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Red Tilapia Could Be Cultured Under Different Salinity Levels, Feeding on Low Protein Diet Under Condition of Biofloc System

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ABSTRACT

An 84-day experiment was conducted using a biofloc system to investigate the effects of two different protein levels and varying water salinity levels on the growth performance, feed utilization, whole body composition, survival rate, and health status of red tilapia. One hundred and forty four fish were divided into six treatments with an average initial weight (12± 0.1g). Two levels of crude protein (CP25, CP30) and three levels of salinity (0ppt, 18ppt. and 36ppt) were designed to establish six treatments of S0CP25, S0CP30, S18CP25, S18CP30, S36CP25 and S36CP30. No significant differences were recorded among treatments regarding fish performance. The experimental factors had no effect ($P > 0.05$) on all water quality parameters, except for TAN and DO values. The results revealed that the feed intake, protein efficiency ratio increased significantly ($P \leq 0.05$) at the salinity level of 18ppt. The survival rate showed no significant differences among treatments. Furthermore, the total heterotrophic bacteria (THB) group was higher in fish intestine. Increasing salinity to 36ppt stimulated the growth of total vibrio counts (TVC) and increased TVC/THB ratios in the water. No significant differences were recorded in blood biochemical analysis, except for GOT values. The lowest significant value of GOT was recorded in S36CP30, while the highest value was observed in S0CP30. The histological sections of the liver and intestines did not show any changes and were normal in all treatments. Regarding our study, red tilapia can be cultured under biofloc condition within different salinity levels, with no significant effect on fish performance feeding on low- protein die (25%).

INTRODUCTION

Aquaculture is among the most rapidly growing agri-business sectors in recent years, with an enormous potential to meet the increasing need for animal protein **(FAO, 2016)**.

The semi-intensive system is the dominant method for fish production in Egypt, requiring continuous water exchange. This practice increases the potential for pathogen spread and has a negative impact on the environment. New aquaculture technologies with more intensified fish production and limit water exchange have been proposed **(Durigon *et***

***al*., 2020)**. Biofloc technology (BFT) is an aquaculture production system that functions actively with little to no water exchange **(Sgnaulin *et al*., 2018)**. The biofloc system relies on microbial aggregates, which help control toxic nitrogen compounds, provide naturally available live food in the form of floc particles, and enhance disease resistance. To activate microbial activity, maintaining a balanced C:N ratio and ensuring continuous aeration are essential **(Emerenciano *et al*., 2017)**.

Numerous studies have demonstrated the potential of the biofloc system in tilapia culture. Research by **Ekasari and Maryam (2012)**, **Lima *et al.* (2015)**, **Brol *et al.* (2017)**, and **Haridas *et al.* (2017)** highlighted its efficacy in grow-out, overwintering, larval phases, fingerlings production, broodstock formation, as well as in maintaining the appropriate C:N ratio and carbon sources **(Avnimelech, 2007; Crab *et al.*, 2009; Ekasari *et al.*, 2015; Pérez-Fuentes *et al*., 2016; Zhang *et al.*, 2016; Pinho *et al.*, 2017; García-Ríos *et al.*, 2019)**. Though some studies **(Azim & Little, 2008; Abdel-Tawwab *et al*., 2010; Silva *et al.*, 2018)** explored crude protein levels in BFT in freshwater, none of them investigated the ideal protein levels under different salinity conditions. Proper usage of ground water resources could be an option for increasing fish culture in the arid region. The salinity of ground water differs largely with the source site. Red tilapia (*Oreochromis* sp.) could thrive in both freshwater and saltwater environments due to its wide salinity tolerance. Remarkably, temperature, water quality, and feed composition are the most critical factors influencing fish growth **(Azim & Little, 2008; Enayati *et al*., 2013; De Verdal *et al*., 2014)**. Yet, water salinity can disrupt fish homeostasis and affect their performance **(Hrubec *et al*., 2000; Chen *et al*., 2003; Moorman *et al*., 2015).** Extra energy may be required to maintain iono-osmotic balance, while adapting fish to salinity changes **(Rahmah *et al*., 2020)**. Protein provides the majority of energy utilized by fish **(Falco *et al.*, 2020)**. The additional energy required for osmoregulation can directly impact the dietary protein needs of fish. Consequently, understanding the nutritional requirements of red tilapia under varying salinity conditions is essential to optimize fish production. This understanding is closely correlated with the specific culture system in use.

The study aimed to assess the effects of different water salinity levels on the growth performance, feed utilization, health status of fish fed different protein level under condition of biofloc system.

MATERIALS AND METHODS

1. Experimental design

The experiment started on 17 September 2019. The experiment is a part of a master thesis where no animal ethical approval was required for master studies by that time in Faculty of Agriculture, Cairo University. One hundred and forty-four hybrid red tilapia (*O. mossambicus* × *O. niloticus*) fingerlings were obtained from the stock raised at the National Institute of Oceanography and Fisheries (NIOF), the Gulfs of Suez and Aqaba's branch, Suez, Egypt. Fish were acclimatized to salinity of 4ppt for 5 hours daily until it reached 18ppt and 36ppt before they were placed in the experimental aquaria. Fish were randomly distributed into 12 aquaria (80× 30× 40cm - 0.096m3 of water) to conduct 6 treatments (two glass aquaria per treatment) with an initial weight of 12± 0.1g. Two levels of crude protein (25 and 30% CP) and three levels of salinity (0, 18, 36ppt) were examined for their effect on fish performance. Air pumps were used to provide oxygen levels no less than 5- 6mg/ L.

Experimental tanks were provided with starch according to the amount of feed to maintain C/ N ratio around 1: 10 to activate the growth of bacterial community (**Avnimelech, 1999**). Fish were fed twice a day and weighed bi-weekly days, where feed was adjusted accordingly. Fingerlings were left under the condition of natural light.

2. The experimental diets

Diets were formulated to contain 25 and 30% crude protein diets (Table 1). Feed ingredients were mixed, pelleted (1mm), oven dried (55°C / 24h) and stocked (-2°C) for further use.

