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Evaluation of Biofloc Technology Effects on White Leg Shrimp, *Litopenaeus vannamei* Growth Performance and Water Quality of Super-intensive Culture Ponds

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Abstract: Six grow-out ponds were designed with an area of 0.3 ha per pond. Each pond was stocked by 500 individual/m^2 of *Litopenaeus vannamei* with an average weight of 0.26 g. Three ponds were used for biofloc technology (BFT) and other three were used for water exchange system (control). Waters of BFT were filtered, aerated, and fertilized by organic and inorganic materials to create blooms of microbial community, while the control waters were stocked without any fertilizers. In a period of over 140 days (20 weeks), the effects of BFT were evaluated on *L. vannamei* growth performance as well as water quality parameters. All physicochemical parameters, shrimp growth parameters, survival, and feed conversion ratio (FCR) were measured. Results showed significant differences ($p < 0.05$) in physicochemical parameters, shrimp growth parameters, FCR, and final production were observed between biofloc and control. The best increase ($p<0.05$) in average final weight (17.7400±0.2389 g), FCR (1.8067±0.0968%) and average final production (13920±516.68 kg/pond) in biofloc ponds compared to average final weight (15.0900±0.3952 g), average feed conversion ratio (2.4033±0.1642%) and average final production (11217±605.29 kg/pond) were recorded in control ponds. *L. vannamei* that reared in biofloc technology, showed better results for super-intensive culture with positive effects on water quality.

Keywords: Super-intensive; Biofloc; *Litopenaeus vannamei*; Growth Performance; Water Quality

1. Introduction:

Litopenaeus vannamei is found abundantly in coastal waters of the eastern Pacific Ocean from the Mexican state of Sonora as far south as northern Peru [1]. There are no native species of *Litopenaeus* at the eastern Mediterranean Sea, Red Sea, Arabian Sea, Persian Gulf, and the northwest Indian Ocean. This species is already presented in marine ecosystems outside of its native range [2,3].

Water quality maintenance in shrimp culture is very important to achieve optimum shrimp production [4]. The water exchange is the common method to reduce the accumulation of ammonia and organic matter [5] and to prevent deterioration in pond water quality [6]. However, aquaculture wastes may contain a variety of constituents, including dissolved or particulate organics, nutrients, and specific organic or inorganic compounds that could cause poor impact when discharged into the environment [7]. Moreover, the new water that was introduced into the ponds will cause high mortality due to the change in water quality suddenly [8]. Therefore, reducing water exchange is an adaptive solution to minimize the possibility of disease transmission in shrimp ponds [9].

Increasing concerns over negative environmental impacts from shrimp farm effluents, along with widespread outbreaks of disease, have led to the development of culture systems with minimal or zero water exchange [10, 11]. With little or no water exchange, properly managed Recirculating Aquaculture Systems (RAS) can reduce or eliminate the amount of nutrients and pathogens released into receiving streams [12].

The intensive development of the aquaculture industry has been accompanied by an increase in environmental impacts. The production process generates substantial amounts of polluted effluent; contains uneaten feed and faces [13]. Discharges from aquaculture into the aquatic environment contain nutrients from various organic and non-organic compounds such as ammonium, phosphorus, dissolved organic carbon, and organic matter [7, 14]. The high level of nutrients causes environmental deterioration of the receiving water bodies. In addition, the drained water may increase the occurrence of pathogenic microorganisms and introduce invading pathogenic species [15].

Biofloc systems are a unique type of recirculation aquaculture system (RAS) that maintains a population of suspended microalgae, autotrophic and heterotrophic bacteria formed in systems of limited-exchange [12, 16]. Operating high-density shrimp culture systems with limited or no water exchange results in the production of large volumes of suspended flocculated organic particles recently referred to as biofloc [6, 16,17].

Biofloc technology (BFT) is a rearing system with little or no water exchange. In such a system, a conglomerate of microbes, algae, and protozoa develops in the water column, along with detritus and dead organic particles [18]. On other words, the biofloc technology is characterized by the conversion of the nitrogen supplied by food, which is not eaten and by the excreta of cultivated organisms which together, with the addition of organic carbon sources will be converted into microbial protein available as additional food in the culture environment and allowing for improvement in the feed conversion rates [19,20]. The advantages that these systems provide are a reduction in the demand for water and a consequent reduction in the emission of effluents, minimizing the impact on the environment. In addition, BFT increases the biosecurity of the culture by reducing the introduction and spread of disease and by providing the nutritional benefits of the natural productivity of biofloc [19,21]. Generally, inorganic nitrogen accumulation is controlled through the addition of carbonaceous substrates, raising the C/N ratio and leading to the immobilization of nitrogen through the production of microbial proteins [22-25].

Although there are several researches and studies concerned on the biofloc technology (BFT) in aquaculture but there has been a few studies about the *L. vannmei* culture under commercial scale with super-intensive. On other words, most studies about BFT were carried out in small trials and under controlled conditions. Therefore, this study is a new trial in commercial-scale to use (500 shrimp/m^2) using biofloc technology to investigate the influences of the biofloc technology on the water quality and shrimp yield performance.

The main aim of this study is to assess the potential of biofloc technology (BFT) in super-intensive culture on enhancement of *L. vannamei* shrimp rearing and to find out its effects on the water quality parameters and shrimp yield performance during the grow-out culture.

