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Bioavailability of crude protein and lipid from biofloc meals produced in an activated sludge system for white shrimp, *Litopenaeus vannamei*

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ABSTRACT - The present study compared the bioavailability of crude protein and lipid from biofloc meals generated with an activated sludge system using two water sources: wastewater from shrimp experimental culture (BFL-W) and, artificially, using clean seawater (BFL-C). The sludge system operated by chemical and organic fertilization three times per week. Sampling of bioflocs occurred every two days during 81 days. To evaluate digestibility, each type of biofloc meal was incorporated into a reference diet (REF) at 300 g/kg. Another diet acted as a negative control (NEG) by using fish waste meal. The apparent digestibility of bioflocs was estimated by the indirect method using chromic oxide (Cr_2O_3) as the inert marker at 10 g/kg of the diet. Juvenile *L. vannamei* of 5.09±0.79 g (n = 440) were stocked at 10 shrimp/tank in 44 tanks of 61 L each that operated under a water recirculating regime. Biofloc meals contained a high ash content (591.0-649.2 g/kg) combined with a low crude protein content (95.9-137.3 g/kg). After 26 days, shrimp achieved a final survival of 93.2±0.8% and a biomass gain of 37.1±1.8 g/tank. Final shrimp body weight ranged from 9.01±0.15 to 9.45±0.13 g. The apparent digestibility coefficient (ADC) of crude protein in the biofloc produced from BFL-W, BFL-C and fish waste meal (NEG) reached 26.0, 25.7, and 64.1%, respectively. Similarly, the lipid ADC was 78.9, 67.9, and 85.8%, respectively. This study indicated that biofloc meals had a low protein availability for *L. vannamei*. However, although low levels of lipid were present, it proved to be available for the species. The dietary inclusion of biofloc meal appears to have a growth-promoting effect on shrimp, which may be associated with trace minerals, or other nutrients not identified in this study.

Key Words: activated sludge bioflocs, digestibility

Introduction

Discharge of aquaculture effluents has been associated with nutrient and organic enrichment of receiving coastal aquatic ecosystems (GESAMP, 1991, 1996; Tho et al., 2014). Attempts to treat aquaculture wastewater have ranged from the use of constructed wetlands (Buhmann and Papenbrock, 2013), settling basis (Jones et al., 2001; Engle and Valderrama, 2003), artificial substrates (Stewart et al., 2006; Arnold et al., 2009), bivalve filtration (Jones et al., 2001), reduction of water discharge (Hopkins et al., 1993), and more recently, use of microbial-based zero or low-water exchange rearing systems (Wasielesky et al., 2006; Avnimelech, 2007; Ballester et al., 2010; Crab et al., 2012; Hende et al., 2014).

Aquaculture wastewater has been applied for irrigation of agricultural crops (McIntosh and Fitzsimmons, 2003; Miranda et al., 2008), fertilization of halophytes (Brown and Glenn, 1999), and production of microbial flocs (also known as bioflocs) used as a dietary food source (Kuhn and Boardman, 2008; Kuhn et al., 2009, 2010). These and other studies have reported that microbial flocs can serve as a nutrient source for marine shrimp and other farmed species (Burford et al., 2004; Ju et al., 2008; Azim and Little, 2008; Crab et al., 2010; Bauer et al., 2012; Hende et al., 2014). Biofloc particles can have between 120 and 490 g/kg of crude protein, but they usually contain lipid levels below 20 g/kg (Emerenciano et al., 2009; Crab et al., 2010).

The production of microbial flocs from aquaculture wastewater has been demonstrated previously. Kuhn et al. (2009, 2010) generated bioflocs with effluents from an inland tilapia farm using a sequencing batch reactor with carbon supplementation and with a membrane biological reactor without carbon supplementation. In these biological wastewater treatment systems, nutrient levels in microbial flocs were high. Kuhn et al. (2009, 2010) reported that the crude protein content exceeded 380 g/kg (dry matter basis), successfully replacing fish meal and soybean protein in diets for the white shrimp, *Litopenaeus vannamei*. If the production of bioflocs can be carried out artificially using wastewater from aquaculture operations, it can be converted into dry biomass to serve as a potential source of protein in shrimp diets, reducing the pressure that effluent discharge

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may have on receiving waters. The present study compared the bioavailability of crude protein and lipid from biofloc meals generated with an activated sludge system using two water sources: wastewater from shrimp experimental culture and, artificially, using clean seawater.

