



The Assessment of the Effective Use of Biofloc Technology on Enhancement of White Leg Shrimp, *Litopenaeus vannamei* Growth Performance and Water Quality of Super-intensive Culture Ponds

Ahmed Ali Balqadi ^{1*}, Yasser Abdul Kader Al-Gahwari ², Ahmed Abdullah Al-Buhaishi ³

¹⁻² Marine Biology Department, College of Environmental Science and Marine Biology, Hadhramout University, Mukalla City, Hadhramout Governorate, Yemen

³ Centre of Oceanography, Mukalla City, Hadhramout Governorate, Yemen

* Corresponding Author: **Ahmed Ali Balqadi**

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Abstract

This experiment was conducted over a culture period of five months (twenty weeks) to assess the effects of using biofloc technology (BFT) on enhancement of white leg shrimp (*Litopenaeus vannamei*) growth yield performance and survival as well as water quality in super-intensive culture ponds. Six ponds were designed with an area 0.3 ha per pond. Each pond was stocked at a density of 500 shrimp/m² of *Litopenaeus vannamei* post larvae with an average initial weight of 0.26 g. Three ponds were used as triplicates for biofloc technology (BFT) treatment and the other three ponds were used as triplicates for water exchange system treatment (control). The results of all physicochemical parameters indicated that the quality of water was an optimal and suitable for *L. vannamei* culture in both treatment ponds. Biofloc ponds had significantly lower levels of TAN and NO₂-N than control ponds. These results showed that there were accepted interactions between TAN and NO₂-N in water since ammonia is converted to nitrite by nitrifying bacteria. On the contrary, the levels of NO₃-N were higher in biofloc ponds than in control ponds. Although, DO range (5.0 - 6.18 mg L⁻¹) recorded at biofloc ponds were slightly lower than control ponds range, but it's still within the optimal limit for the survival and growth of *L. vannamei* shrimp.

Using two-way ANOVA, the main effects of biofloc treatment had shown significant differences on all water quality parameters except on NO₃-N and temperature ($P > 0.05$). The results of two-way ANOVA showed that there was very high significant interaction ($P < 0.001$) in some growth parameters like WG, DWG, SGR and production of biofloc and control ponds throughout the cultural period (months). Whilst, there were no significant interaction in values of PER, FCR and survival ($P > 0.05$) between treatments and period of shrimp culture (months). The values of WG, DWG and production were high (3.55 ± 0.13 g, 0.122 ± 0.01 g d⁻¹ and 13.27 ± 1.6 MT pond⁻¹, respectively) in biofloc ponds and low (3.02 ± 0.19 g, 0.104 ± 0.01 g d⁻¹ and 11.89 ± 5.34 MT pond⁻¹ respectively) in control ponds. The results of growth parameters such as WG, DWG, SGR, PER and FCR in waters treated with biofloc were significantly higher than the results in control treatment. These might be due to the synergistic effects of the enhanced water quality, increasing bacterial and zooplankton densities in ponds waters.

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1. Introduction

Fish culture has grown up so quickly over the last of decade due to considerable increases in the global demand for seafood. Marine shrimp culture is one of the most important marine farm products because of its high market value (FAO, 2010) ^[10]; (Anand *et al.*, 2014) ^[4]. In shrimp culture production, farmers expend the largest expenditure on feeding with commercial shrimp diets. This costly food mostly covers around 40% - 60% of the total production expenses (Bender *et al.*, 2004) ^[11]. Fish meal is an irreplaceable source of food and is considered as an essential demand for shrimp diets due to its balanced content of protein, fat, minerals and vitamins (Suarez *et al.*, 2009) ^[59].

Traditional culture systems with intensive densities are a countable for producing an immense amount of excess organic and inorganic wastes that requires continuous change of pond water (Ahmad *et al.*, 2017) [2].

Studies conducted by Piedrahita (2003) [45] and Avnimelech (2006) [6] reported negative effects on water quality and growth performance in intensive shrimp culture. Nowadays, the biofloc super-intensive technology has protruded as an inordinate promising substitution culture system. Implementing biofloc technology (BFT) to cultivation of shrimp like *Litopenaeus vannamei* has newly become successful system (Cohen *et al.*, 2005; Samocha *et al.*, 2007; Mishra *et al.*, 2008; Correia *et al.*, 2014; Samocha *et al.*, 2015) [12, 28, 29, 19, 30].

The biofloc system is depend on the fundament of rubbish nutrients recycling, where target organism are cultured at high densities (minimum of 150 individual/m²) and little or no water exchange (Hariati *et al.*, 1996; Emerenciano *et al.*, 2017; Bossier and Ekasari, 2017) [16, 22, 8].

Applying the potential of biofloc technology with shrimp culture to dodge or minimize some problems such as the surplus of suspended solids, eutrophication and hypernutrification, were recorded by some investigations (Gaona *et al.*, 2017; and Al-Gahwari and Al-Buhaishi, 2021) [18, 3].

Bioflocs are partly consisted of an immense microorganism's diversity such as heterotrophic and autotrophic bacteria, microalgae, yeast, rotifers, ciliates, protozoa, nematodes, copepods, fungi and zooplankton (Monroy *et al.*, 2013; Collazos *et al.*, 2015) [31, 13].

Microorganisms within biofloc are utilized in aquaculture, taking in consideration their nutritional support and their ecological prominence, because are the essential of all aquatic food chain (Muller, 2000) [33].

Generally, protein content in microalgae may modify from 30 - 65% of dry weight (Becker, 1986) [10].

Saturated fatty acids constitute (15 - 40%) of total fatty acids in chlorophytes and diatoms within biofloc, meantime green microalgae exhibit minimum concentrations of monounsaturated fatty acids and maximum polyunsaturated concentrations (Fernandez-Reiriz *et al.*, 1989) [15].

The determination of composition of zooplankton species that are existed in biofloc show that rotifers contains 54 to 60% of crude protein, whereas cladocerans 50 to 68%, and copepods 70 to 71% (Martínez *et al.*, 2002; Ray *et al.*, 2010) [25, 37].

Study by (Pilotto *et al.*, 2018) [44] confirmed that enhancement of immunity and reduced diseases after biofloc technology was applied in shrimp culture. Moss *et al.* (2001) [32] and Xu and Pan (2012) [66] have also observed that improvement of digestive enzymes of shrimp reared in biofloc system, which lead to elevation of production it (Xu *et al.*, 2012; Silva *et al.*, 2013; Panigrahi *et al.*, 2019) [65, 42, 40].

Biofloc system is a method of improving water quality in marine and fresh water culture through equilibrium carbon and nitrogen in the system (Crab *et al.*, 2012; Martinez-Porchasa *et al.*, 2020) [14, 26].

Previous studies conducted by Avnimelech (1999) [7] and Emerenciano *et al.* (2017) [22] confirmed that ability of some bacteria within biofloc on removing the excess ammoniacal nitrogen (NH₃-N) and preserving stable levels of nutrients in the pond waters.

Many studies have reported the positive effects of biofloc

systems on water quality, growth performance and survival in shrimp and fish culture (Burford *et al.*, 2004; Schram *et al.*, 2009; Crab *et al.*, 2010; Crab *et al.*, 2012; Supono *et al.*, 2014; Kim *et al.*, 2015; Yuniartik *et al.*, 2015; Martinez-Porchasa *et al.*, 2020) [9, 48, 13, 14, 54, 21, 68, 26].

