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Biofloc as a potential natural feed for shrimp postlarvae

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ABSTRACT

In this study, the suitability of excess biofloc that was discarded as waste from *Litopenaeus vannamei* farm effluent was investigated for effectiveness as a dietary replacement in rearing *L. vannamei* postlarvae (PL). A commercial shrimp diet (control) was compared to four diets containing dried waste biofloc at 25%, 50%, 75% and 100% replacement levels and fed to shrimp PL to evaluate the survival rate, growth performance and nutritional composition. Total ammonium nitrogen and nitrite nitrogen were maintained in culture tanks with minimal water exchange throughout the experiment. Results showed that PLs fed with 50% biofloc feed (50% BF) had significantly higher ($p < 0.05$) specific growth rate compared to the other treatments. In addition, PLs fed with 50% and 75% BF had significantly higher survival rate ($p < 0.05$) compared to those fed with commercial feed only. However, protein content of PLs fed with 50% and 75% BF was comparable to those of 100% commercial feed. This study demonstrates that waste biofloc has potential to be used as a cost effective feed for rearing shrimp PLs.

1. Introduction

Aquaculture has grown rapidly over the last couple of decades due to significant increases in the global demand for fish and seafood. Marine shrimp farming is one of the major and important commercial aquaculture activities in terms of production value (FAO, 2010) as shrimps are considered to be a highly valued seafood commodity (Anand et al., 2014). However, intensive shrimp farming has some environmental problems such as poor water quality and low feed utilization (Avnimelech, 2006). White shrimp *Litopenaeus vannamei* is the most commonly cultured species worldwide due to its rapid growth, high survival rate and disease tolerance in intensive shrimp farming (Cuzon et al., 2004). The postlarvae of *Litopenaeus vannamei* require 18–25% of dietary protein (Velasco et al., 2001), 40% carbohydrates (Cousin et al., 1993) and 5–8% lipids (Cuzon et al., 2004). Excess of nutrients and organic matters from shrimp culture ponds may lead to long term environmental problems (Piedrahita, 2003).

Further, the expansion of aquaculture is also restricted due to its strong dependence on fishmeal (Browdy et al., 2001). According to Tan et al. (2005), shrimp feed constitutes 40–60% of the total production costs. This is mainly due to the cost of protein component in commercial diets (Bender et al., 2004). In the commercial culture phase, 30% digestible protein in feed is generally required and is the costliest component of the diet (National Research Council (NRC), 2001). Fishmeal is an essential ingredient in marine shrimp diets due to its balanced content of amino acids, fatty acids, vitamins, and minerals (Suárez et al., 2009). Increase in demand and inconsistent supply of fishmeal has caused a significant increase in fishmeal prices significantly lowering the profit margins of aquaculture farmers and making aquaculture operations unprofitable. The increasing demand for fishmeal has prompted a search for cheaper and sustainable protein ingredients in aquaculture diets (Salze et al., 2010).

There has been research in alternative feed to overcome such issues. Microalgal products (Boonyaratpalin et al., 2001; Supamattaya et al., 2005; Ju et al., 2009), macroalgae (Yeh et al., 2006), probiotics (Ziaei-Nejad et al., 2006; Wang, 2007; Yang et al., 2010), prebiotics (Zhang et al., 2012) and periphyton (Anand et al., 2013) have been tried and many are used as dietary supplements to enhance growth, immune response and digestive enzyme activities in shrimp farming. These alternatives will help in improving water quality as well as reduce cost for sustainable shrimp farming.

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An ingredient that has potential for use in shrimp feed is microbial floc meal (Kuhn et al., 2009, 2010). Microbial floc meal can be obtained from shrimp farm effluents that use biofloc technology system (BFT). Microorganisms present in BFT not only maintain water quality but also helps in lowering feed costs by providing nutrition (Emerenciano et al., 2013). Biofloc encompasses a heterogeneous mixture of diatoms, macroalgae, food and faecal remnants, exoskeletons, bacteria, invertebrates (Jatobá et al., 2014) and other microscopic organisms found in ponds (Hargreaves, 2006). Studies by Arnold et al. (2009) and Megahed (2010) have suggested that bioflocs could enhance growth performance and thereby can be used as a supplemental food source for cultured shrimps (Avnimelech, 1999) thereby reducing feed costs (Wasielesky et al., 2006). The consumption of bioflocs increases feed utilization efficiency and can replace a significant fraction of the nutrition demand (Crab et al., 2010) by recycling feed residues and fecal excrements (Schneider et al., 2005; Hargreaves, 2006). More importantly, bioflocs or its attached microorganisms could aid in the enzymatic activity of shrimp digestion (Moss et al., 2001). The higher digestion rates and improved absorption of the feed resulting from the increase of digestive enzymes in the digestive tissues have contributed to the improvement of growth performance and feed utilization of the shrimp (Xu and Pan, 2012).

