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The effect of different stocking densities of marine shrimp larvae *Litopeneaus vannamei* on growth performance and survival rate using biofloc technology

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ABSTRACT

There is a need to develop diets for shrimp cultured in Egypt in different rearing systems that will provide sufficient protein for shrimp production while minimizing the amount of nitrogen being introduced into the culture medium. Biofloc technology (BFT) is a new application in keeping up good water quality and growth performance of shrimp cultured. The present experiment was conducted to investigate the effect of three different stocking densities of *Litopeneaus vannamei* and the carbon source (sugarcane bagasse) with biofloc and commercial diet (38% CP) with a control system. The impact of the aforementioned variables was recorded with respect to water quality, microbial communities, biofloc composition, growth performance, feed utilization, and survival rates of shrimp. Shrimp were stocked with different densities (12, 14 and16 larvae/l) in 18 tanks with a water volume of 150 L for 90-days; triplicate groups of shrimp for both biofloc treatment and control. Shrimp were fed thrice daily at a ratio of 14% of body weight (initial weight of mean 0.002g) and re-adjusted gradually to 5% at the end of the experiment. The water quality parameters {temperature, salinity, PH, total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), total suspended solids (TSS) and biofloc volume (BFV)} were suitable for culture of *L. vannamei* in biofloc and control treatments. TSS and BFV were significantly higher in biofloc treatments compared to control. Growth performance and survival rate were better in all biofloc treatments. The results suggest that 16 larvae/l BF treatment is the best stocking density according to the economic analysis. The addition of a carbon source into *L. vannamei* culture reduced the total ammonia-N compounds, increasing total heterotrophic performance and survival rate of shrimp. microorganisms. Furthermore, the increase in total heterotrophic bacteria loaded an effect on the increase of the zooplankton community which, in return, improved the growth

INTRODUCTION

Aquaculture continues to pioneer the advancement of intensive cultivation technology in order to increase the production and meet the individual need for protein. Intensification has led to deterioration in culture climate, increase in disease outbreaks,

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and thereby, reduced productivity. Technologies which minimize the impact on the environment and the incidence of disease are highly required. The use of various natural products in aquaculture as immunostimulants has been reported effective in reducing the occurence of fish and shrimp aquaculture diseases and increasing production **(Manoppo *et al.*, 2015)**. Moreover, researches have addressed the use of medicinal plants and spices such as garlic, ginger, etc. to prevent disease and promote growth **(Nya & Austin 2009; Manoppo *et al.*, 2016** ; **Payung *et al.*, 2017)**. Another promising alternative is biofloc application but still in its developing stage. Biofloc systems are used in Indonesia for aquaculture of catfish, tilapia and shrimp, especially of *Litopenaeus vannamei* with high density **(Azim & Little, 2008; Ekasari, 2009; Hermawan *et al*., 2014)**.

The white shrimp, *Litopenaeus vannamei*, is distributed in the Pacific Ocean from Northern Mexico to the coast of Northern Peru, in waters with temperatures usually above 20°C throughout the year **(FAO, 2009)**. Not surprisingly, *L. vannamei* has generally been cultured in tropical regions around the world. The shrimp grows best at temperatures between 28 and 32°C **(Van Wyk & Scarpa, 1999)**. In temperate and subtropical climates, *L. vannamei* culture is strongly affected by low temperatures. Low temperatures may limit shrimp growth and cause mortality during the coldest months **(Krummenauer *et al*., 2011)**. In Northeast Brazil, crop cycles can be repeated in order to ensure production throughout the year. However, in Southern Brazil, low temperatures restrict *L. vannamei* pond culture to periods between 6 and 8 month **(Krummenauer *et al.*, 2010)**.

The heart of the biofloc technology (BFT) system is the formation of microbial aggregates which are initiated by heterotrophic bacterial colonization stimulated by the addition of an external carbon source **(Avnimelech, 2015).** Heterotrophic bacteria incorporate the inorganic nitrogen and orthophosphate by assimilation, turning them into cell biomass, and consequently, reducing water exchange and improving biosecurity **(Poli *et al*., 2018)**. In addition, microbial aggregates can be used as a food source by the cultured animals **(Xu *et al*., 2013)**.

