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INTENSITY AND DENSITY OF *PYIGIDIOPSIS SUMMA* AND *GENETA*, AND THEIR EFFECT ON SOME OF THE SERUM CONSTITUENTS OF *TILAPIA* SP.

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Key words: fish environment, bio-pollution, parasites intensity, biochemical parameters, Tilapia sp.

ABSTRACT

Parasitic infections of *Tilapia* sp. from the agricultural drainage in the eastern region of Saudi Arabia were examined. The identification of the parasites, the histopathological, and the histo-chemical analysis of the infected fish organs were investigated in series of studies. The present work estimated the intensity and the density of the encysted metacercariae of the *Pyigidiopsis summa and geneta* in random samples of different fish organs. The study also investigated the effect of these parasites on the serum glucose, total protein, and the activity of the LDH enzyme. The results indicated that all fish in the examined samples (100%) were infected with the encysted metacercariae of the *Heterophyd* sp. The highest intensity of the metacercariae was found in the liver (43.30%), and its mean density was 17.31±35.68 cyst/cm^2. The intensity of the metacercaria in the intestine was 33.37%, and its mean density was 13.34±15.77 cyst/cm^2. The lowest intensity and density of the parasite was in the skin, 23.33%, and 9.33±8.87 cyst /cm^2 respectively. The biochemical analysis showed a significant increase in the serum glucose, and the activity of the LDH enzyme of the infected fish. However, total protein did not vary much between the infected fish and the uninfected control.

INTRODUCTION

Although fish represent the best animal protein for human and animal consumption, it can be harmful, by transmitting parasitic infections. These parasites infect fish through its environment or food chain. Intensity and density of these parasites in different fish organs

varied according to different factors. Among those factors kind and condition of the infected fish and whether this fish is intermediate or final host, environmental factors, such as water temperature, pH, and salinity, plus the trophic level of the fish. Mansour *et al.* (1987), found that there were variation in the intensity of parasitic infection among Tilapia species which infected by *Pyigidiopsis genata.* While the intensity of the metacercaria in the *Tilapia zillii* were 30.30% it was 37.9% in *Orecchromis niloticus*, 21.6% in *Tilapia simonies and* 21.4% in *Sarotherodon galilaeus.* Mohammed (1983) showed that the average number of encysted metacercariae per gram tissues of Tilapia sp. was 21 cysts while it was 7 in the *Mugil* sp. and 18 in the *Clarias* sp. In another study by Paperna (1980), the average number of the encysted metacercariae per gram of tissues was 100 in the *Clarias lazara*, 300 in the *Tilapia* sp. and 800 in the *Mugil* sp. Makhaloüf *et al.* (1987), recorded the seasonal changes, which affected the intensity of the parasitic infections in *Tilapia* sp. The intensity of the parasites from the Hetrophidae family was more in summer than winter. The intensity and density of the parasites is also affected by fish size and type of infected organs. Ramadan (1991) discovered that big sizes of fish are more susceptible to parasitic infection than small size, and the density of the parasites varied between different organs. Paperna, (1980) found that the highest metacercaria density of Hetrophidae family was in the fish muscle, vescera and gills.

Increase the intensity and density of the parasitic infections could cause physiological and biochemical changes to the fish. The changes will be due to the stress caused by the parasite; its biological or/and biochemical reactions. Different authors studied the effect of the parasitic infections on fish blood biochemical constituents. Awad, (1992) and ElSeify *et al.* (1998) reported a decrease in the total protein and albumin and increase in the hepatic enzymes, ALT, AST, and alkalie phosphatase of the fish infected with several protozoa species.

The present work is a part of a serial studies aimed to identify and examine the effect of a heavily parasitic infection on *Tilapia* sp. This fish were raised in the agriculture drainage in the eastern region of the kingdom of Saudi Arabia. The present study investigated the intensity and density of the pre-identified parasite in different fish organs such as liver, intestine and skin. The effect of the parasitic infections on the physiological functions of some of the infected organs is also studied. The changes in fish serum glucose, total

protein, and in the activity of the lactate dehydrogenase (LDH) enzyme are investigated.

