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Histological alterations in the intestine of threatened Asian catfish, *Clarias batrachus* fed with different types of fats through semi-purified diets

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ABSTRACT

In the present study the intestinal sac method (ex vivo) was used to evaluate the interactions between different fats in the diets in the gastrointestinal (GI) tract of Asian catfish, Clarias batrachus. 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 contains 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, beef tallow, soybean oil, linseed oil. Histological changes following dietary interventions were assessed by microscope. Control samples and samples fed with different fat had a similar appearance to intact intestinal mucosal epithelium, with no signs of cellular damage. However, C. batrachus fed with fish oil showing increased number of villi. Fishes fed with beef tallow showing more space at the base of villi and longitudinal muscles are seen degenerated fishes fed with soybean oil depicting more spaces at the base of the villi and more goblet cells are seen. Linseed oil fed fishes showing appearance of enlarged spaces between circular muscles and base of villi. Intestine of C. batrachus fed with mixed oil showing elongated lumen in villi and enlarged circular muscles are normally seen and less prominent. Intestine of C. batrachus fed with saturated oil showing elongated lumen in villi and increased in numbers of villi and circular muscles less seen, longitudinal muscles are seen normally and serosa layer is reduced showing some detachment of base of villi observed with more spaces and vacuolation. The results suggests that supplementation of different fats has direct relation with the histological alterations in the fish intestinal tissues.

Key words: *Clarias batrachus*, dietary fats, histology, intestine.

INTRODUCTION

Good Nutrition in fish production system is essential to economically produce healthy, high quality fish products. Riche and Garling [1] demonstrated that fish reared in intensive tank systems requires all nutrients in a complete pelleted diet since natural food is limited and fish cannot forage freely for natural foods. The study for the alteration in fish intestine has been elucidated to see the changes, if any, in the tissue on feeding the fish for a longer time on alternative feeds. Due to the constantly increasing world production of fish and other aquatic organisms, it is necessary to replace fishmeal in diets with less expensive raw materials of plant origin. Due to the higher fiber content, increased quantity of CHO-, non-nutritional factors, and imbalance content of amino acids can have deleterious effects on the digestive tract of fish and, therefore, on health and production of cultured fish. The intestines are the most important organ for the digestion and absorption of nutrients from feed. Therefore, examining histological changes of fish intestine is the method of correct choice in determining the effects of nutrient and their mixtures that use materials of plant or animal sources.

Histopathological analysis of the digestive system is seems to be a good parameter for the assessment of the nutritional status of fish [2, 3, 4]. The intestine is the most important organs in digestion and absorption of nutrients from ingested food, and, therefore, examining of these organs is considered to be much necessary [5, 6]. Fishmeal and fish oil as raw material are the preferred choice in aquaculture system due to better quality protein with nearly balanced amino acid profile [7, 8]. The aim of the aquaculture feed production is the partial and/or full replacement of fishmeal and fish oil with less expensive source of protein and fat, generally of plant source. The low content of fibres, CHO- and indigestible non-nutrients, higher protein level, better amino acid profile, high acceptability, palatability and digestibility are better characteristics of plant source ingredients in aquafeed [9,10]. Hardy [11] argues that the industry will soon run out of required quantities of fish oil and fishmeal. Even if all these characteristics are existing in plant, it does not infers that it can be routinely used as to replace fishmeal with balanced amino acids. Recent researches are pointing out the scope of almost 100 % replacement of FM and fish oil in diets for carnivorous fish species like Atlantic salmon and Atlantic cod with proteins of plant origin without deleterious effects on growth performances [12,13]. Soy based by-products exhibiting a major plant source of protein and fat in diets for monogastric animals, and many have demonstrated better potential as fish meal and fish oil replacements in diets for many fish species [14-20]. Soybean is low in methionine [21,22] and need to be supplemented in fish diet. Air- breeding Asian catfish, *Clarias batrachus* (Family: Clariidae), locally known as Magur, is a threatened fish of higher demand and shows the attention of fish farmers for its upcoming market value. Since knowledge of the cellular structure of the intestine is essential to the understanding of physiological and also abnormal conditions, this research was undertaken to describe the intestinal histology of *Clarias batrachus.* This experiment was carried to study the synergistic effects of dietary fats on the histological alteration in the intestine of *Clarias batrachus.*

