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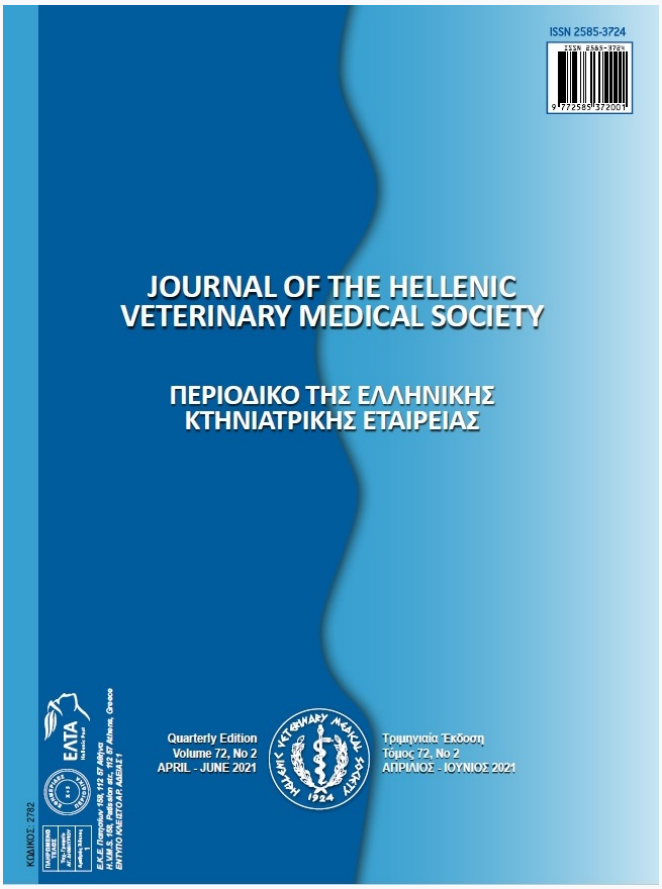

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Research article
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Assessing the Impact of using biofloc system with different feeding rates on Nile Tilapia (*Oreochromis niloticus*) Performance

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ABSTRACT: Biofloc technology is a technique of enhancing water quality in aquaculture through balancing carbon and nitrogen in the system. Randomly designed 3×1 factorial treatments for 90 days was conducted;to assess the effects of biofloc technology in indoor tanks for Nile tilapia (*Oreochromis niloticus*); on the growth performance, digestive enzyme activity, hematology, immune response, intestinal morphometry and chemical composition of Nile tilapia and flocs. In addition, to distinguish the best feeding; through utilizing different feeding rate 0.5%, 1% and 1.5% rate; with zero water change (biofloc technique; BFT) and their impact on aquatic animal rearing. Fish were reared in nine fiber-glass indoor tanks (2 m^3/ each); three replicates / treatment (feeding rate) with 100 fish / tank. Diets were offered twice / day. Results showed that values of water parameters were optimal for tilapia culture and the recorded ammonia, nitrite and nitrate concentrations are created through nitrification process in the BFT system. BFT protein increased positively with increased feeding rate, while BFT lipid and carbohydrates negatively decreased. Growth performance and feed utilization efficiency were significantly improved by increased feeding rates however; the best food conversion rate was recorded in 0.5% feeding rate treatment.There was negative relationship between crude protein and fat contents in fish body; with the highest crude protein content in 1.0% feeding rate treatment. The overall improvement in hae-matological and serum biochemical parameters reflects the positive effect of biofloc system on the physical condition and immune response of tilapia.The total intestinal length and intestinal villi heights were significantly increased with decreased feeding rate; with highest length in 0.5% feeding rate treatment. In conclusion, using BFT in tilapia rearing with 0.5% feeding rate, had beneficial effects on maintaining good water quality, improving feed utilization and growth performance, increasing fish body protein content, physical and immune response promotion as well as increasing the absorptive capacity of the intestine. Subsequently, BFT offers not only economic benefits for fish farmers and safe product for consumers but also, for the sustainability of the fish environment.

***Keywords*:** Biofloc, feeding rates, hematology, Nile tilapia, performance

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INTRODUCTION

Aquaculture is a fundamental industry for supporting the world's interest of seafood protein and will play a much increasingly significant role as the worldwide population continues to increase (Jackson, 2007). To adapt to the issue of lack in protein food supplies, which is mainly located in the developing countries, the current worldwide growth rate of the aquaculture business (8.9-9.1% per year since the 1970s) is highly required (Subasinghe, 2005; Gutierrez-Wing & Malone,2006; Matos et al., 2006). Recently, tilapia has become the sparkling star of aquaculture with beginning of several farms to compensate the progressive consumption rate (Fitzsimmons, 2005; FAO, 2012).The Egyptian aquaculture sector is the largest producer of cultivated fish in Africa and the third largest worldwide producer of cultivated tilapia after China and Indonesia (FAO, 2016; Fitzsimmons, 2016).

Intensive aquaculture constitutes the intelligent key to keep up the tilapia production (FAO, 2012). Intensive aquaculture industry faces two noteworthy issues; (i) Water quality deterioration caused by pollution from waste products (Piedrahita, 2003) (ii) expanded cost burden of artificial feed. Feed is the greatest portion of working expense of aquaculture systems additionally, availability of ingredients limit the growth of fed aquaculture. So as to make aquaculture completely effective, there is an urgent demand to create innovation that will increase financial, environmental sustainability and lessen feed cost and improve production (Sharma *et al.,* 2015).

Recently, biofloc technology (BFT) is considered as a more eco-friendly and sustainable technique for use in zero-water exchange culture systems (Avnimelech, 2007; Azim & Little, 2008; De Schryveret al., 2008; Crab et al., 2009). Utilization of biofloc technology (BFT) offers a solution for both last problems. The system depends on the fact of lower speculation and maintenance costs together with incorporating the possibility to reuse feed together with regular domestic wastewater treatment systems (Timmons & Ebling, 2007). Microbial biomass is developed on fish excreta resulting in evacuation of these undesirable components from the water. The major driving force is the intensive growth of heterotrophic bacteria (De Schryver *et al.,* 2008).

