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Effect of biofloc system on the water quality of the white leg shrimp *Litopenaeus vannamei* reared in zero water exchange culture tanks

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ABSTRACT

A 90-day trial was conducted to study the effects of different protein levels (25, 30 and 35% CP) and different carbon sources (sugarcane bagasse "SB" and wheat flour "WF") with biofloc and one control (45% CP without biofloc), on water quality parameters of the white leg shrimp *Litopenaeus vannamei* juveniles (0.23g ± 0.04) in zero-water exchange culture tanks. Six biofloc treatments and one control without BFT were managed. Temperature and salinity did not show any significant difference between the control and biofloc treatments, and they were at the optimum range for *L. vannamei* cultured. The PH value was unexpected in the bioflocs treatments. BFV and TSS were significantly higher ($P < 0.05$) in biofloc treatments compared to the control. Concentrations of TAN and NO2 at biofloc treatments were lower compared to the control ($P < 0.05$), the addition of different levels of protein and different carbon sources into the zero water exchange system for shrimp culture can effectively increase the activities of nitrogen cycle bacteria, which can thus reduce inorganic nitrogen levels and gradual increases of both BFV and TSS levels.

INTRODUCTION

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, containing uneaten feed and feces. Discharges from aquaculture into the aquatic environment contain nutrients, various organic and inorganic compounds such as ammonium, phosphorus, dissolved organic carbon and organic matter (Piedrahita, 2003 and Sugiura *et al*. 2006). The high levels of nutrients cause environmental deterioration of the receiving water bodies. In addition, the drained water may increase the occurrence of pathogenic microorganisms and introduce invading pathogen species (Thompson *et al*. 2002).

Biofloc technology (BFT) is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system, through an external carbon source or elevated carbon content of the feed. This promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves, 2006).

The application of biofloc technology in shrimp aquaculture has gained great attention recently because it offers a practical solution to effectively control water quality under minimal or zero-water exchange and improves shrimp growth performance, thus achieving efficient and healthy culture of shrimp (De Schryver *et al*. 2008; Stokstad, 2010; Avnimelech, 2012; Crab *et al*. 2012; Xu and Pan, 2013), decrease of food conversion ratio (FCR) and associated costs in feed (Burford *et al*. 2004). Good water quality is the key factor for the success of aquaculture and that ensures the survival, production and growth rate of the cultured animals (Boyd, 1990; Burford, 1997).

The carbon sources applied in BFT are often by-products derived from human and/or animal food industry, preferentially cheap and local available. Cheap sources of carbohydrates such as molasses, glycerol and herbal bulgur (wheat, corn, rice) are used before the introduction of postlarvae and during the growth phase to maintain a high ratio of C/N (15–20) (Taw, 2010). Molasses is widely and successfully used as a promoter of bacterial growth in shrimp culture ponds, probably because of their low cost (Martinez-Cordova *et al*. 2014).

If carbon and nitrogen are well balanced in the solution, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass (Schneider *et al*. 2005). By adding carbohydrates to the pond, heterotrophic bacterial growth is stimulated and nitrogen uptake through the production of microbial proteins takes place (Avnimelech, 1999).

Many studies have show that the carbohydrate addition into the zero water exchange system for shrimp culture can effectively increase the activities of nitrogen cycle bacteria, which can reduce inorganic nitrogen levels. Use of carbohydrates like cane molasses (Emerenciano et at., 2011), sucrose (Gao *et al*., 2012), sugarcane molasses (90%) and wheat bran (10%) (Emerenciano *et al*., 2012), maize starch (Liu *et al*., 2014), sugarcane molasses, dextrose and rice bran (Serra *et al*., 2015), sugarcane molasses, tapioca flour and heat flour (Rajkumar *et al*., 2016), rice flour and molasses (Kumar *et al*., 2017).

BFT was applied by many authors on different species of shrimp e.g. Zhao *et al*.,(2012), Anand *et al*., (2013), Emerenciano *et al*., (2013a), Xu *et al*., (2013b), Kumar *et al*., (2014), wang *et al*., (2015), Hussain *et al*., (2015), Gaona *et al*., (2015 & 2016), Braga *et al*., (2015), Vilani *et al*., (2016) and Xu *et al*., (2016).

