

Histological changes in threatened Asian catfish, *Clarias batrachus* (Linnaeus, 1758) gills following dietary fat interventions

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ABSTRACT

The present study was conducted to evaluate the interactions between different fats in the diets and the gill tissues of threatened Asian catfish, *Clarias batrachus* (average weight 55.83 ± 3.14 g). The catfish was fed with six diets (FISOL, F1; BETAL, F2; SOYAL, F3; LINOL, F4; MIXOL, F5; SATOL, F6) and a control (NATFO, F7) with natural food. FISOL, BETAL, SOYAL, LINOL and SATOL diets contain 10.0% fish oil, beef tallow, soybean oil, linseed oil and saturated oil in F1, F2, F3, F4 and F6 diets, respectively. The MIXOL, F5, contains 2.5% each of fish oil, tallow, soybean oil, linseed oil. Histological changes following dietary interventions were assessed by light microscope after feeding the diets for 12-weeks. *C. batrachus* fed with Natural feed (NATFO, F7) showing normal primary gill lamellae (PGL), secondary gill lamellae (SGL). Fishes fed with fish oil (FISOL, F1) showing swelling in PGL whereas, fishes fed with tallow (BETAL, F2) showing fusion of PGL and SGL. Fishes fed with soybean (SOYAL, F3) showing knob like swelling and cartilaginous cells, enlarged cartilaginous cells and linseed oil (LINOL, F4) fed fishes showing fusion of PGL, SGL and enlarged cartilaginous cells. Gill of *C. batrachus* fed with mixed oil (MIXOL, F5) showing prominent cartilaginous cells, damaged PGL while fishes fed with saturated oil (SATOL, F6) showing fusion of epithelial cells of PGL and SGL and damaged cartilaginous cells. It was deduced that addition of various fats in the diet has impact in the gill tissues; however, cheaper fat source could be used for normal growth and also to reduce the feed cost for aquaculture of this premium commodity. The results suggest that addition of variety of fats has direct relation with the histological changes in the fish gills.

Keywords: *Clarias batrachus*, Gills, Histological Alterations, dietary fats

INTRODUCTION

The Asian catfish (*Clarias batrachus*) is found in south-east Asia and Indian sub-continent. In many studies on dietary supplementation fat in feed had been reported on changes in many tissues of *Clarias batrachus* like liver [1], kidney [2], intestine [3]. Similarly, in *Channa striatus* various cellular changes in various organs like liver [4],

kidney [5], intestine [6], gill [7] have been reported. On feeding various dietary lipids the fatty acid profile of *C. striatus* [8] and *C. batrachus* [9] had been well documented. The growth and survival on adding the various lipid in *Channa striatus* grow-out and fingerling has been documented [10,11] and *Clarias batrachus* [12] also been well reported with same group of researchers. Srivastava *et al.* [13] had reported on the effects of live and artificial feed on Larval Rearing of *C. batrachus*. The gill of the fish apart from being the respiratory organ, and are also responsible for many physiological functions like acid-base balance, excretion of metabolic wastes, and ionic regulation. The changes found in this organ are generally simpler to determine than functional one [7,14], and serve as signs of losses/ damages to animal health [15]. Histological techniques are rapid, sensitive, and inexpensive tools as well reliable for the assessment of stress-response of dietary ingredients.

A feed and feeding in fish production is a item necessary for the higher production of quality and healthier fish. It is demonstrated [16] that fish cultured in intensive culture systems require all energy nutrients in a complete feed. The study to assess the tissue level changes in the gill on feeding any modified diet for a longer time is determined as an useful tool for the observation of its effects. Due to the increasing fish demand, it is need of the hour to increase fish production with dietary manipulations by increasing dietary energy through addition of rich fat contents and protein sources. Since the fish oil (FO) is not only costlier but also less available, there is an urgent requirement to assess the various other potential available sources of fat from both animal and plant sources. In this connection, assessment of gill histology is a method to examine the impacts of various energy nutrient that we use as raw materials of different origin especially various types of dietary fat. Replacement of FO by vegetable oils (VO) has proved in numbers of fishes without impacting on growth [17-20]. Changes in dietary fats may lead to imbalances in the fatty acids (FA), and may be indirectly affecting the cellular architecture of various organs including gills. The present study was to assess the effect of different dietary fats in diets of Asian catfish fed on long-term basis on gill cellular histology. The Asian catfish, *Clarias batrachus*, is one of the most important fish of Indian continent that has a big aquaculture potential and is now under threatened list. The marketed fish feeds have been reported to exhibit rich fat accumulations in the gill of this. However, the effect of dietary fats in order to provide higher dietary energy for better growth and improved health in this fish have not been assessed, through cellular evaluation of gill, therefore, this was the main interest of the study.

