Latin American Journal of Aquatic Research, 51(1): 12-22, 2023
DOI: 10.3856/vol51-issue1-fulltext-2924

Research Article

Variation of dietary protein/lipid levels used in postlarvae of freshwater prawn *Macrobrachium rosenbergii* cultured in a biofloc system

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ABSTRACT. A 70-day experimental trial was performed to evaluate the contribution of biofloc to the nutrition of *Macrobrachium rosenbergii* 15 days old postlarvae (PL) (average 82.00 ± 19.71 mg) fed different crude protein (P) and lipids (L) levels in zero-water exchange culture tanks. Six biofloc treatments (BFT) as experimental diets with 15, 20, or 25% P levels and 5 or 8% L levels were managed: BFT-15/5; BFT-15/8; BFT-20/5; BFT-20/8; BFT-25/5; BFT-25/8, and a clear-water control without biofloc fed with 25% P and 8% L (CW-25/8). The experiment was done in triplicate in 21-60 L plastic tanks containing 10 prawns tank^{-1}. At the end of the experiment, survival of prawns was above 66%, with no significant differences among experimental groups ($P > 0.05$). The best growth performance was observed in the experimental prawns maintained in BFT-20/5, BFT-20/8, BF-25/5, and BFT-25/8 compared to the control ($P < 0.001$). The feed conversion rate of the prawns in BFT-20/5 was significantly lower than that in control ($P < 0.001$); the best P efficiency ratio and productive P value were observed in all BFT compared to control prawns ($P < 0.001$). Results showed that it is feasible to use low concentrations of P (20%) and L (5%) in diets to grow giant freshwater prawn PL in a biofloc system. Results also demonstrated that biofloc contributes to the P and L requirements of cultured freshwater prawn PL, as indicated by improved feed utilization, P retention, and growth performance.

Keywords: *Macrobrachium rosenbergii*; biofloc; growth performance; giant freshwater prawn; protein:lipid ratio

INTRODUCTION

Aquaculture maintains a steady growth as an alternative to open sea fisheries. Projections for 2030 indicate that aquaculture could produce 53% of the total seafood production (FAO 2020). A major challenge of aquaculture is to reduce the water consumption required to maintain the quality and quantity of its effluents and the number of dissolved solids generated during production. Techniques to provide sustainable alternatives that would reduce environmental impact without affecting the health and growth of cultured organisms are essential. Biofloc technology is one option for developing a sustainable aquaculture industry (Avnimelech 1999).

The giant freshwater prawn *Macrobrachium rosenbergii* is an important species and has become an important part of freshwater aquaculture production in developing countries (Schwantes et al. 2009, New & Nair 2012, Banu & Christianus 2016). The annual global production of giant freshwater prawns has significantly increased from 2861 metric tons (mt) in

Corresponding editor: Crisantema Hernández

1980 to 229,419 mt in 2009 (New & Nair 2012). The success of the commercial production of *M. rosenbergii* postlarvae (PL) depends on the efficient use of available food protein (P) and lipid (L) levels (Al-Hafedh 2007, Goda 2008). The cost of aquaculture feed is influenced by P and L contents. High concentrations of these nutrients negatively affect the economic feasibility of prawn farming. Therefore, feed P and L should be optimally utilized to support rapid growth rather than without affecting profitability. Currently, there is limited information on the production of giant freshwater prawn PL with biofloc technology.

Biofloc promoted and developed in the culture-water column can effectively control the accumulation of ammonia (N-NH_3 mg L^{-1}), nitrite (N-NO_2 mg L^{-1}) and nitrate (N-NO_3 mg L^{-1}), in culture systems and make them available as a supplemental food source for cultured species (Correia et al. 2014, Cardona et al. 2015, Ballester et al. 2018). Moreover, the consumption of flocs can increase feed utilization and P efficiency by recovering some fractions of excreted nutrients and stimulating enzymatic activity (Xu & Pan 2012, Xu et al. 2012). The bioflocs within the culture system can be consumed and digested by cultured giant freshwater prawns (Asaduzzaman et al. 2010, Crab et al. 2010, Pérez-Fuentes et al. 2013). However, much is still unknown about the benefits of bioflocs on freshwater prawn performance in this system, particularly their possible contribution to prawn's P and L requirements. Therefore, we performed this study to evaluate the effects of bioflocs on feed utilization, P retention, and growth performance of *M. rosenbergii* postlarvae in zero-water exchange culture tanks through feeding with different dietary P and L levels. Likewise, the reduction of dietary P and L levels without affecting prawn growth in the presence of biofloc was assessed.