Material and Methods

This study was conducted in the northeast region of Brazil. All procedures were performed in compliance with relevant laws and institutional guidelines, including those related to animal welfare. For the study, two activated sludge systems were used to generate microbial flocs with two water sources: (1) wastewater derived from an experimental shrimp nursery system (six tanks of 3 m^3 each, wastewater biofloc); and (2) sand-filtered and disinfected seawater obtained from a nearby estuary (clean seawater biofloc). Activated sludge systems were designed based on Van-Haandel and Marais (1999) with some modifications.

The system was composed of two laboratory-scale activated sludge systems (Figure 1). Each system was placed at the end of the effluent discharge from the marine shrimp experimental rearing facility, but before a a halophyte wetland. Each activated sludge system was equipped with a 1-m^3 tank for storage of clean or wastewater from shrimp rearing tanks, two electromagnetic metering pumps (LMI Milton Roy, Ivyland, USA), one fiberglass reactor, one fiberglass settling chamber, and one 2.5-hp air blower for water aeration. The reactor that operated with clean water was attached to a 20-m^3 tank, where sand-filtered and chemically disinfected seawater was stored.

The system operated by first supplying water (either clean or wastewater) by gravity to a 1-m^3 tank. A metering pump that operated with a flow rate of 180 mL/min moved water from the 1-m^3 tank to the reactor (Figure 1). An additional metering pump with a flow rate of 1.2 mL/min was used to recirculate water between the reactor and the settling chamber. Water was always collected from the tank bottom and mechanically moved to the top.

Initially, the activated sludge systems operated for a 6-month trial period using wastewater derived from shrimp nutrition trials. For the current study, the system was reset by removing all remaining water and activated sludge accumulated in the reactor and settling chamber. The activated sludge and residual water were transferred to a 1-m^3 tank where the supernatant was collected after a 4-h resting period. A total of 80 L of concentrated sludge was split and placed in each one the reactors to serve as an inoculum. The remaining volume of each reactor was filled with previously disinfected seawater (40 g/L salinity) using calcium hypochlorite at 8.0×10^3 g/L of (65% of active chlorine).

Each reactor was chemically and organically fertilized three times per week using a nutrient solution in accordance with Kim and Pagilla (2003), Diez et al. (2005), and Samocha et al. (2007). This mix was composed of 60 g of sugar cane molasses, 2.3 g of urea, 5 g of monocalcium phosphate, and 100 mL of macronutrients. The latter consisted of 16.8 g of ammonium chloride (NH_4Cl), 5.3 g of calcium chloride ($CaCl_2$), 15 g of potassium phosphate (K_2PO_4), 6 g of magnesium sulfate ($MgSO_4$), and 1 mL of micronutrients (Table 1). In the activated sludge system that operated with sterile seawater (clean water biofloc), application of sugar cane molasses was modified to 100 g.

A total of 47 biofloc samples of each type (i.e., wastewater and clean seawater) were collected every two days during 81 days. Biofloc was sampled from the bottom of its settling basin at a volume of 6 L per day. After collection, the material was allowed to rest for 5 min. It was then screened in a 20-micron mesh, rinsed with freshwater, and then kept frozen at –22 °C until drying in a convection

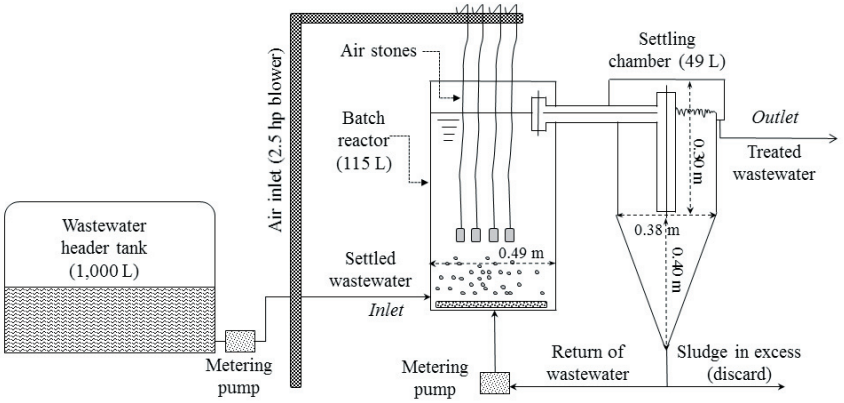


Figure 1 - A schematic diagram of the pilot-scale activated sludge system used in the present study.

oven under 45 °C to achieve a final moisture content of less than 10 g/kg.

