Study on nursery growth performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) under different feeding levels in zero water exchange system

Khanjani M.H. [1]*; Sajjadi M.M.[2], Alizadeh M.[3]; Sourinejad I.[1]

Received: July 2015 Accepted: September 2015

Abstract

Effect of different feeding levels on water quality, growth performance, survival rate and body composition of Pacific white shrimp *Litopenaeus vannamei* post larvae were studied in zero water exchange system. Shrimp post larvae with mean weight of 74.46± 6.17 mg were fed for 32 days in 300L fiberglass tanks containing 130L water at density of 1 post larvae L^{-1}. There were five treatments including control and four biofloc treatments with different feeding levels of 15%, 15%, 12%, 9%, 0% of body weight per day, respectively. The results showed that there were no significant differences in water parameters such as dissolved oxygen and pH between different treatments (p>0.05). There were significant differences in water ammonia level between different treatments (p<0.05). The maximum (0.39 mg/L) and minimum (0.12 mg/L) levels of ammonia were observed in control and biofloc treatment with minimum feeding level (9%BW/day), respectively. The highest body weight gain (1.55g), growth rate (48.50 mg per day), specific growth rate (9.64%/day), biomass gain (182.1g) and body length increase (33.62mm) were observed in biofloc treatment with maximum feeding level. The highest feed conversion ratio and the lowest feed efficiency were obtained in control (p<0.05). The proximate body composition analysis revealed an increase in lipid, protein and ash in biofloc treatments. Results showed that using biofloc technology can decrease water exchange amount and improve feed utilization in nursery culture of Pacific white shrimp. Moreover, presence of biofloc improved the water quality which led to the enhancement in growth performance in nursery stage of shrimp.

Keywords: Biofloc technology, Zero-water exchange system, Water quality, Growth performance, Body composition, Nursery, Pacific white shrimp.

1- Fisheries Department, Faculty of Marine and Atmospheric Sciences and Technologies, Hormozgan University, Iran
2- Fisheries Department, Faculty of Natural Resources, Guilan University, Iran.
3- Iranian Fisheries Research Organization - Inland Salt Water Fishes Research Center, Bafgh-Yazd-Iran
* Corresponding author's Email: : m.h.khanjani@gmail.com

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

Introduction

As human population is increasing, food production industries such as aquaculture need to expand as well as shrimp farming. Several environmental impacts arising from the expansion of intensive shrimp culture such as deteriorated water quality, pathogen spread and the outbreak of diseases are inevitable (Burford and Williams, 2001; Jackson *et al*., 2003; Zhang *et al*., 2012; Liu *et al*., 2014). In shrimp culture systems, an innovative technology which is called as biofloc technology (zero-water exchange system) has been identified for solving the aforesaid impacts in order to achieve the objectives of sustainable aquaculture development by using zero-water exchange (Crab *et al*., 2007; De Schryver *et al*., 2008; Avnimelech, 2008; 2009).

This technology is basically dependent on manipulation and regulation of carbon/ nitrogen (C/N) ratio for developing microbial community, bioflocs (Crab *et al*., 2007; Asaduzzaman *et al*., 2008; De Schryver *et al*., 2008). Adding carbohydrates into zero-water exchange systems and regulating the C/N ratio (10 to 20) (Asaduzzaman *et al*., 2008) lead to microbial harvest from excreted inorganic nitrogen and produce microbial proteins that are then actively consumed by the shrimp (Avnimelech, 2009). These processes would results in the enhancement of water quality, heterotrophic bacterial activities, zooplankton growth and better growth performance for Pacific white shrimp (Gao *et al*., 2012; Xu and Pan, 2012; Xu *et al*., 2012, 2013). The biofloc is a mass rich containing diatoms, bacteria, fungi, protozoa, microalgae, fecal pellets, remains of dead cells, small organic and inorganic particles and other suspended organisms (Hargreaves, 2006; Avnimelech, 2007; De Schryver *et al*., 2008; Valle *et al*., 2015). Biofloc technology, in addition, could provide food source with high value for the cultured shrimp which is available for 24 hours per day in situ as a supplemental food source (Avnimelech, 1999; Avnimelech, 2009; Crab *et al*., 2010). Bioflocs can improve growth performance and enhance feed digestion and utilization of the cultured shrimp in zero-water exchange tanks (Xu and Pan, 2012). They can be used as an important feed supplement in the shrimp diet while contributing as a digestible protein source especially at nursery stage (Otoshi *et al*., 2011; Wasielesky *et al*., 2013). Moreover, as previously reported, bioflocs can be applied as a feed ingredient in shrimp diet enhancing the growth rate of Pacific white shrimp (Kuhn *et al*., 2010).

Many researchers have reported that bioflocs produced within the culture systems can be consumed by the shrimp and fulfill a significant part of nutrition demand, and subsequent reduction in the requirement of formulated feed protein and feed cost (Burford *et al*., 2004; Hari *et al*., 2006; Wasielesky *et al*., 2006; Crab *et al*., 2010; Xu *et al*.,

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

2012). Feed and protein utilization is significantly higher in biofloc systems (Avnimelech, 2009). Bioflocs are effective potential food source for tilapia fish; feeding ratio of tilapia in biofloc ponds is 20% less than conventional ponds (Avnimelech, 2007). In this system, about 20-30% of protein assimilation by shrimp originates actively from biofloc harvesting (Burford *et al*., 2003, 2004). Biofloc could be suggested as a new alternative feed in marine shrimp industry which decreases feeding dependence on fish oil and meal (Megahed and Mohamed, 2014).

The Pacific white shrimp is the most widely farmed shrimp species throughout the world. Typical performance characteristics of this species, together with its tolerance against a wide range of salinities and disease, rapid growth, suitable survival and high-density culture make it be considered as a good candidate for intensive culture (Cuzon *et al*., 2004).

In recent years, biofloc technology has become a popular technology in the farming of Pacific white shrimp, with zero-water exchange (Tacon *et al*., 2002; Burford *et al*., 2004; Wasielesky *et al*., 2006, 2013) and successful operation in nursery phase (Samocha *et al*., 2007; Mishra *et al*., 2008; Wasielesky *et al*., 2013). Nursery phase is the transitional period between hatcheries and grow out systems (Mishra *et al*., 2008). Improvement of this phase helps the production of more shrimp during grow out stage while increasing harvest yields (Arnold *et al*., 2009; Souza *et al*., 2014). However, the advantageous effects of bioflocs on the shrimp performance in zero-water exchange system still are unknown.

In the present study the effects of different feeding levels on nursery culture function of Pacific white shrimp *L. vannamei* (Boone, 1931) in zero-water exchange tanks were investigated. Water quality, densities of total heterotrophic bacteria and body composition of shrimps in different treatments were also studied in the present research.

Materials and methods

Experimental design and system management

The experiment was conducted in Kolahi Shrimp Hatchery located at Hormozgan Province, Iran from August to September 2014. Initial mean body weight and body length of treated post larvae were (74.46± 6.17 mg) and (19.8± 0.88 mm), respectively.

