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EFFECT OF STARVATION ON IMMUNE ORGANS OF FISH LABEO BOGA

Raina S.[1] & Sachar, A.[2]
Department of Zoology, University of Jammu, J&K, India-180006[1, 2]

Abstract

In the present investigation an attempt has been made to study the effect of starvation on the immune organs viz., thymus, head kidney and spleen of fish, *Labeo boga* for the experimental period of 60 days. On the basis of this research, the author has made an observation that this natural stressor (starvation) induced various alterations in the immune organs of the present fish at the cellular level. Prolonged starvation has been found to result in structural abnormalities as well as immunosuppressive effect on immune organs of fish, *Labeo boga*. It therefore means that health status of the fish gets drastically affected under prolonged starvation thereby reducing its quality as well as quantity.

***Key words*:** Starvation, Immune organs, *Labeo boga*.

Introduction

An efficient immune system is a basic requirement to prevent the invasion of harmful substances and safeguard the health of fish (Caruso *et al*., 2010). The main immunocompetent or lymphoid organs which provide immunity in fish are thymus, head kidney and spleen (Sachar, 2011). When fishes get exposed to stressful conditions like starvation, the functioning of their immune organs can be greatly affected (Tiongco *et al*., 2004). Starvation is known to strongly reduce the immune response and antibody production in fishes. These conditions may decrease the fish's resistance to diseases which may eventually prove fatal for them (Wedmeyer, 1990).

Fishes are able to sustain starvation but only for a certain period of time and as the duration of starvation increases, their ability to cope with it decreases. Moreover, prolonged starvation depletes the energy reserves of the fish (Rundles, 2008) due to which their immune system get suppressed and as a result, fishes become susceptible to the infectious diseases. In the lieu of above fact, present work has been undertaken to study the impact of starvation on the immune organs (thymus, head kidney and spleen) of fish *Labeo boga* for an experimental period of 60 days.

Materials and Methods

Fingerlings of fish, *Labeo boga* (about 50 in number) collected with the help of cast net from River Tawi of J&K state, India, were acclimatized to laboratory conditions for two weeks and were fed regularly with artificial diet. After acclimatization, healthy specimens of *Labeo boga* (size ranging between 10-15cm) were divided into control and fasting group in triplicates. Each group contained ten individuals. Control groups were fed with commercially available pellet fish feed twice a day where as the starved groups were deprived of food for an experimental period of 60 days.

For studies related to cellular architecture of immune organs viz., head kidney and spleen, imprinting method was employed. Freshly cut pieces of immune organs (viz., head kidney and spleen) were gently blotted on a clean slide and an imprint was made of the resident cells. Slides so prepared were air dried and stained using leishman-geimsa stain (Anderson, 2003) and cellular components were identified using compound microscope.

Since thymus is not a localised organ, it was not possible to evaluate the alterations in its cellular composition by imprinting method. Therefore the immune cells of thymus were studied by histological method. For histology, thymus tissue was embedded in histowax of 54-56°C. 5-7 µm thick sections were cut on microtome and stained using haematoxylin eosin stain. Slides of immune organs were scanned and photographed with Sony-DC378P-Semi-Digital Camera attached with Olympus CH20i Research microscope.

Results

Microscopic examination of the imprints of immune organs viz., spleen and head kidney of control fish depicted thrombocytes, macrophages and leucocytes comprising of agranulocytes (lymphocytes & monocytes) and granulocytes (neutrophils, eosinophils & basophils) (Figs. 1,2 & 9, 10). Imprints of spleen and head kidney of starved fishes exhibited decrease in agranulocytes viz., lymphocytes & monocytes and granulocytes viz., neutrophils, eosinophils & basophils during the 60 days of starvation period (Figs. 3-8 & 11-16). Macrophages have also been found to exhibit drastic decline in their numbers (Figs. 3-8 & 11-16)) but thrombocytes depicted increase in their count under the influence of prolonged starvation .

The microscopic examination of histology of thymus of control fish (Fig. 17) depicted two types of cells viz., thymocytes (Thy) and lymphocytes (Lym). Thymus of starved fishes exhibited necrosis (N) by 20th day of the starvation (Fig. 18). Necrosis was followed by vacuolation (V) of its tissue after 30 days of experiment (Fig. 19). Necrosis and vacuolation ultimately resulted in loss of normal cellular architecture (DCA) of thymus which became apparent on 40th day of starvation (Fig. 20). Between 50th (Fig. 21) to 60th day (i.e. end of experimental period) there was total degeneration (TD) and consequent decrease in the cellular content of the thymus tissue (Fig. 22).