The dried biofloc was individually ground in a hammer-mill (industrial mill Vieira, model 280, 5 hp, Tatuí, SP, Brazil) fitted with a mesh of 600 microns to produce a meal. The meal was then chemically analyzed following standard procedures (AOAC, 1990).

The apparent digestibility of biofloc meals was estimated by the indirect method using chromic oxide (Cr_2O_3) as the inert marker at 10 g/kg of the diet. Initially, the concentration of Cr_2O_3 in the finished diets and in shrimp feces was used to determine the apparent digestibility coefficient (ADC):

$$ADC = 100 - \left[100\left(\frac{\%Cr_2O_3 f}{\%Cr_2O_3 d}\right) \times \left(\frac{\%Nf}{\%Nd}\right)\right] \quad (1)$$

in which ADC = apparent digestibility coefficient of nutrient (crude protein and lipid); $Cr_2O_3 d$ = concentration (in %) of chromic oxide in the diet; $Cr_2O_3 f$ = concentration (in %) of chromic oxide in shrimp feces; N*d* = concentration (in %) of nutrient (crude protein and lipid) in the diet; and N*f* = concentration (in %) of nutrient (crude protein and lipid) in shrimp feces.

Subsequently, the digestibility of crude protein and lipid of each individual ingredient under evaluation was calculated (Cho and Slinger, 1979; Bureau et al., 1999; Bureau and Hua, 2006):

$$ADC_{test\ ing.} = ADC_{test\ diet} + \left[(ADC_{test\ diet} - ADC_{ref.\ diet}) \times \left(\frac{0.7 \times D_{ref}}{0.3 \times D_{ing.}}\right)\right] \quad (2)$$

in which $ADC_{test\ ing.}$ = apparent digestibility coefficient (crude protein and lipid) of test ingredient; $ADC_{test\ diet}$ = apparent digestibility coefficient (crude protein and lipid) of the test diet; $ADC_{ref.\ diet}$ = apparent digestibility coefficient (crude protein and lipid) of the reference diet; D_{ref} = concentration (in %) of nutrient (crude protein and lipid) in the reference diet; and $D_{ing.}$ = concentration (in %) of nutrient (crude protein and lipid) in the test ingredient.

Table 1 - Composition of the micronutrients, part of the nutrient solution, used to produce bioflocs with sterile seawater and wastewater from shrimp rearing

Nutrient	Concentration (mg/L)
K_2HPO_4	25
NH_4Cl	25
$MgSO_4$	8.6
$CaCl_2$	5.3
$FeCl_2$	0.3
H_3BO_4	0.08
$ZnCl_2$	0.02
$CoCl_2$	0.3
$(NH_4)_6Mo_7O_{24}$	0.2
$CuCl_2$	0.01

A reference diet (REF) was formulated with practical ingredients to fully meet the reported nutrient requirements of *L. vannamei* (NRC, 2011; Table 2). From this diet, three other diets were prepared containing 70% of the reference diet and 30% of the tested ingredient (wastewater and clean seawater biofloc meals). A fourth diet using fish waste meal as the test ingredient was included to act as a negative control (NEG). Diets were prepared with a laboratory extruder as described by Browdy et al. (2012). Sinking pellets of 2.0 mm in diameter were used over the course of the rearing period.