MATERIALS AND METHODS

Experimental design and diets

A two-factor P and L completely randomized design was used in triplicate, for which six experimental diets (Table 1) were formulated to contain three different P levels (15, 20, and 25%) and two L levels (5 and 8%). The control P level (25%) was chosen based on the results obtained by Hari & Kurup (2003). The experimental diets were formulated with low P (%) to ensure that the feed would not cover the P and L requirements of prawns and found a biofloc contribution without affecting the PL's health. A close 1.5:1 plant-animal P ratio was used by a mixture of fish meal and soybean meal based on the results obtained by Hari & Kurup (2003). Cornstarch and wheat flour were used as the main carbon source. L sources were fish oil, canola oil, and soybean lecithin oil. Other dietary ingredients were added to fulfill the nutritional requirements of *Macrobrachium rosenbergii* and were kept at the same levels in all diets (Table 1).

All dry ingredients were ground through 250 μm particle size. The diets were prepared by mixing the dry ingredients in a mixer (Mazal Rotabowl, model JC32, México) for 20 min, followed by supplementation of soybean lecithin oil and fish oil. Then, 35% hot water was added, followed by 15 min of mixing. The wet mash was compressed through a 1.5 mm die using a meat grinder (Moulinex HVO8, model DKA1, Francia). The resulting pellets were dried in a parallel flux forced air drier (Ríos Rocha S.A., model HCF-102, México) at 60°C until the moisture content was reduced to about 10%. After drying, strings were broken, and pellets were sieved in a 2 mm sieve and stored in plastic bags at -20°C until use.

The feeding experiment was conducted indoors in 21-60 L plastic tanks. Six biofloc treatments (BFT) and one control group were managed: BFT-fed diets of 15% P and 5% L (BFT-15/5), 15% P and 8% L (BFT-15/8); 20% P and 5% L (BFT-20/5); 20% P and 8% L (BFT-20/8); 25% P and 5% L (BFT-25/5); 25% P and 8% L (BFT-25/8); and clear water control without biofloc fed with 25% P and 8% L (CW-25/8). Each group consisted of triplicate tanks, and each tank contained 10 freshwater prawns PL. Acclimatized prawns in the intermolt period were selected and weighed to obtain their initial body weights (wet weight; ww) and then randomly stocked into 21 tanks. In addition, 20 prawns were randomly sampled for an initial whole-body analysis of crude protein.

Biofloc system preparation and maintenance

Original concentrated biofloc samples were collected from the *Oreochromis niloticus* indoor biofloc-based culture ponds and inoculated into all BFT tanks at the same amount (2 mL L^{-1} flocs volume) just before stocking the prawns. Every day, molasses was added to the biofloc experimental tanks to maintain a C:N ratio of 20:1 following the recommendations to promote bio-flocculation, bacterial growth, and growth performance in the freshwater prawn culture system (Aasaduzzman et al. 2008). We considered that molasses contains 40% organic carbon (Sierra-De La Rosa 2009) to estimate the daily ratio of molasses and that feed P contains 16% N, of which approximately 70% of the nitrogen intake is excreted as ammonium (Asaduzzaman et al. 2008). Based on the above assumptions, the amount of

Table 1. Ingredients and proximate composition of experimental diets (g kg^{-1}) containing three crude protein levels and two lipid levels for *Macrobrachium rosenbergii* postlarvae. *Premixture of vitamins and minerals: vitamins, minerals, and lutein, Kirkland, Vitae laboratories, Jalisco, Mx.

Ingredients (g kg^{-1})	Treatments (dietary protein/lipid levels (g kg^{-1}))					
	250/50	250/80	200/50	200/80	150/50	150/80
Fish meal	164.38	184.93	143.84	136.99	109.59	109.59
Soybean meal	234.00	210.00	125.00	175.00	116.67	116.67
Wheat meal	525.00	500.00	583.33	520.83	416.67	416.67
Fish oil	0.47	12.93	4.33	19.76	9.04	24.04
Canola oil	11.88	27.50	13.33	26.98	16.67	31.67
Cornstarch	4.27	4.63	70.17	60.44	271.37	241.37
Mineral premix*	10.00	10.00	10.00	10.00	10.00	10.00
Vitamin premix*	10.00	10.00	10.00	10.00	10.00	10.00
Carboxymethyl cellulose	30.00	30.00	30.00	30.00	30.00	30.00
Soybean lecithin	5.00	5.00	5.00	5.00	5.00	5.00
Pigment	5.00	5.00	5.00	5.00	5.00	5.00
Proximate composition (%)						
Crude protein	26.74	25.94	19.82	21.52	15.71	14.61
Crude lipid	5.38	8.71	5.49	8.15	5.04	8.40
Energy kcal 100 g^{-1}	428.04	445.58	430.15	440.79	427.59	443.64

molasses to be supplied per day was determined according to the following formula: molasses (g) = [feed offered (g) × protein in feed (%)] × 0.16 × 0.70 × 20 × 2.50).