Some definitions regarding the notion of biofloc technology have been put forward by some experts. **Crab *et al*. (2012)** stated that biofloc technology is a technique to improve the quality of water for aquaculture activities by adding carbohydrate sources externally through feed. According to **Aiyushirota (2009)**, biofloc is a biological wastewater treatment that activates sludge, while **Ekasari (2009)** claimed that biofloc technology is the conversion of inorganic nitrogen, particularly ammonia, to microbial biomass by heterotrophic bacteria, which can then be consumed by cultivated organisms. Biofloc deals with waste that has the potential to harm the ecosystem that is continually generated by aquaculture activities with a high nutrient content **(Riani *et al.*, 2012)**.

Some factors that make biofloc systems more efficient compared to conventional systems include reducing feed use, preventing spread of disease by minimizing water changes that are environmentally friendly. Biofloc is critical for improving the quality of the water and preventing disease incidence **(Choo & Caipang, 2015; Nurhatijah *et al.*, 2016)**. Furthermore, **Cadiz *et al.* (2016)** found that the use of biofloc could control the presence of *Vibrio* sp. and *Vibrio parahaemolyticus* in intensive culture of *L. vannamei*. Therefore, biofloc can be applied at aquaculture with high densities **(Hargreaves, 2013)**. Generally, protein is only consumed by about 20-25 percent for fish feed, and the remainder is secreted by waste and unfed food containing ammonia and organic nitrogen. To stimulate the growth of heterotrophic bacteria and turn inorganic nitrogen into microbial protein, manipulation of the ratio of C: N in aquaculture environments is recommended **(Avnimelech, 1999)**.

The addition of carbon sources with an increase in the C: N ratio can theoretically increase the conversion of toxic inorganic nitrogen species to microbial biomass available as food for cultivated animals. The optimum C: N ratio in aquaculture systems can be maintained by adding a variety of cheap carbon sources or reducing protein content in feed **(Avnimelech, 1999; Hargreaves, 2006)**. Biofloc utilization is urgently needed for environmentally friendly aquaculture production.

The major objective of the project was to evaluate the effect of different stocking densities of marine shrimp larvae on water quality under the biofloc technology.

MATERIALS AND METHODS

The experiment were performed indoor at Invertebrate laboratory of National Institute Oceanography and Fisheries(NIOF), Suez branch in 8/2018 To 11/2018. Larvae of *L .vannamei* were obtained from a commercial shrimp hatchery in Burj al-Arab, Alexandria, Egypt. Shrimps were transported in oxygenated double – layered polythene bags. At the laboratory shrimps were acclimated in tank filled with seawater (salinity: 32 ‰). Initial samples were taken immediately after reaching larvae from hatchery, and final samples were taken from each tank at the end of experiment for chemical analysis. Prior to experiment, shrimps were acclimated to laboratory condition for two weeks and fed twice daily with commercial feed (38% crude protein, 10% lipid, 3.1% fiber and 19% carbohydrate).

The experiment was carried out in 18 tanks with water volume of 150 L. Tanks were filled with seawater after filtering by plankton net (50µm) to prevent the entry of unwanted materials and suspended particles into the tanks, and was diluted with fresh water to achieve a salinity of (32 ‰). All tanks were supplied with 4 air stone-hoses type of diffuser system which is fitted to air-blower (220w). Aeration was provided 24 hours throughout the experiment for ensuring better bioflocculation. The biofloc was produced in one tank (200 L) using water from shrimp culture pond as an inocula growth according to **Avnimelech (1999)** using sugarcane bagasse (SB) as carbon source. The suspension was incubated for two weeks for development of microbial communities. Proximate composition and organic carbon content in sugar bagasse were determined according to **Jackson (1967)** and **AOAC (1995)** as shown in Table (1).

Table 1: The chemical analysis of sugarcane bagasse.

Parameters (%)	Sugarcane bagasse(SB)
Protein	**1.5**
Lipid	**1.5**
Ash	**7.6**
Fiber	**65**
Carbohydrate	**24.4**
Organic carbon	**39.45**

All tanks were kept covered with plastic sheet to reduce escape of shrimp. In the tanks representing the control system treatments, water was exchanged twice a week. While, biofloc tanks were maintained for 90 days without any water exchange (zero water exchange) except to compensate for evaporation rate.