Table 1. Chemical composition of of the experimental formula

	Dietary protein levels	
Ingredient %	**25% Protein (Diet 1)**	**30% Protein (Diet 2)**
Fish meal	18	22
Soybean meal	15	21
Corn meal	40	30
Wheat bran	16	16
Fish oil	4	4
Starch	2	2
Vitamin mix[1]	2	2
Mineral[2]	2	2
Carboxymethyl cellulose	1	1
Total	100	100
Chemical composition %:		
Dray matter	91.89	93.26
Crude protein	25.03	30.22
Ether extract	7.61	8.12
Crude fiber	9.09	9.31
Ash	6.10	6.77
Nitrogen free extract (NFE)[3]	44.06	38.84
Gross energy (Kcal/100g)[4]	431.23	444.72

[1]Each one kg of vitamin mixture contained: Vit. A 72000IU, Vit.B_1 6mg, Vit. B_3 12000IU, Vit. B6 9mg, B12 0.06mg, Vit E 60mg, Vit. 12mg, Pantothonic acid 60mg, Nicotinic acid 120mg, Folic acid 6mg, Biotin 0.3mg, and Choline chloride 3mg

[2]Each one kg of the mineral mixture contained: Zinc sulfate hepahydrate 3.0mg, Sulfate 0.335, Coppous chloride 0.10, Calcium phosphate monobasic 135.8, Calcium lactate 327.0, Ferric citrate 29.7, Potassium phosphate dibasic anhydrous 239.8, Sodium phosphate monobasic 87.2, Sodium chloride 43.6, Aluminium chloride anhydrous 0.15, Potassium iodide 0.15, Cobalt chloride 1.0, Sodium selenite 0.011 and L-cellulose 132.25 (as g/ kg mineral mix)

[3]NFE= DM-(CP+ EE+ CF+ ASH) , CP: Crude protein , EE: Ether extract , CF: Crude fiber

[4]Calculated according to NRC (1993) using factors 5.64k.cal/ g for protein, 9.44k.cal/ g for fat, and 4.11k.cal/ g for carbohydrates

3. Water quality

Mercury thermometer was suspended at 30cm depth of different experimental tanks to detect water tepmerature. Dissolved oxygen (DO) was reported four times during the experimental period using oxygen meter (YSI model 56), while five values for water pH was determined by using a pH meter. Ammonia, NO2 and (NO3) were registered four times. The total suspended solids (TSS) was noted once at the end of the experiment in a chemistry lab at the NIOF, while the floc volume (FV) was evaluated six times using imhoff cone.

4. Growth performance and feed utilization

Fish performance and feed utilization were calculated using the following equations:

Final body weight (FBW)= Total weight/ fish number

Weight gain (WG) = Final body weight per fish g (W_t) - Initial body weight per fish g (W_o)

Specific growth rate (SGR) = (L_nW_t- L_nW_o) * 100/experimental period (days)

Feed conversion ratio (FCR) = Feed consumption (g)/ WG

Survival rate (%) = (final number of fish/ initial number of fish) * 100

5. Body composition and chemical analysis

Standard **AOAC (1990)** procedures were used to assess moisture, protein, fat, ash, and blood from random samples of the studied meals and whole-fish bodies (5 fish from each treatment). Dietary and fish samples were subjected to moisture, crude protein, lipids, ash, and NFE approximate analysis using **AOAC (1980)** protocols. Dry matter served as the basis for all chemical composition characteristics, with moisture content determined as a percentage after 12 hours of drying at 105°C. Protein content was calculated using the Kjeldahl method (nitrogen 6.25); lipids were measured through soxhlet ether extractives, ash content was determined as the residue after heating at 550°C for 12 hours, and nitrogen-free extractives (NFE) were calculated using appropriate methods.

6. Blood samples and biochemical analysis

Serum of five fish was collected and frozen at -20ºC for future analysis. Serum glucose, total serum protein, albumin content, total globulin were detected according to the methods of **Trinder (1969)**, **Henry (1974)**, **Busher (1990)** and **Young (2000)**, respectively**.** Transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum urea and creatinine were determined according to the methods of **Reitman and Frankel (1957)**, **Henry (1974)** and **Young (2001)**, respectively. The lipid profile included measurements of triglycerides as per **Stein (1987)**. In addition, the measurement of cholesterol levels were determined following the methods outlined by **Ellefson and Caraway (1976)**. Additionally, HDL cholesterol and LDL cholesterol were determined using the techniques described by **Friedewald *et al.* (1972)** and **Warnick and Wood (1995)**, respectively.

7. Assessment of the microbial count

Each replicate's tilapia gut was aseptically removed at the end of the experiment to determine the total number of microorganisms present. After being homogenized in a mortar, they were each washed three times with sterile distilled water to eliminate the non-adherent surface germs. Using the previously established approach by **Draper and Smith (1998)**, we determined the total heterotrophic bacterial count (THB) and the total vibrio count (TVC) in the gut samples. Colony-forming units per milliliter of water (CFU/ ml) were used to quantify the bacterial load **(Draper & Smith, 1998)**. In a nutshell, a sterile polypropylene container containing 30ml of water was taken from the middle of the tank. After serially diluting a 1ml sample by folding it into 9ml of distilled water, the final volume was 1ml. To determine the total number of THB and TVC, 1ml of a suitable dilution was spread in duplicate across plates of tryptone soya agar with 1.0% w/ v NaCl and thiosulfate-citrate-bile salts-sucrose agar (HiMedia). Each colony between 30 and 300 was counted and expressed as CFU, and the ratio of TVC/ THB was found after plates were incubated at 28 and 37°C, respectively, for 24 hours.

8. Assessment of the zooplankton count

Zooplankton samples were collected using a plankton net (20μm mesh size). The collected samples were immediately fixed with 4% formaldehyde. Five subsamples (1ml) were investigated using a binocular research microscope (100× and 400× magnification). The zooplankton's lowest possible taxonomic level (species) was identified according to the taxonomic key's references **(Koste 1978; Shiel & Koste 1979; Ruttner-Kolisko 1989)**. Zooplankton density was calculated according to measurments of **APHA (2005)**. The number of zooplankton was expressed for organisms/ litters depending on the following equation:

$$\text{number of organisms/ litter} = (N \times D)/(S \times C)$$

Where, N= Organisms number; D= Volume of filtered sample; S= Sub-samples numbers, and C= Total volume of the collected sample.