MATERIALS AND METHODS

Diets and Feeding

Six diets with 10% fat (F1, FISOL;F2, BETAL;F3, SOYAL;F4, LINOL;F5, MIXOL;F6, SATOL and F7, NATFO) containing Fish oil, Tallow, Soybean oil, Linseed oil, Mixed oil (i.e. containing in 1:1:1 ratio of Fish oil, tallow, Soybean oil, Linseed oil), Vegetable oil and minced chicken meat as natural food were used (Table-1).

Rearing of Fish

Clarias batrachus (av. weight 55.83 ± 3.14 g) were acclimated to laboratory conditions in a 1500 L capacity FRP tank, feeding on crushed/crumbled feed containing a minimum of 50% crude protein for one week. Further, fishes were accustomed to aerated, 300 L capacity plastic pools with two - thirds filled with bore well water and covered with plastic covers. Four hundred twenty (Replicate 3 X Feed 7 X Fish 20) fishes were randomly sampled and distributed into twenty-one plastic pools containing about 200 L of water. The fishes were fed twice a day *ad libitum*.

Histological study

After 12-weeks of feeding trials with seven feed combinations (Table-1), the fishes were sacrificed. The gill from control and experimental fishes were excised and fixed in 4 % formaldehyde and processed by standard histological techniques ([19] i.e., kept in aqueous Bouin's fluid for 24-hr and washed for 8-hr in running tap water. The organs were routinely processed. Sections of the gill tissue were stained with Haematoxylin and Eosin (HE). Histological slides were observed under microscope (Labomed, Model: Digi 2) for assessment of the gill condition.

Table: 1 Ingredients composition (w/w) of feeds for *Clarias batrachus*

Feed	F-1	F-2	F-3	F-4	F-5	F-6	F-7
Ingredients	FISOL	BETAL	SOYOL	LINOL	MIXOL	SATOL	NATFO
Soybean meal	35.0	35.0	35.0	35.0	35.0	35.0	-
Starch Soluble	29.0	29.0	29.0	29.0	29.0	29.0	-
Casein	19.5	19.5	19.5	19.5	19.5	19.5	-
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.	4.0	4.0	4.0	4.0	4.0	4.0	-
Fish Oil	10.0	-	-	-	2.5	-	-
Tallow	-	10.0	-	-	2.5	-	-
Soybean Oil	-	-	10.0	-	2.5	-	-
Linseed Oil	-	-	-	10.0	2.5	-	-
Saturated Oil	-	-	-	-	-	10.0	-
Live Fish/ Natural Food (minced chicken meat)	-	-	-	-	-	-	100.0

FISOL = Fish Oil; BETAL = Tallow; SOYOL = Soybean Oil; LINOL = Linseed Oil; MIXOL = Mixed Oil (Fish Oil : Tallow : Soybean Oil : Linseed Oil :: 1 : 1 : 1 : 1 w/w); SATOL = Saturated Oil; NATFO = Natural Food

RESULTS AND DISCUSSION

Figures 1 to 7 showed the gill tissues of the *C. batrachus*, a gill arch is very peculiar to teleost fish. The following alterations were observed: fusion of respiratory lamellae, hypertrophied tissues, and destructuring of the gill lamella. Morphological changes in gills of *C. batrachus* collected after 12 – week feeding with various dietary fat showed that fishes were exhibiting some cellular alterations, however, there was no onset of pathological conditions. This indicated that the inclusion of dietary fat exerted a cellular modifications on the excretory system of this fish which may be temporary and diet-dependent. In the present study, the gills of *Clarias batrachus* in control group was shown a normal structure (Fig1) and gills of the lipid fed fishes showed some pathological changes at the end of the experimentation. The changes observed in the gill of *C. batrachus* exposed to various dietary fat induced histological changes such as *C. batrachus* fed with Natural feed (NATFO, F7) showing normal primary gill lamellae (PGL), secondary gill lamellae (SGL) (Fig-1). Fishes fed with fish oil (FISOL, F1) showing swelling in PGL whereas (Fig-2), fishes fed with tallow (BETAL, F2) showing fusion of PGL and SGL (Fig-3). Fishes fed fishes with soybean (SOYAL, F3) showing knob like swelling and cartilaginous cells, enlarged cartilaginous cells(Fig-4) and linseed oil (LINOL, F4) fed fishes showing fusion of PGL, SGL and enlarged cartilaginous cells(Fig-5). Gill of *C. batrachus* fed with mixed oil (MIXOL, F5) showing prominent cartilaginous cells, damaged PGL (Fig-6) while fishes fed with saturated oil (SATOL, F6) showing fusion of epithelial cells of PGL and SGL and damaged cartilaginous cells (Fig-7).

