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Effect of Different Dietary Protein Levels on the Rabbitfish (*Siganus rivulatus*) Performance and Health Status Under Biofloc System Condition During the Nursery Phase

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ABSTRACT

The current study was performed to determine the impact of dietary protein level on the *S. rivulatus* performance under biofloc system conditions during the nursery phase. Three commercial diets with three dietary protein levels were evaluated; 25, 30 and 40 crude proteins (coded as 25CP%; 30CP% and 40 CP% diets, respectively). 135 fries with an initial weight of 0.76 ± 0.03g/fry were randomly stocked into nine experimental tanks at 15 fries/ tank. The experiment extended for 60 days. Results showed that fish fed 40CP and 30CP diets recorded the highest significant weight gain and specific growth rate without significant difference between them. However, fish fed 25CP diet recorded the lowest performance. Histological examinations revealed mild changes in the intestines of fish fed 40CP diet, compared to fish fed 30CP and 25CP diets. While, the gills and liver were not affected by the change in diet crude protein concentration. It could be concluded that 30% crude protein diets may be recommended to cultivate *S. rivulatus* under biofloc system conditions during the nursery phase.

INTRODUCTION

Knowledge about fish nursery phase and the requirements of juvenile habitat optimizes survival and growth; it is critical for population number and activity bottleneck **(Chambers *et al.*, 2012; Grol *et al.*, 2014; Mabroke *et al.*, 2021)**. It is worthy mentioning that, crude protein is the most critical component in fish diets as well as its amino acids (**Lazo *et al.*, 1998**). The protein requirements of *S. rivulatus* fish was not previously well studied under clear water. Previous studies reported that *Siganus rivulatus* requirement under clear water system and the best growth was recorded associated with a 40% crude protein diet **(El-Dakar *et al.*, 2007; Abuo Duaod *et al.*, 2014)**. Biofloc is one of the options to overcome aquaculture problems, such as water scarcity, reduction of protein inputs, nursery management and diseases **(Buttner *et al.*, 1992)**. Biofloc is an eco-friendly sustainable system with limited water exchange; it enhances water quality, fish immunity and acts as a food source reducing FCR **(FAO, 1987; De Schryver *et al.*, 2008; Emerenciano *et al.*, 2017)**. Moreover, it improves the feed nutrient utilization and digestive enzymes efficiency **(Crab *et al.*, 2009; Xu & Pan, 2012; Xu *et al.*, 2012; Mabroke *et al.*, 2021; Suloma *et al.*, 2021).** Additionally, the

biofloc system provides protection against infection via pathogenic organisms **(Crab *et al*., 2010a; Emerenciano *et al*., 2017**; **Bakr *et al*., 2021)**. Fry rearing reaching the fingerling stage and their trade can provide more value and economic return to the farmers, with less capital investment by using biofloc technology **(Sontakke & Haridas, 2018)**. The number and quality of the fries are the main targets of the hatcheries. Our previous study reported that the biofloc system may be regarded as sustainable system for the cultivation of *S. rivulatus* during the nursery phase **(Henish *et al*., 2022)**. However, the high protein levels under the biflocc system may negatively affect water quality, which in turn affect fish performance and its health status due to turbidity elevation resulting from the high amount of external carbon source needed to control the ammonia **(Khalil *et al.*, 2016; El-Shafiey *et al.*, 2018; Suloma *et al*., 2021)**. Therefore, the present study was designed to determine the optimal dietary protein level required for rearing *S. rivulatus* during the nursery phase under the biofloc system conditions.

MATERIALS AND METHODS

The current study was conducted at the Fish Rearing Lab., the National Institute of Oceanography and Fisheries, Hurghada, Egypt.

Experimental fish

The experimental fish were collected from the Red Seashore of the NIOF, and immediately transported in plastic tanks. The fries were acclimatized for two weeks prior the experiment to adapt to laboratory conditions. During acclimatization, fish were fed on a commercial diet containing 40% protein.

Experimental design and rearing conditions

Three commercial diets with three different dietary protein levels were used; 25 %; 30 % and 40 % crude protein (coded at 40CP%; 30CP% and 25CP% diets, respectively) (Table 1).