9. Statistical analysis

A two-way analysis of variance (ANOVA) were applied with a significance of $P< 0.05$. Ranking of means was detected by Duncan`s multiple range using MSTATC.

RESULTS

The effect of dietary protein, salinity, and their interaction on water quality parameters are shown in Table (2). The experimental factors had no effect ($P> 0.05$) on all water quality parameters, except for TAN and dissolved oxygen values. Neither salinity nor dietary protein factors affected ($P> 0.05$) the TAN results, while the S36CP30 recorded a slight increase ($P< 0.05$) for TAN (0.80mgL-1) value among other treatments. Slight increase in the dissolved oxygen levels ($P< 0.05$) was noticed in parallel with increasing both protein and salinity levels. The highest DO value ($P< 0.05$) was recorded for $S_{36}Cp_{30}$ treatment (5.95mgL-1).

Table 2. Effect of water salinity and dietary protein levels on water quality parameters

Treatments		TAN (mgL^{-1})	PH	NO_2 (mgL^{-1})	DO (mgL^{-1})	T.S.S (mgL^{-1})	FV (mlL^{-1})
S*CP	S_0cp_{30}	0.75^{ab}	7.70^{a}	0.05^{a}	5.60^{ab}	83.00^{a}	18.58^{ab}
	S_0cp_{25}	0.65^{b}	7.80^{a}	0.00^{a}	5.45^{b}	82.50^{a}	19.33^{a}
	$S_{18}cp_{30}$	0.65^{b}	7.95^{a}	0.05^{a}	5.75^{ab}	89.00^{a}	17.25^{b}
	$S_{18}cp_{25}$	0.75^{ab}	7.95^{a}	0.10^{a}	5.45^{b}	84.00^{a}	19.42^{a}
	$S_{36}cp_{30}$	0.80^{a}	8.05^{a}	0.05^{a}	5.95^{a}	90.00^{a}	18.25^{ab}
	$S_{36}cp_{25}$	0.70^{ab}	7.90^{a}	0.05^{a}	5.75^{ab}	86.00^{a}	16.84^{b}
±SE		**0.029**	**0.121**	**0.041**	**0.102**	**4.067**	**0.52**
CP	Cp_{30}	0.73^{a}	7.90^{a}	0.050^{a}	5.77^{a}	87.33^{a}	18.03^{a}
	Cp_{25}	0.70^{a}	7.88^{a}	0.050^{a}	5.55^{b}	84.17^{a}	18.53^{a}
±SE		**0.0236**	**0.070**	**0.024**	**0.059**	**2.348**	**0.30**
S	S_0	0.70^{a}	7.75^{a}	0.02^{a}	5.52^{b}	82.75^{a}	18.96^{a}
	S_{18}	0.70^{a}	7.95^{a}	0.07^{a}	5.60^{ab}	86.50^{a}	18.33^{ab}
	S_{36}	0.75^{a}	7.97^{a}	0.05^{a}	5.85^{a}	88.00^{a}	17.54^{b}
±SE		**0.029**	**0.085**	**0.029**	**0.072**	**2.876**	**0.37**

Different superscript letters in the same column are significantly different ($P< 0.05$) TAN: Total ammonia/ nitrogen, NO2: Nitrite, T.S.S: Total suspended solid, DO: Dissolved oxygen, FV: Floc volume.

Growth and feed utilization of red tilapia

The effect of salinity and dietary protein levels and their interaction on growth and feed utilization of red tilapia under biofloc conditions are shown in Table (3). The different protein

and salinity levels showed no significant effect on red tilapia growth performance. The highest values for FBW and gain ($P> 0.05$) were recorded for $S_{18}CP_{25}$ treatment (47.05, 31.25), respectively, while the lowest values were recorded for $S_{36}CP_{30}$ (42.75, 26.95), respectively. In the same context, all feed utilization parameters were not affected by the experimental factors except for feed intake and protein efficiency ratio. The highest FI ($P< 0.05$) was recorded for $S_{18}CP_{25}$ treatment (59.65g), while the lowest value was recorded for the S_0CP_{30} (53.40). The treatment of S_0CP_{25} recorded the worst numerical FCR value (2.10), while $S_{18}Cp_{25}$ treatment recorded the best value (1.90). The highest significant PER value was noticed for $S_{18}CP_{25}$ (2.10) treatment, while the lowest ($P< 0.05$) value was reported for $S_{36}CP_{30}$ (1.60). The improvement in PER values was significantly associated with dietary protein level as fish fed on CP_{25} showed the highest PER value ($P< 0.05$) compared to the CP_{30}. In contrast, salinity factor showed no effect on PER values. Experimental factors and their interaction showed no effect on survival rate. The highest survival rate ($P> 0.05$) was recorded for the treatment of S_0Cp_{30} treatment (83%), while the lowest value ($P> 0.05$) was reported for $S_{18}Cp_{30}$ and $S_{18}CP_{25}$ (75%).

Table 3. Effect of water salinity and dietary protein levels on growth and feed utilization of red tilapia fingerlings