The main target of this article is to evaluate the effects of three different levels of protein (25%, 30% and 35%) and two different carbon sources (sugarcane bagasse and wheat flour) in zero water exchange culture tanks of *L. vannamei*.

MATERIALS AND METHODS

Postlarvae of *L. vannamei* were obtained from a commercial shrimp hatchery in Al Deba triangle, Domietta, Egypt. Shrimps were transported in oxygenated double-layered polythene bags. When the shrimp arrived at the laboratory, they were moved into the acclimation tanks filled with seawater (salinity, 20 ppt). Prior to the start of the experiment, shrimps were acclimated to laboratory conditions for two weeks and fed twice daily with commercial feed (45% CP)

The experiment was carried out in 21 glass aquaria (50×40×80 cm) with water volume 80 Liter. Tanks were filled with seawater after filtered by plankton net (50 µm) and diluted with tap water to achieve a salinity of (20 ppt). There were seven treatments: six biofloc treatments and one control were managed :- 1. BFT1 (25% CP + SB), 2. BFT2 (25% CP + WF), 3. BFT3 (30% CP + SB), 4. BFT4 (30% CP + WF), 5. BFT5 (35% CP + SB), 6. BFT6 (35% CP + WF) and one control without BFT (C). Aeration was provided for 24 hours throughout the experiment for ensuring better bioflocculation. The biofloc was produced in two plastic containers (20 L) using water from shrimp culture pond as a inocula growth according to (Avnimelech, 1999) and different carbon sources (sugarcane bagasse (SB) and wheat flour (WF). The suspension was incubated for two weeks for development of microbial. Proximate composition and organic carbon content in the sugar bagasse and wheat flour were determined according to AOAC (1995) and Jackson (1967) respectively as shown in Table (1).

Table 1: The proximate composition % of carbon sources

Parameters%	Sugarcane bagasse (SB)	Wheat flour (WF)
Protein (%)	1.5	12.2
Lipid (%)	1.5	1.2
Ash (%)	7.6	4.1
Fiber (%)	65	1.3
Carbohydrate (%)	24.4	81.2
Organic carbon (%)	39.45	41

Shrimps were held under natural light (12:12 h, light : dark schedule). All tanks were covered with black plastic sheets. In the tanks representing the control treatments (without biofloc system) water was exchanged two time in week, while for BFT tanks were maintained for 90 days without any water exchange (zero water exchange), except for the addition of dechlorinated freshwater to compensate for evaporation losses.

Acclimatized shrimp (0.23 g ± 0.04) were selected and randomly stocked in each tanks. Each tank contained 10 shrimp and there were three replicate tanks for each treatment. Shrimp were fed with experimental diets at 10% of initial weight and adjusted gradually to 3% at the end of experiment. The daily feeding ration for each treatment was calculated and adjusted by estimating the biweekly sampled mean biomass. The ration was divided and distributed three times daily at 8:00, 14:00 and 20:00 h. Pre-weighed carbon sources Viz., sugarcane bagasse and wheat flour were completely mixed in a glass beaker with tank water sample and spread to the tank surfaces at 14:00h. In BFT treatments C:N ratio was maintained at 16:1 for activate bacterial growth. (approximately calculated based on the carbon and nitrogen content of the daily feed input and the carbon sources addition in biofloc tanks).

Water temperature was recorded daily at 13:00 h using a mercury thermometer. Salinity and PH were measured daily between 09:00 and 10:00 h by using a refractometer and PH meter (Orion pH meter, Abilene, Texas, USA), respectively. Water samples (100 mL) were collected every week from each tank. Half of the water sample was analyzed spectrophotometry for total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^-N) and nitrate nitrogen (NO_3^-N) according to (Parsons *et al*. 1984). Water samples (50ml) were collected biweekly from each tank and filtered under vacuum pressure through pre-dried and pre-weighed GF/C filter paper. The filter paper containing suspended materials was dried at 105°C in an oven until constant weight, and the dried sample was weighed to 0.01 mg. The weight difference was calculated and an estimate of the total suspended solids (TSS) was obtained (Avnimelech and Kochba, 2009). Bioflocs volume (BFV) was determined on site using Imhoff cones every week, registering the volume taken in by the flocs in 1000 mL of tank water after 30 min sedimentation (Avnimelech & Kochba 2009).