MATERIALS AND METHODS

Random samples of *Tilapia* sp. of different sizes and weight were collected at four different times in autumn season from the agricultural drainages in Elhofof area in the eastern region of Saudi Arabia. The sizes and weight of the fish samples were ranged between 9-18 cm & 10-110g. The physical and biological specifications of the study area will be discussed in the results and discussion section. Fish were transferred from the drainge immediately to the king Fiasel University laboratory aquaria. After 2 days of starvation fish were stocked in plastic bags filled with water and O_2 in icebox to minimize wastes and respiration process during their trip by aircraft to Jeddah. At the laboratory of king Abdullaziz University a total number of 40 fish were kept alive in aquarium filled with de-chlorinated water for the further parasitological and biochemical examinations. Blood samples were withdrawn, using hibernated capillaries, from the heart or the caudal artery of random samples of infected and uninfected fish, for the biochemical analysis. Fish were then dissected and their organs were labeled and kept in the refrigerator for the parasitological analysis. Blood glucose was measured directly using Accutrend Gct. device. The rest of the blood was centrifuged and the serum was used to estimate total protein and the activity of the lactate dehydrogenase (LDH) enzyme using (Crescent diagnostics) systems, while the Biuret reaction test was used to estimate the blood total protein. The blood LDH activity was estimated by using the method of Optimized UV-Test Accord. to SCE Mod. (Scandinavian Committee on Enzyme). The principal of this test based on

the following reaction:

$$\text{Pyruvate} + \text{NADH} + \text{H} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ \text{ (Klin, 1970).}$$

Three random samples from each of the infected organs of three fish were taken daily to estimate the intensity and density of the parasitic infection in these organs. The preliminary histopathological

investigations, and the internal and external examination of the infected fish, showed that liver, intestine, and skin were the most infected organs (Figs. 1&2). Therefore each of the named organ samples was first measured then shacked, scraped and washed by saline water in a Petri- dish to collect the parasites. Hand lens and light microscope were used to count the parasites from each tissue sample. Results were statistically analyzed using descriptive analysis and student T- test to compare results of the infected fish with the control (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSIONS

Elhofof area is situated in Alhasa region; it has 16 main irrigation canals and 3 drainages. Present study fish samples were collected from the drainage D^2 (Fig. 3). Temperature range around the drainages area was 14-36C^0, water DO concentration ranged between 6.4-12.8 mg/l, the bicarbonate was between 262 to 280 mg/l, and the salinity was 3.2-4.8 ppt all year around. The dominant snails in the drainages are *Melano tuberculata* and there are also snails from *Planoribs* sp. There are also different species of green algae and crustaceans species. as Ostracods and Cyclopoda. In the drainage area there are about 14 species of birds. The most dominant sp. are *Gallinula chloropas*, *Rallus aguaticus*, and winter immigrate birds as *Gallingo gallingo*, *Actitis hypoleucos*, and *Charadarius dubius*.

Tilapia fish from the drainages were mostly hybrid between *O.niloticus*, *O.aureus* and others (Fig.1). Prior to this study the parasites, which infected the experimental fish were identified. They were *Pyigidiopsis summa and geneta* trematodes from Heterophidae family (Fig.4).

In the present study all fish in the collected samples were 100% infected by the encysted metacercariae of *Pyigidiopsis genata and summa*. The results showed that the highest intensity and density of the parasites in the infected organs were in the liver. Liver was loaded by 43.36%; of all counted metacercariae from the examined organs; and the average density per square cm of tissue were 17.31±35.68 cyst/cm^2 (Fig.5). The second highest infected organ was the intestine, 33.37% and the density of the parasite was 13.34±15.77 cyst/cm^2. The intensity of the parities in the skin was 23.33% and its density was 9.33±8.87cyst/cm^2. While differences, in the intensity and density of the parasites, between the fish liver and skin was

significant ($p < 0.05$) it was not significant between liver and intestine, and between intestine and skin (Table 1) & (Fig .6). Table (1) show the minimum and the maximum number of the metacercariae per gram of the *Tilapia* sp. tissues. The present results are not far from the findings of Mohammed (1983), who showed that the average number of encysted metacercariae per gram of *Tilapia* sp. tissues was 21 cysts. However, the same author in 1990 detected a higher number of encysted metacercariae from the same species of fish; it was 319 cysts/g of tissues. In this respect Paperna (1980), also stated that the number of metacercariae per gram of *Tilapia* sp. was up to 300 cysts respectively.