The digestibility assay was carried out in a water recirculating system composed of 44 tanks of 61 L each

Table 2 - Ingredient composition (as is basis) of experimental diets used to measure *in vivo* digestibility of bioflocs in juvenile marine shrimp

Ingredient	Inclusion (g/kg, as is)/Diet			
	REF	BFL-W	BFL-C	NEG
Soybean meal[1]	349.6	-	-	-
Wheat flour[2]	250.0	-	-	-
Salmon by-product meal[3]	150.0	-	-	-
Soy protein concentrate[4]	80.0	-	-	-
Poultry meal[5]	60.0	-	-	-
Soybean lecithin	25.0	-	-	-
Corn gluten meal	20.0	-	-	-
Krill meal[6]	20.0	-	-	-
Salmon oil	20.0	-	-	
Mineral-vitamin premix[7]	15.0	-	-	
Chromic oxide	10.0	-	-	-
Cholesterol[8]	0.4	-	-	-
Reference diet (REF)	-	700.0	700.0	700.0
Natural biofloc (BFL-W)	-	300.0	-	-
Artificial biofloc (BFL-C)	-	-	300.0	-
Fish waste meal (NEG)[9]	-	-	-	300.0

REF - reference; BFL-W - diet containing 300 g/kg of biofloc produced from wastewater; BFL-C - diet containing 300 g/kg of bioflocs produced from clean seawater; NEG - negative control.

[1] Indústria e Comercio de Rações Dourado Ltda. (Eusébio, Brazil) - 476 g/kg crude protein (CP), 32 g/kg fat, 61 g/kg ash, 42 g/kg crude fiber (CF), 103 g/kg moisture, 3,859 kcal/kg gross energy (GE), 14 g/kg methionine (Met), 35 g/kg lysine (Lys).

[2] Moinhos Cruzeiro do Sul S/A (Olinda, Brazil) - 134 g/kg CP, 22 g/kg fat, 12 g/kg ash, 8 g/kg CF, 110 g/kg moisture, 4,043 kcal/kg GE, 2 g/kg Met, 3 g/kg Lys.

[3] Pesqueira Pacific Star (Puerto Montt, Chile) - 628 g/kg CP, 107 g/kg fat, 160 g/kg ash, 1 g/kg CF, 99 g/kg moisture, 4,559 kcal/kg GE, 17 g/kg Met, 4.6 g/kg Lys.

[4] Sementes Selecta S.A. (Goiânia, Brazil) - 626 g/kg CP, 8 g/kg fat, 42 g/kg ash, 43 g/kg CF, 82 g/kg moisture, 4,017 kcal/kg GE, 13 g/kg Met, 59 g/kg Lys.

[5] Kabsa S.A. (Porto Alegre, Brazil) - 667 g/kg CP, 171 g/kg fat, 97 g/kg ash, 24 g/kg moisture, 5,127 kcal/kg GE, 13 g/kg Met, 32 g/kg Lys.

[6] QRILL™, Aker Biomarine ASA (Oslo, Norway) - 590 CP, 180 g/kg fat, 130 g/kg ash, 60 g/kg CF, 7,1 g/kg moisture, 4,610 kcal/kg GE, 19 g/kg Met, 38 g/kg Lys.

[7] Rovimix Camarao Extensivo. DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). Guaranteed levels per kg of product: vitamin A, 1,000,000 IU; vitamin D3, 300,000 IU; vitamin E, 15,000 IU; vitamin K3, 300 mg; vitamin B1, 3,000 mg; vitamin B2, 2,500 mg; vitamin B6; 3,500 mg; nicotinic acid, 10,000 mg; pantothenic acid, 5,000 mg; biotin, 100 mg; folic acid, 800 mg; vitamin C, 25,000 mg; choline, 40,000 mg; inositol, 20,000 mg; iron 2,000 mg; copper, 3,500 mg; chelate copper, 1,500,0 mg; zinc, 10,500 mg; chelate zinc, 1,500 mg; manganese, 4,000 mg; selenium, 15 mg; chelate selenium, 15 mg; iodine, 150 mg; cobalt, 30 mg; chromium 80 mg; filler, 1,000 g.

[8] Cholesterol XG, Dishman Netherlands B.V. (Veenendaa, the Netherlands) - 91 g/kg of active cholesterol.

[9] Courtesy of InVivo Nutrição e Saúde Animal Ltda. (Paulínia, Brazil). Made from fisheries by-catch and (or) fish residues (trimmings and offal). - 652 g/kg CP, 150 g/kg fat, 184 g/kg ash, 55 g/kg CF, 8.0 g/kg moisture, 4,512 kcal/kg GE, 14 g/kg Met, 3.7 g/kg Lys.