All variables measured of the water quality parameters were analyzed by two-way ANOVA to determine the effect of different protein levels (25, 30 and 35% CP) and different carbon source (sugarcane bagasse and wheat flour) under biofloc system and their interaction. The ANOVA were performed using the SAS v 9.0.0 (2004) program. The ANOVA was followed by Duncan test (1955) at $P < 0.05$ level of significant.

RESULTS

The water quality parameters such as temperature, salinity and PH monitored during the experimental period are shown in Table. (2). Temperature, and salinity did not show significant difference between the control and biofloc treatments, and they were at the optimum range for *litopenaeus vannamei* cultured. During the experiment, the PH was fluctuated in the bioflocs treatments, which was sometimes slightly below the range considered to be optimal, and then corrected for on several occasions. The fluctuations of pH is shown in Fig. (1a). TAN, nitrite-N, nitrate-N, BFV and TSS are shown in Table (2). The biofloc development in terms of BFV and TSS over experimental period (90 days) are shown in Fig (1b) and (1c), respectively. Bioflocs were observed as brown color after the third week in all biofloc treatments, and were composed of suspended organic particles in the form of flocculated aggregates, which were colonized by a number of heterotrophic bacteria, microalgae and protozoa. In the six biofloc treatments, the average of BFV and TSS levels ranging from 18.47 ± 12.21 to 24.6 ± 19.50 mL L^{-1} and 322.8 ± 81.1 to 363.9 ± 99.5 mg L^{-1}, six experimental diets decreased total ammonia nitrogen (TAN) and nitrite (NO_2) in all biofloc treatments. The results of TAN, nitrite-N and nitrate-N concentrations are shown in Fig (1d), (1e) and (1f), respectively. All treatments showed significant reduction ($p<0.05$) in TAN level, the higher level of TAN observed in control (0.153 mg L^{-1} ± 0.06) and BFT6 (0.102 mg L^{-1} ± 0.05). lower TAN level were observed from BFT5 (0.084 mg L^{-1} ± 0.05), BFT2 (0.086 mg L^{-1}± 0.05) and BFT3 (0.089 mg L^{-1}±0.05). However, no significant difference ($P > 0.05$) in nitrite–N and nitrate–N were noticed among the treatments.

Table 2: Effect of biofloc technology on the water quality parameters (Mean± SD) in experimental tanks of *L. vannamei* under different protein levels and different carbon sources for 90 days.

Parameters	Treatments							Interaction		
	C	BFT1	BFT2	BFT3	BFT4	BFT5	BFT6	P	CS	P×CS
Temp °C	27.7± 2.1[a]	27.9± 1.8[a]	27.6 ± 1.7[a]	27.6± 1.7[a]	27.7 ± 1.7[a]	27.4± 1.9[a]	27.5± 1.9[a]	ns	ns	ns
Salinity ppt	20.4± 1.1[a]	20.6± 1.1[a]	20.4 ± 1.2[a]	20.6± 0.9[a]	20.6 ± 1.2[a]	20.6± 1.3[a]	20.5± 1.2[a]	ns	ns	ns
PH	7.54± 0.2[ab]	7.54± 0.2[ab]	7.50± 0.2[abc]	7.52± 0.2[abc]	7.43± 0.25[c]	7.58± 0.2[a]	7.48± 0.2[bc]	ns	ns	*
TAN mg/l	0.153± 0.06[a]	0.09± 0.07[bc]	0.086± 0.05[bc]	0.089± 0.05[bc]	0.091± 0.04[bc]	0.084± 0.05[c]	0.102 ± 0.05[b]	ns	ns	*
NO2 mg/l	0.167± 0.03 [ab]	0.163± 0.04[ab]	0.163± 0.04[ab]	0.160± 0.04[ab]	0.154 ± 0.03[b]	0.168 ± 0.03[ab]	0.172 ± 0.03[a]	ns	ns	ns
NO3 mg/l	0.294± 0.02 [ab]	0.290± 0.02[b]	0.291± 0.02[ab]	0.288± 0.02[b]	0.291 ± 0.02[ab]	0.293± 0.02[ab]	0.298 ± 0.01[a]	ns	ns	ns
BFV ml/l	4.7 ± 2.52 [b]	20.51± 15.10[a]	22.32± 16.86[a]	23.75± 19.39[a]	18.47± 12.21[a]	24.60± 19.50[a]	19.36± 14.3[a]	ns	ns	ns
TSS mg/l	193.9± 10 [b]	328.3 ± 85 [a]	335.6 ± 84.2 [a]	339.4 ± 86.2[a]	322.8 ± 81.1[a]	363.9± 99.5 [a]	325.6± 72.8[a]	ns	ns	ns

