diminuta* and some of changes in host, physiology by Daugherty (1955). Studies on protein complexes of the cestode *Raillientina cesticillus* by Kent (1957 b), amino acids in the hydatid fluid plasma layer of *Echinococcus* by Karvavica et al; (1959 b), urea formation and urea cycle present in the cestode, *Hymenolepis diminuta* by Campbell (1963a) absorption and digestion of amino acid in the tapeworm *Anoplocephala magna* by Karvavica et al; (1959a) and the estimations in *Phyllobothrium foliatum* has worked out. Sidorov (1980) made comparative investigation of proteins composition and *Eubothrium crassum* and the host. Ganzalez (1978) worked on serum in animal, which were infected, with parasitic helminthes.

**PROTEIN ESTIMATION**

**Principle:** (Lowry’s Method)

The first step involves formation of a copper protein complex in alkaline solution. This complex then reduces a phosphomolybdic-phosphotungstate reagent to yield an intense blue colour. This assay procedure is much more sensitive than the burette method but is more time consuming. The only precaution to be observed when performing this assay concern addition of the Folin’s reagent. This reagent is stable only at acidic pH; however, the reduction indicated above occurs only at pH 10. Therefore, when Folin’s reagent is added to the alkaline copper protein solution, mixing must occur immediately so that the reduction can occur before the phosphomolybdic-phosphotungstate (Folin’s)
Reagents:

1) Lowry’s ‘A’ solution: Dissolve 2gms of Na₂CO₃ in 100ml of NaoH solution (400mg of NaoH in 100ml of distilled water = 0.1 N NaoH)

2) Lowry’s ‘B’ solution: B1 = 1% Copper Sulphate (CuSO₄), B2 = 2% Sodium Citrate

3) Lowry’s ‘C’ solution: 1ml of B1 + 2ml of B2 diluted to 100ml with Lowry’s ‘A; solution

4) 10% TCA solution: Dissolve 4gm of Trichloroacetic acid (TCA in 100ml of distilled water).

5) 1 N NaoH: Dissolved 4gm of NaoH (Sodium Hydroxide) in 100ml of distilled water.

6) Folin Phenol reagent: This reagent is diluted by distilled water in preparation of 1:1.

7) Standard stock solution: 10mg of BSA (Bovine Serum Albumin) in 10ml of 1 N NaoH.

8) Blank solution: 0.1 ml (1 N NaoH) + 4ml of Lowry’s ‘C’ solution + 0.4ml of Folin Phenol.

Material and Method (Lowry’s method)

The collected worms were dried on the blotting paper to remove excess of water and the wet weight of tissue was recorded. This material was transferred into previously weight watch glass and kept in oven at 58 to 60°C for twenty-four hours, for drying and then the dry weight of material, prepared a powder with the help of mortar pestle. The powder weight 100.00mg on a sensitive balance and then it is homogenized in a mortar and pestle with 1 ml of 10% TCA solution and transferred in centrifuge tube. Then it is centrifuged for 15 minutes at 3000 RPM.
Supernatant is discarded and residue is taken and added 10ml of N NaOH for dissolving the ppt. Then taken 0.1 ml above solution and add 4 ml Lowry’s ‘C’ solution, then it add 0.4 ml of Folin Phenol reagent and this test tubes was kept for half an hour at dark place, until the blue colour developed. Then this colour was read on the colorimeter with 530μm filter to note the optical density to calculate the protein content.

Bovine Serum albumin was used for standard (10mg of BSA in 10ml of 1 N NaOH) and read the optical densities.
Protein Estimation of *Circumoncobothrium hemlatae* n.sp.

Results

Fifteen fresh water fishes *Mastacembelus armatus* (Lacepede) were brought to the laboratory and dissected carefully. Ten of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the Protein content in them using Lowry’s method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Circumoncobothrium hemlatae* n.sp.

Table 1: Mean (± SD) values of Protein content in *Circumoncobothrium hemlatae* n.sp. Infected and Uninfected intestinal tissue in the *Mastacembelus armatus* (Lacepede).

