Nutritional characterization of biofloc developed with water from the Ecuadorian Pacific for growing *Litopenaeus vannamei*

Caracterización nutricional del biofloc desarrollado con agua del Pacífico ecuatoriano para el cultivo de *Litopenaeus vannamei*

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The objective of this study was to determine the bromatological and amino acid composition of biofloc generated in the macrocosm tank for growing *Litopenaeus vannamei* with water from the Ecuadorian Pacific. *Thalassisira sp*. microalgae was inoculated to form the macrocosm (1.4 x 10^6 mL/L) and 40 shrimp/m^2 with an average weight of 12.7 ± 1.2 g were placed, as well as commercial food with 35 % protein. To guarantee bacterial growth, a C:N ratio of 20:1 was maintained through the contribution of sugar cane molasses and nitrogen from the feed. The biofloc showed high protein levels (36.53 %), low lipid contribution (0.97 %) and high ash values (33.39 %). There was presence of all amino acids, the highest levels were found for leucine, arginine, valine, threonine and phenylalanine. In contrast, tryptophan was the one with the lowest concentration and the lowest chemical score (0.56), resulting in the first limiting amino acid. Likewise, the isoleucine (0.70) and lysine (0.80) scores indicated that they were limiting amino acids of the biofloc that was produced. The index of biofloc essential amino acids was 0.89, which classifies as useful protein material. This study allows to conclude that the biofloc generated with Ecuadorian Pacific water presented an adequate protein quality, in terms of its amino acid composition, chemical score and essential amino acid index, so it can serve as a supplement to the balanced food for growing shrimp with biofloc technology.

Keywords: *flocs, white shrimp, amino acids, bromatological composition*

El trabajo tuvo como objetivo determinar la composición bromatológica y aminoacídica del biofloc generado en el tanque macrocosmos para el cultivo de *Litopenaeus vannamei* con agua del Pacífico ecuatoriano. Para la conformación del macrocosmos se inoculó la microalga *Thalassisira sp*. (1.4 x 10^6 mL/L) y se colocaron 40 camarones/m^2 con peso promedio de 12.7 ± 1.2 g y alimento comercial con 35 % de proteína. Para garantizar el crecimiento bacteriano se mantuvo una relación C: N de 20:1 mediante el aporte de melaza de caña y el nitrógeno procedente del pienso. El biofloc que se generó presentó altos niveles de proteína (36.53 %), bajo aporte en lípidos (0.97 %) y altos valores de cenizas (33.39 %). Hubo presencia de todos los aminoácidos, los mayores tenores se encontraron para la leucina, arginina, valina, treonina y fenilalanina. Por el contrario del triptófano que fue el que presentó la menor concentración y el menor score químico (0.56) resultando el primer aminoácido limitante. De igual forma, los scores de la isoleucina (0.70) y la lisina (0.80) indicaron que fueron aminoácidos limitantes del biofloc que se produjo. El índice de aminoácidos esenciales del biofloc fue de 0.89, lo que clasifica como material proteico útil. Este estudio permite concluir que el biofloc que se generó con agua del Pacífico ecuatoriano presentó una adecuada calidad proteica, en cuanto a su composición aminoacídica, score químico e índice de aminoácidos esenciales de ahí, que puede servir de suplemento al alimento balanceado para el cultivo de camarón con tecnología biofloc.

Palabras clave: *flóculos, camarón blanco, aminoácidos, composición bromatológica*

There are two fundamental premises for the nutrition of water organisms: protein levels and their quality. This last is determined by the composition and availability of amino acids, as well as the level of digestive utilization made by the animal (NRC 2011). In addition, it is the main factor that affects growth, water quality and diet costs during shrimp production (Tacon *et al.* 2013 and Ponce-Palafox *et al.* 2017).

The application of biofloc technology (BFT) provides flocs composed by bacteria, microalgae, protozoa and other organisms, which together with detritus and dead organic matter can increase the availability of food for shrimp, 24 hours a day (Avnimelech *et al.* 2009, Khun *et al.* 2010 and Emerenciano *et al.* 2012). However, there are factors such as the contribution in protein of the concentrated food, biofloc size, associated microorganisms, fertilizer, type of bioreactor and others,

Existen dos premisas fundamentales para la nutrición de organismos acuáticos: los niveles de proteína y su calidad. Esta se determina por la composición y disponibilidad de los aminoácidos, así como el nivel de utilización digestiva que el animal realice (NRC 2011). Además, es el factor principal que influye en el crecimiento, en la calidad del agua y en los costos de las dietas durante la producción de camarón (Tacon *et al.* 2013 y Ponce-Palafox *et al.* 2017).