The experiment was carried out in 300l indoor fiberglass tanks (0.38 m^2 in bottom area). All tanks were filled by sea water throughout a sand-filtered (130l) in 33ppt salinity. Density in each tank was 130 individuals (1 ind./L). Five treatments were set up as clear water (control), maximum feeding level, medium feeding level, minimum feeding level without water exchange (biofloc-based) and water exchange with floc feeding (Without pellet feeding). Daily feeding rates were 15%, 15%, 12%, 9%, 0% BW/day at the

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

beginning of experiment and then gradually decreased to 11%, 11%, 8.8%, 6.6% BW/day at the end of experiment, respectively (Table 1).

Shrimps were fed by commercial feed with 38% crude protein three times per day at 8 a.m., 14 p.m. and 20 p.m. Daily water exchange rate in control group was 35-50%.

Bioflocs were collected from three indoor bioflocs-based shrimp culture tanks (2000 lit. capacity) by filtration of water through a 20µm mesh size nylon bag and then inoculated into all bioflocs-based tanks with the same amount (0.5 mL/L biofloc volume) prior stocking the shrimps.

Molasses (59.48% dry matter, 75.40% carbohydrate) as source of carbohydrate was added to bioflocs-based tanks under zero-water exchange in order to promote the development of bioflocs during the experimental period. Adding molasses was performed once a day based on the calculation as described by Avnimelech (2009). C/N ratio as 15:1 was used in days of the experiment to provide assuming 50% carbon contents of the molasses added assimilated by microbial biomass. The amount of required organic carbon was determined based on the amount of shrimp feed added to the experiment tanks.

The pre-weighed molasses was completely mixed in a beaker with the relevant tank water and uniformly distributed over the tank's surface directly after feed application at 14:00 p.m. In the case of physicochemical parameters of the water used in the beginning of the experiment, the average water salinity, dissolved oxygen, pH and water temperature were 33±0.5ppt, 6±0.2 mg/L, 8.15±0.2 and 31.5±0.5°C, respectively. All tanks were aerated and continuously agitated using 3 air-stones connected to an air pump.

Table 1: Description of treatments based on different feeding levels

Treatments Description	Feeding rate (% body weight)					Water exchange
	W1	W2	W3	W4	4 days end	
Control (clear water)	15	14	13	12	11	35-50% (daily)
Maximum feeding level	15	14	13	12	11	No exchange
Mdium feeding level	12	11.2	10.4	9.6	8.8	No exchange
Minimum feeding level	9	8.4	7.8	7.2	6.6	No exchange
Without pellet feeding	0	only adding floc				35-50% (daily)

W= trail weeks.

Water parameters measurements

Measurements of water temperature (Digital Thermometer), pH (pH Lutron 208, pH meter) and dissolved oxygen (DO) (DO Lutron 510 Oxygen meter) were recorded twice daily (8 a.m. and 16 p.m.). Water salinity was measured daily at 9 a.m. Water transparency was recorded using a Secchi disk three times per week. Settled solids (SS) was determined on site using Imhoff cones (scaled conical hopper) every five days by recording the volume taken in through the bioflocs in 1000 mL of the

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

tank water after 20min sedimentation (Avnimelech and Kochba, 2009). In order to calculate total suspended solids (TSS), water sample was filtered under vacuum pressure through pre-dried and pre-weighed Whatman GF/C filter paper Number 42. The filter paper containing suspended materials was dried at 105°C in oven until constant weight and the dried sample was weighed to 0.01 mg (Azim and Little, 2008). Total Heterotrophic Bacteria (THB) count in the water was estimated according to standard procedures (APHA, 2005) and expressed as colony forming units (CFU). Measurement of ammonia, nitrite and nitrate (mg/L) was performed every week following method adapted from MOOPAM (1999).

Approximate body composition and biofloc

All the shrimp and floc samples were analyzed for proximate composition according to methodologies reported by AOAC (2000). Samples were oven dried (at 105°C for 24h) and then stored in a freezer (-18°C) until analysis.

Growth performance

Growth indices including body weight gain, body length gain (from postorbital edge to tip of telson), body weight, daily growth rate, biomass, specific growth rate, feed conversion ratio, feed efficiency, survival and yield shrimp bioassays were measured every week. Shrimp weight was measured on a weekly basis to determine shrimp growth and the amount of feed and organic carbon offered. Growth parameters were determined based on the following equations (Tacon *et al*., 2002; Khanjani *et al*., 2016).

Body weight gain (mg) = final weight-initial weight

Body length gain (mm) = final length-initial length

Body weight index (BWI) (%) = [(final weight- initial weight)/initial weight] × 100

Growth rate (GR) (mg) = [(final weight- initial weight)/ days of experiment]

Biomass (g) = (final weight- initial weight) × survival rate × number of shrimp

Survival rate (%) = (number of individuals at the end of testing period/initial number of individuals stocked) × 100.

Specific growth rate (SGR in weight) (%/day) = [(ln final weight-ln initial weight) ×100]/days of experiment

Specific growth rate (SGR in length) (%/day) = [(ln final length -ln initial length) ×100]/days of experiment

Feed conversion ratio (FCR) =feed consumed (dry weight)/live weight gain (wet weight)

Feed efficiency (FE) (%) = [Final weight- initial weight]/ feed consumed ×100

Statistical analysis

Results were expressed as the mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA). Mean differences were compared by Duncan's multiple

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

range tests at the level of 0.05. Statistical analyses were carried out using statistical package of SPSS (SPSS, version 21). All the charts and graphs were plotted using Excel version 2013.

Results

Water quality parameters measured during the experimental period are shown in Table 2. No significant differences were observed in values of parameters including temperature, dissolved oxygen and pH among various treatments during culture period (p>0.05). However, there were significant differences between treatments in some indices such as salinity, ammonia, nitrate and nitrite (p<0.05). The amounts of ammonia, nitrite and nitrate concentrations in treatments are demonstrated in Fig.1 A, B, C. There were significant differences in ammonia level of water between different treatments (p<0.05). The maximum (0.39 mg/L) and minimum ammonia (0.12 mg/L) levels were observed in water exchange treatment (control) and biofloc treatment with minimum feeding level, respectively (p<0.05).

The highest concentrations (Mean ± SD) of NO_2 and NO_3 were 5.88± 2.40 mg/l, 6.80 ± 3.40 mg/L in control treatment and biofloc treatment with maximum feeding level, respectively. The amounts of settled solids (SS), total suspended solids (TSS) and water transparency in 32-day experiment period are shown in Figs 2 A, B and C.

Total heterotroph bacteria count of water in different treatments is presented in Fig. 3A. The highest value ($9.1 \times 10^6 \pm 0.28$) and the lowest value ($1.13\times 10^4 \pm 0.15$ cfu mL^{-1}) of total count were measured on 32th day of experiment in control treatment and biofloc treatment with maximum feeding level, respectively.

Values of weight and length at different weeks of trail are presented in Figs 3 B and C.

Shrimp growth performance is shown in Table 3.

Table 2: Water parameters measured in experimental treatments.

