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 host’s gastrointestinal tract 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 gastrointestinal tract 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 sites of lymphocytes (Mc Burney, 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 and Scott, 1994; Edusinghe and Tomkins, 1995; Coop and 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 mechanisms by which malnutrition impair immune-competence (Scott and Koski, 2000).

Nutritional deficiencies as a result of intestinal helminth infection have been the subject of several investigations (Hadju, et al., 1996; Lunn and Nothrop-Clewer, 1996). Intestinal helminth may affect the nutritional status by causing increased nutrient loss, in addition to decreased food intake and nutrient
absorption (Edirisinghe and 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 (Brown 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 parasite 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 responses. Bundy and Golden (1987) described mechanisms by which host nutrition might influence helminth infection: nutritionally mediated changes in host defense 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 and 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. This may be due to difference in the extent to which the parasites comprises nutrient reserves.

The overall aim of the present study was to examine the relationship between cestode infection and nutrient reserves in fresh water fish _Mastacembelus armatus_ (Lacepede) in relation to infection with cestode parasites, and bird _Gallus gallus domesticus_, in parasite _Senga sakurensis_ and _Railletina (Raillietina) tetragona_ Molin respectively.
INTRODUCTION

The carbohydrate, which include low molecular weight (LMW) sugar and various cell wall and storage non-starch polysaccharides are the most important sources for the animal (Bach Knudsen, 1997). It is now clear that dietary carbohydrates are diverse group of substances which varied fats in the gastro-intestinal tract and physiological properties of differing important to animal health (Cumming and 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 et. al., 1997). It has profound influence on the secretary response of the gut to feed intake, the volume flow (Bach Knudsen et. al., 1997), the mucosal architecture (Brunsyaard, 1988), and the development of 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 and Nesheim, 1982: Nesheim, 1984).

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.

Daugherty et al. (1956), Falbairn, Werthein, Harpur and Schiller (1961), Markov (1939), 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, Reid (1942).
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 it is unobtainable from its media.

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 (1889) and Foster (1856) demonstrated the occurrence of glycogen in helminthes.

In cestode *Hymenolepis diminuta* the absence or restriction of availability of dietary carbohydrates resulted in decreased establishment, growth and reproduction Roberts, 1981; Dunkley and Metrick (1969) have found that in rats, 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 worm’s reproductive system. Additionally, it was found that *Hymenolepis diminuta* worms from high starch diet rat were bigger than low starch diet rats, which are bigger than from sucrose diet rats (Roberts, 1981), *T. taeniaeformis* and *Caliobothrium vertiallantum* (Fisher, 1965) as well as the Acanthocephalan *Polymorphus minutes* (Crompton and lackword, 1968) absorb glucose against 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).

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 development 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 CO₂ 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% (Fairbirm et al., 1961) 16% in *Schistocephalus solidus* larva (Hopkins, 1950) respectively.

**GLYCOGEN ESTIMATION**

**Principle: (Kemp, 1954)**

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

**Reagents:**

1) Anthrone reagent: - Dissolve 200mg anthrone in 100ml of 95% sulphuric acid.
2) 95% Ethanol: - 95ml ethanol added in 5ml of Distilled water.
3) 30% KOH: - 30gm of KOH in100ml Distilled water.
4) Standard glucose solution: - 100mg of glucose in 100ml Distilled water.
5) Blank solution: - 1ml distilled water + 5ml Anthrone reagent.

**Material and Method (Kemp 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.

Grounded that material in mortar and pestle then add 1ml of 30% KOH to it and transferred in centrifuge tube, kept in boiling water for 20 minutes, cooled at room temperature, then adds 1.5ml of 90% ethanol by stirring gently boil in hot water bath. This solution centrifuged for 15 min at 2000 RPM and discards the
supernatant (glycogen settled at bottom) and dissolve residue by adding 5ml of distilled water. Take 0.1ml of solution add 0.9ml of distilled water, add 5ml of Anthrone reagent, heat for 10min and reading were taken with the help of colorimeter at 620µm filter.

Fresh water fishes *Mastecembelus armatus* were brought to the laboratory, dissected carefully. Some of them were infected with cestode parasites; small pieces of infected and non-infected intestines were also collected to find the glycogen content in respective parasites and their hosts by using Kemp 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 taxonomic identification. These were later stained with Harris Haematoxylin and identified species is *Senga sakurensis* sp. nov.

The biochemical estimation of the above species is observed.

**Glycogen Estimation in *Raillietina (Raillietina) tetragona* Molin**

Percentage of glycogen = \[ \frac{100 \times U}{1.11 \times S} \]

Where U = O.D. of the unknown test solution

S = O.D. of the 100 mg. of glucose standard.

1.11 = conversion factor of glucose to glycogen.