La aplicación de la tecnología biofloc (TBF) proporciona flóculos formados por bacterias, microalgas, protozoos y otros organismos, que unidos con detritus y materia orgánica muerta pueden incrementar la disponibilidad de alimento para el camarón las 24 h (Avnimelech y Kochba2009, Khun *et al.* 2010 y Emerenciano *et al.* 2012). No obstante, hay factores como el aporte en proteína del alimento concentrado, el which influence on the quality and quantity of generated flocs (Ju *et al.* 2008, Kuhn *et al.* 2010 and Ekasari *et al.* 2014).

Therefore, the objective of this study was to determine the bromatological and amino acid composition of the biofloc of the macrocosm tank, which was generated with water from the Ecuadorian Pacific, for growing *L. vannamei* in an intensive system.

Materials and Methods

In this study, a circular fiberglass tank with a capacity of 3000 L was used for the macrocosm. It had an air diffuser in the center to guarantee oxygenation, continuous movement of water and resuspension of particles.

The *Thalassisira sp.* microalgae (1.4 x 10^6 mL/L) was inoculated to form the macrocosm, and 40 shrimp/m^2 with an average weight of 12.7 ± 1.2 g were placed. A commercial 35% protein food (Alimentasa) was daily provided. To guarantee bacterial growth, a C: N ratio of 20:1 was maintained through the contribution of sugar cane molasses and nitrogen from feed, as recommended by Avnimelech (2012).

Bromatological composition of biofloc. Dry matter (DM), crude protein (CP), ether extract (EE) and ashes were determined to the biofloc contained in the macrocosm tank, according to AOAC (2016). Three macrocosm tanks were formed and a water sample (10 L) was taken from each tank for filtration (n = 3). Dried and ground samples were stored at -20 °C its analysis.

Amino acid profile. Samples were analyzed by triplicate to determine the amino acid profile by high performance liquid chromatography (HPLC) with sodium ion exchange column and post-column derivatization with ninhydrin in a visible-ultraviolet detector, according to the 994.12 regulation (AOAC 2016). For the quantification of the amino acids, samples were hydrolyzed with 6N hydrochloric acid, for 22 h at 110 °C, according to the method described by Moore and Stein (1963). For tryptophan, samples were hydrolyzed with 4N lithium hydroxide according to methodology described by Lucas and Sotelo (1980).

Chemical score and essential amino acid index (EAAI). The chemical score was determined as the relation of the value of each essential amino acid (EAA) of the experimental food (biofloc) with the corresponding amino acid value of a pattern protein as described by Peñaflorida (1989). The recommended essential amino acid requirements for shrimp (*L. vannamei* and *P. monodon*) were used as the standard protein (Xie *et al.* 2012, Zhou *et al.* 2013 and Lin *et al.* 2015).

The essential amino acid index (EAAI) evaluates the degree of EAA of the entire sample in relation to the composition or requirements of the EAA of the animal (Ju *et al.* 2008). The formula proposed by Peñaflorida (1989) was used to calculate the EAAI:

tamaño del biofloc, los microorganismos asociados, el fertilizante, tipo de bioreactor y otros, que influyen en la calidad y cantidad de flóculos que se generan (Ju *et al.* 2008, Kuhn *et al.* 2010 y Ekasari *et al.* 2014).

Por lo tanto, el objetivo de este trabajo consistió en determinar la composición bromatológica y aminoacídica del biofloc del tanque macrocosmos, que se generó con agua del Pacífico ecuatoriano para la alimentación de *L. vannamei* en sistema intensivo.

Materiales y Métodos

En este estudio se utilizó un tanque de fibra de vidrio circular de 3000 L de capacidad para el macrocosmos. Este contó con un difusor de aire en el centro para garantizar la oxigenación, el movimiento continuo del agua y la resuspensión de partículas.