2. Materials and Methods:

2.1 The Experimental Background:

The entire experiment was carried out at national aquaculture group (NAQUA) which is located 160 Km south of Jeddah, Saudi Arabia. The culture period was over 140 days (20 weeks). The hatchery at NAQUA provided the experiment by post larvae of *L. vannamei* (0.25 – 0.28 g) transferred to grow-out ponds (experimental ponds) using trucks with fiber glass tanks filled by fresh seawater, provided by oxygen cylinders, one technician to monitor the dissolved oxygen level and shrimp condition.

2.1.1 Pond Design:

The experimental setup was designed to classify the ponds into two sections, three ponds were marked as biofloc (B1, B2, and B3), and the other three ponds were marked for water exchange system (C1, C2, and C3). All ponds were lined with high-density polyethylene (HDPE), with an area of 0.3 ha and 1.4 m average depth for each pond, the slope designed toward the harvest gate, and the center of the pond for concentrating the sludge. The drainage pipe was located in the center to drain the wastes and sludge.

2.1.2 Filtration System:

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

2.1.3 Fertilization:

The fertilization was done only in B1, B2 and B3 (biofloc technology ponds) using diamonium phosphate (DAP), urea, and molasses; every fertilizer was mixed with seawater and separated it manually in pond waters. The dosages of fertilizer were applied according to [18].

2.1.4 Feeding Management:

Water exchange and biofloc systems ponds were fed with the same feed 35% crud protein, at the beginning of the experiment, the shrimp had an average weight 0.26 g. The rearing units (ponds) consist of 6 grow out ponds and were stocked at an initial stocking density 500 individual/m^2.

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. Feed was given in a daily four meals using auto-feeder [26].

Molasses was also added at a rate 50% of feed applied to each biofloc pond to maintain an optimum C:N ratio (10:4)

[12,27]. The Molasses spread to the water surface in the afternoon, and completely mixed with the water of each biofloc pond using a strong aeration system.

Water exchange system ponds was done with regular water exchange daily at the rate of 20% of the existing water level, whereas biofloc ponds water level also maintained by adding new seawater compensate the evaporation loss and salinity increment.

2.1.5 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 [21], 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 wastes toward the central drain pipe where the siphoning pipe there.

2.2 Measurements of Parameters:

2.2.1 Water Quality Parameters :
This is a considerable step in getting a successful of aquaculture. Physico-chemical parameters during the experimental period were monitored according to APHA [28], temperature and dissolved oxygen were measured twice a day (5 am and 5 pm) using D.O meter YSI model 58. The pH was measured used a digital pen pH meter AT130. The salinity was measured once a day at 11 am using refractometer model 101 ATC, and transparency was measured by Secchi disc. According to APHA [28], total ammonium nitrogen (TAN), nitrite-nitrogen (NO_2–N) and nitrate-nitrogen (NO_3-N) concentrations (mg/L) were measured every week at central analytical service (CAS) in national aquaculture group (NAQUA).

2.2.2 Shrimp Performance Indicators:
Sampling was carried out every 15 days to estimate survival rate (populations counting). 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. The calculation was done based on the following formula:

Pop. 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. The calculations were done based on the following equations:

Mean weight gain (g/shrimp) = final mean weight – initial mean weight [29].

Average daily weight gain (ADG) (g/d) = (Final mean weight (g) - Initial mean weight (g) / rearing period in days [30].

Specific growth rate (SGR) (%/d) = [(ln final weight - ln initial weight) x 100 / rearing period in days [31].

Final mean production (Kg/pond) = Total biomass at harvest / numbers of ponds [30].

Feed conversion ratio (FCR) = Dry weight of feed given (kg) / weight gain (kg) [31].

Survival (%) = final number of shrimp harvested x 100 / initial number of shrimps stocked.

2.3 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.

2.4 Statistical Analysis:
Water quality and performance parameters of shrimp in different treatments were subjected to analysis of repeated measure ANOVA ($P < 0.05$) considering the assumptions required for their application (have normality and homoscedasticity of the data). The other parameters were subjected to one-way ANOVA ($P < 0.05$). For the parameters that were detected with significant difference, the Tukey test for separation of means was applied ($P < 0.05$).

3. Results:

3.1 Water Quality Parameters:
The weekly data of all physicochemical parameters at the surface water for all sampling ponds of *L. vannamei* culture is shown in Table 1. The values of means are the triplicate results of the three ponds of both biofloc and water exchange (control) treatments during the period of culture (Table 2).

All ponds showed more or less the same level of variation in the range values of temperature (22 - 32 ºC). The range of dissolved oxygen during this study was (2.3 – 9.9 mg/L). The lowest value was recorded in biofloc pond (2.3 mg/L) and the highest value (9.9 mg/L) was found in control pond. It appears that the maximum value of dissolved oxygen in control treatment ponds were slightly higher than the maximum value of dissolved oxygen in biofloc treatment ponds. While the minimum value of dissolved oxygen in control treatment ponds were almost near to the same levels of the minimum value of dissolved oxygen in biofloc treatment ponds (Table 1). One-way ANOVA analysis showed that the dissolved oxygen results were significantly different ($P < 0.05$) among sampling weeks.