Biofloc can acclimatize the nitrogen wastes and recycle it into microbial protein, where the last made by means of floccules of bacteria that attracts other organisms as micro/macro invertebrates, filamentous organisms fungi, ciliates, flagellates, rotifers, nematodes, metazoans and detritus which known as flocs. Bacterial flocs contribute to diminishing the requirements for artificial food for fish species such as tilapia, carp and shrimp where, it's considered as optional feed source, according to its composition and nutritional value (Wasielesky *et al.,* 2006; Emerencia*no et al.,* 2012). Biofloc combines the expulsions of nutrients from the water with encourage growth of microbial biomass (heterotrophic bacteria) which consume ammonia for growth leading to diminishing the pond water exchange. Subsequently, few investigations have examined different feeding management under biofloc system (Sharma et al., 2015; Lara et al., 2017). However, there are several advantages of a biofloc system for aquaculture. Nevertheless, there are some practical disadvantages of implementing a BFT system to culture fish includes the additional requirement of organic carbon delivery to maintain a C:N ratio above 10 and relatively high energy costs associated with intense mixing and aeration to prevent active bioflocs from settling out of suspension and to meet the additional biological oxygen demand (BOD) caused by elevated microbial respiration. Excessive suspended solid concentration in the rearing environment can also clog the gills of fish, resulting in growth and welfare depression (Luo *et al.,* 2014). Moreover, the most obvious disadvantage is the need for high oxygenation and hence high energy cost in order to keep the fish as well as the microbiotas in optimal condition, any prolonged power failure in the scale of minutes is highly lethal to the biofloc system. Besides; a biofloc system is slow to develop as it may take more than 4 weeks for the nitrifying bacterial community to establish (Thong & Yong, 2014). Hargreaves & John (2013) stated that increased energy requirement for mixing and aeration as well as reduced response time because water respiration rates are elevated.

The current experiment was conducted to assess the effects of biofloc technology in indoor tanks for Nile tilapia (*Oreochromis niloticus*); on the growth performance, digestive enzyme activity, hematology, immune response, intestinal morphometry and chemical composition of Nile tilapia and flocs. In addition, to distinguish the best feeding; through utilizing different feeding rate 0.5%, 1% and 1.5% rate; with zero water change (biofloc technique; BFT) and their impact on aquatic animal rearing.

MATERIALS AND METHODS

Ethical Approval

All handlings of fish were directed according to the guidelines for animal care and use for scientific purposes built up by the Ethics Committee of the Faculty of Agriculture, Kafrelsheikh University, Egypt (Approval Date: 18-03-2018).

Fish and experimental conditions

The experiment was performed using 900 mono-sex male Nile tilapia (*O. niloticus*) fish weighing an average of 53.45±1.49 g. They were obtained from National institute of Oceanography and fisheries (NIOF), El-Serw Research farm, Damietta Governorate, Egypt. All collected fish were accommodated in three fiberglass tanks for two weeks at the laboratory of El-Serw Research farm, Damietta Governorate. During the accommodation period, fish were fed a commercial diet (25% crude protein, Skretting Company; Egypt) at a rate of 3% of biomass, which provided twice/day of equal rations at 09:00 am and 3:00 pm to adapt the artificial diet and conditions of the trial.

After the accommodation period, randomly designed 3×1 factorial treatments were applied. The fish were randomly divided into 3 groups (treatments) of 300 fish / each group allotted into three replicates of 100 fish / each replicate. Fish were distributed into the experimental fiberglass tanks (2 m^3 in size for each) contained 2000 L of water, (100 fish/ fiberglass tanks) and were equipped with effective aeration system. Treatments are based on different feeding ratio of 0.5, 1.0, and 1.5% of fish body weight under zero water exchange; referring to T1, T2 and T3, respectively.

The experimental diets was admitted to fish twice daily at (09:00 am and 3:00 pm) for 90 days. Fish were weighed at fortnightly intervals along the experimental period and the feed amounts were adjusted by the change in live body weight. Aeration was continuously provided using an air blower (model 2BH7-520-0AH-8) made in Germany.

Assessment of water quality parameters

Water temperature and dissolved oxygen were recorded daily at one o'clock utilizing thermometer and dissolved oxygen meter (HI 9146-HANNA interment, USA). The pH values were recorded twice a week (Orion pH meter, Abilene, Texas, USA). Ammonia, nitrite, and nitrate were measured bi-weekly according to APHA (1998).

Biofloc precipitation

Subsequent to setting the fish in the tanks, providing the feed and calculating the amount of remaining ammonia, the molasses were added as a source of carbon to control the proportion among carbon and nitrogen. The amount of molasses required was determined according to De Schryver *et al.*, 2008. Using the Imhoff cone, the volume of floc on the bottom of the cone was estimated after 15 minutes of sedimentation three times weekly (Avnimelech, 2009). Suspended material was precipitated twice when floc volume reached to 20 ml L^{-1}. Naylon net 55 µ mesh size, 25 cm diameter and 80 cm length was utilized for filtering the suspended material. Thin layers of collected biofloc were exposed directly to the sunlight to diminish the moisture.

Growth indices

Random fish samples representing the whole tank (around half of the tank) were totally weighed (50 fish/each replicate) using an electronic balance.

Final body weight (FBW), weight gain (WG), relative growth rate (RGR), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated using the following equations:

WG = Final body weight (g) - Initial body weight (g)(Annet, 1985)

RGR = 100 ×(Final body weight (g) - Initial body weight (g))/ Initial body weight (g)

Specific growth rate (SGR % / day) = SGR= 100 (Ln FBW-Ln IBW)/ T

(Pouomonge & Mbonglang, 1993)

FCR = feed intake (g) /weight gain (g) (De Silva & Anderson, 1995)

PER = weight gain (g) /protein intake (g)(De Silva & Anderson, 1995).

Chemical composition of fish and biofloc

Dry matter, crude protein, crude lipid and ash contents of the fish (five fish/each tank), the experimental diets and bifloc were all performed according to AOAC (1990). Fish samples were dried in an oven at 80 ºC till steady weight than were grounded and stored at -20ºC for subsequent analysis; while, precipitate flocs were solar dried. Ash determined by incineration at 550 ºC for 4-6 h (Azim & Little, 2008). Crude protein was estimated by the micro-Kjeldahl method, %N× 6.25 (utilizing

Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat were additionally estimated by Soxhlet extraction with diethyl ether (40 - 60 ºC).