Parameters	Control	Maximum feeding level	Medium feeding level	Minimum feeding level	Without pellet feeding
Temperature a.m. (˚C)	30.12 ± 0.76^{a}	30.20 ± 0.71^{a}	30.09 ± 0.72^{a}	30.06 ± 0.76^{a}	30.17 ± 0.82^{a}
Temperature p.m. (˚C)	30.56 ± 0.56^{a}	30.61 ± 0.52^{a}	30.51 ± 0.55^{a}	30.53 ± 0.56^{a}	30.60 ± 0.53^{a}
Dissolved oxygen a.m. (mg/L)	6.36 ± 0.54^{a}	6.24 ± 0.51^{a}	6.27 ± 0.62^{a}	6.33 ± 0.57^{a}	6.17 ± 0.66^{a}
Dissolved oxygen p.m. (mg/L)	6.05 ± 0.64^{a}	5.79 ± 0.54^{b}	6.00 ± 0.63^{ab}	5.96 ± 0.58^{a}	5.84 ± 0.73^{a}
pH a.m.	8.30 ± 0.09^{a}	8.25 ± 0.10^{a}	8.26 ± 0.10^{a}	8.26 ± 0.08^{a}	8.27 ± 0.13^{a}
pH p.m.	8.22 ± 0.08^{a}	8.18 ± 0.07^{a}	8.20 ± 0.06^{a}	8.20 ± 0.08^{a}	8.23 ± 0.08^{a}
Salinity (ppt)	32.65 ± 0.47^{b}	33.56 ± 0.90^{a}	33.45 ± 0.93^{a}	33.50 ± 0.97^{a}	32.72 ± 0.59^{b}
Ammonia (mg/L)	0.39 ± 0.36^{a}	0.15 ± 0.12^{b}	0.14 ± 0.11^{b}	0.12 ± 0.1^{b}	0.21 ± 0.18^{ab}
Nitrite (mg/L)	5.88 ± 2.4^{a}	4.65 ± 1.83^{ab}	4.2 ± 1.5^{b}	4.08 ± 1.6^{b}	1.98 ± 0.84^{c}
Nitrate (mg/L)	3.38 ± 1.4^{bc}	6. 80 ± 3.40^{a}	5.79 ± 2.67^{ab}	4.77 ± 2.12^{abc}	2.72 ± 1.03^{c}

a.m. - before midday; p.m. - after midday

Values are expressed as mean±SD. Values in the same row with different letters are significantly different (p<0.05).

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

Table 2: Water parameters measured in experimental treatments.

Parameters	Control	Maximum feeding level	Medium feeding level	Minimum feeding level	Without pellet feeding
Temperature a.m. (˚C)	30.12 ± 0.76[a]	30.20 ± 0.71[a]	30.09 ± 0.72[a]	30.06 ± 0.76[a]	30.17 ± 0.82[a]
Temperature p.m. (˚C)	30.56 ± 0.56[a]	30.61 ± 0.52[a]	30.51 ± 0.55[a]	30.53 ± 0.56[a]	30.60 ± 0.53[a]
Dissolved oxygen a.m. (mg/L)	6.36 ± 0.54[a]	6.24 ± 0.51[a]	6.27 ± 0.62[a]	6.33 ± 0.57[a]	6.17 ± 0.66[a]
Dissolved oxygen p.m. (mg/L)	6.05 ± 0.64[a]	5.79 ± 0.54[b]	6.00 ± 0.63[ab]	5.96 ± 0.58[a]	5.84 ± 0.73[a]
pH a.m.	8.30 ± 0.09[a]	8.25 ± 0.10[a]	8.26 ± 0.10[a]	8.26 ± 0.08[a]	8.27 ± 0.13[a]
pH p.m.	8.22 ± 0.08[a]	8.18 ± 0.07[a]	8.20 ± 0.06[a]	8.20 ± 0.08[a]	8.23 ± 0.08[a]
Salinity (ppt)	32.65 ± 0.47[b]	33.56 ± 0.90[a]	33.45 ± 0.93[a]	33.50 ± 0.97[a]	32.72 ± 0.59[b]
Ammonia (mg/L)	0.39 ± 0.36[a]	0.15 ± 0.12[b]	0.14 ± 0.11[b]	0.12 ± 0.1[b]	0.21 ± 0.18[ab]
Nitrite (mg/L)	5.88 ± 2.4[a]	4.65 ± 1.83[ab]	4.2 ± 1.5[b]	4.08 ± 1.6[b]	1.98 ± 0.84[c]
Nitrate (mg/L)	3.38 ± 1.4[bc]	6. 80 ± 3.40[a]	5.79 ± 2.67[ab]	4.77 ± 2.12[abc]	2.72 ± 1.03[c]

a.m. - before midday; p.m. - after midday

Values are expressed as mean±SD. Values in the same row with different letters are significantly different (p<0.05).

Table 3: Growth performance *Litopenaeus vannamei* nursery in treatments based on different feeding levels in 32-days culture period.

Parameters	Control	Maximum feeding level	Medium feeding level	Minimum feeding level	Without pellet feeding
Weight gain (g)	1.361 ± 0.115[b]	1.552 ± 0.167[a]	1.527 ± 0.130[a]	1.292 ± 0.109[c]	0.654 ± 0.060[d]
Length gain (mm)	30.50±1.86[b]	33.62±2.08[a]	33.38±1.68[a]	29.33±1.70[c]	18.40±1.44[d]
Body weight index (%)	1828.7± 154.9[b]	2084.2±224.47[a]	2051.4±175.49[a]	1735.3±146.6[c]	879.14±81.28[d]
Growth rate (mg/day)	42.55 ± 3.60[b]	48.50 ± 5.22[a]	47.74 ± 4.08[a]	40.38 ± 3.41[c]	20.46 ± 1.89[d]
Survival rate (%)	86.66± 0.88[c]	90.25± 1. 7[b]	90± 1.33[b]	84.87± 1.17[c]	98.20± 0.44[a]
Biomass gain (g)	153.4± 12.99[b]	182.1± 19.61[a]	178.7± 15.29[a]	142.6± 12.04[c]	83.58± 7.72[d]
SGR in weight (%/day)	9.25±0.25[b]	9.64±0.32[a]	9.59±0.25[a]	9.09±0.26[c]	7.13±0.27[d]
SGR in length (%/day)	2.91±0.11[b]	3.10±0.12[a]	3.08±0.09[a]	2.84±0.10[c]	2.05±0.12[d]
Feed conversion ratio	1.51± 0.13[a]	1.27± 0.14[b]	1.04± 0.09[c]	0.98± 0.08[c]	-
Feed efficiency (%)	65.95± 5.58[a]	78.27± 8.43[b]	96.03± 8.21[c]	101.99 ± 8.61[c]	-

Values are expressed as mean±SD. Values in the same row with different letters are significantly different (p<0.05).

values of the parameters including weight gain, length gain, body weight index, growth rate, survival rate, biomass gain, specific growth rate (SGR) in weight, SGR in length, feed conversion ratio (FCR) and feed efficiency (FE) indicated significant differences among biofloc treatments and control treatment (p<0.05). There was no difference in Pacific white shrimp growth between maximum feeding level (15% BW/day) and medium feeding level (12% BW/day) treatments (p>0.05) in biofloc system.