<table>
<thead>
<tr>
<th>Protein content</th>
<th>Cestode parasite</th>
<th>Intestinal tissue</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><em>Circumoncobothrium hemlatae</em> n.sp.</td>
<td>Infected</td>
</tr>
<tr>
<td>16.48 ± 0.98 mg/100mg</td>
<td>10.29 ± 0.24 mg/100mg</td>
<td>15.14 ± 0.84 mg/100mg</td>
</tr>
</tbody>
</table>

Table 1 shows the mean percentage of protein in *Circumoncobothrium hemlatae* n.sp. (16.48 ± 0.98) was lower than in the infected (10.29 ± 0.24) and uninfected (15.14 ± 0.84) intestinal tissues. The result when compared showed that the worm *Circumoncobothrium*
The result when compared showed that the worm *Circumoncobothrium hemlatae* n.sp. obtained 4.85% of protein from the intestinal tissue of its respective host; the protein content in the infected intestinal tissue was low as compare to the normal fish intestinal tissue of *Mastacembelus armatus* (Lacepede).

Goodchild (1961) reported protein content in *Hymenolepis diminuta* isolated from adequately fed rats contained 32% protein, while the corresponding figure for worms taken from starved rats was 59.5%.

In the present study protein content in *Circumoncobothrium hemlatae* n.sp. is more than the infected and uninfected intestinal tissue of their host (*Mastacembelus armatus*).
Protein Estimation of *Lytocestus teesgaonensis* n.sp.

**Results**

Ten fresh water fishes *Clarias batrachus* (Linnaeus) were brought to the laboratory, killed by pithing brain and dissected carefully. Six of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the protein content in them using Lowry’s method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Lytocestus teesgaonensis* n.sp.

**Table 2**: Mean (± SD) values of Protein content in *Lytocestus teesgaonensis* n.sp. infected and uninfected intestinal tissue in the *Clarias batrachus* (Linnaeus)

<table>
<thead>
<tr>
<th>Protein content</th>
<th>Cestode parasite</th>
<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Lytocestus teesgaonensis</em> n.sp.</td>
<td>Infected</td>
</tr>
<tr>
<td>27.05 ± 0.75 mg/100mg</td>
<td>10.16 ± 0.75 mg/100mg</td>
<td>19.25 ± 1.49 mg/100mg</td>
</tr>
</tbody>
</table>

Table 2 shows mean percentage of protein in *Lytocestus teesgaonensis* n.sp. (27.05 ± 0.75) was higher than in the infected (10.16 ± 0.75) and uninfected (19.25 ± 1.49) intestinal tissues of *Clarias batrachus* (Linnaeus).
Protein Estimation of *Gangesia bensurensis* n.sp.

Result

Twelve fresh water fishes *Wallago attu* (Bleeker) were brought to the laboratory and dissected carefully, out of which six of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the Lipid content in them using **Lowry's method**

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Gangesia bensurensis* n.sp.

**Table 3:** Mean (± SD) values of Protein content in *Gangesia bensurensis* n.sp, infected and uninfected intestinal tissue in the *Wallago attu* (Bloch).

<table>
<thead>
<tr>
<th>Protein content</th>
<th>Cestode parasite</th>
<th>Intestinal tissue</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gangesia bensurensis n.sp</td>
<td>Infected</td>
<td>Uninfected</td>
<td></td>
</tr>
<tr>
<td>20.19 ± 0.80 mg/100mg</td>
<td>9.20 ± 0.74 mg/100mg</td>
<td>16.32 ± 1.58 mg/100mg</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows the mean percentage of protein in *Gangesia bensurensis* n.sp. (20.19 ± 0.80) was higher than in the infected (9.20 ± 0.74) and uninfected (16.32 ± 1.58) intestinal tissues. The result when compared
Fig. No. 6. Protein content in *C. hemlatae* n.s.p., infected and uninfected intestinal tissue of *Mastacembelus armatus*

![Bar chart showing protein content in C. hemlatae n.s.p., infected and uninfected tissues.](image)

Fig. No. 7. Protein content in *L. teesgaonensis* n.s.p., infected and uninfected intestinal tissue of *Clarias batrachus*

![Bar chart showing protein content in L. teesgaonensis n.s.p., infected and uninfected tissues.](image)

Fig. No. 8. Protein content in *G. bendsurensis* n.s.p., infected and uninfected intestinal tissue of *Wallago attu*

![Bar chart showing protein content in G. bendsurensis n.s.p., infected and uninfected tissues.](image)
showed that the worm *Gangesia bendsurensis* n.sp. has a high protein content than then intestinal tissue, were as protein content in the infected intestinal tissue was lower as compare to the normal fish *Wallago attu* (Bloch).