Table 1. Proximate composition of the experimental diets

Variable	25% CP	30% CP	40% CP
Moisture (%)	8.03	11.91	6.12
Crude protein (%)	24.02	32.61	41.23
Crude lipids (%)	6.66	4.06	7.50
Ash (%)	8.96	7.18	7.20
Total carbohydrates	52.33	44.24	37.95
Gross energy (kcal/Kg)	416.90	406.59	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 replicates of 35L 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. 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 that was 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 spreading on the tanks. No water exchange was done except for the compensation of the evaporated water. The overall experiment lasted for 60 days.

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 weekly collected to determine 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 1000ml of the tank water after 30min of sedimentation **(Avnimelech & Kochba, 2009)**.

Chemical analysis

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

Growth performance parameters

Fish samples were taken every 15 days to determine total body weight (g) and total body length (cm). Feed quantity was always re-adjusted 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) and feed conversion ratio protein (FCR) were determined according to **Castell and Tiews (1980)** as follows: TWG = [FBW(Final body weight) – IBW(Initial body weight)], ADG= TWG/ time, SGR % = 100 X (Ln FBW - Ln IBW) / (t) Where, Ln: Natural log, and t is the duration period, (SR: Survival rate %)=(No. of fish at the end/ No. of fish at start) x 100, FCR= FI/WG, PER= WG/ PI, PPV= 100 and K_C= W/L^3*100.

Histological examinations

Samples for histopathological examination originated from the whole intestine, liver and gills of fish from different groups. Intestinal samples were collected from different groups, 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).

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 at ($P<0.05$) level (**SPSS, 1997**).

RESULTS AND DISCUSSION

Water quality

Water quality parameters are presented in Table (2). There is no significant difference among the three treatments regarding the water quality parameters levels and it was within the suitable ranges for rearing of *Siganus rivulatus* **(ANZECC, 2000; EPA, 2003; Saoud *et al*., 2007b; Saoud *et al*., 2008)**. Moreover, it was noticed that the water quality was not affected by the protein levels. The recorded temperature varied between 26.7and 29.7^0 C. **Saoud *et al*.(2008)** reported that the 27^0 C is suitable for *Siganus rivulatus* rearing. The salinity varied from 32.67 to 41.7mg/L. The level of pH throughout theexperiment ranged from 7.6 to 8.2. On the other hand, the dissolved oxygen fluctuated from 7.6 to 8.2 mg/L. Ammonia level in all the treatments varied from 0.26 and 0.3 mg/L. Consequently, all the water parameter values are within the acceptable ranges of rabbitfish cultivation as reported in previous studies **(ANZECC, 2000; EPA, 2003; Saoud *et al*., 2007b; Saoud *et al*., 2008)**.

Table 2. Water quality parameters in the different experimental tanks

Parameters	**25% CP**	**30% CP**	**40% CP**
Temperature(ºC)	28.20±0.67(26.70-29.40)	28.40±0.60(27.30-29.70)	28.40±0.53(26.90-29.30)
pH	7.90±0.20 (7.70-8.20)	7.90±0.20 (7.60-8. 10)	8.00±0.30 (7.60-8.20)
TAN (mg/L)	0.30±0.01(0.26-0.30)	0.30±0.01(0.26-0.30)	0.30±0.01 (0.27-0.30)
DO(mg/L)	7.75±0.05(7.70-8.20)	7.60±0.07(7.60-8.10)	7.60±0.05(7.60-8.20)
salinity(mg/L)	35.50±2.10(32.67-41.70)	36.20±0.80(34.45-38.50)	37.01±1.40(33.38-39.70)
Floc volume (mg/L)	19.38±9.10(5.50-45.90)	18.23±8.01(5.40- 41.00)	18.30±8.10(5.40-42.00)