Para la conformación del macrocosmos se inoculó la microalga *Thalassisira sp.* (1.4 x 10^6 mL/L) y se colocaron 40 camarones/m2 con peso promedio de 12.7 ± 1.2 g. Diariamente se suministró un alimento comercial de 35 % de proteína (Alimentasa). Para garantizar el crecimiento bacteriano se mantuvo una relación C: N de 20:1 mediante el aporte de melaza de caña y el nitrógeno procedente del pienso según lo recomendado por Avnimelech (2012).

Composición bromatológica del biofloc. Se determinó materia seca (MS), proteína bruta (PB), extracto etéreo (EE) y cenizas al biofloc contenido en el tanque macrocosmos según AOAC (2016). Se conformaron tres tanques macrocosmos y se tomó una muestra de agua (10 L) de cada tanque para su filtración (n=3). Las muestras secas y molidas se almacenaron a -20 °C para su análisis.

Perfil de aminoácidos. Las muestras por triplicado se analizaron para determinar el perfil de aminoácidos por cromatografía líquida de alta resolución (HPLC) con columna de intercambio iónico de sodio y derivatización post-columna con ninhidrina en un detector ultravioleta-visible según la norma 994.12 (AOAC 2016). Para la cuantificación de los aminoácidos, las muestras se hidrolizaron con ácido clorhídrico 6N, por 22 h a 110 °C según el método descrito por Moore y Stein (1963). Para el triptófano, las muestras se hidrolizaron con hidróxido de litio 4N según metodología descrita por Lucas y Sotelo (1980).

"Score" químico e índice de aminoácidos esenciales (EAAIE). El "score" químico se determinó como la relación del valor de cada aminoácido esencial (AAE) del alimento experimental (biofloc) con el valor del aminoácido correspondiente de una proteína patrón según lo descrito por Peñaflorida (1989). Se utilizó, como proteína patrón, los requerimientos de aminoácidos esenciales recomendados para el camarón (*L. vannamei* y *P. monodon*) (Xie *et al.* 2012, Zhou *et al.* 2013 y Lin *et al.* 2015).

El índice de aminoácido esencial (EAAI) evalúa el grado de AAE de la muestra entera con relación a la composición o requerimientos de AAE del animal (Ju *et al.* 2008). Para calcular el EAAI se empleó la fórmula propuesta por Peñaflorida (1989):

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} X \frac{aa_2}{AA_2} X \dots X \frac{aa_n}{AA_n}}$$

Where: aa_1 = content of each amino acid in the food; AA_1 = requirement of each amino acid of shrimp, n = amount of determined essential amino acids.

Results and Discussion

Bromatological composition of biofloc. The biofloc developed in the macrocosm showed high protein levels (table 1). These values were comparable to those found by Wasielesky *et al.* (2006) and Li *et al.* (2018) although lower than 38.41 % of CP, reported by Luo *et al.* (2014) and 43 % of CP found by Maicá *et al.* (2011). Meanwhile, lipid contribution was low when compared with 2.6 % found by Tacon *et al.* (2002) and 7.5% reported by Ekasari *et al.* (2014).

Table 1. Proximal composition of the biofloc of the macrocosm tank with white shrimp culture (g/100g DM, n = 3)

Indicator	Mean	DS	VC (%)
Dry matter	97.46	0.74	0.76
Crude protein	36.53	1.75	4.79
Ether extract	0.97	1.14	14.18
Ashes	33.39	2.60	7.79

According to Azim and Little (2008) and Hargreaves (2006), dry weight of protein content can vary from 25 to 50 %, although the most common is between 30 and 45 %. Fat content varies from 0.5 to 10 % and the most common range is between 1.0 to 5 %. These authors also agreed that biofloc is a good source of vitamins, minerals and has a probiotic effect.

Reports on CP levels (about 33%) in microbial flocs by Emerenciano *et al.* (2011) and Suita *et al.* (2015) were lower than those found in this study, which could be associated with the composition of the microorganism communities present in the biofloc, that are more productive in the waters of the Pacific Ocean (Maridueña 2004). On the other hand, the lipid contents were slightly higher compared to those reported by Wasielesky *et al.* (2006) and Emerenciano *et al.* (2011), with 0.47 and 0.49 %, respectively, and similar to those of Suita *et al.* (2015), of 1.6 %. These authors attribute these lipid levels to the presence of ciliates and microalgae, specifically diatoms that are a source of essential fatty acids and lipids.