It was also found that growth and survival in the medium feeding level zero- water exchange is better than control group with more feeding level.

Proximate analysis of shrimp body composition and biofloc samples are demonstrated in Table 4. Significant differences were observed between biofloc treatments and the control (p<0.05).

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

Table 4: Mean (± SD, n=3) values of proximate composition of shrimp body and biofloc (% dry weight) in different treatments at the end of experiment

Treatment	Dry matter (%)	Crude protein (% DW)	Crude lipid (% DW)	Ash (% DW)
		Shrimp		
Initial	25.55± 0.38	75.91± 0.17	6.86± 0.06	10.98± 0.01
Control	25.29± 0.18^{bc}	75.65± 0.20^{a}	7.07± 0.07^{c}	10.93± 0.02^{a}
Maximum feeding level	24.82± 0.17^{b}	75.84± 0.08^{a}	7.67± 0.06^{a}	11.12± 0.03^{b}
Medium feeding level	25.33± 0.77^{bc}	75.81± 0.19^{a}	7.59± 0.05^{ab}	11.26± 0.06^{bc}
Minimum feeding level	25.96± 0.36^{c}	75.75± 0.28^{a}	7.47± 0.04^{b}	11.37± 0.02^{c}
Without pellet feeding	22.79± 0.35^{a}	74.03± 0.35^{b}	4.57± 0.09^{d}	15.91± 0.07^{d}
		Bioflocs		
Control				-
Maximum feeding level	24.40± 0.10^{a}	28.33± 1.52^{a}	0.91± 0.04^{a}	40.63± 1.06^{a}
Medium feeding level	24.18± 0.30^{a}	27.1± 1.15^{a}	0.71± 0.03^{b}	41.78± 0.39^{a}
Minimum feeding level	24.88± 0.27^{a}	27.53± 1.74^{a}	0.53± 0.05^{c}	40.59± 0.69^{a}
Without pellet feeding	20.44± 1.07^{b}	21.37± 1.47^{b}	0.39± 0.08^{d}	42.61± 0.50^{b}
initial biofloc added	23.31± 1.47	28.77± 2.348	0.57± 0.105	38.12± 0.34

Values are expressed as mean±SD. Values in the same column with different letters are significantly different (p<0.05).

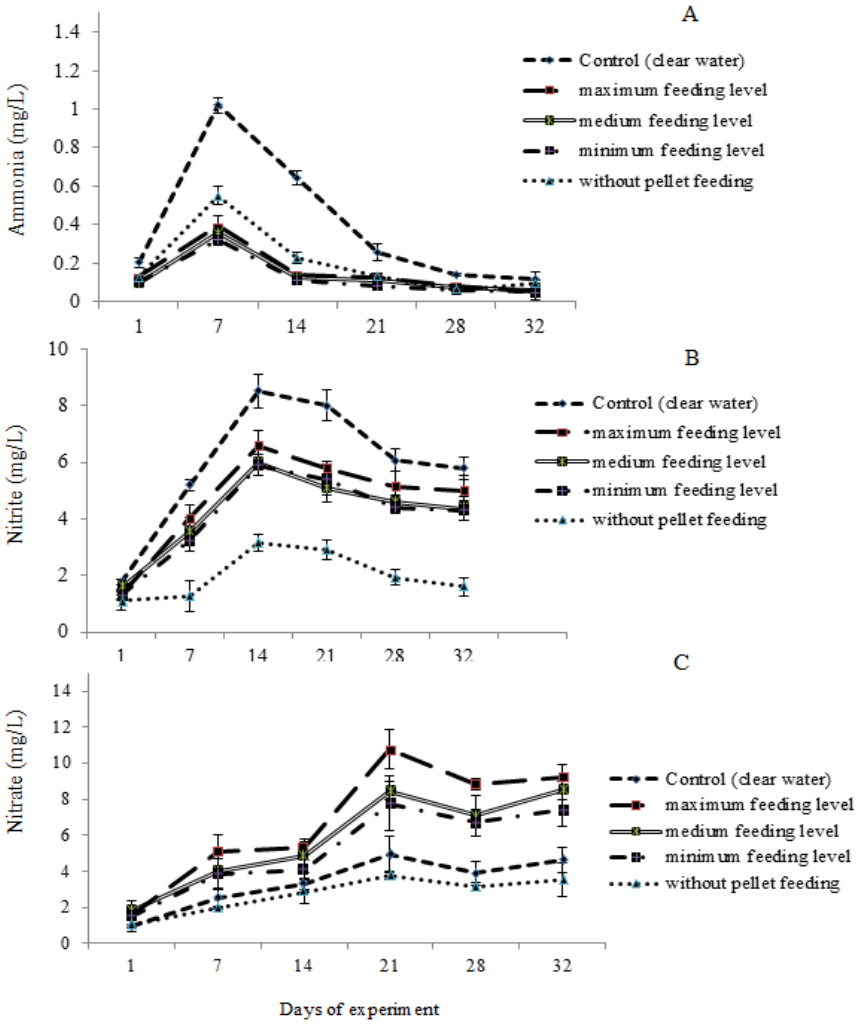


Figure 1: Mean values (±SD, N=3) of ammonia (A), nitrite (B) and nitrate (C) concentrations in water tanks of Pacific white shrimp cultivated at different feeding levels during the rearing period.

