The occurrence of fat liver disease (FLD) in Rainbow trout due to side effects of dietary lipids at cultured salmonids farms in North of Iran

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Abstract
Industrially cultured fish of rainbow trout (*Oncorhynchus mykiss*) are the most sensitive fish of salmonids to the fat degenerative disease of the liver, which may be the result of feeding with high-fat diets or inadequate nutritional dietary regimens at low temperatures. The particular important problem contributed to the provision of fat requirements in fishes is the prevention of O$_2$ auto oxidation of lipids or unsaturated fatty acids chemically comprised chains of hydrogenated carbon atoms with long chains (4 to 6 carbon atoms in the chain). In this work, the qualitative histopathological tests were performed on liver, kidney, intestines and muscle tissues specimens of the rainbow trout to revealed fat accumulation. The severity of fatty infiltrations was judged by the relative quantities of intracellular fat droplets. The cellular changes in the following cells were obvious; sinusoidal endothelial lining cells, hepatocytes, the epithelial cells of biliary, and hepatic ducts. The histopathological examination in rainbow trout after using the lipids which are the most effective source of energies in fish after proteins, showed steatites in the visceral fat area. Increasing the fat rate up to %17 in the dietary regimens is economical and will save protein usage, will reduces N$_2$ excretion and water pollution.

Keywords: Fatty Liver Disease (FLD), Rainbow trout, liver

Introduction:
A salmonids are susceptible to lipid liver degeneration but it is a particularly Significant problem in Rainbow trout culture and, recently Rainbow trout as an important farming cold water fish, has been cultured in large number.

Since, the dietary regimens have a great real role in the costs for a breeding system of fish, changing of the regimens and having a proper formula is very important and essential, since it could provide the nutritional requirements. Using the lipids in a proper percentage, as the most significant source of energy in fish, is very important.

In this study, the fishes collected by the laboratory of fish pathology were examined histopathologically for the occurrence of fat liver disease. The supplementary of the fish feed usually contained (more than 17% by the weight) either Kilka Oil (K.O) or Soy Oil (S.O) were used, the lipids comprised of polysaturated fatty acids (PUFA) (with four or more double bands) at 17% of diet for K.O and S.O respectively.

In fish culture, using a proper rate of fat is very important because of the quality of the product and with a view of containing fat in muscles and making histopathological alterations in the fish liver, so the fat should be used in rates with definite limits to prevent mortality in fishes.

The purpose of this study was to show the histopathological changes of the fatty liver disease in Rainbow trout, due to dietary lipids. The rate of 17% fat could be recommended regarding growth which not produce damages or mortality in fish.

Materials &Methods;
In 1999, the occurrence of FLD in the specimens of 100 Rainbow trout at cultured salmonids farms from northern of Iran, received by the laboratory of fish histopathology, was examined microscopically. The autopsied samples of the liver, kidney, intestine, muscle and spleen were fixed in 10% formaline and then the Hematoxylen & Eosine (H&E) staining method was performed.

**Results:**

In contribution to the histopathological effects of fat in diets of fishes various investigations have been conducted. As Randall *et al* reported in 1997 that the hybrids of white bass were fed an experimental fat diet up to 205% by weight; there were no significant effects from lipid storage point of view in the tissue of fish. Guarda, Hellou *et al* (1997) reviewed the effects of lipids in fish.

In this work the purpose of study was to show the histopathological lesions of FLD (Fatty liver Disease) and to demonstrate the liver changes.

The rate of IDF (intra peritoneal fat) was significantly high. The FLD effects on the normal metabolism of liver, the liver enlarged and showed microscopic changes. The lesions were as follows,

1. **Liver,**
   In the liver vacualation was increased, predominantly due to accumulation of fat. Cellular edema and infiltration of fat in the hepatocytes were occurred because of the lack of the metabolism and cellular oxidation resulted from existence of toxicants in food and anemia produced by disease. In some hepatic cells coagulation necrosis induced by the effects of toxicants were observed and finally substitution by fats were obvious. Accordingly, histopathologically, the Main feature was the extreme infiltration of hepatocytes by lipid, which caused loss of cytoplasm staining and distortion of hepatic muralia.

Kidney,

Hemosidrine was present. The hematopoetic tissue was degenerated.

Spleen,

Congestion, hemosidrosis and pale – staining fragment in the melanomacrophage centers were observed.

Intestines,

Congestion and infiltration of monocytes in the parrine mucusal section were noticed. Also, Steatites or infiltration and substitution by the inflammatory cells and the extensive deposits of hyaline intercellular material and thickening of the cell membrane were cleared, which was due to the high rate of lipids or the effects of toxicants resulted from the food or the fat oxidation consumed.
Fig. 1: Gross lesions of FLD in Rainbow trout at the necropsy examination time.
Fig 2: Microscopic views of the liver in the FLD.

Fig 3: Cross section of liver bile ducts with melanomacrophaghs around them and the accumulation of hemosidrine in hepatic tissue.

Fig 4: The vacuolated hepatocytes’ cytoplasm’s, precipitation of hemosidrine in liver tissue.
Fig 5: In kidney, the existence of hemosidrine and proliferation hematopoetic tissue and vacuolated cytoplasm of urinary ducts epithelial cells.

Fig 6: In spleen, hemosidrine and presence of light pink cerroid liquids in the macrophages of some areas.
Fig 7: Proliferation of inter peritoneal stocked fat tissues.

Fig 8: Steatitis of IPF.
Discussion:
The main changes were noticed in the liver, and the IPF (intaperitoneal fat tissue). In this study the aim of the work was to show the microscopically features of FLD in Rainbow trout fish. Invasion and infiltration of lipids followed by hepatocyte cell swelling occurred.
Anemia after failure of the liver to secrete hemopoetine was induced and at last mortality in fish could be seen. In IPF thickening of the cell membrane and substitution of fat tissue by hyaline intercellular material and inflammatory cells were seen. Also, steatitis or infiltration and substitution by the inflammatory cells and the extensive deposits of hyaline intercellular material and thickening of the cell membrane were cleared, which was due to the high rate of lipids or the effects of toxicants resulted from the nutritional effects of lipid dietary and was related to the consumed fat oxidation.

In conclusion, it can be said that increasing the fat rate in the regimens is partly cast benefited and will save protein usage, reduce N₂ exertion and water pollution.

The results show that the fishes whit nutritional problems, show edema of hepatocytes and after lipid invasion and after impairment of liver in secretion of hemopoetin, the anemia will occur.

In IPF (Intaperitoneal Fat tissue), the increase of intracellular hyaline and thickening of membrane cells will be seen.

The fishes show anemia, the gills are pale, and the liver with fatty vacuoles is yellow and pale in color.

Microscopically in FLD the infiltration of fat into hepatocytes caused pale cytoplasm staining and the wary maralia. The changes spleen and the hematopoetic tissue of kidneys were present. The macrophages containing ceroid liquid produced by metabolism and ligation of phospholipids, were different.

The other changes such as steatitis of fat in the IPF, darkening, hemosidrosis of spleen, atrophy of muscles, the substitution by fat tissue, the degeneration of hematopoetic tissue in kidneys were seen, compared with controls.

Accordingly, it can be emphasized that increasing the rate of fat in dietary can improve the growth in a limited rates and adding inappropriate rate of fat in diet can make the diet economical and inhibit water pollution due to reduction of N₂ excretion but can causes mortality in fish.

References: