



Effect of dietary lipid sources on rabbitfish (*Siganus rivulatus*) performance and health status under biofloc system condition during the nursery phase

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ABSTRACT

This study was conducted to estimate the negative effect of feeding a commercial diet containing vegetable oils on *S. rivulatus* performance during the nursery phase under biofloc system conditions. Three experimental diets were formulated; a control diet supplemented with 4% cod liver oil (CLO), a diet supplemented with 4% coconut oil (CO), and a diet supplemented with 4% sunflower oil (SO). Fry were randomly stocked into nine experimental tanks of 15 fry/tank with an initial weight of 0.76 ± 0.03 g/fry. The experiment was extended for 60 days. The fish fed on the CLO diet recorded the highest significant growth performance parameters, while no significant difference was recorded between SO diet and the CO diet. The survival rate showed no significant difference among the three treatments. Gill sections of *S. rivulatus* fed CLO and CO diets showed mild adhesion of the secondary gill lamellae. Hepatic sections of *S. rivulatus* fed CLO diet showed mild hepatic vacuolation. Gastric sections of *S. rivulatus* fed CO diet showed mild granular degenerative changes covering the mucosal and lining epithelium of the gastric glands. It was concluded that the tropical fish nursery farmers should analyze the feed to ensure the HUFA level. Moreover, the biofloc system may be considered a promising system for tropical marine fish during the nursery phase.

INTRODUCTION

Rabbitfish (*Siganus rivulatus*) is an indo-west pacific warm water and herbivorous marine and brackish water fish (Woodland, 1983; Saoud *et al.*, 2008). Rabbitfish is suitable for cultivation because of its high tolerance to handling; crowding and environmental factors (Saoud *et al.*, 2007). Rabbitfish have a high market value in the Eastern Mediterranean countries (Stephanou and Georgiou, 2000). Nursery phase is a crucial and transitional stage, thence it can optimize profitability, if it is well controlled and designed (Mabroke *et al.*, 2021). Nursery is an important phase of the life history of many marine fishes (Staples, 1980; Haedrich, 1983). Accordingly, the availability of appropriate juvenile habitats can have strong impacts on populations of adults (Nagelkerken *et al.*, 2017). Biofloc technology (BFT) has been introduced as environmental friendly effective alternatives for traditional fish rearing (Avnimelech,

1999; Gutierrez-Wing and Malone, 2006). This technology could minimize water exchange (0.5–1 per cent per day). In the same context, the active bacterial community of biofloc enhances the treatment of organic wastes and, in turn, recycle essential nutrients (Azim and Little, 2008; Day, 2015; Zhao *et al.*, 2014). Microbial biofilms and bioflocs are used as food sources which improved growth performance, reduce the cost of conventional feed and provide high economic benefits for aquaculture (Panjaitan, 2010; Martínez-Porchas and Vargas-Albores, 2017; Luna-González *et al.*, 2017).

Dietary lipids serve as energy source and provide essential fatty acids, furthermore they serve as a source of sterols and phospholipids necessary for survival, growth, and maintenance and proper physiological functions (Corbin *et al.*, 1983). Fish oil is very important source in aquafeed production. It is the most commonly used as marine lipid source for marine fish feed due to its high digestibility, essential fatty acids, in particular long chain polyunsaturated fatty acid (Nasopoulo and Zabetakis, 2012) and fat soluble nutrients for normal growth and development of fish (Turchini *et al.*, 2009). Highly unsaturated fatty acids (HUFA), have been identified as major nutrients required for the early growth of marine fish to increase growth and survival of larvae and juveniles, promote ovarian maturation in broodstock and promote production of better quality eggs (Parakarma *et al.*, 2009). It is worth mentioning that the low quality seed will affect the final production of any farm (Parakarma *et al.*, 2009).

