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Effect of bioflocs on growth performance and survival of the white-leg shrimp *Litopenaeus vannamei* raised in zero-water exchange culture tanks

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ABSTRACT

A 90-day trial was conducted to evaluate the effects of three different protein levels (25%, 30% and 35% CP) and two different carbon sources (sugarcane bagasse and wheat flour) on the growth performance, feed utilization and survival rate of the white-leg shrimp *Litopenaeus vannamei* in zero –water exchange tanks. Six biofloc treatments and one control (45% CP) without biofloc were managed. Final body weight, weight gain, specific growth rate and protein efficiency ratio were highly affected " interaction "($P < 0.01$) by different protein levels (25%, 30% and 35% CP) and different carbon sources (sugarcane bagasse and wheat flour). Survival rates ranging from 72.2% to 83.3%, and there were no significant difference ($P > 0.05$) among all treatments. There were no significant differences ($P > 0.05$) in moisture, crude protein and lipid among six biofloc and control treatments.

INTRODUCTION

Shrimp are the most valuable commercial crustaceans in the world. Shrimp now ranks second in value terms and 6th in terms of quantity amongst all the taxonomic groups of aquatic cultivated animals. Shrimp aquaculture can help in the reduction of the pressure on the overexploited wild stocks, in terms of natural resources protection **(FAO, 2016)**.

The white-leg shrimp, *Litopenaeus vannamei*, primarily comes from Asia and the Americas **(FAO, 2016)**. This species dominates shrimp culture in the Western hemisphere **(Laramore *et al*., 2001)**. but Thailand and China are the top two producers **(Cao, 2012)**. Similar to global aquaculture trends, white-leg shrimp culture production has undergone a great expansion, from just 8,000 tons in 1980 to over three million tons in 2016 **(FAO, 2016)**.

Shrimp growth depends on the nutritional quality of dietary protein. Artificial diet in shrimp aquaculture accounts 50% –70% of total operating cost in the extensive, semi-intensive and intensive farms. Therefore, attention has been paid towards reducing the feed cost by using of less expensive and highly nutritive ingredients or by better consumption and assimilation of feeds by the shrimps **(Varghese, 2007)**.

Formulated diets fed to shrimp consist of high amounts of protein (35% –50%), nitrogen (6%) and phosphorus (2%). However, only 20% -22% of feed assimilated in to shrimp tissues while the remaining is discharged as dissolved and particulate waste **(Hanh *et al*., 2005)**.

Biofloc technology (BFT) is an emerging alternative towards more environment friendly aquaculture production systems. This technology was developed to create economical and environment benefits via reduced water use, effluent discharges, artificial feed supply and improved biosecurity **(Wasielesky *et al*., 2006; Avnimelech, 2007; Mishra *et al*., 2008)**. It 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. The addition of carbon materials such as molasses into shrimp culture with manipulating the carbon: nitrogen (C/N) ratio level is one of the best strategies of controlling ammonia and nitrite in shrimp culture with Limited Water Exchange Model **(Panjaitan, 2011; Paiva Maia *et al*., 2016)**. The C/N ratio in an aquaculture system can be increased by adding different locally available cheap carbon sources (agricultural by-products) and also by the reduction of protein content in the feed **(Avnimelech, 1999; Hargreaves, 2006)**. Different organic carbon sources (glucose, cassava, molasses, wheat, corn, sugar bagasse, sorghum meal, etc.) are used to enhance production and to improve the nutrient dynamics through altered C/N ratio in shrimp culture **(Avnimelech, 1999)**. C/N ratio is also widely used as a guide for analyzing the decomposition of organic matter and reduction of toxic nitrogenous compounds **(Browdy *et al*., 2001)**. The biofloc system maintained with C/N ratio of higher than 15–20 will be developing sufficient microbial floc to assimilate toxic nitrogenous species under intensive farming with limited discharge.

Simple carbohydrates such as sugar and molasses are immediately decomposed by bacteria and quickly react so they must continuously be added to the tank to maintain optimum activity of heterotrophic bacteria. However, complex carbohydrates such as cellulose and starch grains are slowly decomposed by bacteria, resulting in stable levels of carbohydrates in the tank and slower response time **(Avnimelech, 2009** and **Serra *et al.*, 2015)**. 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)**.