Discussion

The trend of the immune cells in the presently studied starved fishes very clearly suggests that prolonged starvation has caused marked reduction in the leucocytes (leucocytopenia) in both of the immune organs viz., spleen and head kidney. Leucocytopenia surfaced after 10 days of starvation which became more pronounced with the advancement of the experiment in both spleen and head kidney. Nutrients regulate the functioning of the immune organs in an organism (Rundles, 2008). Their non-availability during prolonged food deprivation, according to present author, may result in functional impairment of these organs. This ultimately by hampering the process of leucopoiesis in immune organs rather results in leucocytopenia in the presently studied starved fishes. Among leucocytes, lymphocytes are regarded as the most prevalent and immunocompetent leucocytes in the immune organs of fish (Ellis, 1981). Presently, lymphocytes have been observed to exhibit marked decline (lymphocytopenia) under the influence of prolonged starvation. Lymphocytopenia must have resulted in reduced antibody production in starved fishes and thus can make such fishes more susceptible to infections and stress of xenobiotics. Ham (1974) and Olabuenaga (2004) also reported that prolonged starvation suppresses the immune response of the fish by reducing the population of lymphocytes in immune organs and hence affect their antibody producing capacity.

Presently it appears that prolonged starvation may inhibit lymphopoiesis in the immune organs and hence result in lymphocytopenia in spleen and head kidney of fishes. Lymphocytopenia as earlier stated by Tort *et al*. (1998) causes immunosuppression and thus may increase susceptibility of starved fishes to various diseases and infections.

Other immune cells viz., monocytes, neutrophils, eosinophils, basophils, thrombocytes and macrophages are usually involved in phagocytic functions (Anderson, 2003). Phagocytes serve as the first line of defense against toxicants (Safahieh *et al*., 2010). Chronic starvation generally finds relation to reduced phagocytic activity of starved fishes (Rundles, 2008). Decrease in the phagocytic activity of starved fish can be supported by the significant decline in all the phagocytic cells (monocytes, neutrophils, eosinophils and basophils) in starved fish *L. boga* of the present studies. In tune to present findings, Rundles (2008) and Fard and Woo (2008) also reported starvation related decline in phagocytic activity of fishes they studied. Moreover, macrophages, the other important phagocytic cell have also been observed to exhibit decrease in their number due to prolonged food deprivation. This clearly indicates that starvation drastically influence the phagocytic machinery of the fishes. Compared to other leucocytes, thrombocytes are the only cells which have been found to exhibit an appreciable increase in their number. These being phagocytic in nature (besides their involvement in blood clotting), appears to maintain the phagocytic machinery of the starved *L. boga*.

Thymus is the primary lymphoid organ in fishes (Xie *et al*., 2006). Its major function is to provide an appropriate microenvironment for the development, proliferation and maturation of lymphocytes.Thymus tissue has been found to exhibit necrosis by 20^{th} day of starvation. Necrosis by causing premature death of the living tissue in thymus appears to be the primary cause of degeneration of a tissue. Presently, necrosis appeared to result in death of thymic cells and leads to its vacuolation by 30^{th} day of the starvation. Vacuolation by causing sparse distribution of cells in a way exhibited the signs of initiation of loss of normal cellular architecture of the thymus by 40^{th} day of the experiment. This was observed to get more intense with the advancement of the starvation period. By the end of the experiment, thymic tissue revealed almost complete atrophy. It simply implies that prolonged starvation has caused the destruction of the histological architecture of thymic tissue. Starvation hence appears to be causative of structural as well as functional impairment of the thymus and may drastically suppress its immune functioning. Rundles (2008) reported that nutrient insufficiency leads to reduced thymic function and this is directly associated with decreased host defense mechanism. Since thymus is central to the development of controlled adaptive immunity in an organism, starvation related atrophy of this primary lymphoid organ can definitely cause profound immune malfunctioning leading to susceptibility of fishes to pathogens, foreign agents/toxicants and opportunistic infections.

Conclusion

From the overall discussion on immune organs (thymus, spleen and head kidney), it can be therefore be concluded that prolonged food deprivation has resulted in the loss or reduction in the immune defense mechanism of fish *L. boga* by causing hypocellularity of these immune organs. Because the immune system is vital for the fish's defense against infections/harmful agents, their impairment in starved fishes can critically affect not only their health but their growth and quality as well. Present findings are thus suggestive that nutrition is the physical base of immune system like other systems and that it is good nutrition which helps in maintaining the immunological functioning of the fishes. It is therefore strongly recommended that fish should not remain under starvational stress for a longer duration as it reduces the effectiveness of their immune system thereby increasing the opportunities for disease causing organisms.

