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RESEARCH ARTICLE

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Effects of two commercial feeds with high and low crude protein content on the performance of white shrimp *Litopenaeus vannamei* raised in an integrated biofloc system with the seaweed *Gracilaria birdiae*

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Abstract

A trial was conducted for 42 days to evaluate the effects of two commercial feeds with high and low crude protein content on the performance of white shrimp *Litopenaeus vannamei* cultivated in an integrated biofloc system with the seaweed *Gracilaria birdiae*. The experiment had a 2 × 2 factorial design (a biofloc monoculture or an integrated system with 32% (low) or 40% (high) crude protein content) with the following treatments: IS32 (an integrated system using low protein commercial feed); IS40 (an integrated system using high protein commercial feed); M32 (a monoculture system using low protein commercial feed); and M40 (a monoculture system using high protein commercial feed), all in triplicate. Shrimp individuals (0.23 ± 0.04 g) were stocked at a density of 500 shrimp/m^3 and no water exchange was carried out during the experimental period. No significant influence ($p > 0.05$) was found to be caused by the integrated system or the crude protein levels on water quality. However, a significant influence ($p < 0.05$) was found for final weight (3.21–4.12 g), weight gain (2.97–3.89 g), yield (1.39–1.96 kg/m^3) and feed conversion ratio (1.47–1.74). Growth was similar in IS32, M40 and IS40, indicating that crude protein levels can be reduced with no adverse effect on shrimp performance variables in integrated biofloc systems with *G. birdiae*.

Additional keywords: growth; nutrition; seaweed; shrimp; water quality.

Abbreviations used: FCR (feed conversion ratio); IS32 (an integrated system using low protein commercial feed); IS40 (an integrated system using high protein commercial feed); M32 (a monoculture system using low protein commercial feed); M40 (a monoculture system using high protein commercial feed); PL (postlarvae); TSS (total suspended solids).

Authors' contributions: All authors contributed equally to this work (conception; acquisition, analysis, interpretation of data; drafting of the manuscript; critical revision of the manuscript and statistical analysis).

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Introduction

The crude protein levels of shrimp feed depend on many factors, such as species (Shiau, 1998), size (Van Wyk, 1999), the energy:protein ratio (Martinez-Córdova *et al.*, 2010), and salinity (Perez-Velazquez *et al.*, 2007). The levels vary from 30 to 57% (Shiau, 1998; Van Wyk, 1999), and are one of the primary cost components of prepared feed (Kureshy & Davis, 2002). Commercial feeds with higher crude protein levels have lower organic carbohydrate:nitrogen ratios (C:N <10), which discourage ammonia decomposition by heterotrophic bacteria, resulting in its accumulation in intensive culture systems (Avnimelech, 2009). Moreover, excess protein will be metabolized by the shrimp as a source of energy, and nitrogen will be excreted as ammonia (Van Wyk, 1999).

In biofloc systems, the addition of carbohydrates causes an increase in heterotrophic bacteria which can partially transform feed waste into microbial protein for shrimp nutrition, improving digestive enzyme activity of protease, amylase, cellulase and lipase (Xu *et al.*, 2013)

and immunity response (Xu & Pan, 2013; Becerra-Dorame *et al.*, 2012; Ekasari *et al.*, 2014), therefore improving the zootechnical variables (Avnimelech, 2009).

The use of seaweed, fish and mollusks in the aquaculture ponds can minimize nitrogen and phosphorus waste, reduce risk of disease and increase the farmers' income, as well as mitigate environmental problems caused by effluents (Ren *et al.*, 2012; Lander *et al.*, 2013; Shpigel *et al.*, 2016). This method is extremely relevant because aquaculture production has increased significantly worldwide in recent years. Intensive and semi-intensive systems have often resulted in increased waste discharge and nutrient concentrations in effluents, generating environmental and social challenges. In this sense, integrated systems can mitigate some of these problems associated with monoculture systems (Troell *et al.*, 2009; Chopin *et al.*, 2012).