After two weeks, all tanks were stocked with shrimp larvae at three different stocking densities of shrimp (12, 14 and16 larvae/l) in each tank (x^3). Before stocking, the shrimps were weighed, and the initial body weight (Mean, 0.002g) was determined. Shrimp were fed with experimental diets at 14% of body weight and adjusted gradually to 5% at the end of the 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 and pre-weighed SB was completely mixed in a glass beaker with tank water sample and spread to tanks surfaces. In biofloc treatments, C:N ratio was maintained at 16:1 for activate bacterial growth which, however, approximately calculated based on carbon and nitrogen content of the daily feed input and the carbon sources addition in biofloc tanks.

Water quality parameter

To maintain water quality at optimum range for shrimps the following parameters were monitored during the experiment to follow the effect of biofloc system and state it in comparison with the control system.

Daily parameters: water temperature (°C) was measured daily at 13:00h, salinity and pH were measured daily at 10:00h using multiparameter analyzer.

Biweekly parameters: water sample (100 ml) were collected from each tank and filtered by filter papers to analyze total ammonium nitrogen (TAN), total suspended solid (TSS), nitrite-N (NO_2-N), nitrate-N (NO_3-N) and phosphate-P (PO_4-P) using spectrophotometer model (JENWAY 6100). Biofloc volume was estimated on a weekly basis using Imhoff cone, and measured in 1000 mL tank water after 15-20 min of sedimentation **(Avnimelech & Kochba, 2009)**.

Growth parameters

Shrimp larvae weight (g) was measured at the initial of the experiment and biweekly by collected randomize number of shrimp from each tank and weighted in an analytical digital balance and then returned back to their tanks and length (mm) was be measured during the experiment. shrimp Weight gain (WG), Final body Length (FBL), Condition factor (K), Specific growth rate (SGR) and Survival rate% (SR) were calculated using the given formula below:

- Weight gain (WG) = Final body weight (g) - Initial body weight (g).
- Final body length (FBL) = Final length (cm) - Initial length (cm).
- Specific growth rate % (SGR) = [(Ln FBW - Ln IBW) /day of experiment] ×100
- Survival rate % (SR) = (Final number of shrimp / Initial number of shrimp) ×100.

Determination of microbial community

Levels of viable heterotrophic bacteria were determined biweekly by counting the colonies that grew on Trypticase soya agar plates with supplemented with (50:50) marine water: distilled water. Before plating each sample onto the agar medium, serial dilutions were made with 9 ml distilled water. Levels of bacteria were detected in colony forming units per ml of water (CFU/ml).

Proximate composition of shrimp

At the beginning of the trial, a random pooled sample of shrimps was be collected and sacrificed for determination of initial whole-body proximate composition. At the termination of the feeding trial, 5 shrimp will be randomly selected from each tank, to determine the proximate whole body composition. The Chemical composition of shrimp samples will be determined according to AOAC (1995).

Economic analysis

Feed cost, shrimp cost, total cost, total production, total income and net return were calculated using the given formula below:

- Feed cost = Amount of feed consumed kg × Price of 1kg
- Shrimp cost/1000larvae = Amount of shrimp × Price
- Total production = Weight gain × Survival number

- Total income/Tank = Total production × Price of 1kg
- Net return/Tank = Total income – Total cost

Statistical analysis

The ANOVA was performed using the **SAS** v9.0.0 **(2004)** program. The ANOVA was followed by **Duncan test (1955)** at $P<0.05$ level of significance.

RESULTS

Water quality

The water quality parameters such as temperature, salinity, pH, TAN, nitrite-N, nitrate-N, phosphate, total suspended solids (TSS) and Biofloc volume (BFV) monitored during the experimental period are shown in Table (2). Temperature, salinity and pH were at the optimum range for *Litopenaeus vannamei* cultured. While the biofloc development in terms of TSS and BFV over experimental period (90-days). Bioflocs were brown color after the third week in all biofloc treatments, and composed of suspended organic particles in the form of flocculated aggregates, and colonized by a number of heterotrophic bacteria, microalgae and protozoa. In the biofloc treatments, BFV and TSS ranged from7.8±4.66 to 9.4±4.67 ml/L and 284±58.9 to 338±62.1 mg/l, respectively. The addition of the carbon sources in the experimental diets decreased total ammonia nitrogen (TAN), nitrite-N (NO_2), nitrate-N (NO_3) and Phosphate (PO_4) that ranged from, 0.01±0.01 to 0.02±0.01, 0.02±0.01 to 0.03±0.03, 0.021±0.007 to 0.02±0.006 and 0.30±0.12 to 0.40±0.18 in all biofloc treatments, respectively.