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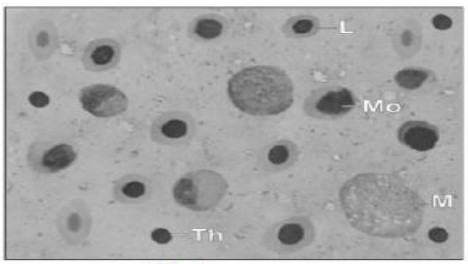
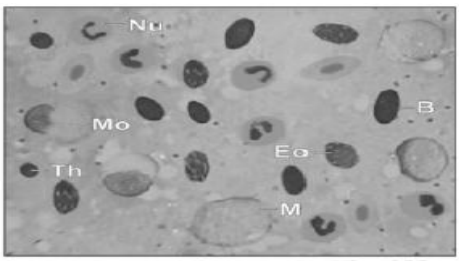


Fig.1



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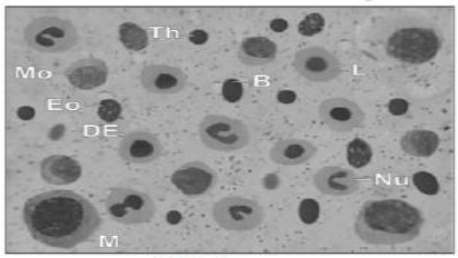

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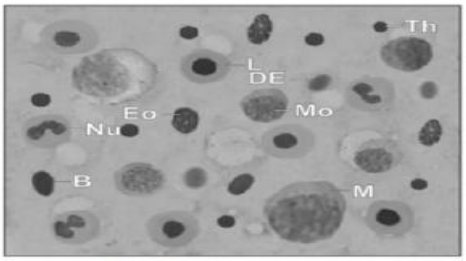

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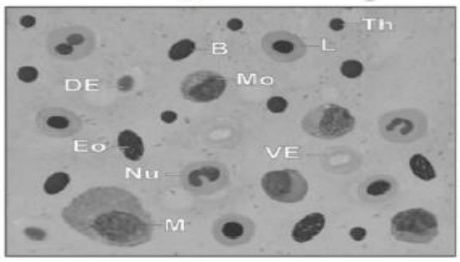

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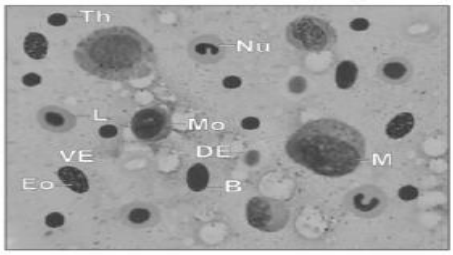

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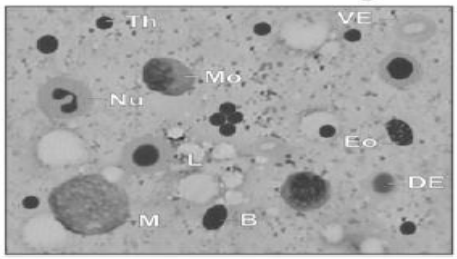

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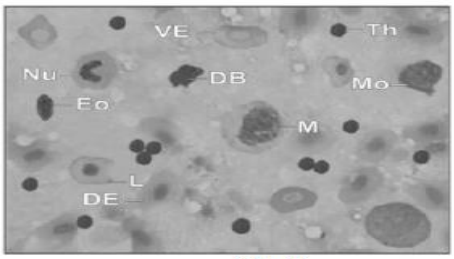
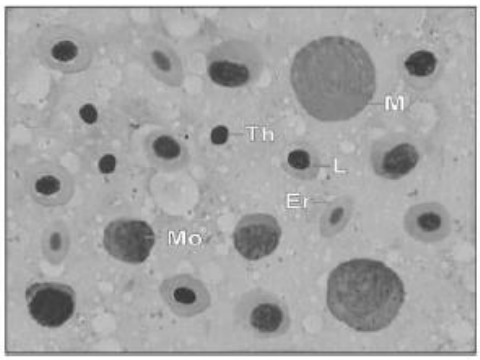


Fig. 8

Fig. 1 and 2 Microphotograph of imprint of splenic tissue of *L. boga* from control showing normal Erythrocytes (E), Lymphocytes (Lym), Monocytes (Mo), Macrophages (M), Neutrophils (Nu), Eosinophils (Eo), Basophils (B) and Thrombocytes (Th).