Treatment		FBW	Gain	SGR	FI	FCR	PER	SR %
S*CP	S_0cp_{30}	43.45^{a}	27.65^{a}	1.00^{a}	53.40^{b}	1.95^{a}	1.73^{bc}	83.35^{a}
	S_0cp_{25}	43.10^{a}	27.30^{a}	0.90^{a}	56.80^{ab}	2.10^{a}	1.93^{ab}	79.15^{a}
	$S_{18}cp_{30}$	43.70^{a}	27.90^{a}	0.85^{a}	56.85^{ab}	2.05^{a}	1.64^{c}	75.00^{a}
	$S_{18}cp_{25}$	47.05^{a}	31.25^{a}	0.95^{a}	59.65^{a}	1.90^{a}	2.10^{a}	75.00^{a}
	$S_{36}cp_{30}$	42.75^{a}	26.95^{a}	0.90^{a}	54.75^{ab}	2.05^{a}	1.64^{c}	79.15^{a}
	$S_{36}cp_{25}$	43.40^{a}	27.60^{a}	0.95^{a}	54.15^{ab}	2.00^{a}	2.00^{a}	79.15^{a}
±SE		**1.515**	**1.432**	**0.089**	**1.542**	**0.089**	**0.079**	**5.631**
CP	Cp_{30}	43.30^{a}	27.50^{a}	0.92^{a}	55.00^{a}	2.02^{a}	1.87^{b}	79.16^{a}
	Cp_{25}	44.52^{a}	28.72^{a}	0.93^{a}	56.87^{a}	2.00^{a}	2.00^{a}	77.76^{a}
±SE		**0.875**	**0.832**	**0.051**	**0.890**	**0.051**	**0.046**	**3.251**
S	S_0	43.27^{a}	27.47^{a}	0.95^{a}	55.10^{a}	2.02^{a}	1.82^{a}	81.25^{a}
	S_{18}	45.37^{a}	29.57^{a}	0.90^{a}	58.25^{a}	1.97^{a}	1.87^{a}	75.00^{a}
	S_{36}	43.07^{a}	27.27^{a}	0.92^{a}	54.45^{a}	2.02^{a}	1.80^{a}	79.15^{a}
±SE		**1.071**	**1.441**	**0.063**	**1.090**	**0.063**	**0.056**	**3.982**

Different superscript letters in the same column are significantly different ($P< 0.05$).

FBW: Final body weight, SGR: Specific growth rate, FI: Feed intake, FCR: Feed conversion ratio, PER: Protein efficiency ratio.

Body composition

The effect of salinity and dietary protein levels and their interaction on the chemical composition of red tilapia carcass are displayed in Table (4). The highest significant value of moisture content was recorded for S_0CP_{30} (74.77), followed by $S_{18}Cp_{30}$ (74.73) and $S_{18}CP_{25}$ (74.47) treatments, while the lowest significant value was recorded for the S_0CP_{25} (72.86) treatment. Fish fed on CP_{30} recorded a higher significant ($P< 0.0.05$) moisture content than those provided with CP_{25}. The salinity factor affected carcass moisture significantly, as S_{18} recorded the highest moisture value compared to the other salinity levels (S_0 and S_{36}). Neither of the experimental factors nor their interaction affected ($P> 0.05$) the protein content of fish carcasses. The S0CP30 treatments recorded the highest significant ($P< 0.05$) value for ether extract (20.72%), while the S18CP25 treatment recorded the lowest value (15.98). Fish fed high dietary protein level (CP30) significantly deposited more ether extract. The lower the salinity level (S0), the higher the deposition of ether extract in red tilapia carcass. Treatments of S18Cp30, S36Cp30, and S36CP25 recorded higher values for ash content ($P< 0.05$) than other treatments. Regarding the protein effect, fish fed high protein-diet (CP_{30}) recorded the

highest significant ($P< 0.05$) ash content. As for the salinity factor, the highest significant ash content was noticed in fish cultured under S_{36}.

Table 4. Effect of water salinity and dietary protein levels on chemical composition of tilapia Bacterial abundance in water and tilapia intestine

Treatments		Moisture	Crud protein	Ether extract	Ash
S*CP	S_0cp_{30}	74.77[a]	56.23[a]	20.72[a]	15.46[b]
	S_0cp_{25}	72.86[b]	56.20[a]	18.91[b]	14.59[c]
	$S_{18}cp_{30}$	74.73[a]	56.63[a]	17.60[c]	16.11[a]
	$S_{18}cp_{25}$	74.47[a]	56.80[a]	15.98[d]	14.12[c]
	$S_{36}cp_{30}$	73.77[ab]	56.50[a]	17.06[cd]	16.36[a]
	$S_{36}cp_{25}$	73.81[ab]	57.23[a]	17.30[c]	16.15[a]
SE±		**0.3270**	**0.405**	**0.409**	**0.191**
CP	Cp_{30}	74.42[a]	56.46[a]	18.46[a]	15.98[a]
	Cp_{25}	73.71[b]	56.74[a]	17.39[b]	14.95[b]
SE±		**0.189**	**0.405**	**0.236**	**0.110**
S	S_0	73.81[b]	56.22[a]	19.81[a]	15.02[b]
	S_{18}	74.60[a]	56.72[a]	16.79[b]	15.12[b]
	S_{36}	73.79[b]	56.87[a]	17.18[b]	16.26[a]
SE±		**0.231**	**0.496**	**0.289**	**0.135**

Different superscript letters in the same column are significantly different ($P< 0.05$).

The water and intestine bacterial abundance of Heterotrophic bacteria (THB) and Vibrio bacteria (TVC) under condition of different dietary protein and salinity level by the end of the experiment are tabulated in Table (5). Generally, water bacterial counts of THB group were higher than those in fish intestine, where the highest significant count was noticed in S_{18} treatment in both water and intestine. Increasing the salinity up to 36ppt resulted in growth activation of TVC and increasing the TVC/ THB ratios in water. The water bacterial count of THB, TVC and TVC/ THB ratio were increased under condition of high protein-diet (370×103 CFU/ ml, 23424 CFU/ ml, and 0.778%, respectively). The bacterial profile of intestine did not reflect the bacterial count of the water, where no significant differences were noticed among the treatment regarding the TVC group. The intestinal TVC/ THB ratio was affected significantly by dietary protein and salinity factors, where the highest ration recorded for fish fed high protein diet (CP_{30}) and those under low salinity S_0.