Values for each treatment are means ± SD. Means in the same row having superscript differ significantly. *$P < 0.05$, ns = not significantly. Results from two-way ANOVA: P = protein levels (25%, 30% and 35% CP); CS = carbon sources (sugarcane bagasse and wheat flour); P×CS = interaction of different protein levels and carbon sources . C = control without BFT, BFT1 = 25% CP + SB, BFT2 = 25% CP + WF, BFT3 = 30% CP + SB, BFT4 = 30% CP + WF, BFT5 = 35% CP + SB, BFT6 = 35% CP + WF. *Total ammonia nitrogen (TAN), Nitrite (NO2), Nitrate (NO3), Biofloc volume (BFV), Total suspended solids (TSS).

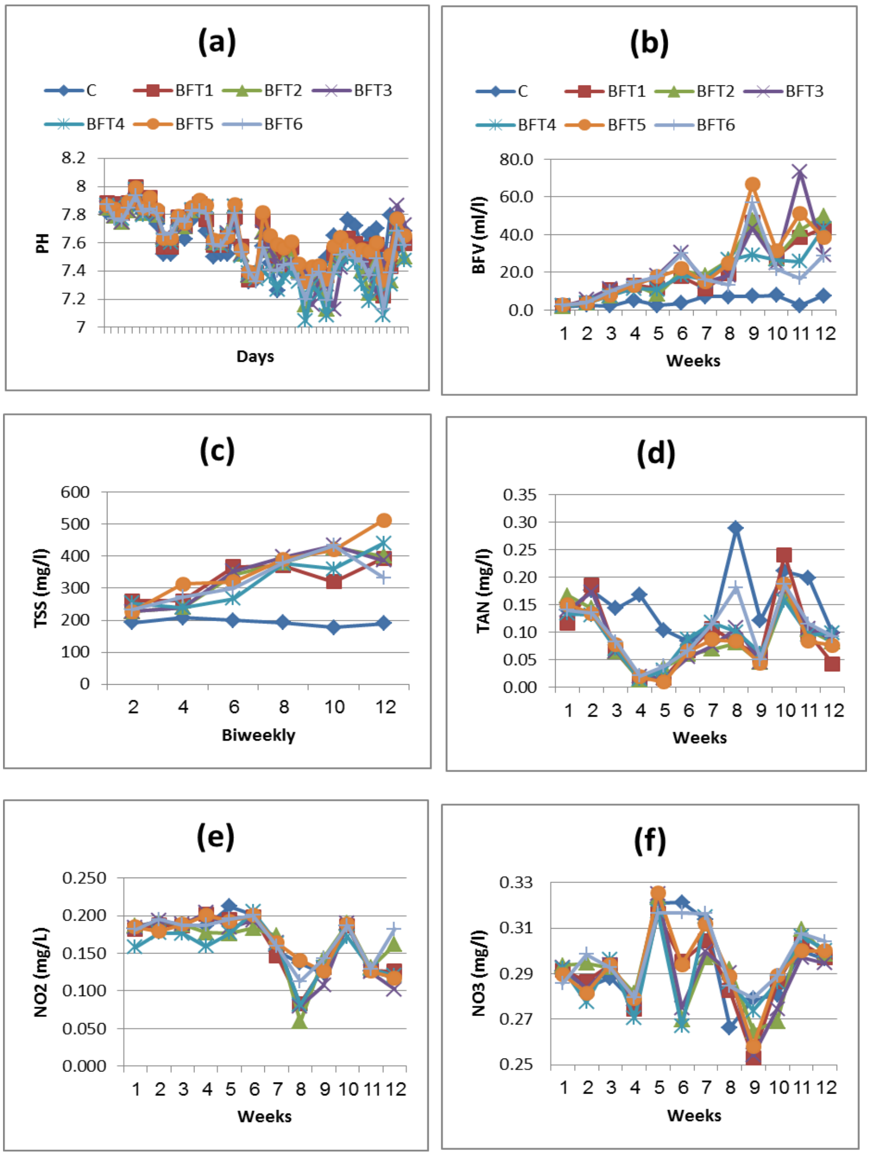

Fig. 1: The effect of biofloc technology on (a) PH, (b) BFV, (c) TSS, (d) TAN, (e) NO2 and (f) NO3 in experimental tanks of L. vannamei under different protein levels and different carbon sources for 90 days.

DISCUSSION

Bioflocs technology aims to improve the water quality in aquaculture systems by carefully balancing carbon and nitrogen. In this study, the effect of biofloc technology under different levels of protein (25, 30 and 35% CP) and different carbon sources (sugarcane bagasse and wheat flour) for white shrimp (*Litopenaeus vannamei*) were studied. During the experimental period (90-d), temperature and salinity did not show any significant difference between the control and biofloc treatments, and they were at the optimum range for *L. vannamei* culture as reported by Fast & Lester (1992); Boyd and Gautier (2000) and Cuzon *et al*. (2004). The PH was unexpected in the bioflocs treatments, which was sometimes slightly below the range considered to be optimal, and then corrected for, on several occasions. The values of pH in the present study were within the preferable range for penaeid shrimps as cited by Van Wyk *et al*. (1999). Increasing C/N ratio (16:1) in biofloc treatments created the decrease in pH in these treatments resulting from the increased CO_2 production by the higher biomass of heterotrophic bacteria (Xu *et al*. 2016).