Pyigidiopsis summa and *geneta* are, from Heterophidae family; usually invade muscle, viscera, and gills of the fish (Paperna, 1980). However, internal and external examinations of the infected fish in the present study, showed that these parasites were invaded the whole body of the fish. Bouls (1979) and Mansour *et al.* (1987) found that the metacercariae of the *Pyigidiopsis geneta* in *Tilapia* sp. was found mostly in the head region followed by the tail then the body of the fish. However, (Mahdy, 1991), and (Mahdy *et al.* 1995), found that the Heterophidae metacercariae were mainly encysted in between muscle bundles and dermis. Jong *et al.* (1986) declared that *Pyigidiopsis summa* metacercaria were found in gills and esophagointestine of Mullet. Present results revealed that the highest intensity and density of the parasites were in the fish liver followed by the intestine then the skin. This may indicate that the metacercariae migrated through the skin to the blood vessels to reach the liver and other organs, and it may reach the intestine also through the food chain. Gharib and Hamdy (1969) found that the metacercariae of heterophyes reached even the eyes of the fish. The intensity of the parasites in the studied organs in the present study is not very far from the findings of Mahdy (1991), Jihan (1993), El-Dally (1983), and Mohamoud (1983).

Present results also show that there were significant differences of the fish serum glucose and the activity of the LDH enzyme between the infected and non-infected fish ($P<0.05$). However, blood total protein did not varied much between both infected fish and the control (Fig.7) and (Table 2). Mean serum glucose was almost five times higher in the infected fish (590.90 ± 4.32mg/dl) compared with the control (120.48 ± 30.76mg/dl). The

activity of the LDH of the infected fish (407.76±45.34 u/l) was elevated to 1.3 times the activity of the control (292.55±88.03u/l). While serum total protein of the infected fish was (5.00±1.25g/dl), it was (3.92±0.65g/dl) for the control fish.

Kudryshova (1970), Vonzi (1979) and El-seify *et al.* (1998) investigated the effect of naturally infected Tilapia with different parasites species on the fish blood total protein, and different enzymes. Their results showed a decrease in the fish blood total protein and increase in the transeaminase enzymes such as ALT, AST and the alkaline phosphatase. The present results showed no significant changes in the serum protein between the infected and non-infected fish. Fish might compensate their protein losses due to the parasitic infection by restoring protein synthetic potentials (Sahib, 1984). The stress and the cell damage caused by any foreign body, either chemical or biological, can enhance the liver to synthesized high levels of protein to compensate for enzymes loss (Gill *et al.*, 1990), or produce more antibodies.

The internal and external examination of the fish showed that fish was completely invaded by the parasite. The infection of the fish respiratory system, in addition to the stress caused by the infestation of the parasites to all fish organs may lead to hypoxic or anoxic conditions. It is known that these conditions enhanced glycogenolysis and gluconeogensis processes, which caused the rise in the serum glucose level of the infected fish. The present findings agreed with various researchers such as Oruc and Uner (1998&1999), Al-Akel and Shamsi (2000), and Al-Kahem (1996).

In the present study, results also indicated that the activity of the lactic dehydrogenase enzyme (LDH) was initially elevated in the infected fish. The increase of the LDH activity could be explained by the elevation in the anaerobic catabolism of blood glucose or/and due to the damage of the liver and muscle tissues (Rui and Zuzuki, 1997), (Van-Raaij *et al.* 1996), and (Oruc and Uner, 1998&1999). The present results showed that the fish liver, intestine and skin were the most infected organs with the *Pyigidiopsis geneta* and *Pyigidiopsis summa* parasites. This caused damage to these tissues, stress and elevation to the serum glucose.

Agricultural drainages in Elhofof area are source of fish production for the area residents. Therefore, we would like to recommend further studies for the fish in these drainages. These studies can investigate the effect of the different environmental, and

seasonal variations on the intensity and density of the parasites that invade the drainages fish. Further studies could be also Carried out on the effect of these parasites on fish mortality rates, fish growth, immunity, and on the function of fish different organs.

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Table-1. Density cyst/cm2 of the *Pyigidiopsis geneta and summa* in the liver, intestine, and skin of Tilapia sp.

Organ name	Minimum	Maximum	Mean	Std.deviation
Liver	5	185	17.31	35.68
Intestine	2	98	13.34	15.77
Skin	3	50	9.33	8.87

Table-2. Serum glucose mg/dl, activity of LDH u/l, and total protein g/dl of the infected (infc.) and control (c.) fish.