U = 0.46

S =2

\[ \text{Mg. of glycogen} = \frac{100 \times 0.46}{1.11 \times 2} \]

=20.72 mg /100ml. solution
So also same technique has been used for the estimation of glycogen from the host intestine, when calculate infected intestine of the host has 22.07 mg. of glycogen /100 ml. solution

By studying the result obtained it is seen that the worm Raillillina (R.) tetragona Molin is quite successful in obtaining a sufficient amount of glycogen from the environment. Percentage different is seen as the environment contains 22.07 ml. of glycogen /100ml. of solution where as the parasite has acquired 20.72 mg./100ml. solution.

**Glycogen Estimation in Senga sakurensis Sp. Nov.**

Percentage of glycogen = \( \frac{100 \times U}{1.11 \times S} \)

Where U = O.D. of the unknown test solution

S = O.D. of the 100 mg. of glucose standard.

1.11 = conversion factor of glucose to glycogen.

U = 0.46

S = 2

Mg. of glycogen = \( \frac{100 \times 0.46}{1.11 \times 2} \)

=20.72 mg /100ml. solution

So also same technique has been used for the estimation of glycogen from the host intestine, when calculate infected intestine of the host has 22.07 mg. of glycogen /100 ml. solution
By studying the result obtained it is seen that the worm *Senga sakurensis* is quite successful in obtaining a sufficient amount of glycogen from the environment. Percentage different is seen as the environment contains 22.07 ml. of glycogen /100ml. of solution where as the parasite has acquired 20.72 mg./100ml. solution.

**CONCLUSION**

From the above it can be conclude that the worms could maintain a good balance in glycogen content.
The main sources of energy reserves in fishes and birds 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 consists 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.

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.

The relative contributions of protein to energy production in fishes and birds depend on a 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.

In parasitic helminthes, protein usually constitutes 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 host tissue as there is no media to acquire proteins in parasites these proteins 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 hosts proteins are removed from the diet, even those 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 favouring the migratory phase of *Nippostrogylus basiliensis* proved somewhat unfavourable to the adult worm (Clarke, 1968) but indirect rather than direct influence was probably involved Clarke,(1968).

Many workers have gone through the studies of protein metabolism in various cestode parasites. 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 (1963 a) absorption and digestion of amino acid in the tapeworm *Anoplocephala magna* by Karvavica et al.; (1959 a) and the estimations in *Phyllobothrium foliatum* has worked out. Sodorov (1980) made comparative investigation of proteins composition of *Eubothrium crassum* and the host. Ganzales (1978) worked on serum in animal, which were infected, with parasitic helminthes.

The essential and non essential amino acid are required, proteins are also digested at the host parasites interface by the activity of proteolytic enzyme and the cestode tegument secrets these amino acides 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 contents 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 scientists (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 parameter is changed (Mettrick, 1971b) this can have 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 hosts.

**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-phosphotungastate reagent to yield an intense blue colour. This assay procedure is much more sensitive than the biurette method but is more time consuming. The only precaution to be observed when performing this assay concern addition of the Folins reagent. This reagent is stable only at acidic pH; however, reduction indicated above occurs only at pH 10. Therefore, when Folins reagent is added to the alkaline copper protein solution, mixing must occur immediately so that the reduction can occur before the phosphomolybdic-phosphotungastate (Folins).

**Reagents:**

1) Lowry’s ‘A’ solution: Dissolve 2gms of Na$_2$Co$_3$ 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 (CuSo$_4$), 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 Trichloro-acetic acid (TCA in 100ml of distilled water).
5) 1N 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 (Bovin Serum Albumin) in 10ml of 1 N NaOH.

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

**Material and Method (Lowry’s method)**

The cestode 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 10 ml of NaOH for dissolving the ppt. then taken 0.1ml above solution and adds 4ml Lowry’s ‘C’ solution, then it adds 0.4ml 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.

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 taxonomic identification. These were later stained with Harris Haematoxylin and identified species are *Senga sakurensis Sp.Nov.*
PROTEIN ESTIMATION IN RAILLIETINA

The amount of protein in the worm was calculated by the formula:

\[
\text{% of protein} = \left( \frac{O.D. \text{ of unknown tissue} \times \text{known tissue protein}}{O.D. \text{ of standard}} \right) \times 100
\]

O.D. of unknown tissue = 1.20
O.D. of known tissue = 0.7 mg/ml
O.D. standard = 2.5 mg/ml

\[
\text{% of protein} = \left( \frac{1.20 \times 0.7}{2.5} \right) \times 100
\]

= 33.60 mg/gm wet. Weight of tissue

The protein percentage of the host intestine *Gallus gallus domesticus* was also estimated by the same procedure. The obtained results showed that the intestine posses 35.00 mg/gm of wet weight tissue of protein.

The results compared, showed that the worm Raillientina (Raillietina) tetragona Molin obtained 33.60 mg/gm of wet weight tissue of protein from the environment which, contained mg/gm of wet weight tissue of protein.

