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Impact of dietary fats on histological alterations in the kidney tissue of Asian catfish, *Clarias batrachus* (Linnaeus, 1758)

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ABSTRACT
Various fats in the diets were used to evaluate their impact on the kidney tissues of Asian catfish (*Clarias batrachus*). There were seven (F1-F7) treatments (FISOL, BETAL, SOYAL, LINOL, MIXOL, SATOL and NATFO containing Fish oil, Tallow, Soybean oil, Linseed oil, Mixed oil (i.e. containing 1:1:1:1 ratio of Fish oil, tallow, Soybean oil and Linseed oil), Vegetable oil and minced chicken carcass as natural food, respectively, each having 3 replications, stocked @ 30 grow-out with an initial average weight 55.83 $\pm$ 3.14 g in a circular plastic pools (cap. 300 L). Kidney of 3 fishes from each treatment were excised and processed for routine histological evaluation. The cellular changes in kidney tissues, following dietary fat incorporation, were assessed under light microscopy. F-1, fed fishes showing normal renal tubules and glomeruli; F-2, showing normal renal tubules, glomeruli and some vacuolation; F-3, exhibiting vacuolation, normal renal tubules and shrunken glomeruli with Bowman's space; F-4, fed fishes exhibiting normal renal tubule, vacuolization and larger cavity in Bowman's space; F-5, showing normal renal tubule, vacuolization and Bowman's space and F-6, fed fishes showing disrupted renal tubules, more vacuolization and Bowman's space. Kidney of *C. batrachus* fed with natural food (NATFO, F7) showing normal renal tubules and glomeruli. The results exhibit some cellular changes in the kidney tissue like vacuolization, disrupted renal tubules and shrunken glomeruli which were recorded with minimum cellular effects, on addition of different dietary fats through feed. It was concluded that addition of dietary fats has some role in the cellular changes, in the kidney tissues in this fish however, the fat could be safely be used for better flesh quality and to reduce the feed cost.

Keywords: Histopathology, kidney, *Clarias batrachus*, grow-out, dietary fats

1. Introduction

Kidney of the teleostean fish is an organ having endocrine, reticuloendothelial, hematopoietic, and excretory components. The main function of the kidney in fish is the osmotic regulation of salts and water than the excretion of nitrogenous wastes as in case of mammalian system. In fish, the major of nitrogenous excretory substances are excreted from the gills. In freshwater, the kidney does the conservation of salts and eliminate excessive water. This is accomplished by a higher rate of glomerular filtration, reabsorption of salts, and dilution of urine in the distal tubule. The histological alterations in the kidney tissue of vertebrates on dietary interventions are important bio-markers for the evaluation of the effects of dietary changes.

A balanced feed and feeding in fish production system is necessary for the better production of quality fish. It is reported [1] that fish cultured in intensive systems require all nutrients in a complete aquaculture diet. The study to assess the cellular changes in the fish kidney on feeding any formulated diet is actually considered as a tool for the observations of its impact. Since the fish oil is not only costlier but becoming less and less available day by day, there is an urgent requirement to assess the potential of other available sources of fat from both animal and/or plant origin. In this connection, assessment of histological tissues of fish kidney is the method to assess the effects of various nutrient raw materials of plant and/or animal origin including fat contents. Fish oil replacement by vegetable oils has proved in many fishes without hampering on growth [2-5]. Variation in dietary oils may lead to changes in the fatty acids profile of fish and may be affecting cellular architecture as well.

Histopathological changes have been widely used as biomarkers in the assessment of the fish health exposed to various chemicals/contaminants in lab [6, 7] and field studies [8-10]. One of the main advantages of using histopathological assessment is that this category of markers allows to study the target organs, including kidney, gills, and liver, that are responsible for important physiological functions, such as deposition and bio-magnifications of chemicals and excretion in the fish [11]. The changes recorded are, in general, simpler to identify than functional ones [12], and serve as signs of deleterious effects to animal health [13].

The objective of the present study was to compare the effects of dietary fats on Asian catfish, *Clarias batrachus* fed on long-term basis on kidney tissue histology. The Asian catfish, (Family: Claridae), locally known as magur, is one of the important fish of Indian sub-continent that has a great aquaculture potential. The effect of lipids in order to provide higher dietary energy for better growth and improved health in this fish have not been much assessed, through evaluation of kidney tissue architecture and, therefore, was the main interest in present study.