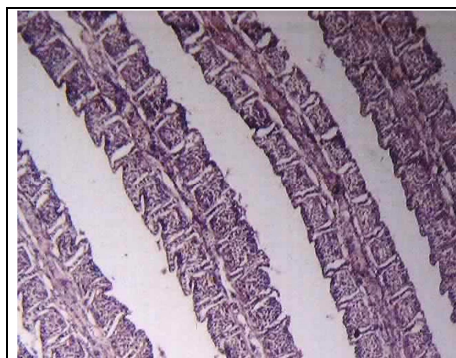


Fig.-1 Control Intestine of *C. batrachus* fed with natural food (F7).

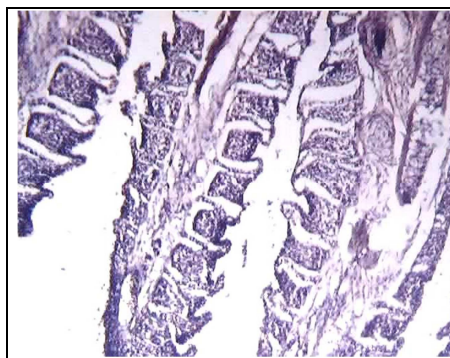


Fig.-2 Intestine of *C. batrachus* fed with FISOL (F-1).

