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Research Article

Physical and chemical characteristics of lyophilized biofloc produced in whiteleg shrimp cultures with different fishmeal inclusion into the diets

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ABSTRACT. Physical and chemical characteristics of lyophilized biofloc produced into the culture of whiteleg shrimp was determinated. The study consisted in the evaluation of biofloc produced with four experimental diets, isoproteic (35%) and isolipidic (8%), with different fishmeal content: 0 g kg^{-1} (T0), 100 g kg^{-1} (T1), 200 g kg^{-1} (T2), 300 g kg^{-1} (T3), and a commercial diet with 300 g kg^{-1} (TC) as a control. The shrimp was cultured in low salinity (5 g L^{-1}) at a density of 600 ind m^{-3}. The bioflocs were manually collected at day 28, lyophilized, and processed. Proximal composition was determined. To analyze morphology and particle size, photomicrographs were obtained using a Scanning Electron Microscope (SEM). Molecular weights of the protein hydrolysates were determined, and finally the bioflocs protein surface hydrophobicity (S_0) was measured. No significant differences were detected for protein (360-404 g kg^{-1}), lipid (6-8 g kg^{-1}) and fiber (5-9 g kg^{-1}) contents, but the ashes (205-284 g kg^{-1}) were different. The hydrolysate protein molecular weights were similar, in all cases varied from 22 to 200 kDa. The 50% of lyophilized particles had sizes from 3 to 15 μm. The fluorescence spectra slopes indicated differences in protein surface hydrophobicity (S_0) between the treatments. In general, the physical and chemical characteristics of the bioflocs were independent of the used diet. The lyophilized biofloc has properties that allow its use as a protein source or raw material for biotechnological processes.

Keywords: whiteleg shrimp, biofloc, shrimp nutrition, protein molecular weight, vegetable protein, SEM analysis.

Características físicas y químicas de biofloc liofilizado producido en cultivos de camarón blanco con diferente inclusión de harina de pescado en la dieta

RESUMEN. Se determinaron las características físicas y químicas del biofloc liofilizado producido en cultivos de camarón blanco. Los tratamientos consistieron en evaluar el biofloc producido con cuatro dietas experimentales, isoproteicas (35%) e isolipídicas (8%), con diferente contenido de harina de pescado: 0 g kg^{-1} (T0), 100 g kg^{-1} (T1), 200 g kg^{-1} (T2), 300 g kg^{-1} (T3), y una dieta comercial con 300 g kg^{-1} (TC) como control. El camarón se cultivó a baja salinidad (5 g L^{-1}) en densidades de 600 ind m^{-3}. Los bioflóculos fueron colectados al día 28, liofilizados y procesados para evaluar sus características físicas y químicas y determinar su composición proximal. Se analizó la morfología y tamaño de las partículas con un microscopio electrónico de barrido (SEM). Se determinaron los pesos moleculares de los hidrolizados de proteína y se midió la hidrofobicidad de superficie (S_0) de las proteínas. No se encontraron diferencias significativas en el contenido de proteínas (360-404 g kg^{-1}), lípidos (6-8 g kg^{-1}) y fibra (5-9 g kg^{-1}), pero las cenizas (205-284 g kg^{-1}) fueron diferentes. Los pesos moleculares del hidrolizado de las proteínas fueron similares y en todos los casos variaron de 22 a 200 kDa. El 50% de las partículas tuvo tamaños de 3 a 15 μm. Las pendientes del espectro de fluorescencia indicaron diferencias en la hidrofobicidad de superficie (S_0). En general, las características físicas y químicas de los bioflóculos fueron independientes de las dietas utilizadas. El biofloc liofilizado tiene propiedades físicas y químicas que permiten su uso como fuente de proteína o materia prima para procesos biotecnológicos.

Palabras clave: camarón blanco, biofloc, nutrición camarón, peso molecular de proteínas, proteína vegetal, análisis SEM.

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INTRODUCTION

Shrimp is one of the most popular seafood worldwide. Increased demand for shrimp has encouraged the expansion of its aquaculture (Yano *et al.*, 2015). The use of biofloc technology (BFT) has increased, especially to culture tilapia and shrimp. The BFT allows the drastic reduction of water exchanges, and promotes the live feed development, which contributes to cover the nutritional requirements of farmed organisms (Avnimelech, 2014).