In shrimp industry BFT has been used with great success (Bauer et al., 2012) and waste biofloc can be obtained from the effluent of super-intensive shrimp farms that use BFT. Instead of discarding the nutrient rich waste biofloc from BFT, it can be used as a feed for the aquaculture industry while minimizing environmental problems. In this study, the suitability of excess biofloc that is discarded as waste from BFT was investigated as a dietary replacement for commercial feed. The study focussed on survival, growth performance and nutritional composition while rearing *L. vannamei* postlarvae (PL).

2. Methods

2.1. Collection of waste biofloc

Waste biofloc was collected from *L. vannamei* farm effluents that utilizes BFT in Setiu, Terengganu, Malaysia. Water from the shrimp waste pond containing biofloc was taken and poured into Imhoff Cone and the biofloc was allowed to settle for 1 h. Harvested biofloc was kept in sterile bottles and transported (refrigerated with ice) to laboratory for further processing.

2.2. Feed formulation from waste biofloc

Harvested biofloc was dried in oven at 40 °C and subsequently ground using a stand mixer (KitchenAid® Professional 600 Series, Michigan, USA). The dried samples were milled to 300 μm, stored in plastic bags and kept at −18 °C for further use. The treatment consisted of five diets with 0, 25, 50, 75 and 100% replacement of commercial feed with waste biofloc. A commercial feed commonly used for production of *L. vannamei* at this rearing phase was used as control diet.

2.3. Experimental design

The experiment was conducted at the Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia. Five treatments consisting of 100% commercial feed (100% CF) as control; commercial feed with 25% biofloc replacement (25% BF); commercial feed with 50% biofloc replacement (50% BF); commercial feed with 75% biofloc replacement (75% BF) and 100% biofloc (100% BF) were evaluated in triplicate using a complete randomized design. Fifteen rectangular glass aquaria each containing 40 L clean, filtered (5 μm filter bag) and chlorinated seawater were used in this experiment. *L. vannamei* postlarvae stage 1 (PL1) were obtained from a commercial hatchery and were stocked into the glass aquaria at a density of 50 PL1 L^{-1}. Postlarvae were taken from the same broodstock that were hatched from the same batch at the same time and were stocked on the same day. Thus the PLs in all the treatment and the control tanks were considered to be at the same PL stage at the end of the study. Constant aeration was provided to each aquarium using an air compressor. The hatchery tanks were maintained under a 12 h light − 12 h dark cycle. Postlarvae in all tanks were fed with formulated diet and *Artemia* (Golden Dolphin, Malaysia) for four times in a day at 6 h intervals. The excess feed was removed from the tanks 1 h before the next feeding. The experiment was conducted for a period of 12 days.

2.4. Physico–chemical parameters

Temperature, salinity, pH, and dissolved oxygen level in the culture tanks were measured daily using a YSI 556 MPS (YSI, New Jersey, USA). Total ammonia nitrogen, nitrite nitrogen and phosphorus were analysed on alternate days following the method of Parsons et al. (1984).

2.5. Biological parameters

Specific growth rate (dry weight basis) was calculated from the body weight (mg) based on the formula of Ricker (1979): $G = (\ln w_2 - \ln w_1)/(t_2 - t_1)$, where w_2 and w_1 represent the final and initial weight, respectively, and $(t_2 - t_1)$, the duration of the experimental period. The survival of the PL was also determined at the end of the experiment. Survival was calculated as the percentage of shrimp remaining in each tank from the estimated number stocked initially.

2.6. Proximate composition analysis

Proximate composition such as protein, lipid, carbohydrate, moisture and ash of waste biofloc, experimental feed and the shrimp postlarvae (PL12) were carried out using freeze–dried samples.

2.6.1. Protein

Protein was analysed according to Lowry et al. (1951) by adding 1 N sodium hydroxide followed by alkaline copper solution and Folin-Ciocalteau reagent. Standard solution was prepared using bovine serum albumin at different concentrations. The absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at a wavelength of 750 nm.

2.6.2. Lipid

Lipid was determined by the sulphuric acid-charring technique of Marsh and Weinstein (1966) following the carbonization method using tripalmitin as the standard after extracting lipids according to the methods of Bligh and Dyer (1959). The optical density was measured at 375 nm using a spectrophotometer (Shimadzu UV-1601).

2.6.3. Carbohydrate

Carbohydrate analysis was conducted following the method of Dubois et al. (1956). Samples were analysed by adding 1 mL of a 5% phenolic solution and 5 mL of concentrated sulphuric acid. Standard solution was prepared using glucose. The optical density was measured at 488 nm using a spectrophotometer (Shimadzu UV-1601).

2.6.4. Moisture

Two grams of freeze–dried samples were weighed accurately using a calibrated microbalance in an ash-free filter paper (pre-dried at 100 °C to constant weight) placed in a glass Petri plate (pre-dried at 100 °C to constant weight). The samples were then dried again in an oven at 100° C for 6–12 h or until constant weight was observed. After drying, the plates were cooled inside a desiccator and weighed. The moisture content was calculated using the formula below (Horwitz, 1984):

% Moisture = (wt. of oven dried samples/wt. of original samples) × 100

% Dry matter = 100 − % moisture

2.6.5. Ash

The crucible containing dried sample obtained from the moisture determination exercise was placed in a muffle furnace and heated at 550 °C to 600 °C for 6 h and till the sample reduced to ash. The ash together with the crucible was cooled down in a desiccator and weighed. The ash content was calculated using the formula below:

% Ash = (Weight of ash/Weight of dried samples) × 100

2.7. Statistical analysis

The collected data were analysed using one-way analysis of variance (ANOVA). Significant differences amongst treatments were determined using Duncan's multiple range test at 0.05 level. All the data which were expressed in percentages were arcsine-transformed to satisfy the condition of homogeneity of variance. Statistical analyses were accomplished using the Statistical Analysis System (SAS, 2002) computer software.

3. Results

3.1. Physico–chemical parameters

There were no significant differences ($p > 0.05$) found in water with regards to salinity (ppt), dissolved oxygen (mg L^{-1}) and temperature (°C) between the treatments and the control tanks as shown in Table 1. The concentrations of TAN and NO_2-N were low in the beginning of the culture period until day 2 for all treatments as seen in Fig. 1. However, the 100% CF tanks showed increasing levels of TAN and NO_2-N beginning day 4 and reaching maximum values on day 10 for TAN, and day 12 for NO_2-N which was significantly higher ($p < 0.05$) than other treatments. For NO_2-N, high values were observed at day 12 in 100% CF tanks. TAN and NO_2-N levels were maintained at low concentrations in 25, 50, 75 and 100% BF treated tanks throughout the culture period. Amongst all the treatments, tanks with 100% BF had the lowest concentrations of TAN (0.20 ± 0.01 mg L^{-1}) and NO_2-N (0.03 ± 0.02 mg L^{-1}) than those tanks with 25, 50 and 75% BF and the 100% CF as shown in Fig. 1.

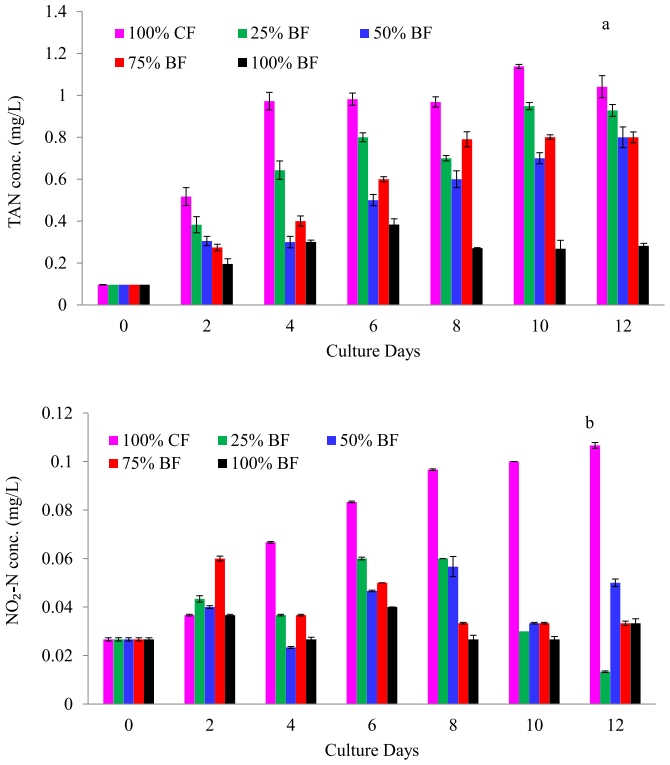

Fig. 1. Concentration of total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N) in shrimp postlarvae rearing tanks during the experimental period.

3.2. Proximate composition of biofloc and experimental feed

The proximate composition of waste biofloc and experimental feed fed to shrimp PL are presented in Tables 2 and 3 respectively. The dried biofloc contained 30.4 ± 0.21% protein, 4.2 ± 0.10% lipid, 18.5 ± 0.10% carbohydrate, 12.3 ± 0.31 moisture and 31.2 ± 0.21 ash. Result showed that waste biofloc contained adequate amount of

Table 2
Mean ± standard error of proximate composition of waste biofloc.

Proximate composition	Content
Protein (% dry weight)	30.4 ± 0.2
Lipid (% dry weight)	4.2 ± 0.1
Carbohydrate (% dry weight)	18.5 ± 0.1
Moisture (%)	12.3 ± 0.3
Ash (%)	31.2 ± 0.2

Table 1
Mean values ± standard error of temperature, dissolved oxygen, salinity and pH range in control and treated tanks during experimental period.

Treatments	Parameters			
	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Salinity (ppt)
100% CF	28.0^a ± 0.3	6.0^a ± 0.1	7.2–8.1	28.0^a ± 0.2
25% BF	27.1^a ± 0.2	6.3^a ± 0.2	7.1–8.2	27.9^a ± 0.3
50% BF	28.0^a ± 0.1	6.5^a ± 0.2	7.0–8.1	27.1^a ± 0.1
75% BF	27.1^a ± 0.2	6.4^a ± 0.3	7.2–8.2	28.1^a ± 0.1
100% BF	28.2^a ± 0.4	6.5^a ± 0.2	7.5–8.3	28.0^a ± 0.3

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

Table 3
Mean ± standard error of proximate composition of experimental feed.

Treatments	Proximate composition (% dry weight)				
	Protein	Lipid	Carbohydrate	Moisture	Ash
100% CF	$36.7^{b} \pm 0.7$	$9.5^{a} \pm 0.2$	$23.8^{a} \pm 0.3$	$10.3^{b} \pm 0.3$	$17.3^{c} \pm 0.1$
25% BF	$37.7^{b} \pm 0.2$	$5.4^{b} \pm 0.3$	$20.2^{b} \pm 0.2$	$10.2^{b} \pm 0.1$	$17.5^{c} \pm 0.1$
50% BF	$43.8^{a} \pm 0.4$	$5.6^{b} \pm 0.1$	$18.8^{c} \pm 0.3$	$11.5^{a} \pm 0.3$	$20.2^{b} \pm 0.2$
75% BF	$43.0^{a} \pm 0.2$	$5.8^{b} \pm 0.2$	$15.1^{d} \pm 0.2$	$12.2^{a} \pm 0.1$	$18.2^{c} \pm 0.2$
100% BF	$30.4^{c} \pm 0.2$	$4.2^{c} \pm 0.1$	$18.5^{c} \pm 0.1$	$12.3^{a} \pm 0.3$	$31.2^{a} \pm 0.2$

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

protein and carbohydrate. On the other hand, lipid content was low.

In this experiment waste biofloc was replaced with different percentage of commercial feed to get adequate nutrition for shrimp PL. Experimental diets showed significant difference ($p < 0.05$) in protein for various percentages of biofloc as shown in Table 3. The highest protein ($p < 0.05$) (43.8% and 43.0% of dry weight) was observed in 50% BF and 75% BF, respectively, followed by 25% BF (37.7% of dry weight), 100% CF (36.7% of dry weight) and 100% BF (30.1% of dry weight). However, the lipid content was significantly higher ($p < 0.05$) in 100% CF compared to biofloc mixed diet. The carbohydrate content ranged from 15.1 to 23.8% in the experimental diets as shown in Table 3.

3.3. Biological parameters

Fig. 2(a) shows the survival percentages of shrimp PLs fed with different percentage of waste biofloc. Shrimp PLs fed with 50% BF showed significantly higher ($p < 0.05$) survival (95.4 ± 0.6%) than 100% CF (67.7 ± 0.3%) followed by 75% BF (90.1 ± 0.1%), 25% BF (82.2 ± 0.2%) and 100% BF (37.7 ± 0.4%). Fig. 2(b) shows the specific growth rate (SGR) of the shrimp PLs fed with different percentage of waste biofloc. The SGR of the PLs were significantly different ($P < 0.05$) in all the treatments and control, with the highest rate in PL fed with 50% BF (17.5 ± 0.01%) followed by the 75% BF (15.3 ± 0.03%), 25% BF (14.8 ± 0.01%), 100% CF (13.3 ± 0.04%) and the 100% BF (10.3 ± 0.05%).

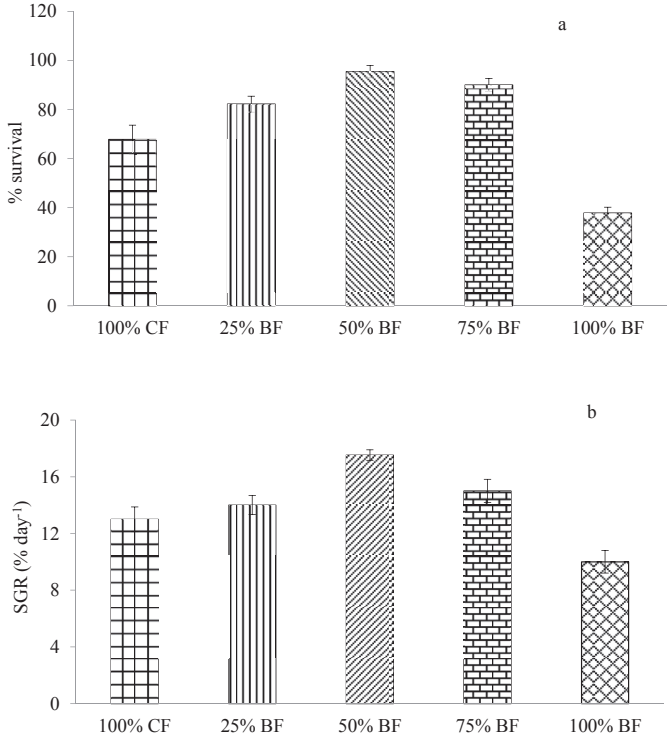

Fig. 2. Survival (a) and specific growth rate (b) of postlarvae fed with different replacement levels of waste biofloc and commercial feed.

3.4. Proximate composition of shrimp postlarvae

The highest ($p < 0.05$) protein content was found in the PLs fed with 50% BF (34 ± 0.2%), followed by 75% BF (30 ± 0.3%), 100% BF (28 ± 0.1%), 25% BF (26 ± 0.4%) and 100% CF (24 ± 0.5%) as shown in Fig. 3(a). Likewise, PL fed with 50% BF (9.5 ± 0.3%) were found to contain the highest ($p < 0.05$) percentage of lipid compared to the other diets. Whereas lowest percentage of lipid was found in PL fed with 25% BF (6 ± 0.2%) and 100% BF (6.0 ± 0.5%) as shown in Fig. 3(b). Carbohydrate content of shrimp PL was high when PLs were fed with 50% BF (28 ± 0.2%) in comparison to those fed with 100% BF (17 ± 0.1%) (Fig. 3(c)).

4. Discussion

In the present experiment, the PLs reared in tanks fed with BF had significantly low levels of TAN and NO2−N compared to control tank. Apart from forming food for the PLs, the BF maintained low levels of ammonia concentration (Yun et al., 2015), implying the usefulness of biofloc in improving water quality in the tanks. The low TAN and NO_2-N would have also favoured high growth and survival of PLs in the BF tanks. Referring to Fig. 1, it should be noted that TAN and nitrite nitrogen level were maintained below the

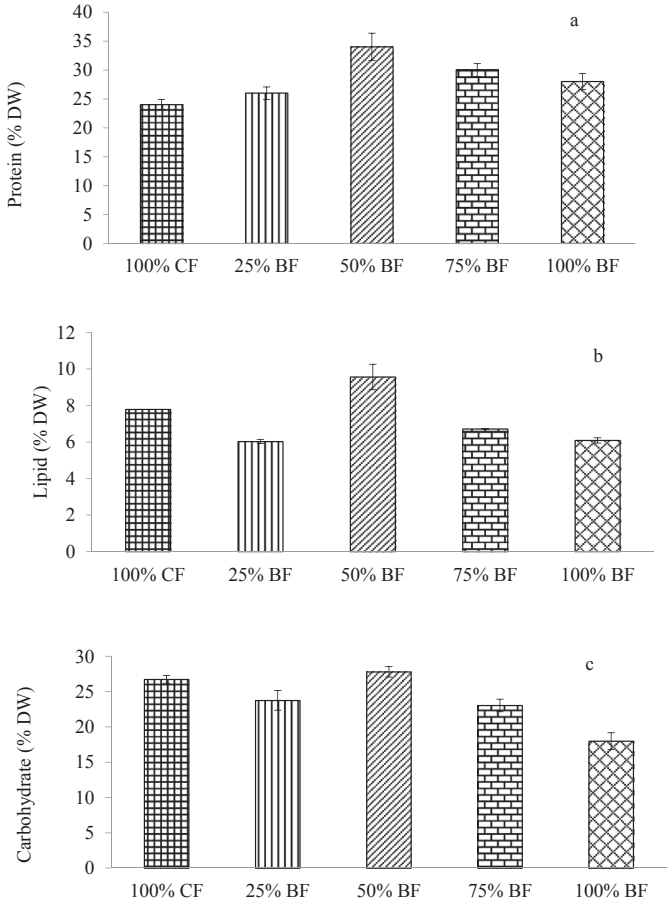

Fig. 3. Protein (a), lipid (b) and carbohydrate (c) content of postlarvae fed with different replacement levels of waste biofloc and commercial feed.

recommended range for shrimp culture in the BF tanks even though minimal water exchange in the BF tanks had occurred during the 12 days trials. The physical parameters of the water (temperature, pH, dissolved oxygen ammonia nitrites and nitrates) were also suitable for the culture of *L. vannamei* (Boyd and Gautier, 2000) in all the culture treatments.

While employing BFT, the water quality in the pond or culture tank can be maintained to good standards. Da Silva et al. (2013) also reported that microbiota in biofloc are able to maintain the water quality of *L. vannamei* juveniles cultured in fiberglass tanks. Besides that, the microorganisms in the biofloc can be used as an additional feed for organism culture. According to Kuhn et al. (2009; 2010), excess biofloc which is discarded as waste from BFT can be used as microbial floc meal. Microorganisms present in the bioflocs contain protein, lipid, and carbohydrate, which are similar to artificial diet for the growth of cultured organisms (Emerenciano et al., 2011). Biofloc could be used as additional feed for the cultured organisms as it has an adequate protein, lipid, carbohydrate and ash content for use as an aquaculture feed (Crab et al., 2010). Earlier studies (Moss et al., 2001; Wasielesky et al., 2006) have reported increase in growth rates, general welfare and survival of shrimps in microbial floc-based systems.

Protein content of the biofloc in the present study is in agreement with the findings of Ballester et al. (2010) who reported 30.4% crude protein when the carbohydrate source is wheat flour and molasses. In the present experiment, the waste biofloc collected from the shrimp farm also used wheat flour and molasses in the BFT. However, the nutritional composition of biofloc is dependent on the carbohydrate source. Study done by Crab et al. (2010) reported that biofloc developed from glycerol, acetate and glucose had 42–43% and 28% crude protein, respectively, compared to *Bacillus* inoculated glycerol which had 58% crude protein. In another study, Mahanand et al. (2013) showed that biofloc developed from wheat flour containing approximately 50% organic carbon had 35.4%, 1.1%, and 44.0% crude protein, crude lipid, and carbohydrate, respectively. Emerenciano et al. (2013) showed that the crude protein, crude lipid, and carbohydrates in the shrimp (*Farfantepenaeus duorarum*) pond was 24.7%, 0.6%, and 26.3%, respectively. Lipids present in bioflocs can influence animal growth (Izquierdo et al., 2006). In the present study, lipid was found to be comparatively higher than other studies (Emerenciano et al., 2013; Mahanand et al., 2013) but lower than the commercial feed. In the present study, ash content was found to be high in the biofloc collected from *L. vannamei* BFT. Bauer et al. (2012) and Ju et al. (2008b) also had reported high ash content of microbial floc meal collected from *L. vannamei* tanks due to the salt content of the biofloc culture system.

Growth performance of *L. vannamei* improved with the inclusion of biofloc as a dietary ingredient in shrimp diet (Ju et al., 2008b; Kuhn et al., 2009, 2010). Similarly, Kuhn et al. (2009) and Xu and Pan (2012) reported that addition of biofloc to the diet of shrimp could help in enhancement of growth performance of *L. vannamei*. Studies conducted by Ju et al. (2008a) have demonstrated that bioflocs are rich source of carotenoids, chlorophylls, phytosterols, bromophenols and amino sugars. In addition, they contain anti-bacterial compounds (Crab et al., 2010). In the present study, *L. vannamei* PLs fed with 50% biofloc feed significantly enhanced the survival and specific growth rate compared to the other treatments and the control.

These results and earlier research indicate that microbial components, unknown growth factors or probiotic microorganisms may have contributed to significantly higher survival and growth rate in shrimp fed with biofloc incorporated diet. However, biofloc replacement at 75% level and 100% biofloc did not result in increase in survival and specific growth rate compared to control. This may be due to the fact that microbial products at higher level render the feed less palatable and digestible (Kiessling and Askbrandit, 1993). At the end of the experiment, proximate analysis revealed that shrimp PLs reared in tanks containing 50% BF had higher amount of protein, lipid and carbohydrate compared to the other treatments and control. Colvin and Brand (1977) reported on the importance of high protein levels for the growth and survival of the early PL stages of *P. stylilostris* and *P. californiensis*. The minimum requirement of protein, lipid and carbohydrate for shrimp is 40–45% (Akiyama and Chwang, 1989), 5–10% (Akiyama and Chwang, 1989; Chen, 1993) and 20% (Alava and Pascual, 1987), respectively.

In the current study, the BF had sufficient levels of protein, lipid and carbohydrates in natural form that enhanced growth and survival of the shrimp PLs. However, Ju et al. (2008b) reported that high survival and growth rates are not always linked to specific nutrients present in the bioflocs. It may be due to the effects of feed intake rate, digestibility, absorption, assimilation and animal health. It may also be inferred that waste bioflocs provide favourable digestibility, absorption and assimilation enhancing the growth and survival of shrimp PLs. From the results it can be identified that inclusion of waste biofloc at 50% level would be economically beneficial in improving growth performance of shrimp PL.

Hari et al. (2006) and Xu and Pan (2012) have also elucidated the useful role of biofloc system in penaeid shrimp. However, there are only few studies on the use of waste biofloc particularly as feed replacement for aquaculture organisms. Kuhn et al. (2009) reported enhancement of *L. vannamei* growth when biofloc was used as dietary ingredient in shrimp diet. The present study has demonstrated the suitability of replacing commercial feed with excess biofloc that is discarded as waste from BFT.

5. Conclusion

The study had demonstrated that dietary replacement of commercial diet with waste biofloc at 50% level had beneficial effects on survival and growth performance of *L. vannamei* PL. The results obtained for *L. vannamei* performance confirm that under experimental conditions, replacement of commercial feed with an alternative source such as waste biofloc as an ingredient in feed formulation is possible, which can translate into reduction in feed costs for shrimp production. In addition, utilization of waste biofloc prevents the release of nutrient rich effluent. This form of waste management brings sustainability benefits for the environment. These findings may encourage feed manufacturers to consider biofloc as a viable alternative diet for aquaculture organisms. Future studies are required to find suitable techniques for collecting large amount of wet biofloc and processing them as feed. In addition, fatty acids, amino acid profile and non-protein content of the biofloc with respect to nutritional requirement of shrimp PL also need to be studied.

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References

Akiyama, D.M., Chwang, L.M., 1989. Shrimp feed requirement and feed management. In: Akiyama, D.M. (Ed.), Proceedings of the Southeast Asia Shrimp Farm Management Workshop. American Soybean Association, Singapore, pp. 75–82.