Table 5. Total counts of heterotrophic bacteria, vibrio bacteria and V/ H bacterial ratio in red tilapia intestine and water for different experimental treatment

Treatments		Water			Intestine		
		THB	TVC	(TVC/ THB)	THB	TVC	TVC/ THB
S*CP	S_0cp_{30}	200×10^{3} [c]	130[b]	0.001[b]	10.0×10^{3} [e]	125.667[a]	0.013[a]
	S_0cp_{25}	120×10^{3} [d]	124[b]	0.001[b]	30.0×10^{3} [c]	129.000[a]	0.004[bc]
	$S_{18}cp_{30}$	900×10^{3} [a]	131[b]	0.000[b]	10.0×10^{3} [e]	123.333[a]	0.012[a]
	$S_{18}cp_{25}$	730×10^{3} [b]	124[b]	0.000[b]	500.0×10^{3} [a]	132.000[a]	0.000[d]
	$S_{36}cp_{30}$	30×10^{3} [f]	70×10^{3} [a]	2.333[a]	20.0×10^{3} [d]	121.667[a]	0.006[b]
	$S_{36}cp_{25}$	60×10^{3} [e]	120[b]	0.002[b]	40.0×10^{3} [b]	133.333[a]	0.003[c]
SE±		86.5	50.20	0.0007	122.1695	9.6215	0.0007
CP	Cp_{30}	370×10^{3} [a]	23×10^{3}[a]	0.778[a]	13×10^{3}[b]	123.556 [a]	0.010[a]
	Cp_{25}	300×10^{3} [b]	123[b]	0.001[b]	190×10^{3}[a]	131.444 [a]	0.003[b]
SE±		49.9	28.98	0.0004	70.5346	5.5550	0.0004
S	S_0	160×10^{3} [b]	127[b]	0.001[b]	20×10^{3}[c]	127.333 [a]	0.009[a]
	S_{18}	810×10^{3} [a]	128[b]	0.000[b]	255×10^{3}[a]	127.667 [a]	0.006[b]
	S_{36}	40×10^{3} [c]	35×10^{3}[a]	1.167[a]	30×10^{3}[b]	127.500 [a]	0.005[b]
SE±		61.14	35.50	0.0005	86.3869	6.8035	0.0005

Different superscript letters in the same column are significantly different ($P< 0.05$); THB: Total heterotrophic bacteria, and TVC:Total vibrio counts.

The blood biochemical analysis

The values of blood biochemical analysis are showed in Table (6). No significant differences were recorded among treatments regarding the blood parameters, except for the GOT values. Glucose values ranged between 31.2 and 102.9mg/ dl, while protein fluctuated between 2.5 and 3.2g/ dl (S_0CP_{30}, $S_{36}CP_{25}$), respectively. Albumin values ranged between 3.2 and 2.3g/ dl ($S_{18}CP_{25}$, S_0CP_{30}), respectively. The lowest significant value for GOT 39.8u/ l was recorded in treatment $S_{36}CP_{30}$, while the highest value was noticed in S_0CP_{30}.

Assessment of kidney function can be done by detecting urea and creatinine values in blood that showed no significant difference among treatments. Urea showed the highest value 12.2mg/ dl ($P> 0.05$) in S_0CP_{25}, while the lowest value was recorded in treatment $S_{36}CP_{25}$ (5.8mg/ dl). Creatinine ranged between 0.66 and 0.38 ($S_{18}CP_{30}$, $S_{18}cp_{25)}$, respectively. Treatment of S18CP30 showed the highest numerical value for total cholesterol, triglycerides and LDL (247.8, 114.3, 59.79mg/ dl), respectively.

Table 6. Blood biochemical analysis of red tilapia under different experimental treatments

		GLU (mg/ dl)	Prot. (g/ dl)	Albu. (g/ dl)	GPT (ALT) (u/ l)	GOT (AST) (u/ l)	Urea (mg/ dl)	Creat. (mg/ dl)	TRG (mg/ dl)	Chol. (mg/ dl)	Hdl (mg/ dl)	LdL (mg/ dl)
S*CP	**S_0cp_{30}**	35.3^{a}	3.2^{a}	3.2^{a}	73.9^{a}	109.8^{a}	12.2^{a}	0.49^{a}	155.9^{a}	98.07^{a}	13.00^{a}	53.89^{a}
	S_0cp_{25}	102.9^{a}	2.9^{a}	2.6^{a}	38.4^{a}	83.6^{abc}	7.8^{a}	0.40^{a}	174.6^{a}	53.44^{a}	7.50^{a}	23.94^{a}
	$S_{18}cp_{30}$	31.2 ^{a}	2.9^{a}	2.5^{a}	69.7^{a}	100.9^{ab}	9.3^{a}	0.66^{a}	247.8^{a}	114.3^{a}	5.00^{a}	59.79^{a}
	$S_{18}cp_{25}$	39.9 ^{a}	3.0^{a}	2.3^{a}	60.3^{a}	55.3^{bc}	8.4^{a}	0.38^{a}	93.7^{a}	75.48^{a}	16.25^{a}	40.49^{a}
	$S_{36}cp_{30}$	51.4 ^{a}	2.5^{a}	2.5^{a}	54.0^{a}	39.8^{c}	10.6^{a}	0.47^{a}	142.3^{a}	86.23^{a}	12.00^{a}	45.77^{a}
	$S_{36}cp_{25}$	50.8 ^{a}	2.5^{a}	2.6^{a}	36.5^{a}	82.3^{abc}	5.8^{a}	0.56 ^{a}	224.3^{a}	99.72^{a}	17.25^{a}	37.62^{a}
SE±		30.3	0.4	0.4	11.9	15.1	2.4	0.17	57.11	22.10	6.35	27.83
CP	**Cp_{30}**	39.3^{a}	2.9^{a}	2.7^{a}	65.9^{a}	83.5^{a}	10.7^{a}	0.54^{a}	182.0^{a}	99.54^{a}	10.00^{a}	53.15^{a}
	Cp_{25}	64.5^{a}	2.8^{a}	2.5^{a}	45.0^{a}	73.7^{a}	7.3^{a}	0.45^{a}	164.2^{a}	76.22^{a}	13.67^{a}	34.02^{a}
SE±		17.5	0.2	0.2	6.8	8.7	1.4	0.10	32.97	12.76	3.66	16.07
S	**S_0**	69.1^{a}	3.0^{a}	2.9^{a}	56.1^{a}	96.7^{a}	10.0^{a}	0.44^{a}	165.3^{a}	75.76^{a}	10.25^{a}	38.91^{a}
	S_{18}	35.6^{a}	3.0^{a}	2.4^{a}	65.0^{a}	78.1^{a}	8.8^{a}	0.52^{a}	170.7^{a}	94.90^{a}	10.62^{a}	50.14^{a}
	S_{36}	51.1^{a}	2.5^{a}	2.6^{a}	45.3^{a}	61.1^{a}	8.2^{a}	0.52^{a}	183.3^{a}	92.97^{a}	14.62^{a}	41.70^{a}
SE±		21.4	0.31	0.3	8.4	10.6	1.7	0.12	40.38	15.63	4.49	19.68

Different superscript letters in the same column are significantly different ($P< 0.05$).