Table 1. Maximum and minimum values of physicochemical parameters performed during *L. vannamei* grow-out in biofloc and water exchange ponds

	Systems			
Parameters	Biofloc		W. Exchange (Control)	
	Max.	*Min.*	*Max.*	*Min.*
Temperature (ºC)	32	22	32	22
Dissolved Oxygen (mg/L)	6.5	2.3	9.9	3.1
pH	8.8	7.2	8.8	7.5
Salinity (ppt)	50	40	45	40
Transparency (cm)	90	15	20	130
TAN (mg/L)	1.31	0.01	5.31	0.01
NO_2–N (mg/L)	0.43	0.01	0.85	0.01
NO_3–N (mg/L)	6.30	1.37	8.20	0.41

The highest and lowest values of pH in biofloc ponds were (8.8) and (7.2), respectively. While water exchange ponds recorded the highest value of pH (8.8) and the lowest one was (7.5) (Table 1). Generally, the observations and statistical analysis showed that the changes in pH values between the biofloc treatment ponds and control ponds were not significantly different ($P > 0.05$) (Table 2).

The range of salinity was (40– 50 ppt) with a mean value (44.26 ppt) in biofloc treatment ponds and (42.11 ppt) in water exchange ponds. The maximum concentration (50 ppt) was recorded in biofloc pond. This is to indicate that an increasing trend in salinity was observed in biofloc ponds compared to water exchange ponds and it reached to a maximum of 50 ppt during the period of culture. One-way ANOVA indicated to different significantly ($p < 0.05$) in values salinity between control and biofloc treatment (Table 2).

Water transparency values during culture period were ranged from 15 to 90 cm in biofloc treatment ponds with the mean value (31.13 cm), and the range in water exchange ponds was (20 – 130 cm) with the mean value (54.30 cm) (Table 1&2). A significantly difference in transparency values were found in pond waters among control and biofloc treatments. Although transparency values, recorded in this study, were higher but they still within the acceptable ranges for most penaeid species surviving in conditions of biofloc system.

Table 2. Mean, standard error (±SE) values of physicochemical parameters performed during *L. vannamei* grow-out in biofloc and water exchange ponds

	Systems			
Parameters	Biofloc		W. Exchange (Control)	
	Mean	SE	Mean	SE
Temperature (ºC)	29.08	± 0.16 [a]	29.08	± 0.16 [a]
Disso. Oxygen (mg/L)	4.53	± 0.06 [a]	6.29	± 0.05 [b]
pH	7.90	± 0.20 [a]	8.06	± 0.01 [a]
Salinity (ppt)	44.26	± 0.19 [a]	42.11	± 0.08 [b]
Transparency (cm)	31.13	± 1.20 [a]	54.30	± 2.20 [b]
TAN (mg/L)	0.27	± 0.04 [a]	1.12	± 0.11 [b]
NO_2–N (mg/L)	0.12	± 0.01 [a]	0.27	± 0.01 [b]
NO_3–N (mg/L)	2.31	± 0.07 [a]	2.28	± 0.10 [a]

Mean ±SE. Means with different superscripts in columns are significantly different ($p < 0.05$).

TAN concentration mean (1.12 mg/L) recorded for control ponds was significantly higher ($P < 0.05$) than the mean value (0.27 mg/L) for biofloc ponds. The concentration of TAN was low at the first period of the rearing in all ponds as it's clear seen in Figure 1a &b. The biofloc ponds showed significantly increasing ($P < 0.05$) levels of TAN beginning week 4 and reaching maximum value (1.31 mg/L) in week 15. For control ponds, high fluctuation was clearly seen from week 4 to week 18, and the highest TAN concentration (5.31 mg/L) recorded in week 10.

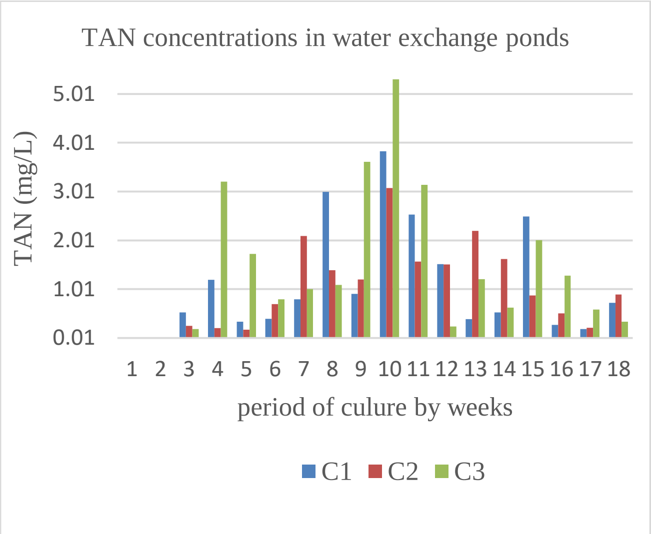

Figure 1.a The total ammonium nitrogen (TAN) concentrations (mg/L) in BFT ponds during the culture period.

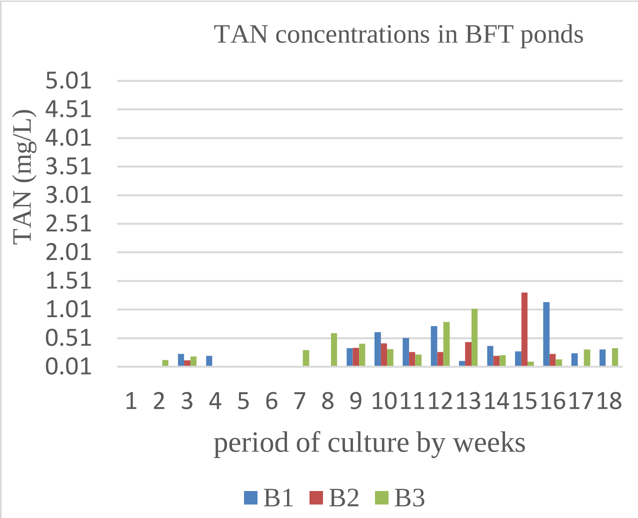

Figure 1.b The total ammonium nitrogen (TAN) concentrations (mg/L) in water exchange ponds during the culture period.