Vegetable Oil (VO) used as the ideal alternative of fish oil (FO) in aquafeed formulations, due to their availability and relatively low price. VO lacks LC-PUFA, but rich in polyunsaturated fatty acids with 18 carbon atoms (C18 PUFA), namely linolenic acid (LNA; 18:3n-3) and linoleic acid (LA; 18:2n-6) (Turchini *et al.*, 2010; Suloma *et al.*, 2022). C18 PUFA can satisfy the essential fatty acid (EFA) requirements of some vertebrates, which possess the complement of fatty acyl desaturase (Fads) and elongation of very long-chain fatty acids (Elovl) enzymes (Torstensen and Tocher, 2010). Marine fishes especially larvae and fry are unable to biosynthesize HUFA specially DHA and EPA from linolenic acid, therefore its highly recommended to not use vegetable oils in marine fish diet (Torstensen and Tocher, 2010; Fonseca-Madrigal *et al.*, 2014). Some aquafeed factories supplement vegetable oils to marine feeds without informing the farmers. This may affect the performance of marine fish especially during the nursery phase. Therefore, the objective of this study was to evaluate the effect of dietary vegetable oils on the *S. revolutuse* growth performance and health status during the nursery phase under biofloc system conditions.

MATERIALS AND METHODS

The current study was carried out at the Fish Rearing Lab., National Institute of Oceanography and Fisheries (NIOF), Hurghada, Egypt.

Experimental fish

Experimental fish were collected from the Red Seashore of the NIOF, and immediately transported into plastic tanks filled 35 L with seawater. The fry were acclimatized for one week to be adapted to the laboratory conditions. During acclimation, fish were fed a commercial diet containing 40% crude protein and 4% crude fats.

Experimental design and conditions

Three experimental diets were formulated by adding (4%) of three different oils to the commercial diet containing 40% crude protein and 4% crude lipid; control diet supplemented with 4% cod liver oil (CLO), diet supplemented with 4% coconut oil (CO) and diet supplemented with 4% sunflower oil (SO). The overall experiment lasted for 60 days (Table 1).

Table 1. Proximate composition of the experimental diets

Parameters	CLO	CO	SO
Moisture (%)	5.55	5.58	6.12
Crude protein (%)	37.76	39.18	41.23
Crude lipids (%)	8.93	8.36	7.50
Ash (%)	7.19	7.27	7.20
Total carbohydrates	40.5	39.61	37.95
Gross energy (kcal/Kg)	465.50	464.35	460.77

Total carbohydrate content was calculated as follows: total carbohydrate=100-(% crude protein+% crude fat+% total ash+%moisture);dietary gross energy was calculated using the conversion factors of 5.6, 9.4, and 4.2 kcal/kg for protein, lipids and carbohydrates, respectively (**Hepher et al., 1983**).

Each treatment included three replicate plastic tanks (48 X 35 X 20 cm). Tanks were aerated by air stones connected with 0.5HP ring blower to supply oxygen with a minimum level of (5-6 mg/L). Fry were randomly stocked into nine experimental tank of 15 fry / tank (430 fish/m³) with an initial weight 0.76 ± 0.03g/fry. During the first 3 days of the experiment, dead fish were replaced with individuals of the same size. Starch was used as a carbon source and added daily to maintain the C/N proportion at 1:10 to activate the heterotrophic bacteria (**Avnimelech, 1999**). Starch was completely mixed with water cultured tank in a beaker before spread to the tanks. No water exchange was done except for the compensation of the evaporated water.

Water quality

During the experimental period water temperature, salinity, dissolved oxygen (DO) and pH were measured using Lovibond® Tintometer® water testing device and Milwaukee

ph600 pocket pen, respectively. Water samples were collected weekly for total ammonia nitrogen (TAN) following the standard methods for the examination of water and wastewater (APHA, 1998). Biofloc volume (FV) was determined on site using Imhoff cones weekly, registering the volume taken in by the flocs in 1000 ml of the tank water after 30 min sedimentation (Avnimelech and Kochba, 2009)

Chemical analysis

At the end of each experiment, samples of fish and feed were taken. Fish were dissected to take a piece of fish whole body in closed containers and stored in the deep freezer for chemical analysis to determine the proximate composition analysis of the frozen piece of fish body and diets including dry matter (DM), for crude protein by the Kjeldahl method using a Kjeltech auto-analyzer (Model 1030, Tecator, Hoganas, Sweden) (Bligh and Dyer, 1959). Ether extracts (EE) and ash contents were determined according to AOAC (1995).