Moreover, *Gracilaria birdiae* has a total lipid content (as a % dry weight) of 1.3%; 1.0% fatty acid; 7.1% soluble protein, 9.1% amino acid and 22.5% ash (Gressler *et al.*, 2010), as well as essential vitamins and minerals (Tabarsa *et al.*, 2012; Syad *et al.*, 2013). In this sense, *G. birdae* may also improve yield and growth of *Litopenaeus vannamei* shrimp (Brito *et al.*, 2014; 2016) because it can serve as a food source for shrimp through direct grazing on the seaweed, and on the biofilm that forms on its surface.

In this context, this study evaluated the effects of two commercial feeds with high and low crude protein content on the performance of juvenile white shrimp *Litopenaeus vannamei* raised in integration with the seaweed *Gracilaria birdiae*.

Material and methods

Experimental conditions

A 42-day indoor trial was conducted at the Sustainable Mariculture Laboratory of the Department of Fisheries and Aquaculture, Federal Rural University, Pernambuco Recife, Brazil (08°01'00.16"S, 034°56'57.74"W). The experiment had a 2 × 2 factorial design (monoculture or integrated system with 32% (low) or 40% (high) crude protein content) with the following treatments: IS32 (an integrated system using low protein commercial feed); IS40 (an integrated system using high protein commercial feed); M32 (a monoculture system using low protein commercial feed); and M40 (a monoculture system using high protein commercial feed), all in triplicate. The main ingredients of the feed were soybean meal, wheat bran, meat and bone meal, fish meal, salmon meal, blood meal, viscera meal, rice bran, soy lecithin and fish oil; urea-formaldehyde, propionic acid (preservative) and ethoxyquin (preservative) were also added to the feeds.

Five days prior to stocking, water from an indoor biofloc matrix tank [TAN (total ammonia nitrogen) 0.3 mg/L; NO_2-N nitrite-nitrogen 0.1 mg/L; NO_3 nitrate 3.08 mg/L; alkalinity 100 mg $CaCO_3$/L, pH 8.1; PO_4^3 orthophosphate 2.16 mg/L; TSS total suspended solids 320 mg/L; and salinity 28 g/L] was mixed and equally distributed to fill twelve experimental black-plastic tanks (the useful dimensions of the tanks were 50 × 35 × 23 cm, equivalent to 40 L). The experimental units were maintained under constant aeration by using three cylindrical airstones (diameter 2.4 cm and length 2.6 cm) per tank. No water exchange was carried out during the experimental period, except for the addition of dechlorinated freshwater (2% weekly) to compensate for evaporation losses. Light intensity was maintained at ~ 1000 lux using a fluorescent lamp with a 12 h light / 12 h dark regime.

Molasses were added once a day to maintain the carbohydrate:nitrogen ratio at 12:1. Hydrated lime ($Ca(OH)_2$) was added at 10% (by weight) of the daily feed allotment throughout the study to maintain the alkalinity at about 100 mg/L and pH 7.5 (Furtado *et al.*, 2013).

Water quality

Dissolved oxygen and temperature (YSI model 55, Yellow Springs, OH, USA) were monitored twice a day (at 08:00 and 16:00 h). Salinity (YSI model 30, Yellow Springs, OH, USA) and pH (YSI model 100, Yellow Springs, OH, USA) were monitored twice a week. TAN (Koroleff, 1976), NO_2-N (Golterman *et al.*, 1978), NO_3 (Mackereth *et al.*, 1978), TSS (APHA, 2005), PO_4^3 (APHA, 2005) and alkalinity (mg/L $CaCO_3$) (Felföldy *et al.*, 1987) were monitored once a week.

Shrimp stocking, feeding and monitoring

Specific pathogen-free postlarvae (PL_{10}, 0.001 g) of *L. vannamei* were obtained from a commercial laboratory (Potiporã, RN, Brazil). PLs were raised in two 400L rectangular tanks until 20 days (average final weight of 0.23 g), at a shrimp stocking density of 2,500 PLs/m^3 (1,000 shrimp per tank) in salinity of 35 g/L. The cultivation water was prepared by adding *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricornutum* at 15 × 10^4 cells/mL (5 × 10^4 cells/mL for each diatom species) every five days during the culture period. The postlarvae were fed five times a day (at 08:00, 11:00, 13:00, 15:00 and 18:00 h), with a commercial shrimp feed (0.4 to 1 mm in diameter) composed of 40% crude protein and 8% lipids (Aquavita Premiun, Guaraves, Brazil). The daily feeding rate was 35% of body weight at the start of the culture, which was gradually reduced to 20% of body weight after 20 days based on the Van Wyk table (1999).