Experimental prawn husbandry

Freshwater prawns *M. rosenbergii* PL (15 days after metamorphosis, 82.00 ± 19.71 mg weight) were obtained from the genetic improvement and aquaculture production area of the Instituto Tecnológico de Boca del Río, Veracruz, Mexico. During the acclimation period, half of the water was renewed daily, and prawns were fed twice daily with the shrimp commercial feed Pedregal, Silver Cup: 35% P and 8% L.

Feeding management and water quality monitoring

The experimental diets were fed to prawns for 70 days. Feeding was done by hand to apparent satiation two times per day at 08:00 and 16:00 h following the feeding strategy used by Goda (2008) with modifications in each ration time. The food level was adjusted every 10 days based on growth after the biometric analysis and growth increase data. The daily feeding rate was slowly reduced from approximately 10% of total body weight to 5% by the end of the feeding experiment.

During the experimental period, dissolved oxygen (DO; mg L^{-1}), temperature (°C), and pH of the water in all tanks were recorded daily at 09:00 h (YSI556 MPS multi-probe, YSI, Yellow Springs, OH). Concentrations (mg L^{-1}) of ammonia, nitrite, and nitrate were determined weekly by colorimetric tests (Tetra Easy Strips™). Biofloc volume was determined on site using Imhoff cones every 10 days, recording the volume taken in by the flocs in 1000 mL of the tank water after 30 min sedimentation (Avnimelech & Kochba 2009). No water exchange was done in all the BFT tanks, and half of the water was renewed daily in the control tanks during the experimental period. All tanks were always aerated continuously using air stones connected to an air pump, and dechlorinated freshwater was added to maintain the water level.

Biofloc collection and prawn sampling

At the end (day 70) of the experiment, biofloc samples were collected by passing BFT tank water through a 50 µm mesh-size nylon bag. Two concentrated biofloc samples from each tank were dried in an oven at 105°C until constant weight, pooled together, and then stored at -20°C until chemical composition analysis. The proximate composition of the biofloc was determined to obtain information on its primary nutritional values for prawns. Every 10 days, the individual weight and length of prawns were evaluated. At the end of the experiment, survival (%) from each tank was determined, and each individual's final body weight (ww) was recorded.

Proximal composition of prawns and biofloc

Seven prawns from each tank were randomly sampled for the final whole-body analysis. Each sample was

dried at 60°C for 24 h in a convection oven (Memmert UNP 400, Memmer GmbH + Co., Schwabach, Germany); subsequently, samples were kept refrigerated at 5°C until analysis. The proximate composition (dry weight basis) of experimental prawns and the biofloc was evaluated as follows: P (%) and energy (kcal kg^{-1}) were determined using an elemental analyzer (Flash 2000, Thermo Fisher Scientific, UK), for P (%) N × 5 considered as a conversion factor following Gnaiger & Bitterlich (1984), for samples of marine origin; total L (%) were analyzed using the Soxhlet method with methanol-chloroform, in a ratio of 2:1, as a solvent; ash (%) was analyzed through incinerating the dried sample in a furnace muffler (Felisa, Jalisco, Mexico) at 650°C for 5 h; nitrogen-free extract (NFE) (%) was determined by the difference of 100 concerning the summative values of P, L, and ash, each analysis was triplicated (AOAC 1990).

Diets suitability was evaluated based on: survival rate (SR), specific growth rate (SGR), apparent feed conversion rate (AFCR), and protein efficiency ratio (PER) were calculated using the following equations: SR (%) = 100 × (final prawn count / initial prawn count); SGR (% d^{-1}) = 100 × [ln(final body weight) - ln(initial body weight)] / experimental duration (d); AFCR = total dry weight of feed offered / total prawn wet weight gained; PER = total prawn wet weight gained / total dry weight of feed protein offered. In the present study, AFCR was used instead of feed conversion rate FCR because there is a natural food supply given by the system's productivity (biofloc), and the impact of cannibalism and biofloc consumption by the shrimp could not be directly measured, which makes FCR variable or not measurable (Tacon et al. 2002).

