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Histopathological alteration in the livers of striped snakehead murrel (*Channa striatus*) grow-out fed with different dietary fats

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ABSTRACT

The livers of teleost fish are susceptible to numerous disturbances including metabolic changes. Different types of fat in the diets were evaluated to observe their impact on the hepatic tissues of saul (Channa striatus). The saulgrow-out having an initial average weight 27.36 ± 0.09 to 32.54 ± 0.41 g were fed with seven experimental diets (F1, F2, F3, F4, F5, F6 and a control, F7 of natural foodstuffs, NATFO for 12-weeks). F1 (L3HUF) contains 0.5% n-3 fatty acid& 7.5% saturated oil; F2 (H3HUF) contains 1.0% n-3 fatty acid& 7.0% saturated oil; F3 (MUSOL) contains 8.0% mustard oil; F4 (LINOL) contains 8.0% linseed oil; F5 (MIXOL) contains 4.0% mustard oil and 4.0 % linseed oil; F6 (SATOL) contains 8.0% saturated oil. The control fishes were fed with live natural food. Livers of 3 fishes from each treatment were excised and processed for routine histo-pathological evaluation. The histo-architectural changes in hepatic tissue, following dietary fat interventions, were assessed under light microscopy. F-1, showing vacuolization in cells; F-2, showing normal hepatocytes with some vacuolization; F-3, showing normal hepatocytes and central vein; F-4, showing necrotic patches and enlarged cells; F-5, showing necrotic normal cells; F-6, showing normal cells and central vein with vacuolization. Liver of Channa striatus fed with natural food (NATFO, F7) showing normal architecture of liver with nucleated hepatocytes with cytoplasm and connective tissue cells. The results revealed some pathological changes in the liver tissue like fatty degeneration, cellular necrosis, vacuolization and which was found with least effects, observed with addition of various dietary fats. It was concluded that supplementation of various fats has role in the histological alterations, at cellular level, in the hepatic tissues in this carnivore fish and the fat could be safely used for better fish quality in terms of fatty acid deposition and economizing the feed cost.

Keywords: Histopathology, liver, *Channa striatus*, grow-out, dietary fats

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INTRODUCTION

The livers of fish plays an important role in various metabolic processes and their work can be broadly classified as storage, synthesis and excretion. The histological changes in the liver tissue in vertebrates on account of dietary supplementation are important markers for the assessment of the effects of dietary alterations. A balanced feed and feeding in fish production system is necessary for the better production of healthy and quality fish. It is reported[1]

that fish reared in intensive culture systems require all nutrients in a complete diet. The study to record the cellular changes in the fish liver on feeding any manipulative diet for a longer time is actually considered as an useful tool for the observation of its impact. Due to the regularly increasing fish demand, it is necessary to increase aquaculture production on dietary manipulations with increased dietary energy by adding rich fat contents and protein sources. Since the fish oil is not only costly but becoming less available, there is an urgent need to assess the dietary potential of various other available sources of fat from both animal and plant sources. In global scenario, the emphasis is being given to dietary replacement of animal fat with less expensive plant fat. In this connection, monitoring histological tissues of fish liver is the method to assess the impacts of various energy nutrient that we use as raw materials of plant and/or animal origin especially various types of fat contents. Replacement of fish oil by vegetable oils has proved in many fishes without impacting on growth performances [2,3,4,5]. However, the impacts of various dietary oils on lipid metabolism of fish are still not very, particularly where fish oil provide the only source of highly unsaturated fatty acids, very much essential for catfishes. Variation in dietary oils may lead to imbalances in the essential or non-essential fatty acids, and may be differently affecting cellular architecture.

The objective of the present study was to compare the effect of different sources of dietary fats of both plant and animal origin in practical diets of striped murrel fed on long-term basis on liver histology. The striped snakehead murrel, *Channa striatus*(Family: Channidae), locally known as 'Saul', is one of the most important fish of Indian continent that has a great aquaculture potential. High-energy commercial feeds have been reported to exhibit trich fat accumulations in the liver tissue of this fish which is a major concern to aquaculturist. However, the effect of lipids in order to provide higher dietary energy for better growth and improved health in this fish have not been evaluated, through evaluation of liver tissue and, therefore, was the main interest of present study.