The observed changes in the proximate composition of the intestinal tissue of infected fish, simulating the picture of depletion of energy reserves to meet the energy requirements of both the host and the parasite, and because of the inability of the host to replenish the reserves by way of food owing to the diminished absorptive power of the intestine caused by the worms, especially in heavy infection. It is noteworthy that heavy infection causes reduction in the protein value of the fish.
GLYCOGEN

Introduction

The carbohydrate, which include low molecular weight (LMW) sugar and various cell wall and storage non-starch polysaccharides (NSP) are the most important energy sources for animal (Bach Knudsen, 1997). It is now clear that dietary carbohydrates are diverse group of substances with varied fates in the gastro-intestinal tract and physiological properties of differing important to animal health (Cummings & Englyst, 1995). The composition of the carbohydrate fraction influences the digestion and absorption process of carbohydrate and other nutrients in various parts of the gastro-intestinal tract (Bach Knudsen & Jorgensen, 2001). It has profound influence on the secretory response of the gut to feed intake (Low, 1989), the volume flow (Bach Knudsen et al., 1993), the mucosal architecture (Brunsyaard, 1998), composition of the gut flora (Jensen & Jorgensen, 1994) and the development of the gastrointestinal tract (Jorgensen et al., 1996). Studies on the influence of carbohydrates on growth of parasite and establishment have been limited mainly to cestode and acanthocephalan (Crompton & Nesheim, 1982; Nesheim, 1984).

The main carbohydrate reserve in parasitic helminth is “Glycogen” which is a typical energy reserve of helminthes inhabiting biotopes with low oxygen tension. The main polysaccharide in cestode is glycogen, closely resembling mammalian glycogen. The early work of Bernard Claude (1859) and Foster (1856) demonstrated the occurrence of glycogen in helminthes.

Glucose is said to be an important energy source for helminthes inhabiting the alimentary tract of vertebrates. It is generally believed that helminthes absorbed glucose against a concentration gradient and use their
endogenous carbohydrates only as an energy source when unobtainable from its media.

Daugherty et al. (1956), Fairbairn, Werthein, Harpur and Read (1961), Markov (1939, Read, Rothman (1957 b) have pointed out that the cestode have high rate of transport of exogenous glucose into the body, high rate of utilization of endogenous carbohydrate and high rate of glycogenesis, the cestode parasites as a group store relatively large quantities of polysaccharides, which in most cases have assumed to be glycogen, Read (1949 b), Reid (1942).

