Discussion
DISCUSSION

The present findings revealed that infection caused by the progenetic metacercariae (larva) of *Clinostomum complanatum* induced metabolic alterations in liver and intestine of *Trichogaster fasciatus*, which serve as second intermediate host. The metacercariae of *C. complanatum* found in the peritoneal cavity also caused some physiological changes in the liver and intestine of the host during the present study.

In general the protein content in infected host tissues was depleted. Pronounced depletion in protein content was noticed in liver and intestine of infected forage fish as compared to tissues of normal fish. Our finding is in agreement with Chopra *et al.*, (1983). Loss of protein contents in the host tissues due to the parasitic infections has also been reported by Kuttler and Marble (1960), Turner and Wilson (1962) Joshi (1977) and Uppal and Rai (1978). The protein was observed to be significantly low (p<0.05) in liver and intestine of infected fishes during the present study.

In order to perform various metabolic processes, an organism requires a supply of energy which is obtained by breakdown of a number of macromolecules like carbohydrate, lipids and proteins which are ultimately incorporated into intermediates of the TCA (Tricarboxylic acid) cycle. The integration among the metabolic pathways of these macromolecules is under the control of several enzymes, and among these enzymes aminotransferases serve as an important link between carbohydrate and protein metabolic pathways. Some helminths incorporate ammonia directly into pyruvate and 2-ketoglutarate to form alanine and glutamate respectively. Thus, amino acids are readily metabolized by transamination and are very important because they interact with pathways of energy metabolism, catalysed by aminotransferases. Pathophysiological study includes the effect of parasite on enzymes like GOT, GPT, alkaline phosphatase and acid phosphatase which are commonly known as pathological marker enzymes. Aspartate transaminase (AST), also called as serum glutamic oxaloacetate transaminase (SGOT) or aspartate aminotransferase (AST) is an enzyme associated mainly with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells, cardiac and skeletal muscle and is therefore not specific only to liver. AST enzyme has also been
used as a cardiac marker. Alanine transaminase (ALT) also called serum glutamic pyruvate transaminase (SGPT) or Alanine aminotransferase (ALT) is an enzyme which is present in hepatocytes (liver cells) and when the cells are damaged it leaks this enzyme into blood where it can be measured and the degree of damage can be determined. ALT is raised dramatically in acute liver damage such as viral hepatitis etc.

Alkaline phosphatase (ALP) is another important enzyme present in the cells lining biliary ducts of the liver. Generally ALP level in plasma increases due to duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. It is also present in bone and placental tissues.

**GOT** (L-Aspartate 2-oxoglutarate aminotransferase E.C. (2.6.1.1)) has been demonstrated in all animal and human tissues including microorganisms, most active in heart, muscles, brain, liver, gastric mucosa, adipose tissues, skeletal muscle, kidney etc. and finally serum with considerable smaller amounts.

**GPT** (EC 2.6.1.2) also found in liver, kidney, heart, skeletal muscle, pancreas, lung and serum.

The GOT and GPT enzymes play a key role in transferring amino group of amino acids from alpha amino acid to alpha-keto acids, therefore named as transaminases, stored mostly in liver. GOT enzymes mainly exist in liver and myocardium and in fewer amounts in kidney and pancreas.

Phosphatases catalyse the hydrolytic cleavage of phosphoric acid esters. They are designated either “acid” or “alkaline phosphatases” according to their pH optima. **Alkaline phosphatases** (orthophosphoric monoester phosphohydrolase, alkaline optimum E.C. (3.1.3.1)) occur in all animal and human tissues. Bile and osteoblasts show particularly high activity of these enzymes and their raised values in serum always arises due to diseases accompanied by involvement of liver or bile ducts.

**Acid phosphatases** (orthophosphoric monoester phosphohydrolase, acid optimum, E.C. (3.1.3.2)) are also found in all animal cells and its high activity found in erythrocytes. The diseased condition caused by the parasite *Clinostomum complanatum* in fishes mark the presence of these enzymes in parasites and affected organs such as liver and intestine of the fish. Alkaline phosphatase is a hydrolase
enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. The process of removing the phosphate group is called dephosphorylation. It is most effective in an alkaline environment.

When body tissue or an organ such as heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in blood is directly related to extent of tissue damage. After severe damage, AST levels rise in 6-10 hours and remain high for about 4 days. The ratio of AST to ALT sometimes may help to determine whether the liver or another organ has been damaged. Both AST and ALT levels can be used as a test for liver damage.