The weekly NO_2-N concentration results for all ponds is shown in Figure 2a &b. Higher fluctuations of NO_2-N were observed from week1 to week 20. The analysis of one-way ANOVA showed that NO_2-N results at the surface water was significantly different among sampling weeks ($P < 0.05$). The gradually increasing of NO_2-N concentration started from week 4 to reach to the maximum concentration (0.43 mg/L) in week 14 for biofloc ponds and (0.85 mg/L) for control ponds in week 14 too. Although, the minimum concentrations of NO_2-N were similar for biofloc and control ponds, the mean and the maximum values for control ponds were double the mean and the maximum values for biofloc ponds. In general, NO_2-N concentration seemed to be slightly affected by the treatment.

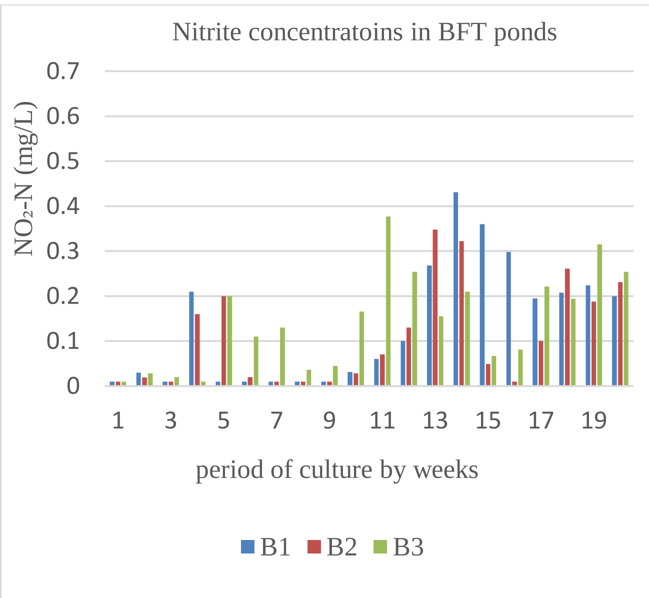

Figure 2.a The nitrite concentrations (mg/L) in BFT ponds during the culture period

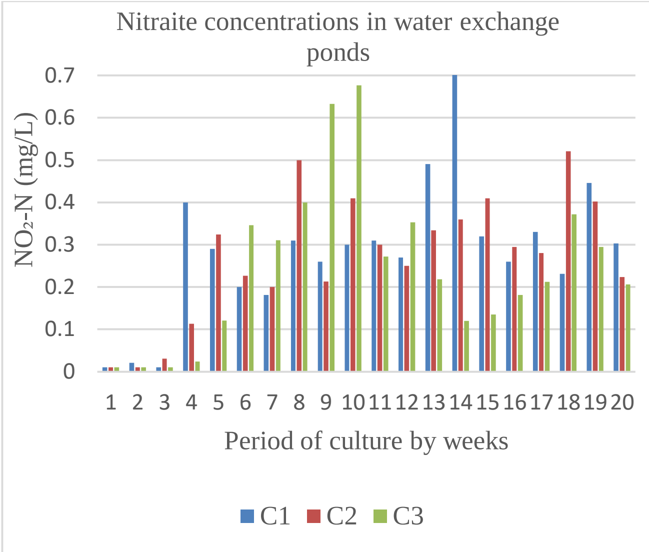

Figure 2.b The nitrite concentrations (mg/L) in water exchange ponds during the culture period.

The range of NO_3-N concentrations in biofloc ponds were (1.37 – 6.30 mg/L) with a mean value (2.31 mg/L). While the range in water exchange ponds were (0.41 – 8.20 mg/L) with a mean value (2.28 mg/L) (Figure 3a &b). The highest value of NO_3-N (6.30 mg/L) was observed in week 6 in biofloc pond (B1), while the highest value (8.20 mg/L) in water exchange pond (C3) was observed in week 14. One-way ANOVA showed that NO_3-N results in biofloc ponds were no significantly different with the results of the same parameter in water exchange ponds.

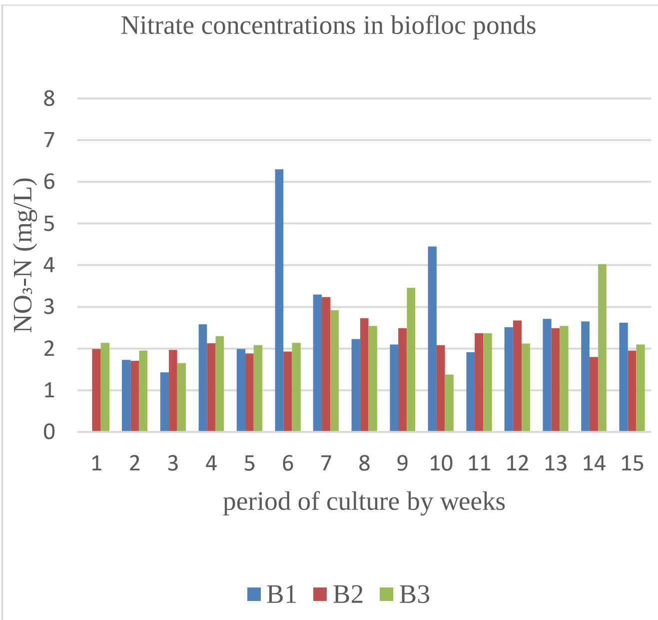

Figure 3.a The nitrate concentrations (mg/L) in BFT ponds during the culture period