The literature at our disposal discloses that the carbohydrates play an important role in cestode, than most of other parasitic worms, which are distinguished by different growth patterns. These carbohydrates are utilized exogenously, their mechanism of the uptake is not known but the evidence indicates that the active mechanism undoubtedly is entangled in the carbohydrate transport of helminthes. In cestode Hymenolepis diminuta the absence or restriction of availability of dietary carbohydrates resulted in decreased establishment, growth and reproduction (Robert, 1980; Keymer et al; 1983 a). Dunkley and Metrick (1969) have found that in rat, fed by sucrose containing diet were found smaller Hymenolepis diminuta worms than in rats on glucose or maltose diets and Roberts and Platzer (1967) pointed that absence of carbohydrates in the rat diet injured the worms reproduction system. Additionally, it was found that Hymenolepis diminuta worms from high starch diet rat were bigger than low starch rats, which are bigger than from sucrose diet rats (Roberts, 1966), T. taeniae formis (Von Brand et al., 1964) and Caliobothrium vertiallatum (Fisher, 1965) as well as the Acanthocephalan Polymorphus minutus (Crompton and Lackwood, 1968) absorb glucose against concentration gradients and the survival, growth and reproduction of Moniliformis moniliformis are dependent on the carbohydrates liberated at
concentration gradients and the survival, growth and reproduction of *Moniliformis moniliformis* are dependent on the carbohydrates liberated at different rates from the intestinal tract of the host during digestion and absorption (Nesheim et al., 1977, 1978). In addition, Parshad et al. (1980) have found *Moniliformis moniliformis* worm dry mass at five weeks was greatest to smallest in order, maltose, fructose and galactose 3% diets fed rats. Further more typical inhibitors of active transport e.g. philoridizin interferes effective with the glucose uptake of cestodes (Phifer, 1960 a., Lauris, 1961., Von Brand et al., 1964) and at least in *Taenia taeniaeformis*, glucose absorption has an absolute sodium requirement (Von Brand et al., 1964, Von Brand and Gibbs 1966), apparently corresponding closely to the sodium pump of vertebrate tissue.

The glycogen content of cestodes fluctuates over a wide range due to factors such as season, physiological state of the host, the time of autopsy, strain of the host, rate of infection and to some extent on the stage of the life cycle, In few cestodes developmental history changes, the growth of parasites is rapid at the first 18-24 hrs and then slows down, even if the concentration is very high as it was the early phase. It has been observed the same in *Hymenolepis diminuta* increase from 15% of the dry substance in 5 and 7 day old worms to 37% in 13 and 16 days old specimens (Mettric and Cannon, 1970),, it has been observed that the uptake of glucose is very much effective when Co$_2$ is present in the surrounding than it is absent.

A number of workers have reported the content of glycogen in different helminth parasites. In *Taenia taeniaeformis* 2.5 to 5.6% (Brand von et al., 1968), in *Hymenolepis diminuta* from 1.1 to 9.3% (Fairbairn et al., 1961) 16% in *Schistocephalus solidus* larva (Hopkins, 1950) respectively.
ESTIMATION OF GLYCOGEN (DeZwaan and Zandee (1972) method)

Principle:

The Anthrone reaction is a rapid method for the determination of hexoses, aldopentoses and hexuronic acids, either free or present in polysaccharides. The blue green solution shows absorption maximum at 620μm.

Reagents:

1) 30% KOH: Dissolved 30gms of KOH (Potassium Hydroxide) in 100ml of distilled water.

2) 2% Na₂SO₄ Solution: Dissolved 2 gms of Sodium Sulphate (NaSO₄) in 100ml of distilled water.

3) 100% ethyl solution

4) Anthrone reagent: Dissolved 150mg of Anthrone in 100ml of concentrated H₂SO₄ (88%).

5) Standard stock solution: 100mg of glucose in 100ml of distilled water.

6) Blank solution: 1ml distilled water + 5ml Anthrone reagent.
Material and Method

Glycogen Estimation DeZwaan and Zandee (1972) method

The collected worms were dried on the blotting paper to remove excess water and transferred in a previously weighed watch glass, weighted on a sensitive balance.

The wet weight of the tissue taken and kept in oven at 58 to 60°C for twenty-four hours to make the material dry. The dry weight of the material was taken and prepared the powder. The powder was weighted 100mg on a sensitive balance and was homogenized by mortal pestle adding 1ml of 30% KOH to it and transferred in centrifuge tube kept in boiling water bath for 3 to 5 minutes, cooled at room temperature, then adds 0.2ml of 2% Na₂ So₄ solution. Add to the same 6ml of absolute alcohol by stirring with a glass rod and keep it in refrigerator overnight for formation of carbohydrate ppt. This carbohydrate ppt solution centrifuged for 15minutes at 3000 RPM and discards the supernatant (glycogen settled at bottom) and dissolve residue by adding 10ml of distilled water bath for 10 minutes and immediately cooled and reading were taken with help of Colorimeter at 620μm filter.

Similarly for standard glucose concentration was used (100mg of glucose in 200ml of distilled water) and read the optical densities.
Glycogen Estimation of *Circumoncobothrium hemlatae* n.sp.