Table 2: Effect of biofloc technology on water quality (Mean±SD) in experimental tanks of *L. vannamei* under different stocking densities for 90 days.

Parameter	Stocking densities					
	12 larvae/l		14 larvae/l		16 larvae/l	
	C	BF	C	BF	C	BF
Temperature	28.5± 0.53	28.4± 0.47	28.2± 0.58	28.3± 0.57	28.4± 0.43	28.3± 0.57
Salinity	32.0± 0.57	32.7± 0.44	31.8± 0.58	32.6± 0.55	32.1± 0.42	32.7± 0.44
pH	7.8± 0.33	7.9± 0.21	7.9± 0.30	7.9± 0.23	7.9± 0.31	7.9± 0.39
TAN	0.03± 0.012	0.02± 0.01	0.02± 0.01	0.01± 0.01	0.03± 0.01	0.02± 0.008
NO_2	0.03± 0.02	0.02± 0.01	0.04± 0.03	0.03± 0.02	0.04± 0.03	0.03± 0.03
NO_3	0.022± 0.008	0.021± 0.007	0.03± 0.01	0.02± 0.006	0.03± 0.01	0.02± 0.006
PO_4	0.36± 0.13	0.30± 0.12	0.43± 0.20	0.38± 0.18	0.44± 0.21	0.40± 0.18
TSS	246± 96.6	284± 58.9	257± 91.7	299± 96.3	315± 48.5	338± 62.1
BFV	Zero	7.8± 4.66	Zero	8.1± 4.88	Zero	9.4± 4.67

Biofloc technology aims to improve the quality of water in aquaculture systems by combining carbon and nitrogen with care. In this study, the impact of biofloc technology was studied on white-leg shrimp (*L. vannamei*) under various storage densities (12, 14 and 16 larvae/l) and one carbon source (sugarcane bagasse). During the experimental period (90 days), there was no significant difference in temperature or salinity between control and biofloc treatments while recording the optimum range for *L. vannamei* cultivated **(Foes *et al*., 2011; krummenauer *et al*., 2011; Wasielesky *et al.*, 2013; DaSilva *et al.*, 2015; Ali *et al.*, 2020)**. In temperate and subtropical climates, *L. vannamei* community is hit hard by low temperatures. Low temperatures can curb shrimp growth and cause death in the coldest months **(Peixoto *et al*., 2003)**. In the treatment of biofloc, the pH was unexpected, sometimes slightly below the assumed optimal range and then corrected for several times. In the present study, the pH values for penaeid shrimps were within the preferred range as determined by **Van Wyk and Scarpa (1999)**. Increasing C: N ratio in the treatment of biofloc resulted in a decrease in pH in those treatments due to the increased production of CO_2 by higher biomass of heterotrophic bacteria **(Xu *et al*., 2016)**.

The results of the present study are supported by **Hussain *et al*. (2015)**, who found that the increasing levels of the C:N ratios (16:1) in 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 in the values of pH. However, pH usually declines when the redox potential declines as a result of microbial activity **(Ritvo *et al*., 1998; Ali *et al.*, 2020)**. Additionaly, **Ebeling *et al*. (2006)** stated that nitrogen uptake by heterotrophic process, that was likely to dominate BFT system, consumes alkalinity half than nitrification (3.57g alkalinity/g NH_4^+-N). The previous authors 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.

It is wothy to mention that, **Avnimelech *et al*. (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 the acceptable ranges, and the biofloc treatments recorded significantly higher BFV and TSS compared to control. It is possible that sugarcane bagasse, a kind of a high fiber and slightly soluble substance, was poorly utilized by biofloc.

The present results are similar to those of **Rajkumar *et al*. (2016)**, who found that TSS was within the recommended level of <500 mg/L for penaeid shrimps **(Samocha *et al*., 2007)**. Several authors have indicated that a similar trend of concentration of TSS is beneficial for both the shrimp and the system stability **(De Schryver *et al*., 2008; Baloia *et al*., 2013)**.