The experimental units were stocked with Pacific white shrimp *L. vannamei* (0.23 ± 0.04 g initial weight) at a density of 500 shrimp/m^3 (20 shrimp per experimental unit). The juvenile shrimp were fed three times a day (at 08:00, 12:00 and 16:00 h), with a commercial shrimp feed (Evialis, Presence, Brazil, 1 to 2.5 mm in diameter) (Table 1). The daily feeding rate was 20% of body weight at the start of the experiment, gradually reduced to 6% of body weight at the end of the 42-day experiment based on the Van Wyk table (1999).

Once a week, 10 shrimp were randomly sampled from each unit and individually weighed on a digital balance (M523 Series Milligram Balances, BEL Engineering, Italy), and put back in the tank. Mean weights were used to determine shrimp growth and adjust the amount of feed and organic carbon offered. At the end of the trial, the total shrimp biomass and the individual shrimp weights from each tank were recorded to determine: final average weight (g) = final biomass (g) / number of individuals at the end; weight gain (g) = final weight (g) – initial weight (g); yield (kg/m^3) = final biomass (kg) / volume of the experimental unit (m^3); survival (%) = (number of individuals at the end of the evaluation period / initial number of individuals) × 100; and FCR = feed supplied / biomass gain.

Seaweed stocking

Samples of *Gracilaria* biomass were collected at the Pau Amarelo beach, Paulista, Pernambuco, Brazil (07º54'54.74¨S, 034º49'12.07"W), and stored in plastic bags for laboratory analysis. Water was drained from all the samples, the material was carefully inspected to eliminate encrusted organisms and then weighed. Seaweed with reproductive structures, signs of depigmentation and necrosis were discarded (Tsutsui *et al.*, 2010; Marinho-Soriano *et al.*, 2011). Experimental units were stocked with 2.5 kg wet weight of seaweed per cubic meter.

Table 1. Proximate composition for the two commercial feeds used for the shrimp *Litopenaeus vannamei* juveniles according to manufacturer information.

Proximate composition (g/kg feed)	High 40% crude protein	Low 32% crude protein
Moisture	130	130
Crude protein	400	320
Lipid	80	80
Fiber	40	40
Ash	120	120

Calcium 30 g/kg; phosphorus 13 g/kg; magnesium 500 mg/kg; potassium 10 g/kg; cobalt 0.1 mg/kg; copper 46 mg/kg; iodine 2 mg/kg; manganese 13 mg/kg; selenium 0.25 mg/kg; sodium 4,000 mg/kg; zinc 200 mg/kg; folic acid 9 mg/kg; pantothenic acid 120 mg/kg; biotin 2.25 mg/kg; choline 660 mg/kg; niacin 210 mg/kg; vitamin A 9,000 UI/kg; vitamin B 190 mg/kg; vitamin B12 150 mg/kg; vitamin B2 60 mg/kg; vitamin B6 60 mg/kg; vitamin C 500 mg/kg; vitamin D3 3,000 UI/kg; vitamin E 210 UI/kg; vitamin K3 42 mg/kg.

Statistical analysis

A two-way analysis of variance (ANOVA) was used to determine the effect of the integrated biofloc system and crude protein content (low and high) and their interaction, after confirming homocedasticity (Cochran $p < 0.05$) and normality (Shapiro-Wilk $p < 0.05$). Tukey's test was used when differences between factors and treatments were detected ($p < 0.05$). Water quality variables were analyzed by performing repeated ANOVA measures. Data analyses were performed using ASSISTAT Version 7.7 (Assistat Analytical Software, Campina Grande, Paraíba, Brazil).