Various investigations on the advantage of biofloc in fish and shrimp culture have placed the potential of biofloc technology (BFT) on enhancement of *Litopenaeus vannamei* shrimp yield performance growth within the culture systems (Gaona *et al.*, 2012; Xu and Pan, 2012; Baloi *et al.*, 2013; Al-Gahwari and Al-Buhaishi, 2021) [17, 66, 5, 3].

In this context, this study was aimed to assess the effective use of biofloc technology (BFT) on enhancement of *Litopenaeus vannamei* shrimp growth performance and water quality in super-intensive culture ponds.

Materials and Methods

Culture Site and Condition

The entire experiment was carried out in six rearing ponds within 20 weeks (5 months) of culture period at national aquaculture group (NAQUA) located 160 Km south of Jeddah, Saudi Arabia. Post larvae of *Litopenaeus vannamei* with an initial weight range from 0.25 g to 0.28 g and an average weight 0.26 g transferred from the hatchery at NAQUA to the experimental ponds. According to the transferring protocol, the post larvae were carried from the hatchery to the experimental ponds by trucks with fiber glass tanks filled by fresh supplies oxygenated seawater and supported by a technician to monitor the dissolved oxygen levels and shrimp conditions. An initial stocking density amount of 500/m² of *Litopenaeus vannamei* post larvae was stocked in each experimental pond.

Pond Design

The experimental six ponds set up were grouped into two treatment systems: control and biofloc. Both control and biofloc put in triplicate using completely randomized design (CRD). Control treatment, as the first pond system, was water exchange system without fertilization and the other treatment pond system was biofloc technology (BFT) with fertilization. The biofloc treatment ponds were fertilized by mixed fertilization consists of diamonium phosphate (DAP), urea and molasses. The dosages of fertilizer were applied according to Avnimelech (2009) [5].

Each pond with an area of 0.3 ha and an average depth of 1.4 m, was lined with high-density polyethylene (HDPE). The slope in pond was designed toward the harvest gate and the center of pond to accumulate the sludge. The drainage pipe was located in the center to drain the wastes and sludge.

Filtration System

Supplied water were filtered before filling the ponds through slow sand filter and 250 μ of bag net to ensure that there were no waste or small fish and soil enter to the experimental ponds.

Feeding and Water Management

Water exchange and biofloc pond systems were fed with the same feed rate 35% of crude protein. Feeds were given from 1.5% to 10% of the shrimp biomass, rely on many factors: weather condition, dissolved oxygen, animal molting, population estimation, average body weight (ABW), feed on check tray and TAN level. Daily, four meals were given in a using auto-feeder (Clifford, 1992).

Molasses was also added at a rate 50% of feed applied to each biofloc pond to maintain an optimum C:N ratio of 10:4 (Avnimelech, 1999 and Hargreaves, 2006) [7, 20].

The Molasses spread to the water surface in the afternoon, and completely mixed with the water of each biofloc pond using strong aeration system.

Daily, seawater was changed regularly in water exchange (control) ponds in a rate of 20% of the existing water level, whereas biofloc ponds water levels were also replaced by adding new seawater to compensate the evaporation loss and salinity increment.

Aeration System

Sixteen paddle wheels were installed on each pond of biofloc and control ponds. Every paddle wheel has been 1 horse power (hp) for 550 kg of shrimp biomass (Mc Intosh, 2000), they were running in one direction to sustain the suspended solids in the column of water and to keep the dissolved oxygen at an optimum level, also to push all waste toward the central drainage pip where the siphoning pipe there.

Measurements of Parameters

Water Quality Parameters

Water quality monitoring is a considerable step in getting a successful of aquaculture. During the experimental period, physico-chemical parameters in the culture systems were determined by several standard methods according to the procedures described APHA (1998). Temperature and dissolved oxygen were recorded twice a day (5 am and 5 pm) using a YSI model 58 probe. pH readings were obtained by a digital pen pH meter AT 130. A ATC 101 refractometer and a Secchi disc were used once a day at 11 am to measure salinity and transparency, respectively. Total suspended solids (TSS) values were evaluated by the volatilization gravimetry method adapted from APHA (1998). According to the method described by APHA (1998), the alkalinity measurements were determined following neutralization titrimetric method every week at central analysis service (CAS) at NAQUA. Total ammonia nitrogen (TAN), Nitrite nitrogen (NO₂-N) and Nitrate nitrogen (NO₃-N) concentration levels were analyzed spectrophotometrically every week according to standard methods (APHA, 1998) acquired from CAS at NAQUA.

Growth Performance Parameters

Sampling was carried out every 15 days to estimate the survival rate (population count). Population estimation was done from 5 g as a minimum of average body weight (ABW) onward, throws number was 4 throws per pond using the cast net. Calculation was done based on the following formula: Population count = (no. shrimp captured) / (no. throws x area of cast net)

The estimation of average body weight (ABW) was done once every week using the cast net and digital weighing scale to evaluate growth performance and feed conversion ratio during the culture. The calculation was done based on the following equation:

Average body weight gained (ABW) (g/shrimp) = (weight of shrimp captured) / (no. shrimp cultured).

At the end of 140 days (20 weeks) experiment, the shrimp performance indicators were used to assess biofloc technology effects on shrimp performance. Computed based on the following equations:

Let me know if you have the next section (e.g., Results,

Discussion, or more paragraphs) ready — I'll continue adding the references in the exact same way.

- Mean weight gain (g/shrimp) = Final mean weight - Initial mean weight (Zaid and Sogbesan, 2010).
- Average daily weight gain (ADG) (g d⁻¹) = (Final mean weight (g) - Initial mean weight (g) / rearing period in days (Varghese, 2007).
- Specific growth rate (SGR) (% d⁻¹) = [(lnWf - lnWi) x 100/T] (Raj *et al.*, 2008),

Where:

lnWf = the natural logarithm of the final weight

lnWi = the natural logarithm of the initial weight

T = time (days) between lnWf and lnWi (rearing period in days).

- Final production (MT/pond) = Total biomass at harvest / numbers of ponds (Varghese, 2007).
- Feed conversion ratio (FCR) = Dry weight of feed given (kg) / weight gain (kg) (Raj *et al.*, 2008).
- Protein efficiency ratio (PER) = Weight gain / weight of protein consumed (protein intake) (Shahkar *et al.*, 2014).
- Survival (%) = 100 x (final number of shrimp survived / initial number of shrimps stocked) (Pérrin *et al.*, 2015).

Harvest of Production

The yield harvest was done when the shrimp size reached to around 17 g using long drag net to drop the shrimp toward harvest gate where the long harvest net installed, then, the final shrimp yield parameters was done by weighing it at the field. In addition, putting the harvested shrimp in the plastic tubes contain on ice mixed with water to preserve the shrimp at suitable temperature (0 - 4°C) until the trucks arrived to the processing plant.

Statistical Analysis

The difference in water quality parameters between treatments (Biofloc and control) and period of culture (5 months) was examined using a two-way ANOVA (analysis of variance). Two-way ANOVA was also performed to determine the variation in growth parameters between treatments (biofloc and control) and period of culture (5 months) as factors. At the end of the experiment, the difference in growth parameters between biofloc and control was analyzed by ANCOVA (analysis of covariance). All statistical analyses were performed using SPSS (version 20); P < 0.05 was considered as significant.