Growth performance parameters

Fish samples were taken every 15 day to determine total body weight (g) and total body length (cm). Feed quantity was always readjusted according to the increase in the body weight of the fish.

Total weight gain (TWG) (g), average daily body weight gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein productive value (PPV) and conditional factor (KC) were determined according to Castell and Tiews (1980) as follows: $TWG = [FBW(\text{final body weight}) - IBW(\text{initial body weight})]$, $ADG = TWG / \text{time}$, $SGR \% = 100 \times (\ln FBW - \ln IBW) / (t)$ Where: Ln: Natural log, and t is the duration of the experiment, (SR survival rate %)= (No. of fish at the end/ No. of fish at the start) x 100, $FCR = \text{feed intake (FI)} / \text{WG}$, $PER = \text{WG} / \text{protein intake (PI)}$, $PPV = 100$ and $KC = W/L^3 \times 100$

Histological examination

5 fish samples from each treatment were dissected for histological examination. specimens were obtained from the whole intestine, liver and gills of fish from different experimental treatments. Intestinal; gills and liver samples were collected from different groups, and then fixed in 10% neutral buffered formalin. After dehydration and clearance, the tissues were embedded in paraffin and sectioned in 5 μm thickness. The serial sections were subjected to staining with hematoxylin and eosin (Bancroft and Layton, 2013). The Histomorphometric analysis was performed using ImageJ analysis software (National Institutes of Health, MD, USA), whereas the intestinal villi length, width and the inter-villi space were measured by ImageJ analysis software and expressed as μm . This software is free on web under <https://imagej.nih.gov/ij/download.html>.

Statistical analysis

Data of the experiment were analyzed by one-way analysis of variance ANOVA. Significant differences were considered at $P < 0.05$. When significant differences were found, Duncan's multiple range tests was used to identify differences among experimental groups. All statistical analyses were performed using Duncan multiple range test (Duncan, 1955) at ($P < 0.05$) level (SPSS, 1997).

RESULTS AND DISCUSSION

Water quality

The results of the water quality parameters are presented in Table (2). The average of water temperature ranged between 28.4°C and 28.8°C. Dissolved oxygen levels varied from 7 to 7.8 mg/L. The pH ranged between 8.0 and 8.2. The salinity varied from 35.9 to 36.8 mg/L and the total ammonia (NH₃-N) was around 0.3 mg/L. These parameters were within the suitable range for nursing of rabbitfish (*S. rivulatus*) (Saoud *et al.*, 2008).

Table 2. Water quality parameters in the different experimental tanks

Parameters	CLO	SO	CO
Temperature(°C)	28.40±1.86(25.6-29.6)	28.50±0.96(25.8-30.2)	28.80±2.00(25.8-30.4)
pH	8.20±0.03 (8.2-8.3)	8.00±0.08 (7.9-8.2)	8.18±0.05 (8.1-8.3)
TAN (mg/L)	0.30±0.01 (0.26-0.29)	0.30±0.01(0.25-0.30)	0.30±0.00(0.27-0.28)
DO(mg/L)	7.80±0.10(7.5-8.1)	7.70±0.06(7.6-7.9)	7.60±0.07(7.5-7.8)
Salinity(mg/L)	36.80±0.30(32.16-45.6)	35.90±2.02 (32.89-41.8)	35.90±2.30(32.31-42.8)
Floc volume (mg/L)	14.50±5.60(2.3-27.7)	14.20±5.60(2.1-27.4)	14.80±5.80(2.5-28.5)