GLU: Glucose, PROT: Protein, CREAT: Creatinine, ALT: Aspartate amino transferase, AST: Alanine amino transferase, HDL: High density lipoprotein, and LDL: Low density lipoprotein.

The results of histological sections

The change in gills shape, size and structure can be easily observed in all treatments (S_0CP_{25}, S_0CP_{30}, $S_{18}CP_{25}$, $S_{18}CP_{30,}$ $S_{36}CP_{25}$, $S_{36}CP_{30}$) (Fig. 1), leading to an apparent lamellar fusions, hypertrophy and edema within the epithelial cells. However, thinning of secondary lamellae was observed in fish of treatment $S_{18}CP_{25}$, $S_{18}CP_{30,}$ $S_{36}CP_{25}$, and $S_{36}CP_{30}$. Nonetheless, shortening of lamellae was observed in treatments cultured under a salinity of 36ppt.

On the other hand, histological sections of liver and gut did not show any histological changes and seems to be normal, as seen in Figs. (2, 3).The histological structure of liver is similar to a normal fish. Polyhydric hepatocytes with many hepatic sinusoids in between can

be observed regularly arrayed out of hepatic blood venules forming a cord-like structure (Fig. 2). Many normal hepatopancreatic acini are also obviously detected. Similarly, the gut of the experimental fish has normal serosa and muscularis of both longitudinal and circular muscle layers which is followed internally by the sub-mucosa and mucosa (Fig. 3).

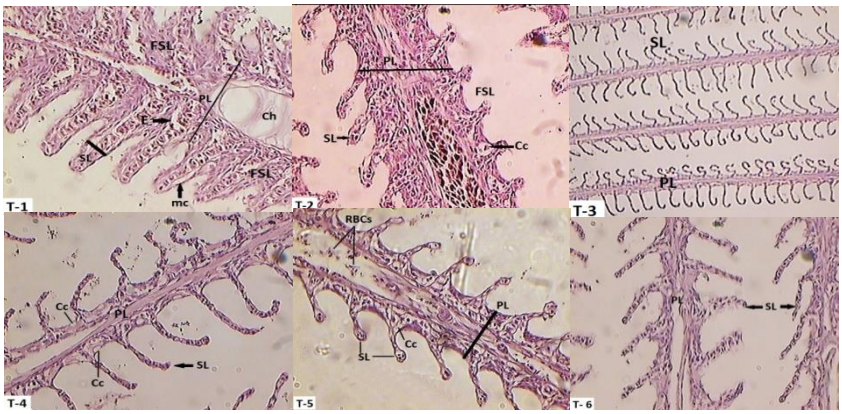


Fig. 1. Histology of gills in red tilapia treatment T1 (S_0CP_{25}), T2 (S_0CP_{30}) , T3($S_{18}CP_{25}$) , T4 ($S_{18}CP_{30}$), T5($S_{36}CP_{25}$), and T6 ($S_{36}CP_{30}$). Primary lamella (PL); Secondary lamella (SL); Mucous cell (mc); Chloride cell (Cc); Edema (E); Fusion of several lamella (FSL), and Chondrocytes (Ch).

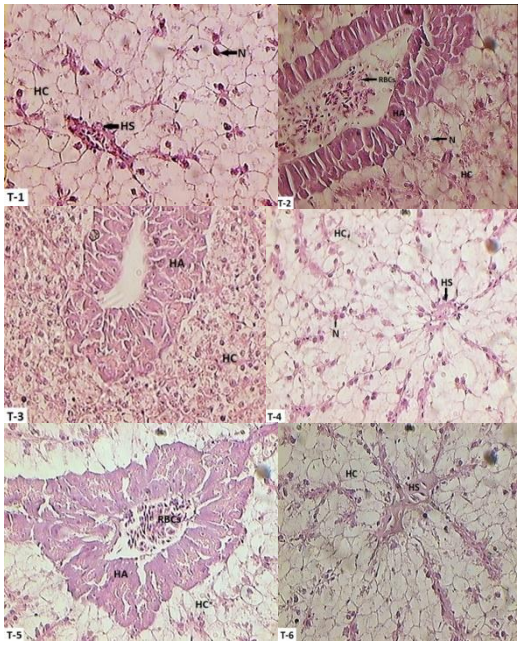


Fig. 2.
Histology of liver in red tilapia treatment; T1 (S_0CP_{25}), T2 (S_0CP_{30}) , T3 ($S_{18}CP_{25}$) , T4 ($S_{18}CP_{30}$) , T5 ($S_{36}CP_{25}$), and T6 ($S_{36}CP_{30}$). Hepatocytes (Hc); Hepatic sinusoid (HS); Hepatic vein (HV); Hepatopancreatic acini (HA), and Nucleus (N).

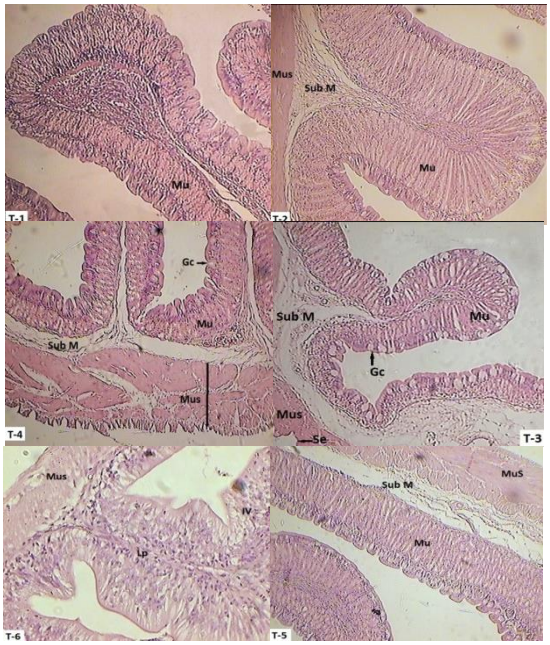


Fig 3
Histology of gut in red tilapia control (C) treatment (T1(S_0CP_{25}), T2 (S_0CP_{30}), T3 ($S_{18}CP_{25}$), T4 ($S_{18}CP_{30}$), T5 ($S_{36}CP_{25}$), and T6 ($S_{36}CP_{30}$). Mucosa (Mu); Sub-mucosa (Sub M); Muscularis (Mus); Serosa layer (Se), Venul (Ven); Intestinal villi (IV); Lamina propria (Lp), and Goblet cell (Gc).