In recent years, the increasing demand and the high prices of fishmeal has caused increases in the price of formulated feed, making necessary to continue with the evaluation of alternative protein sources. To supply the demand of aquafeeds, the replacement or reduction of fishmeal use is of great interest for the aquaculture industry (Kuhn *et al.*, 2009).

The main problems related with fishmeal substitution include: deficiency of some essential amino acids, presence of anti-nutritional factors, palatability and digestibility (Forster *et al.*, 2003; Naylor *et al.*, 2000). However, the use of low fishmeal diets combined with biofloc technology, could be a successful strategy for culturing aquatic species without negative effects in their production response (López-Elías *et al.*, 2015), since the bioflocs contain essential amino acids, fatty acids, vitamins and other nutritional components which are mainly derived from the microorganism present in the culture systems (Ju *et al.*, 2008; Martínez-Córdova *et al.*, 2014).

The importance of the nutrients presents in the biofloc is not only due to the amount of protein, but also by the presence of diverse elements, which are not present in the formulated food (Barrows & Hardy, 2000; Martínez-Córdova *et al.*, 2016). The physical and chemical characteristics of the feed ingredients determine their biochemical properties (Lian *et al.*, 2005), hydrophobic characteristics (Mahn *et al.*, 2009) and size of clusters formed (Brendonck., 1993; More *et al.*, 2014). Thus, the knowledge of these properties can explain their nutritional bioavailability as well as their biotechnological potential.

Several researchers have used biofloc as a supplementary protein source. Kuhn *et al.* (2009) evaluated the microbial floc meal produced in bioreactors as replacement ingredient of fishmeal in shrimp feed; they reported significant improvement in gain weight per week, compared with the control diets. In an experimental culture, Bauer *et al.* (2012) substituted the fishmeal with soybean concentrate and biofloc meal and did not found a negative effect on the productive parameters of the Pacific white shrimp. Nunes *et al.* (2010) used an experimental feed (less than 250 g kg^{-1} crude protein; mix of a commercial diet and commercial poultry feed) in a biofloc technology (BFT), demonstrating that the Pacific white shrimp (*L. vannamei*) had a better performance in the BFT system compared with a traditional diet (370 g kg^{-1} crude protein) in traditional culture. Valle *et al.* (2015) evaluate the effect of the gradual replacement (100, 200, 300 and 400 g kg^{-1}) of fishmeal by fish protein hydrolysate and biofloc flour in *L. vannamei* postlarvae; they concluded that it is possible to replace fishmeal by either of these two ingredients.

The shrimp culture in low water salinity is growing in several countries as United States, Ecuador, Brazil, Thailand, China and Australia (Roy *et al.*, 2010). The biofloc produced in low- salinity water, facilitates their manipulation; with an appropriate processing, can be used as a high quality ingredient for animal diets and other biotechnological purposes. Under this approach, the biofloc ponds can be used as bioreactors to produce microbial biomass. In general, exist three types of biofloc: heterotrophic, mixotrophic and autotrophic (Avnimelech, 2014). The conservation of the nutritional properties of bioflocs could be achieved through lyophylization. This widely used process consists of isolating a solid substance from solution by freezing the solution and vaporizing the ice away under vacuum conditions. This process is highly efficient to conserve the biochemical properties of the organic matter (enzymes, proteins, bioactive compounds, etc.).

In a traditional BFT farm, at the end of the culture period the pond water is discharged. The discharges can cause an overload of organic matter into the natural water bodies. The storage and processing of bioflocs could contribute to diminish the environmental impact, and promote the use of alternative protein sources to feed aquatic or terrestrial animals. The aim of this study was to determine the physical and chemical characteristics of lyophilized biofloc produced in the culture of whiteleg shrimp fed diets with different fishmeal inclusion, in order to assess the potential use of microbial bioflocs as protein source in the animal nutrition or in biotechnological applications.