Values are mean \pm SD range

Growth performance

The growth performance parameters are presented in Table (3). The fish fed CLO diet recorded the highest significant weight gain, specific growth rate and average daily gain values, while no significant difference was recorded between SO diet and CO diet. The survival rate showed no significant difference among the three treatments. Many studies reported that the survival rate of hybrid grouper was not affected by dietary oil source (Firdaus *et al.*, 2016; Ismail *et al.*, 2018). Biofloc contains high levels of proteins, lipids, minerals, vitamins, amino acids, and fatty acids as well as enzymes, immunostimulants and probiotics (Hosain *et al.*, 2021). These nutrients may enhance the survival rate for all the treatments (El-Shafiey *et al.*, 2018; Mabroke *et al.*, 2019, 2021). The cod liver oil showed a significant improvement in the growth performance, which may attributed to the inability of *S. rivulatus* fry to convert linolenic acid to DHA and EPA. Growth and

survival were significantly higher in milkfish fed n-3HUFA enriched (**Borlongan *et al.*, 2000; Ogata *et al.*, 2006; Suloma and Ogata, 2012**). On the other hand, it was reported that dietary sunflower oil improved the survival and growth of some fresh water fish species (**Miller *et al.*, 2007; Kim *et al.*, 2012; Bhavan *et al.*, 2013**).

Table 3. Growth performance and survival rate of *Siganus rivulatus* fed three different lipid sources.

Parameters	CLO	CO	SO
IBW	0.76±0.01	0.76±0.01	0.75±0.00
FBW	3.33±0.03 ^a	2.34±0.017 ^b	2.35±0.02 ^b
TWG	2.57±0.03 ^a	1.58±0.04 ^b	1.59±0.02 ^b
ADG	0.04±0.00 ^a	0.03±0.00 ^b	0.03±0.00 ^b
SGR	2.46±0.02 ^a	1.88±0.05 ^b	1.89±0.03 ^b
SR	97.78±2.20	93.33±0.01	93.33±3.80

The values in the same row with different superscripts indicate statically significant difference.

Feed and nutrient utilization

The results of the feed nutrient utilization parameters are shown in Table (4). The SO showed the lowest significant FI, while there is no significant difference between CO and CLO. CLO showed the best significant FCR, whereas there is no significant difference between CO and SO. The results showed no significant difference in PER among all the experimental treatments. The CLO showed the highest significant PPV, on the contrary there is no significant difference between CO and SO. **Fitriyani *et al.* (2015)** reported that feed utilization of hybrid grouper fed with coconut oil had better FCR than fish fed FO. Moreover, **Yong *et al.* (2019)** reported that feed utilization of milk fish fed with coconut oil had better FCR than fish fed fish oil. On the other hand, dietary fish enhances the feed utilization of fresh fishes (**Bransden *et al.*, 2003; Miller *et al.*, 2007; Piedecausa *et al.*, 2007**). The aforementioned studies proved the ample that the marine fish can't biosynthesize DHA and EPA from linolenic acid which have critical function on composition of cell membranes.

Table 4. Feed and nutrient utilization values of *Siganus rivulatus* fed three different lipid sources

Variables	CLO	CO	SO
FI	2.89±0.01 ^a	2.88±0.00 ^a	2.86±0.00 ^b
FCR	1.12±0.01 ^b	1.82±0.03 ^a	1.79±0.02 ^a
PER	2.23±0.03	1.95±0.12	2.10±0.1
PPV	81.34±0.18 ^a	81.47±0.06 ^b	82.05 ±0.06 ^b

The values in the same row with different superscripts indicate statically significant difference.

The conditional factor (**KC**) is represented in Table (5). The CLO showed the highest significant **KC**, while SO showed the lowest significant **KC**. It was reported that there are length gain significant elevations on fresh water fish and prawn fed diet containing vegetable oils (**Kamarudin et al., 2011; Kim et al., 2012; Bhavan et al., 2013**).