Blood sampling and serum separation

At the end of the experiment, all fish were anesthetized using 150 mg/l MS222 (Argent Laboratories, Redmond, Washington). Blood samples were gathered from the caudal blood vessels (v. caudalis) from 12 randomly sampled fish from each group (4 fish/each replicate) using a sterile syringe (Urbinate & Carneiro, 2006). Each sample was divided into two portions; the first portion was transferred into a 2-mL sterile test tube with anticoagulant (10% ethylene diamine tetra acetate-EDTA) for haematological assay and the second portion was kept in a 2-mL plain Eppendorff tube for serum separation. Blood was left to clot at 4°C for 6 min. After that, tubes were centrifuged at 704 g/ 10 min using an Eppendorff centrifuge for serum separation. The serum was collected in Eppendorff tubes and stored at -40 °C until analyses.

Haematological analysis

Red blood cells (RBCs) and WBCs were counted immediately with a hemocytometer after dilution with Natt& Herrick's solution (Houston, 1990). For hematocrit (Hct) determination capillary tubes were filled with blood and spun in a hematocrit centrifuge at 12,000 g for 5 min and hematocrit values were read as percentage (Karimi *et al.,* 2013). For haemoglobin assay Drabkin's solution was added to blood and then solution was centrifuged (3500 g for 6 min) to remove interferents, afterwards blood haemoglobin concentration was determined with a spectrophotometer (Model RA 1000, Technicon Corporation, USA) at 540 nm using the method of Blaxhall & Daisley (1973).

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated (Houston 1990) according to the following formulas:

MCV (fl) = 10 × (PCV per RBC)

MCH (pg) = 10 × (Hb per RBC)

MCHV (%) = 100 × (Hb per PCV)

Biochemical analysis

Serum total proteins (biodiagnostic, Egypt cat No TP20 20) was estimated colorimetricaly at wave length 550) according to Doymas *et al.,* (1981). Serum albumin (Diamond, Egypt) was estimated colorimetricly at wave length 550 nm according to Dumas & Biggs (1972). Activities of aspartate amninotransferase (AST) (biodiagnostic, Egypt cat No AS 1061 (45)) and alanine aminotransferase (ALT)(biodiagnostic, Egypt cat No AL 1031(45)) were determined calorimetrically at the wave length 540 nm, according to Reitman & Frankel (1957). Glucose level (mg/100 ml) was determined using glucose enzymatic PAP kits obtained from Bio-Merieux (France) (Trinder, 1969).Serum creatinine (biodiagnostic, Egypt cat No Cr 1251) was colorimetrically determined according to Heinegard & Tiderstrom (1973). Cholesterol (Cholesterol colorimetric assay kit CHOD-PAP method Elabscience, USA), triglyceride (T.G colorimetric assay kit GPO-PAP method Elabscience, USA) and uric acid (colorimetric assay kit uricase-POD, Spinreact, Spain at wave length 520)were determined colorimetrically according to the manufacturer's instructions using the commercial kits purchased from the Laboratory Biodiagnostics Company (Cairo, Egypt).

Intestinal Morphometry

Five fish were randomly selected from each treatment. After deep anaesthesia using 40% ethyl alcohol, the belly was dissected and specimens from anterior (hepatic loop) of the intestine were sampled. The tissue samples were fixed in Bouin's solution for 18-24 hours. After fixation, the samples were dehydrated by using ascending concentrations of ethyl alcohol (70% to absolute alcohol) then cleared in xylene and prepared for histological investigations. Sections of 4-5 µm thickness were stained with hematoxylin and eosin for morphometric analysis according to Bancroft & Gamble (2007). The length, width of intestinal villi and crypt depth in addition to goblet cells count was measured by using image analysis software (NIH, Bethesda, MD). A total of six random villi and villus-associated crypts from 5 intestinal cross-sections were selected from each and the average was calculated (±SE)

Statistical analysis

All data are presented as means± standard error (SE). Growth, hematology, blood chemistry and hormones data were analyzed using one-way ANOVA, followed by Duncan's multiple range tests (Duncan, 1955) which was used to compare differences among individual means, with statistical software SAS ANOVA procedure (statistical analysis system, 2006). A probability of 0.05 was utilized to account for the statistical difference between the means. Before the

analysis, percentage data were normalized by arc-sine-transformation.

RESULTS

Water quality parameters

Water quality parameters; pH, temperature, dissolved oxygen, ammonia, nitrite and nitrate was estimated and summarized in table 1. The 1.5% feeding rate group showed the highest values in temperature, ammonia and nitrate values. However, the 1.0% feeding rate group showed the highest values in dissolved oxygen and nitrite values. On the other side, the 0.5% feeding rate group showed the highest values in pH value.

The chemical composition of biofloc

As demonstrated in table2,the highest values of protein of biofloc were recorded in treatment with a feeding rate of 1.5%. Feeding rate 0.5 % treatment has the highest carbohydrates and ash of biofloc content. In general, fat content of biofloc was low in different feeding rate groups except for 1.0 % feeding rate.

Table 1: Physicochemical parameters of rearing water of biofloc technique at different feeding rates

	pH	Temperature	O_2	NH_4	NO_2	NO_3
0.5	7.66±0.21	26.16±1.69	6.08±0.65	0.344±0.14	0.264±0.15	0.280±0.20
1.0	7.58±0.21	26.15±1.70	6.10±0.88	0.331±0.16	0.281±0.17	0.295±0.17
1.5	7.57±0.22	26.21±1.68	5.93±0.38	0.396±0.15	0.267±0.19	0.437±0.31

Table 2: Chemical composition of biofloc at different feeding rates on dry matter base

Feeding levels (% of biomass)	Chemical composition (%)			
	Crude lipid	Crud protein	Ash	Carbohydrate
0.5	3.03±0.03^{b}	10.87±0.32^{b}	13.70±0.87^{a}	72.38±0.80^{a}
1.0	10.20±0.12^{a}	12.87±1.59^{ab}	6.10±0.23^{b}	70.83±1.62^{ab}
1.5	3.90±0.52^{b}	16.03±0.26^{a}	12.00±0.40^{a}	68.06±0.68^{b}

* Data shown are Means (±SD) in each row; different superscript letters indicate significant difference ($P \leq 0.05$)

Floc volume

The impact of different feeding rate treatments on floc volume was shown in fig.1. Floc volume was increased with the increase of the feeding rate of tilapia. The highest floc volume was recorded in1.5 % feeding rate treatment while, the lowest floc volume was recorded in 0.5% feeding rate treatment.