MATERIALS AND METHODS

Experimental diets

Four experimental feeds (isoproteic and isocaloric; 350 g kg^{-1} crude protein; 80 g kg^{-1} of crude fat; 17 kJ g^{-1} gross energy) were elaborated based on previous formulation provided by Moreno-Arias *et al.* (2016). Each diet contained different percentages of fishmeal (0, 10, 20 and 30), and a vegetable meal mix (soybean, corn, wheat and sorghum meal) were used to get the established protein and energy levels. One commercial

diet was used as control (Camaronina; Agribrands Purina Mexico, S.A. de C.V; labeled information indicates: 350 g kg^{-1} of crude protein and 80 g kg^{-1} of crude fat).

Experimental shrimp

Shrimp poslarvae were obtained from SRY Promotora Acuícola S.A. de C.V at Las Bocas, Sonora, Mexico. The sanitary certificate indicates absence of pathogens including white spot syndrome virus (WSSV), yellow head virus (YHV), Taura syndrome virus (TSV), hematopoietic necrosis virus (IHHNV) and acute hepatopancreatic necrosis disease (AHPND). The postlarvae (PL 15) were acclimated from 35 g L^{-1} to 5 g L^{-1} during 96 h and fed with frozen *Artemia*.

Experimental design

The experiment was developed under laboratory conditions. A single factor randomly design with three replicates per treatment was performed. The five experimental treatments consisted of diets with different content of fishmeal: 0 g kg^{-1} (T0), 100 g kg^{-1} (T1), 200 g kg^{-1} (T2), 300 g kg^{-1} (T3), and commercial diet with 300 g kg^{-1} as a control (TC). The control diet is a standard feed used into the shrimp farming, and was used to compare the physical and chemical characteristics of biofloc with those produced with the experimental diets. The experimental tanks (250 L) were filled with 200 L of filtered water (10 µm) with 5 g L^{-1} of salinity. Acclimated shrimp postlarvae (PL19) with a mean individual weight of 24.2 mg were randomly distributed into the tanks at a density of 600 ind m^{-3}. The feeding rate was around 25% of their biomass, distributed in four rations per day. The water was not exchanged and temperature was not controlled. The illumination was minimum (from dark to 100-200 lux) to induce the heterotrophic biofloc. The carbon: nitrogen ratio was 12:1. All tanks were aerated continuously using a blower (0.3 HP) and one air stone (6 cm^3) per container.

Bioflocs collection

The biofloc was manually collected at day 28 after culture. The aeration was suspended for 10 min to concentrate the total suspended solids in the bottom. A total of 100 L of water were siphoned and settled externally by successive sedimentation. The biofloc biomass were immediately placed in an ultrafreezer at -80°C (Thermo Fisher Scientific; Waltham, MA, USA). Posteriorly the samples were processed in a Telstar, lyoquest-55 lyophilizer (Telstar Co. D.F., Mexico) and stored until analysis.

Proximate composition of bioflocs and molecular weight of protein

Moisture, crude protein, ash, lipid and crude fiber were determined according to the AOAC (2002) methods.

The molecular weight distribution for the protein hydrolysates was determined using sodium dodecyl sulfate (SDS) poly-acrylamide gel electrophoresis (Laemmli, 1970). Electrophoresis was performed using a MiniProtean III (Bio-Rad Laboratories Chemical, Hercules, CA, USA) vertical electrophoresis device. The stacking and separating gels were prepared using 4 and 10% of acrylamide, respectively. Gel wells were loaded with 12 µL aliquots of the biofloc protein extract, and electrophoresis was performed at 4°C and run at 120 volts. The protein bands were stained with 0.1% coomassie brilliant blue R250 and distained with 10% acetic acid. Protein bands were identified by comparing with the broad-range molecular weight standards (Sigma Chemical Company, St. Louis, MO, USA): myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase-b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa). Gels were analyzed using a Bio-Rad Molecular Imager Universal Hood II - S.N. 76S/02460 Gel Doc XR System.

Preparation to photomicrograph and particle size measurements

Micrographs of the five lyophilized bioflocs were generated to investigate the bioflocs morphological characteristics and size. After freeze drying and layering the samples with 13 mm carbon paper tape and 20 nm gold coating, the photomicrograph was obtained using a Scanning Electron Microscope (SEM) (JSM 5400LV scanning electron microscope, Peabody, MA, USA) at an acceleration voltage of 15 kV. The particle size and morphology of bioflocs, were analyzed using an image analyzer software Image Pro Premier (Media Cybernetics, Inc. Rockville, MD, USA).