Table 5. Initial, final body length and conditional factor (**KC**) values of *Siganus rivulatus* fed three different lipid sources under biofloc system.

Parameters	CLO	CO	SO
IBL(cm)	3.90±0.012	3.90±0.01	3.90±0.02
FBL(cm)	6.15±0.013 ^c	6.56±0.07 ^a	6.36±0.02 ^b
KC	1.42 ±0.00 ^a	0.93±0.02 ^c	0.92±0.01 ^b

The values in the same row with different superscripts indicate statically significant difference.

Body chemical composition:

The body chemical composition results are showed in Table (6). The fish fed CO recorded the highest significant moisture level followed by fish fed SO then fish fed CLO diets. On the other hand, the fish fed CLO showed the highest significant protein level followed by fish fed CO then fish fed SO diets. On the contrary, the fish fed SO showed the highest significant lipid and ash levels followed by fish fed CLO then fish fed CO diet. The aforementioned results indicated that the chemical body composition was affected by the dietary oil sources which agree with **Yong et al. (2018)** who reported that the different vegetable oil influenced the whole body proximate composition of hybrid grouper. Body chemical composition is very important for organisms' nutritive values, a good indicator for physiological condition and the cultivation (**Vijayavel and Balasubramanian, 2006**). Additionally, **Muralisankar et al. (2014)** reported that the body chemical composition significantly affected the dietary oil sources supplemented to *M. rosenbergii* diet. **Kim et al. (2012)** and **Bhavan et al. (2013)** found changes in fish body chemical composition when compared between the diet supplemented sunflower oil and fish oil. In addition, the vegetable oil supplemented feed fed to *Diplodus puntazzo* and *C. gariepinus* gained a significant improvement in body carcass composition (**Piedecausa et al., 2007; Aderolu and Akinrem, 2009**).

Table 6. Chemical composition of *Siganus rivulatus* whole fish fed three different lipid sources.

Parameters	CLO	CO	SO
Moisture (%)	72.71±0.12 ^c	77.85±0.043 ^a	75.95±0.01 ^b
Protein (%)	72.74±0.20 ^a	71.39±0.12 ^b	50.39±0.45 ^c
Lipids (%)	9.18 ±0.03 ^b	8.24 ±0.21	12.54 ±0.37 ^a
Ash (%)	8.92 ±0.03 ^b	8.23 ±0.08 ^c	16.02 ±0.13 ^a

The values in the same row with different superscripts indicate statically significant difference.

Histological studies

Gill sections *S. rivulatus* fed CLO and CO diets showed normal primary and secondary gill lamellae with mild adhesion of the secondary gill lamellae, On the other hand, the fish fed SO diet treatment showed normal primary and secondary gill lamellae (Fig. 1).

Hepatic sections of *S. rivulatus* fed SO and CO diets showed normal hepatic architecture including both hepatic and portal tissues, while CLO diet showed mild hepatic vacuolation. These vacuoles may be lipid vacuolation consequently, cod liver oil accumulates fats on liver (Fig. 2). Similarly, **Aziza *et al.* (2013)** stated that the dietary lipid supplementation at 3% level may induce lipid accumulation in the liver which may cause steatosis. In addition, **Spisni *et al.* (1998)** reported that different degrees of lipid accumulations in livers of catfish and common carp fed diets containing alternative lipid source: soyacid oil and yellow grease.

Gastric sections of *S. rivulatus* fed SO and CO diets showed normal gastric glands, on the other hand CLO diet showed mild granular degenerative changes covering mucosal and liningepithelium of the gastric glands (Fig. 3). **Azizia *et al.* (2014)** detected changes in the intestinal epithelium due to the dietary substitution of CLO with vegetable oils. Anomalies in the intestinal structure can disturb dietary fat metabolism which may affect the immune system and lead to poor fish health (**Tacom, 1992; Caballero *et al.*, 2020**).