It is done to:

1. Check liver damage.
2. Help to identify liver diseases.
3. Check on success of treatment for liver disease.
4. Find out whether any disease is caused by blood disorder or due to liver disease.

Considering the significance and important roles of the transaminases as well as phosphatase enzymes (Acid and alkaline phosphatases) during diseased condition, present study was designed to check the extent of pathological damage in the *Trichogaster* fish which was chosen as a model fish host. GOT, GPT, acid as well as alkaline phosphatase enzymes are considered as important pathological marker enzymes in higher animals. Determination of these pathological marker enzymes become an established diagnostic procedure during the last two or three decades. Particularly, the transaminases can give important hints in evaluating the possible existence of necroses, diagnoses of certain disease related to any infection and conditions increasing permeability. Increased transaminase level evidently is a common occurrence from protozoa to helminthic infections (von Brand, 1979).

In the present study, GOT, GPT, Alkaline and Acid phosphatases enzyme levels were determined in parasite as well as in liver and intestine of infected fishes and compared with non-infected fish.
We observed that level of these enzymes was elevated in parasitized *Trichogaster* liver as compared to normal liver tissues. In contrast the level of GOT and GPT enzymes in normal intestine of *Trichogaster fasciatus* was found higher as compared to intestine of parasitized *Trichogaster* during the present study. Similarly elevation of acid and alkaline phosphatase enzymes was observed in the liver of parasitized *Trichogaster*. The elevation of acid and alkaline phosphatase enzymes has also been reported in *Schizothorax* species (Chopra *et al*., 1986) infected by metacercariae of *Diplostomum tetrai* which infest skin, gills, fins and operculum of this species. Although the results varied in case of intestine of parasitized *Trichogaster fasciatus*, the level of acid as well as alkaline phosphatases was found to be elevated in intestine of normal *Trichogaster* rather in parasitized fish.

On comparing transaminases activity the level of GOT was found to be higher than GPT in *Clinostomum complanatum* metacercariae which is a causative agent of pathological changes in the *Trichogaster* fish. In other trematodes like adult *Fasciolopsis buski* higher level of GOT than GPT have been reported by Tandon and Mishra (1984). Higher GOT level was also observed in *Hymenolepis nana* by Abusen (1966) in *Stephenurus dentalus* and in *F. buski* by Sharma and Singh (1977).

In the present study, level of acid and alkaline phosphatase enzymes was found to be significantly higher (p<0.05) in the liver of diseased fishes. This may be attributed to the fact that phosphatases play an important role in active transportation of materials through phosphorylated intermediates (von Brand, 1973). Therefore the high metabolic activities might have raised the levels of phosphatases in liver during infection. But the depleted level of these enzymes in intestine of infected fishes suggest that during infection, the metabolic activities might have been lowered leading to secretion of low amount of enzymes in intestine as compared to intestine of normal fish which is not in accordance with von Brand, (1973). The activity of acid and alkaline phosphatases has been studied in liver and blood serum of *Schizothorax richardsonii* fish, infected with black spot disease by Chopra *et al*., (1986). The data showed that in infected fishes there was significant increase of acid and alkaline phosphatase in the liver.

It can be concluded from the above discussions, that the nature and degree of effects cannot be generalized and these are related to a number of factors which
include degree of infection, size and age of the host, developmental pattern of the larvae and the physiological condition of the organs invaded. Pathological changes occurring in the host may also involve a range of parasitic larval stages differing in their physiological requirements, mode of feeding and morphological complexities. Further, the host-parasite relationship becomes more complex because of a regular flux in climatic conditions which ultimately affect the physiological as well as nutritional state of the host. The exact nature of this relationship therefore, varies as the life cycle unfolds and progresses from host to host (Erasmus, 1972). Therefore, it would be stated that during infection host show adaptive elevation in the activity levels of GOT and GPT enzymes, particularly in liver thereby aiding gluconeogenesis through transamination of glucogenic amino acids to meet the energy demand under state of infection.