Statistical analysis

Data obtained were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov and Bartlett tests. Before analyses, percentage data were arcsine transformed, AFCR transformed to Log_{10}, and PER transformed to square root, but only untransformed means are given. Homoscedastic and normal distribution data (water quality parameters, survival, SGR, AFCR, PER, and proximal composition of prawns and biofloc) were first analyzed by two-way ANOVA. Since there was no significant effect of the P and L factors, both effects were pooled into a single treatment and analyzed by one-way ANOVA followed by Tukey multiple range test. Final weight and total length results were analyzed by the Kruskal-Wallace test (for non-normal distributions) followed by the Mann-Whitney U test. Significant differences were considered at $P < 0.05$. All statistical analyses were performed using Statistica v.7 software (Statsoft, Tulsa, OK, USA).

RESULTS

Water quality and biofloc development

The measured water quality in all experimental groups remained within recommended levels for giant freshwater prawn *Macrobrachium rosenbergii* culture throughout the experimental period (Table 2). Dissolved oxygen (DO) was constant and higher than 5.50 mg L^{-1}, the temperature was maintained at 25.27 ± 1.65°C (mean ± standard deviation, SD), pH was 8.11 ± 0.28 (mean ± SD), ammonia, nitrite and nitrate remained constant and within the appropriate values for Malayan prawn culture.

The biofloc volume levels increased gradually throughout the experimental period. The biofloc volume increased in the BFT tanks with average levels of around 11 mL L^{-1} (Fig. 1).

Survival and growth performance

In general, the mean SR were above 66%, with no significant differences ($P > 0.05$) among all experimental groups, with the lowest survival rate for the control, BFT-20/5, and BFT-25/5 treatments, and the highest SR for the BFT-25/8 treatment (Table 3).

Growth performance in the experimental prawns was evaluated through final weight, total length, and SGR. The growth of prawns subjected to biofloc treatments was higher than that obtained in the control treatment (Table 3).

The SGR in the prawns subjected to BFT was significantly higher ($P < 0.001$) than that obtained in the control treatment, and no significant differences ($P > 0.05$) in the SGR of prawns were observed among BFT (Table 3). The growth of prawns in BFT-20/5 and BFT-25/5 (in terms of final weight and total length) was significantly better ($P < 0.001$) than that obtained in the control treatment, and there were no significant differences ($P > 0.05$) between BFT-20/8 and BFT-25/8 (Table 3).

Feed utilization and protein retention

The AFCR of prawns in BFT-20/5, BFT-20/8, and BFT-25/5 were significantly lower ($P < 0.001$) than that obtained in control (Table 3). No significant differences ($P > 0.05$) were found among BFT-15/5, BFT-15/8, BFT-25/8, and the control group (Table 3). As for PER, higher values ($P < 0.001$) were observed in the BFT

Table 2. The overall means ± standard deviation and range values (minimum, maximum) of water quality parameters in the control and biofloc treatments during the 70-day experimental period. DO: dissolved oxygen, $N\text{-}NH_3$: ammonia, $N\text{-}NO_3$: nitrate, $N\text{-}NO_2$: nitrite.

Treatment	Parameter					
	Temperature (°C)	DO (mg L^{-1})	pH	$N\text{-}NH_3$ (mg L^{-1})	$N\text{-}NO_3$ (mg L^{-1})	$N\text{-}NO_2$ (mg L^{-1})
C-25/8	25.34 ± 1.69 (21.70, 29.00)	5.44 ± 0.54 (4.46, 6.16)	8.17 ± 0.29 (7.50, 8.50)	0.06 ± 0.02 (0, 0.12)	10.46 ± 2.05 (0, 20.00)	0.24 ± 0.08 (0, 0.8)
BFT-15/5	25.28 ± 1.55 (21.50, 29.00)	5.47 ± 0.53 (4.44, 6.37)	8.09 ± 0.31 (7.40, 8.40)	0.07 ± 0.02 (0, 0.12)	14.29 ± 9.76 (0, 20.00)	0.03 ± 0.11 (0, 0.4)
BFT-15/8	25.10 ± 1.55 (21.50, 27.00)	5.48 ± 0.48 (4.51, 6.26)	8.12 ± 0.27 (7.60, 8.50)	0.06 ± 0.02 (0, 0.12)	15.71 ± 11.34 (0, 20.00)	0.06 ± 0.12 (0, 0.4)
BFT-20/5	25.25 ± 1.54 (22.00, 27.50)	5.58 ± 0.43 (4.96, 6.37)	8.10 ± 0.26 (7.60, 8.40)	0.06 ± 0.01 (0, 0.12)	14.24 ± 9.76 (0, 20.00)	0.08 ± 0.12 (0, 0.4)
BFT-20/8	25.24 ± 1.71 (22.00, 28.00)	5.51 ± 0.45 (4.71, 6.30)	8.10 ± 0.28 (7.50, 8.50)	0.07 ± 0.02 (0, 0.12)	14.24 ± 9.76 (0, 20.00)	0.03 ± 0.11 (0, 0.4)
BFT-25/5	25.33 ± 1.89 (22.00, 28.70)	5.48 ± 0.48 (4.57, 6.37)	8.10 ± 0.27 (7.50, 8.40)	0.06 ± 0.02 (0, 0.12)	14.24 ± 9.76 (0, 20.00)	0.04 ± 0.11 (0, 0.4)
BFT-25/8	25.34 ± 1.81 (22.00, 28.70)	5.55 ± 0.43 (4.93, 6.16)	8.06 ± 0.32 (7.20, 8.40)	0.06 ± 0.02 (0, 0.12)	14.24 ± 9.76 (0, 20.00)	0.04 ± 0.11 (0, 0.4)