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

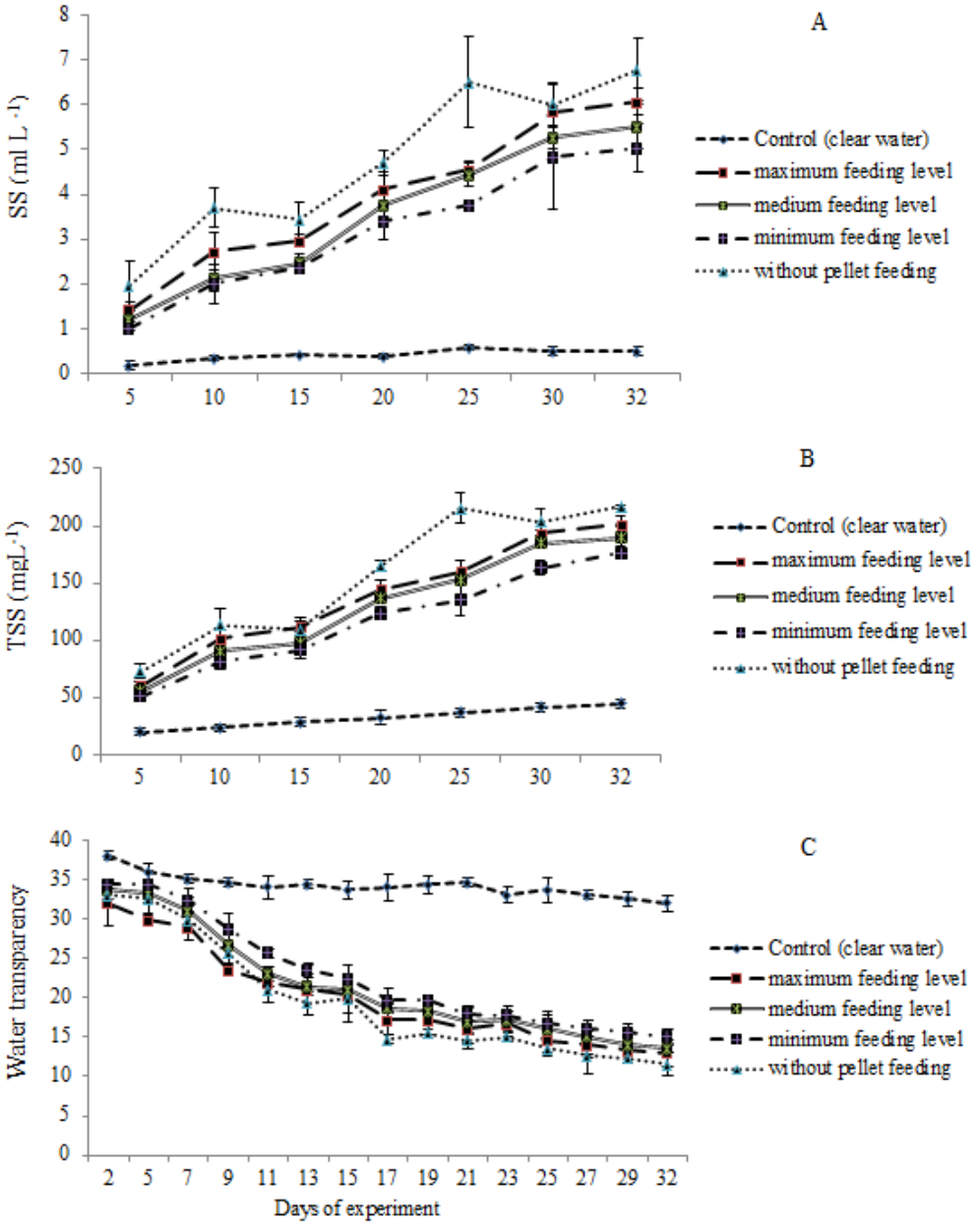


Figure 2: Mean (±SD, N=3) values of settled solids, SS (A), Total suspended solids, TSS (B) and water transparency (C) in tanks of Pacific white shrimp cultivated at different feeding levels during the rearing period (32 days).

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

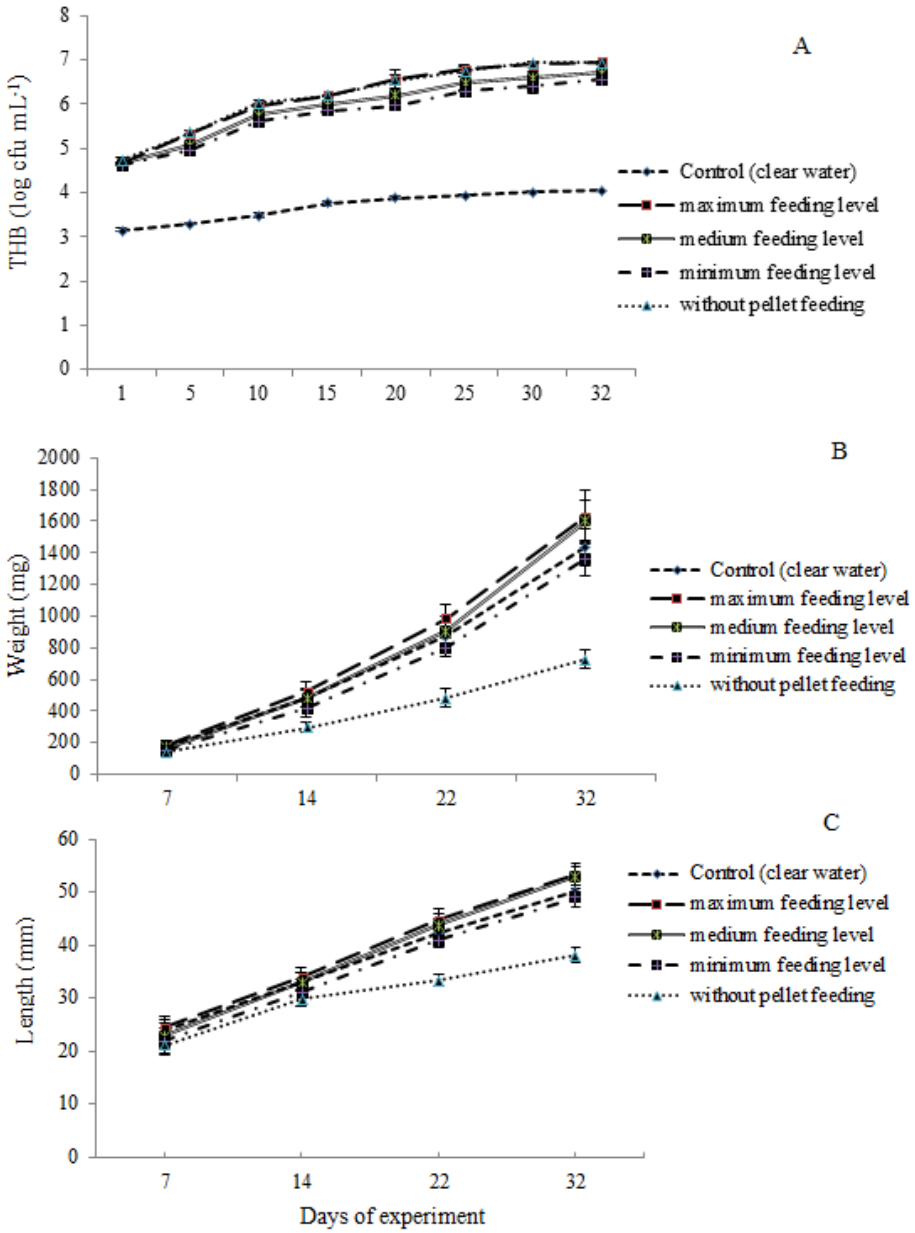


Figure 3: Mean (±SD, N=3) values of Total Heterotroph Bacteria (THB) count (A) in water tanks, Weight (mg) (B) and Length (mm) (C) of Pacific white shrimp cultivated at different feeding levels during the rearing period of 32 days.

Discussion

At the present study, the recorded water temperature, salinity, dissolved oxygen and pH remained within the optimal environmental ranges for Pacific white shrimp culture (Cohen *et al*., 2005). Values of dissolved oxygen and pH in biofloc treatments were lower than the control. This might be as a result of higher respiration rate due to the presence of a heterotrophic community that increased the carbon dioxide concentration in zero- water exchange systems (Wasielesky *et al*., 2006; Emerenciano *et al*., 2012). The proper value of pH for optimal performance in

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

penaeids family is 7–9 (Cohen *et al*., 2005) which is consistent with the result obtained from the present experiment. Dose not mentioned the results are agree with the references or not.