The present study accepted with Hussain *et al*. (2015) who found that increasing the levels of the C:N ratios (16:1) in the biofloc tanks have significantly influenced the values of pH during the culture period by keeping them more or less constant. This could be related to the presence of heterotrophic bacteria which consume organic matter and cause the increase in the level of water inorganic carbon (CO_2) and decrease the values of pH. pH usually declines as the redox potential declines as a result of microbial activity (Ritvo *et al*. 1998). Ebeling *et al*. (2006), stated that nitrogen uptake by heterotrophic process that likely to dominate BFT system consumes alkalinity half than nitrification (3.57g alkalinity/g NH_4^+-^{N}). They also concluded that as alkalinity concentration relates to the buffering capacity of water, the effect of the high concentration of CO_2 resulted from organisms cultured and microbial respiration on water pH could sufficiently buffered in BFT systems.

Avnimelech (2012) reported that floc volume (FV) and total suspended solids (TSS) are the true indicators of biofloc formation. In the present study, the biofloc development in terms of biofloc volume (BFV) and total suspended solids (TSS) during the experimental period were kept within acceptable ranges and the biofloc treatments recorded significantly higher BFV and TSS compared to control. Furthermore, the BFV and TSS levels were higher in treatments with sugarcane bagasse treatments (BFT 5 "35% CP + SB" and BFT 3 "30% CP + SB") compared to wheat flour treatments (BFT 4 "30% CP + WF" and BFT 6 "35% CP + WF"), but it was not significantly different between the biofloc treatments. It was possible that sugarcane bagasse, a kind of high fiber and slightly soluble substance, was poorly utilized by biofloc. Hari *et al*. (2004) and De Schryver *et al*. (2008), found that wheat flour, maize, tapioca powder, molasses etc. are widely used carbon sources in aquaculture. Thomsen (2005) found that microbes are capable to utilize the diverse range of carbon sources originating from the agricultural products. Zhou *et al*. (2002) found that the type of carbon sources seems to affect the biofloc production rate as molasses containing sucrose, a disaccharide was more effective compared to rice flour having starch, a polysaccharide. This indicates that nature of carbohydrate affects the quantum of biofloc production with higher level of production by application of simple sugar.