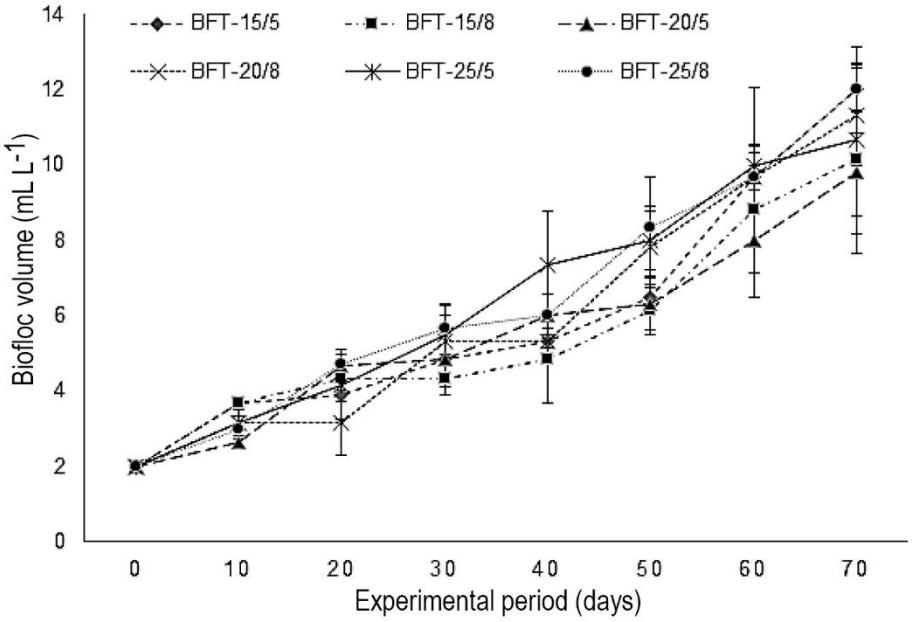

Figure 1. Changes in bioflocs volume (mean ± standard error) of six bioflocs treatments throughout the 70 days experimental period.

compared to those in the control group (Table 3), and there were no significant differences ($P > 0.05$) among BFT-15/5, BFT-15/8, BFT-20/5, BFT-20/8, and BFT-25/5 (Table 3).

Proximate composition of prawns and biofloc

No significant differences were observed in crude P, crude L, ash, and moisture contents of experimental prawns and biofloc from the water among all treatments ($P > 0.05$) (Tables 4-5).

Table 3. Growth performance, feed utilization, and survival (mean ± standard deviation) in *Macrobrachium rosenbergii* of different experimental groups at the end of the 70-day experimental period. Values in the same column with different superscripts are significantly different ($P < 0.01$). SGR: specific growth rate, AFCR: apparent feed conversion rate, PER: protein efficiency rate.