The available information on transaminases indicates that these enzymes occur in polymorphic forms (von Brand, 1979) and respond differently to metabolic inhibitors or stimulators (Siddiqui and Nizami, 1982). Enhancement of GOT and GPT activities by EDTA have been reported in parasitic nematode, *Bunostomum trigonocephalum* and *T. ovis* by Gupta and Trivedi (1986). While iodoacetamide appeared as a weak inhibitor or had no effect on the activity level of GOT and GPT, (Goil, 1978) but our findings suggest that iodoacetamide, in most of the cases acted as stimulator for GOT and GPT activity.

Goil (1978) reported that EDTA acts as activator for both GOT and GPT in *Paramphistomum explanatum* which is an amphistome parasite. In the present study, similar response of GOT and GPT in presence of EDTA was observed in the metacercariae of *C. complanatum*. The presence of transaminases have been demonstrated in some helminth species such as *Hymenolepis diminuta* (Aldrich, et al., 1954, Goodchild, 1958 and Werthein, et al., 1960) *Schistosoma mansoni* (Garson and Williams, 1957), *S. japonium* (Huang, et al., 1961) and in *Fasciola hepatica* (Daugherty 1952; Connolly and Downey, 1968). In the present study it has been observed that iodoacetamide serves as a stimulator of GOT in the liver of normal and infected fish, while it acts as an inhibitor of GPT in liver of infected fish. Further, the iodoacetamide activated GPT activity in liver of normal fish. In intestine of infected fish both EDTA and iodoacetamide observed to be as an inhibitor of GOT but in
intestine of normal fish both EDTA and iodoacetamide acted as stimulator of GOT, while EDTA appeared as a strong activator for GPT in intestine of normal and infected fish. Further, iodoacetamide was observed as an activator for GPT in intestine of normal and infected fish.

In the present study, EDTA has been observed be as an inhibitor for alkaline phosphatase in livers of infected and normal fish which is in agreement with Yora et al., (1986). But in intestine, EDTA acted as a stimulator for alkaline phosphatase enzyme which is not in agreement with Yora et al., (1986). It can be suggested that in liver tissue alkaline phosphatase is more sensitive towards EDTA. Therefore it appeared as an inhibitor, while in intestine due to less sensitivity towards EDTA it acted as stimulator of alkaline phosphatase. Alkaline phosphatase of liver of normal fish was slightly activated by iodoacetamide and iodoacetamide also acted as a stimulator for alkaline phosphatase in intestine of infected fish while EDTA inhibited acid phosphatases in intestine of infected and normal Trichogaster.

Apart from pathophysiological and pathobiochemical studies, a monthly survey of C. complanatum infection in T. fasciatus was recorded. Morphometric studies were also performed to observe correlation between worm burden and morphological change if any. Data related to parasite prevalence revealed that the highest prevalence of infection was found in summer season as compared to winters. The highest rate of prevalence was observed in April, June September and October. Although highest incidence of infection was observed in summer season but somewhat higher prevalence has been seen in other months in winter for e.g. in December 83.33% prevalence was observed. Higher parasitic index value has also been reported in summer season by Souza and Ludwig (2005). Current data on morphometric analysis did not show any correlation between prevalence of parasitic infection and morphological changes like the total length, standard length, head length, pre-dorsal length and snout length of infected and normal fishes. Therefore only determination of the length related factors cannot be an indicator of parasitic infection. Similar observation has been reported by Vianna (2001).

Thus, the variation in length of T. fasciatus does not seem to a valid parameter to determine the parasitic infection. Prevalence of infection can vary due to some environmental conditions, or some physiological conditions of the host.
In the present study, effect of parasitism was also checked on the polypeptide profile. The Coomassie stained gels showed differences in the resolved polypeptides in parasite, liver and intestine of normal and infected *Trichogaster fasciatus*. Analysis of SDS-PAGE profile revealed quantitative as well as qualitative variation in the expression of polypeptides. The polypeptides resolved may be categorized as common polypeptides, specific and variable polypeptides. We reported 132 and 114 kDa as “conserved polypeptides”. It was observed that parasitism affected expression of polypeptides. The infected tissues expressed low number of polypeptides as compared to normal tissue samples.

Thus, we can conclude that helminth infections damage tissue and alter the physiology and biochemistry of the host. Screening a wide variety of biomolecules can predict the degree of pathological damage and establish basis for host-parasite relationship. In future, histopathological studies can provide strong evidences related to physiological, biochemical and immunological disturbances. Consequently, opening some new chemotherapeutic targets to control parasitic infections.