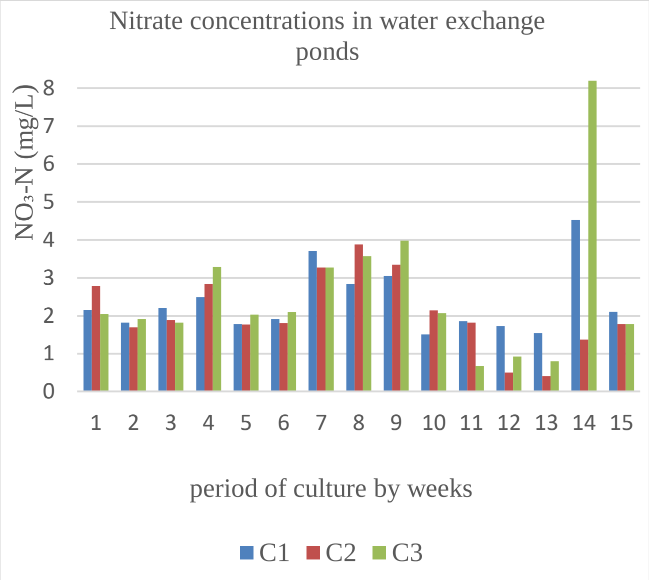

Figure 3.b The nitrate concentrations (mg/L) in water exchange ponds during the culture period.

3.2 Growth Performance:

The mean values result of growth performance and feed utilization from commercial experiment ponds with and without biofloc technology are shown in Table 3.

Table 3. The ffect of biofloc on the growth performance and nutritional parameters of *L. vannamei* for culture period.

Parameters	Treatments	
	Biofloc	Water exchange
Average initial weight (g)	0.2700 ± 0.0057 [a]	0.2567 ± 0.0088 [a]
Average final weight (g)	17.7400 ± 0.2389 [a]	15.0900 ± 0.3952 [b]
Daily growth rate (g/d)	0.1260 ± 0.0021 [a]	0.1010 ± 0.0015 b
Specific growth rate (%/d)	3.0200 ± 0.0416 [a]	2.8133± 0.0353 b
Final production (kg/pond)	13920 ± 516.68 [a]	11217 ± 605.29 [b]
Feed conversion ratio	1.8067 ± 0.0968 [a]	2.4033 ± 0.1642 [b]
Survival (%)	52.3800 ± 2.5259 [a]	49.6233 ± 3.0235 [a]

Mean ± SE. Means followed by the same letter in row are not significantly different ($P > 0.05$).

3.2.1 Average Final Weight Gained:

Significantly, the higher average of *L.vannamei* final weight gained was recorded in biofloc treatment pond B1 followed by B2 and B3 (18.21 g, 17.58 g and 17.43 g) respectively, than in water exchange ponds C1, C2 and C3 (15.88 g, 14.67 g and 14.72 g) respectively, (Figure 4). The mean final weight of shrimp in biofloc and water exchange ponds are presented in Table 3. The mean final weight in biofloc ponds was (17.74 g) and in water exchange ponds was (15.09 g).

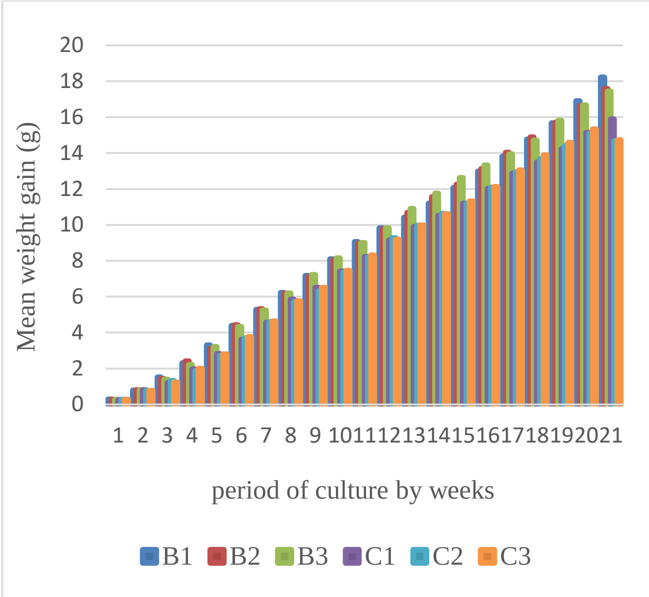

Figure 4. The average shrimp body weight gained (g) during the culture period in all treatment ponds.

3.2.2 Average Daily Growth Rate and Specific Growth Rate:

The results from one-way ANOVA showed that the average daily growth rate (ADGR) of biofloc ponds were significantly different ($P < 0.05$) from water exchange ponds. Statically, the biofloc ponds showed significantly ($P < 0.05$) higher specific growth rate (SGR) value than the water exchange ponds (Figure 5).

In present study, water exchange system gave the lowest response on the growth of shrimp. The best SGR was obtained in shrimp treated with BFT (3.0 and 3.10%) at B1and B2 respectively, compared to water exchange ponds C1, C2 and C3 (2.88, 2.76 and 2.80 %) respectively. This to indicate that molasses as a carbohydrate source of biofloc technology used in shrimp aquaculture *L.vannamei* had a significant effect on the specific growth rate ($P < 0.05$).