Results

Water Quality Parameters

The water quality parameters results in experimental ponds during the study period are presented in Figure 1 and 2. Some physico-chemical parameters monitored in this study such as total ammonia nitrogen (TAN mg L⁻¹), nitrite nitrogen (NO₂-N mg L⁻¹), dissolved oxygen (DO mg L⁻¹), pH and transparency (cm) showed lower mean values (0.27±0.04, 0.13±0.02, 4.5±3.4, 7.96±0.14 and 30.58±5.98, respectively) in biofloc treatment compared to control treatment mean values (Figure 1a, b, e and f and 2c). Two-way ANOVA showed very high significantly differences (P < 0.001) in values of TAN, DO, pH and high significant differences (P < 0.01) in nitrite values between biofloc treatment and control (Table 1 and Figure 1a, 1b, 1e and 1f). In contrast, the higher mean values (2.38±0.11 mg L⁻¹, 29.04±1.12 °C, 189.27±7.28

mg L⁻¹, 151.47±5.98 mg L⁻¹ and 44.45±1.27 ppt) for nitrate nitrogen (NO₃-N mg L⁻¹), temperature, total suspended solids (TSS), alkalinity and salinity, respectively were recorded in biofloc treatment compared to the lower mean values (2.28±0.15 mg L⁻¹, 28.85±1.28 °C, 91.13±5.83 mg L⁻¹, 144.09±9.27 mg L⁻¹ and 42.35±0.66 ppt, respectively) were recorded in control (Figure 1c, 1d and 2a, 2b and 2d). Two-way ANOVA also showed significant differences ($P < 0.05$) in values of total suspended solids, alkalinity, and very high significant differences ($P < 0.001$) in salinity values between biofloc treatment and control (Table 1 and Figure 2 a, 2b and 2d). Thus, biofloc treatment effect clearly appeared on all water quality parameters ($P < 0.001$; $P < 0.01$; $P > 0.05$) except on nitrate and temperature ($P > 0.05$). Two-way ANOVA also showed significant monthly variation in all values of water quality parameters ($P < 0.001$; $P < 0.01$; $P > 0.05$) except in values of nitrate which did not show any significant difference among months culture period ($P > 0.05$)

(Table 1).

The monthly TAN values, as shown in Figure 3a, increased gradually for the first three months of culture period and then this increasing trend did not find for the 4th and 5th months. The maximum mean value of TAN (1.41±0.29 mg L⁻¹) recorded in the 3th month of culture (Figure 1a). Two-way ANOVA confirmed that there was a significant effect of culture period on TAN values especially in the 3th month which had a significant higher value than the other monthly periods (1st, 2nd, 4th and 5th months) (Figure 3a).

Similar to TAN, the monthly NO₂⁻ concentration in ponds waters also increased with culture period. This gradually increasing appeared throughout the whole culture months except the 5th month. The maximum mean value (0.27±0.04 mg L⁻¹) of NO₂⁻ in ponds waters was recorded after 4th month (Figure 3b). Two-way ANOVA indicated that NO₂⁻ having significantly higher values after 4th month than other months (Figure 3b).

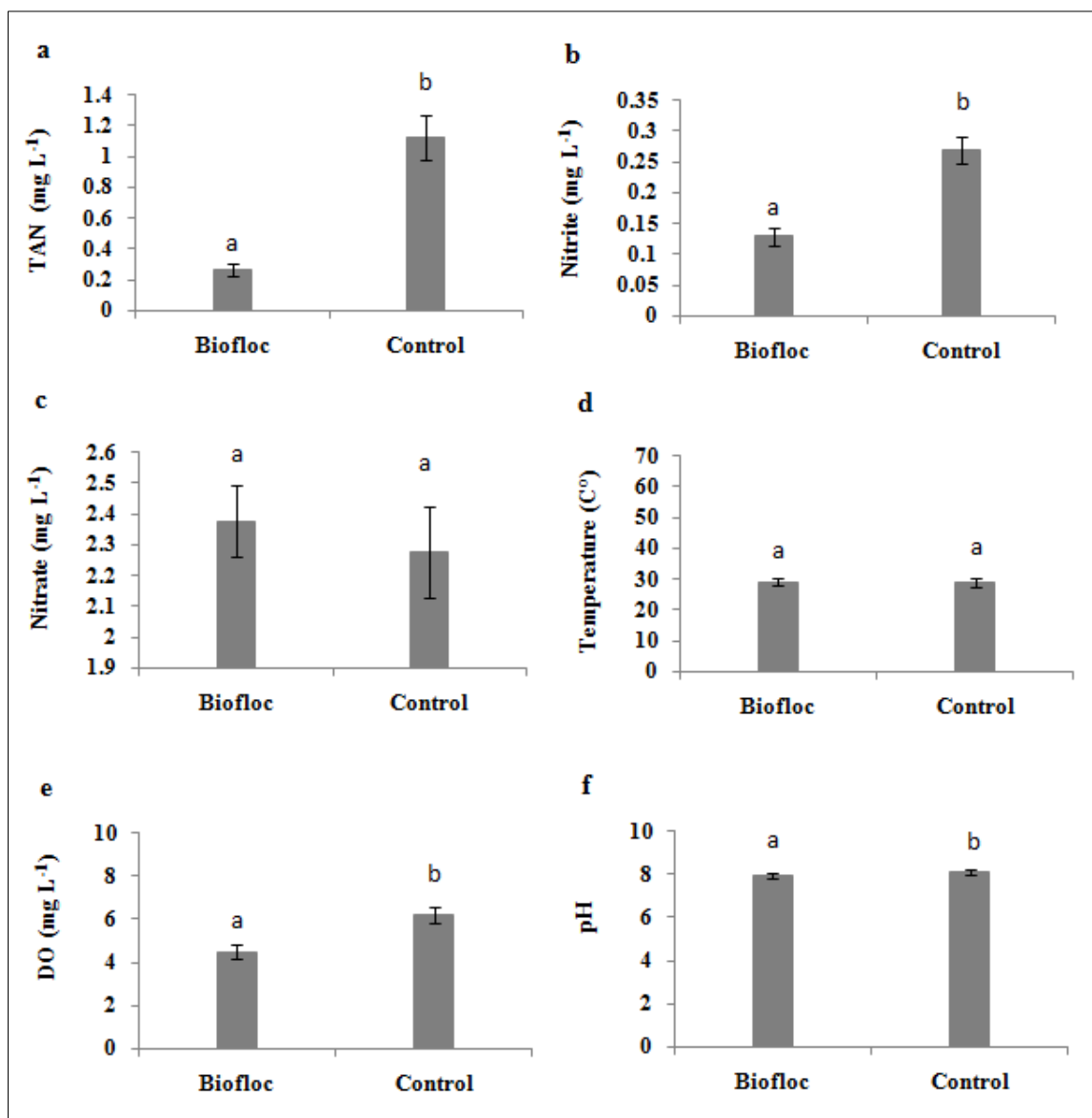


Fig 1: Water quality parameters in shrimp *L. vannamei* rearing ponds treated with biofloc produced from mixture of molasses, urea and DAP compared to control (mean±SE, n=3). a) total ammonia nitrogen (TAN mg L⁻¹), b) nitrite (NO₂⁻ mg L⁻¹), c) nitrate (NO₃⁻ mg L⁻¹), d) temperature (°C), e) dissolved oxygen (DO mg L⁻¹) and f) pH. Same letters on the bars indicate no significant difference ($P > 0.05$).