MATERIALS AND METHODS

Experimental diets
Six semi-purified experimental diets were formulated with iso-energetic (19.55 kJ/g, F1-F6) diets. Weighed dry ingredients and some water were poured into a mixer and the resulting dough processed in a hand pelletizer to make 2 mm diameter pellets. Compounded feed pellets were dried in an oven at 60°C, packed separately and stored at -20°C until used during the feeding trial. The dietary treatments were designated as FISOL (Fish oil), BETAL (Beef tallow), SOYAL (Soybean oil), LINOL (Linseed oil), MIXOL and SATOL (Vegetable oil) containing lipid source @ 10% lipid source in all the five feeds except in MIXOL (containing FISOL, BETAL, SOYAL, LINOL in the ratio of 1: 1: 1: 1 w/w) and results are compared with natural food (NATFO). Table 1 gives the summary of ingredients used in the formulation of experimental.

Fish rearing and feeding trials
Clarias batrachus grow-outs (Av. initial weight 55.83 $\pm$ 3.14 g) were hatchery bred at National Bureau of Fish Genetic Resources (NBFGR), Lucknow and shifted to the wet laboratory. Fishes were acclimated to laboratory conditions in a 1500 L capacity Fibre Reinforced Plastic (FRP) tank, feeding on crumbled pelleted feed containing a minimum of 500 g per kg crude protein for one week. Further, fishes were accustomed to aerated, 300 L capacity plastic pools with two - thirds filled with water and covered with plastic covers. Four hundred twenty (Replicate 3 X Feed 7 X Fish 20) grow-out were randomly sampled and distributed into 21 plastic pools containing about 200 L of water. During the experiment, the fishes were fed twice a day at 10:00 and 17:00 hours *ad libitum*.

Histological studies
After twelve weeks at the end of experiment of feeding trials (Table -1) the animals were sacrificed. The distal intestine from (NATFO) and experimental fishes fed with various fats were excised and fixed in 4 % formaldehyde and processed by standard histological techniques ie., kept in aqueous Bouin's fluid for 24-h and washed for 8-hr in running tap water. The organs were routinely processed (dehydrated in ethanol series, embedded in paraffin, serially sectioned sectioned at 6µ). Sections of the intestine were stained with Haematoxylin and Eosin (HE), Humason, [23]. Histological slides were observed under microscope (Labomed, Model : Digi 2).

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

Feed / Ingredients	F-1 FISOL	F-2 BETAL	F-3 SOYOL	F-4 LINOL	F-5 MIXOL	F-6 SATOL	F-7 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	-	-	-	-	-	-	100.0

FISOL = Fish Oil; BETAL = Beef 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

Intestine of *C. batrachus* fed with Natural feed (NATFO, F7) showing normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi (Fig.-1). Intestine of *C. batrachus* fed with fish oil (FISOL) (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. Number of villi also increased (Fig. – 2) Intestine of *C. batrachus* fed with beef tallow (BETAL) (F2) showing normal appearance of circular muscles, serosa and villi. There are more space at the base of villi and longitudinal muscles are seen degenerated (Fig. – 3) Intestine of *C. batrachus* fed with soybean oil in the diet (SOYOL)(F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. More spaces at the base of the villi and more goblet cells are seen(Fig. – 4).

Intestine of C. *batrachus* fed with linseed oil (LINOL) (F4) showing appearance of enlarged spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen (Fig. – 5). Intestine of *C. batrachus* fed with mixed oil (MIXOL) (F5) showing elongated lumen in villi. Circular muscles are enlarged, longitudinal muscles and serosa are normally seen and less prominent (Fig.- 6).