In addition, Schneider *et al.* (2005) pointed out that the intake of bioflocs can increase food utilization efficiency by recovering a fraction of the excreted nutrient and retaining nitrogen from aggregate foods between 7 and 13 %. In this sense, Xu *et al.* (2012) confirmed the contribution of bioflocs in the protein

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} X \frac{aa_2}{AA_2} X \dots X \frac{aa_n}{AA_n}}$$

Donde: aa_1 = contenido de cada aminoácido en el alimento; AA_1= requerimiento de cada aminoácido del camarón, n= cantidad de aminoácidos esenciales determinados.

Resultados y Discusión

El biofloc que se desarrolló en el macrocosmos presentó altos niveles de proteína (tabla 1). Valores comparables con los encontrados por Wasielesky *et al.* (2006) y Li *et al.* (2018) aunque inferiores a 38.41 % PB informado por Luo *et al.* (2014) y 43 % PB, hallado por Maicá *et al.* (2011). En tanto, el aporte en lípidos fue bajo al comparar con 2.6 % encontrado por Tacon *et al.* (2002) y 7.5 % informado por Ekasari *et al.* (2014).

Según Azim y Little (2008) y Hargreaves (2006) el peso seco del contenido de proteína puede variar de 25 a 50 %, aunque lo más común es entre 30 y 45 %. El contenido de grasas varía de 0.5 a 10 % y el rango más común se encuentra entre 1.0 a 5 %. Estos autores también coincidieron que el biofloc es una buena fuente de vitaminas, minerales y tiene efecto probiótico.

Reportes sobre niveles de PB (alrededor de 33 %) en flóculos microbianos por Emerenciano *et al.* (2011) y Suita *et al.* (2015) fueron inferiores a los encontrados en este estudio, lo que se pudiera asociar a la composición de las comunidades de microorganismos presentes en el biofloc, que en las aguas del océano Pacífico son de mayor productividad (Maridueña 2004). Por otra parte, los contenidos de lípidos fueron ligeramente superiores en comparación con los reportados por Wasielesky *et al.* (2006) y Emerenciano *et al.* (2011) (0.47 y 0.49 %, respectivamente) y similares a los de Suita *et al.* (2015) de 1.6 %. Estos autores atribuyen estos niveles de lípidos a la presencia de ciliados y microalgas, específicamente diatomeas que son fuente de lípidos y ácidos grasos esenciales.

Además, Schneider *et al.* (2005) señalaron que el consumo de bioflocs puede aumentar la eficiencia de utilización de los alimentos mediante la recuperación de una fracción del nutriente excretado y la retención de nitrógeno de los alimentos agregados entre un 7 y nutrition of *L. vannamei* in culture tanks with BFT. These authors concluded that the formation and development of bioflocs can have an important function as in situ supplementary food source and in the improvement of food utilization, protein digestion and retention by the shrimp, contributing even more to improve growth.

The ash content of this study (table 1) reached high values (33.39%) and are similar to other studies, where ash content varied from 7 to 32 % of DM (Ju *et al.* 2008, Ekasari *et al.* 2010 and Xu *et al.* 2012). In this regard, Tacon *et al.* (2002) stated that the high ash content in bioflocs is probably related to the presence of acid soluble oxides and mixed silicates. In addition, these authors stated that they are a good source of essential minerals and trace elements.

Maia *et al.* (2016) reported that, in commercial ponds with TBF, the use of molasses influenced on water quality and on the natural food source (biofloc), with good nutritional composition for shrimp. At the same time, it should be mentioned that sugar cane molasses used for fertilizing favored the increase of ashes since it is rich in this indicator (Valdivié *et al.* 2012).

Li *et al.* (2018) reported that the choice of carbon source is of paramount importance when using biofloc as a food source to reach optimal nutritional indicators in shrimp farming. In this sense, Kuhn *et al.* (2010) stated that molasses is the most used carbon source to produce biofloc. This is because it is a source of simple carbohydrates, easily assimilated by microorganisms that develop in the biofloc and can promote the growth of culture species (Martínez-Córdova *et al.* 2016). In addition, it is a source of easy acquisition and low costs (Moss *et al.* 2010).