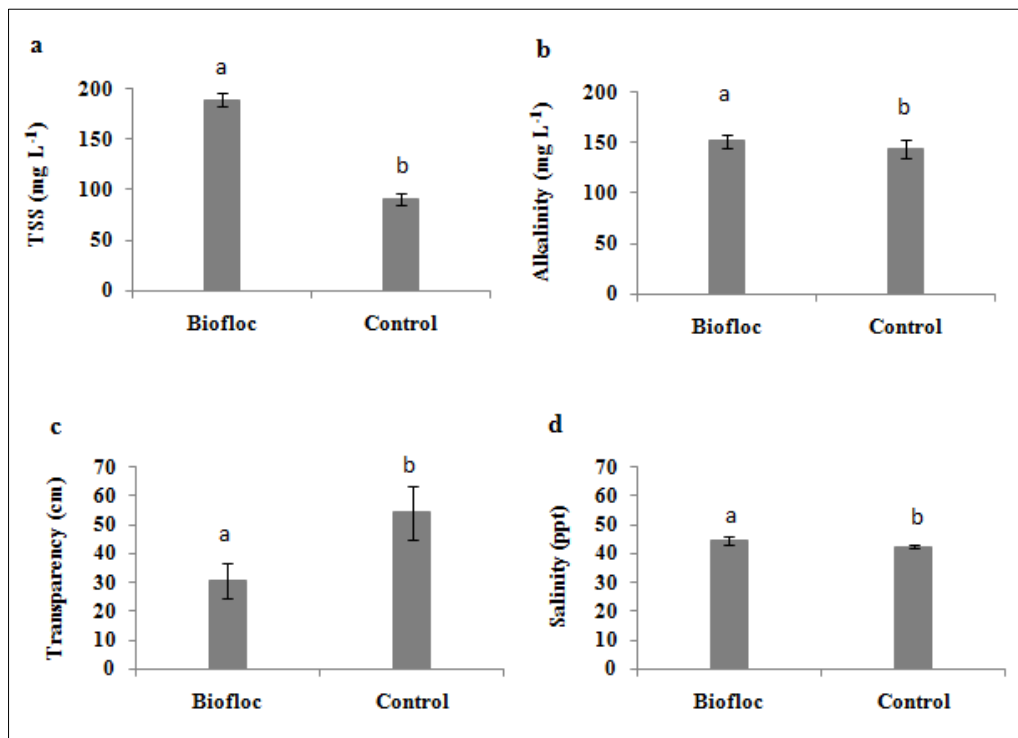


Fig 2: Effects of adding biofloc (mixture of molasses, urea and DAP) for ponds waters of shrimp *L. vannamei* on results of four water quality parameters a) total suspended solids (TSS mg L⁻¹), b) alkalinity (mg L⁻¹), transparency (cm) and salinity (ppt) (mean±SE, n=3). Different superscripted letters on the bars denote significant differences between biofloc and control ($P > 0.05$).

Figure 3c and Table 1 show that there were fluctuations in monthly NO₃⁻ concentrations among the biofloc and control systems and between them. However, there was no positive effect of rearing period on NO₃⁻ values, although, NO₃⁻ mean value was lower (2.13±0.17 mg L⁻¹) after 3rd month and higher (2.44±0.31 mg L⁻¹) after 5th month (Figure 3c). TSS and alkalinity increased with culture period, this increasing was not found for 4th and 5th months. After 3rd month had higher values (152.13±13.06 mg L⁻¹ and 157±2.13 mg L⁻¹ for TSS and alkalinity respectively. In addition, after 3rd month exhibited higher significantly in values of TSS

than 1st month (Figure 4a). However, there was higher significantly in alkalinity values after 3rd month than 1st and 5th months (Figure 4b).

The maximum mean value of DO in ponds waters reached to 5.54±0.16 mg L⁻¹ after 3rd month (Figure 3e). While, pH mean value reached to 8.30±0.03 after 4th month (Figure 3f). Two-way ANOVA also did find significant differences in DO levels in ponds waters among 4th month and 1st, 2nd and 3rd months (Figure 3e). Whilst, higher significant value of pH was recorded in 4th month of culture period comparing with 1st, 2nd, and 3rd months (Figure 3f).

Table 1: Two-way ANOVA (analysis of variance) of the water quality parameters during this study. Treatments (biofloc and control) and periods of culture (5 months) were used as factors for ANOVA. Means (±SE, n=3), very high significant = $P < 0.001$, high significant = $P < 0.01$, significant = $P < 0.05$ and not significant = $P > 0.05$.

Parameters	Periods (one month each)	Treatment		Two-way ANOVA		
		Biofloc	Control	Periods (P value)	Treatment (P value)	Periods X Treatment (P value)
TAN (mg L ⁻¹)	1	0.079±0.019	0.472±0.184	0.000	0.000	0.000
	2	0.086±0.064	1.130±0.033			
	3	0.435±0.056	2.376±0.635			
	4	0.463±0.045	1.173±0.213			
	5	0.269±0.089	0.457±0.216			
NO ₂ - (mg L ⁻¹)	1	0.044±0.019	0.055±0.033	0.000	0.000	0.005
	2	0.063±0.022	0.284±0.030			
	3	0.107±0.032	0.354±0.043			
	4	0.217±0.015	0.331±0.057			
	5	0.216±0.015	0.319±0.029			
NO ₃ - (mg L ⁻¹)	1	1.799±0.186	2.246±0.145	0.203	0.585	0.483
	2	2.773±0.352	2.661±0.243			
	3	2.493±0.228	1.966±0.306			
	4	2.282±0.215	2.350±0.604			
	5	2.538±0.204	2.160±0.081			
TSS (mg L ⁻¹)	1	160.75±19.64	57.58±9.96	0.000	0.000	0.017
	2	202.83±12.62	99.5±7.23			

Alkalinity (mg L ⁻¹)	3	207.5±11.94	96.75±7.68	0.000	0.000	0.056
	4	193.83±8.34	94.25±9.90			
	5	181.42±12.22	107.58±7.50			
	1	144.58±6.21	127.83±23.06			
	2	151.83±4.19	153.58±3.01			
Transparency (cm)	3	161.58±4.62	152.42±6.27	0.000	0.000	0.000
	4	152.83±5.40	148.75±3.96			
	5	144.89±3.38	135.78±4.52			
	1	33.33±12.51	109.17±9.36			
	2	26.25±6.64	67.08±8.92			
DO (mg L ⁻¹)	3	36.67±12.14	35.00±8.70	0.000	0.000	0.000
	4	30.00±5.90	29.58±4.17			
	5	26.67±3.10	30.42±8.83			
	1	4.89±0.36	6.1±0.17			
	2	4.08±0.41	6.93±0.45			
pH	3	4.81±0.15	6.28±0.15	0.000	0.000	0.000
	4	4.18±0.22	6.05±0.24			
	5	4.52±0.20	5.54±0.31			
	1	7.70±0.11	8.11±0.05			
	2	7.93±0.05	8.01±0.05			
Salinity (ppt)	3	7.83±0.04	7.86±0.05	0.000	0.000	0.000
	4	8.29±0.08	8.32±0.08			
	5	8.03±0.06	8.19±0.06			
	1	43.33±0.75	41.00±0.43			
	2	46.42±1.49	42.17±0.41			
Temperature (C ^o)	3	45.83±0.54	42.58±0.76	0.000	0.106	0.305
	4	44.58±0.83	43.00±0.49			
	5	42.08±0.52	43.00±0.43			
	1	27.28±1.40	26.58±1.68			
	2	28.63±0.57	28.42±0.57			
	3	29.02±0.98	29.02±1.00			
	4	30.03±1.00	30.00±1.02			
	5	30.23±0.44	30.21±0.46			