The formation and development of the biofloc in the BFT treatments water is 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; Ebeling *et al.*, 2006)**, and simultaneously, over all water quality, especially low levels of TAN and NO_2-N, could be maintained within recommended range for shrimp culture due to the carbon source addition **(Xu & Pan, 2012)**.

In the current results, the concentrations of TAN and NO_2 at biofloc treatments were lower compared with those in the control ($p<0.05$). This finding coincides with that of **Gaona *et al*. (2011)**. They have shown that the carbohydrate addition 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.

By adding carbohydrates to the water and regulating the C: N ratio, the heterotrophic bacterial assimilation of nutrients, and the formation of biofloc could be optimized, consequently leading to the removal of TAN and NO_2 **(Avnimelech, 1999).** The organic carbon source of choice would greatly determine the composition of the floc produced, considering the type and amount of storage polymers **(De Schryver *et al*., 2008)**, and those findings agree with the present results.

The community structure of biofloc and its development affect the microbial process of metabolite assimilation and nutrient cycling, creating different water quality dynamics in the culture system. In the present study, the effect of addition of carbon source in the experimental diets decreased the total ammonia nitrogen (TAN) and nitrite (NO_2) significantly in all biofloc treatments. The concentration of TAN was significantly higher ($P<0.05$) in the control. The present results concur with those of **Wasielesky *et al*. (2013)**, who found that the concentration of total ammonia was maintained at low levels during the experiment which most likely resulting from the development of the microbial community that was established in the culture water.

It was noticed that, TAN and NO_2-N in the treatment without biofloc (control) were at the optimal ranges for *L. vannamei*, due to the change of water twice a week during the experimental period. Those 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 heterotrophic assimilation (e.g., TAN assimilation to nitrite and then to nitrate) maintaining them at acceptable ranges for shrimp culture.

The present results of TAN and NO_2 concentrations at biofloc treatments were lower compared to the control ($P<0.05$), which was also agreed with other studies **(Kuhn *et al*., 2009; Gaona *et al*., 2011** and **Wasielesky *et al*., 2013).** They observed that carbon supplementation enhanced the removal rates of TAN at 26% per hour compared to 1% per hour in a control system.

Hussain *et al*. (2015) found that concentration of ammonia and nitrite in the biofloc tanks decreased as the levels of C: N ratio (16:1) increased. This result implies that addition of carbon source had an obvious effect on the inorganic nitrogen reduction through stimulation of the bacterial growth.

Generally, the water quality parameters; particularly ammonia, nitrite and total level of ammonia nitrogen are the primary limiting factors in the survivability of shrimps **(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-or low water exchange system which is caused by adding organic carbon source to the system.

Thus, addition of sugarcane bagasse reduced significantly the total ammonia nitrogen compared to the control.

Growth performance

Details of growth shrimp harvested from experimental tanks with and without biofloc system are presented in Table (3). The significant highvalues in final body weights (FBW) 8.48, 9.84, 9.85 g, weight gain (WG) 8.49, 9.83, 9.82 g, specific growth rate (SGR) 9.48, 9.65, 9.65 g, length gain (FBL) 10.37, 10.81, 11.31 cm were recorded in 12BF, 14BF, 16BF, respectively, compared to control treatments (C) with FBW values of 7.28, 7.97, 8.01 g, (WG) 7.27, 7.96, 8.03 g, (SGR) 9.31, 9.41, 9.42 g, (FBL) 9.98, 9.99, 10.16 cm; all were recorded in 12C, 14C,16C, respectively.

Overall, weight gain (WG), final body weight (FBW), specific growth rate (SGR) and final body length (FBL) were significantly ($P< 0.05$) higher in biofloc treatments compared to the control treatments. Survival rates (SR%) were above 80%, and they were significantly higher in biofloc treatments compared to control treatments.

Table 3: Effect of biofloc technology system and different stocking densities on growth performance and survival rate % of *L. vannamei* larvae (Mean± SD) in experimental tanks under different stocking densities for 90 days.