Salinity levels in biofloc treatments were higher than salinity levels in water exchange treatments, probably due to higher evaporation in zero- water exchange systems (Emerenciano *et al*., 2012). Salinity and temperature are the factors that affect the concentration of formed biofloc (Decamp *et al*., 2003) as with increasing salinity, particles tend to accumulate more and biofloc size increases (Hakanson, 2006; Avnimelech, 2007). In many reports, successful reduction of ammonia in limited/zero-exchange culture systems was demonstrated by the manipulation of the carbon to nitrogen ratio, implicating the assimilation of the inorganic nitrogen compounds and the production of microbial biomass (Ebeling *et al*., 2006; Samocha *et al*., 2007; Avnimelech 2009; Gao *et al*., 2012; Krummenauer *et al*., 2014). In the present study, the lowest ammonia concentration was found in biofloc treatment with minimum feeding level and a reduction in ammonia and nitrite levels was observed in biofloc treatment which was also quoted by Gaona *et al*., (2011).

Concentration reduction can be described as a major process in the uptake of inorganic nitrogen by heterotrophic and nitrifying bacteria that increased into the culture water with the biofloc. At the end of the experiment, total heterotroph bacteria count in biofloc treatments was significantly higher than the control.

Heterotroph bacteria in biofloc system are tiny; their assemblage with algae, protozoa and organic particles altogether create bioflocs that are unique ecosystems. These rich and energetic particles that are suspended in relatively poor water are porous, light and have a diameter of 0.1 to a few mm (Avnimelech, 2009). In the present study, results showed that the goal of molasses addition achieved by stimulating the growth of heterotrophic bacteria in the water, as evidenced by keeping ammonia and nitrite at low levels throughout the experiment. Addition of molasses could effectively increase the C/N ratios in tank culture which is suitable for bacterial growth (Emerenciano *et al*., 2012; Gao *et al*., 2012). As previously demonstrated, nitrifying and heterotrophic bacteria could play a main role in controlling ammonia emission in a super intensive shrimp culture (Vinatea *et al*., 2010; Gao *et al*., 2012).

Results of the present work on water quality parameter proved the results reported by Crab *et al*., 2010; Emerenciano *et al*., 2012; Gao *et al*., 2012 and Krummenauer *et al*., 2014.

The maximum SS and TSS concentration were observed in without pellet feeding treatment. The value of SS and TSS showed a steep decline on some days of the experiment which might be attributed to the consumption

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

of a large fraction of the biofloc available in the culture tank or changes in the composition and abundance of microbial organisms in the food chain as a result of ecological succession (Emerenciano *et al*., 2012). In zero-water exchange tanks, SS and TSS concentrations tend to increase over time which is primarily due to an increase in microbial biomass. The observed decrease in TSS content is associated with a decrease in settle solids (SS) concentration and increased water transparency. As recommended by previous researchers, the reasonable values of TSS are below 300 mg/L in an intensive shrimp nursery system (Mishra *et al.*, 2008) and 123 and 414 mg/L for the minimum and maximum recorded values of TSS, respectively, in a limited water exchange nursery system (Emerenciano *et al*., 2012) that result of the present study was in accordance with the above proposed values.

There were significant differences between biofloc treatments and the control in growth performance. In general, the performance of Pacific white shrimp in zero- water exchange system was better than water exchange aquaculture system. This finding confirmed the results of different researches that showed improved shrimp performance and specific growth rate (Ballester *et al*., 2007; Khanjani *et al*., 2016), enhanced growth rate and weight gain (Xu and Pan, 2012), decreased feed conversion ratio (Wasielesky *et al*., 2006; Tidwell, *et al*., 2007; Xu and Pan, 2013, 2014), reduced feeding costs (Burford *et al*., 2004), improved feed utilization (Xu and Pan 2012), high survival rate (Kuhn *et al*., 2008; Mishra *et al*., 2008) and water quality improvement (Asaduzzaman *et al*., 2008; Arnold *et al*., 2009; Gao *et al*., 2012).

Commercial pelleted feeds may not provide the nutrients needed to grow shrimp.. Some of the nutrients (vitamins and minerals) can be obtained from the biofloc generated in the tank. Therefore, the biofloc as a supplemental food source is used for shrimp (Xu and Pan 2012). Biofloc, as an alternative and rich food source produced in zero-water exchange nursery system, is available overtime 24 hours per day (Avnimelech, 2007) with highly diverse components consisting bacterial protein (Ballester *et al*., 2010; Hargreaves, 2013), and poly-β-hydroxybutyrate (PHB) created by bacteria (De Schryver *et al*., 2010), microalgae, protozoa, nematodes (Azim and Little 2007; Valle *et al*., 2015), copepods and rotifers (Ray *et al*., 2010).

The bacterial storage compound, poly-β-hydroxybutyrate (PHB) is a biodegradable polymer with several advantages including improved digestibility in intestine, increased unsaturated fatty acid and growth enhancement in fish and shrimp (Crab *et al*., 2007; Emerenciano *et al*., 2013). The maximum survival rate (98.20± 0.44 %) of Pacific white shrimp was observed in treatment of without pellet feeding and containing only floc. The

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

recorded survival rates for Pacific white shrimp at nursery stage ranged from 55.9% to 100 (Mishra *et al*., 2008) and 97% to 100% (Cohen *et al*., 2005) in biofloc system. Improved survival rates due to biofloc consumption with nutritional benefits, which is provided by high natural productivity characteristic, have been proposed by several researchers (Wasielesky *et al*., 2006; Supono *et al*., 2014). In the present study, there was no difference in shrimp growth between maximum feeding level and medium feeding level treatments. It was also found that over 20% of daily feed intake can be replaced with biofloc in Pacific white shrimp culture.

Previous researches have shown that microbial biomass could include up to 29% of daily food consumption in shrimp (Burford *et al*., 2004). In biofloc treatments cleared using microbial proteins as a partial source of protein and not as the only one feed for shrimp.

Megahed and Mohamed (2014) reported that the dietary protein level could be reduced from 45% to 25% without affecting the growth by using the present biofloc in Indian white shrimp (*Fenneropenaeus indicus)* culture.

Biochemical composition of biofloc could provide important nutrient contents such as protein, lipids, and minerals. Other researchers reported that biofloc could provide amino acids, fatty acids and vitamins for shrimp (Izquierdo, *et al*., 2006; Logan *et al*., 2010; Emerenciano *et al*., 2012). Proximate analysis of biofloc including crude protein, lipids and ash content were recorded 30.4%, 1.9% and 38.9%, respectively, by Ju *et al* (2008) and 18.2-29.3 %, 0.4-0.7% and 43.7-51.8%, respectively, by Emerenciano *et al.*, (2013) which was in agreement with the result of the present work. In microbial particles, protein, lipid and ash content could vary substantially (12- 49 %, 0.5-12.5 % and 13 to 46%, respectively) (Emerenciano *et al.*, 2013).