Surface hydrophobicity measurements

The biofloc protein surface hydrophobicity was measured using 1-anilino-8-naphthalenesulfonate (ANS) as a hydrophobic probe (Hayakawa & Nakai, 1985). For the five bioflocs type, a 1 mg mL^{-1} protein solution was prepared in 10 mM phosphate buffer at pH 7.0. These solutions were diluted to 0, 0.5, 0.25, and 0.125 mg mL^{-1}, and 10 µl of a 10 mM ANS solution in 0.1 M phosphate buffer at pH 7.0 was added to each dilution. The fluorescence intensity (FI) of the protein dilutions was measured using a Cary Eclipse Fluorescence Spectrophotometer recording data at the emission of 485 nm and excitation of 375. The FI readings were calibrated using an ANS standard (2 mL methanol added

directly to 10 µL of ANS), and the reading was adjusted to 80% of the full scale. The blank was FI in the sample without ANS. The slope of FI *vs* the protein concentration was determined using least-squares linear regression and was reported as the surface hydrophobicity (S_0).

Data analysis

The assumptions of normality and homogeneity of variance were verified. Then, a one-way analysis of variance (ANOVA) (Zar, 1996) was performed to compare the water conditions in biofloc production, and proximate composition of bioflocs (carbohydrates, lipids, proteins and ashes) produced with the different experimental diets. In cases where the statistic test were significant ($P < 0.05$) *a posteriori* multiple comparison Tukey's tests were applied. Reported data of particle characterization were based on the average from three determinations, the variation among replicates was <5%. Statistica for Windows 5.1, StatSoft Inc. ® software was used for data processing.

RESULTS

Water parameters during biofloc production

Global means of temperature (25.33 ± 0.13°C to 25.43 ± 0.10°C) and dissolved oxygen (5.78 ± 0.07 to 5.96 ± 0.25 mg L^{-1}). The pH interval varied from 8.42 to 8.47. The means of chlorophyll-*a* varied from 19.09 ± 0.88 to 23.32 ± 3.00 µg L^{-1}; the total ammonia nitrogen (TAN) varied from 0.40 ± 0.14 to 0.89 ± 0.16 mg L^{-1} and NO_2-N from 4.86 ± 0.23 to 6.31 ± 0.67 mg L^{-1}, in all cases no significant diffe-rences among treatments were determined ($P > 0.05$).

Characterization of bioflocs

Proximal composition

The biofloc content of protein varied from 360 to 400 g kg^{-1}, lipid from 6 to 8 g kg^{-1} and fiber from 5 to 9 g kg^{-1}. There were no significant differences among treatments ($P > 0.05$) (Table 1). The ashes varied from 205-284 g kg^{-1}, the lowest corresponded to T3 and the highest to T0, with significant differences among treatments ($P < 0.05$).

Molecular weight of biofloc protein

Some engineering properties of feed and presence of bioactive compounds has been attributable to molecular size (Rao *et al*., 2014). The protein molecular weights detected by SDS-PAGE, did not show differences in the banding pattern. Bioflocs produced with high content of fishmeal (300 g kg^{-1}) were similar to those produced with 0 g kg^{-1} (Fig. 1). Numerous fractions at approximately 200, 110, 66, 36 and 22 kDa were obtained from all bioflocs types.

Table 1. Proximate composition (mean ± SD) in g kg^{-1} of lyophilized biofloc produced in whiteleg shrimp culture with different fishmeal inclusion into the diets (0, 100, 200 and 300 g kg^{-1} of fishmeal). Data are average of triplicate determinations. Means with different script indicates significant difference ($P < 0.05$).

	Treatment (g kg^{-1})			
	0	100	200	300
Protein	364 ± 83	404 ± 55	389 ± 42	394 ± 35
Lipid	8 ± 5	6 ± 1	6 ± 1	7 ± 4
Ash	284 ± 31^{bc}	243 ± 11^{b}	231 ± 11^{a}	205 ± 20^{a}
Fiber	9 ± 5	7 ± 5	6 ± 2	5 ± 3

Morphology and particle size of lyophilized biofloc

The particle size analysis is important to determine their physical associations (Goldan *et al.*, 1997) and biotechnological applications. The morphological analysis of the biofloc was conducted to establish the particles size after lyophilization process. The bioflocs microstructure examined by SEM showed no significant difference between the treatments. The biofloc shapes were similar among treatments (Fig. 2).