Tested Component-name	Minimum	Maximum	Mean	Std. deviation
Glu-infc	537.10	637.45	590.92	40.32
Glu-c	89.39	156.09	120.48	30.76
Ldh-infc	339.75	465.40	407.76	45.34
Ldh-c	230.30	354.80	292.55	88.03
Prot-infc	3.53	6.45	5.00	1.25
Prot-c	3.21	4.49	3.92	0.65

LEGENDS OF FIGURES

Fig. 1. Show the infected Tilapia. Sp. with some black spots in the skin (b), hemorrhage(s) and fins erosions (arrows)

Fig. 2. Show some of the internal organs of the infected fish, liver (L), spleen (arrow), and intestine (I).

Fig. 3. Show the agricultural drainage D2, in Elhofof area; the source of the study fish samples

Fig. 4. Show the mature stages of the *P. summa* (1) and *P. genata* (2), which were identified in another part of the present study.

Fig. 5. Show liver tissue from the infected fish loaded with the encysted metacercariae.

Fig.6. Intensity of the parasite in the liver, intestine, and skin of the infected fish.

Fig. 7. Shows the differences in the serum glucose(glu), activity of the lactate dehydrogenase enzyme(LDH), and total protein(prot), between the infected fish and the control.

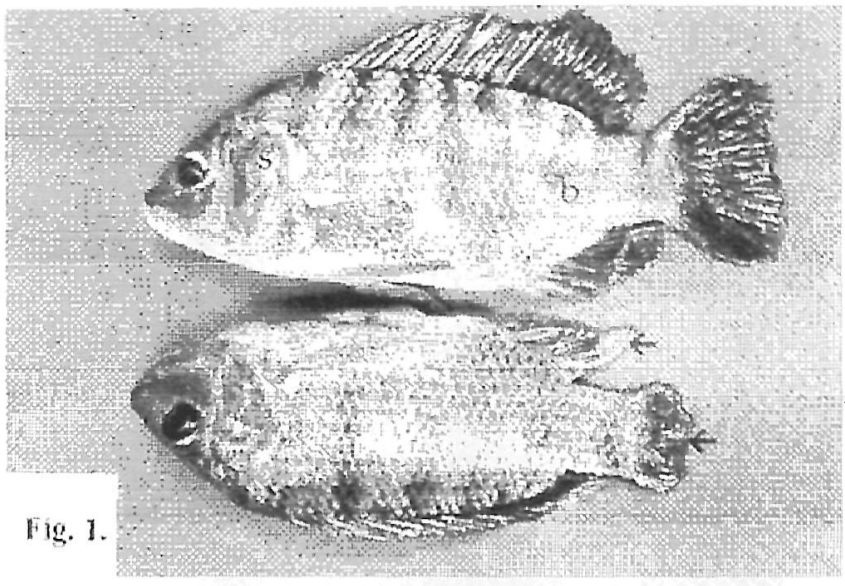

Fig. 1.

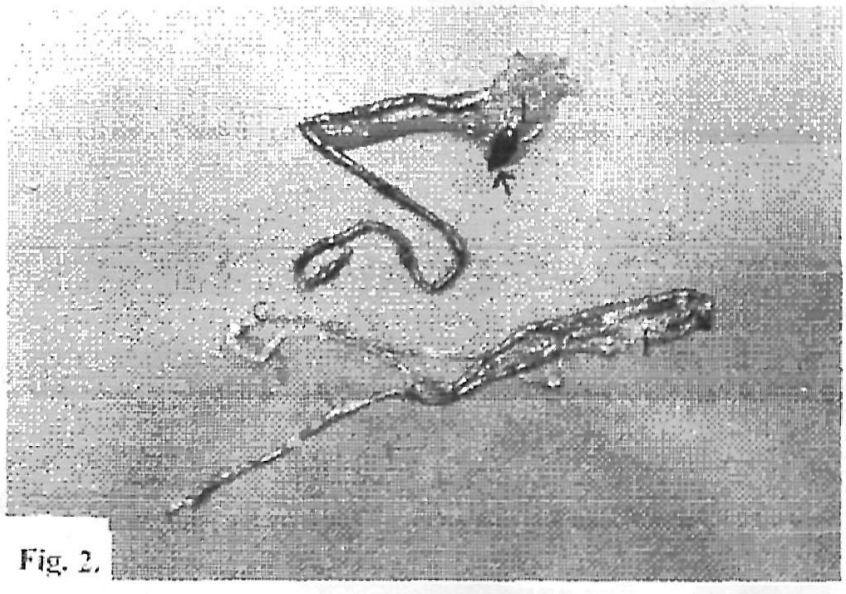

Fig. 2.

Fig. 3.