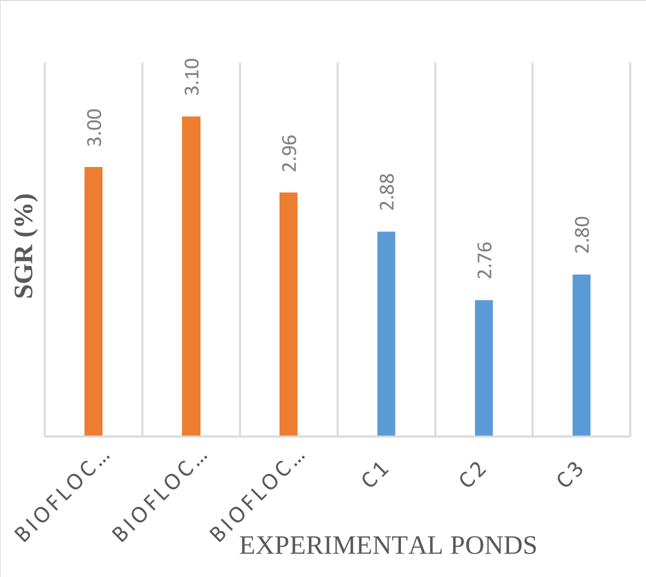

Figure 5. The specific growth rate (%) of *L. vannamei* in all culture ponds.

The results indicated that supplementation of several sources of carbohydrates had significant effect on the SGR of the shrimp. The growth of shrimp from 0.26 g post larvae to an average of 18.21 g, 17.58 g and 17.43 g white leg shrimp in treatment B1, B2 and B3 (with BFT) was resulted from the nutritional content of the biofloc.

3.2.3 Final Production :

The final production ranged from (12891 kg) to (14514 kg) in biofloc ponds with the mean value (13920) kg, where the final production in water exchange ponds was between (12240 kg) and (10145 kg) with the mean value (11217 kg). One-way ANOVA showed that there was a significant difference ($P < 0.05$) between the mean final production in both biofloc and water exchange ponds. The results of final production in biofloc ponds and water exchange ponds are presented in Figure 6.

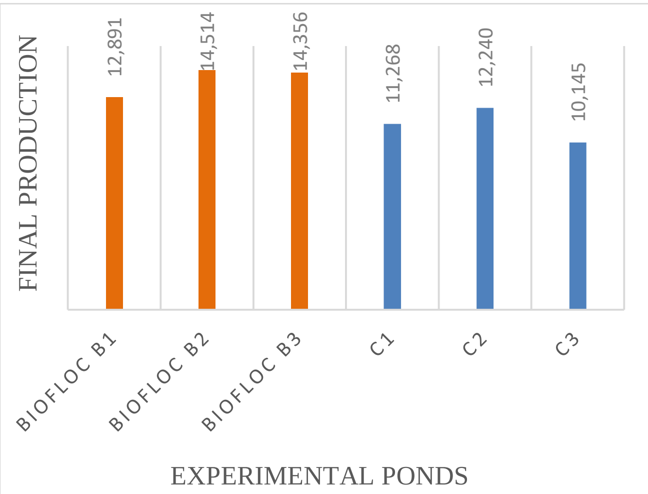

Figure 6. The results of final production (Kg/pond) in biofloc and water exchange ponds.

The biofloc technology leads to yield and performance improvement as compared to water exchange system in present study as shown in figure 6. Yield of shrimp found in biofloc ponds was more than 4.3 kg/m^2. This result was indeed significantly higher than the result of shrimp yield in water exchange ponds which was recorded as less than 3.7 kg/m^2.

3.2.4 Feed Conversion Ratio (FCR):

Significantly, lower feed conversion ratio (FCR) values (1.85 %, 1.87 %) were recoded from the treatment ponds B3, B2 respectively, while the higher values were ranged from 2.68 % to 2.36 % in water exchange ponds.

Effect of biofloc application on feed conversion ratio of *L.vannamei* shrimp reared in ponds presented in Figure 7. The best FCR results (1.85 % and 1.87 %) was observed in shrimp treated with BFT. According to [32], supplementation of different carbon sources to biofloc media could increase the growth rate and reduce the value of FCR for tiger shrimp.

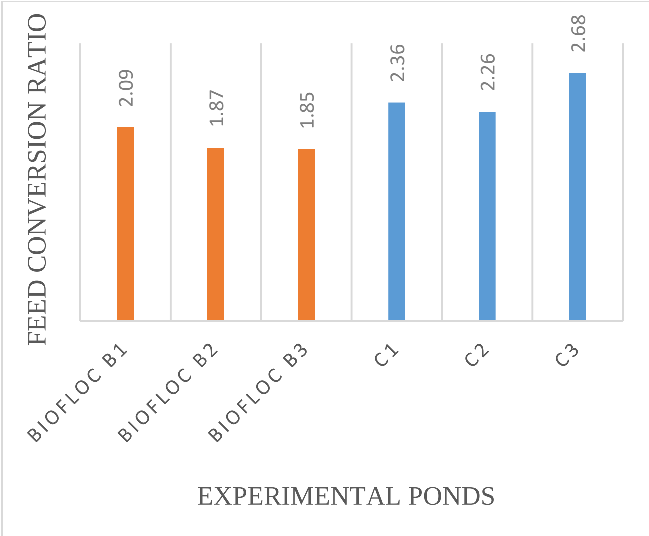

Figure 7. The effect biofloc application on feed conversion ratio of *L. vannamei* shrimp reared in grow out ponds.