Parameter	Stocking densities					
	12 larvae/l		14 larvae/l		16 larvae/l	
	C	BF	C	BF	C	BF
IBW	0.002	0.002	0.002	0.002	0.002	0.002
FBW	7.28±0.51[b]	8.48±0.67[a]	7.97±1.52[b]	9.84±1.08[a]	8.01±0.71[b]	9.85±1.70[a]
WG(g/l)	7.278±0.73[b]	8.478±0.45[a]	7.968±2.72[b]	9.838±1.18[a]	8.008±0.82[b]	9.848±1.60[a]
FBL(cm)	9.98±0.93[b]	10.37±0.74[a]	9.99±0.86[b]	10.81±0.86[a]	10.16±0.98[b]	11.31±0.90[a]
SGR%	9.31±0.69[b]	9.48±0.76[a]	9.41±0.67[b]	9.65±0.69[a]	9.42±0.67[b]	9.65±0.69[a]
S%	86±8.56[a]	92±4.65[a]	78±9.76[a]	86±11.11[a]	88±6.42[a]	89±3.49[a]

***** Means in the same row having different superscript are significantly varied ($P<0.05$).

There is a need to develop diets for shrimp cultured in intensive farming systems to provide sufficient protein for shrimp production while minimizing the amount of nitrogen being introduced into the culture medium **(McIntosh *et al.*, 2001)**. Shrimp typically have a higher dietary protein requirement during the different growth phases **(Chen *et al.*, 1985; Velasco *et al.*, 2000)**. However, there is a wide range in reported dietary protein requirements for *L. vannamei* , typically from 300 to 480 g Kg-1 (30-48%). In intensive nursery systems, *L. vannamei* have been fed diets with protein levels as high as 40-55% **(Samocha *et al.*, 1993; Velasco *et al.*, 2000)**.

In our results, supplementation of sugarcane bagasse enhanced the growth performance of shrimp on the basis of higher significant difference in FBW, WG and SGR between the biofloc and control treatments throughout the experimental period (90 days).

Productivity is an important parameter because it combines growth and survival, and enables the evaluation of the system's capacity to support high stocking densities **(Fóes *et al*., 2011)**.

Results coincide with those of **Costa *et al.* (2018)**, the densities of 100 and 150 shrimp/m^2 tested in this study did not present this relationship, since the highest density showed highest productivity resulting in a significant difference. This also means that those densities are suitable in the culture. Although the BFT system supports higher densities than presented here, until the moment, those densities were the highest tested in ponds in this region.

The success of these cultures is related to high-technology resources that include supplemental dissolved oxygen, filters, specific pathogen-free larvae, and feed specifically formulated for the BFT culture system **(Krummenauer *et al*., 2011)**.

Remarkably, the reduced growth and survival of cultured *penaeid* shrimp at high densities is related to a combination of factors such as reduced availability of space and natural productivity, degradation of water quality and sediment accumulation **(Da Silva *et al*., 2015)**.

Overall, shrimp survival of lower densities (1,500 and 3,000 shrimp/m^2) is similar to that reported (70-99%) in studies that evaluated the nursery phase of *L. vannamei* in a biofloc system with limited water exchange **(Samocha *et al*., 2007; Mishra *et al*., 2008; Wasielesky *et al*., 2013**). In addition, **Foes *et al.*, (2011)** detected a decrease in survival of *Farfantepenaeus paulensis* post larvae when reared in biofloc system at the highest density treatment (2000 larvae/m^2).

Furthermore, **Arnold *et al.* (2006)** argued that survival is the most important parameter to be considered in intensive production of *Penaeus monodon* during the nursery.

Determination of microbial community

Total heterotrophic bacteria (THB) developed over the experimental period in all biofloc treatments compared with control treatments. The results of THB are shown in Table (4).

Table 4:- Effect of biofloc technology system and different stocking densities on Total heterotrophic bacteria (THB) (Mean±SD) in experimental tanks of *L.vannamei* larvae under different stocking densities for 90 days

Parameters	Stocking densities					
	12 larvae/l		14 larvae/l		16 larvae/l	
	C	BF	C	BF	C	BF
THB (cfu/ml) ($x10^{-5}$)	157.84 ± 8.33^b	225.78 ± 26.58^a	143.76 ± 29.54^b	178.68 ± 145.43^a	203.48 ± 16.23^b	341.18 ± 26.14^a

The importance of natural feed and the benefits achieved through the adequate promotion and utilization of biota in different aquaculture systems have been documented

(Ballester *et al*. 2010). Biofloc system usually transition from phytoplankton dominated to bacterial dominated as feed input, biomass and suspended solids increase (Hargreaves, 2006).