MATERIALS AND METHODS

Six type of feeds were formulated having similar feed ingredients in same quantities excepting different source of fat namely low level of highly unsaturated fatty acid (L3HUF, F1); high level of highly unsaturated fatty acid (H3HUF, F2); mustard oil (MUSOL, F3); linseed oil (LINOL, F4); mixed oil (MIXOL, F5); saturated fat (SATOL, F6) and a control (NATFO, F7) comprising of natural food stuffs (Table 1). Six diets (L3HUF, F1; H3HUF, F2; MUSOL, F3; LINOL, F4; MIXOL, F5; SATOL, F6) and a control (NATFO, F7) with natural food. F1, contains 0.5% n-3 fatty acid and 7.5% saturated oil; F2, contains 1.0% n-3 fatty acid and 7.0% saturated oil; F3, contains 8.0% mustard oil; F4, contains 8.0% linseed oil; F5, contains 4.0% mustard oil and 4%

linseed oil; F6, contains 8% saturated oils. (Table 1).In order to evaluate the effect of different oil sources on the liver of *C. striatus*, the experiment was conducted in indoor condition in 14 (7 types of feed, 2 replicates) round plastic pools of 300 litre capacity, each filled-up with 100 litre tube well water. Each having two replications, stocked with 20 grow-out having an initial average weight 27.36±0.09g to 32.54±0.41g were plotted in each of the plastic pool after proper acclimatization. The tanks were provided aeration from a portable aerator round the clock. During the experiment, the fishes were fed twice a day at 10:00 and 17:00 hours ad libitum per day. Rearing pools were cleaned every second day and about half of the water was replaced with fresh bore-well water to reduce the nitrogenous waste accumulated as debris and faecal matters. After 12-weeks of feeding trials with seven feed combinations (Table-1) the animals were sacrificed. The liver from control(F7,NATFO) and experimental fishes (feed with different fats (F1 to F6) were excised and fixed in 4 % formaldehyde and processed by standard histological techniques ie., kept in aqueous Bouin's fluid for 24-hrand washed for 8-hr in running tap water. The organs were routinely processed (dehydrated in ethanol series, embedded in paraffin, serially sectioned at 6µ). Sections of the liver tissue were stained with Haematoxylin and Eosin(HE). Histological slides were observed under microscope (Labomed, Model :Digi 2) for cellular evaluation.

RESULTS

The feeds tested did not have a deleterious impact on survival, growth rate and condition on *C. striatus*. The H/E sections of liver of control fish fed with natural feedstuffs (NATFO, F7) showed normal architecture of liver tissue with hepatocytes cells. The hepatocytes were normal looking with polygonal shape, abundant cytoplasm and dark centrally rounded vesicular nucleus with nucleoli. There was no sign of any kind of necrosis or inflammations (Fig 1). Liver tissue of F-1(L3HUF) fed *C.striatus*, showing vacuolization in cells and no kind of necrosis were seen (Fig. 2).The liver tissue of high level of HUFA (H3HUF, F2) fed fish also showed normal architecture of

hepatocytes and no fatty degeneration of parenchymal cells, however, some vacuolization noticed (Fig.-3). F-3 (MUSOL) fed fishes showing normal hepatocytes and central vein (Fig-4). The fish fed linseed oil (F-4, LINOL) showing necrotic patches and enlarged cells (Fig-5). The fish fed mixed oil (F5, MIXOL) showing necrotic normal cells (Fig-6). Liver of *C.striatus* fed with saturated oil (SATOL, F6) showing normal cells and central vein with vacuolization (Fig. 7). The above results were compared with the control(F7, NATFO).