In this study, particles sizes were similar in all treatments (Fig. 3). The most common biofloc particle size varied from 3 to 15 µm (>50%). The maximum particle size was of 800 µm, but the sizes from 80 to 800 were less than 10% of the total particles.

Protein surface hydrophobicity

The fluorescence spectra slopes indicate the S_0 differences between the treatments (Fig. 4). The treatment with 300 g kg^{-1} of fishmeal inclusion had the S_0 highest value, while the rest of the biofloc samples were similar.

DISCUSSION

Parameters during biofloc production

Temperature and dissolved oxygen were into the interval to the white leg shrimp culture (Martínez-Córdova, 1999). The pH values were similar from 7.0 to 9.0 previously reported (Esparza-Leal *et al*., 2010) for shrimp culture in low salinity and from 7.9 to 8.1 reported for shrimp culture in biofloc and low salinity (Maicá *et al*., 2012). The chlorophyll-*a* concentrations were similar to the reported 61.3 µg L^{-1} at salinity of 4 g L^{-1} (Maicá *et al*., 2012), the low chlorophyll-*a* values are consistent with the heterotopic biofloc type since the bacteria dominate over the microalgae.

At certain C:N ratio, heterotrophic bacteria immobilize inorganic nitrogen, converting it into microbial

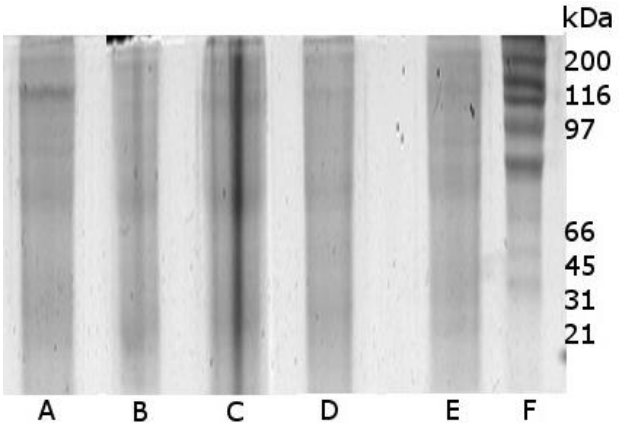

Figure 1. Electrophoretic analysis of the lyophilized bioflocs. Lines: A) treatment with 0 g kg^{-1} of fishmeal inclusion, B) treatment with 100 g kg^{-1} of fishmeal inclusion, C) treatment with 200 g kg^{-1} of fishmeal inclusion, D) treatment with 300 g kg^{-1} of fishmeal inclusion, E) control (commercial diet with 300 g kg^{-1} of fishmeal), and F: molecular weight markers.

biomass with the subsequent formation of bioflocs (Yuniasari & Ekasari, 2010; Martínez-Córdova *et al*., 2014). In this study it was shown that the low CN ratio (12:1) combined with low illumination allowed the development of heterotrophic biofloc. The heterotrophic and nitrifying bacteria were able to maintain the nitrogenous compounds in low levels, which were similar to other biofloc studies (Megahed, 2010; Xu *et al*., 2013).

Proximal determination

The proximate composition of biofloc may vary according to several factors such as cultured specie, salinity, aeration intensity, dissolved oxygen, carbon source, temperature, as well as microbial community developed (heterotrophic, mixotrophic or autotrophic) (Decamp *et al*., 2007; De Schryver *et al*., 2008; Martínez-Córdova *et al*., 2014). In this study, the lack of significant differences among treatments, indicates the biofloc nutritional quality was independent of fishmeal level present in the diet. It was remarkable that the microbial community was able to use the unconsumed food, feces as well as dead animals to produce live biomass with similar nutritional quality, independently of the supplied diet.

Molecular weight distribution

The bands of protein molecular weights (200, 110, 66, 36 and 22 kDa) obtained from bioflocs could be related with micromolecules and macromolecules present in microorganisms as well as in shrimp feed (Wingender *et al*., 1999).