Bioflocs are aggregates (flocs) bacteria, algae, protozoa and zooplankton and other kinds of particulate organic matter such as feces and uneaten feed **(Hargreaves 2006; 2013)**. Each floc is held together in a loose matrix of mucus that is secreted by bacteria, bound by filamentous microorganisms, or held by electrostatic attraction.

Bioflocs were applied by many authors on different species of shrimp in different areas of the world e.g. **Emerenciano *et al*. (2012) ; Gao *et al*. (2012) ; Xu *et al*. (2012) ; Zhao *et al*. (2012) ; Anand *et al*. (2014) ; Ekasari *et al*. (2014) ; Kumar *et al*. (2014) ; Xu and Pan (2014a) ; Hussain *et al*. (2015) ; Luis-villasenor *et al*. (2015) ; Serra *et al*. (2015) ; Yun *et al*. (2015) ; Wang, *et al*. (2016) ; Khatoon *et al*. (2016) ; Paiva Maia *et al*. (2016) ; Zhao *et al*. (2016) ; Khanjani *et al*. (2017) and Yassien *et al*. (2019).**

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

MATERIALS AND METHODS

Three replicates for each of the seven treatments, (three different levels of protein "25%, 30% and 35% CP" and two different carbon sources sugarcane bagasse "SB" and wheat flour "WF") under biofloc system and one control 45% CP without biofloc system). Experiment was carried out for 90 days, in 21 glass tanks (50×40×80 cm) with filtered seawater volume 80 Liter and was diluted with tap water to achieve a salinity of (20 ppt). Aeration was provided for 24 hours to ensuring better bioflocculation. All glass tanks were always covered with black plastic sheets to reduce the light intensity, escapees of the shrimp and evaporation. In the control treatment tanks (without biofloc system) water was exchanged two times in a week, while BFT tanks were maintained for 90 days without any water exchange (zero water exchange), except for the addition of dechlorinated freshwater to compensate the evaporation. The biofloc was produced in two plastic containers (20 L) using water from the commercial shrimp culture pond as an 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)** as shown in **Table 1** .

Table 1. The proximate composition % of the two 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

Six treatments of biofloc system with different levels of protein (25%, 30% and 35% CP) and two different carbon sources (sugarcane bagasse and wheat flour), in addition to one control (45% CP) without biofloc system. Details of tank allocation and experimental code are given in **Table 2**.

Table 2. Experimental design of marine shrimp *L. vannamei* in the BFT

NO	Treatments	Experimental Code
1	Control shrimps were fed with commercial diet (45% CP) without biofloc system	**C**
2	Shrimps were fed with commercial diet (25% CP) + sugarcane bagasse (SB) as carbon source	**BFT1**
3	Shrimps were fed with commercial diet (25% CP) + wheat flour (WF) as carbon source	**BFT2**
4	Shrimps were fed with commercial diet (30% CP) + sugarcane bagasse (SB) as carbon source	**BFT3**
5	Shrimps were fed with commercial diet (30% CP) + wheat flour (WF) as carbon source	**BFT4**
6	Shrimps were fed with commercial diet (35% CP) + sugarcane bagasse (SB) as carbon source	**BFT5**
7	Shrimps were fed with commercial diet (35% CP) + wheat flour (WF) as carbon source	**BFT6**

Post larvae (Juveniles) of *L. vannamei* were obtained from a commercial shrimp hatchery, acclimatized in tanks of 20 ppt. salinity for 2 weeks and fed twice daily with commercial feed 45% CP. After two weeks, all tanks were stocked with juveniles at the rate of 10 shrimps in each glass tank with a mean initial body weight (0.23 g ± 0.04). Commercial diets with three dietary protein levels, viz., 25%, 30% and 35% with biofloc system and one control without biofloc (45% CP) were selected as experimental diets. The composition of the dietary ingredients is shown in **Table 3**. 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).

