4.7.2.3 Effect of starvation on the immune organs of *Garra gotyla gotyla* (Tables 40-41 and Figures 207-226)

Main function of the immune system is to protect an organism against infection in order to minimize the fitness costs of being infected. An efficient immune system thus is the basic requirement of any organism so as to prevent the invasion of harmful substances in a bid to safeguard the health of fish. Although in fishes, like mammals a strong relationship exists between starvation and immune system but this has received very little attention. Starvation is known to reduce the immune defense mechanism in fish but it definitely depends upon the length of extent of starvation period. Previous studies have shown that food deprived for short period did not cause severe consequences to fish but it was only after exposure to prolonged starvation that the fish’s immune system shows the failure of such functions.
Presently an attempt has been made to evaluate the effect of starvation (for nine weeks) on the immune organs viz. head kidney, spleen and thymus of fish *Garra gotyla gotyla* when starved for a period of 9 weeks (63 days). Changes in cellular architecture through imprint method for spleen and head kidney and by microtechnique in thymus have been analyzed to assess the gravity of effect.

It is clearly evident from the Tables (40 & 41) and Figures (207, 208, 213 & 214) that imprints of spleen and head kidney of control *Garra gotyla gotyla* showed the presence of both agranulocytes (lymphocytes and monocytes) and granulocytes (neutrophils, basophils and eosinophils) besides thrombocytes and macrophages. Starved fishes on the other hand were observed to depict variations in these cells at different time intervals (Tables 40 & 41 and Figures 209-212 & 215-218). As evident from Tables (40 & 41) and Figures (225 & 226) a decline in number of agranulocytes viz. lymphocytes and monocytes and granulocytes viz. eosinophils and basophils was observed. Neutrophils however exhibited an increase during the starvation period. An increase in the number of thrombocytes has been observed during the starvation period of 9 weeks whereas macrophages exhibited a decline in their number under the stress of starvation.

Lymphocytes, which are the known site for antibody production, play an important role in the defense mechanism of fish like other vertebrates. During present investigation marked decline has been observed in the count of lymphocytes (lymphocytopenia) due to stress of starvation. This decline in lymphocyte count indicates that there will be decrease in the rate of antibody production in starved fish. Hence an immunosuppressive response gets evoked in starved fishes which make them more susceptible to infections and diseases. Bagley *et al.* (1991), Blazer (1992), Lopez (2004), Olabuenaga (2004) and Raina (2011) also reported immunosuppressive state in fish due to decline in the population of lymphocytes in immune organs of fish. They further added that this ultimately affect their antibody producing capacity. Tort *et al.* (1998) also reported that immunosuppression in fish due to lymphocytopenia results in increase in susceptibility of starved fishes to various diseases and infections. Starvation appears to affect the lymphopoietic process and hence become responsible for a decline in rate of lymphocyte production in general blood circulation.
Other constituents of immune system viz. monocytes, neutrophils, basophils, eosinophils, thrombocytes and macrophages through the process of phagocytosis play a key role in both non specific and specific immune responses of fishes and represent the first line of defense against invading agents (Ellis, 1977; Anderson, 2003 and Safahieh et al., 2010). Significant decline was observed in monocytes, basophils, eosinophils and macrophages presently in starved fishes which author proposes resulted in reduction in their phagocytic activity. Fard and Woo (2008), Rundles (2008) and Rawadeh (2010) too supported present viewpoint on the basis of observed reduced phagocytic activity in starved fishes they studied.

Neutrophils, also one of major phagocytic cell, however, showed a gradual increase during the experimental period. This increase may seemingly be an attempt on the part of fish to compensate the effect of decreased phagocytic activity of fish, because of decline in number of all other phagocytic cells (monocytes, basophils, eosinophils and macrophages). Rios et al. (2005) also reported increase in phagocytic activity due to increase in the population of neutrophil cells to combat the stress caused by starvation. Wedemeyer et al. (1990) also reported stress related changes in the differential leucocyte count of fishes. Besides neutrophils, thrombocytes also manifested an increase throughout the period of starvation (Tables 40 & 41 and Figures 225 & 226). Thrombocytes being phagocytic in nature may only be helping the otherwise deteriorating phagocytic machinery of starved fishes. Thrombocytes also play an important role in blood clotting and to arrest internal bleeding caused by stress of starvation.

From above discussion it becomes amply clear that food deprivation drastically affects the differential leucocyte count in both of the immune organs viz. spleen and head kidney.

**Thymus**

The thymus plays a pivotal role in the development of the adaptive immune system, an important factor that separates higher vertebrate from the rest of animal phyla (Chilmonczyk 1992 and Trede and Zon, 1998). In fishes, thymus is primary lymphoid organ and plays a key role in the maturation of lymphocytes (Romano et al., 2004 and Xie et al., 2006).
Microscopic examination of the thymus of control fish depicted the presence of (1) lymphocytes i.e. cell involved in antibody production and (2) thymocytes i.e. cell involved in the production of thymosin hormone (Figure 219).

Thymus of starved fish exhibited various histopathological changes including necrosis, vacuolation, loss of normal cellular architecture and degeneration of thymic tissue during the experimental period of 9 weeks (Figures 220-224).

Thymus of starved fish showed the initiation of process of necrosis by 3rd (Figure 220) week of starvation. Necrosis is an advanced and irreversible stage of degeneration and leads to premature death of the thymic tissue. Necrosis by disrupting the normal functioning of thymic tissue leads to its vacuolation. Vacuolation which surfaced during the 4th week of starvation (Figure 221), become more intense by 5th week of experimental period. Vacuolation impair the synthesis and subsequent release of substances into the general blood circulation.

Both necrosis and vacuolation by resulting in loss of normal cellular architecture of thymic tissue by 6th week finally has been observed to lead to degeneration of thymic tissue by the end of 9 week of experimental duration (Figures 222-224). These cellular changes by causing total loss of thymic tissue cellular architecture may affect its functional efficiency. Tiongco et al. (2004) advocated that food deprivation by resulting in the degeneration of thymic tissue, finally lead to fish mortality due to their continued immunosuppressive state.

Functioning of thymus, like that of any other organ of body also depends upon the nutritional status of fish. Deficiency of any nutrient can create crucial state in the immune organ (thymus) by altering its functional efficiency and thereby increase their susceptibility to pathogens and opportunistic infections. Rundles (2008) also reported that nutrient insufficiency leads to reduced thymic function and this is directly associated with decreased host defense mechanism.

On the basis of histopathological observations in the thymus tissue, it can be stated that starvation period of 9 weeks has resulted in the immunosuppressive state in fish Garra gotyla gotyla. Because of such condition fish become immunologically too weak and no longer appear to combat the stress caused by starvation.