Alava, V.R., Pascual, F.P., 1987. Carbohydrate requirements of *Penaeus monodon* (Fabricius) juveniles. Aquaculture 61, 211–217.

Anand, P.S., Kohli, M.P.S., Roy, S.D., Sundaray, J.K., Kumar, S., Sinha, A., Pailan, G.H., 2013. Effect of dietary supplementation of periphyton on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture 392, 59–68.
Anand, P.S., Kohli, M.P.S., Sundaray, J.K., Roy, S.D., Venkateshwarlu, G., Sinha, A., Pailan, G.H., 2014. Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture 418–419, 108–115.
Arnold, S.J., Coman, F.E., Jackson, C.J., Groves, S.A., 2009. High-intensity, zero water exchange production of juvenile tiger shrimp, *Penaeus monodon*: an evaluation of artificial substrates and stocking density. Aquaculture 293, 42–48.
Avnimelech, Y., 1999. Carbon nitrogen ratio as a control element in aquaculture systems. Aquaculture 176, 227–235.
Avnimelech, Y., 2006. Biofloc Technology – a Practical Guide Book. The World Aquaculture Society, Baton Rouge, Louisiana, United State, p. 182.
Ballester, E.L.C., Abreu, P.C., Cavalli, R.O., Emerenciano, M., de Abreu, L., Wasielesky, J.W., 2010. Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. Aquacult. Nutr. 16, 163–172.
Bauer, W., Prentice-Hernandez, C., Tesser Jr., M.B., WasieleskyWilson, P., Luís, H.S., 2012. Substitution of fishmeal with microbial flocmeal and soy protein concentrate in diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342, 112–116.
Bender, J., Lee, R., Sheppard, M., Brinkley, K., Philips, P., Yeboah, Y., Wah, R.C., 2004. A waste effluent treatment system based on microbial mats for black sea bass *Centropristis* striata recycled water mariculture. Aquacult. Eng. 31, 73–82.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. J. Biochem. Physiol. 37, 911–917.
Boonyaratpalin, M., Thongrod, S., Supamattaya, K., Britton, G., Schlipalius, L.E., 2001. Effects of β-carotene source, *Dunaliella salina*, and astaxanthin on pigmentation, growth, survival and health of *Penaeus monodon*. Aquac. Res. 32, 182–190.
Boyd, C.E., Gautier, D., 2000. Effluent composition and water quality standard. Glob. Aquac. Advocate 3, 61–66.
Browdy, C.L., Bratvold, D., Stokes, A.D., Mcinstosh, R.P., 2001. Perspectives on the application of closed shrimp culture systems. In: Jory, E.D., Browdt, C.L. (Eds.), The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture. The World Aquaculture Society, Baton Rouge, LA, USA, pp. 20–34.
Chen, H.Y., 1993. Recent advances in nutrition of Penaeus monodon. J. World Aquac. Soc. 24, 231–240.
Colvin, L.B., Brand, C.W., 1977. The protein requirement of penaeid shrimp at various life cycle stages in controlled environment systems. Proc. World Maric. Soc. 8, 821–840.
Cousin, M., Cuzon, G., Blanchet, E., Ruelle, F., 1993. Protein requirements following an optimum dietary energy to protein ration for *Penaeus vannamei* juveniles. In: Kaushik, S.J., Luquet, P. (Eds.), Proceedings of the Aquaculture Feed Processing and Nutrition Workshop. Fish Nutrition in Practice, INRA, Paris, pp. 599–606.
Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W., 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquac. Res. 41, 559–567.
Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C., Guillaume, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture 235, 513–551.
Da Silva, K.R., Wasielesky, W., Abreu, P.C., 2013. Nitrogen and phosphorus dynamics in the biofloc production of the pacific white shrimp, *Litopenaeus vannamei*. J. World Aquacult. Soc. 44, 30–41.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colometric method for determination of sugars and related substances. Anal. Chem. 18, 350–356.
Emerenciano, M., Ballester, Eduardo, L., Cavalli, C., Ronaldo, O., Wasielesky, W., 2011. Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance. Aquacult. Int. 19, 891–901.
Emerenciano, M., Cuzon, G., Paredes, A., Gaxiola, G., 2013. Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: growth performance, water quality, microorganisms profile and proximate analysis of biofloc. Aquacult. Int. 21, 1381–1394.
FAO, 2010. The State of World Fisheries and Aquaculture 2010. Food and Agriculture Organization of the United Nations, Rome, Italy, p. 197.
Hargreaves, J.A., 2006. Photosynthetic suspended-growth system in aquaculture. Aquacult. Eng. 34, 334–363.
Hari, B., Madhusoodana Kurup, B., Varghese, J.T., Schrama, J.W., Verdegem, M.C.J., 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 252, 248–263.
Horwitz, W., 1984. Official Method of Analysis of the Association of Official Analytical Chemist, fourteenth ed. Association of Official Analytical Chemist, Washington, p. 1018.
Izquierdo, M., Forster, I., Divakaran, S., Conquest, L., Decamp, O., Tacon, A., 2006. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp Litopenaeus vannamei. Aquacult. Nutr. 12, 192–202.