Results

The water quality variables of temperature (25°C), dissolved oxygen (5.8–6.1 mg/L), salinity (28.7–29 g/L), TAN (0.10 mg/L), NO_2-N (0.17–0.21 mg/L), NO_3 (4.21–4.42 mg/L), alkalinity (115.1–136.8 mg/L $CaCO_3$), pH (8.1–8.2), PO_4^3 (1.99–2.10 mg/L) and TSS (478.4–501.7 mg/L) were not significantly affected ($p > 0.05$) by use of a monoculture or integrated system or by the crude protein content (Table 2).

Shrimp survival rates were all above 80% during the 42-day experimental period and did not differ significantly ($p > 0.05$) between the treatments. Growth was more accelerated with the high (40%) crude protein content until 21 days, however, at the end of the experimental period, the final weight was similar in the IS32, IS40 and M40 treatments, with significant effects ($p < 0.05$) caused by the use of an integrated system and by differences in crude protein content (Fig. 1 and Table 3).

The yields in the integrated systems were 1.71 and 1.96 kg/m^3, and in the monoculture systems were 1.39 and 1.80 kg/m^3, with significant effects ($p < 0.05$) caused by use of the integrated system and differences in crude protein content (Table 3). The FCR in the integrated systems were 1.47 and 1.67, and in the monoculture system were 1.49 and 1.74, with significant effects ($p < 0.05$) caused by the differences in crude protein content (Table 3).

Discussion

The water temperature (25 °C) was lower than recommended (28 to 32°C) by Van Wyk & Scarpa

Table 2. Effects on water quality of two commercial feeds with high and low crude protein content given to white shrimp *Litopenaeus vannamei* raised in an integrated biofloc system with the seaweed *Gracilaria birdiae* during a 42-day experimental period.

Variables	Treatments[1]				Significance (p value)[2]		
	IS32	M32	IS40	M40	I	P	I×P
Temperature (ºC)	25.55 ± 0.04[a]	25.69 ± 0.13[a]	25.59 ± 0.04[a]	25.75 ± 0.15[a]	ns	ns	ns
Dissolved oxygen (mg/L)	5.91 ± 0.04[a]	5.90 ± 0.04[a]	5.86 ± 0.02[a]	6.10 ± 0.41[a]	ns	ns	ns
Salinity (g/L)	28.75 ± 0.41[a]	29.01 ± 0.23[a]	28.91 ± 0.01[a]	28.98 ± 0.33[a]	ns	ns	ns
Total ammonia nitrogen (mg/L)	0.19 ± 0.01[a]	0.19 ± 0.02[a]	0.18 ± 0.03[a]	0.10 ± 0.05[a]	ns	ns	ns
Nitrite–nitrogen (mg/L)	0.21 ± 0.13[a]	0.18 ± 0.03[a]	0.18 ± 0.11[a]	0.17 ± 0.06[a]	ns	ns	ns
Nitrate (mg/L)	4.20 ± 1.04[a]	4.24 ± 0.79[a]	4.42 ± 0.83[a]	4.21 ± 0.68[a]	ns	ns	ns
Alkalinity (mg $CaCO_3$/L)	136.81 ± 11.20[a]	134.07 ± 15.24[a]	125.55 ± 17.16[a]	115.08 ± 13.30[a]	ns	ns	ns
pH	8.20 ± 0.01[a]	8.21 ± 0.01[a]	8.15 ± 0.02[a]	8.12 ± 0.02[a]	ns	ns	ns
Orthophosphate (mg/L)	2.08 ± 0.33[a]	1.99 ± 0.19[a]	2.11 ± 0.24[a]	2.02 ± 0.17[a]	ns	ns	ns
Total suspended solids (mg/L)	499.45 ± 83.45[a]	501.69 ± 119.61[a]	479.45 ± 64.18[a]	478.48 ± 101.99[a]	ns	ns	ns

[1] IS32 (an integrated system using low protein commercial feed); IS40 (an integrated system using high protein commercial feed); M32 (a monoculture system using low protein commercial feed) and M40 (a monoculture system using high protein commercial feed). The data correspond to the mean of three replicates ± standard deviation. Mean values on the same row with different superscript letters differ significantly ($p < 0.05$). [2] Results from split-plot two-way ANOVA and Tukey's test; I = integrated or monoculture system; P = crude protein; I × P = integrated or monoculture system and crude protein interaction; ns = not significant ($p > 0.05$); *$p < 0.05$.