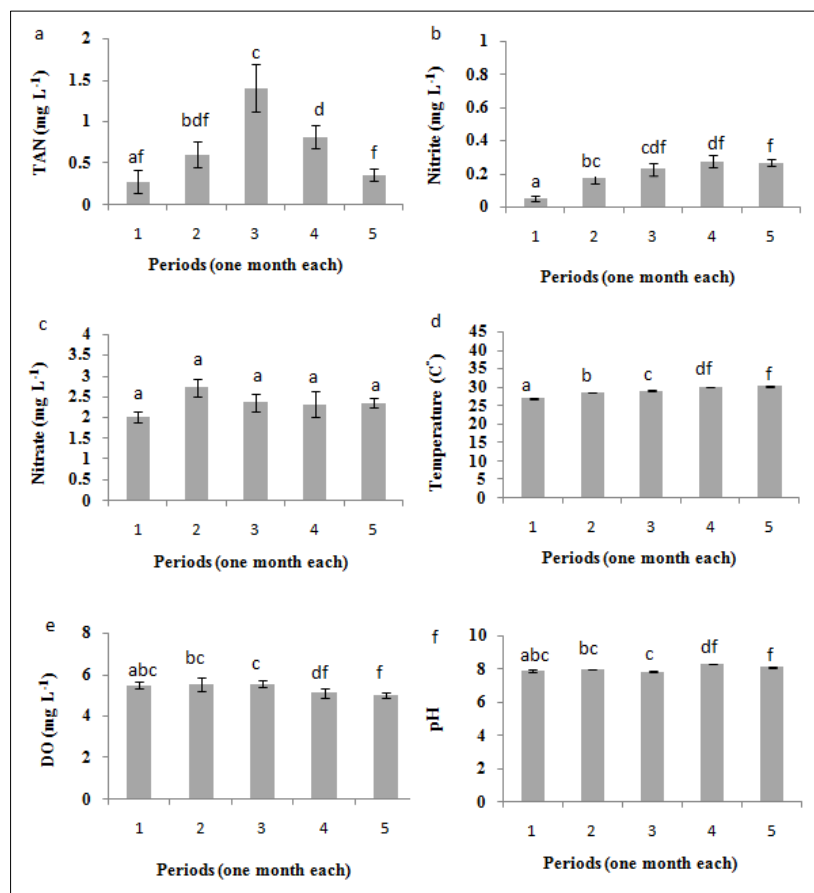


Fig 3: The concentration levels (mean±SE, n=3) of a) total ammonia nitrogen (TAN, mg L⁻¹), b) nitrite (NO₂⁻ mg L⁻¹), c) nitrate (NO₃⁻ mg L⁻¹), d) temperature (°C), e) dissolved oxygen (DO mg L⁻¹) and f) pH for ponds waters of shrimp *L. vannamei* throughout 5 months of culture period. Different superscripted letters on the bars indicate significant differences during 5 months of culture (P > 0.05).

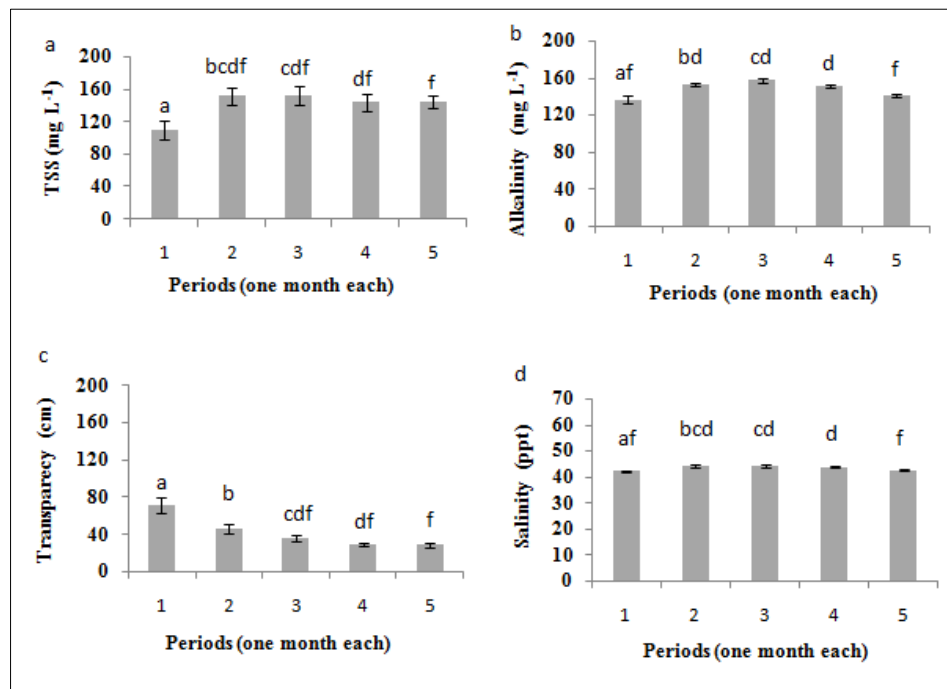


Fig 4: The variation (mean±SE, n=3) of a) total suspended solids (TSS mg L⁻¹), b) alkalinity (mg L⁻¹), c) transparency (cm) and d) salinity (ppt) in ponds waters of shrimp *L. vannamei* throughout 5 months of culture period. Different superscripted letters on the bars indicate significant differences between months ($P > 0.05$).

The highest mean values of temperature (30.22 ± 0.06 °C) and salinity (44.29 ± 0.64 ppt) were observed after 2nd and 5th months, respectively (Figure 3d and 4d). Whereas, lowest values were 26.93 ± 0.06 °C and 42.17 ± 0.64 ppt, for temperature and salinity respectively after 1st month (Figure 3d and 4d). It seemed that salinity levels perhaps affected by temperature values of water during measurement time. The gradually increasing of temperature started from 1st month to reach to the maximum value in last month of culture in both biofloc and control treatments ponds (Table 1). Temperature differed significantly in values among 5th month and both 1st, 2nd and 3rd months (Figure 3d). Whilst, the significantly variation in salinity was observed among 2nd, 1st and 5th months (Figure 4d). The range of salinity was (42.08– 46.62 ppt) in biofloc treatment ponds and (41.00– 43.00 ppt) in water exchange ponds (Table 1). An increasing trend in salinity was observed in biofloc ponds compared to water exchange ponds throughout cultural period except last month. At the end of experiment (last month), salinity mean value (42.08 ± 0.52 ppt) in biofloc pond was controlled by biofloc treatment and became less than mean value (43.00 ± 0.43 ppt) in water exchange pond (Table 1).

Generally, the observations and two-way ANOVA statistical analysis showed very high significant differences ($P < 0.001$) in transparency mean levels between the biofloc treatment ponds and control ponds. The maximum level of transparency in biofloc pond was 36.67 ± 12.14 cm recorded in mid culture period (3rd month) while in control pond was 109.17 ± 9.36 cm recorded at the beginning of culture period (1st month) (Table 1). Figure 4c shows decreasing trend in transparency value with rearing period. The maximum level of transparency was 71.25 ± 8.78 cm after 1st month of culture and showed minimum level of 28.67 ± 12.32 cm (Figure 4c). Two-way ANOVA found clear significant differences in transparency levels in the first month of culture rather than other months (Figure 4c).

Also, statistically high significant two-way interaction in

most parameters of water quality such as concentrations of TAN ($P < 0.001$), nitrite ($P < 0.01$), DO ($P < 0.001$), pH ($P < 0.001$), salinity ($P < 0.001$) and transparency level ($P < 0.05$) between treatments and period of culture (months) (Table 1). In contrast, two-way ANOVA indicated that statistically significant interaction in TSS value ($P < 0.05$) (Table 1). However, no statistically significant two-way interaction in concentrations of nitrate ($P > 0.05$), alkalinity ($P > 0.05$) and temperature ($P > 0.05$) between treatments and period of shrimp rearing (months) (Table 1).