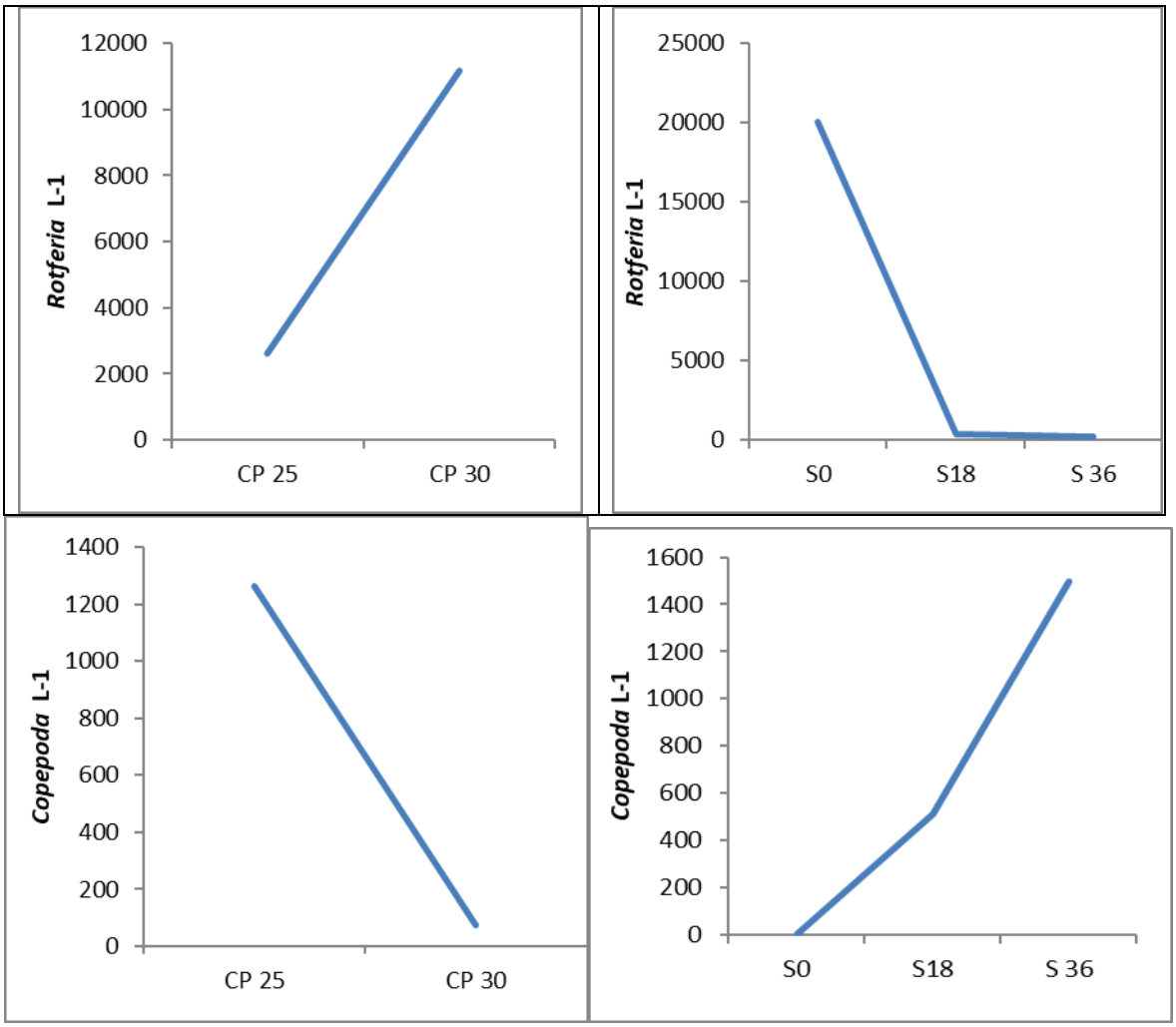
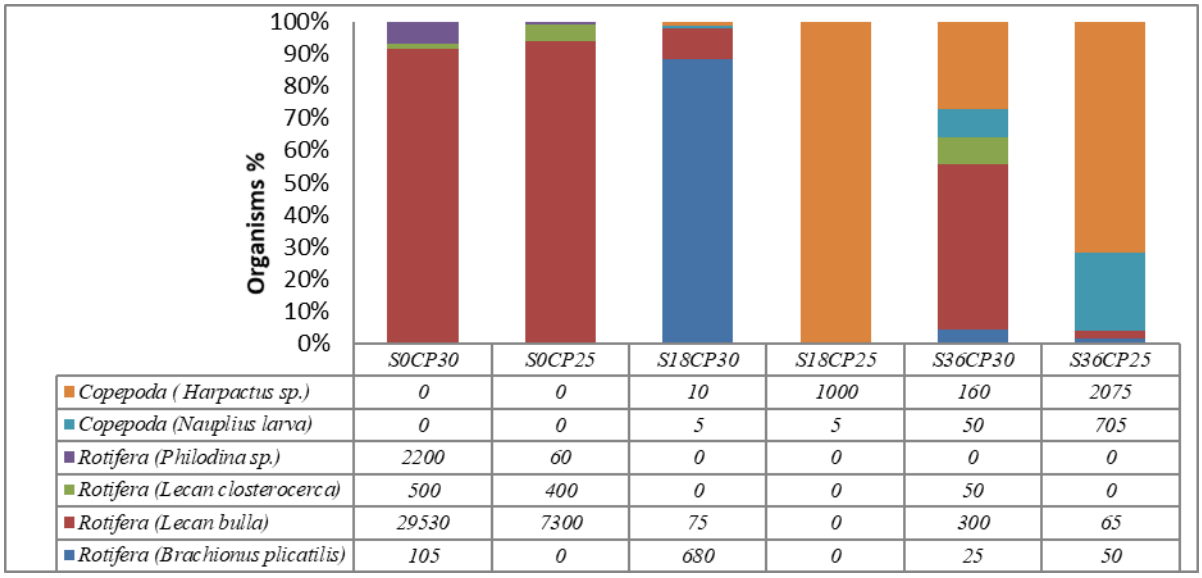