Values are mean 1 ±SD range

Growth performance

The growth performance results are presented in Table (3). Fish fed 40CP and 30CP diets recorded the highest significant weight gain, specific growth rate and average daily gain values without significant difference between them. On the other hand, fish fed 25CP diet showed the lowest performance. **El-Dakar *et al.* (2011)** found enhanced rabbitfish performance, under clear water conditions, with the increasing in dietary protein level and the highest performance was recorded in fish fed 40% crude protein diet. The results of our previous study Henish et al. (2022) indicated that under biofloc system conditions the contribution of microbial protein may reduce the dietary protein level needed in commercial diet for rabbitfish cultivation. It was noticed that the impact of insertion of dietary protein levels on the growth performance, under the same rearing conditions, were controversial; **Khalil *et al.* (2016)** reported that crude protein levels had significantly higher effect on the growth performance parameters of *liza carinata* under biofloc system conditions. **Xu *et al.* (2012)** postulated that their is no significant difference in the final weight and SGR of *L.vannamei* fed on three different diets concentrations 25%, 30% and 35% CP under biofloc system conditions.

Moreover, the rearing condition affects the growth performance; **Wasielesky *et al.* (2006)** demonstrated that *L.vannamei* juveniles grown in a biofloc technology (BFT) had higher growth rate than that grown in clear water system. Similarly, **Megahed (2010)** reported that *Penaeus semisulcatus* fed on dietary protein 16.25% under biofloc system

could even show better growth rate than shrimp fed on 42.95% CP without bioflocs**. Furthermore, Ballester *et al.* (2010)** demonstrated that the dietary protein content delivered to *Farfantepenaeus paulensi*s can be reduced up to 10% (from 45% to 35%) without affecting the shrimps' growth performance under bioflocs system. Reduction of dietary protein levels without affecting shrimp growth has been reported by several authors and microbial proteins from biofloc system have been provided as an crucial source of protein available for shrimp in these systems (**Decamp *et al.*, 2002; Hari *et al.*, 2004; Ballester *et al.*, 2010).** Elevating the protein level in tilapia feed from 25% to 35% under biofloc system conditions did not show any difference in growth **(Avnimelech *et al*., 1994; Azim and Little, 2008)**.

In the present study fish fed 40%CP diet showed highest significant survival rates of the *Siganus rivulatus* and there was no significant difference between fish fed 25%CP and fish fed 30%CP diets. The survival values are presented in Table (3). Fish fed 40CP diet recorded the highest significant survival rate (93.3%) without a significant difference with fish fed 25CP diet (80.3%) and fish fed 30CP diets (73.3%), respectively. **Dakar *et al.* (2011)** noted that the survival rate of the *Siganus rivulatus* was more than 90% in different protein concentration with no significant differences observed among treatments under clear water conditions. **Suresh and Lin (1992), Rostika (2014), Faizullah *et al.* (2015**; **Khalil *et al.*, 2016** recorded that Survival rates was 100% 93.56%; 94%; 91.8% for *Liza carinata*, Tilapia, *L.vannamei*, and goldfish, respectively, under biofloc system.

Table 3. Growth performance and survival rate of S. rivulatus fed three different protein levels under biofloc system conditions.

Parameters	25% CP	30% CP	40% CP
IBW	0.76±0.01^{a}	0.75±0.01^{a}	0.76±0.01^{a}
FBW	2.50±0.69^{b}	3.30±0.11^{a}	3.40±0.15^{a}
TWG	1.74±0.11^{b}	2.54±0.11^{a}	2.60±0.06^{a}
ADG	0.03±0.01^{b}	0.043±0.00^{a}	0.043±0.01^{a}
SGR	1.98±0.056^{b}	2.45±0.09^{a}	2.49±0. 44^{a}
SR	80.30±0.00^{b}	73.30±0.00^{b}	93.33±0.81^{a}

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 fish fed 40%CP showed the best FI, FCR, PER and PPV values which agree with **El-Dakar *et al.* (2011)** who found that the feed utilization of the *Siganus rivulatus* was enhanced, under clear water conditions, until 40% crude protein inclusion then didn't change. **Parazo (1990)** reported that dietary protein requirement of *S. rivulatus* under clear water system were higher than 35%**.**

Table 4. Feed and nutrient utilization values of *S. rivulatus* fed three different protein levels under biofloc system conditions.

Parameters	25% CP	30% CP	40% CP
FI	2.83±0.01^{c}	2.86±0.01^{b}	2.89±0.01^{a}
FCR	1.63 ±0.06^{a}	1.31 ±0.09^{b}	1.09 ±0.01^{b}
PER	2.19±0.21^{b}	3.37±0.33^{a}	2.28±0.028^{b}
PPV	81.71 ±0.06^{b}	95.41 ±0.07^{a}	77.46±0.17^{c}

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

The conditional factor is presented in Table (5). Fish fed 30CP showed the highest significant K_C followed by fish fed 40CP and 25CP diets. This is in accordance with **Khalil *et al.* (2016)** who found that condition factor of keeled mullet fish was significantly affected by various dietary protein levels. On the other hand, **Wassef *et al.* (2001)** reported that the condition factor for *Mugil cephalus* was not significantly affected by different dietary protein levels.

Table 5. Initial, final body length and conditional factor (KC) values of *S. rivulatus* fed three different protein levels under biofloc system conditions.

Parameters	25% CP	30% CP	40% CP
IBL	3.90±0.02 ^{a}	3.90±0.03 ^{a}	3.90±0.02^{a}
FBL	6.39±0.06^{c}	6.70±0.10^{b}	7.52±0.06^{a}
KC	0.96±0.01^{b}	1.09±0.02^{a}	0.80±0.01^{c}

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

Body chemical composition

The body chemical composition of the fish muscles are presented in Table (6). Fish fed 25% CP diet recorded the lowest significant moisture level without significant difference neither with those fed 40% CP diet nor 30% CP diets.. On the other hand,fish fed 25% CP showed the highest significant lipid levels followed by those fed 30% CP and 40% CP diets. Moreover, fish fed 40% CP showed the highest significant protein levels followed by those fed 30% CP and 25% CP diets. The aforementioned observations agree with the findings of **El-Dakar *et al.* (2011)** who found that crude protein content of fish carcass was significantly greater in siganids offered the10% protein diet than in siganids offered the diets with greater protein content. Moreover, **Xu and Pan (2012)** found a significant difference in the protein content when juvenile *Litopenaeus vannamei* fed different dietary protein levels.

Table 6. Chemical composition of S. rivulatus whole fish fed three different protein levels under biofloc system conditions.

Parameters	25% CP	30% CP	40% CP
Miosture%	72.68±0.016^{b}	75.45±0.17^{a}	75.78±0.17^{a}
Protien%	72.86±0.06^{c}	76.56±0.25^{b}	78.45±0.18 ^{a}
Lipid%	11.90 ±0.09^{a}	7.75 ±0.35^{b}	6.20 ±0.19^{c}
Ash	5.74 ±0.01^{a}	5.78 ±0.07^{a}	5.43 ±0.14^{a}

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

Histological examinations

The gills and liver of *S. rivulatus* were not affected by the protein inclusion; where the gills of *S. rivulatus* fed 40% CP; 30% CP and 25% CP diets, respectively, showed normal primary and secondary gill lamellae (Fig. 1). In addition, the hepatic sections of *S. rivulatus* fed 40% CP; 30% CP and 25% CP diets, respectively, showed normal hepatic architecture including both hepatic and portal tissues. (Fig. 2).

On the other hand, it was noticed that protein inlusion affected the histology of the gut; where the gastric sections of *S. rivulatus* fed 25% CP and 30% CP diets, respectively, showed normal histological architecture of the gastric glands. On the other hand, 40% CP treatment showed marked degenerative changes of the gastric glands (arrows) (Fig. 3). Consequently, the more crude protein levels the more histopathological alterations of the gut was noticed. In the same context, **Şahin and Gürkan (2022)** reported that *Ancistrus cirrhosus* fries fed diets containing over 40% protein (45%, 50% and 55%) had more histopathological defects in the intestine.