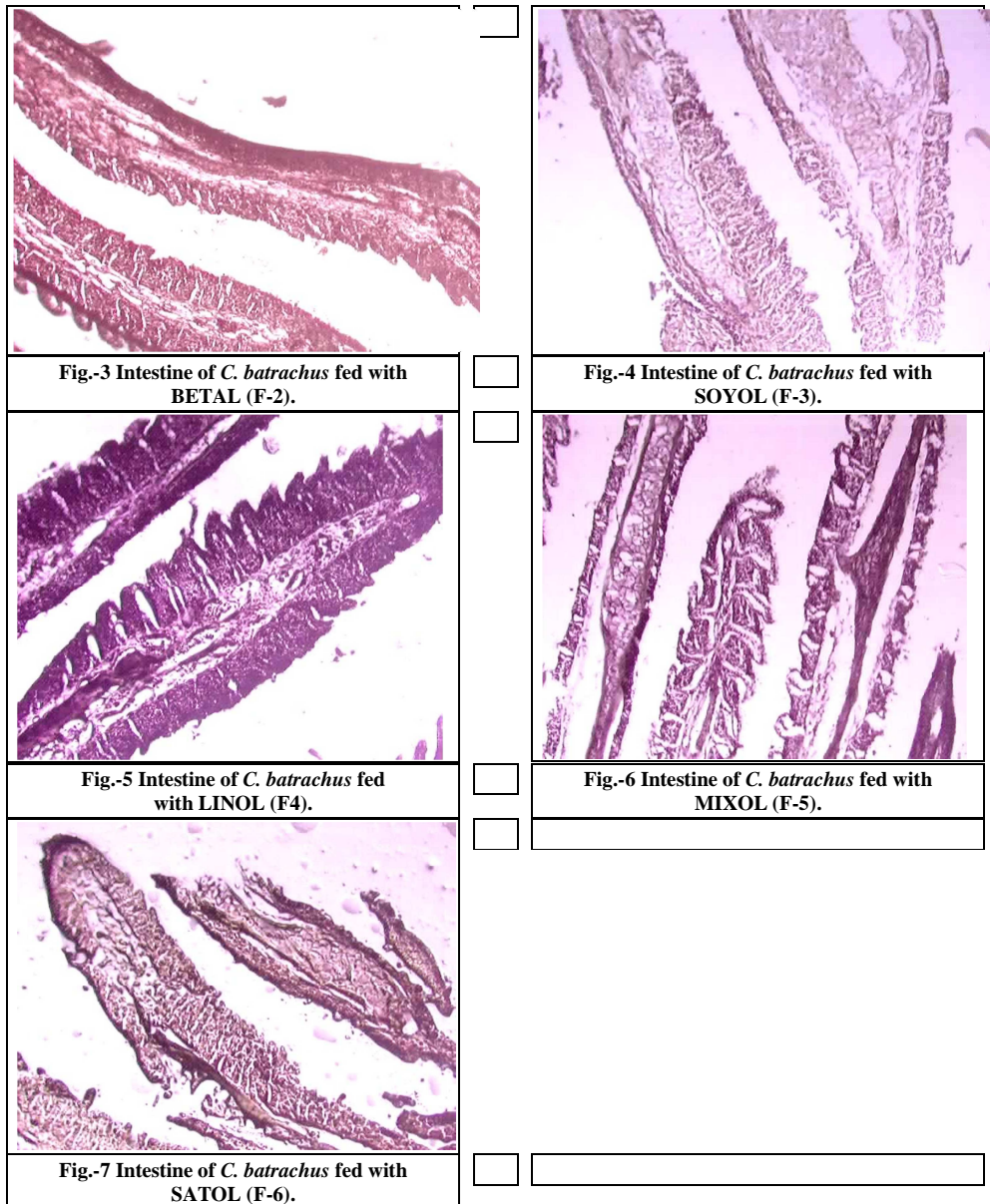


Figure-1 Gill of *C. batrachus* fed with Natural feed (NATFO, F7) showing normal PGL, SGL (H/E 20X).

Figure-2 Gill of *C. batrachus* fed with fish oil (FISOL, F1) showing swelling in PGL (H/E 20X).

Figure-3 Gill of *C. batrachus* fed tallow (BETAL, F2) showing fusion of PGL and SGL (H/E 20X).

Figure-4 Gill of *C. batrachus* fed fishes with soybean (SOYAL, F3) showing knob like swelling and cartilaginous cells, enlarged cartilaginous cells (H/E 20X).

Figure-5 Gill of *C. batrachus* fed with linseed oil (LINOL, F4) showing fusion of PGL, SGL and enlarged cartilaginous cells (H/E 20X).

Figure-6 Gill of *C. batrachus* fed with mixed oil (MIXOL, F5) showing prominent cartilaginous cells, damaged PGL (H/E 20X).

Figure-7 Gill of *C. batrachus* fed with saturated oil (SATOL, F6) showing fusion of epithelial cells of PGL and SGL and damaged cartilaginous cells (H/E 20X).

It has been reported that the dietary stress caused by the variations in the fats quality and pathological agents induced the proliferation of mucus cells and increased secretion [21,22]. The findings on many tissues of *C. batrachus* like liver [1], kidney [2], intestine [3] is well documented on addition of various dietary lipids. Same way, in *C. striatus* different cellular changes also recorded in various tissues like liver [4], kidney [5], intestine [6], gill [7]. Furthermore, on feeding various lipids, the fatty acid profile of *Clarias batrachus* [9] and *Channa striatus* [8] had been recently reported. The effects of various fats, supplemented in diet, on growth in *C. striatus* fingerling and grow-out has been demonstrated [10,11] and similarly in *C. batrachus* it is well documented by researchers [12]. Clubbed lamellae are an example of degeneration in the gills. In the present study, the epithelial hyperplasia could be a consequence of the epithelial detachment [23] and lamellar damage/ fusion could be a result of both hyperplasia of epithelial cells [24]. Vacuolization of secondary lamellae was predominant and this might be due to temporary inflammation condition due to different dietary fats. Since gills are not used as food item there is no worry as the growth of the fish was at par with control fishes. In conclusion, the histological alterations in the gill tissue of *C. batrachus* exhibit that the fish were responding to the direct effects of the dietary lipids. The dietary fats have shown very mild to moderate level of alterations in the gill tissue at 10% addition in the diet in a 12-week feeding trial. The observations suggest that supplementing with different fat/lipid sources in the diet has direct impact on cellular level modifications in the gills of *C. batrachus*. Further, studies are required at molecular level to assess the deleterious effects of various fats.

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