(1999) for white shrimp culture. This variable influences oxygen consumption, molt cycle, growth, survival, feed consumption rates, metabolic rate and ammonia excretion of shrimp, but did not limit the *L. vannamei* growth, because the performance variables were considered satisfactory for these conditions. Concerning the water quality variables, mean values of oxygen concentration, salinity, pH, total ammonia nitrogen, nitrite, nitrate and alkalinity were within the range recommended for marine shrimp culture (Table 2) (Van Wyk & Scarpa, 1999).

Similar results for higher concentrations of nitrate, as compared to nitrite and TAN, were also observed in other studies of integrated biofloc systems (using seaweed and shrimp) (Brito *et al.*, 2016, 2014), thus indicating that nitrifying bacteria were also present in the system. No effect from the use of an integrated biofloc system was observed on dissolved inorganic nitrogen removal, probably due to a lower photoperiod (12:12 light/dark), and lower biomass rate of shrimp:seaweed (1.27 – 1.79) than those used by Sánchez-Romero *et al.* (2013) (12:12 light/dark and shrimp:seaweed 1:8), and higher TSS levels of about 500 mg/L as compared to Brito *et al.* (2016), who used 223 to 253 mg/L.

No significant effect was observed on alkalinity and pH levels from the use of an integrated biofloc system or because of crude protein content, because hydrated lime inputs positively influenced alkalinity and pH levels, despite nitrification processes and the conversion of ammonium nitrogen into heterotrophic microbial biomass. No significant effect was observed either on orthophosphate levels from use of an integrated biofloc system, corroborating similar results observed by Brito *et al.* (2014, 2016). Du *et al.* (2013) found better phosphate removal by *G. asiatica* at a 10:1 N:P ratio, however, in this study a lower N:P (2:1) ratio in the water was observed.

Final weight, weight gain, and yield were significantly influenced by use of an integrated biofloc system (Table 3), while the substitution of a high (40%) for a low (32%) crude protein content commercial feed in integrated biofloc system has no adverse effect on zootechnical variables of shrimp juveniles for these conditions. Correia *et al.* (2014) and Yun *et al.* (2016) demonstrated

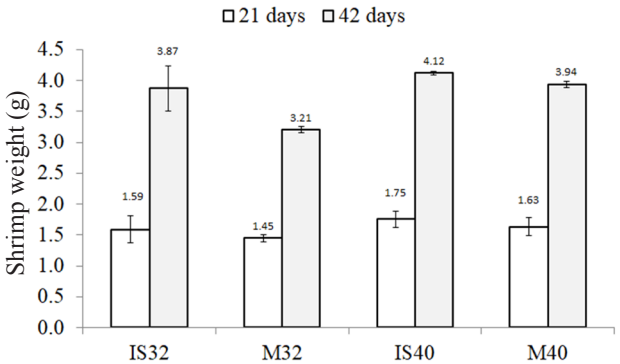

Figure 1. Growth of *Litopenaeus vannamei* juveniles in an integrated biofloc system (IS) and in monoculture (M) using feed with two crude protein contents (low and high) in a 42-day experimental period. The data correspond to the mean of three replicates ± sd. Abbreviation for treatments as in Table 2.