Table 3. Ingredients and proximate composition of the experimental diets (% of DM basis)

Ingredients	**45%**	**35%**	**30%**	**25%**
Fish meal	450	270	270	90
Soybean meal	330	330	230	330
Yellow corn	90	205	250	280
Wheat bran	45	100	155	195
Fish oil	40	50	50	60
Cholesterol	5	5	5	5
Di-calcium phosphate	20	20	20	20
Vit&Min	20	20	20	20
Proximate analysis				
Dry matter%	90.60	90.18	89.96	89.35
Crude protein%	45.17	35.21	31.96	25.62
Crude lipid%	8.12	8.93	9.26	7.18
Ash content%	11.23	9.01	8.12	7.81
Crude fiber %	3.38	3.98	3.93	4.98
Nitrogen free extract %	32.1	42.87	46.79	54.41
Gross energy (MJ kg^{-1} diet)	19.38	19.20	19.22	18.70

At the end of the experiment, the nutritive quality of shrimp's flesh was determined according to **AOAC (1995)**. Upon termination of the 90-days trial, final weights of the remaining shrimp were obtained as shrimp weight per tank (FBW). Weight gain (WG) = final body weight (FBW) - Initial body weight (IBW), Specific growth rate (SGR) = 100 x (ln FBW – ln IBW) / rearing period in days, Feed conversion ratio (FCR)=dry feed consumed / WG, Protein efficiency ratio (PER) = WG / protein consumed and Survival rate = (final shrimp number / initial shrimp number) x100.

All variables of shrimp growth performance and feed utilization 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

Details of the growth of shrimp harvested from experimental tanks with and without biofloc technology are presented in **Table 4**. The significantly higher ($P < 0.01$)

final body weights (FBW) 10.6 g ± 0.1, 10.40 g ± 0.1 and 9.23 g ± 0.06 were recorded in BFT6, BFT5 and BFT4, respectively, compared to C treatment (7.51 g ± 0.12) and BFT3 (8.14 g ± 0.11). Moreover, there were no significant difference between BFT2 (8.75 g ± 0.06) and BFT1 (8.68g ± 0.1). Final body weight (FBW), weight gain (WG), specific growth rate (SGR) and protein efficiency ratio (PER) were high affected " interaction "($P < 0.01$) by different protein levels (25%, 30% and 35% CP) and different carbon sources (sugarcane bagasse and wheat flour). Overall, weight gain (WG) and specific growth rate (SGR %) were significantly better ($P < 0.01$) at 35% protein level i.e. BFT5 &BFT6 and different carbon source (sugarcane bagasse and wheat flour) compared to other treatments. BFT2 (3.50 g ± 0.01) and BFT1 (3.47 g ± 0.01) were significantly higher ($P < 0.01$) protein efficiency ratio (PER) than the other treatments. The results also showed that there were no significant differences ($P > 0.05$) feed conversion ratio (FCR) found among the treatments, although the lower FCR value in BFT4 (1.36 g ± 0.08) and BFT6 (1.37 g ± 0.01), and the higher in BFT1 (1.57 g ± 0.09) and BFT2 (1.54 g ± 0.33). Survival rates (SR%) ranging from 72.2% to 83.3%, and there were no significant difference ($P > 0.05$) between treatments.

Table (4) Effect of biofloc technology on growth performance and feed utilization of *L. vannamei* (Mean ± SD) in experimental tanks under different protein levels and different carbon sources for 90 days.