The microorganisms produced some of extracellular polymeric substances with chemical characteristics related to formation and properties of microbial aggregates (floc) (More *et al*., 2014). These substances, are attributed to microorganisms in natural environments and occurs in prokaryotic as well as in eukaryotic microorganisms (Wingender *et al*., 1999; Flemming & Wingender, 2001).

The formation of flocs is related to the size of extracellular polymeric substances, high molecular weight bands involves higher flocculating activity (More *et al*., 2014). The important engineering properties (based on structural characteristics) such as adsorption, biodegradability, hydrophilicity/hydrophobicity of extracellular polymeric substances has been attributable (More *et al*., 2014).

In biofloc system, the mix of food, extracellular polymeric substances, and feces, can be associated with properties of cell surface. These properties are connected with presence of extracellular carbohydrates, binding proteins, structural proteins or non-enzymatic proteins and constitute a link between bacterial surface and extracellular surface (Flemming & Wingender, 2010).

Numerous fractions at approximately 200 to 66 kDa can be considered as particles of medium and high molecular weight. The particles around of 200 kDa can come from microorganism's membrane (More *et al.*, 2014), the units of molecular weight around 110 and 66 kDa are related with sub units of αβ-heterodimers of some nitrobacterium (Spieck, 1996). The fractions from 21 to 36 kDa were grouped like tropomyosin, myosin related with muscular protein (Mignino *et al*., 2008), probably from feed and shrimp carcass. It is also necessary to consider, that the presence of small particles are related with process of freeze-dried (Spieck, 1996).

Morphology and particle size

The particle size varied from 3 to 800 μm, it is possible that the collection and process of bioflocs did not permit to detect small bacteria which common diameter is less than 1 μm (Robertson & Button, 1989). The most abundant particles observed were consistent with the ciliates and flagellates sizes, which can varied from 2 to 10 μm (Gonzalez *et al*., 1990), and have reported as common microorganism in biofloc (Decamp *et al*., 2007; Monroy-Dosta *et al*., 2013; Martínez-Córdova *et al*., 2014).

Otherwise, the differences between particles sizes are mostly attributed to the nature of the aggregates, like organic macromolecules that are formed by polymerization of similar or identical building block; many polysaccharides may contain no-polymeric subs-