Intestine of C. *batrachus* fed with saturated oil (SATOL) (F6). Showing elongated lumen in villi and increased in numbers of villi. Circular muscles less seen, longitudinal muscles are seen normally and serosa layer is reduced showing some detachment of base of villi observed with more spaces and vacuolation (Fig. – 7). The *Clarias batrachus* fed F7 with natural feed ie., *Artemia nauplii* and minced chicken alimentary canal meat showing normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi (Photo-1). Results of fishes fed with F2-F6 were compared with the control (F7).

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

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

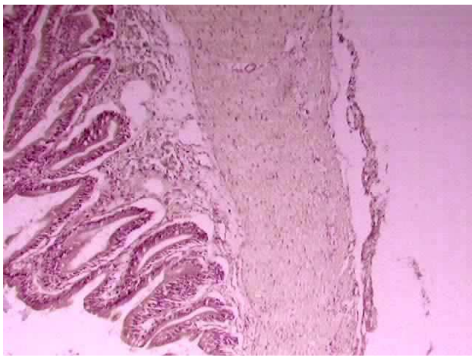

Fig.-3 Intestine of *C. batrachus* fed with BETAL (F-2)

Fig.-4 Intestine of *C. batrachus* fed with SOYOL (F-3)

Fig.– 1 Intestine of *C. batrachus* fed with Natural feed (NATFO, F7) showing normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi. H/E X 125.

Fig.- 2 Intestine of *C. batrachus* fed with fish oil (FISOL) (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. Number of villi also increased. H/E X 125.

Fig.-3 Intestine of *C. batrachus* fed with beef tallow (BETAL) (F2) showing normal appearance of circular muscles, serosa and villi. There are more space at the base of villi and longitudinal muscles are seen degenerated. H/E X 125.

Fig.– 4 Intestine of *C. batrachus* fed with soybean oil in the diet (SOYOL)(F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. More spaces at the base of the villi and more goblet cells are seen. H/E X 125.

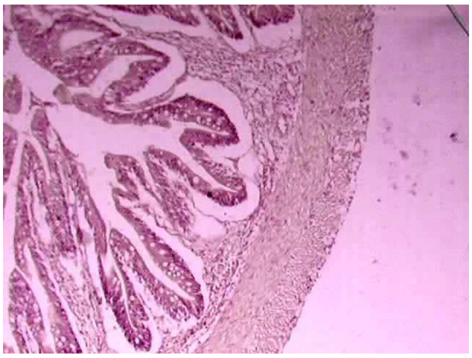

Fig.-5 Intestine of *C. batrachus* fed with LINOL (F4)

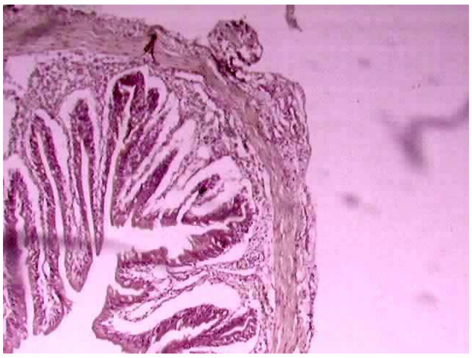

Fig.-6 Intestine of *C. batrachus* fed with MIXOL (F-5)

Fig.-7 Intestine of *C. batrachus* fed with SATOL (F-6)

Fig.-5 Intestine of *C. batrachus* fed with linseed oil (LINOL) (F4) showing appearance of enlarged spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen. H/E X 125.

Fig.-6 Intestine of *C. batrachus* fed with mixed oil (MIXOL) (F5) showing elongated lumen in villi. Circular muscles are enlarged, longitudinal muscles and serosa are normally seen and less prominent. H/E X 125.

Fig.-7 Intestine of *C. batrachus* fed with saturated oil (SATOL) (F6). Showing elongated lumen in villi and increased in numbers of villi. Circular muscles less seen, longitudinal muscles are seen normally and serosa layer is reduced showing some detachment of base of villi observed with more spaces and vacuolation. H/E X 125.