2. Materials and Methods

2.1. Experimental diets

Six experimental diets were prepared with iso-energetic (19.55 kJ/g) diets (F1-F6). Dry ingredients and appropriate water quantity were poured into a mixer-cum-blender and the final dough processed in a hand pelletizer to make 2 mm dia. pellets. Compounded feed pellets were dried in an oven at 60 °C, for 24 hours and packed separately and stored at -20 °C until used. The dietary treatments were designated as FISOL (Fish oil), BETAL (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 2.5% each FISOL, BETAL, SOYAL, LINOL in the ratio of 1: 1: 1: 1 w/w) and results are compared with natural food (NATFO). The summary of feed ingredients used in the experimental diets are shown in Table-1.

2.2. Fish Rearing

Clarias batrachus (av. weight 55.83 ± 3.14 g) were hatchery bred at National Bureau of Fish Genetic Resources (NBFGR), Lucknow and reared in the wet laboratory. The fishes were acclimated to laboratory conditions in a 1500 L capacity Fibre Reinforced Plastic (FRP) tank, feeding on crushed/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 borewell 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. During the experiment, the fishes were fed twice a day at 10:00 and 17:00 hours *ad libitum*.

2.3 Histological Studies

After 84 days of feeding experimental diets (Table-1) the animals were sacrificed. The kidney from (NATFO) and experimental fishes were excised and fixed in 4% formaldehyde and processed by standard histological techniques i.e., 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 6 µ). Sections of the kidney were stained with Haematoxylin and Eosin (HE), Humason [14]. Histological slides were observed under light microscope (Labomed, Model: Digi 2).

Table1: 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 = 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

3. Results and Discussion

The feeds tested in this experiment did not have negative impact on survival, growth rate and condition on *C. batrachus*. The sections of kidney tissue of control fish fed with natural feedstuffs (NATFO, F7) showed normal structure of kidney tissue with normal glomerulus and tubules. The tubules were normal with circular shape, dark centrally rounded vesicular nucleus with nucleoli with abundant cytoplasm. There was no sign of any necrosis and/or inflammations observed (Fig 1). The normal architecture of kidney tissue in this case may be due to availability of natural food of animal origin, most suitable to this fish, as being an omnivore but mainly carnivore and has preference for meaty aquatic living food. F-1, fed fishes showing normal renal tubules and glomerulus (Fig 2); F-2, showing normal renal tubules, glomeruli and some vacuolation (Fig 3); F-3, exhibiting vacuolation, normal renal tubules and shrunken glomeruli with Bowman's space (Fig 4); F-4, fed fishes exhibiting normal renal tubule, vacuolization and larger cavity in Bowman's space (Fig 5); F-5, showing normal renal tubule, vacuolization and Bowman's space (Fig 6) and F-6, fed fishes showing disrupted renal tubules, more vacuolization and Bowman's space (Fig 7). The results exhibit some cellular alterations in the kidney like vacuolization, disrupted renal tubules and some shrunken glomeruli and which was recorded with bare minimum effects, after addition of various dietary fats in the feed. These changes are reversible in nature on changing the dietary compositions. In all the feeding trials, the most of the cellular features were the presence of well-organized structure of renal tubules, glomeruli, nucleus and cellular structures, of kidney, which indicated that all types of oils tested in the study were not much harmful to Asian catfish, *Clarias batrachus* at their levels of maximum supplementation of 10%. Hence, they may be used in combination which did not create significant changes in the kidney architecture.

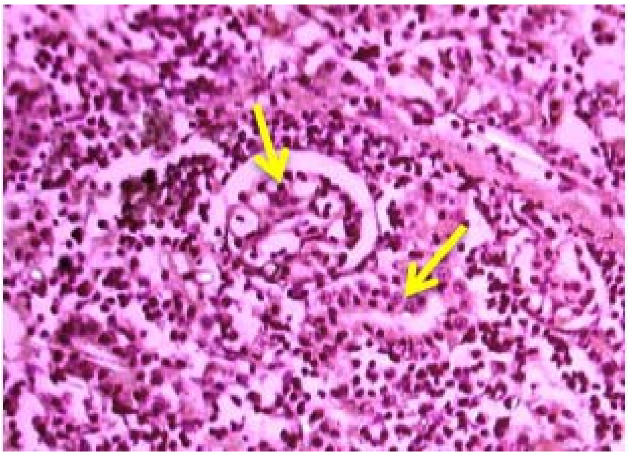

Fig 1: Kidney of *C. batrachus* fed with natural feed (NATFO, F7) showed normal structure of kidney tissue with normal glomerulus and tubules (H/E 40X).