Table 3. Effects of two commercial feeds with high and low crude protein content on the performance of zootechnical variables of white shrimp *Litopenaeus vannamei* raised in an integrated biofloc system with the seaweed *Gracilaria birdiae* during a 42-day experimental period

Variables	Treatments[1]				Significance (p value)[2]		
	IS32	M32	IS40	M40	I	P	I×P
Final weight (g)	3.87 ± 0.37[a]	3.21 ± 0.05[b]	4.12 ± 0.03[a]	3.94 ± 0.05[a]	*	*	ns
Weight gain (g)	3.64 ± 0.38[a]	2.97 ± 0.05[b]	3.89 ± 0.05[a]	3.71 ± 0.05[a]	*	*	ns
Yield (kg/m^3)	1.71 ± 0.22[ab]	1.39 ± 0.06[b]	1.96 ± 0.10[a]	1.80 ± 0.09[a]	*	*	ns
Survival (%)	93.3 ± 6.7[ab]	83.3 ± 5.7[b]	98.3 ± 1.7[a]	90.0 ± 8.6[ab]	ns	ns	ns
FCR	1.67 ± 0.07[a]	1.74 ± 0.02[a]	1.47 ± 0.07[b]	1.49 ± 0.06[b]	ns	*	ns

that for *L. vannamei* reared in biofloc systems, the dietary protein level could be reduced with no adverse effect on shrimp growth and body composition. According to Xu & Pan (2014), a reduction of dietary protein level (35% for 25%) in biofloc systems improves the biofloc proximate composition and the extracellular enzyme activities (amylase), which probably contributes to shrimp growth.

According to Angell *et al.* (2016), seaweeds have relatively high quality proteins (essential amino acids as a proportion of total amino acids) compared to fishmeal and soybean meal. Moreover, Brito *et al.* (2016) showed that *G. birdiae* tissue in a biofloc system had high crude protein levels. These beneficial nutrition components of seaweed may act as nutritional supplements and/or improve the shrimps' utilization of nutrients and can therefore partially substitute artificial feed (Cruz-Suárez *et al.*, 2010; Gamboa-Delgado *et al.*, 2011; Pallaoro *et al.*, 2016; Peña-Rodríguez *et al.*, 2017).

The data suggest that substituting high-protein (40%) with low-protein (30%) feed in an integrated biofloc system (for 0.2 to 4 g shrimp) can reduce shrimp production costs because of reduced use of crude protein (feed cost). Shrimp growth variables are highly sensitive to protein quality, especially to adequate supply of essential amino acids (Tacon, 1987). Brito *et al.* (2016) recorded increased crude protein levels in shrimp (in whole bodies) reared in integrated biofloc systems. However, Jatobá *et al.* (2014) and Yun *et al.* (2016) did not find significantly different ($p > 0.05$) results for protein in whole bodies and muscle tissue in juvenile shrimp reared in a biofloc monoculture system and fed diets containing varying levels of protein.

In addition, Cruz-Suárez *et al.* (2010) found that shrimps' fatty acid profile was clearly modified by use of integrated treatments, with a much higher docosahexaenoic acid content in shrimp raised under integrated conditions. This modification is very important because docosahexaenoic acid is part of the cell membrane components of steroid hormone precursors important to homeostatic balance, brain and nervous system tissue, blood clotting and the immune responses (Tacon, 1987).

Other important compounds found in seaweed are β-glucan, carotenoids, tocopherols, polyphenols, polysaccharides - which act as antioxidants and promote bioactivity against virulent antibiotic resistance (Peso-Echarri *et al.*, 2012; Kolanjinathan *et al.*, 2014) - balanced minerals (sodium, potassium, calcium, iron, magnesium, zinc, nickel, copper, cobalt) and vitamins (A, E, C B1, and B2) (Tabarsa *et al.*, 2012; Syad *et al.*, 2013), which may improve shrimp production in integrated biofloc systems. It is important to remember that according to Tacon (1987), reduction in the shrimps' ability to absorb certain minerals and vitamins from the water may arise under intensive shrimp rearing.

In summary, the results of this study demonstrate that use of an integrated biofloc system can improve feed utilization and growth performance of *L. vannamei* juveniles. The results also showed that dietary protein content could be reduced to 32% without affecting the zootechnical variables of shrimp juveniles. This suggests that the use of integrated biofloc systems is a sustainable strategy for supporting shrimp growth, as it can allow reducing the crude protein content of their feed. Future studies are needed to fully understand the potential role (protein and lipid retention on shrimp tissue) of seaweed for promoting shrimp nutrition when provided as a supplemental food source in integrated biofloc systems.

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