Fig. 3-8 Microphotograph of Imprint of splenic tissue of starved *L. boga* showing Distorted and Vacuolated Erythrocytes (DE, VE), decline in Lymphocytes (Lym), Monocytes (Mo), Neutrophils (Nu), Eosinophils (Eo), Basophils (B) and Macrophages (M) with increase in thrombocytes (Th) of the fish after 10, 20, 30, 40, 50 and 60 days of the experiment respectively.



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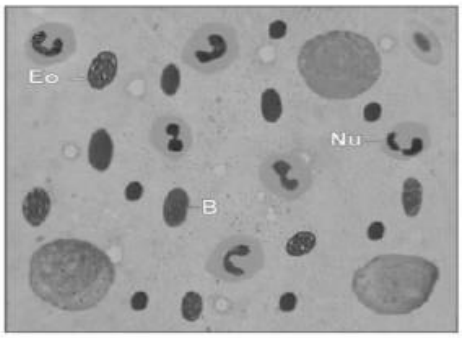

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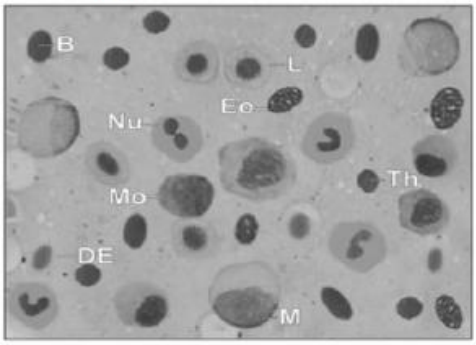

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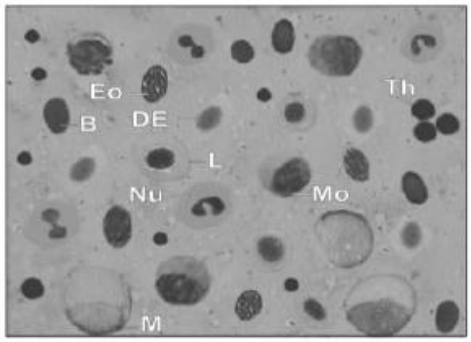

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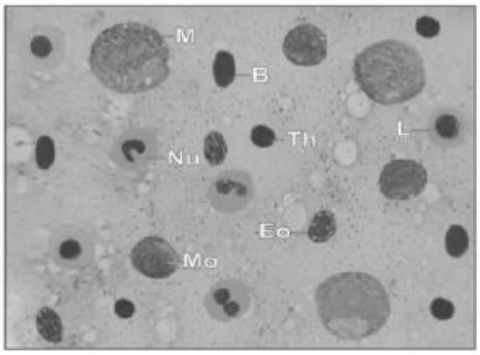

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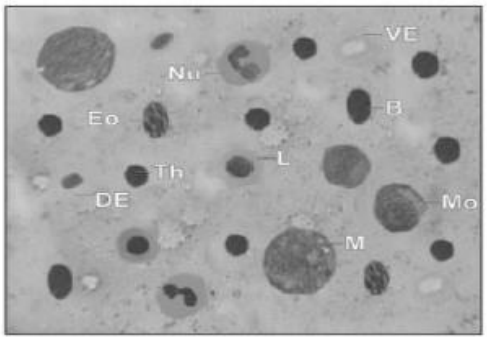

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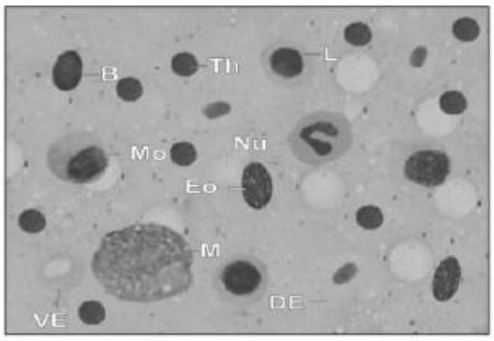

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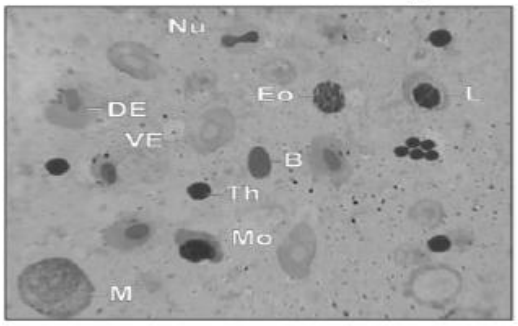


Fig. 16

Fig. 9 and 10 Microphotograph of imprint of kidney tissue of *L. boga* from control showing normal Erythrocytes (E), Lymphocytes (Lym), Monocytes (Mo), Macrophages (M), Neutrophils (Nu), Eosinophils (Eo), Basophils (B) and Thrombocytes (Th).