According to Avnimelech, (2012), the use of a simple sugar, such as glucose or molasses, results in fast removal of free ammonia ,while more complex carbohydrates require more time for degradation to simple sugars, resulting in slower reduction of ammonia. These results indicated that supplementation of various combinations of carbon sources could affect the development of biofloc in zero-water exchange culture systems in which the water quality had no obvious changes.

The present study similar to that of Rajkumar *et al*. (2016), who found that the total suspended solids (TSS) was within the recommended level of <500 mg L^{-1} for penaeid shrimps (Samocha *et al*. 2007). Several authors have indicated that a similar trend of concentration of TSS which is beneficial to the shrimp and to the system stability (Schryver *et al*. 2008 and Baloia *et al*. 2013).

The present results accepted with study of Xu and Pan, (2014) who reported that no significant effects (P>0.05) of dietary protein level, C:N ratio or their interaction were observed on BFV or TSS levels at biofloc treatments. This indicates that whatever dietary protein level was applied within tested ranges (25% ~ 35%), suitable supplementation of carbon source could offer enough favorable organic substrates for the development of bioflocs as long as C/N ratio was manipulated above 15. So, the biofloc system had the capacity to buffer against input changes in dietary protein levels and carbohydrate additions under given conditions.

Mishra *et al*. (2008), found that intensive shrimp nursery culture a total amount of 300 mg/L TSS was considered to be optimal while using foam fractionators to remove solids. The results of this study concurred with the study of Wang *et al*. (2016), in which adding varied levels of carbon sources into *L. vannamei* culture system could result in gradual increases of both BFV and TSS levels, which was also observed in our study. Zhao *et al*. (2016), observed that supplying different carbon sources into the zero-water exchange system for *L. vannamei* culture did not significantly impact water quality parameters.

The formation and development of the biofloc in the BFT treatments water was likely to be linked with the direct assimilation of dissolved nitrogenous matters (especially ammonia–nitrogen) from diets and shrimp excretions by heterotrophic bacteria (Avnimelech, 1999; Schneider *et al*. 2005 and Ebeling *et al*. 2006), and simultaneously, overall water quality, especially low levels of TAN and NO2−-N, could be maintained within recommended range for shrimp culture due to the carbon source addition (Xu *et al*. 2012).

The community structure of biofloc and its development affect the microbial processes of metabolite assimilation and nutrient cycling, creating different water quality dynamics in the culture system. In the present study, the effect of addition of different carbon sources and different levels of protein in experimental diets decreased total ammonia nitrogen (TAN) and nitrite (NO_2) in all biofloc treatments. The concentration of TAN was significantly ($P < 0.05$) in control (45% CP). The present study similar to that of Gao *et al*. (2012), who found that the concentrations of TAN and NO_2-N of the water were kept in significantly lower levels with the certain adding quantity of carbon source sucrose (75% and 100%) during the whole culture period (90-d). These results revealed that controlling C/N ratios could effectively decrease the accumulation of TAN and NO_2^-N of the water. Our results were inconsistent with those of Samocha *et al*. (2007), who reported that the molasses addition does not result in a significant effect on *L. vannamei* culture system (81 ind m^{-2}). This might be due to their performing the culture in a limited-water exchange system and the relatively short culture period. We found that TAN and NO_2-N in the treatment without biofloc (control) on optimal ranges for *L. vannamei*, that due to change the water biweekly during the experimental period. These results suggest that once a mature biofloc community is established in the culture water, TAN and NO_2^-–N concentrations can be effectively controlled by either heterotrophic assimilation (e.g., TAN assimilation into microbial biomass) or autotrophic nitrification (e.g., TAN assimilation to nitrite and then to nitrate) maintaining them at acceptable ranges for shrimp culture.