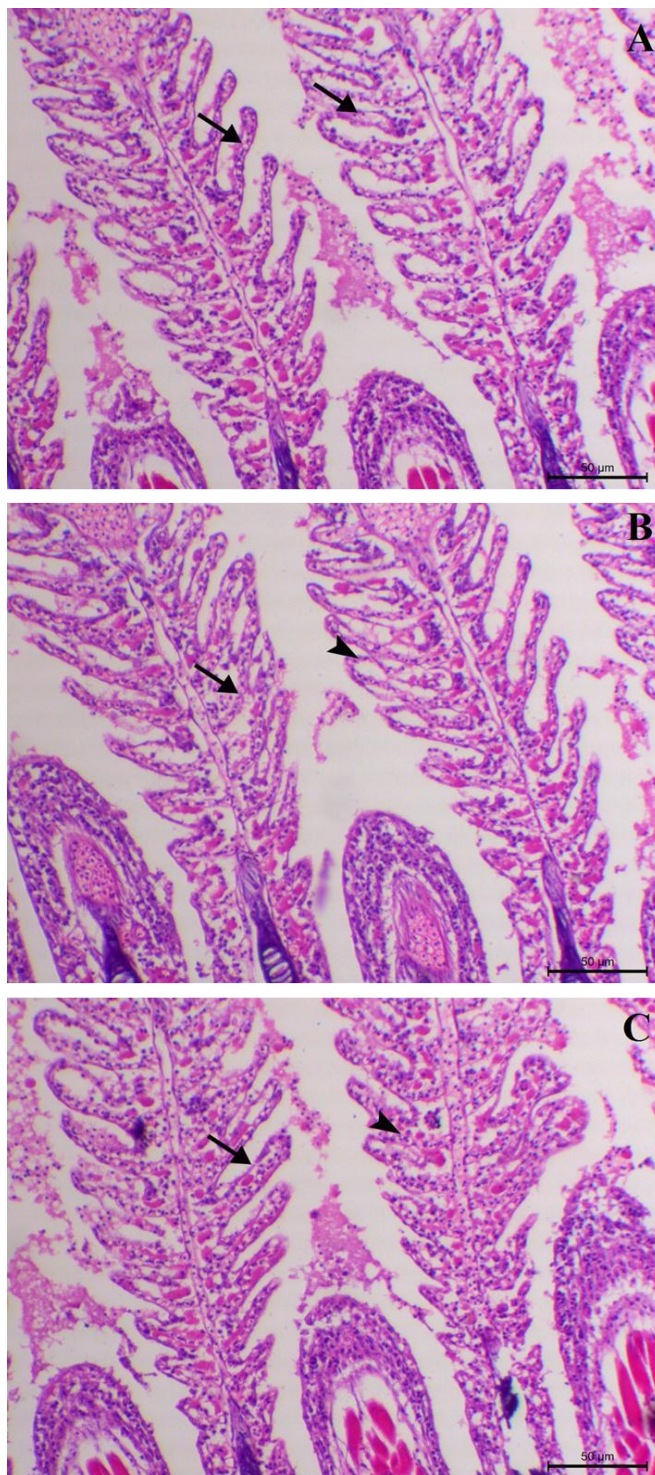


Fig. 1: Photomicrographs gills sections of *Siganus rivulatus* fed three different lipid sources (A) sunflower oil; (B) coconut oil and (C) cod liver oil showing normal primary and secondary gill lamellae (arrow) with mild adhesion of the secondary gill lamellae (arrowhead), H&E stain, X200, bar= 50 µm.