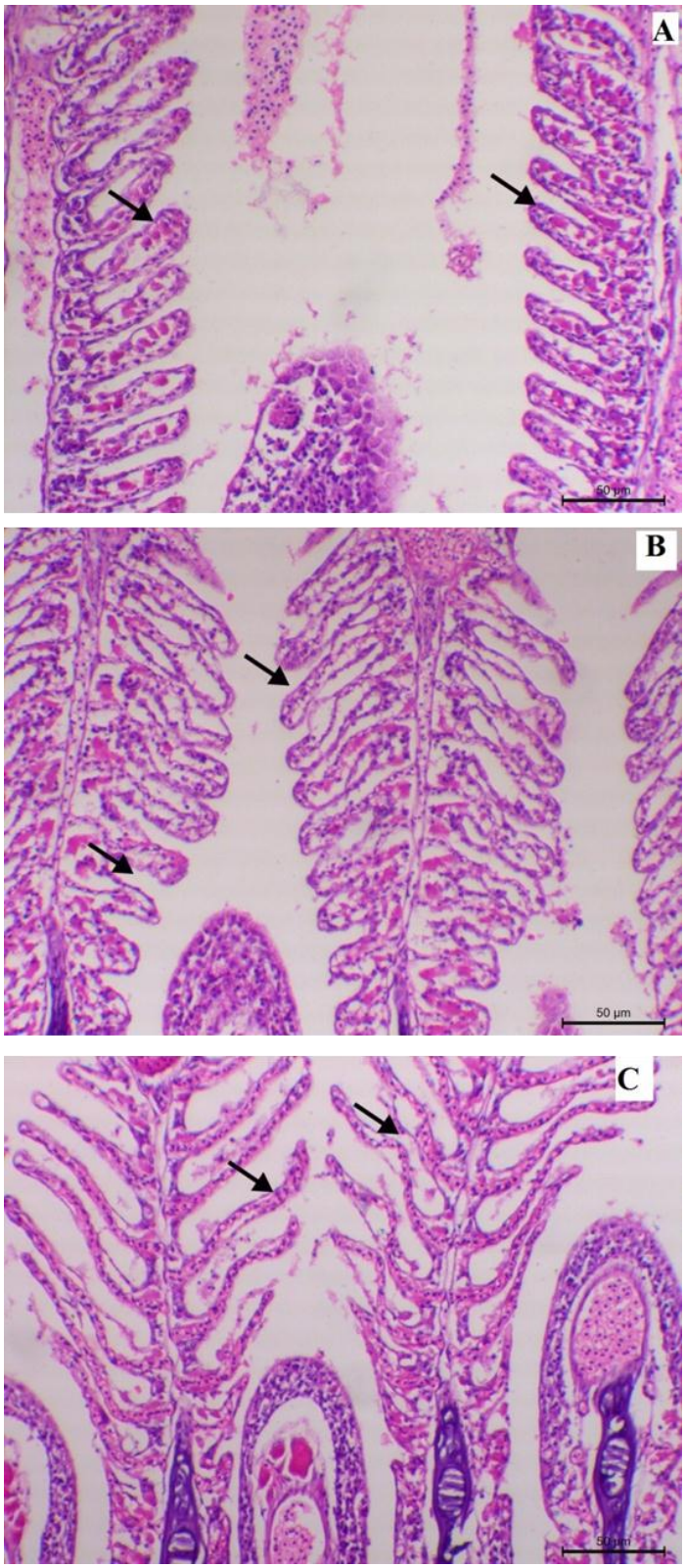

Fig. 1: Photomicrographs gills sections of *Siganus rivulatus* fed three protein levels (A) 25CP; (B) 30CP and (C) 40CP showing normal primary and secondary gill lamellae (arrows), H&E, X200, bar= 50 µm.