The results indicated that FCR of shrimp treated with molasses had a good response. The FCR values were not much different as compared to water exchange ponds. According to [25] [27], single cell proteins synthesized by heterotrophic bacteria could be directly used as a source of feed for cultured fish, thereby reducing the demand for fish feed protein. In addition [33] reported that the low value of the FCR was obtained because the bacteria in the form of probiotics could produce extracellular enzymes that increase the digestibility of food in shrimp intestine, thus increase nutritional absorption. Therefore, reduction in the FCR in BFT ponds.

In a culture without application of bioflocs technology (water exchange), the mean value FCR was 2.4 % with 35% crude protein (CP), which means 2.4 kg of feed, is required to produce 1 kg shrimp. 2.4 x 0.35 = 0.84 kg protein is given per kg shrimp produced. According to [34] only 20% of the feed is taken up by the shrimp. Thus, 0.24 x 0.84 = 0.20 kg protein is taken up per kg shrimp produced.

In a culture system with application of biofloc, the mean FCR was 1.8 % with 35% CP, which means 1.8 kg of feed, is needed to produce 1 kg shrimp. Part of the feed will be recycled into flocs, which the shrimp can also use it as a source of feed. Therefore, less artificial feed will be applied to the system. If BFT is applied, the amount of artificial feed added to the system.

3.2.5 Survival Rate (SR):

Survival rates of shrimp in biofloc treatment ponds and water exchange ponds were presented in Figure 8. Survival of the shrimp did not vary significantly ($P < 0.05$), but raw data and direct observation showed relatively lower survival from C3. The lowest and highest survival was recorded from the ponds C2 (55.68 %) and C3 (45.95 %), respectively.

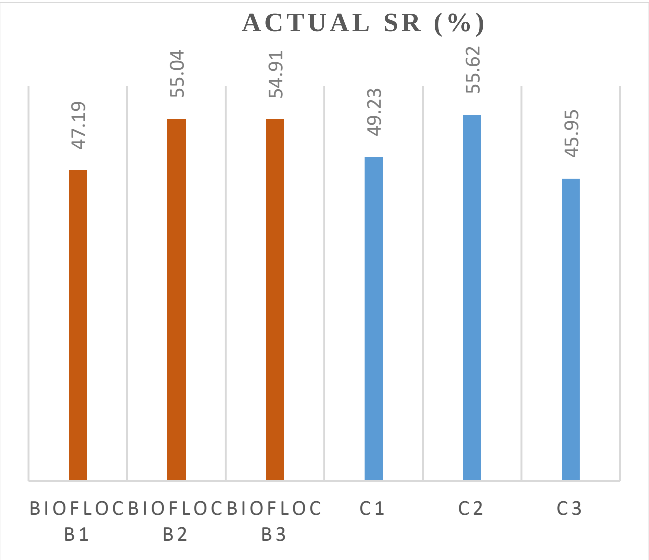

Figure 8. The survival rate (%) of shrimp in biofloc treatment ponds and water exchange ponds.

The highest survival rate mean of *L. vannamei* was achieved in shrimp at biofloc ponds. As stated in [35], the high density might cause cultivation failure in the early stages of post larval development. Agustina et al. [36] reported the level of stocking density affected the survival of *L. vannamei* due to the competition occurred between shrimp.

The higher stocking density, the smaller space available for each individual. It was found in this study that the shrimp were stocked with super high density (500 individual /m^2) of white leg shrimp, this reason caused of low survival in this study. Varghese et al. [30] stated that high stocking density did not affect shrimp growth but the availability of quality feed in sufficient quantities will reduce the percentage of shrimp larvae mortality. As mentioned by [20] that immunostimulants from biofloc lead to increased immunity and antioxidant status of shrimp and fish to provide broad-based resistance to many infections. Thus, shrimp that are cultivated with biofloc systems have a high level of tolerance to the environment, especially those that use carbohydrate sources. In contrast, as our observation the low survival rate in present study causes essentially by two reasons: first, the super-intensive density of shrimp that cause evident competition. Second, due to bird predation such as seagull.

4. Discussion:

In this study, most parameters of water quality and growth performance appeared improving using biofloc technology system. This treatment demonstrated better survival, yield and final production. The results of temperature in this study are acceptable since all the ponds were exposed and affected to the monthly and seasonally changes in weather. Water temperature in both biofloc and water exchange ponds was typically for survival and growth of *L. vannamei* in tropical areas. According to [37], the water temperature ranged from 26 to 30 ºC during the cultivation period is optimal to shrimp culture. Temperature has had a significant impact on the overall activity, food consumption and growth. Shrimp at 22 ºC water temperature were relatively inactive and had low intake of food consumption compared with hyperactive shrimp at 32 ºC. Shrimp at 32 ºC had the highest rate of food consumption when given unlimited food. The values of DO supposed to be higher than these results of water exchange treatment since there were air exchange as well as high doses of aeration was produced to these culture ponds. The decreasing of the values of DO in biofloc treatment ponds may be because of the organic matter in the ponds that oxidized and gave rise to many secondary organic compounds and some inorganic compounds and causes a decrease in the concentration of dissolved oxygen. Dissolved oxygen content of any water supply is an important criterion to assess its suitability for a variety of purposes. It is often taken as an index of organic pollution. pH range in this study is a typical range for *L. vannamei* culture. Previous studies such as [38] found similar range of pH and others recommended the range 6.5 – 9.0 for aquaculture [39]. Salinity values seemed to be highly affected by the treatments. An increasing trend in salinity was observed in biofloc ponds compared to water exchange ponds and it reached to a maximum of 50 ppt. This was due to the evaporation and zero water exchange in the first weeks of culture. Transparency levels did not differ ($P < 0.05$) among biofloc treatment ponds and these levels were within the acceptable ranges for most *penaeid* species surviving in the conditions of biofloc system.