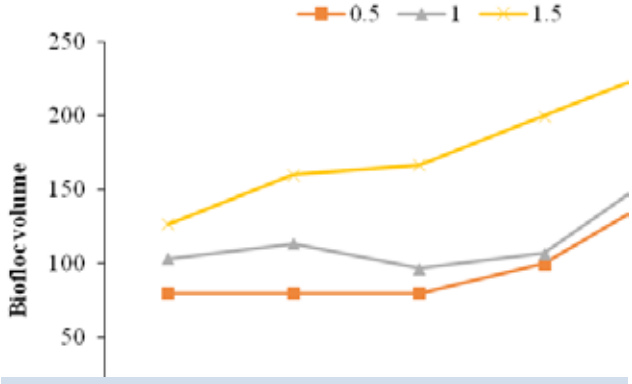

Fig. 1: Biofloc volume in different treatments throughout the experimental period

Tilapia performance

As shown in table 3, under biofloc condition, fish fed at feeding rate 1.5% of fish body weight recorded the highest significant values of growth performance parameters (FW, TWG, ADG, RGR, and SGR) and FI. However, fish fed at feeding rate of 0.5% under biofloc condition showed the best significant food conversion rate values ($P \leq 0.05$). However, no significant differences ($P \geq 0.05$) were recorded to survival rate among different treatments.

Chemical composition of tilapia fish

Proximate chemical analysis of the whole fish body is well demonstrated in table 4. Highest significant ($P < 0.05$) dry matter, fat, and energy contents were recorded in fish group fed at 1.5% feeding rate under biofloc condition. Whereas, the highest significant ($P < 0.05$) crude protein content in the fish body were recorded in 1.0% feeding rate fish group.

Hematological parameters:

Results of haematological analysis are summarized in table 5. The highest feeding rate (1.5%) fish group reflected the highest RBCs and WBCs counts, Hb concentrations, MCH and HCT %; however, 1.0 % feeding rate fish group reported the highest MCHC (%).

Blood serum biochemical parameters:

Blood serum biochemical parameters were summarized in table 6. The 1.5% feeding rate group showed the highest values in TCH, GLU and AST values; whereas, 1.0% feeding rate group showed the highest values in total protein, albumin and uric acid values. On the other side, 0.5% feeding rate group recorded the highest values in creatinine and ALT values.

Table 3: Growth performance and feed efficiency of Nile tilapia under biofloc technique at different feeding rates throughout the experimental period

Parameters	Feeding levels (% of biomass)		
	0.5	1.0	1.5
Final weight (FW, g/fish)	130.2±3.19^{b}	133.3±0.88^{b}	157.7±2.33^{a}
Weight gain (TWG, g/fish)	75.50±3.18^{b}	80.33±1.20^{b}	104.0±1.53^{a}
Average daily gain (ADG, g/fish/day)	0.84±0.04^{b}	0.89±0.01^{b}	1.16±0.02^{a}
Relative growth rate (RGR, %)	138.1±5.83^{c}	151.6±3.63^{b}	193.8±1.57^{a}
Specific growth rate (SGR, %/day)	0.96±0.03^{c}	1.02±0.02^{b}	1.20±0.01^{a}
Condition factor	2.68±0.09^{c}	2.96±0.03^{b}	3.26±0.11^{a}
Intestine length	157.5±4.33^{a}	128.5±0.87^{b}	108.0±3.46^{c}
Villi length	342.2±10.92^{a}	217.5±13.19^{b}	132.3±7.9^{3c}
Villi width	54.88±4.18^{a}	51.31±2.52^{a}	38.77±2.01^{b}
Crypt depth	17.13±1.46^{b}	25.05±3.45^{a}	14.19±0.95^{b}
Goblet cells /mm2	35.80±0.57^{a}	25.90±0.52^{b}	22.70±0.51^{c}
Intestine length:bodylength	7.74±0.43^{a}	6.57±0.23^{b}	5.11±0.04^{c}
HIS	2.32±0.17^{b}	3.50±0.65^{ab}	4.45±0.65^{a}
Feed intake (g/fish)	36.28±0.45^{d}	73.64±0.90^{c}	128.8±1.36^{a}
Feed conversion ratio	0.48±0.05^{d}	0.92±0.04^{c}	1.24±0.01^{b}
Feed efficiency (%)	207.9±5.14^{a}	109.0±4.42^{b}	80.74±1.17^{c}
Survival (%)	97.67±0.33	96.00±0.58	94.67±0.33

Data shown are Means (±SD) in each row; different superscript letters indicate significant difference (P ≤0.05).