There was a significant increase in the total number of heterotrophic bacteria with increased levels of C:N ratio (16:1) in the biofloc tanks of penaeus semisulcatus cultured (Hussain *et al*. 2015).This indicates that bacteria required carbon from the carbon source in order to multiply their cells.

However Middelboe *et al*. (1995), studied bacterial utilization of dissolved free amino acids, dissolved combined amino acids and ammonium in the Delaware Bay estuary in the US and stated that the addition of glucose increased the number of heterotrophic bacteria in water. Similarly, other investigators (Avnimelech *et al*. 1992; Hari *et al*. 2004) found that the numbers of heterotrophic bacteria increased in response to increasing levels of C:N ratio in penaeid shrimp culture.

The microorganisms play important roles in water quality control and nutrient cycling of the culture systems (McIntosh, 2001). Adding organic carbon sources into the culture systems has been proven to be an effective management to promote the growth of heterotrophic bacteria and the formation of biofloc (Tranvik, 1988). Microorganisms associated with biofilm and biofloc are known to have an excellent nutritional composition, i.e. high quality proteins with adequate amino acid profile, highly unsaturated fatty acids and vitamins (Avnimelech, 2009).

Proximate composition of shrimp

Body composition of the shrimp are shown in Table (5). There were no significant differences ($p \geq 0.05$) in Crud protein, lipid, ash, fiber and total carbohydrates among biofloc treatments and their controls. In the biofloc treatments, Crud protein, lipid, ash, fiber and total carbohydrates contents from 55.2 to 56.4%, 16.2 to 17.4%, 11.4 to 12.4%, 5.4 to 5.8% and 9.5 to 9.8% respectively.

Table.(5):- Effect of biofloc technology system on chemical composition of *L.vannamei* and nutritional value of biofloc g/100g diet (Mean± SD) in experimental tanks under different stocking densities for 90 days

Stocking densities	Dry Matter	Crude Protein	Lipid	ASH	Fiber	Total carbohydrates
12C	26.3±1.3	56.1±1.9	17.4±0.9	11.5±0.6	5.5±0.15	9.5±0.35
12BF	26.8±0.6	56.2±1.2	16.2±0.8	12.2±0.3	5.8±0.12	9.6±0.30
14C	25.4±1.0	55.2±1.2	17.1±0.9	12.4±0.2	5.7±0.10	9.6±0.34
14BF	26.9±0.7	56.4±1.3	16.9±0.8	11.4±0.6	5.6±0.16	9.7±0.31
16C	26.3±1.0	55.5±1.6	16.8±0.4	12.1±0.4	5.8±0.14	9.8±0.33
16BF	25.9±1.4	56.3±1.5	17.2±0.1	11.6±0.6	5.4±0.13	9.5±0.36

In the present study, shrimps in biofloc treatment and control had suitable moisture contents 73.7% to 74.6%, and this results coincide with Yulianty *et al*.(2019), who found that the moisture of fresh shrimp is generally reported as 75% to80%. Protein was found as the major constituent indicating that shrimp flesh can be a good source of amino acids (Puga-lopez *et al*. 2013).

Crude protein levels showed a tendency to increase in the shrimp reared in the biofloc treatments. Protein, lipid, ash, fiber and total carbohydrate contents were no significantly difference ($p<0.05$) in biofloc treatments and control, representing that the quality of flesh was not affected by any of the culture conditions used in this study. Proximate compositions in shrimps flesh are affected by several factors such as species, growth stage, feed and season (Karakoltsidis *et al*. 1995).

Economic analysis

Table(6):-Brief indication of economic analysis for L.vannamei production under different stocking densities treated with biofloc and control systems in experimental tanks for 90 days

Stocking densities	Feed cost (L.E)	Shrimp larvae cost/1000 larvae (L.E)	Total operating cost (L.E)	Total net production (Kg)	Total income/Each treatment (L.E)	Net return/Each treatment (L.E)
12C	1.022	1.620	2.717	34	3394.61	678.09
12BF	36	1.620	1.656	42	4189.14	2533.51
14C	1.097	1.890	2.912	39	3923.61	1011.99
14BF	46	1.890	1.954	53	5340.25	3386.01
16C	1.112	2.160	3.272	51	5086.09	1813.62
16BF	64	2.160	2.206	63	6275.36	4068.99

The price of each diet was determined by multiplying the amount of feed consumed in all days of the experiment by their respective cost per Kg. The price of each ingredient in August 2018 was as follows: Shrimp feed =21.5 L.E / 1 Kg ; sugarcane bagasse =1 Pound / 1Kg. According to the net return of this economic the best stocking density was 16BF (larvae/l).