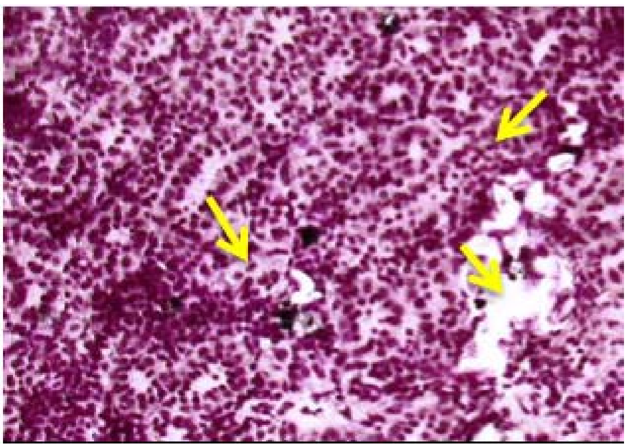

Fig 2: Kidney of *C. batrachus* fed with F-1 (FISOL), showing normal renal tubules and glomerulus (H/E 40X).

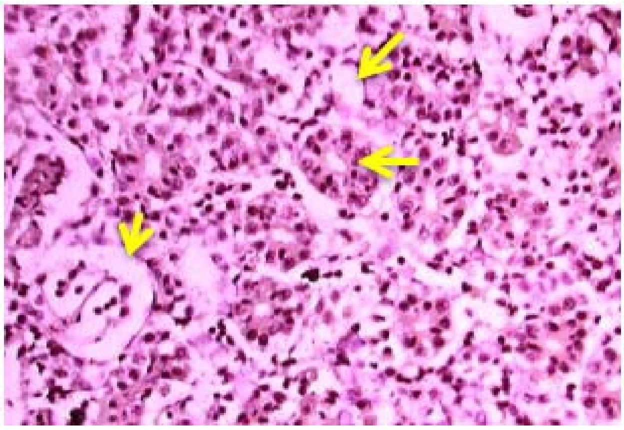

Fig 3: Kidney of *C. batrachus* fed with F-2 (BETAL), showing normal renal tubules, glomeruli and some vacuolation (H/E 40X).

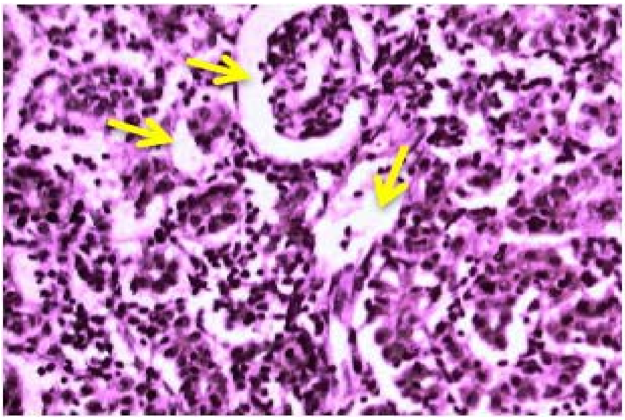

Fig 4: Kidney of *C. batrachus* fed fishes with F-3 (SOYAL), exhibiting vacuolation, normal renal tubules and shrunken glomeruli with Bowman's space (H/E 40X).

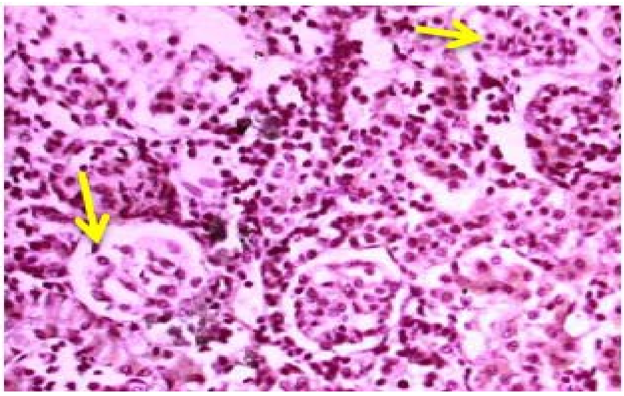

Fig 5: Kidney of *C. batrachus* fed with F-4 (LINOL), exhibiting normal renal tubule, vacuolization and larger cavity in Bowman's space (H/E 40X).