Results and Discussion

Fifteen fresh water fishes *Mastacembelus armatus* were brought to the laboratory and dissected carefully. Ten of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the Glycogen content in them using DeZwaan and Zandee (1972) method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Circumoncobothrium hemlatae* n.sp.

Table 4: Mean (± SD) values of Glycogen content in *Circumoncobothrium hemlatae* n.sp. infected and uninfected intestinal tissue in the *Mastacembelus armatus* (L.).

<table>
<thead>
<tr>
<th>Glycogen content</th>
<th>Cestode parasite</th>
<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Circumoncobothrium hemlatae</em> n.sp.</td>
<td>Infected</td>
<td>Uninfected</td>
</tr>
<tr>
<td>21.80 ± 0.26 mg/100mg</td>
<td>34.51 ± 2.52 mg/100mg</td>
<td>36.51 ± 1.25 mg/100mg</td>
</tr>
</tbody>
</table>

Table 4 shows the mean percentage of glycogen in *Circumoncobothrium hemlatae* n.sp. (21.80 ± 0.26) was higher then in the infected (34.51 ± 2.52) and uninfected (36.51 ± 1.25) mg/100mg intestinal tissues. The result when compared showed that the worm *Circumoncobothrium hemlatae* n.sp. obtained 3.99 % of glycogen from
the intestinal tissue of its respective host; the glycogen content in the infected intestinal tissue was low as compare to the normal fish *Mastacembelus armatus* (Lacepede).

It is now well established that glycogen is the main reserve food product in cestodes and Markov (1939, 1943) used the glycogen as criteria of starvation or nutrition.

Brand von (1973) suggested that the tapeworm obtain their nutrient supply either from intestinal mucosa or exocrine-interic circulation of the host. Hence it is considered view of the author that the observed fall result in glycogen content in the infected host tissue might have occurs due to physiological dysfunction occurring in the intestinal tissue of host which thereby results into decrease rate of glucose supply to the intestine.
Glycogen Estimation of *Lytocestus teesgaonensis* n.sp.

Results and Discussion

Ten fresh water fishes *Clarias batrachus* (Linnaeus) were brought to the laboratory, killed by pithing brain and dissected carefully. Six of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the glycogen content in them using DeZwaan and Zande (1972) method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Lytocestus teesgaonensis* n.sp.

**Table 5**: Mean (± SD) values of glycogen content in *Lytocestus teesgaonensis* n.sp, Infected and Uninfected intestinal tissue in the *Clarias batrachus* (L.).

<table>
<thead>
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<th>Glycogen content</th>
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<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Lytocestus teesgaonensis</em> n.sp.</td>
<td>Infected</td>
</tr>
<tr>
<td>33.2 ± 3.12 mg/100mg</td>
<td>15.78 ± 1.05 mg/100mg</td>
<td>21.57 ± 2.10 mg/100mg</td>
</tr>
</tbody>
</table>

Table 5 shows mean percentage of glycogen in *Lytocestus teesgaonensis* n.sp (33.2±3.12) was higher than in the infected (15.78 ± 1.05) and uninfected (21.57 ± 2.10) intestinal tissues of *Clarias batrachus* (Linnaeus).
Glycogen Estimation of *Gangesia bendsurensis* n.sp.

**Results and Discussion**

Freshwater fishes *Wallago attu* (Bloch) were brought to the laboratory and dissected carefully. The infected, uninfected and intestine were also collected to find the Glycogen content in them using DeZwaan and Zande (1972) method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Gangesia bendsurensis* n.sp.

**Table 6:** Mean (± SD) values of glycogen content in *Gangesia bendsurensis* n.sp, infected and uninfected intestinal tissue in the *Wallago attu* (Bloch).