Xu *et al*. (2012) reported that water quality parameters were not much affected by the dietary protein level fed to the shrimp in bioflocs tanks. On the other hand, Correia *et al*. (2014) found only difference in TAN, nitrite, nitrate and phosphate content, which were significantly higher in the bioflocs tank fed with high dietary CP (40%) than that of low CP (30%). Yun *et al*. (2015), observed that the nitrate, nitrite and TAN content showed increasing pattern with the corresponding increase in dietary protein level, since high-protein feed would have generated more TAN than the low protein feed ammonia oxidizing bacteria developed faster than nitrite oxidizing bacteria.

The present results of concentrations of TAN and NO_2 at biofloc treatments were lower compared to the control ($P < 0.05$), which was also quoted by other researchers such as Gaona *et al*. (2011). Kuhn *et al*., (2009) observed that carbon supplementation enhanced the removal rates of TAN at 26% per hour compared to

1% per hour in a control system.Our results accepted with study of Xu and Pan, (2013), was found no significant effects between dietary protein level (25 and 35%) and C/N (15 and 20 ratio) for TAN, NO_2-N and NO_3-N concentrations during the time of the experiment.

Hussain *et al*. (2015), found that concentrations of ammonia and nitrate in the biofloc tanks decreased as the levels of C: N ratio (16:1) increased. This result implies that rice meal addition (as a carbon source) had an obvious effect on the inorganic nitrogen reduction through stimulation of the bacterial growth. Kumar *et al*. (2017), found that addition of different carbon sources (rice flour–R and molasses-M) and protein levels (32 and 40%) in black tiger shrimp *P. monodon* cultured significantly reduced the total ammonia–N compared to controls. The nature of carbon sources influenced the amount of biofloc generation as molasses was more effective compared to rice flour.

Our results accepted with study of Xu *et al*. (2013), the addition of carbon source (brown sugar) as a way to raise the C/N ratios in *L. vannamei* in zero-water exchange culture tanks to promote bioflocs development and was found to be efficient in maintaining low levels of TAN and NO_2^- -N throughout the experimental period.

Water quality parameters particularly ammonia, nitrite and total level of ammonia nitrogen are the primary limiting factors in shrimp survival (Santacruz-Reyes & Chien, 2012). Better growth and survival may be due to the decreased production of toxic metabolites as a result of biofloculation in zero-water exchange system which is caused by adding organic carbon source to the system. So, both sugarcane bagasse and wheat flour addition significantly reduced the total ammonia-N compared to the control.

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ARABIC SUMMARY

تأثير نظام البيوفلوك على جودة المياه المستزرع فيها الجمبرى ذو الارجل البيضاء (*Litopenaeus vannamei*)

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اجريت هذه الدراسة لتقييم اثار ثلاثة مستويات مختلفة من البروتين (٢٥ و٣٠ و ٣٥٪) واثنين من مصادر الكربون المختلفة (مصاصة القصب ودقيق القمح) مع نظام البيوفلوك وكنترول واحد (٤٥ ٪ بدون نظام البيوفلوك) ، فى احواض استزراع الجمبرى ذو الاجل البيضاء (الفانمى) على جودة المياه . وجد ان درجة حرارة المياه والملوحة لم تظهر اى تغييرات معنوية بين كل من معاملات البيوفلوك والكنترول .

اضافة كلا من مصاصة القصب ودقيق القمح يعمل على انخفاض كبير فى نسبة الامونيا الكلية والنيتريت مقارنة بالكنترول ، واظهرت النتائج ان حجم البيوفلوك والمواد الصلبة المعلقة ، بهم فروق معنوية عالية فى معاملات البيوفلوك مقارنة بالكنترول.