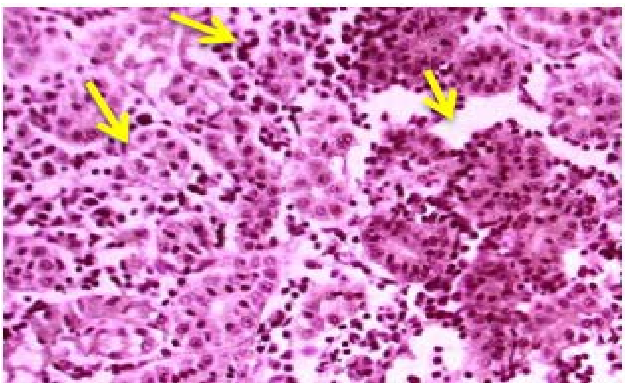

Fig 6: Kidney of *C. batrachus* fed with F-5 (MIXOL), showing normal renal tubule, vacuolization and Bowman's space (H/E 40X).

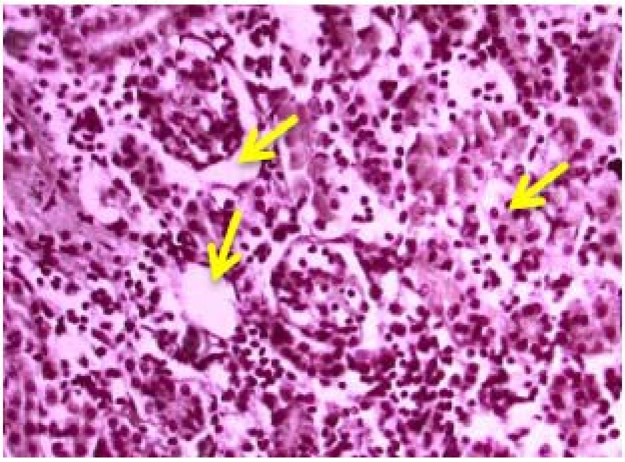

Fig 7: Kidney of *C. batrachus* fed with F-6 (SATOL), showing disrupted renal tubules, more vacuolization and Bowman's space (H/E 40X).

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) maximum recoded in saturated oil (F-6) with similar changes and showing enlarged cells (Fig.-7). The degenerative changes in kidney tissues with all fat sources were comparatively very milder and therefore, could be routinely used in the diet of this species at comparatively up to 10%. Barring some shrinkage of vacuolization in Bowman's capsule and increased Bowman's space no other deformity recorded.

The teleostean kidney is one of the most susceptible organs to be affected by contaminants in the water [7]. Most common alterations found in the kidney of fishes are tubule degeneration and dilation of capillaries in the glomerulus and reduction of Bowman´s space [15]. Exposure to chemicals frequently causes alterations in the glomerulus and tubules, as described by Thophon *et al.* [7] for the perch (*Lates calcarifer*); Handy & Penrice [16] found swollen Bowman´s capsule cells and melanomacrophages in the kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*). Similar alterations were found in fishes exposed to organic chemicals [17] and mixed environmental contaminants [9, 18]. In the present study, kidney of the fish often showed swelling in tubule cells. This cellular alterations can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle [13, 15]. In more severe cases, the degenerative process can lead to tissue necrosis [15]. The presence of tubule disruption, with the absence of necrosis in the kidney in the present study indicates that the kidney suffered some damage after exposure to the different dietary fats.

4. Conclusion

It was concluded that out of six types of fats used in the present study, it is demonstrated that fats have shown milder to moderate level of alterations in the kidney tissue at 10% addition in the diet in a 12-week trial. The observations, in the present study, suggests that manipulation with various fat sources in the feed has direct relation with cellular level changes in the kidney of *Clarias batrachus*. In overall conclusion, this study showed that *C. batrachus* performed optimally on diets containing FISOL, LINOL and MIXOL with respect to weight gain, SGR, feed intake and FCR (published elsewhere). Therefore, it is recommended that addition of various fats to the diet of *C. batrachus* can be used for improved growth performance and better nutrient utilization showing minimum harmful effects on the kidney tissues.

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