<table>
<thead>
<tr>
<th>Glycogen content</th>
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<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Gangesia bendsurensis</em> n.sp</td>
<td>Infected</td>
</tr>
<tr>
<td>29.40 ± 1.05 mg/100mg</td>
<td>21.50 ± 0.26 mg/100mg</td>
<td>25.49 ± 0.22 mg/100mg</td>
</tr>
</tbody>
</table>

Table 6 shows the mean percentage of glycogen in *Gangesia bendsurensis* n.sp, (29.40±1.05) was higher than in the infected (21.50±0.26) and uninfected (25.49±0.22) intestinal tissues. The result when compared showed that the worm *Gangesia bendsurensis* n.sp, obtained 3.99 % of Glycogen from the intestinal tissue of its respective
Fig. No. 9. Glycogen content in *C. hemlatae* n.sp., infected and uninfected intestinal tissue of *Mastacembelus armatus*

Fig. No. 10. Glycogen content in *L. teesgaonensis* n.sp., infected and uninfected intestinal tissue of *Clarias batrachus*

Fig. No. 11. Glycogen content in *G. bensurensis* n.sp., infected and uninfected intestinal tissue of *Wallago attu*
host; the glycogen content in the infected intestinal tissue was low as compare to the normal fish *Wallago attu* (Bloch).

The glycogen reserve is in fact a sensitive indicator of variations in metabolic conditions is well shown by the experiments of Brand (1933) and Reid (1942) the former found tha in *Taenia marginata* the glycogen content was increased from 4.99% to 8.38% when the host dog was fed on a carbohydrate- rich diet. Conversely Reid found the glycogen reserved fell by over 90 per cent in *Raillietina cesticillus* after 20 hours starvation of the host fowl.
ESTIMATION OF LIPIDS

Introduction

Cestodes are incapable of de novo synthesis of nonvolatile saturated and unsaturated fatty acids (Meyer et al., 1966, Jacobsen and Fairbairn 1967) and have been shown to rely upon their hosts to supply fatty acids for lipid biosynthesis. Thus, the fatty acids of these worms reflect to varying degrees of host intestinal contents (Ginger and Fairbairn, 1966a; Overturf and Dryer, 1968; Botero and Reid, 1969).

Lipids are heterogeneous group of compound with similar physical properties, being relatively insoluble in water but soluble in organic solvents. The total lipid content of helminth parasites is very variable, but is usually between 10-305 of the dry weight. Lipids have a variety of functions in tissues.

There is a considerable variation in lipids from species to species, in the degree of lipid content. Variation is also seen in the segments and region of the worms being experimented, thus total lipid to be some what meaning less, unless the degree of maturity is known. The lipid content of some species grown in different hosts may vary substantially. In *Hymenolepis diminuta* the lipid tend to be more abundant in the most posterior proglottids (Feirbairn Wetheim, Harpur and Schiller, 1961).

In older proglottids the higher content of lipid has led to the view that much of this lipid largely represents waste products, of metabolism (Brand T, Von, 1952). One of the more unusual features of the composition of cestode lipid in the fact that unsaponifiable material and phospholipids often account for more then 20% of the total lipids.
Principle:
Lipid contains a smaller proportion of oxygen than is present in the other types of food stuffs and therefore provide, per gram, more combustible carbon and hydrogen. Then energy released in oxidation of one gram of fat is over twice that obtained from the same weight of either carbohydrate or protein. They are roughly divided into those which serve primarily as a store of oxidisable substance and those, which are part of the structural, like methanol, chloroform therefore they are estimated.

Reagents:
1) 30% KOH: Dissolved 30gms of KOH (Potassium Hydroxide) in 100 ml of distilled water.
2) 2% Na₂SO₄ solution: Dissolved 2 gms of Sodium Sulphate (NaSO₄) in 100 ml of distilled water.
3) 100 % ethyl solution
4) Anthrone reagent: Dissolve 150 mg of Anthrone in 100 ml of Conc H₂SO₄(88%).
5) Standard stock solution: 100 mg of glucose in 100 ml of distilled water.
6) Blank solution: 1 ml distilled water + 5 ml Anthrone reagent.