Table: 1 Ingredients composition (w/w) of feeds for *Channa striatus*

Feed Ingredients	F-1 (L3HUF)	F-2 (H3HUF)	F-3 (MUSOL)	F-4 (LINOL)	F-5 (MIXOL)	F-6 (SATOL)	F-7 (NATFO)
Soybean meal	41.0	41.0	41.0	41.0	41.0	41.0	-
Starch Soluble	25.0	25.0	25.0	25.0	25.0	25.0	-
Casein	20.0	20.0	20.0	20.0	20.0	20.0	-
Carboxy Methyl Cellulose	2.0	2.0	2.0	2.0	2.0	2.0	-
Papain	0.5	0.5	0.5	0.5	0.5	0.5	-
Vitamin & Mineral Mix.	3.5	3.5	3.5	3.5	3.5	3.5	-
Omega – 3 HUFA	0.5	1.0	-	-	-	-	-
Saturated Oil	7.5	7.0	-	-	-	8.0	-
Mustard Oil	-	-	8.0	-	4.0	-	-
Linseed Oil	-	-	-	8.0	4.0	-	-
Live Fish/ NATFO	-	-	-	-	-	-	100.0

L3HUF = Low Omega – 3 HUFA; H3HUF = High Omega – 3 HUFA; MUSOL = Mustard Oil; LINOL = Linseed Oil; MIXOL = Mixed Oil (Mustard Oil : Linseed Oil :: 1 : 1 w/w) ; SATOL = Saturated Oil; NATFO = Natural Food

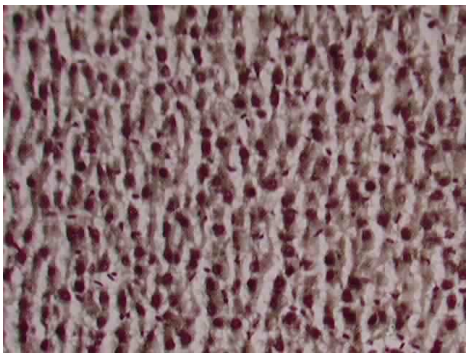

Fig.-1 Control liver of *C. striatus* fed with natural food (F7)

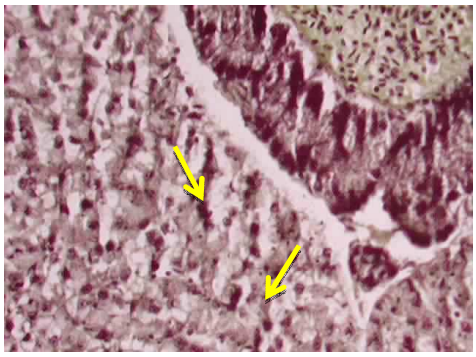

Fig.-2 Liver of *C. striatus* fed with L3HUF (F-1)

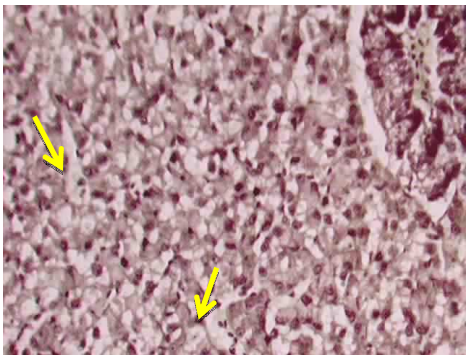

Fig.-3 Liver of *C. striatus* fed with H3HUF (F-2)

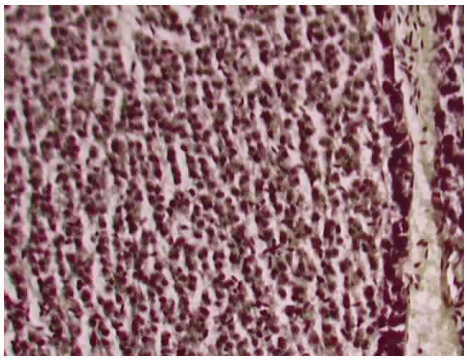

Fig.-4 Liver of *C. striatus* fed with MUSOL (F-3)

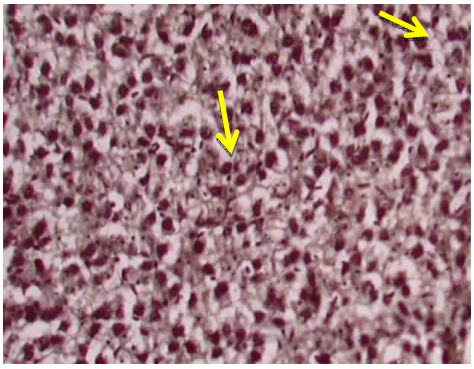

Fig.-5 Liver of *C. striatus* fed with LINOL (F4)