Parameters	C	BFT1	BFT2	BFT3	BFT4	BFT5	BFT6	P	CS	P × CS
FBW	7.51±0.12 [f]	8.68 ± 0.1d	8.75±0.06d	8.14 ±0.11e	9.23±0.06c	10.40±0.1b	10.6±0.1 a	**	**	**
WG	7.28 ± 0.6 f	8.45 ± 0.7 d	8.52 ± 0.6 d	7.91 ± 0.4 e	9.00 ± 0.6 c	10.17 ± 0.4 b	10.38±0.5[a]	**	**	**
SGR %	3.87 ± 0.01 f	4.03 ± 0.03 d	4.04 ± 0.01 d	3.96 ± 0.01 e	4.10 ± 0.01 c	4.24 ± 0.03 b	4.26 ± 0.01 a	**	**	**
FCR	1.43±0.78 a	1.57 ± 0.09 a	1.54 ± 0.33 a	1.48 ± 0.18 a	1.36 ± 0.08 a	1.40 ± 0.09 a	1.37 ± 0.01 a	ns	ns	ns
PER	0.167 ± 0.01 g	0.347 ± 0.01 b	0.350 ± 0.01 a	0.272 ± 0.003 f	0.308 ± 0.02 c	0.297 ± 0.001 e	0.304 ± 0.001 d	**	**	**
SR%	80.6 ± 7.5 a	72.2 ± 10.1 a	75 ± 9.2 a	77.8 ± 8 a	80.6 ± 8.3 a	80.6 ± 7.7 a	83.3 ± 6.4 a	ns	ns	ns

Values for each treatment are means ± SD (range) of three replicate tanks. Means in the same row having superscript differ significantly. $*P < 0.05$, $**P < 0.01$, 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. *Initial body weight (IBW) = 0.23gm. Shrimp final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR%).

Body composition of the shrimp is shown in **Table 5**. There were no significant differences ($P > 0.05$) in moisture, crude protein and lipid among six biofloc and control treatments. Crude ash contents were significantly ($P < 0.05$) higher in all biofloc treatments compared to control treatment. In six biofloc treatments, crude protein content ranging from 58.31% to 61.72%. While, crude lipid and ash contents ranging from 19.0% to 21.19% and 18.19% to 23.14%, respectively. Generally, the flesh of shrimp raised in the biofloc tanks showed relatively higher values of crude protein, lipid and ash than the control treatment. The moisture was the highest component in the shrimp's flesh.

Table (5) Effect of biofloc technology on chemical composition of *L. vannamei* g/100 g diet (Mean ± SD) in experimental tanks under different protein levels and different carbon sources for 90 days.

Treatments	Moisture	Crude protein	Lipid	Ash
C	80.13±0.6	58.15±1.97	19.23±0.68	17.20±0.62c
BFT1	72.16±0.52	60.74±1.98	20.19±0.62	21.13±0.56a
BFT2	71.43±0.5	60.12±1.15	19.0±0.82	19.18±0.56b
BFT3	76.62±0.1	61.72±2.23	21.19±0.98	22.90±0.93a
BFT4	73.46±0.7	58.31±2.52	21.13±0.64	18.19±0.72b
BFT5	70.90±0.5	60.98±1.56	20.89±0.06	23.14±0.68a
BFT6	78.18±0.5	59.30±2.63	20.19±0.54	19.19±0.43b

DISCUSSION

Shrimp typically have a higher dietary protein requirement during the different growth phases **(Chen *et al*. 1985)**. However, there is a wide range in reported dietary protein requirements for *L. vannamei*, typically from 300 to 480 g kg−1 (30–48%), with an optimum for post larvae (PL) of 340 g kg−1 (34%) **(Hu *et al*. 2008)**.

In the present study, supplementation of different levels of protein (25%, 30% and 35% CP) and different carbon sources (sugarcane bagasse and wheat flour) enhanced the growth performance of shrimp on the basis of significant increase in FBW, WG, SGR and PER on six biofloc treatments throughout the experimental period (90-d). Different combinations or levels of carbon sources added to a biofloc-based system would change the biofloc, which could play important roles in maintaining good water quality **(Yassien *et al*. 2019)**, supplementing food source and contributing to the improved growth performance **(Wang *et al*. 2016** and **Zhao *et al*. 2016)**.

If carbon and nitrogen are well balanced according to either the use of lower protein diet and/ or supplying additional carbon sources, e.g. glucose, sucrose, and starch to the pond, the inorganic nitrogen components (ammonia, nitrite, and nitrate) in pond will be converted into bacterial biomass **(Avnimelech, 1999)**. As such, nutrients from excretion and remnant feed are recycled into bacterial biomass and formed biofloc which can be taken up as an additional feed for aquatic animals **(Avnimelech, 2006)**.