Treatment	Parameters							
	Survival rate (%)	Initial weight (mg)	Initial length (cm)	Final weight (mg)	Total length (cm)	SGR (% d^{-1})	AFCR	PER
C-25/8	66.67 ± 5.77	82.00 ± 19.71	2.36 ± 0.21	321.00 ± 99.41^{c}	3.56 ± 0.44^{d}	0.80 ± 0.18^{b}	5.55 ± 2.36^{a}	0.81 ± 0.33^{c}
BFT-15/5	73.33 ± 15.28	82.00 ± 19.71	2.36 ± 0.21	500.90 ± 169.64^{b}	4.10 ± 0.57^{c}	1.06 ± 0.23^{a}	3.78 ± 2.09^{ab}	2.08 ± 0.86^{ab}
BFT-15/8	73.33 ± 20.82	82.00 ± 19.71	2.36 ± 0.21	528.63 ± 210.62^{b}	4.17 ± 0.67^{bc}	1.09 ± 0.24^{a}	3.58 ± 1.95^{ab}	2.40 ± 1.15^{ab}
BFT-20/5	66.67 ± 5.77	82.00 ± 19.71	2.36 ± 0.21	729.00 ± 235.50^{a}	4.75 ± 0.69^{a}	1.29 ± 0.24^{a}	2.58 ± 1.59^{b}	2.42 ± 0.88^{a}
BFT-20/8	73.33 ± 15.28	82.00 ± 19.71	2.36 ± 0.21	664.54 ± 240.68^{ab}	4.60 ± 0.59^{ab}	1.23 ± 026^{a}	2.85 ± 1.71^{b}	2.09 ± 0.89^{ab}
BFT-25/5	66.67 ± 11.55	82.00 ± 19.71	2.36 ± 0.21	709.00 ± 311.24^{ab}	4.51 ± 0.74^{ab}	1.25 ± 0.30^{a}	2.93 ± 1.87^{b}	1.75 ± 0.86^{ab}
BFT-25/8	83.33 ± 15.28	82.00 ± 19.71	2.36 ± 0.21	564.00 ± 263.73^{ab}	4.17 ± 0.78^{ab}	1.11 ± 0.28^{a}	3.55 ± 2.18^{ab}	1.49 ± 0.84^{bc}

Table 4. Proximate composition (mean ± standard deviation) of whole-body *Macrobrachium rosenbergii* of different experimental groups at the end of the 70-day experimental period. Protein, lipid, and ash are based on the dry weight.

Treatment	Parameter (%)			
	Moisture	Crude protein	Total lipid	Ash
C-25/8	73.04 ± 1.40	65.92 ± 0.48	11.29 ± 0.69	16.80 ± 0.42
BFT-15/5	73.63 ± 2.32	64.92 ± 2.76	9.69 ± 1.65	16.36 ± 1.50
BFT-15/8	73.55 ± 0.76	63.21 ± 2.91	11.01 ± 0.66	18.11 ± 0.66
BFT-20/5	73.55 ± 1.82	64.97 ± 2.05	11.54 ± 0.85	17.91 ± 2.46
BFT-20/8	74.56 ± 1.08	65.60 ± 1.19	9.80 ± 0.48	16.05 ± 1.19
BFT-25/5	73.98 ± 1.65	64.78 ± 2.35	11.04 ± 0.60	18.06 ± 1.02
BFT-25/8	73.54 ± 0.34	64.96 ± 0.95	9.86 ± 1.09	17.93 ± 0.47

Table 5. Proximate composition (mean ± standard deviation) of the biofloc production in *Macrobrachium rosenbergii* experimental feeding with cane molasses as a carbon source. Protein, lipid, and ash are based on the dry weight.

Treatment	Parameter (%)			
	Moisture	Crude protein	Total lipid	Ash
FT-15/5	87.85 ± 1.63	24.66 ± 1.80	8.28 ± 1.03	36.79 ± 0.01
BFT-15/8	90.07 ± 1.05	28.66 ± 0.93	9.39 ± 1.60	36.81 ± 3.32
BFT-20/5	90.33 ± 0.68	27.82 ± 5.55	7.80 ± 2.53	35.50 ± 3.52
BFT-20/8	88.29 ± 3.60	25.04 ± 4.02	6.41 ± 1.57	35.79 ± 6.36
BFT-25/5	87.11 ± 1.92	30.08 ± 0.62	7.45 ± 2.09	35.27 ± 3.83
BFT-25/8	90.63 ± 1.58	26.87 ± 0.41	6.14 ± 2.31	30.93 ± 6.82

DISCUSSION

In the present study, a BFT system was used to evaluate the effects of biofloc on feed utilization, P retention, and growth performance of *Macrobrachium rosenbergii* PL in zero-water exchange culture tanks through feeding with different dietary P and L levels. According to Bautista (1988), *M. rosenbergii* PL supports temperature ranges that oscillate between 15 and 35ºC in the waters it inhabits; however, temperatures below 23ºC can reduce survival (Ortega-Salas & Arana-Magallón 2006), feed consumption and SGR (Niu et al. 2003). Besides the temperature, physicochemical parameters were within the range recommended for nursery-reared giant freshwater prawn PL (New & Singholka 1984). At different times of the study, the temperature was less than 23ºC, without significant differences between treatments and the control group. In our experiment, periods with low temperatures could have reduced feed consumption and low survival in both treatments and control. Nevertheless, the effect was general, and we consider it was not an impairment

to evaluate the contribution of biofloc on P and L nutrition of *M. rosenbergii* PL.