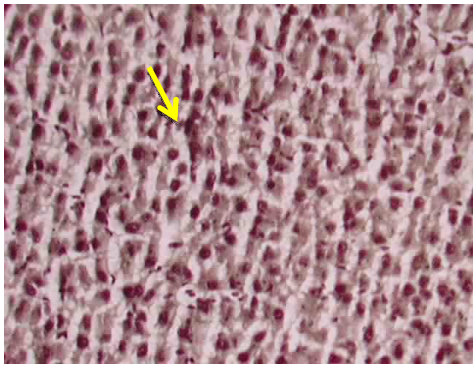

Fig.-6 Liver of *C. striatus* fed with MIXOL (F-5)

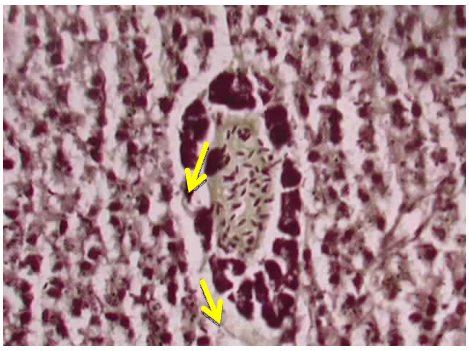

Fig.-7 Liver of *C. striatus*fed with SATOL (F-6)

Figure-1 Liver of *C. striatus* fed with Natural feed (NATFO, F7) showing normal hepatic parenchymal cells in tissue and cytoplasm and dark basophylic centrally rounded vesicular nucleus with nucleoli (H/E **40X)**.
Figure-2 Liver of *C. striatus* fed with low unsaturated fatty acid (L3HUF) (F1) showing normal appearance of hepatic parenchymal cells with more dense and prominent nucleus and showing vacuolization in cells, no kind of necrosis were seen (H/E **40X)**.
Figure-3 Liver of *C. striatus* fed with high unsaturated fatty acid (H3HUF) (F2) normal architecture of hepatocytes and no fatty degeneration of parenchymal cells, however, some vacuolization noticed (H/E **40X)**.
Figure-4 Liver of *C. striatus* fed fishes with F-3 (MUSOL) showing normal hepatocytes and central vein (H/E **40X)**.
Figure-5 Liver of *C. striatus* fed with linseed oil (LINOL, F4) showing necrotic patches and enlarged cells (H/E **40X)**.
Figure-6 Liver of *C. striatus* fed with mixed oil (MIXOL, F5) showing necrotic normal cells (H/E **40X)**.
Figure-7 Liver of *C.striatus* fed with saturated oil (SATOL, F6) showing normal cells and central vein with vacuolization (H/E **40X)**.

DISCUSSION

The effects of different dietary fats on the histology of the liver of *C. batrachus* have been investigated in the present study. It is well known that the liver is the main organ affected by fat deficiency or in excess, lipid type, presence of essential fatty acids, etc in the diet. Feeding fish with natural feedstuffs (NATFO) showed normal architecture of hepatic parenchyma arranged in cord like fashion with clear visible intact nuclei and nucleoli, well packed sinusoidal spaces and no vacuolization (Fig.1). The fish fed with low unsaturated fatty acid (L3HUF) (F1) showing normal appearance of hepatic parenchymal cells with more dense and prominent nucleus and showing vacuolization in cells, no kind of necrosis were seen (Fig.2). The normal architecture of liver tissue in both these cases was due to availability of natural foodstuffs of animal origin in the diet most suitable to this fish as being an omnivore but primarily carnivore and has preference for meaty aquatic food stuffs. The fish fed with other sources of oils,

however, showed some degenerative changes in the form of development of necrosis, vacuolization in the hepatic parenchyma and cellular necrosis. In all the treatments, the most of the cellular features were the presence of well-organized architecture of the hepatic parenchyma, nucleus and cellular structures which indicated that all types of oils tested in the study were not much harmful to striped murrel, *Channa strtiatus* at their levels of maximum supplementation of 8%. Hence they may be used in combination which did not create significant changes in liver structure.