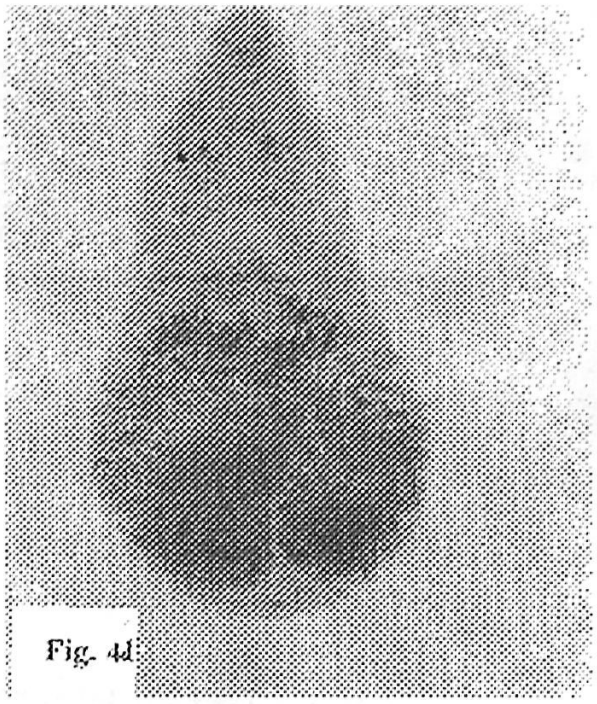

Fig. 4d

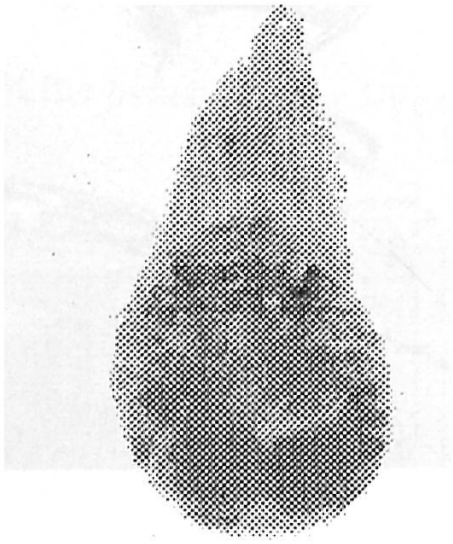

Fig. 4.2

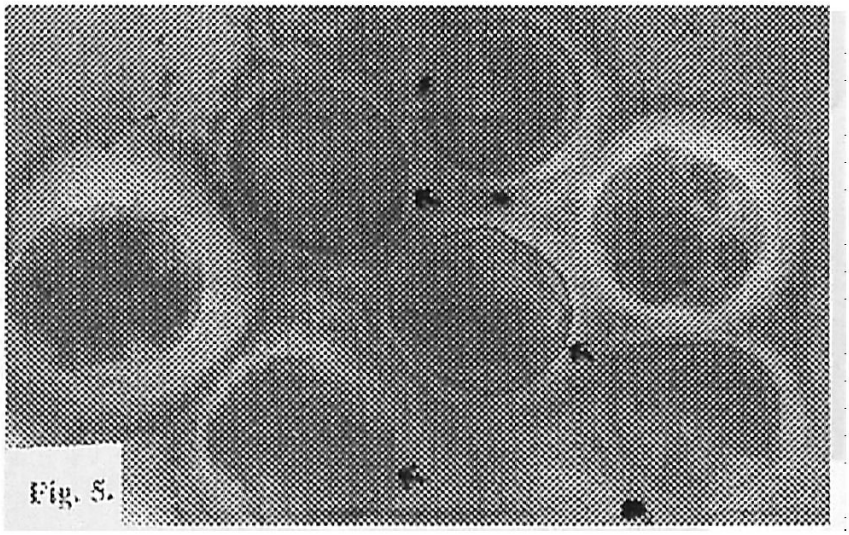

Fig. 5.

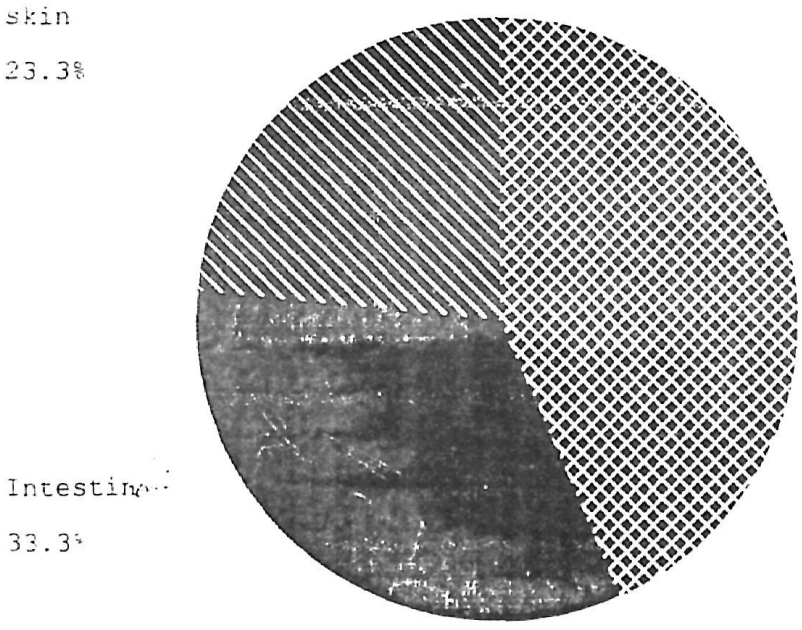
skin
23.3%
Intestin
33.3%

Fig.6

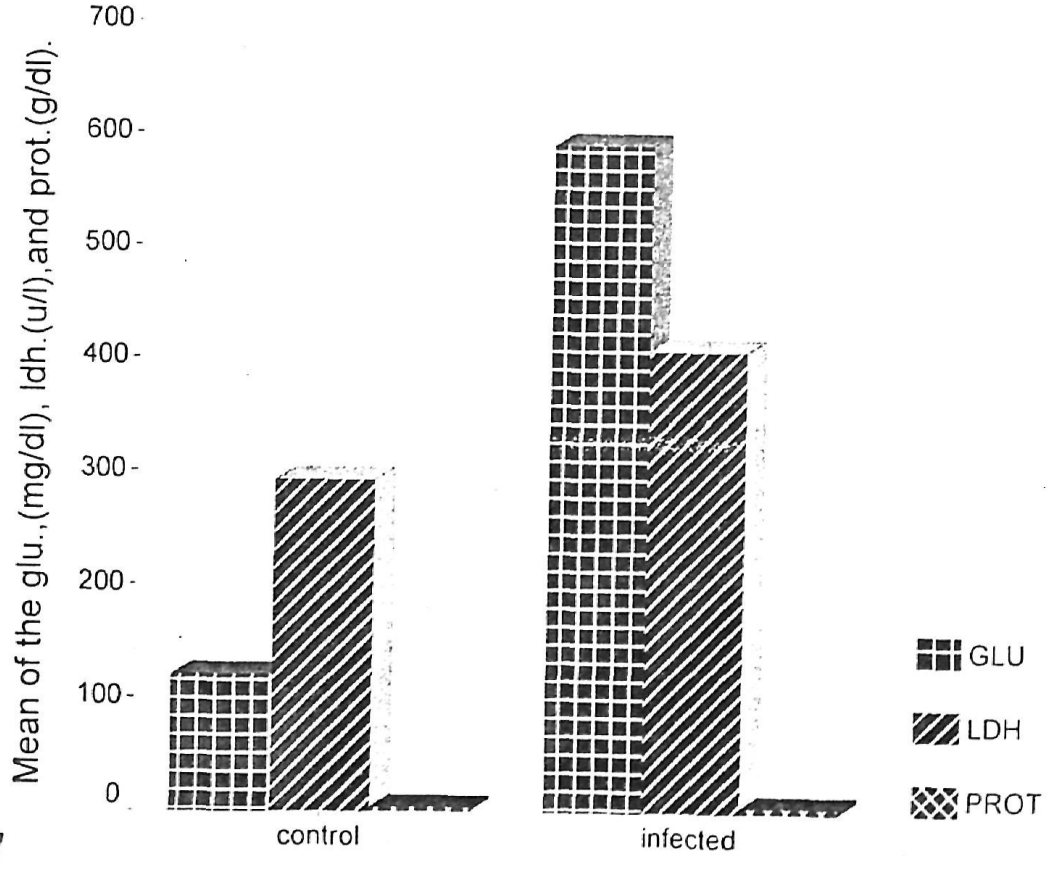
Mean of the glu.,(mg/dl), ldh.(u/l),and prot.(g/dl).
700
600
500
400
300
200
100
0
control
infected
GLU
LDH
PROT

Fig.7