Amino acid profile. The amino acid composition of biofloc that occurred in the macrocosms is presented in table 2. It can be seen that the 12 limiting amino acids were present and at levels according to the literature (Ekasari *et al.* 2010 and Khun *et al.* 2016). Similarly, the concentration of amino acids found corresponds to the shrimp requirements (Xie *et al.* 2012 and Lin *et al.* 2015). This suggests that this natural food circulating in the culture medium may be a good supplementary source of EAA for white shrimp (Sabry-Neto *et al.* 2015).

Out of the EAA, the highest levels were found for leucine, arginine, valine, threonine and phenylalanine, in contrast to tryptophan, which was the one with the lowest level. These results generally coincide with other studies that analyzed the EAA present in biofloc (Ju *et al.* 2008).

It is important to state that one of the main reasons that reduce the growth of water organisms in studies where fish meal is partially or totally replaced by alternative protein sources is attributed to an inadequate balance of EAA that lead to a decreased of protein synthesis,

13 %. En este sentido, Xu *et al.* (2012) confirmaron la contribución de los bioflocs en la nutrición proteica de *L. vannamei* en tanques de cultivo con TBF. Concluyeron que la formación y el desarrollo de bioflocs puede desempeñar un roll importante como una fuente de alimento suplementario in situ y en la mejora de la utilización del alimento, la digestión de proteínas y la retención por el camarón, contribuyendo aún más en mejorar el crecimiento.

El contenido de cenizas en este estudio (tabla 1) alcanzó valores altos (33.39 %) y se corresponden con otros estudios, donde el contenido de cenizas varió de 7 a 32 % de MS (Ju *et al.* 2008, Ekasari *et al.* 2010 y Xu *et al.* 2012). Al respecto, Tacon *et al.* (2002) plantearon que el alto contenido de cenizas en los bioflocs probablemente se relaciona con la presencia de óxidos solubles en ácido y silicatos mixtos. Además, plantearon que son una buena fuente de minerales esenciales y elementos traza.

Maia *et al.* (2016) informaron que en estanques comerciales con TBF el uso de melaza influyó en la calidad del agua y en la fuente natural de alimento (biofloc) con buena composición nutricional para el camarón. A su vez podemos atribuir que la melaza de caña que se utilizó para fertilizar favoreció el incremento de cenizas ya que es rica en esta (Valdivié *et al.* 2012).

Li *et al.* (2018) refieren que la elección de la fuente de carbono es de importancia primordial al emplear el biofloc como fuente de alimento para alcanzar indicadores nutricionales óptimos en el cultivo de camarón. En este sentido, Kuhn *et al.* (2010) plantearon que la melaza es la fuente de carbono más utilizada para producir biofloc. Esto se debe a que es una fuente de carbohidratos simples, de fácil asimilación por los microorganismos que se desarrollan en el biofloc y puede promover el crecimiento de especies de cultivo (Martínez-Córdova *et al.* 2016). Además, es una fuente de fácil adquisición y bajos costos (Moss *et al.* 2010).

Perfil de aminoácidos. La composición en aminoácidos del biofloc que se produjo en los macrocosmos se presenta en la tabla 2. Se puede observar que estuvieron presente los 12 aminoácidos limitantes y en niveles acorde a la literatura (Ekasari *et al.* 2010 y Khun *et al.* 2016). De igual forma, la concentración de aminoácidos encontrados se corresponde con los requerimientos del camarón (Xie *et al.* 2012, Lin *et al.* 2015). Lo que sugiere que este alimento natural circulante en el medio de cultivo puede ser buena fuente suplementaria de AAE para el camarón blanco (Sabry-Neto *et al.* 2015).

De los AAE, los mayores tenores se encontraron para la leucina, arginina, valina, treonina y fenilalanina, por el contrario del triptófano que fue el que presentó menor nivel. Estos resultados de forma general coinciden con otros estudios que analizaron los AAE presentes en biofloc (Ju *et al.* 2008).