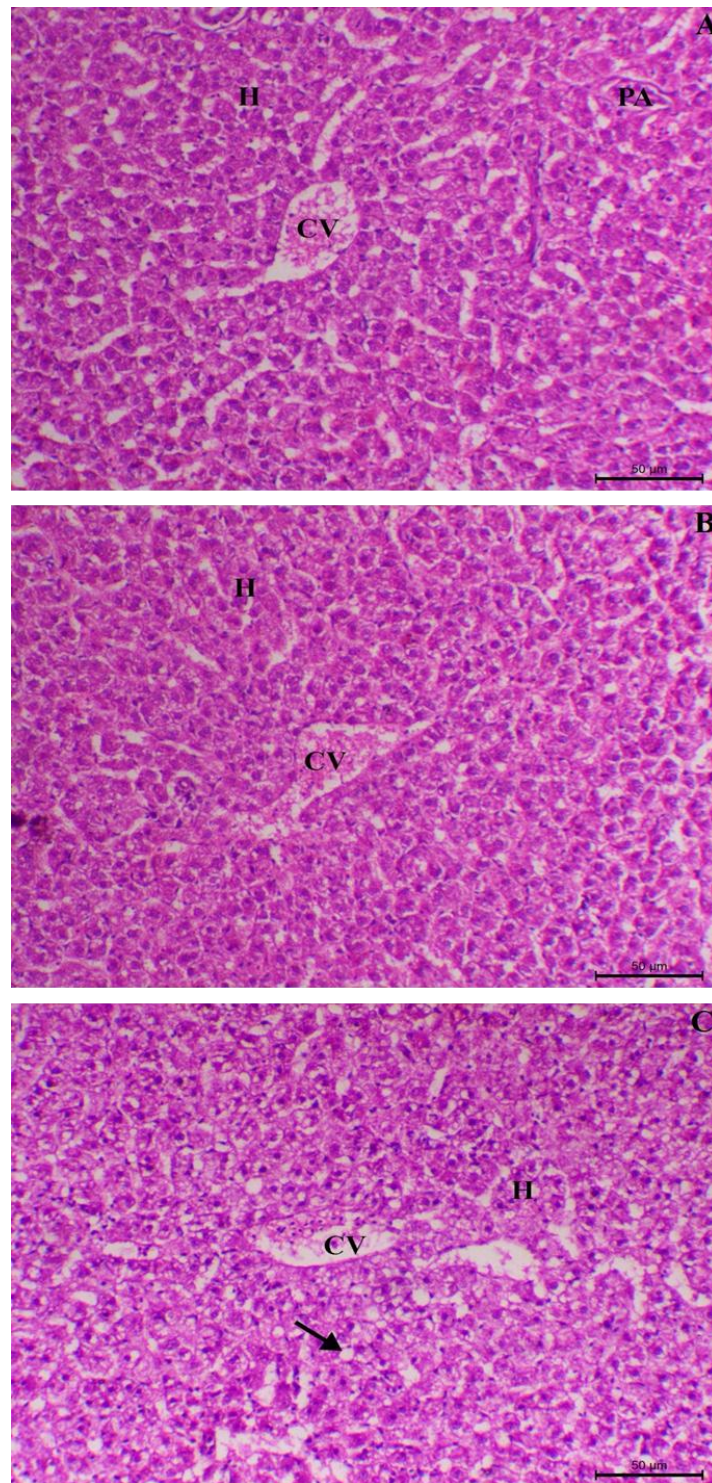


Fig. 2: Photomicrographs liver sections of *Siganus rivulatus* fed three different lipid sources (A) sunflower oil; (C) coconut oil and (B) cod liver oil showing normal hepatic tissues including both hepatic and portal tissues (arrow) (H letter indicates hepatocyte, CV indicates central vein and PA indicates portal area), H&E stain, X200, bar= 50 µm.