Growth Performance Parameters

There were very high significant differences ($P < 0.001$) found in growth parameters such as weight gain (WG), daily weight gain (DWG) and production between biofloc treatment and control (Table 2). In contrast, two-way ANOVA revealed a statistically significant variation in specific growth rate (SGR) between biofloc and control. The values of weight gain (g), daily weight gain (g d⁻¹) and production (MT pond⁻¹) were high (3.55 ± 0.13 g, 0.122 ± 0.01 g d⁻¹ and 13.27 ± 1.6 MT pond⁻¹, respectively) for shrimp reared in ponds waters treated with biofloc and low (3.02 ± 0.19 g, 0.104 ± 0.01 g d⁻¹ and 11.89 ± 5.34 MT pond⁻¹, respectively) in control (Table 2). Shrimp reared in ponds waters treated with biofloc also showed higher value ($4.97 \pm 0.53\%$ d⁻¹) of specific growth rate than control value ($4.77 \pm 0.52\%$ d⁻¹) (Table 2). However, treatments did not show significant effect ($P > 0.05$) on both feed conversion ratio (FCR), protein efficiency ratio (PER) and survival (Table 2). Although, FCR was lower (0.77 ± 0.13) in biofloc treatment and higher (0.797 ± 0.11) in control (Table 2). In contrast, protein efficiency ratio (PER) and survival were elevated ($3.20 \pm 0.32\%$ and $88.4 \pm 2\%$, respectively) in biofloc and reduced in control (3.09 ± 0.32 and $87.4 \pm 2.03\%$, respectively) (Table 2). Results of this study revealed very high significantly differences ($P < 0.001$) in all growth parameters among periods culture (months) (Table 2).

The highest significant ($P < 0.001$) weight gain and daily weight gain results (4.08 ± 0.08 g and 0.14 ± 0.003 g d⁻¹, respectively) were recorded for *L. vannamei* post larvae after 2nd month of culture, then after 3rd month (3.50 ± 0.07 g and 0.121 ± 0.002 g d⁻¹, respectively), while after 4th month (3.14 ± 0.08 g and 0.11 ± 0.003 g d⁻¹, respectively), after 5th month (2.98 ± 0.44 g and 0.103 ± 0.02 g d⁻¹, respectively) and after 1st month (2.72 ± 0.10 g and 0.09 ± 0.003 g d⁻¹, respectively) (Table 2).

Specific growth rate (SGR) values decreased significantly ($P < 0.001$) with culture period, with a maximum value ($8.38 \pm 0.11\%$ d⁻¹) for shrimp after 1st month of culture (Table 2). While, production values increased significantly ($P < 0.001$) with culture period (months), with highest value (19.944 ± 762.5 MT pond⁻¹) in shrimp after 5th months of rearing period (Table 2).

Very high significant difference ($P < 0.001$) was also observed in protein efficiency ratio (PER) among post larvae rearing periods. The maximum value (5.26 ± 0.46) was recorded after 5th month, followed after 1st month (3.22 ± 0.07), then after 4th month (2.73 ± 0.18) and after 3rd month (2.30 ± 0.18) and after 2nd month of culture (2.22 ± 0.25).

The lowest results ($P < 0.001$) of FCR (0.43 ± 0.04 and 0.81 ± 0.04 g d⁻¹) were recorded in shrimp after 5th and 4th months of culture respectively and the highest value (1.02 ± 0.08) was observed after 2nd month (Table 2).

Survival percentage decreased significantly ($P < 0.001$) with culture period, with a maximum value (100%) was recorded after 1st month of culture, and a minimum value ($81 \pm 0.93\%$) after 5th months (Table 2).

The results of two-way ANOVA showed that statistically high significant interaction in some growth parameters. Among of them, weight gain, daily weight gain, specific growth rate and production between treatments (biofloc and

control) and culture period (months) (Table 2). Whilst, no statistically significant two-way interaction in values of protein efficiency ratio (PER), feed conversion ratio (FCR) and survival ($P > 0.05$) between treatments and period of shrimp culture (months) (Table 2).

The end of experiment (after 5th months) recorded the highest growth in biofloc treatment compared with control as shown in Table 3. Mean weight gain, mean daily weight gain and specific growth rate were higher (17.47 ± 0.24 g, 0.117 ± 0.002 g d⁻¹ and $1.98 \pm 0.008\%$ d⁻¹, respectively) in biofloc treatment than control (14.83 ± 0.40 g, 0.0995 ± 0.003 g d⁻¹ and $1.88 \pm 0.017\%$ d⁻¹, respectively) (Table 3). ANCOVA analysis also indicated to find significantly values of both weight gain ($df = 1; f = 27.52; P < 0.05$), daily weight gain ($df = 1; f = 27.66; P < 0.05$), and specific growth rate ($df = 1; f = 28.01; P < 0.05$) between biofloc and control. At the end of the experiment, shrimp production in pond was also elevated (21620.93 ± 356.3 kg pond⁻¹) in biofloc treatment and reduced to (18300.77 ± 495.7 kg pond⁻¹) in control (Table 3). Results of ANCOVA analysis also showed that rearing of shrimp in waters treated with biofloc had significant effect on production ($df = 1; f = 22.55; P < 0.05$) (Table 3). After 5th months of experiment, the protein efficiency ratio was higher (1.70 ± 0.031) in biofloc treatment and lower (1.49 ± 0.043) in control. In contrast, FCR value (1.28 ± 0.023) in biofloc was lower than (1.46 ± 0.040) in control. So, treatment of shrimp ponds culture with biofloc had significant effect on FCR ($df = 1; f = 14.04; P < 0.05$) and PER ($df = 1; f = 12.93; P < 0.05$). However, results of ANCOVA analysis did not show significant differences in survival between shrimp in ponds treated with biofloc and control ($df = 1; f = 0.107; P > 0.05$). Although, survival was higher ($81.33 \pm 0.88\%$) in biofloc treatment and lower (80.33 ± 1.45) in control.

Table 2: Two-way ANOVA (analysis of variance) of growth parameters during study. Treatments (biofloc and control) and periods of culture (5 months) were considered as factors for ANOVA. Means (\pm SE, n=3), very high significant = $P < 0.001$, high significant = $P < 0.01$, significant = $P < 0.05$ and not significant = $P > 0.05$.