Es importante anotar, que una de las principales razones que reducen el crecimiento de organismos acuáticos en estudios donde se sustituye parcial o

Table 2. Amino acid composition of biofloc in the macrocosm tank for growing white shrimp (%, n=3) with waters from the Ecuadorian Pacific

Amino acids	Mean	SD
Essential amino acids		
Lysine	1.31	0.10
Methionine	0.69	0.15
Arginine	1.89	0.21
Leucine	2.17	0.57
Valine	1.68	0.39
Threonine	1.6	0.02
Histidine	0.86	0.22
Isoleucine	1.22	0.38
Phenylalanine	1.45	0.18
Tryptophan	0.28	0.12
Non essential amino acids		
Glutamic acid	3.08	0.18
Aspartic acid	2.61	0.52
Alanine	2.30	0.37
Serine	1.45	0.29
Proline	1.58	0.10
Glycine	2.01	0.24
Tyrosine	1.20	0.07
Cystine	0.52	0.17

essential for animal growth (Toledo *et al.* 2015). Therefore, the biofloc that is produced in the culture medium should not affect the productive performance of shrimp when the protein levels in the balanced foods are reduced, given the contribution of essential amino acids that it presents.

Ju *et al.* (2008) reported that the flocs have a considerable amount of non-amino acid nitrogen as ammonium salts, but also possibly urea, nucleotides and amino sugar, which may arise from the metabolic activity of microorganisms and shrimp. These same authors compared the EAA profile of three biofloc samples, two dominated by microalgae (chlorophytes and diatoms) and the other by bacteria versus the EAA profile of two balanced shrimp feeds, and reported that they were similar. This indicates that protein quality of the organisms present in the biofloc was high.

Chemical score and essential amino acid index (EAAI). Table 3 shows the chemical score and the EAAI of amino acids present in the biofloc. The chemical score of tryptophan was the lowest (0.56), resulting in the first limiting amino acid, which means that biofloc can only meet approximately half of the shrimp requirement for this amino acid. Similarly, the isoleucine (0.70) and lysine (0.80) scores were found to be second and third limiting amino acids in the macrocosm biofloc sample.

Some factors influence on the quality of protein

totalmente la harina de pescado por fuentes alternativas de proteína se atribuyen a un inadecuado equilibrio de AAE que conducen a la disminución de la síntesis de proteína, esencial para el crecimiento del animal (Toledo *et al.* 2015). Por lo tanto, el biofloc que se produce en el medio de cultivo no debe afectar el desempeño productivo del camarón cuando se disminuyen los niveles de proteína en los alimentos balanceados dado al aporte de aminoácidos esenciales que presenta.

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Table 3. Chemical score and essential amino acid index (EAAI) of the biofloc generated in the macrocosm tank with water from the Ecuadorian Pacific.

EAA	Biofloc	Shrimp requirements	Chemical score[1]
Lysine	1.31	1.64	0.80
Methionine	0.69	0.66	1.00
Arginine	1.89	1.96	0.96
Leucine	2.17	2.37	0.91
Valine	1.68	1.40	1.00
Threonine	1.6	1.18	1.00
Histidine	0.86	0.80	1.00
Isoleucine	1.22	1.73	0.70
Phenylalanine	1.45	1.40	1.00
Tryptophan	0.28	0.50	0.56
EAAI	-	-	0.89

[1]EAA of the sample / EAA of shrimp requirements. An amount of 1.00 was taken as maximum

sources, such as amino acid profile (EAA score) and digestibility (Toledo *et al.* 2015). The chemical score of EAA evaluates the level of each EAA individually and the value 1.00 indicates that the level of the AEA, particularly within the dietary protein, is identical to the requirement of that essential amino acid of the animal. The lowest value, indicates the first limiting amino acid. On the other hand, the essential amino acid index (EAAI) evaluates the degree of EAA of the entire sample in relation to the composition or requirements of EAA of the animal (Montoya-Martínez *et al.* 2016).

The EAAI found in this study was 0.89, which classifies as useful protein material according to Castell and Tiews (1980). This EAAI was equal to the biofloc preferably of bacteria reported by Ju *et al.* (2008) and higher than that shown by soy meal (0.87) in *Penaeus monodon*, according to Peñaflorida (1989). However, they were inferior to bioflocs dominated by microalgae (0.91 and 0.92) (Ju *et al.* 2008). These values suggest that the experimental biofloc can provide a high quality protein source for shrimp growth and may supplement a balanced food.