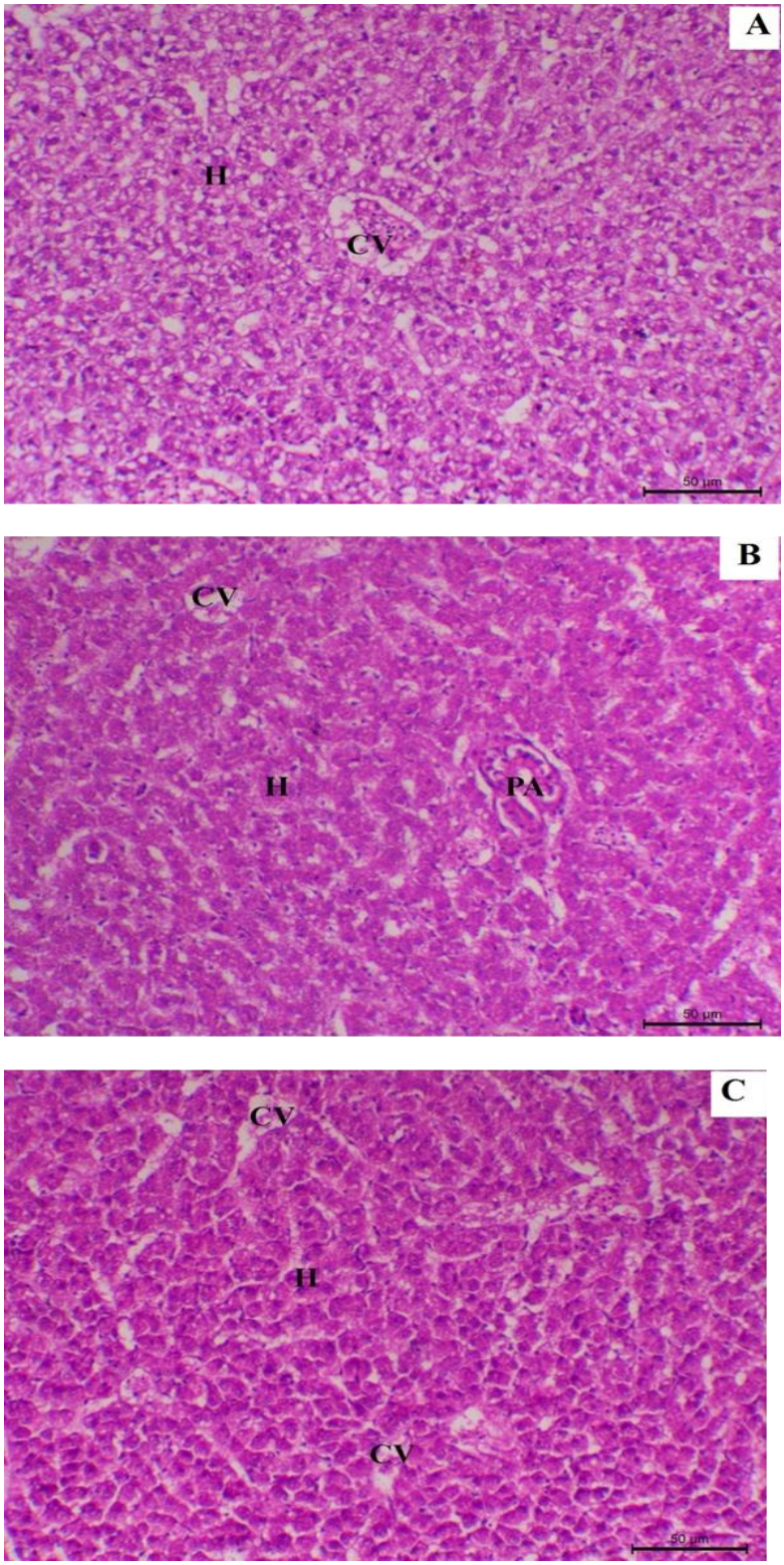

Fig. 2: Photomicrographs gills sections of *Siganus rivulatus* fed three protein levels (A) 25CP; (B) 30CP and (C) 40CP diet showing normal hepatic tissues including both hepatic and portal tissues (H letter indiacates hepatocyte and CV indicates c central vein),H&E, X200, bar= 50 µm.