In this study, BFT6 (35% CP and wheat flour) and BFT5 (35% CP + sugarcane bagasse) have better growth compared to the control treatment fed with 45% CP. This supports the earlier findings that feed with lower protein level with biofloc could replace the higher protein diet **(Hari *et al*. 2004** and **Ballester *et al*. 2010)**. Our results were in agreement with the study of **Rajkumar *et al*. (2016)**, who found that growth rate and survival rate of *L. vannamei* were significantly higher in the treatment BFTW (34.5% CP + wheat flour) than those of others BFTS (34.5% CP + sugarcane molasses) and BFT (34.5% CP + tapioca flour), although no significant differences were observed on survival rate between all the treatments. Indicating that the appropriate quantity of carbohydrate addition was helpful in good growth and survival of *L. vannamei*. **Wasielesky *et al*. (2006)** demonstrated that *L. vannamei* juveniles grown in a bioflocs-based system had higher growth rates compared to juveniles grown in a clear water system. Studies have indicated that carbohydrate addition can result in the production and accumulation of bioflocs **(Avnimelech, 2007; Emerenciano *et al*. 2011** and **Gao *et al*. 2012),** which could serve as an important food source for the zooplankton and thus could increase the growth of the shrimp.

In the present study, the final weight, weight gain and SGR of *L. vannamei* in the bioflocs treatments fed the diets with 25%, 30% and 35% CP were significantly higher than those obtained in the control fed the diet with 45% CP. Also, it can be concluding that feeding high-protein diet could be unnecessary and uneconomical when a high abundance of bioflocs are present in the culture system. That is a relatively low level of dietary protein could be compensated for by ingestion of bioflocs, allowing for reducing protein content of the diet **(McIntosh, 2000)**. Reduction of dietary protein levels without affecting shrimp growth has been reported by several authors and microbial proteins have been provided as an important source of protein available for shrimp in these systems **(Hari *et al*. 2004** and **Ballester *et al*. 2010)**. Also, the results of the present study accepted with **Hamidoghli, *et al*. (2018)**, who reported that shrimp fed diets containing 35% protein had higher WG, SGR and FE than those of shrimp fed 30% protein. The previous investigations for protein requirements of white-leg shrimp cultured in biofloc system indicated that the amount of protein can be reduced from 40% to 35% **(Yun *et al*. 2015)**. **Xu, *et al*. (2012),** reported that dietary protein level could be reduced to 25% without affecting the growth of juvenile white-leg shrimp reared in bioflocs system. **Xu and Pan (2014a)**, reported that dietary protein level can be reduced from 35% to 25% without affecting survival, growth, FCR, and physiological status of immune response and antioxidant capability, indicating the potential contribution of biofloc in the protein nutrition and physiological health of cultured shrimp.

This results show that better values of protein efficiency ratio (PER) were observed in the bioflocs treatments compared to the control and this result coincide with that of **Xu *et al*. (2012)**. Moreover, the results agreed with the study of **Wang *et al*. (2016)**, who reported that the growth performance (in terms of final weight, weight gain and specific growth rate) of the shrimp in bioflocs treatments was significantly higher than that obtained in the control, while the feed conversion rate was significantly decreased. As well as **Kumar *et al*. (2014)**, found that better growth and immune responses were observed in rice flour added groups compared to molasses. Moreover, rice flour added group at 32% protein had better growth and immune response compared to control at 40% protein diet.

In the present study, shrimps in biofloc treatment and control had suitable moisture contents 70.9% to 80.13%, and these results coincide with **Yanar** and **Celik (2006)**, who found that the moisture of fresh shrimp is generally reported as 75% to 80%. Protein was found as the major constituent indicating that shrimp flesh can be a good source of amino acids **(Puga-lópez *et al*., 2013)**. Crude protein levels showed a tendency to increase in the shrimp reared in the biofloc treatments. Protein contents were no significantly difference ($P < 0.05$) in biofloc treatments and control, ranging from 58.15% to 61.72%. Only slight differences were found in the ash contents among shrimp grown in the control and biofloc treatments, representing that the quality of flesh was not affected by any of the culture conditions used in this study.

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