Table 4: Chemical composition of Nile tilapia under biofloc technique at different feeding rates throughout the experimental period

Feeding rate %	Chemical composition of whole-body fish (% dry matter basis)				
	DM¶	Ash	Protein	Lipid	EC (kcal/100g)
0.5	27.72±0.28^{d}	21.59 ±0.65^{a}	64.90 ±0.33^{b}	13.51 ±0.56^{c}	493.5 ±5.59^{c}
1.0	28.90 ±0.17^{c}	17.22 ±0.35^{b}	68.65 ±0.46^{a}	14.13 ±0.80^{c}	520.6 ±5.00^{b}
1.5	31.30 ±0.21^{a}	15.57 ±0.34^{c}	64.40 ±0.67^{b}	20.02 ±0.41^{a}	552.2 ±1.61^{a}

Data shown are Means (±SD) in each column; different superscript letters indicate significant difference (P ≤0.05).
¶**DM** = dry matter

Table 5: Hematological parameters of Nile tilapia under biofloc technique at different feeding rates throughout the experimental period

Parameters	Feeding rates %		
	0.5	1.0	1.5
RBCs (×10^{6} mm^{-3})	1.57 ±0.18^{b}	1.53±0.18^{b}	1.80±0.06^{a}
Hb (g/dL)	5.55 ±0.26^{b}	5.15±0.20^{b}	7.43±0.41^{a}
Hct (%)	22.93±1.41^{b}	17.13±0.32^{c}	34.50±2.02^{a}
MCV (μ^{3})	146.7±5.46^{b}	143.8±0.72^{b}	152.0±2.40^{ab}
MCH (pg)	38.33±1.73^{b}	41.30±0.29^{ab}	43.37±1.88^{a}
MCHC (%)	25.50±0.29^{ab}	30.00±0.58^{a}	24.33±1.86^{b}
WBCs (×10^{3} mm^{-3})	24.30±1.15^{b}	23.30±1.41^{b}	36.67±1.66^{a}

Data shown are Means (±SD) in each row; different superscript letters indicate significant difference (P ≤0.05).
RBCs: Red blood cells
Hb: Hemoglobin
PCT: Hematocrit
MCV: Mean corpuscular volume
MCH: Mean corpuscular hemoglobin
MCHC: Mean corpuscular hemoglobin concentration
WBCs: White blood cells

Table 6: Serum biochemical parameters of Nile tilapia under biofloc technique at different feeding rates throughout the experimental period

Parameters	Feeding rates %		
	0.5	1.0	1.5
TCH	38.50±0.29^{c}	41.00±1.73^{c}	72.50±3.18^{a}
TG / Mg/dl	257.5±19.34	231.0±10.97	259.0±18.56
Total protein, g/dl	2.60±0.12^{ab}	3.43±0.48^{a}	2.77±0.03^{ab}
Albumin, g/l	0.80±0.06	0.90±0.06	0.83±0.03
GLU/ Mg/dl	70.00±4.04^{d}	86.00±1.15^{c}	148.0±4.04^{a}
Creatinine, mg/dl	0.33±0.07^{b}	0.30±0.00^{b}	0.30±0.06^{b}
ALT, u/l	16.00±1.53	13.67±2.19	13.00±0.00
AST, u/l	125.0±2.89	133.0±0.58	150.0±15.01
Uric acid, mg/dl	1.87±0.26^{b}	5.27±0.09^{a}	1.20±0.00^{c}

Data shown are Means (±SD) in each row; different superscript letters indicate significant difference (P ≤0.05).
TCH: Total cholesterol
TG: Triglyceride
GLU: Glucose
AST: aspartate aminotransferase
ALT: aspartate aminotransferase

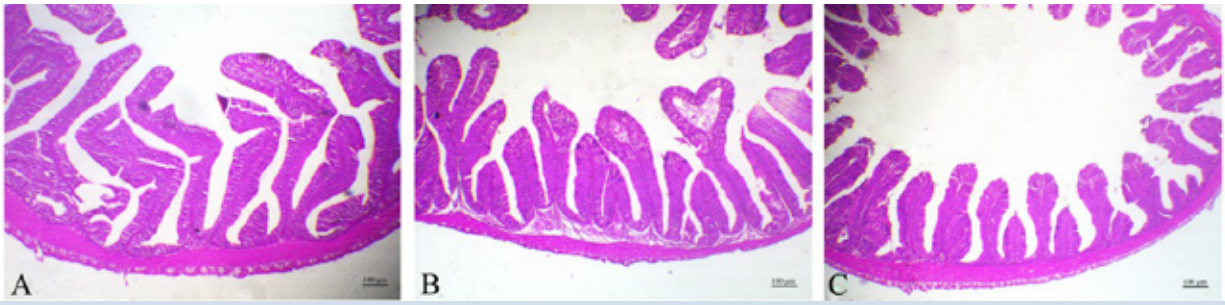

Fig. 2: photomicrograph of anterior part of small intestine of *Oreochromis niloticus* showing:A: intestinal villi of 0.5% feeding rate treated group, B: intestinal villi of 1.0% feeding rate treated group and C: intestinal villi of 1.5% feeding rate treated group. H&E, Bar=100µm

Morphometric analysis

The morphometric analysis of anterior part of intestine revealed significant increase in the intestinal villi length and width besides increase in goblet cells count in 0.5% group compared with the other groups. On contrast to the previous findings, the crypt depth of 0.5% group was significantly decreased compared with 1% group (table 1).

The results of the morphological analysis are summarized in Figure 2 and table 3. The total length of the intestine was significantly increased with decreased feeding rate level; the highest intestinal length was reported in 0.5% feeding rate fish group and the lowest intestinal length was in 1.5 % feeding rate fish group. In addition, the height of the intestinal villi in the anterior part of the intestine was significantly increased with decreased feeding rate. The treatment of 1.5% feeding rate showed the least intestinal villi length. However, the treatment of 1% feeding rate showed moderate increase in the villi length. On the other side, there was marked increase in the intestinal villi length in the treatment of 0.5% feeding rate.

DISCUSSION

Biofloc is clustered aggregations of microbial communities like phytoplankton, bacteria, and particulate organic matter. Biofloc Technology (BFT) is a new potentially revolutionary technology that is particularly productive for tilapia culture (Prajith, 2011). Under favorable economies, tilapia production using biofloc technology represents an attractive investment proposition. BFT is an environmental-friendly technique in aquaculture that controls both water quality and pathogens besides, providing microbial protein feed for the aquatic farm; so, reducing feed costs and feed conversion ratio (De Schryver *et al.,* 2008 and Abdelhamid, 2009a& b, 2019a, b & c and Kourie, 2018a). BFT leads to ammonia removal from water. The bio-flocs technology has received considerable attention because it results in low cost, high production yields, feed protein recycling, water quality, and bacterial infection control (Avnimelech, 2006; Crab

et al., 2007; Little *et al.,* 2008).