The damage caused here in this study are by the F2 and F6 was larger with large part of parenchyma depicting vacuolization (Fig. 2 &7) followed by linseed oil (F-4) with similar deformities and showing necrotic patches and enlarged cells (Fig.-5).The degenerative changes in hepatic tissues with all fat sources were comparatively very mild and therefore could be used in the diet of this species at comparatively up to 8%. Barring fatty degeneration of hepatic parenchyma, some necrosis and vacuolization no other deformity observed. Fatty degeneration of liver has been observed to be associated with nutritional supplies in cultured fish [7] and similarly it is also documented in the present study. In sea bream, steatosis has been observed as a result of an increase in the dietary lipid content [8], decrease in the essential fatty acid [9], due to use of artificial diets [10] and the inclusion of vegetable oils [11]; though their effect in the correct functioning of the liver and its possible reversibility is not well studied. Some authors consider steatosis as a physiological adaptation to the diet [8, 12] whereas others correlate this to some stress situations [13] and feeding synthetic diets for longer periods which might even cause permanent damages to the tissue even though necrosis or cellular damage is not found in these cases. In the present study, impact of the dietary fat sources may be obvious because this fish is carnivore and mainly consume protein rich natural food of aquatic animal origin and change in diet rich in different fats have, therefore, impacted on cellular levels changes in the liver.

Dietary fatty acid composition also influenced the fat composition of the neutral lipids of the fish. This finding is in accordance with studies for other fishes [14, 15]. The consumption of only plant oils as dietary lipids reduces fish growth. Similar findings were also obtained with low levels of dietary fish oil [16]. For growth, the requirements of different fish species for n-3 HUFA have been shown to vary considerably; rainbow trout, 0.5% of the diet as EPA and DHA; channel catfish, 0.5 - 0.75% of n- 3 HUFA for best growth [17,18]; turbot, 0.57-0.8% of the diet without hepatic cellular changes [19,20], and red sea bream, 0.5 DHA or 1% EPA [21]. At low levels of fatty acid supplementation the growth and the physiological and biochemical conditions of sea bream were affected; the most characteristic change being the accumulation of lipid in the liver and an increase in the hepatosomatic index of fish. Similar effects have also been observed for other fishes[22]. The symptoms were exhibiting with much lower degree, within fish fed the low fish oil diets. Dietary fatty acid composition also influenced the fat composition of the neutral lipids of the fish. This finding is in accordance with studies for other fishes[23, 24]. The response to dietary treatment resulted in a considerable change in liver. A similar response has also been found by some other authors[23] for *Oncorhyn chusmykiss* fed different fat sources; these authors reported an increase of total n-6 fatty acids up to 40% of body neutral lipids when dietary manipulation reached 50% of the dietary lipids.

The findings of present study of increase in the cytoplasmic vacuolation and the marked nuclear displacement observed in the livers of seabream after six months of feeding the experimental diets confirmed the accumulation of lipids in the livers of other cultured fishes[23-24]. However, after feeding only fish oil, there was a reduction in the lipid content of the livers, with a decrease in the cytoplasmic vacuolation of the hepatocytes and a lower nuclear displacement, livers recovering their original cellular structure[24-25]. Therefore, the lowering of the lipid content, and the minimizing of the cytoplasmic vacuolation and nuclear displacement recorded in the livers of the washout period could be a consequence of the physiological and metabolic changes associated with the EPA and DHA in sea bream. The investigation on the sea bream showing the similar findings with the present study where vacuolation increased on feeding other lipid sources than fish oil. The cellular changes/proliferation, necrosis and cell enlargement of liver have been reported by many authors in *Channa punctatus*[26,27] and in *Anabas testudineus* [28] against exposure to different chemicals.

CONCLUSION

It was concluded that out of six types of fats used in the present study, the essential fatty acid (H3HUF, F2) is considered best as a feed substitute in the artificial diets. The other fats have shown very mild to moderate level of changes in the hepatic tissue at 8% addition in the diet in a 12-week trial. Therefore, these fatty acids may be used in combination with other fats to cut down the feed price and without effecting the survival and growth of *C. striatus*.

The observations, in the present study, suggest that manipulation with different fat sources in the feed has direct relation with cellular level modifications in the intestine of *Channa striatus.*

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