In proximate analysis, nutritional characteristics of biofloc could be affected by environmental condition, carbon source used, TSS level, salinity, stocking density, light intensity, phytoplankton, zooplankton and bacterial communities (Emerenciano *et al.*, 2013). The results showed that proximate analysis of body composition in shrimp is influenced by the presence of biofloc and confirmed that crude lipid and ash content of shrimp in biofloc treatments tended to increase as compared to the control. The body color of the shrimp fed on biofloc was darker than shrimp clear water treatment. In a previous study by Xu and Pan (2012), the recorded proximate analysis of the whole body composition (% wet weight) in juvenile Pacific white shrimp in the control and two bioflocs treatments with C/N ratios of 15 and 20, respectively, were 17.96, 18.78, 18.53 for crude protein; 2.65, 2.82, 2.85 for ash and 1.80, 1.91, 1.96 for crude lipid, respectively, at the end of 30-day feeding experiment. More increase in lipid content of body

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

composition might be attributed to the assimilation of several essential fatty acids (PUFA and HUFA), amino acids (methionine, lysine) from the bioflocs that were consumed by the shrimp (Izquierdo *et al*., 2006; Ju *et al*., 2008; Supono, *et al*., 2014). Increased ash content of the shrimp body composition might be due to continuous availability of abundant minerals and trace elements especially phosphorus from the biofloc (Tacon *et al*., 2002; Xu and Pan 2012) that was confirmed by high ash content observed in the present study.

Natural microbial community can affect biochemical composition of the floc and shrimp. Protozoan present in biofloc contain sterols in their chemical composition, and a large portion of these sterols is converted to cholesterol or other lipid forms (Loureiro *et al*., 2012).

In conclusion, results of the present study indicated that BFT system was beneficial for Pacific white shrimp by the maintenance of water quality, improving growth performance, increasing feed efficiency, biosecurity and survival rate, reducing FCR, feeding costs, water use, and daily food intake compared with commercial pellet. Also, this study confirmed that shrimp body composition by increasing nutrient retention could be influenced with bioflocs consumption. According to the results, the necessity of using this new technique can be felt in the country's aquaculture industry and especially in the Pacific white shrimp intensive culture in greenhouse-based farms.

Acknowledgements

We would like to thank and appreciate the Director General and Deputy of Hormozgan Fisheries Organization (Iran), the management department and all staff working at the propagation and cultivation aquatic center, Bandar Kolahi- Minab- Hormozgan- Iran. We also appreciate aquaculture engineers, Masandani, Sirpur, Darvishi, Mohammadi and Eslami for providing research facilities which ultimately helped us execute successfully this experiment.

References

AOAC., 2000. Official methods of analysis. 16th ed. AOAC International, Washington, DC, USA.

APHA., 2005. Standard methods for the examination of water and wastewater. 21st ed. American Public Health Association, Washington, DC, USA.

Arnold, S.J., Coman, F.E., Jackson, C.J. and Groves, S.A., 2009. High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: an evaluation of artificial substrates and stocking density. *Aquaculture*, 293, 42-48.

Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Huque, S., Salam, M.A. and Azim, M.E., 2008. C/N ratio control and substrate addition for periphyton development

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*, 280, 117-123.

Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176, 227–235.

Avnimelech, Y., 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture*, 264, 140–147.

Avnimelech, Y., 2008. Sustainable land-based aquaculture—rational utilization of water, land and feed. *Mediterranean Aquaculture Journal*, 1, 45–55.

Avnimelech, Y. and Kochba, M., 2009. Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using N-15 tracing. *Aquaculture*, 287, 163–168.

Avnimelech, Y., 2009. Biofloc technology – A practical guide book, 1st ed., *The World Aquaculture Society, Baton Rouge, Louisiana, USA.*182P.

Azim, M.E., Little, D.C. and Bron, J.E., 2007. Microbial protein production in activated suspension tanks manipulating C/N ratio in feed and implications for fish culture. *Bioresource Technology*, 99, 3590-3599.

Azim, M.E. and Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 283, 29–35.

Ballester, E.L.C., Wasielesky, W.Jr., Cavalli, R.O. and Abreu, P.C., 2007. Nursery of the pink shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: biofilm composition and shrimp performance. *Aquaculture*, 265, 355-362.

Ballester, E.L.C., Abreu, P.C., Cavalli, R.O., Emerenciano, M., Abreu, L. and Wasielesky, 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. *Aquaculture Nutrition*, 16, 163-172.

Burford, M.A. and Williams, K.C., 2001. The fate of nitrogenous waste from shrimp feeding. *Aquaculture*, 198, 79–93.

Burford, M. A., Thompson, P. J., Mc Intosh, R.P., Bauman, R. H. and Pearson, D. C., 2003. Nutrient and microbial dynamics in high intensity, zero exchange shrimp ponds in Belize. *Aquaculture*, 219, 393 - 411.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. and Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high intensity, zero-exchange system. *Aquaculture*, 232, 525–537.

Cohen, J., Samocha, T.M., Fox, J.M., Gandy, R.L. and Lawrence, A.L., 2005. Characterization of water quality factors during intensive

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

raceway production of juvenile *Litopenaeus vannamei* using limited discharge and biosecure management tools. *Aquacultural Engineering*, 32, 425-442.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P. and Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture* , 270, 1–14.

Crab, R., Chielens, B., Wille, M., Bossier, P. and Verstraete, W., 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research*, 41, 559-567.

Cuzon, G., Addison, L., Gaxiola, G., Rosas, C. and Guillaume, J., 2004. Nutrition of *Litopenaeus vannamie* reared in tank or in ponds. *Aquaculture*, 235, 513-551.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N. and Verstraete, W., 2008. The basics of bio-flocs technology: the added value for aquaculture. *Aquaculture*, 277, 125–137.

Decamp, O., Cody, J., Conquest, L., Delanoy, G. and Tacon, A. G. J., 2003. Effect of salinity on natural community and production of *Litopenaeus vannamei* (Boone) within experimental zero-water exchange culture systems. *Aquaculture Research*, 34, 345-355.

De Schryver, P., Sinha, A.K., Kunwar, P.S., Baruah, K. and Verstraete, W., 2010. Poly-beta-hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range-weighted richness in juvenile European sea bass, *Dicentrarchus labrax*. *Applied Microbiology and Biotechnology*, 86, 1535-1541.

Ebeling, J.M., Timmons, M.B. and Bisogni, J. J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic control of ammonia-nitrogen in aquaculture production systems. *Aquaculture*, 257, 346–358.

Emerenciano, M., Ballester, E.L.C., Cavalli, R.O. and Wasielesky, W., 2012. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture Research*, 43, 447–457.

Emerenciano, M., Gaxiola, G. and Cuzon, G., 2013. Biofloc technology (BFT): a review for aquaculture application and animal food industry. In: Matovic MD (ed.) Biomass now – Cultivation and utilization, In Tech, Queen's University, Belfast, Canada, pp. 301–328.

Gao, L., Shan, H.W., Zhang, T.W., Bao, W.Z. and Ma, S.J., 2012. Effects of carbohydrate addition on *Litopenaeus vannamei* intensive culture in a zero-water exchange system. *Aquaculture*, 342, 89–96.