(31.0 × 35.5 × 55.5 cm; height × width × length). A total of 11 tanks were designated for each dietary treatment. Each tank was covered with a net and equipped with aeration, and its own water inlet and outlet system.

Shrimp were purchased from a licensed commercial hatchery (Aquatec Aquacultura Ltda., Canguaretama, Brazil) as post-larvae 10 and raised until juvenile size in the laboratory. Juvenile *L. vannamei* of 5.09±0.79 g (n = 440) body weight were stocked at 10 shrimp/tank and acclimated for three days with the reference diet prior to the start of the study.

Diets were delivered daily at 07.00 h, 13.00 h, and 16.00 h, in a small feeding tray released slowly in the water column. Shrimp were fed in excess over the entire rearing period. Feces were recovered by the syphoning method (Cruz-Suárez et al., 2008). One hour after feed delivery, the feeding trays were removed, the uneaten feed was discarded, and the tank bottom was cleaned. This was followed by two consecutive fecal collections, 1.25 and 2.15 h after feed delivery at 07.00 h, and 13.00 h. This interval is sufficient to allow the gastric evacuation of more than half of a meal ingested by shrimp (Nunes and Parsons, 2000).

No feces were collected in tanks where exuviae were observed due to molting. Feces collection lasted 26 consecutive days, when a minimum amount of dried feces (10 g) was obtained for chemical analysis. At harvest, shrimp were weighed, and counted. Water quality was kept consistent throughout the study, at 35±3 g/L (n = 64) salinity, 7.45±0.36 (n = 64) pH, and 28.1±0.2 °C (n = 64) temperature.

Data were analyzed through one-way ANOVA for completely randomized experiments. When significant differences were detected between the means, they were compared two-by-two with Tukey's HSD test. The significant level of 5% was set in all statistical analyses. The statistical package SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used.

Results and Discussion

A total of 1,965 and 1,145 g of dried biofloc meal (wastewater and clean seawater types, respectively) were obtained over the course of 81 days. Both types of biofloc meals displayed a chemical profile characterized by a high ash content (649.2-591.0 g/kg) combined with low crude protein (95.9-137.3 g/kg). These results differ from studies performed with microbial flocs produced in batch reactors using effluent from tilapia culture and sugar as a culture medium (Kuhn et al., 2009). In their work, Kuhn et al. (2009) found crude protein, ash, lipid, and crude fiber contents of 490±15, 134±6, 11.3±0.9, and 126±1 g/kg, respectively. Although collection of microbial flocs in the present study and Kuhn et al. (2009)'s work were similar, further work is required to reduce the amount of ash present in the samples from the present study and thus concentrate the protein portion of the samples.

In another work, Kunh et al. (2010) produced bioflocs from tilapia effluents with sequencing batch reactors (SBR) using carbon supplementation (sucrose) and with a membrane biological reactor (MBR) without carbon supplementation. For bioflocs produced under SBR and MBR treatments, crude protein and ash reached 388 and 405 g/kg, and 247 and 118 g/kg, respectively. These values also deviate from results obtained in this study. Azim and Little (2008) reported that bioflocs obtained in rearing tanks of Nile tilapia, *Oreochromis niloticus*, contained between 379.3±23.8 and 384.1±36.2 g/kg crude protein, 31.6±3.1 and 32.3±2.1 g/kg lipid, 133.8±13.5 and 118.3±8.0 g/kg ash, and 62.7±4.4 and 57.2±18.6 g/kg fiber. The authors concluded that bioflocs were a suitable food source for tilapia, except with respect to their low lipid levels.

Agreeing with the present study, Hende et al. (2014) also found high ash levels in dried bioflocs obtained from wastewater of pikeperch culture treated through a pilot reactor. While crude protein varied from 158 to 277 g/kg (dry matter basis), ash ranged from 503 to 702 g/kg. Due to the low protein content of biofloc meals, Hende et al. (2014) replaced 17 to 49% of the wheat flour in *L. vannamei* diets. The authors were able to successfully include as much as 80 g/kg of biofloc meal without deleterious effect to shrimp growth, survival, and FCR.