DISCUSSION

Histology of the intestine was investigated on dietary manipulations with various fats. The histological architecture of the digestive tract is: mucosa, which consists of the lamina epithelialis and lamina propria (connective tissue); sub-mucosae, consisting of two layers; muscular layer and serosa [24]. This histopathological structure was not changed even in the experiments in which fish were fed food containing metals [25,26]. Histopathological changes in the intestine may differ from species to species and feed ingredients used in the experimentation. There is no much reported information on the negative effects of dietary fat on the histological alterations of intestine. The adjustment of the fish gastrointestinal tract to a flexi situation on availability of different food are well documented like cod with the high feed intake had comparatively higher weight of different sections of the intestinal tract in comparison to cod with the less feed intake [27]. The findings of Gomez-Requeni et al. [28], where weight was depressed up to 30% in the group that had 100% replacement of FM with plant lipid and proteins was well demonstrated. Hansen *et al*. [13] found a low trend decline in feed intake with complete replacement of meal and fish oil with plant ingredients in the diet for cod. Reduced intake may be a consequence of replacement of FM by lipid and proteins of plant origin[7]. The results of Chowdhary et al. [29] indicates that animal lipid and protein rich feeds with glucosamine were more acceptable than plant origin feeds for Asian catfish, *Clarias batrachus*. Uran et al. [30] reported that carp show significant demonstration of enteritis when fed high levels of soy oil through diet.

In the present study, histopathological changes in the intestine of *Clarias batrachus* have been observed on feeding different oils, proliferation of villi, and serosa, mucosa and sub-mucosa as well as space in villi. The intestinal wall of *Clarias batrachus* comprised of four distinct layers, viz. mucosa, submucosa, muscularis and serosa. The mucosal layer being thrown into finger like villi, which is made up of simple, long columnar cells and numerous goblet cells (mucous cells) with centrally placed nuclei. Sub-mucosa is thin and projected into mucosal folds constituting the lamina propria. This layer is composed of loose connective tissue with numerous collagen fibres and blood cells. Muscularis consists of inner, thick, circular, and outer, thin, longitudinal muscular layers. Serosa is formed of peritorial layer and blood capillaries.

Intestine of *C. batrachus* fed with Natural feed (NATFO, F7) showing normal architecture of intestine. Fishes fed with fish oil (FISOL) (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. Number of villi also increased. And fishes fed with beef tallow (BETAL) (F2) showing normal appearance of circular muscles, serosa and villi. There are more space at the base of villi and longitudinal muscles are seen degenerated. Intestine of *C. batrachus* fed with soybean oil in the diet (SOYAL)(F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. More spaces at the base of the villi and more goblet cells are seen. More spaces at the base of the villi and more goblet cells are also seen after feeding with linseed oil (LINOL) (F4) showing appearance of enlarged spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen. Fish fed with mixed oil (MIXOL) (F5) showing elongated lumen in villi. Circular muscles are enlarged, longitudinal muscles and serosa are normally seen and less prominent. Intestine of *C. batrachus* fed with saturated oil (SATOL) (F6) showing elongated lumen in villi and increased in numbers of villi. Circular muscles less seen, longitudinal muscles are seen normally and serosa layer is reduced showing some detachment of base of villi observed with more spaces and vacuolation. However, showing some detachment of base of villi observed with more spaces. Similar finding have been reported by few authors [31-33]. Observations made by earlier workers relating to histopathological changes in intestine in response to various fat and lipid and protein are being enumerated here. The histological changes in intestine can also reduce growth performance on feeding plant lipid and proteins [34-37]. The plant protein fed fishes showed an alteration in the intestinal architecture in this threatened fish[38]. These authors[38] suggests that supplementation of glucosamine has no direct relation with the histological alterations in the fish intestinal tissues. The growth performance in the same experiment was demonstrated by Yadav et al.[39]. In conclusion, the histological changes observed in the intestine of *C. batrachus* indicate that the fish were responding to the direct effects of the dietary lipids/ fat. Hence safety measures must be taken into account when fat is being used in fish through dietary manipulations. 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 *C. batrachus.*

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