Average measured values of water parameters in the current experiment were within the optimal range suitable for tilapia culture (Boyd & Tucker, 1998, El-Sayed, 2006; & Delong *et al*., 2009). Moreover, differences in ammonia, nitrite and nitrate concentrations seem to be typical features of biofloc systems (Azim & Little, 2008 & Luo *et al.,* 2014). Nitrite and nitrate are created through nitrification in the BFT system; nitrate may experience inadequate denitrification to deliver nitrite and dissimilatory nitrate reduction to ammonia may occur (Azim *et al.,* 2008; & Wu *et al.,* 2012). Nitrite accumulation might be due to free ammonia inhibition during nitrification and denitrification (Shi *et al.,* 2011).

It was suggested that 25-30% crude protein in diets is appropriate for tilapia growth (Chou & Shiau, 1996, Jauncey, 2000). The crude lipid content was sufficient according to thedietary lipid requirement of 5-12% for tilapia (Lim *et al.,* 2009). Floc volume was increased with the increase of the feeding rate of tilapia and settled to the bottom of the tanks. This means that uptake of biofloc by the fish was insufficient to prevent its build-up and the need to remove it regularly from the system was clear. Therefore, characterization of floc and improved approaches to its removal are a pre-requisite for effective management of BFT system. Protein is increased positively with increased feeding rate, while lipid and carbohydrates negatively decreased. The results coincided with previous reports (Azim & Little, 2008; Azim*et al.,* 2008; Crab *et al.,* 2010) but disagreed with Emerenciano *et al.* (2012).

Growth performance and feed utilization efficiency were significantly improved by different feeding rates, implying a potential role of Biofloc system in mitigating stress factors and promoting fish welfare. Biofloc system substantially contributes to tilapia growth and production where wastes turned over into natural food particles such as suspended bacteria (Avnimelech, 2007; Azim & Little, 2008; Beveridge & Baird, 2000; Little *et al.,* 2008; Yuan *et al.,* 2010). The best significant food conversion rate was observed in 0.5% feeding rate where decreased amount of feed was necessary for producing one unit of fish leading consequently to production cost reduction. The results are similar to Avnimelech *et al.,* 1994 and Luo *et al.,* 2014.

In the current study, there is a negative relationship between crude protein and fat contents in the fish body. According to Xu & Pan (2012), biofloc can influence the whole body composition of cultured shrimp with increased lipid content in the whole shrimp body. Izquierdo *et al.* (2006) also found that the whole body lipid content of shrimp growing in mesocosms systems with biofloc exhibited an increasing trend. This might be due to the essential amino acids, fatty acids (PUFA and HUFA) and other nutritional elements provided by the biofloc in the BFT treatment (Izquierdo *et al.,* 2006; Ju *et al.,* 2008b).

Hematological parameters reflect the health status of fish (Harikrishnan *et al.,* 2011). The overall improvement in haematological characteristics reported in 1.5% feeding rate group, used in the current study, indicate that the biofloc system had positive effect on the physical conditions of the tilapia. The results are similar to many previous records (Öz *et al.*, 2018 &2020 a, b; Xu & Pan (2013) and disagree with some reports (Azim & Little, 2008; Souza *et al.,* 2014 and Xu & Pan, 2014). This difference might be attributed to different experimental conditions and culture species

The results of the fish serum biochemical analysis in this study reflected a significant increase in serum total proteins and Albumin in 0.1% feeding rate group. The results indicate the contribution of the biofloc system in improving the immune response of Nile tilapia (Ballester et al., 2010; Long et al., 2015). Biofloc is not only a source of additional nutrition, such as proteins, lipids, minerals and vitamins (Izquierdo *et al.,* 2006; Moss *et al.,* 2006; Ju *et al.,* 2008b; & Xu *et al.,* 2012a), but also provides abundant natural microbes and bioactive compounds such as carotenoids and fat-soluble vitamins (Ju *et al.,* 2008a), and other immune-stimulatory compounds (Crab *et al.,* 2012) that may stimulate the immune response of cultured fish.

The total intestinal length and the heights of the intestinal villi were significantly increased with decreased feeding rate. The role of intestine in nutrient digestion and absorption is well-known in herbivorous fish as tilapia (Grosell *et al.,* 2010). Moreover, the intestinal villi height and the muscular layer thickness are good indicators of a healthy intestine (Khojasteh, 2012). Therefore, the increased intestinal absorptive area with a subsequent increase in nutrient absorption and retention highlight the observed improvement in growth performance, immune response and stress resistance in Nile tilapia in the current study. German and Horn (2006) confirmed the present results; they

found that intestine lengths of herbivores were longer than those of omnivores, and these were longer than those of carnivores.

CONCLUSION

Based on the current results, it could be concluded that using biofloc technique in tilapia rearing had beneficial effects on maintaining good water quality, improving the feed utilization and growth performance, increasing fish body protein content, physical and immune response promotion as well as increasing the absorptive capacity of the intestine. Subsequently, biofloc offers not only excellent benefits for fish farmers (economically) and consumers (safe product) but also, for the sustainability of the environment (treating wastes which turned over into natural feed stuff for fish saving the artificial food cost). Further research efforts should be made to improve the biofloc uptake by the fish to prevent its accumulation.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

REFERENCES

Abdelhamid AM (2009a) Fundamentals of Fish Production and Aquaculture. The New Universal Office, Alexandria, 654p., 977-438-052-5, Deposition No. 24400/2008.

Abdelhamid AM (2009b) New Trends in Aquaculture. The New Universal Office, Alexandria, 600p., 977-438-053-3, Deposition No. 24409/2008.

Abdelhamid AM (2019a) New Methods of Aquaculture.718p., Deposition No. 25438/2018.

Abdelhamid AM (2019b) Fish Nutrition.469p., Deposition No. 25442/2018.

Abdelhamid AM (2019c) Husbandry, Breeding, Physiology, and Fish Diseases.Deposition No. 25440/2018.

Annet CS (1985) A model to facilitated optimal aquaculture production by quantitatively relating fish growth to feed and other environmental resources. Ph.D., Thesis, Michigan. State University, U.S.A.

AOAC (1990) Official methods of Analysis. *In*: Association of Official Analysis of Chemists, Washington D.C. 15th Ed.