CONCLUSION

1- Sugarcane bagasse addition significantly reduced the total ammonia nitrogen, nitrite, nitrate and phosphate compared to control**.**
2- The total heterotrophic bacteria count were higher in all biofloc treatments compared to control treatments.
3- According to the economic analysis the density 16BF (larvae/l) is the best stocking density compared with other densities.

4- Biofloc system proved to increase growth rate of *L.vannamei* as the shrimps that feed on the biofloc would get the additional supplemental nutrition from the assimilated nutritious planktons, bacteria and organic compounds. Consequently, the use of by-agricultural product; as a carbon source in biofloc system, is recommended as a cheap source that enhances the water quality and growth performance of shrimp as well.

5- The results of this study confirmed that the biofloc system can play a key role in developing a sustainable aquaculture via better water quality maintenance. Hence, a decrease in feed requirement, a reduced use of fishmeal or other costly protein sources in shrimp feeding are thereby attained beside sustaining a higher production to achieve more profit in shrimp farming in Egypt.

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ARABIC SUMMARY

تاثيرالكثافات التخزينية المختلفة ليرقات الجمبرى البحرى الفانمى على أداء النمو و معدل البقاء على قيد الحياه باستخدام تقنيه البيوفلوك

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دائما هناك حاجة لتطوير أعلاف الجمبرى المستزرع في انظمة التربية المختلفة داخل مصر وذلك لتوفر بروتينً كافي لإنتاج الجمبرى مع تقليل كمية النيتروجين التي يتم إدخالها في بيئه الاستزراع. تعتبر تقنيه البيوفلوك تطبيق جديد للحفاظ على جودة المياه وأداء نمو الجمبرى المستزرع.

أجريت هذه التجربه لدراسه تاثير ثلاث كثافات تخزين مختلفة و مصدر الكربون (مصاصة القصب) مع نظام البيوفلوك وعلف تجارى نسبة البروتين به (38%) مع نظام الكنترول في احواض استزراع الجمبرى الفانمى على جودة المياة والمجتمعات الميكروبيه وتركيب البيوفلوك وأداء النمو وكفاءه الاستفادة من العلف ومعدلات الاعاشه للجمبرى. تم تخزين الجمبرى بكثافات مختلفه في 18 تانك بمعدل (12 و 14 و16يرقه/اللتر) بحجم مياه 150 لتر لمدة 90 يوما. تم تغذية الثلاث مكررات من الجمبرى (متوسط وزن الجسم ، 0.002) ثلاث مرات يوميا بمعدل 14% من وزن الجسم الابتدائى وتعديلها تدريجيا الى 5% فى نهاية التجربة.

قياسات جوده المياه من حيث درجة الحرارة والملوحة والاس الهيدروجينى والامونيا والنيتريت والنترات و الفوسفات كانت مناسبة لاستزراع الجمبرى في معاملات البيوفلوك والكنترول، الا أن حجم البيوفلوك والمواد الصلبة المعلقة بهم فروق معنوية عالية بين معاملات البيوفلوك مقارنة بالكنترول. وان معدلات النمو والبقاء على قيد الحياة افضل في معاملات البيوفلوك. و وفقًا لنتائج التحليل الاقتصادي، تعد كثافة ال 16يرقه /اللتر بيوفلوك هي أفضل كثافة تخزين مقارنة بالكثافات الأخرى. أدت إضافة مصدرالكربون الى تنكات استزراع الجمبرى الفانمى الى خفض مركبات الامونيا الكلية و زيادة نمو الكائنات الدقيقة الغير ذاتية التغذية والزيادة في عدد البكتريا الغير ذاتيه التغذيه تؤثر على مجتمع الزؤوبلانكتون والذى يؤدى الى تحسين أداء النمو و معدلات الاعاشة للجمبرى.