Biofloc treatment was found to be very effective in the production of inorganic nitrogen at very low levels, besides showing better survival and higher shrimp yield. The effect of biofloc on total ammonium nitrogen (TAN) concentration was observed in the results. Biofloc system with carbohydrates (molasses) addition showed significant reduction in the production of inorganic nitrogen in water. With the help of a series of experiments, researchers of [18] and [27] articles proved that addition of carbohydrates reduces the total ammonium nitrogen concentration. TAN converted into the less toxic nitrite and nitrate in BFT ponds comparing with control ponds, where water exchange regularly was done for water exchange system ponds to reduce or control ammonia.

Molasses and strong aeration may facilitate biofloculation [25] and increase in the C/N ratio which may resulted in high total heterotrophic bacteria (THB) count, which immobilized TAN and helped in the synthesis of new bacterial cell [40,41]. As is for the natural water ecosystem, nitrite always recorded the lowest concentrations. The results of nitrite revealed that addition of molasses as source of carbohydrates to water column is significantly effective in reducing the nitrite levels in BFT ponds during the rearing period. The naturally occurring nitrate, nitrite and ammonia may be the major contributing sources of organic substances in the ponds. The results of nitrate indicated that nitrate is not a limiting factor for *L. vannamei*. The rates of nitrate results were variable throughout the experiment, slightly increasing as expected, considering that nitrate is the end product of nitrification. The removal of nitrate can be accomplished by the consumption of microalgae, renewals of water or use of bioreactors for the denitrification process [12]. This reveals that the concentrations of nitrate and ammonia in this study were related to the denitrification and ammonification process.

The results of this study showed that the growth of *L. vannamei* was affected by adding molasses to water ponds. As mentioned earlier by Cleasby [42] that the nutritional value of 100 g molasses was 75 g carbohydrates, 20 mg of calcium, 35 mg phosphorus and iron 26 mg with total energy of 290 cal. According to [27,43], heterotrophic bacteria will grow optimally by increasing C:N ratio through continuous supplementation of organic carbon such as molasses.

Biofloc also provides additional proteins, lipids, minerals, vitamins and many bioactive compounds such as carotenoids, chlorophyll, phytosterols, bromophenol and amino sugars [27,44,45]. As suggested in [46], biofloc could provide high quality protein and essential fatty acids *in situ* to compensate the partial fishmeal substitution. In addition, biofloc has a positive effect on the activity of shrimp digestive enzymes thus enhance the growth of shrimp [47]. The improvement of yield and growth performance of *L. vannamei* in biofloc ponds was clearly appeared. This is consistent with report by [48] who indicated that the use of biofloc technology is highly beneficial for white leg shrimp *L. vannamei* culture, because good water quality is maintained and the production performance is increased. Ray et al. [49] studied the mechanism behind the suspended solids removal to improve production of *L. vannamei* and part of the same study the team has evaluated a plant based feed in minimal exchange, super-intensive culture systems. According to their study, shrimp biomass production (kg/m^2) was increased 41% when biofloc concentration was managed through the use of external settling chamber. In shrimp culture systems phytoplankton and bacteria play a crucial role in the processing of nitrogenous waste [50]. BFT was found to be very effective in the production of inorganic nitrogen at very low levels, besides showing better survival and higher shrimp yield. Lower feed conversion ratio (FCR) values were recorded in biofloc treatment ponds.

These results indicated that FCR of shrimp treated with molasses gave a good response. As reported in [36] the level of stocking density affected the survival of *L. vannamei* due to the competition occurred between shrimp. The higher stocking density, the smaller space available for each individual. It was found in this study that the shrimp were stocked with super high density ($500/m^2$) of white leg shrimp, caused of low survival in this study. The main reasons for the low survival rate in present study were essentially marked by two reasons: first, the super-intensive density of shrimp that cause evident competition. Second, due to bird predation such as seagull.

5. Conclusion:

Shrimp aquaculture and other industries constantly require new techniques in order to increase yield. Modern technologies and other sciences such as biotechnology and microbiology are important tools that can lead to a higher products quality. The application of biofloc technology allows for minimal or zero water exchange practice during the cultivation period, and can thus improve sustainability, biosecurity and production in shrimp aquaculture [11,51]. The driving force of biofloc technology systems is the development of biofloc, which is responsible for water quality control, waste assimilation, and nutrient cycling that contributes to enhanced performance of cultivated shrimp [17].

It could be concluded in present study that the survival of white leg shrimp *L. vannamei* has decreased at higher stocking densities (super-intensive $500/m^2$). Therefore, when considered the number of the larvae suitable for settlement, the biofloc systems at densities less than 500 larvae per square meter is more efficient for commercial production and to achieve good survival.

It is clear that the using biofloc technology in shrimp culture during this study lead to performance growth, increase yield production and decrease in the feed conversion ratio (FCR). In addition, it was observed that the administration of water exchange system at high densities could not significantly enhance growth parameters and feed conversion ratio, comparing to biofloc system through this experiment.

Thus, present study leads to the conclusion once a mature biofloc community is established, TAN and NO_2–N concentrations can be effectively controlled by either heterotrophic assimilation or autotrophic nitrification that helps maintain their concentrations at acceptable ranges for shrimp culture even at high stocking densities.

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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