Gaona, C.A., Poersch, P.L., Krummenauer, H.D., Foes, G.K. and Wasielesky, W., 2011. The effect of solids removal on water

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

quality, growth and survival of *Litopenaeus vannamei* in a biofloc technology culture system. *International Journal of Recirculating Aquaculture*, 12, 54–73.

Hakanson L., 2006. The relationship between salinity, suspended particulate matter and water clarity in aquatic systems. *Ecological Research*, 21, 75-90.

Hargreaves, J.A., 2006. Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering*, 34, 344–363.

Hargreaves, J.A., 2013. Biofloc production system for aquaculture. *Southern Regional Aquaculture Center Publication No*, 4503.

Hari, B., Kurup, B.M., Varghese, J.T., Schrama, J.W. and Verdegem, M.C.J., 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. *Aquaculture*, 252, 248–263.

Izquierdo, M., Forster, I., Divakaran, S., Conquest, L., Decamp, O. and Tacon, A., 2006. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*, 12, 192–202.

Jackson, C., Preston, N., Thompson, P.J. and Burford, M., 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. *Aquaculture*, 218, 397–411.

Ju, Z.Y., Forster, I., Conquest, L. and Dominy, W., 2008. Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. *Aquaculture Nutrition*, 14, 533–543.

Khanjani, M.H., Sajjadi, M.M., Alizadeh, M. and Sourinejad, I., 2016. Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. *Aquaculture Research*, 1–11, doi:10.1111/are.12985.

Krummenauer, D., Samocha, T., Poersch, L., Lara, G. and Wasielesky, W. Jr., 2014. The reuse of water on the culture of pacific white shrimp, *Litopenaeus vannamei*, in BFT system. *Journal of the World Aquaculture Society*, 45(**1**), 3-14.

Kuhn, D.D., Boardman, G.D., Craig, S. R., Flick, G.J. and McLean, E., 2008. Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp *Litopenaeus vannamei* in recirculating aquaculture systems. *Journal of the World Aquaculture Society*, 39, 72-82.

Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L. and Flick, G.J., 2010. Evaluation of two types of biofloc derived from biological treatment of fish effluent as feed ingredients for

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 303, 28–33.

Liu, L., Hu, Z., Dai, X. and Avnimelech, Y., 2014. Effects of addition of maize starch on the yield, water quality and formation of bioflocs in an integrated shrimp culture system. *Aquaculture*, 418–419, 79–86.

Logan, A.J., Lawrence, A., Dominy, W. and Tacon, A.G.J., 2010. Single-cell proteins from food byproducts provide protein in aqua feed. *Global Advocate*, 13, 56-57.

Loureiro, C.K., Wasielesky, W.JR., Abreu, P.C., 2012. The use of protozoan, rotifers and nematodes as live food for shrimp raised in BFT system. *Atlantica, Rio Grande*, 34(**1**), 5-12.

Megahed, M.E. and Mohamed, K., 2014. Sustainable growth of shrimp aquaculture through biofloc production as alternative to fishmeal in shrimp feeds. *Journal of Agricultural Science*, 6(**6**), 176-188.

Mishra, J.K., Samocha, T.M., Patnaik, S., Speed, M., Gandy, R.L. and Ali, A., 2008. Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei*, under limited discharge condition. *Aquacultural Engineering*, 38, 2–15.

MOOPAM., 1999. Manual of oceanographic observations and pollutants analysis methods (3rd ed.). The Regional Organization for the Protection of the Marine Environment (ROPME), Kuwait. 284P.

Otoshi, C. A., Moss, D. R. and Moss, S. M., 2011. Growth-enhancing effect of pond water on four size classes of Pacific white shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society*, 42, 417-422.

Ray, J.A., Lewis, B.L., Browdy, C.L. and Leffler, J.W., 2010. Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, super intensive culture systems. *Aquaculture*, 299, 89-98.

Samocha, T.M., Patnaik, S., Speed, M., Ali, A.M., Burger, J.M., Almeida, R.V., Ayub, Z., Harisanto, M., Horowitz, A. and Brock, D.L., 2007. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering*, 36, 184–191.

Souza, D.M.D., Suita, S.M., Romano, L.A.Jr.W. and Ballester, E.L.C., 2014. Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus Brasiliensis* (Latreille, 1817) in a biofloc technology system. *Aquaculture Research*, 45, 270–277.

Supono, Hutabarat, J., Prayitno, S.B. and Darmanto, YS., 2014. White shrimp (*Litopenaeus vannamei*) culture using heterotrophic aquaculture system on nursery

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

phase. *International Journal of Waste Resources*, 4 (**2**), 142-145.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P. and Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition*, 8, 121–139.

Tidwell, J.H., Coyle, S., Arnum, A.V. and Weibel, C., 2007. Production response of freshwater prawns *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. *Journal of World Aquaculture Society*, 31, 452-458.

Valle, B.C.S., Dantas, JR., Silva, J.F.X., Bezerra, R.S., Correia, E.S., Peixoto, S.R.M. and Soares, R.B., 2015. Replacement of fish meal by fish protein hydrolysate and biofloc in diets of *Litopenaeus vannamei* postlarvae. *Aquaculture Nutrition*, 21, 105–112.

Vinatea, L., Galvez, A.O., Browdy, C.L., Stokes, A., Venero, J., Haveman, J., Lewis, B.L., Lawson, A., Shuler, A. and Leffler, J.W., 2010. Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: interaction of water quality variables. *Aquacultural Engineering*, 42, 17–24.

Wasielesky, W.Jr., Atwood, H., Stokes, A. and Browdy, C.L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture*, 258, 396–403.

Wasielesky, W.Jr., Froes, C., Fóes, G., Krummenauer, D., Lara, G., and Poersch, L., 2013. Nursery of *Litopenaeus vannamei* reared in a biofloc system: the effect of stocking densities and compensatory growth. *Journal of Shellfish Research*, 32(**3**), 799-806.

Xu, W.J., Pan, L.Q., Zhao, D.H. and Huang, J., 2012. Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. *Aquaculture*, 350, 147-153.

Xu, W.J. and Pan, L.Q., 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. *Aquaculture*, 356, 147–152.

Xu, W.J., Pan, L.Q., Sun, X.H. and Huang, J., 2013. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. *Aquaculture Research*, 44, 1093-1102.

Xu, W.J. and Pan, L.Q., 2014. Dietary protein level and C/N ratio manipulation in zero‐exchange culture of *Litopenaeus vannamei*: Evaluation of inorganic nitrogen

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]

control, biofloc composition and shrimp performance. *Aquaculture Research*, 45, 1842–1851.

Xu, W.J. and Pan, L.Q., 2014. Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. *Aquaculture*, 426, 181-188.

Zhang, Z.F., Zhang, W.B. and Liu, L.P., 2012. Status and sustainability problems of white leg shrimp *Litopenaeus vannamei* culture in Zhanjiang (In Chinese with English abstract). *Guangdong Agricultural Sciences*, 14, 158–160.

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.4.22.2]