Fig. 11-16 Microphotograph of Imprint of kidney tissue of starved *L. boga* showing Distorted and Vacuolated Erythrocytes (DE, VE), decline in Lymphocytes (Lym), Monocytes (Mo), Neutrophils (Nu), Eosinophils (Eo), Basophils (B) and Macrophages (M) with increase in thrombocytes (Th) of the fish after 10, 20, 30, 40, 50 and 60 days of the experiment respectively.

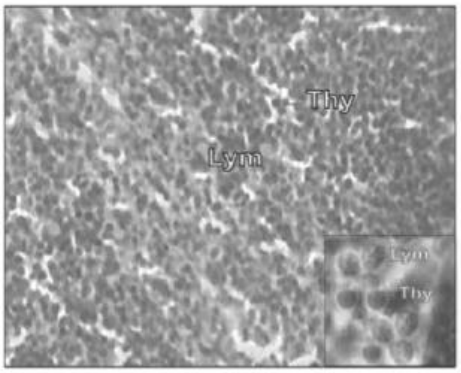
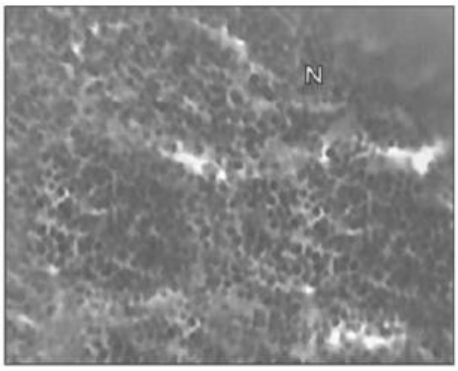


Fig. 17



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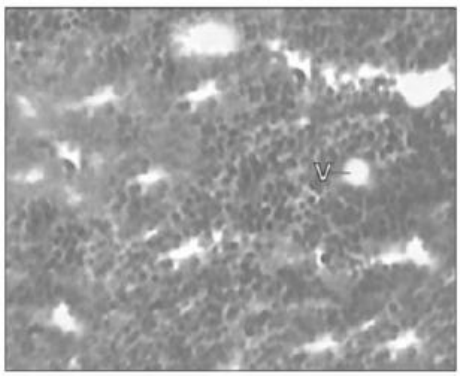

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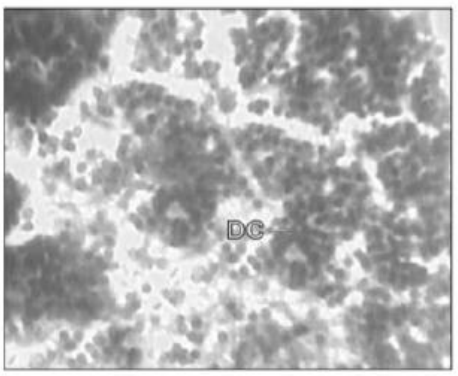

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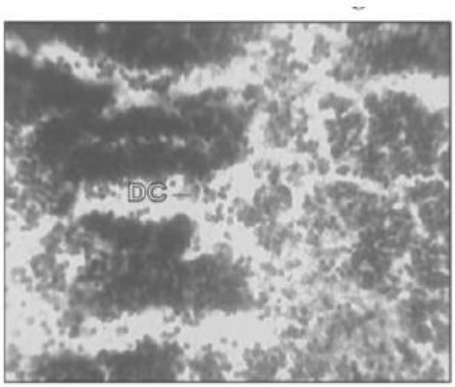

Fig. 21

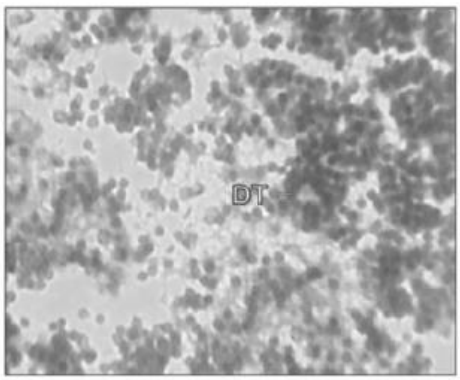

Fig. 22

Fig.17 Microphotograph of Thymus tissue of *L. boga* from control showing Lymphocytes (Lym) and Thymocytes (Thy).

Fig. 18-22 Microphotograph of Thymus tissue from starved *L. boga* showing Necrosis (N), Vacuolation(V), Degenerative changes (DC), Severe Degenerative changes (DC) and Total degeneration (TD) of the tissue after 20, 30, 40, 50 and 60 days of the experiment respectively.