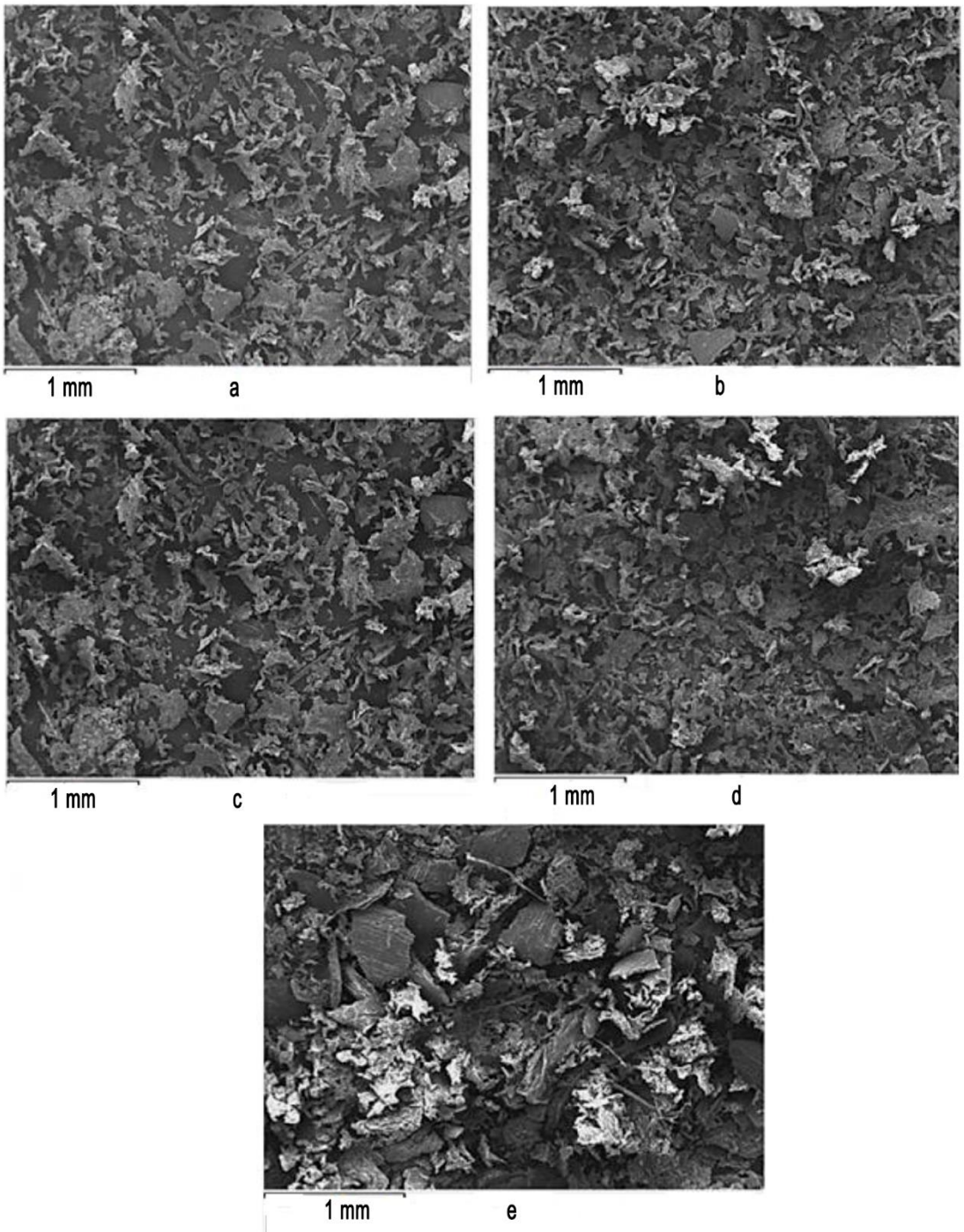

Figure 2. Scanning electron microscope (SEM) images of lyophilized bioflocs samples. a) Treatment with 0 g kg^{-1} of fishmeal inclusion, b) treatment with 100 g kg^{-1} of fishmeal inclusion, c) treatment with 200 g kg^{-1} of fishmeal inclusion, d) treatment with 300 g kg^{-1} of fishmeal inclusion, e) control (commercial diet). Resolution 1 mm.

titutions such acetyl, succinyl or pyruvyl groups or inorganic substituents such as sulfate; proteins can be glycosylated with oligosaccharides to form glycoproteins or can be substituted with fatty acids to form lipoproteins (Wingender *et al*., 1999; Bhaskar & Bhosle, 2005).

The micrographics of biofloc had similar pattern of extracellular polymeric substances.

Their composition may be the result of different processes: active secretion, shedding of cell surface material, cell lysis, and adsorption from the environment (Wingender *et al*., 1999). Therefore, the particle

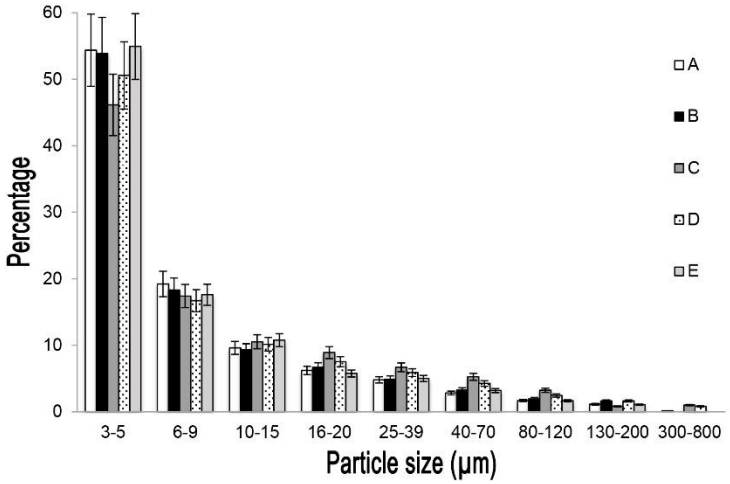

Figure 3. Particle size distribution of the lyophilized bioflocs samples. A) Treatment with 0 g kg^{-1} of fishmeal inclusion, B) treatment with 100 g kg^{-1} of fishmeal inclusion, C) treatment with 200 g kg^{-1} of fishmeal inclusion, D) treatment with 300 g kg^{-1} of fishmeal inclusion, E) control (commercial diet with 300 g kg^{-1} of fishmeal).