Incorporating molasses to keep a 20:1 C:N relationship favored the formation of biofloc in the experimental treatments; the increased biofloc volume in BFT tanks could observe this. In BFT tanks, ammonia and nitrite concentration were maintained at <0.1 mg L^{-1} without flushing. According to Asaduzzaman et al. (2008) and Pérez-Fuentes et al. (2013), the incorporation of molasses in the system at a 20:1 C:N relation promotes the formation of heterotrophic bacterial communities (biofloc) that can assimilate dissolved nitrogenous matter (ammonium and nitrite). Our results reinforce the notion that the BFT system is an alternative to control nitrogenous waste in giant freshwater prawn culture in zero-water exchange.

The pH of the water was within the range recommended for nursery-reared giant freshwater prawn PL (Chen & Chen 2003). At the beginning of the experiment, the global mean pH was 7.56 ± 0.11 without significant differences between treatments and the control. The pH gradually increased in treatments and control until reaching, at the end of the experiment, a global mean of 8.42 ± 0.05. This trend contrasts with the reports of some authors, who observed a gradual pH decrease in the BFT system (Ebeling et al. 2006, Furtado et al. 2011, 2014). According to Ebeling et al. (2006), for each gram of ammoniacal nitrogen assimilated by heterotrophic bacteria, 4.71 g of DO, 3.57 g of alkalinity, and 15.17 g of carbohydrates are consumed, producing 8.07 g of bacterial biomass and 9.65 g of carbon dioxide. In this study, we utilized oyster shells to provide shelter to the prawns; the shells probably released calcium carbonate in the water, increasing the alkalinity to a good level to meet the demand of the microbial community.

In general, the prawn's survival was low compared with the results obtained by other research with similar initial age prawns (Balazs & Ross1976, Goda 2008); it could be caused by low temperature on some days of our bioassay. Ortega-Salas & Arana-Magallón (2006) reported that the survival of 50 days old prawns was 81% at 33°C and 67% at 20°C after two months of culture. There were no differences in prawn's survival rate between treatments and control, which is remarkable considering that we used diets with low P and L levels, but biofloc contributed to cover nutrient requirements. In contrast, Hari & Kurup (2003) recorded an effect of dietary P on survival in prawns cultured in a clear water system; they recorded low survival using diets with 20% P compared with 25, 30, and 35% of P. It is important to consider that bioflocs are soft and fluffy, like detritus, the natural food of *M. rosenbergii* PL.

For this reason, prawns may prefer to eat biofloc, and this food contributes to covering the physiological requirements of these organisms. According to Crab et al. (2010), *M. rosenbergii* PL can feed biofloc and survive 15 days without the addition of commercial feed; unfortunately, growth and feed utilization were not investigated during the trial. On the other hand, a biofloc system supports prawns to have stronger adaptive antioxidant and antibacterial responses and increases their resistance to environmental stress, contributing to their survival (Miao et al. 2020).

We observed that prawns cultured in the BFT system were high-growth performers compared with clear-water system prawns. Our results are consistent with previous studies, showing that biofloc technology improves the growth of *M. rosenbergii* (Asaduzzaman et al. 2010, Perez-Fuentes et al. 2013, Miao et al. 2017) and of the white shrimp *Penaeus vannamei* (Xu et al. 2012, Jatoba et al. 2014, Cardona et al. 2015). The high growth performance of prawns in biofloc could be correlated with increased nutrient absorption ability. According to Miao et al. (2017), biofloc technology combined with the addition of probiotics produced a higher fold height of the distal intestine in giant freshwater prawns, which may be related to the presence of probiotic bacteria, *Bacillus subtilis* and *Lactobacillus* sp., in the rearing water and intestine. Fold height shows nutrient absorption ability and growth in aquatic animals (Reyes-Becerril et al. 2014).

In clear water systems, the higher growth of prawns occurs with 25 to 35% of P (Balaz & Ross 1976, Hari & Kurup 2003, Al-Hafedh 2007). Therefore, the dietary P levels used in the present study (15 and 20%) can be considered insufficient for prawns to correctly grow and survive when cultured in freshwater (Hari & Kurup 2003). However, our results showed that the P composition in the diets did not affect the survival of prawn culture in the BFT system; moreover, in the BFT system, we obtained similar growth performance using 20 or 25% P diets. The biofloc proximal composition in the water of the experimental systems had 27.19 ± 3.15% of mean P concentration (dry base), and 7.58 ± 1.95% mean L concentration (dry base). The proximal composition of the biofloc in this study, considered an important source of nutrients, was in the range recommended for prawn's artificial feed (Hari & Kurup 2003, Al-Hafedh 2007). It not only allowed prawns to maintain an efficient growth rate but also allowed us to suppose that the P and L level in commercial diets for freshwater prawn PL and juveniles is adequate. Thus, it

could help reduce proteins of commercial diets because our prawns grew and survived in a BFT system with only 20% of the P in the diet.