Ash is invariably present in feedstuffs used to manufacture aquatic feeds. However, depending on their nature and origin, it has limited nutritional value since fish and shrimp do not digest it. High levels of ash are often found in meals produced from waste obtained in the slaughtering of terrestrial animals or from fish processing. However, ash values rarely exceed 250 g/kg (NRC, 2011), while the protein content may be greater than 400 g/kg (on dry matter basis).

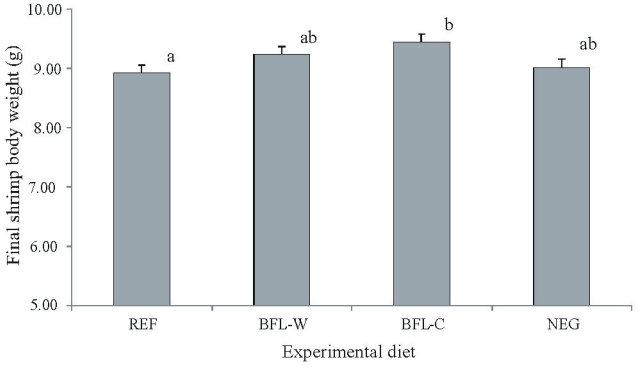
The crude protein content in the biofloc meal produced from clean seawater was superior to that of the wastewater biofloc meal. This may be the result of urea application during the start-up phase of the biofloc produced using clean seawater. The protein analysis is based on the Kjeldahl method, wherein the total nitrogen content of the sample is converted to crude protein by multiplying by a theoretical factor of 6.25 (assuming that the protein contains around 160 g/kg of N). Therefore, excess nitrogen in these biofloc meals resulting from urea application would reflect in

higher levels of crude protein. However, this does imply this protein is biologically available for shrimp. Based on these preliminary chemical analyses (high levels of ash and low protein content), both types of biofloc produced in this study would not be considered of interest as an ingredient for shrimp feeds.

Other studies have successfully included biofloc meal in *L. vannamei* diets as a source of protein. Bauer et al. (2012) produced a biofloc meal from an effluent of a super-intensive shrimp farm. The authors used soy protein concentrate in combination with microbial floc meal to fully replace a Brazilian fish meal in shrimp diets. This occurred despite a reduced amount of sulfur-containing amino acids (methionine + cysteine) in diets with microbial flocs. Their microbial floc meal contained 233.9 g/kg of crude protein and a high ash content, 366.0 g/kg.

In the present study, the shrimp achieved a final survival of 93.2±0.8% and a biomass gain of 37.1±1.8 g/tank ($P>0.05$, ANOVA). There was a statistically significant difference in final shrimp body weight, particular between dietary treatments REF and BFL-C ($P<0.05$, Tukey's HSD; Figure 2). Shrimp fed BFL-C attained 9.45±0.13 g compared with the 8.93±0.14 g for shrimp fed the REF diet.

The NEG diet contained the highest crude protein and lipid levels of all tested diets (Table 3). However, this diet displayed a lower apparent digestibility coefficient (ADC) for crude protein (74.7%), but comparable to the levels obtained with BFL-W (75.0%) and BFL-C (73.1%). Conversely, the REF diet showed the highest ADC for both crude protein and lipid (Table 4). Bioflocs contain low lipid levels and are poor in highly unsaturated fatty acids, such as EPA (eicosapentaenoic, 20:5n-3) and DHA (docosahexaenoic, 22:6n-3; Crab et al., 2010). These fatty acids are considered essential nutrients, playing a major role in penaeid shrimp nutrition (NRC, 2011).



Shrimp of 5.09±0.79 g (n = 440) were stocked under 10 animals/tank and fed four diets: REF (reference), BFL-W (diet containing 300 g/kg of biofloc produced from wastewater), BFL-C (diet containing 300 g/kg of bioflocs produced from clean seawater), and NEG (negative control).
Columns with same letters indicate no statistically significant differences according to Tukey's HSD test at the 0.05 significance level.

Figure 2 - Final body weight of *L. vannamei* juveniles after 26 days in a clear-water recirculation system.