Material and Method
Barnes and Blackstock (1973) method
The collected worms were dried on the blotting paper to remove excess water and wet weight of tissue was recorded. This material was transferred into previously weight watch glass and kept in oven at 58 to 60°C for twenty hours for drying. The dried weight of material was recorded and powder was prepared with the help of mortar pestle, 100 mgs of the tissue was weighted on a sensitive balance, homogenized in a
mortar and pestle by adding 10 ml of 2:1 chloroform: methanol solution, filter the mixture by Whatman filter paper No.41. Then 1 ml of filtrate was pipette out, dried for 3-4 days at room temperature, then 1ml of concentrated H₂SO₄ was added and kept in boiling water bath for 10 minutes and cooled rapidly under water, 0.2 ml of above solution is added in 5ml of vanilline reagent and the test tube were kept for half an hour at room temperature until the pink colour develop. The optical density was calculated by using calorimeter with 530µm filter to note the lipid content.

For the standard of lipid, 5mg of cholesterol is added in 100ml of 2:1 chloroform: methanol. Take 1ml of this solution and dried for two days at 37°C. Then 1ml of concentrated H₂SO₄ boil in water bath for 10 min and cool. Then add 5ml of vanilline reagent and prepare the 10 test tube stock solution ranging from 0.01 to 0.1 ml and read the optical densities.
Lipid Estimation of *Circumoncobothrium hemlatae* n.sp.

**Results and Discussion**

Fifteen fresh water fishes *Mastacembelus armatus* were brought to the laboratory and dissected carefully. Ten of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the lipid content in them using **Barnes and Blackstock (1973) method**

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Circumoncobothrium hemlatae* n.sp.

**Table 7:** Mean (± SD) values of lipid content in *Circumoncobothrium hemlatae* n.sp. infected and uninfected intestinal tissue in the *Mastacembelus armatus* (L).

<table>
<thead>
<tr>
<th>Lipid content</th>
<th>Cestode parasite</th>
<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Circumoncobothrium hemlatae</em> n.sp.</td>
<td>Infected</td>
</tr>
<tr>
<td>5.98 ± 1.38 mg/100mg</td>
<td>6.48 ± 0.30 mg/100mg</td>
<td>8.32 ± 0.48 mg/100mg</td>
</tr>
</tbody>
</table>

Table 7 shows the mean percentage of glycogen in *Circumoncobothrium hemlatae* n.sp. (5.98 ± 1.38) was lower than in the infected (6.48 ± 0.30) and uninfected (8.32 ± 0.48) intestinal tissues. The result when compared showed that the worm *Circumoncobothrium*
*hemlatae* n.sp. obtained 2.32% of lipid from the intestinal tissue of its respective host; the lipid content in the infected intestinal tissue was low as compare to the normal fish *Mastacembelus armatus* (L).
Lipid Estimation of *Lytocestus teesgaonensis n.sp*

Results and Discussion

Ten fresh water fishes *Clarias batrachus* (Linnaeus) were brought to the laboratory, killed by pithing brain and dissected carefully. Six of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the lipid content in them using **Barnes and Blackstock (1973)** method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin.

**Table 8:** Mean (± SD) values of lipid content in *Lytocestus teesgaonensis* n.sp, infected and uninfected intestinal tissue in the *Clarias batrachus* (L.).

<table>
<thead>
<tr>
<th>Lipid content</th>
<th>Cestode parasite</th>
<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Lytocestus</em></td>
<td>Infected</td>
</tr>
<tr>
<td></td>
<td><em>teesgaonensis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.sp.</td>
<td></td>
</tr>
<tr>
<td>6.22 ± 1.50 Mg/100mg</td>
<td>3.52 ± 0.20 mg/100mg</td>
<td>4.58 ± 0.10 mg/100mg</td>
</tr>
</tbody>
</table>

Table 8 shows mean percentage of glycogen in *Lytocestus teesgaonensis* n.sp (6.22 ± 1.50) was higher then in the infected (3.52 ± 0.20) and uninfected (4.58 ± 0.10) intestinal tissues of *Clarias batrachus* (L.).

The present study co-relates to the results recorded by Vykhrestiuk et al. (1981) were they observed cestode parasites *Raillientina tetragona* and *Raillientina echinobothria* infection affects the lipid content of
chicken intestine resulting in the decrease of triglycerides on oleic acid quantities and the increase of the amount of free acids and satiric acids.