Parameters	Treatment			Periods (one month each)					Treatment*period	
	Biofloc	Control	P value	1	2	3	4	5	P value	P value
Weight gain (g)	3.55 ± 0.13^a	3.02 ± 0.19^b	0.000	2.73 ± 0.10^a	4.08 ± 0.08^b	3.50 ± 0.07^{cd}	3.14 ± 0.08^{df}	2.98 ± 0.44^{af}	0.000	0.000
Daily weight gain (g d ⁻¹)	0.122 ± 0.01^a	0.104 ± 0.01^b	0.000	0.09 ± 0.003^a	0.14 ± 0.003^b	0.121 ± 0.002^c	0.11 ± 0.003^{cdf}	0.103 ± 0.02^{af}	0.000	0.000
Specific growth rate SGR (% d ⁻¹)	4.97 ± 0.53^a	4.77 ± 0.52^b	0.013	8.38 ± 0.11^a	5.5 ± 0.06^b	4.12 ± 0.02^c	3.31 ± 0.03^d	3.02 ± 0.04^f	0.000	0.000
Production (MT pond ⁻¹)	13.27 ± 1.6^a	11.89 ± 5.34^b	0.000	4.216 ± 138.7^a	9.080 ± 220.8^b	13.130 ± 217.31^b	16.527 ± 274.94^a	19.944 ± 762.5^c	0.000	0.000
Protein efficiency ratio (PER)	3.20 ± 0.32^a	3.09 ± 0.34^a	0.673	3.22 ± 0.07^a	2.22 ± 0.25^b	2.30 ± 0.18^b	2.73 ± 0.18^{ab}	5.26 ± 0.46^c	0.000	0.772
Feed conversion ratio (FCR)	0.77 ± 0.127^a	0.797 ± 0.110^a	0.662	0.678 ± 0.015^a	1.02 ± 0.08^b	0.98 ± 0.10^{ab}	0.81 ± 0.04^a	0.43 ± 0.04^c	0.000	0.718
Survival (%)	88.4 ± 2.00^a	87.4 ± 2.03^a	0.980	100 ± 0.00^a	94.00 ± 0.58^b	85.00 ± 0.58^c	82.00 ± 0.73^d	81.00 ± 0.93^d	0.000	0.980

Table 3: ANCOVA (analysis of covariance) to determine the effect of adding the biofloc in ponds waters of shrimp on growth parameters after 5 months of culture. (Mean±SE, n=3), very high significant = $P < 0.001$, high significant = $P < 0.01$, significant = $P < 0.05$ and not significant = $P > 0.05$.

Growth parameter	Treatment		ANCOVA analysis		
	Biofloc	Control	df	f	P value
Initial weight (g)	0.27±0.006	0.257±0.009			
Final weight (g)	17.74±0.24 ^a	15.09±0.40 ^b			
Weight gain (WG) (g)	17.47±0.24 ^a	14.83±0.40 ^b	1	27.52	0.013
Daily weight gain (DWG) (g d ⁻¹)	0.117±0.002 ^a	0.995±0.003 ^b	1	27.66	0.013
Specific growth rate (SGR) (% d ⁻¹)	1.98±0.008 ^a	1.88±0.017 ^b	1	28.01	0.013
Production (MT pond-1)	21.621±356.3 ^a	18.300.77±495.7 ^b	1	22.55	0.018
Feed conversion ratio (FCR)	1.28±0.023 ^a	1.46±0.040 ^b	1	14.04	0.033
Protein efficiency ratio (PER)	1.70±0.031 ^a	1.49±0.043 ^b	1	12.93	0.037
Survival (%)	81.33±0.88 ^a	80.33±1.45 ^a	1	0.107	0.765

Discussion

Biofloc forms in shrimp and fish pond culture consist of diverse microbial community which plays an important role in decrease the toxic levels of nitrogen resources such as TAN and NO₂-N to low levels and decrease hazards of diseases and improve growth and survival (Vinatea *et al.*, 2010)^[66]; (Rajkumar *et al.*, 2015)^[52]; (Yun *et al.*, 2015) [not in list]; (Adipu *et al.*, 2019)^[1]. In recent study, the shrimp reared in ponds treated with biofloc had significantly lower levels of TAN and NO₂-N than control ponds. On the contrary, the levels of NO₃-N were higher in biofloc than in control. Previous study by Yuniartik *et al.* (2015)^[69] also observed that nitrate (NO₃-N) in biofloc ponds were significantly higher than control ponds. The higher levels of NO₃-N was expected since NO₃-N is the end product of nitrification. These results indicated that NO₃-N was not a limiting growth factor for *L. vannamei*.

Regardless of forming food for shrimp, the biofloc preserved low levels of TAN and NO₂-N (Yun *et al.*, 2015) [not in list]; (Panigrahi *et al.*, 2019)^[46]. Yuniartik *et al.* (2015)^[69] confirmed that TAN and NO₂-N in biofloc ponds were significantly lower than control. These results showed that there were interactions between TAN and nitrite in water and this correlation is accepted since ammonia is converted to nitrite by nitrifying bacteria. Results of present study reported that levels of TAN and NO₂-N were maintained below save levels (6.1 and 5.1 mg L⁻¹, respectively) for shrimp *L. vannamei* culture (Lin and Chen, 2003)^[38]. Implying the benefit of biofloc in enhancing water quality due to bacterial community activity in the pond culture (Silva *et al.*, 2013)^[59]; (Panigrahi *et al.*, 2019)^[46]. The low values of TAN and NO₂-N, recorded during this study may be due to oxidation of ammonia to nitrate by heterotrophic and autotrophic bacteria within biofloc (Cohen *et al.*, 2005)^[21]; (Supono *et al.*, 2014)^[63, 64]. So, ammoniacal nitrogen and nitrite in control pond (without biofloc system), will be preserved at elevated level due to more feed given into this system. This lead to unbalanced water quality. On the contrary, the microbial floc has a positive influence on culture pond throughout maintenance of water quality and improving productivity through serving as a supplementary food source (Wasielesky *et al.*, 2006)^[67]; (Xu *et al.*, 2012)^[68, 69]; (Emerenciano *et al.*, 2012)^[27]; (Silva *et al.*, 2013)^[59]; (Yuniartik *et al.*, 2015)^[69]; (Panigrahi *et al.*, 2019)^[46].

The current study observed that dissolved oxygen at biofloc ponds was significantly lower ($P < 0.05$) than the control. The previous studies also showed an increase in biological oxidation of waste material within biofloc by bacterial floc which need more oxygen in order to degrade it (Azim and

Little, 2008)^[8]; (Azim *et al.*, 2008)^[9]. Degradation of this waste material in biofloc, that is fundamentally protein, certainly need carbon source such as molasses, coffee waste, rice bran, grain pellets, flour or calcium carbonate to conserve the balance C/N ratio (Yuniartik *et al.*, 2015)^[69]; (Becerril-Cortés *et al.*, 2018)^[11]. Despite the levels of dissolved oxygen at biofloc ponds were slightly lower than control ponds levels, but its levels of dissolved oxygen was maintained between 5 and 6.18 mg L⁻¹ in biofloc and control treatments. These levels were within the optimal limits for the survival and growth of *L. vannamei* shrimp (McGraw *et al.*, 2001)^[41].

Results of current study did not differ significant in survival rate among biofloc and control treatment. However, shrimp reared in waters treated with biofloc had significant effect on growth parameters during period of study. These may be due to contribution of biofloc in enhancing productivity include phytoplankton and zooplankton as additional food sources in cultural ponds (Chen and Chen, 1992)^[20]; (Xu *et al.*, 2012)^[68, 69]; (Silva *et al.*, 2013)^[59]; (Panigrahi *et al.*, 2019)^[46].