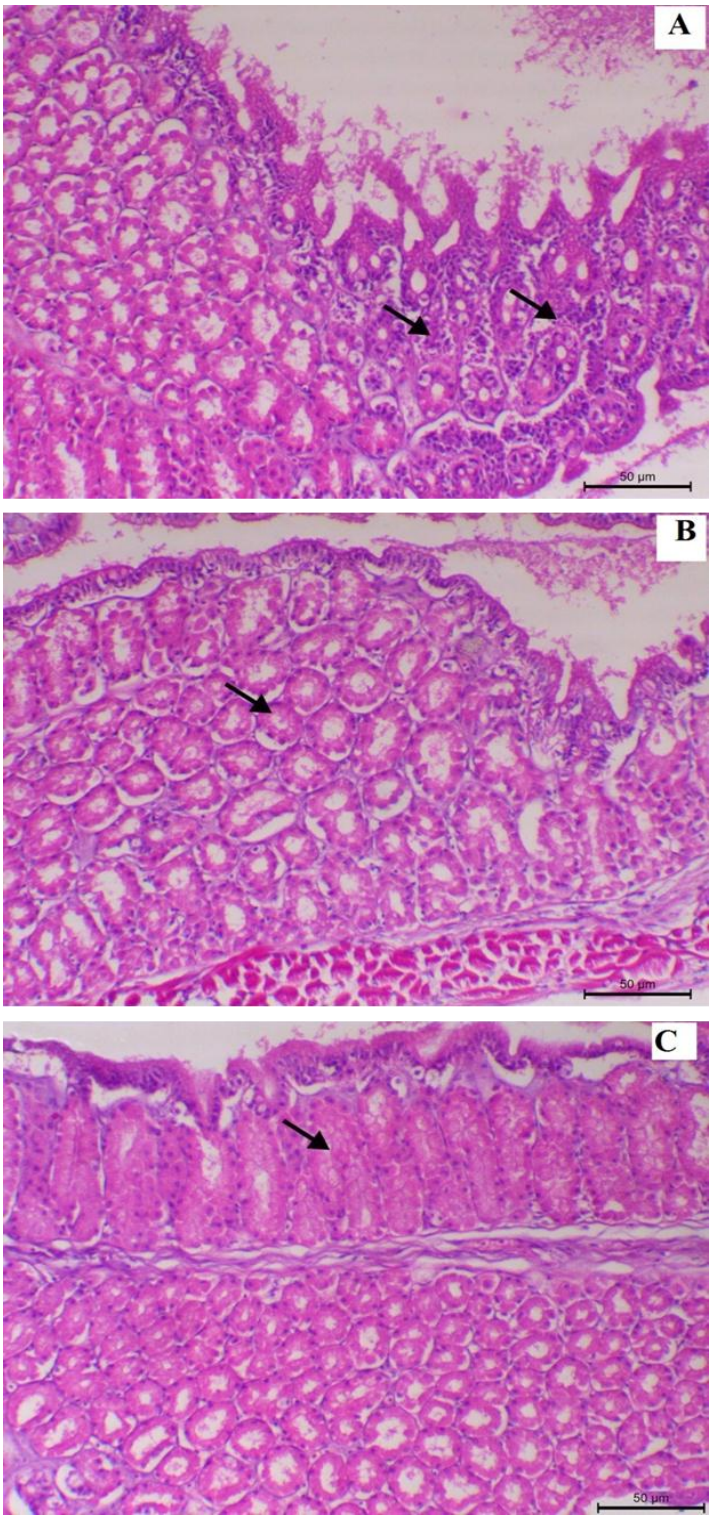

Fig. 3: Photomicrographs gills sections of *Siganus rivulatus* fed three protein levels (A) 25CP and (B) 30CP normal Gastric glands (arrow), while(C) 40CP diet showing marked degenerative chanes of the gastric glands ,H&E, X200, bar= 50 µm.

CONCLUSION

In conclusion, no significant differences between fish groups fed 40% crude protein diet and 30% crude protein diets were noticed concerning the growth performance but theire is maked degenerative change in fish groups fed 40% crude protein gut histopathological sections. It could be concluded that the 30% crude protein diet may be recommended for cultivation of *S. revulatus* under biofloc system conditions during nursery phase.

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