APHA (1998) Standard Methods for the Examination of Water and Wastewater. AmericanPublic Health Association, Washington D C.

Avnimelech Y, Kochva M and Diab S (1994) Development of controlled intensive aquaculture systems with a limited water exchange and adjusted C to N ratio. Isr J Aquac.-Bamid46:119-131.

Avnimelech Y (2006) Bio-filters: the need for a new comprehensive approach. AquacEng 34:172-178.

Avnimelech Y (2007) Feeding with microbial flocs by tilapia in minimal discharge-flocs technology ponds. Aquac 264: 140-147.

Avnimelech Y (2009) Biofloc Technology - A Practical Guide Book. The World Aquaculture Society, Baton Rouge, Louisiana, United States.182 pp.

Azim ME and Little DC (2008) The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquac 283: 29-35.

Azim ME, Little DC and Bron JE (2008) Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. Bio Tech.99:3590-3599.

Ballester E, Abreu P, Cavalli R, Emerenciano M, De Abreu L and Wasielesky Jr W (2010) Effect of practical diets with different protein levels on the performance of Farfantepenaeus paulensis juveniles nursed in a zero exchange suspended microbial flocs intensive system. Aquac Nut 16: 163-172.

Bancroft JD and Gamble M (2007) Theory and practice of Histological Techniques. 5thed; Churchill Livingstone, London, UK. P 125-138.

Beveridge M and Baird D (2000) Diet, feeding and digestive physiology. Tilapias: Biology andExploitation.Springer 59-87.

Blaxhall PC and Daisley KW (1973) Routine hematological methods for use with fish blood. J Fish Biol 5(6):771-781.

Boyd CE and Tucker CS (1998) Pond aquaculture water quality management. Springer Science Business Media. New York, USA, pp. 87-152.

Chou BS and Shiau SY (1996) Optimal dietary lipid level for growth of juvenile hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*. Aquac143:185-195.

Crab R, Avnimelech Y,Defoirdt T, Bossier P and Verstraete W (2007)Nitrogen removal techniques in aquaculture for sustainable production. Aquac 270: 1-14.

Crab R,Kochva M,Verstraete W and Avnimelech Y (2009) Bio-flocs technology application in over-wintering of tilapia. Aquac Eng 40:105-112.

Crab R, Chielens B,Wille M, Bossier P and Verstraete W (2010)The effect of different carbonsources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae.Aquac Eng 41:559-567.

Crab R, Defoirdt T, Bossier P and Verstraete W (2012)Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquac 356-357: 351-356.

De Schryver P, Crab R,Defoirdt T, Boon N and Verstraete W (2008) The basics of bio-flocs technology: the added value for aquaculture. Aquac 277(3-4):125-137.

De-Silva SS and Anderson TA (1995) Fish Nutrition in Aquaculture, Ed., Champman and Hall, 2-6 Bouday Raw, London SEI8 FIN, UK.

DeLong DP, Losordo T and Rakocy J (2009) Tank culture of tilapia. Southern Regional Aquaculture Center. Number 87-CRSR-2-3218.

Doymas BT,Bayso DD, Carter RJ& Schaffer R (1981) Determination of total serum protein. ClinChem 27:1642-1643.

Dumas BT and Biggs HG (1972) Standard Methods of Clinical Chemistry. ed., Academic Press, New York.

Duncan DB (1955) Multiple ranges and multiple F-tests. Biomet 11: 1-42.

El-Sayed AEM (2006) Tilapia culture. CAB International. Oxfordshire, UK, pp. 33-46.

Emerenciano M, Ballester ELC, Cavalli RO and Wasielesky W (2012) Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfante penaeus brasiliensis* (Latreille, 1817). AquacEng43:447-457.

FAO (Food and Agriculture Organization) (2012) The state of world fisheries and aquaculture, Food and Agriculture Organization FAO, Rome, Pp 209.

FAO (Food and Agriculture Organization) (2016) GLOBE FISH-Analysis and information on world fish trade: Tilapia - September 2015.

Fitzsimmons K (2005) Tilapia culture. In: Kelly A.M. and Silverstein J. (eds). Aquaculture in the 21st Century. American Fisheries Society, Symposium 46, Bethesda, Maryland. pp. 563-590.

Fitzsimmons K (2016) Supply and demand in global tilapia markets, Aquaculture, Las Vegas, Nevada, 23-26 Feb 2016.

German DP and Horn MH (2006) Gut length and mass in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. MarBiol1 48(5):1123-1134.

Grosell M, Farrell AP and Colin JB (2010) The multifunctional gut of fish. Oxford, UK: Academic Press.

Gutierrez-Wing MT and Malone RF (2006) Biological filters in aquaculture: trends and research directions for freshwater and marine appli-