The lipid content is more in the parasitic tissue as compared to their host. It seems that the parasites taking benefit from its host and is thus diabsorbing most of lipid content from the host. The parasites are fulfilling its needs from the host and it is in a way disturbing the development of the host. This high level of lipid content may be because the parasites often absorbing the lipid store from the further processes and lipid exhausted very slowly.
Lipid Estimation of *Gangesia bendsurensis* n.sp.

Results and Discussion

Twelve fresh water fishes *Wallago attu* (Bleeker) were brought to the laboratory and dissected carefully, out of which six of them were infected with cestode parasites; small pieces of infected and uninfected intestines were also collected to find the lipid content in them using **Barnes and Blackstock (1973) method**.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification. These were later stained with Harris Haematoxylin and identified as *Gangesia teesgaonensis* n.sp.

**Table 9**: Mean (± SD) values of lipid content in *Gangesia teesgaonensis* n.sp. Infected and uninfected intestinal tissue in the *Wallago attu* (B.).

<table>
<thead>
<tr>
<th>Lipid content</th>
<th>Cestode parasite</th>
<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Gangesia</em></td>
<td>Infected</td>
</tr>
<tr>
<td></td>
<td><em>bendsurensis</em> n.sp</td>
<td>9.20 ± 0.28</td>
</tr>
<tr>
<td>5.60 ± 0.37</td>
<td>mg/100mg</td>
<td>mg/100mg</td>
</tr>
</tbody>
</table>

Table 9 shows the mean percentage of lipid in *Gangesia bendsurensis* n.sp., (5.60 ± 0.37) was lower than in the infected (9.20 ± 0.28) and uninfected (6.82 ± 0.38) intestinal tissues. The result when compared showed that the worm *Gangesia bendsurensis* n.sp., obtained shows that
Fig. No. 12. Lipid content in *C. hemlatae* n.sp., infected and uninfected intestinal tissue of *Mastacembelus armatus*

![Bar chart showing lipid content in C. hemlatae n.sp., infected and uninfected intestinal tissue of Mastacembelus armatus.](chart1)

- **C. hemlatae n.sp.**
- Infected
- Uninfected

Fig. No. 13. Lipid content in *L. teesgaonensis* n.sp., infected and uninfected intestinal tissue of *Clarias batrachus*

![Bar chart showing lipid content in L. teesgaonensis n.sp., infected and uninfected intestinal tissue of Clarias batrachus.](chart2)

- **L. teesgaonensis n.sp.**
- Infected
- Uninfected

Fig. No. 14. Lipid content in *G. bendsurensis* n.sp., infected and uninfected intestinal tissue of *Wallago attu*

![Bar chart showing lipid content in G. bendsurensis n.sp., infected and uninfected intestinal tissue of Wallago attu.](chart3)

- **G. bendsurensis n.sp.**
- Infected
- Uninfected
the lipid content in the infected intestinal tissue was higher as compare to
the normal fish *Wallago attu* (Bleeker).

The present result confound to the observation done by George et
al. (1971), were he reported low content of lipid in cestode parasites
*Calliobithrium verticillatum*, *Lacistorhynchus tenuis* from both Pacific
and Atlantic coast triakid sharks, *Triakis semisfasciata* and *Rhinotriakis
henlei*.

**Conclusion**

The biochemical estimation of protein, glycogen and lipid from the
cestode parasites, *Circumoncobothrium hemlatae* n.sp., *Lyrocestus
teesgaonensis* n.sp. and *Gangesia bensurensis* n.sp, from the host
*Mastacembelus armatus*, *Clarias batrachus* and *Wallago attu* and the
infected and uninfected intestinal tissue of the host were carried out, from
the result it can be concluded that the fish species examined could have
suffered malnutrition due to cestode infection. This condition may result
toa devaluation in protein, glycogen and lipid content in the body of the
fish. Invariably protein deficiency impairs normal metabolism of the liver
particularly in man. Therefore the infected fish can transmit disease to
man resulting to poor public health