Fig. 4. The effect of experimental factors (dietary protein, salinity) on zooplankton community

The zooplankton community

On numerical basis it was recognized that, rotifera group decreased with increasing salinity, while a positive relation was noticed upon increasing the dietary protein. The opposite trend was observed for the copepoda group, as a positive relation was recorded with increasing the salinity, while higher numbers were noted under low protein diet. Thus, copepod group was the dominant group under the condition of $S_{18}CP_{25}$ and $S_{36}CP_{25}$, while rotifera group was the dominant group in S_0CP_{30}, S_0CP_{25} and $S_{18}CP_{30}$ (Figs. 4, 5).



	S0CP30	S0CP25	S18CP30	S18CP25	S36CP30	S36CP25
Copepoda (Harpactus sp.)	0	0	10	1000	160	2075
Copepoda (Nauplius larva)	0	0	5	5	50	705
Rotifera (Philodina sp.)	2200	60	0	0	0	0
Rotifera (Lecan closterocerca)	500	400	0	0	50	0
Rotifera (Lecan bulla)	29530	7300	75	0	300	65
Rotifera (Brachionus plicatilis)	105	0	680	0	25	50

Fig. 5. The most dominant biofloc zooplankton community of different the experimental groups

DISCUSSION

In regard to our results, red tilapia could be cultured at different salinity levels up to 36ppt with no significant effect on the fish performance. It was suggested by **Graci-Ulloa *et al*. (2001)** that the hybrid tilapia can be easily introduced to fluctuating saline waters. Hybrid red tilapia cultured in salinity level of 26ppt showed a significant improvement in growth performance and feed utilization **(Nassar *et al*. 2021)**. In the same context, **Suresh and Lin (1992)** suggested that under salinity range of 10- 20ppt, tilapia species showed remarkable growth. Maximum egg production and larval survival rate were obtained from red tilapia breed under different salinity levels up to 20% **(Malik *et al*., 2017)**. Based on our results, low protein-diet 25% was sufficient to cover the needed protein for red tilapia under biofloc condition of different salinities. Fish fed dietary protein of 25% gained the same growth as those fed on high protein-diet (30%). The protein utilization results supported the latter, as fish fed on low-protein diet showed better PER ($P< 0.05$) under all salinity conditions. The bacterial growth, bacterial assimilation of nitrogen, and the production of microbial proteins could be activated by adding carbohydrates to the pond **(Avnimelech, 1999)**. Additionally, the current results showed an increased number of copepoda (large sized zooplankton group) under the condition of low protein-diet, which may assisted the tilapia growth. The biofloc system has been shown to enhance fish growth even with a low-protein diet, as demonstrated by **Zablon *et al.* (2022)**. Flocs in the system are considered an additional source of protein. Studies such as that of **Hisano *et al.* (2019)** suggested that different levels of dietary protein (36, 32, and 28%) had no influence on the growth performance of the Nile tilapia fry. Furthermore, a reduction in dietary protein to 28% can save production costs and limit the environmental negative impact of dietary nitrogen. In the present study, no significant differences were noted in survival rate among different fish groups, and the same trend was noticed regarding the effect of protein and salinity factors. The findings of **Crab *et al*. (2009)** supported our result, as no significant difference in survival rate was noticed between hybrid

tilapia fed either 23 or 30% protein in their study. In the context of the biofloc system, the survival rate of the Nile tilapia did not significantly differ between fish fed a low-protein diet of 24% CP compared to 35% CP, as shown in the study of **Azim and Little (2008)** or between fish fed 28% to 36%, as observed in the study of **Zablon *et al.* (2022)**. Similar suggestions were made in the study of **Nguyen *et al*. (2021)** for the Nile tilapia fed different protein levels (23, 27, 31 or 35%) under biofloc (biofoc- RAS) condition. Upon our results, different salinities showed no effect on the survival rate of tilapia. Florida red tilapia broodstock (*Oreochromis* sp.) reared under different salinity of (9%, 18%, 24% and 36%) did not show significant differences in the survival rate **(Sallam *et al*., 2017)**.

Fish fed high dietary protein level (CP_{30}), significantly deposit more ether extract (Table 6). This could be related to the catabolism of excesses in amino acids and accumulation of their carbon skelton in the form of lipids. The lower the salinity level (S_0), the higher the deposition of ether extract in red tilapia carcass. It seems that osmoregulation mechanism did not consume great portion of dietary energy under such low salinity, and energy was saved in the form of lipids. Generally, salinity and dietary protein levels showed no effect on blood parameters which support the growth performance results.

Our results regarding the histological sections anticipated that treatments mostly affected gills structures but not liver or gut sections. Fish fed low protein diet showed the most negatively affected gills inform of blood clustress and shortness in lammela length. The latter could be attributed to the high activity of grazing flocs from water column. Salinity may also cause alterations in gill structures, which are observed at high salinity as a result of acclimatization. Fish gills not only involved in gas exchange but also play a very important role in osmoregulation, nitrogenous waste excretion and pH regulation **(Evans *et al*., 2005)**. Gills are significantly affected by chemical and physical changes in its aquatic environment as they have large surface area and a continuous direct contact with the surrounding environment **(Yoon *et al*., 2015; Neuraste *et al*., 2017)**. The normal villus structures of the fish gut emphasize that different salinities had no predominant effect on intestinal histology. This was also observed by **Tran-Ngoc *et al.* (2017)**. It seems that red tilapia in different experimental treatments adapted well to the surrounded conditions despite the changes in gill histological structures.

CONCLUSION

Red tilapia can be cultured under biofloc conditions at various salinity levels up to 36 ppt, without compromising growth performance and health status, even when fed a low-protein diet of 25%.

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