cations. Aquac Eng 34 (3):163-171.
Hargreaves and John A (2013) *Biofloc production systems for aquaculture*. Vol. 4503. Stoneville, MS: Southern Regional Aquaculture Center.
Harikrishnan R, Kim MC, Kim JS, Balasundaram C and Heo MS (2011) Protective effect of herbal and probiotics enriched diet on haematological and immunity status of *Oplegnathus fasciatus* (Temminck& Schlegel) against *Edwardsiella tarda*. Fish Shellfish Immun 30:886-893.
Heinegard D and Tiderstrom G (1973) Determination of serum creatinine by a direct colorimetric method. Acta Int J Clin Chem 43:305-310.
Houston A (1990) Blood and circulation, Methods for fish biology. Pp: 273-334.
Izquierdo M, Forster I, Divakaran S, Conquest L and Decamp O (2006) Effect of green and clearwater and lipid source on survival, growth and biochemical composition of pacific white shrimp *Litopenaeus vannamei*. Aquac Nut 12:192-202.
Jackson A(2007) Challenges and opportunities for the fishmeal and fish oil industry. Feed Technology Updates: Solutions for the Global Feed Industry Vol.2, Issue 1. Honolulu, Hawaii, US.
Jauncey K (2000) Nutritional requirements. Tilapias: Biology and Exploitation.Springer 327-375.
Ju ZY, Forster I, Conquest L and Dominy W (2008a) Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. Aquac Nut 14:533-543.
Ju ZY, Forster I, Conquest L, Dominy W, Kuo WC and Horgen FD (2008b) Determination ofmicrobial community structures of shrimp floc cultures by biomarkers and analysis of floc amino-acid profiles. Aquac Res 39:118-133.
Karimi S,Kochinian P and Salati AP (2013) The effect of sexuality on some haematological parameters of the yellowfin seabream, *Acanthopagrus latus* in Persian Gulf. Iran J Vet Res 14(1):65-68.
Khojasteh SMB (2012) The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. Int J AquatSci 3:71-88.
Kourie R (2018a) Large-scale biofloc tank culture of tilapia in Malawi - a technical success story. Engormix/ Aquaculture/ Technical articles (This article was originally published in World Aquaculture).
Lara G, Honda M, Poersch L and Wasielesky W (2017) The use of biofilm and different feeding rates in biofloc culture system: the effects in shrimp growth Parameters. AquacInt. 25(5):1959-1970.
Lim C,Yildirim-Aksoy M, Li MH, Welker TL and Klesius PH(2009) Influence of dietary levels of lipid and vitamin E on growth and resistance of Nile tilapia to *Streptococcus iniae* challenge.Aquac 298:76-82.
Little DC, Murray FJ, Azim E,Leschen W, Boyd K, Watterson A and Young JA (2008) Options for producing a warm-water fish in the UK: limits to "Green Growth"? Trends Food Sci Tech 19:255-264.
Long L, Yang J, Li Y, Guan C and Wu F (2015) Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquac 448: 135-141.
Luo G, Gao Q, Wang Ch, LiuW, Sun D, Li L and Tan H (2014) Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia *(Oreochromis niloticus)* cultured in a recirculating aquaculture system and an indoor biofloc system.Aquac 422-423:1-7.
Matos J, Costa S, Rodrigues A, Pereira R and Pinto IS (2006) Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. Aquac 252 (1):31-42.
Moss SM, Forster IPdand Tacon AGJ (2006) Sparing effect of pond water on vitamins in shrimp diets. Aquac 258:388-395.
Öz M, Inanan B E and Dikel S (2018) Effect of boric acid in rainbow trout *(Oncorhynchus mykiss)* growth performance. J Appli Animal Res 46(1): 990-993.
Öz M, Karaşahın T, Aksoy N, Inanan B and Dikel S (2020a) Harmful effects of dietary supplementation of boron on blood parameters of Rainbow Trout *(Oncorhynchus mykiss)*. Hellenic Vet Med Soc 71(2): 2225-2232.
Öz M, Yavuz O and Bolukbas F (2020b) Histopathology changes in the rainbow trout *(Onchorhyncus mykiss)* consuming boric acid supplemented fishfodder. Journal of Trace Elements in Medicine and Biology, 126581.
Piedrahita RH (2003) Reducing the potential environmental impact of tank aquaculture effluentsthrough intensification and recirculation. Aquac.226:35-44.
Pouomonge V and Mbonglang M (1993) Effect of feeding rate on the growth of tilapia (*O. niloticus*) in earthen ponds. Isr J aquac 45:147-153.
Prajith KK (2011) Application of Biofloc Technology (BFT) in the nursery rearing and farming of giant freshwater prawn, *Macrobrachium rosenbergii* (deMan). Ph.D. Thesis under the Faculty of Marine Sciences. School of Industrial Fisheries, Cochin University of Science and Technology, Kochi - 682016.
Reitman S and Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalo-acetic and glutamic pyruvic transaminases. Amer J Clin Pathol 28:56-63.
Roselien C et al (2012) Biofloc technology in aquaculture: beneficial effects and future challenges. Aquac 356: 351-356.
SAS (2006) SAS/STAT Guide for personal computer. SAS Inst. Cary, N. C.
Sharma DA, Sharma K and Sangotra R(2015)Biofloc culture and its utilisation as feed in limited water exchange system for the culture of *labeorohita*. J IntAcad Res Multidis 3(2):185-193.
Shi YJ, Wang XH, Yu HB,Xie HJ,Teng SX, Sun XF, Tian BH and Wang SG (2011) Aerobic granulation for nitrogen removal via nitrite in a sequencing batch reactorand the emission of nitrous oxide. Biores Tech 102:2536-2541.
Souza DMD, Suita SM, Romano LA, Jr WW and Ballester ELC (2014) Use of molasses as acarbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a Biofloc technology system. AquacEng 45:270-277.
Subasinghe RP (2005) Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. Prev Vet Med 67(2-3):117-124.
Thong and Yong Poh (2014) Biofloc technology in shrimp farming: success and failure. Aquaculture Asia Pacific Magazine 10: 13-16.
Timmons MB and Ebeling JM (2007) Recirculating Aquaculture. NRAC Publ. No. 01- 007. Cayuga Aqua Ventures, Ithaca, NY, 975p.
Trinder P (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Bioch (6):24-27.
Urbinate EC and Carneiro PCF (2006) Sodium chloride added to transport water and physiological responses of Matrinxã *Brycon amazonicus*(Teleost: Caracidae). Act Amaz 36(4):569-572.
Wasielesky W, Atwood H, Stokes A and Browdy CL (2006) Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquac 258:396-403.
Wu W, Yang F and Yang L(2012) Biological denitrification with a novel biodegradable polymer as carbon source and biofilm carrier. BioresourTechnol 118:136-140.
Xu WJ and Pan LQ (2012) Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquac 356-357:147-152.
Xu WJ and Pan LQ (2013) Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input.Aquac 412-413:117-124.
Xu WJ and Pan LQ (2014) Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared inzero-water exchange biofloc-based culture tanks. Aquac 426-427:181-188.
Xu WJ, Pan LQ, Sun X and Huang J (2012a) Effects of bioflocs on water quality, and survival,growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquac Res 44:1093-1102.
Yuan D, Yi Y, Yakupitiyage A, Fitzimmons K and Diana JS (2010) Effects of addition of red tilapia (*Oreochromis sp.*) at different densities and sizes on production,water quality and nutrient recovery of intensive culture of white shrimp *(Litopenaeus vannamei)* in cement tanks. Aquac 298:226-238.