In clear water systems, the lowest value of AFCR has been observed in prawns fed 25 to 32% dietary P and increases in prawns fed less than 25% dietary P (Hary & Kurub 2003, Goda 2008, Sagar et al. 2019), about the increase of energy requirement for prawns with low P in the diets. In the present study, the AFCR of the prawns in BFT treatments was low compared with the control, possibly related to the nutrients obtained from the biofloc. Nevertheless, we estimated the AFCR based on the feed offered and the contribution of natural food because actual consumption of the diets could not be monitored in BFT tanks. The low temperatures in the bioassay probably reduced the prawn's feed consumption (Niu et al. 2003), but we did not record uneaten feed, which could be the reason for the high AFCR in both BFT treatments and control. In clear water systems, the best PER has been observed in prawns fed 25 to 30% dietary P (Hary & Kurop 2003, Goda 2008). In our experiment, the PER values were better in BFT treatments than in the control. Probably because of more efficient feed utilization, the best PER values were observed in prawns fed 15 and 20% dietary P with biofloc. Previous studies with white shrimp suggest that the promoted biofloc can improve feed utilization and P efficiency in shrimp PL culture, probably by providing supplemental food and improving the activity of protease enzymes (Xu et al. 2012, Xu & Pan 2012). For that, we considered that prawn's consumption of biofloc can increase feed utilization and P efficiency due to the nutritional value of the flocs.

L are the major metabolic energy source for growing *M. rosenbergii* PL (Roustaian et al. 2001). We observed that dietary L concentration (5-8%) did not affect growth and feed efficiency in prawns of the BFT system. According to D'Abramo & Sheen (1994), less than 6% of L in the diet could be slightly inadequate for optimal prawn growth, and they observed the highest growth and survival in prawns fed with 6 to 8% L diets. Our biofloc had an average L concentration of 7.58%; thus, the biofloc L would be an additional source of L for prawn nutrition.

Previous trials observed the effect of dietary P and L levels on prawn composition in clear water culture systems. In these conditions, high P in the diet has a positive effect on the prawn's protein composition, and low P in the diet has a positive effect on the prawn's L composition (Goda 2008, Chowdhury et al. 2008). In the present study, the biofloc allowed for similar prawn proximate composition, independently from the dietary P and L levels. Likewise, in white shrimp cultured in biofloc, a similar proximate composition has been observed independently from the P composition in the diet (Jatobá et al. 2014). We suppose those prawns can use biofloc as an additional source of P to conserve and build muscle, keeping similar proximate composition and compensating for the P deficiencies of the experimental diets with the lower P levels.

In our research, bioflocs had high P (27.19 ± 3.15%, overall means of all BFT treatments) and L (7.54 ± 1.97%, overall means of all BFT treatments) levels without differences between diets. The proximate composition of the biofloc is strongly related to the microbial community's composition (Ju et al. 2008, Gallardo-Collí et al. 2019). In contrast, an inverse relation between diet's P composition and biofloc P composition in white shrimp culture has been reported previously (Xu et al. 2012, Xu & Pan 2014). Xu & Pan (2014) inferred that differences in nutritional compositions between biofloc and those resulting from the different dietary P levels were probably due to differences in the microbial community. However, this was only a supposition because the microbial community structures of the bioflocs collected in that study were not determined. Therefore, the microbial community's composition could be relegated to the diet's P composition. We did not observe an effect of dietary P on biofloc composition, perhaps because low P concentrations were used in all diets, implying low nitrogen availability in all treatments.

CONCLUSIONS

Regardless of the concentration of P and L used, giant freshwater prawns PL cultured in biofloc systems achieve better growth and feed efficiency than the system. Organisms fed 20% P, and 5 % L cultured in biofloc had the best growth, feed conversion, and P efficiency rate. These concentrations can therefore be used in Malayan prawn biofloc culture without affecting the organisms' survival, growth, and welfare. The presence of biofloc in the culture system of giant freshwater prawn PL can contribute partially covering to feeding their P and L requirements when a low P diet is used.

ACKNOWLEDGMENTS

We thank Tecnológico Nacional de México (TecNM) and Instituto de Tecnología de Boca del Río (México), for their facilities and the Consejo Nacional de Ciencia y Tecnología (Conacyt) for scholarship N°581441.

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Received: April 4, 2022; Accepted: October 12, 2022

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