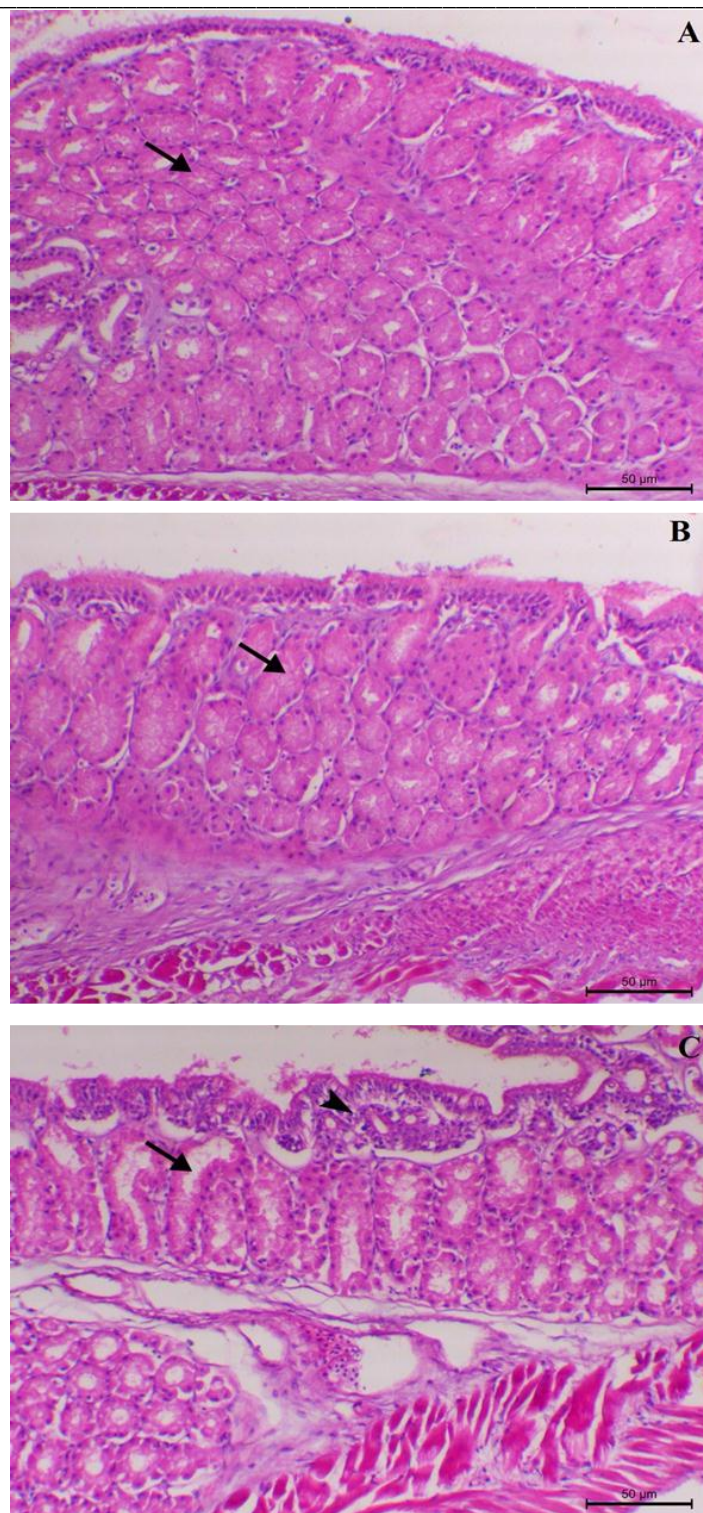


Fig. 3: Photomicrographs of the stomach section of *Siganus rivulatus* fed three different lipid sources (A) sunflower oil; (B) coconut oil showing normal structure of gastric glands and (C) cod liver oil showing mild granular degenerative changes within the both covering mucosal epithelium (arrowhead) and lining epithelium of the gastric glands (arrow), H&E stain, X200, bar= 50 µm.

CONCLUSION

The tropical fish nursery farmers should analyze the feed to insure the concentration of HUFA in the commercial diets. Fish in the BF system appeared to have harvested some of their food from biofloc communities. Because of their lower start-up costs and saving the water farmers should consider using biofloc system for nursery production. Further research is needed to study the negative effect of CLO on the histopathology of fish.

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