Jatobá, A., Corrêa da Silva, B., Souza da Silva, J., Vieira, F.S., Mouriño, J.L.P., Seiffert, W.Q., Toledo, T.M., 2014. Protein levels for *Litopenaeus vannamei* in semi-intensive and biofloc systems. Aquaculture 432, 365–371.
Ju, Z.Y., Forster, I.P., Conquest, L., Dominy, W., Kuo, W.C., David Horgen, F., 2008a. Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. Aquac. Res. 39, 118–133.
Ju, Z.Y., Forster, I., Conquest, L., Dominy, W., 2008b. Enhanced growth effects on shrimp (Litopenaeus vannamei) from inclusion of whole shrimp floc fractions to a formulated diet. Aquac. Nutr. 14, 533–543.
Ju, Z.Y., Forster, I.P., Dominy, W.G., 2009. Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). Aquaculture 292, 237–243.
Kiessling, A., Askbrandit, S., 1993. Nutritive value of two bacterial strains of single-cell protein for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 109, 119–130.
Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick, G.J., 2009. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture 296, 51–57.
Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, G.J., 2010. Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303, 28–33.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
Mahanand, Sudhansu Shekhar, Moulick, Sanjib, Srinivasa Rao, P., 2013. Optimum formulation of feed for rohu, *Labeo rohita* (Hamilton), with biofloc as a component. Aquacult. Int. 21, 347–360.
Marsh, J.B., Weinstein, D.B., 1966. Simple charring method for determination of lipids. J. Lipid Res. 7, 574–576.
Megahed, M.E., 2010. The effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus semisulcatus*) fed with different crude protein levels. J. Arab. Aquacult. Soc. 5, 119–142.
Moss, S.M., Divakaran, S., Kim, B.G., 2001. Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp, *Litopenaeus vannamei* (Boone). Aquac. Res. 32, 125–131.
National Research Council (NRC), 2001. Nutrient Requirements of Fish and Shrimp. Washington DC, USA, p. 306.
Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, p. 173.
Piedrahita, R.H., 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226, 35–44.
Ricker, W.E., 1979. Growth rates and models. Vol. VIII. Bio-energetics and Growth. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology. Academic Press, New York, pp. 677–743.
Salze, G., Mclean, E., Battle, P.R., Schwarz, M.H., Craig, S.R., 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. Aquaculture 298, 294–299.
SAS, 2002. Statistical Analysis System, Version 9.1. SAS Institute Inc., Cary.
Schneider, O., Sereti, V., Eding, E.H., Verreth, J.A.J., 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. Aquacult. Eng. 32, 379–401.
Suárez, J.A., Gaxiola, G., Mendoza, R., Cadavid, S., Garcia, G., Alanis, G., Suárez, A., Faillace, J., Cuzon, G., 2009. Substitution of fish meal with plant protein sources and energy budget for white shrimp Litopenaeus vannamei (Boone, 1931). Aquaculture 289, 118–123.
Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M., Borowitzka, L., 2005. Effect of a *Dunaliella* extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). Aquaculture 248, 207–216.
Tan, B., Kangsen, M., Shixuan, Z., Qicun, Z., Lihe, L., Yu, Y., 2005. Replacement of fish meal by meat and bone meal in practical diets for the white shrimp *Litopenaeus vannamei*. Aquac. Res. 36, 439–444.
Velasco, M., Lawrence, A.L., Neil, W.H., 2001. Comparison of survival and growth of *Litopenaeus vannamei* (Crustacea:Decapoda) postlarvae reared in static and recirculating culture systems. Tex. J. Sci. 53, 227–238.
Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture 269, 259–264.
Wasielesky, W., Atwood, H., Stokes, A., Browdy, C.L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture 258, 396–403.
Xu, W.J., Pan, L.Q., 2012. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture 356–357, 147–152.
Yang, S.P., Wu, Z.H., Jian, J.C., Zhang, X.Z., 2010. Effect of marine red yeast *Rhodosporidium paludigenum* on growth and antioxidant competence of *Liptopenaeus vannamei*. Aquaculture 309, 62–65.
Yeh, S.T., Lee, C.S., Chen, J.C., 2006. Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune resistance of white shrimp *Litopenaeus vannamei*. Fish. Shellfish Immunol. 20, 332–345.
Yun, H., Shahkar, E., Katya, E., Jang, I., Kim, S.K., Bai, S.C., 2015. Effect of biofloc on dietary protein requirement in juvenile whiteleg shrimp, Litopenaeus vannamei. Aquaculture 1–12.
Zhang, J., Liu, Y., Tian, L., Yang, H., Liang, G., Xu, D., 2012. Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Fish. Shellfish Immunol. 33, 1027–1032.
Ziaei-Nejad, S., Rezaei, M.H., Takami, G.A., Lovett, D.L., Mirvaghefi, A.R., Shakouri, M., 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture 252, 516–524.