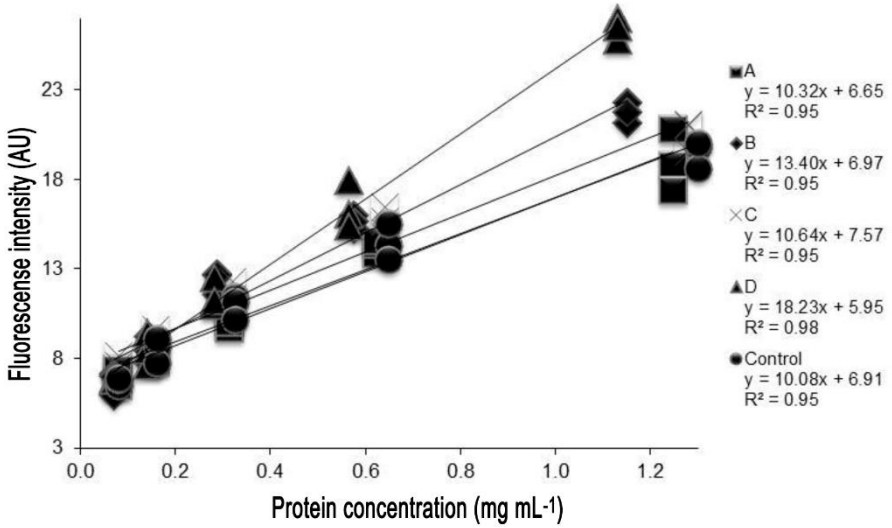

Figure 4. Biofloc samples fluorescence intensity. A) 0 g kg^{-1} of fishmeal inclusion (square), B) 100 g kg^{-1} of fishmeal inclusion (rhombus), C) 200 g kg^{-1} of fishmeal inclusion (cross), D) 300 g kg^{-1} of fishmeal inclusion (triangle), and control: commercial diet (circle).

size variations might be attributed to processes like shrimp digestion, molting and activity of bacteria.

Protein surface hydrophobicity

Surface hydrophobicity (S_0) indicates the character hydrophobic groups on the protein surface that contact the polar aqueous environment (Arias-Moscoso *et al*., 2015). In this study, the fluorescence spectra slopes were different among treatments. This phenomenon could be related to bacterial surface charge (More *et al*., 2014; Muda *et al*., 2014). The surface hydrophobicity depends on the bacterial cell exoproteins net surface charge, which ultimately depends on the type of protein in the cell wall, which could be affected by pH in the aquatic environment (Wang *et al*., 2006).

Other factor determining the charge of the cell surface is the ratio of carbohydrates to protein of the mix in biofloc (feed, extracellular polymeric substances and feces) (Shin *et al*., 2001). This mix has many charged groups (carboxyl, phosphoric, sulfhydryl, and phenolic and hydroxyl groups) and polar groups (aromatic, aliphatic in proteins, and hydrophobic regions in carbohydrates) (Flemming & Leis, 2003). Also can be attributed to the hydrophobic amino acid levels, in the

same way the hydrophobic amino acid levels are related to the level of bitterness (Mahn *et al*., 2009).

The importance of hydrophobic contacts in protein folding and unfolding is related by the protein-protein interactions (Chanphai *et al*., 2015). References of several studies on food protein structure function relationships have emphasized the importance of protein hydrophobicity for protein functional properties after different treatments and/or processes (Mignino *et al*., 2008).

CONCLUSIONS

We concluded that the biofloc nutritional quality was independent of fishmeal included in the diet. The microbial community was able to use the unconsumed food, and feces, as well as dead animals to produce live biomass with similar nutritional quality.

This characterization enhances the understanding of the molecular, and consequently, physical and chemical properties of these bioflocs to establish their proper use. The application of these results could have important benefits to reduce the dependence of fishmeal in the culture of whiteleg shrimp in biofloc systems with low salinity and at the same time promoting the reuse of the suspended solids as a high quality source of protein. Further studies are necessary to improve the bioflocs processing and their possible applications.

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