Commonly, the acceptable variation of temperature values in study area was expected since all the ponds were exposed and affected on the monthly and seasonally changes in weather. The highest value (30.22 °C) and lowest value (26.93 °C) of temperature were recorded during this study, which were also suitable for survival and growth of shrimp (Ponce-Palafox *et al.*, 1997)^[50]. In contrast, results of this study did not show any differ significant in temperature among biofloc and control treatment. Whereas, value of pH at control treatment was significantly higher than biofloc treatment. Also, significantly monthly variation in pH value was observed in this study. This result was degree with result of study conducted by Yuniartik *et al.* (2015)^[69]. These results also confirmed that biofloc could provide optimal water quality for aquaculture (Crab *et al.*, 2012)^[24]. Therefore, pH values in this study were within the recommended range of 7.5-8.5 for penaeid shrimp (Chanratchakool *et al.*, 1995) [not in list]. Although, very high significant differences were found, biofloc seemed to be adequate to control salinity fluctuations when compared to water exchange treatment that depends on changes of water. The higher values of salinity in biofloc ponds were expected. Water evaporation with less compensation is considered the main raising factor of salinity in biofloc ponds while the continually seawater change process in control ponds leads to decrease salinity. Salinity is the major variable could be used for understanding how renew water input affects salinity amount. At the end of experiment, salinity in biofloc pond was less in water exchange pond and this to indicate that biofloc was more

efficient to control salinity variation (Table 1). In present study, the salinity mean values were in an acceptable range for *L. vannamei* culture. According to Briggs *et al.* (2004)^[17], *L. vannamei* tolerates a wide range of salinities (0.5-45 ppt) and more suitable to inland culture sites than *P. monodon* or *L. stylirostris*. In both treatment ponds with higher TSS, the lower values of transparency were observed and vice versa (Figure 2a & 2b). Transparency levels were found for both treatments remained within considered ideal level for growth of *L. vannamei*.

Alkalinity recorded (151.47 and 144.08 mg L⁻¹) in both biofloc and control treatment respectively (Figure 2b). These values of alkalinity were considered higher than the recommended value (130 mg L⁻¹) for provide growth and survival of shrimp culture (Boyd *et al.*, 2002)^[15]. Therefore, this study did not show significantly difference in survival rate between biofloc and control treatment. However, significant differences observed between biofloc and control treatment in growth parameters. It might be due to higher value of alkalinity (151.47 mg L⁻¹) in biofloc system more than control (144.08 mg L⁻¹). During this study, the lower values of pH recorded in both treatments where the alkalinity levels were decreased by consuming. The higher alkalinity and pH values presented in biofloc ponds may have contributed to an increase in TSS content that may formed of microbial aggregates. High accumulation of total suspended solids in biofloc ponds compared to control ponds was suspected because of limited water exchange and molasses additions, which create blooms and microbial community. Although, TSS had its peak in biofloc ponds, but the highest level was still less than 200 mg L⁻¹ (Figure 2a). According to Samocha *et al.* (2007)^[56] and Gaona *et al.* (2012)^[32], the recommended levels of TSS is in the range of 200-500 mg L⁻¹, while the consumption of the best feed in shrimp biofloc systems takes place at good solids concentration of 100 to 300 mg L⁻¹ (Hargreaves, 2013)^[35]. Therefore, TSS values in present study were in optimal range at biofloc production systems for aquaculture.

The use of biofloc system in shrimp *L. vannamei* aquaculture had a significant effect on the growth performance and final individual shrimp weight ($P < 0.05$). This significant effect did not find in survival rate ($P > 0.05$). The results of growth parameters such as WG, DWG, SGR, PER and FCR in waters treated with biofloc were significantly higher than the results in control treatment. These might be due to the synergistic effects of the enhanced water quality, increasing bacterial and zooplankton densities in ponds waters. Yuniartik *et al.* (2015)^[69] noted that the biofloc system have offers benefits in improving water quality and conserve it optimal to support shrimp growth. Previous study by Ray *et al.* (2010)^[53] also confirmed that biofloc particles that form in ponds waters contains a variety of microorganisms such as bacteria, microalgae, protozoa and zooplankton. These bacteria may be belonging to heterotrophic and autotrophic organisms that oxidize ammonia-nitrogen and nitrite into nitrate and increase of accumulate protein in bacterial cell (Supono *et al.*, 2014)^[63, 64]; (Becerril-Cortés *et al.*, 2018)^[11]. Microbial floc does not only enhance water quality but also leads to reducing feed costs by providing nutrition (Emerenciano *et al.*, 2013)^[28]. The results of this study showed that the growth of shrimp was affected by adding carbon, nitrogenous and

phosphorus sources such as molasses, urea, diamonium phosphate (DAP), and to develop biofloc in pond waters. Crab *et al.* (2012)^[24] reported that the carbon source added for the improving of the biofloc encourages specific bacteria, protozoa and microalgae and subsequently influences the microbial composition of the biofloc in additional their nutritional properties. Urea and ammonium Diammonium phosphate are also used separately or together as nitrogen and phosphorus resources in aquaculture (Chakrabarty *et al.*, 1975)^[19]; (Boyd, 1990)^[16]; (Subosa and Bautista, 1991)^[62]; (Suwanpakdee *et al.*, 2010)^[65]. To embed microalgae communities that are a primary food of zooplankton, larval mollusca and crustaceans (Preston *et al.*, 1992)^[51]. Saha and Chatterjee (1975)^[54] also found the increase of zooplankton density with urea was added into the cultural ponds. Shrimp may not devour microalgae and zooplankton directly. However, when zooplankton and algal cells die and they become integrated with some microorganisms such as bacteria, protozoan and so on, to make the detritus masses large enough to be picked up and consumed by shrimp, this detritus is entire of nutritive nutrients for shrimp growth (Burford *et al.*, 2004)^[18].

During the period of culture, the better FCR was observed for shrimp reared in waters treated by biofloc with significantly higher in weight gain (g), daily weight gain (g d⁻¹) and SGR (% d⁻¹) than control. These might be due to positive effect of microbial community in this system like improving the productivity by serving as a complementary food source. Several authors have reported an important role of microbial community in biofloc culture system among of them enhancing the water quality and improving natural production by serving as a complementary food source (Wasielesky *et al.*, 2006)^[67]; (Emerenciano *et al.*, 2012)^[27]; (Xu *et al.*, 2012)^[68, 69]; (Silva *et al.*, 2013)^[59]; (Panigrahi *et al.*, 2019)^[46].

Previous study conducted by Yuniartik *et al.* (2015)^[69] was also found that significantly higher shrimp growth (SGR) in biofloc systems than the control treatment. This denotes that microbes within biofloc provide in situ proteinaceous feed for shrimp (Schram *et al.*, 2009)^[58]; (crab *et al.*, 2010)^[25]; (crab *et al.*, 2012)^[24]. The highest shrimp production at the end of culture period comes from enhancing growth parameters such as SGR, WG, DWG and PER. These might be due to the ability of bacterial floc to degrade organic matter from the excess of feed and feces of shrimp (Ekasari *et al.*, 2014)^[26]; (Anand *et al.*, 2014)^[4].

At the end of culture period, the lowest feed conversion ratio was observed. It may be due to the improvement of the digestive system of the cultured shrimp in biofloc systems as a result of high rates of digestion and absorption of the food provided to it. Moss *et al.* (2001)^[44] and Xu and Pan (2012)^[69] have suggested that microbial floc could assist in activities enzymes of shrimp digestion and lead to elevate digestion and absorption of shrimp feed. Generally, it exhibited that biofloc system could effectively utilize the feed presented for shrimp. In addition, biofloc system is one of the most useful resolutions to aquaculture problems because it can convert uneaten feed and other organic matters into protein which collected in microbial cell and consumed by the shrimp. So, biofloc has been demonstrated that provides multi-useful tasks for target shrimp such as improvement of water quality, higher shrimp growth and increasing of feed efficiency.

Conclusion

It is clear that the using biofloc technology in shrimp culture during this study lead to increase yield production. It was observed that the administration of water exchange system at high densities could not significantly enhance growth parameters comparing to biofloc system through this experiment. This study concluded that the biofloc system increases aquaculture production with less impact on the environment, which in turn contributes to sustainable development. In general, the results of present study suggest that the implementing biofloc technology is feasible for shrimp production in the desert environmental conditions of the Kingdom of Saudi Arabia.

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