Hence it can be concluded that Raillientina (Raillietina) tetragona Molin could maintain balance in protein content and the histopathological relation with the host *Gallus gallus domesticus*. 
PROTEIN ESTIMATION IN SENGA SAKURENSIS

The amount of protein in the worm was calculated by the formula:

\[
\% \text{ of protein} = \frac{O.D. \text{ of unknown tissue} \times O.D. \text{ of known tissue}}{O.D. \text{ of standard}} \times 100
\]

\[
= \frac{1.34 \times 0.7}{2.5} \times 100
\]

\[
= 37.52 \text{ mg/gm wet. Weight of tissue}
\]

O.D. of unknown tissue = 1.34
O.D. of known tissue = 0.7
O. D. of standard = 2.5

The protein percentage of the host intestine *M. armatus* was also estimated by the same procedure. The obtained results showed that the intestine posses 44.52 mg/gm of wet weight tissue of protein.

The results compared, showed that the worm *Senga sakurensis* obtained 37.52 mg/gm of wet weight tissue of protein from the environment which, contained mg/gm of wet weight tissue of protein.

Hence it can be concluded that *Senga sakurensis* could maintain balance in protein content and the histopathological relation with the host *M. armatus*. 
LIPID

INTRODUCTION

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 10-30% 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 somewhat meaningless, 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 unsaponable material and phospholipids often account for more than 20% of the total lipids.

LIPID ESTIMATION

**Principle: (Folch et al. 1957)**

The quantitative determination of lipid by sulphophosphovanilin method depends upon the reaction of lipid (Extracted from sample using chloroform methanol method) with sulphuric acid and Vanillin to give red colour complex.

**Reagents:**

1) Chloroform and Methanol (2:1).

2) Sodium chloride (0.9%) was prepared by dissolving 900mg sodium chloride crystal in 100ml of D.W.
3) Phosphovanillin reagent.

4) 300ml of orthophosphoric acid was mixed with 200ml D.W. in which 2gm Vallin was dissolved standard stock solution.

5) Standard Stock solution: - Dissolve eight million of cholesterol in 4ml of chloroform: methanol (2:1) mixture. Take 1ml of this mixture and dry for 2 days at 37°C add 1ml of conc. H2So4 boil in water for 10 min and cool prepare the 10 tubes.

6) Blank: - an aliquot of 0.2ml of choloform was taken in blank.

Material and Method (Folch et al. 1957)

Weighed 100ml of tissue, homogenate it with the help of Mortar and Pestle by adding 10ml of Chloroform: Methanol (2:1) then by use of Whatmans filter paper filter the mixture. Pipette out 1ml of filter keep for drying at 37°C or 3-4 days at room temp, add 1ml of conc. H2So4 in filter. Keep it in boiling water bath for 10 min and cool rapidly under tap water. Take 0.2ml solution from that and add 5ml Vanillin reagent in it, keep for 30 min at room temp which develop in purple colour and reading were taken with the help of colorimeter at 530µm filter.

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 taxonomic identification. These were later stained with Harris Haematoxylin and identified species is  *Raillietina (Raillietina)tetragona* Molin and  *Senga sakurensis* Sp.Nov.
LIPID ESTIMATION IN RAILLIETINA

\[ \% \text{ of lipid} = \frac{O.D. \text{ of sample} \times \text{conc. of standard}}{O.D. \text{ of standard}} \times 100 \]

\[ = \frac{0.60 \times 0.07}{0.25} \times 100 \]

\[ = 16.80 \text{ mg/gm wet. of Weight tissue} \]

O.D. of unknown tissue = 0.60
O.D. of known tissue = 0.07
O. D. of standard = 0.25

The protein percentage of the host intestine Gallus gallus domesticus was also estimated by the same procedure. The obtained results showed that the intestine posses 19.60 mg/gm of wet weight tissue of protein.

The results compared, showed that the worm Raillietina (Raillietina) tetragona obtained 16.80 mg/gm of wet weight tissue of protein from the environment which, contained mg/gm of wet weight tissue of protein.

Hence it can be concluded that Raillietina (Raillietina) tetragona could maintain balance in protein content and the histopathological relation with the host Gallus gallus domesticus.
LIPID ESTIMATION IN SENG A SAKURENSIS

\[
\% \text{ of lipid} = \frac{O.D. \text{ of sample} \times \text{conc. of standard}}{O.D. \text{ of standard}} \times 100
\]

\[
= \frac{0.72 \times 0.07}{0.25} \times 100
\]

\[= 20.16 \text{ mg/gm wet. Weight tissue}\]

O.D. of unknown tissue = 0.72
O.D. of known tissue = 0.07
O. D. of standard = 0.25

The protein percentage of the host intestine *M. armatus* was also estimated by the same procedure. The obtained results showed that the intestine posses 22.96 mg/gm of wet weight tissue of protein.

The results compared, showed that the worm *Senga sakurensis* obtained 20.16 mg/gm of wet weight tissue of protein from the environment which, contained mg/gm of wet weight tissue of protein.

Hence it can be concluded that *Senga sakurensis* could maintain balance in protein content and the histopathological relation with the host *M. armatus*. 