The low ADC for crude protein observed for diets BFL-W, BFL-C, and NEG reflect the bioavailability of the tested ingredients. The ADC of crude protein in the biofloc meal produced from wastewater (BFL-W), from clean seawater (BFL-C), and for the fish waste meal (NEG) reached 26.0, 25.7, and 64.1%, respectively. Similarly, the lipid ADC were 78.9, 67.9, and 85.8%, respectively. In general, the ADC of crude protein in feedstuffs used in shrimp diets vary between 58 and 97% (NRC, 2011); these include blood meal (ADC = 66-71%), canola meal (80%), corn gluten meal (59%), cottonseed meal (83%), anchovy meal (83-89%), krill meal (81%), meat and bone meal (60-88%), poultry by-product meal (79%), shrimp meal (58%), soybean meal (89-97%), and soy protein concentrate (93%). Feedstuffs with crude protein ADC below 50% are not considered suitable for use as a protein source in shrimp diets.

In the present study, both types of biofloc meals displayed a very poor digestibility for crude protein lower than the negative control (NEG). To date, no ADC values for crude protein and lipid have been reported in the literature for bioflocs. However, Kuhn et al. (2009) succeeded in

Table 3 - Proximate analysis of bioflocs produced in batch reactors using wastewater from shrimp rearing tanks and clean seawater

Parameter	Proximate content (g/kg, dry matter basis)/Biofloc	
	Wastewater	Clean seawater
Dried matter	928.6	913.2
Crude protein	95.9	137.3
Fat	7.2	9.1
Fiber	< 5	< 5
Ash	649.2	591.0
Calcium	23.5	21.1
Total phosphorus	10.1	9.3

Table 4 - Crude protein, fat content, and apparent digestibility coefficients (ADC) for diets containing bioflocs produced with wastewater derived from shrimp culture (BFL-W) and with clean seawater (BFL-C)[1]

Nutrient/ADC	Apparent digestibility coefficient (%, dry matter basis)/Diet			
	REF	BFL-W	BFL-C	NEG
Crude protein (g/kg)	413	331	342	489
ADC diet (%)	80.9	75.0	73.1	74.7
ADC ingredient (%)	-	26.0	25.7	64.1
Fat (g/kg)	85.7	49.1	50.9	97.9
ADC diet (%)	93.8	93.1	92.6	90.2
ADC ingredient (%)	-	78.9	67.9	85.8

[1] The negative control (NEG) contained 70% of the reference diet (REF) and 30% of fish waste meal.

replacing soybean meal and fish meal in *L. vannamei* feed with bioflocs produced with tilapia farming effluents. Kuhn et al. (2010) worked with dietary inclusions of bioflocs between 10 and 30% in diets for juvenile *L. vannamei* and achieved a higher shrimp growth rate between 1.44 and 1.66 g/week when the animals were fed diets containing bioflocs compared with those without it. Ju et al. (2008) produced several laboratory diets for *L. vannamei* juveniles containing bioflocs (intact or in powder) and found that shrimp fed diets containing 20% of the intact biofloc for eight weeks reached the highest growth rates, even when compared with a commercial control.

In this study, a higher final shrimp body weight was observed when the shrimp were fed a diet containing 30% of the bioflocs produced from clean seawater and 70% of the reference diet. This suggests that bioflocs may have had a growth-promoting effect on *L. vannamei*. It is possible that this effect is not directly associated with the protein present in the bioflocs, but another nutrient, possibly macro- (calcium, phosphorus, potassium, and magnesium) and (or) micro-minerals (copper, iron, manganese, and zinc). However, these results must be confirmed by a longer rearing period (typically 10 weeks). More in-depth chemical analysis must be performed in bioflocs produced from aquaculture wastewater to determine what essential nutrients available may have enhanced shrimp growth.

Conclusions

Bioflocs produced with wastewater from shrimp farming or clean seawater through an activated sludge system have a low protein content available for juvenile *L. vannamei*. However, although low levels of lipid were present, it proved to be highly available for the species. The nutritional profile of biofloc meals is characterized by a high mineral content. The dietary inclusion of biofloc meals appears to have a growth-promoting effect, which may be associated with trace minerals, or other nutrients not identified in this study.

Acknowledgments

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