Ju *et al.* (2008) stated that an EAAI close or equal to 1.0 indicates that the diet contains an amino acid profile similar to that found throughout the body of the shrimp and it is assumed to be equivalent to its diet requirements. They also refer that, in general, ingredients or feedstuffs with an EAAI of more than 0.90 are considered to be foods of good protein quality or protein materials, with an EAAI of 0.80 are useful, and below 0.70 are inadequate, according to the classification described by Oser (1959).

However, the ability of a food to meet the amino acid needs of an animal depends on the intake rate, bioavailability (digestibility) and its composition

limiting amino acids in the macrocosm biofloc sample.

Some factors influence on the quality of protein sources, such as amino acid profile (EAA score) and digestibility (Toledo *et al.* 2015). The chemical score of EAA evaluates the level of each EAA individually and the value 1.00 indicates that the level of the AEA, particularly within the dietary protein, is identical to the requirement of that essential amino acid of the animal. The lowest value, indicates the first limiting amino acid. On the other hand, the essential amino acid index (EAAI) evaluates the degree of EAA of the entire sample in relation to the composition or requirements of EAA of the animal (Montoya-Martínez *et al.* 2016).

El EAAI que se halló en este estudio fue de 0.89, lo que clasifica como material proteico útil según Castell & Tiews (1980). Este EAAI fue igual al biofloc preferentemente de bacterias reportado por Ju *et al.* (2008) y superiores al que exhibió la harina de soya (0.87) en *Penaeus monodon* según Peñaflorida (1989). No obstante, fueron inferiores a bioflocs dominados por microalgas (0.91 y 0.92) (Ju *et al.* 2008). Estos valores sugieren que el biofloc experimental puede proporcionar una fuente de proteína de alta calidad para el crecimiento del camarón y puede suplementar un alimento balanceado.

Ju *et al.* (2008) plantearon que un EAAI cercano o igual a 1.0 indica que la dieta contiene un perfil aminoácidos similar al que se encuentra en todo el cuerpo del camarón y se asume que es equivalente a los requisitos dietéticos de este. Refieren, además, que en general los ingredientes o los piensos con un EAAI de más de 0.90 se consideran alimentos de buena calidad proteica o materiales proteicos, con un EAAI de 0.80 son útiles, y por debajo de 0.70 son inadecuados, según la clasificación descrita por Oser (1959).

No obstante, la capacidad de un alimento de satisfacer las necesidades de aminoácidos de un animal depende de la

(Toledo *et al.* 2015). In this regard, Forster and Dominy (2005) report that foods with a low EAA score will require their addition, either by other sources of proteins or synthetic amino acid supplements, to obtain an animal yield comparable to that of a diet of high quality. Biofloc, however, arises naturally from the by-products of shrimp farming (metabolic residues, feces and nutrients lixiviated from feed, etc.), providing a renewable source of nutrients for shrimps at no additional cost to fish farmers.

Conclusions

Biofloc generated with water from the Ecuadorian Pacific presented an adequate protein quality, in terms of its amino acid composition, chemical score and index of essential amino acids. Hence, it can be used as a supplement to the balanced feed for shrimp culture with biofloc technology.

tasa de consumo, la biodisponibilidad (digestibilidad) y su composición (Toledo *et al.* 2015). En tal sentido, Forster y Dominy (2005) refieren que los alimentos con un puntaje bajo de AAE requerirán la adición de estos, ya sea por otras fuentes de proteínas o suplementos de aminoácidos sintéticos, para obtener un rendimiento animal comparable al de una dieta de alta calidad. El biofloc, sin embargo, surge naturalmente de los subproductos del cultivo de camarón (residuos metabólicos, heces y nutrientes lixiviados de los piensos, etc.), proporcionando una fuente renovable de nutrientes para los camarones sin costo adicional para los acuicultores.

Conclusiones

El biofloc que se generó con agua del Pacífico ecuatoriano presentó una adecuada calidad proteica, en cuanto a su composición aminoacídica, score químico e índice de aminoácidos esenciales, de ahí que puede servir de suplemento al alimento balanceado para el cultivo de camarón con tecnología biofloc.

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