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STUDY ABOUT THE USE OF AQUACULTURE BINDER WITH TUNA ATTRACTANT IN THE FEEDING OF WHITE SHRIMP (*Litopenaeus vannamei*)

ESTUDIO SOBRE EL USO DE LIGANTE ACUÍCOLA CON ATRACTANTE DE ATUN EN LA ALIMENTACIÓN DE CAMARÓN BLANCO (*Litopenaeus vannamei*)

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Abstract

The consumption of feed aquaculture (pellets) in juvenile shrimp (*Litopenaeus vannamei*) was determined, 3 formulations were used: aquaculture binder with tuna attractant with pellets (M1), commercial binder with pellets (M2), and only pellets (M3). In the study was put 200 g of pellets mixed with 2 types of aquaculture binders and only pellets, and were put in alternating feeders during the experimentation. Using M1 was obtained a consumption of 83% of pellets in the feeders of the aquaculture pool, using the mixture 2 (M2) was obtained a consumption of 62% of shrimp foods in the feeders, while only using pellets (M3) a consumption of 79% was obtained. Differences were found between the results of the 3 mixtures tested for *Litopenaeus vannamei*, being the mixture 1 (M1) that presented the greatest consumption of pellets in the aquaculture shrimp. Also the aquaculture binder with tuna attractant (AQUAPEGA ATUN "LA") presented the highest content of protein (1.90%) and lysine content of 0.37%. The results showed that the aquaculture binder with tuna attraction presented superior organoleptic and nutritional characteristics that are directly related with the feeding of white shrimp (*Litopenaeus vannamei*).

Keywords: Shrimp, aquaculture binder, *Litopenaeus vannamei*, pellets.

Resumen

Se determinó el consumo de alimentos acuícolas (pellets) en camarones juveniles (*Litopenaeus vannamei*), se utilizaron 3 diferentes formulaciones: ligante acuícola con atractante de atún con pellets (M1), ligante comercial con pellets (M2), y solamente pellets (M3). Se colocaron 200 g de pellets mezclados con 2 tipos de ligantes acuícolas y solamente pellets, que fueron colocados en comederos alternados durante la experimentación. Utilizando M1 se obtuvo un consumo del 83% de pellets en los comedores de la piscina acuícola, usando la fórmula 2 (M2) se obtuvo un consumo del 62% del alimento acuícola en los comedores, mientras solamente usando pellets (M3) se obtuvo un consumo del 79% de pellets. Se encontraron diferencias significativas entre los resultados de las 3 mezclas probadas para *Litopenaeus vannamei*, siendo la fórmula 1 (M1) la que presentó el mayor consumo de pellets en la piscina acuícola. También el ligante acuícola con atractante atún (AQUAPEGA ATUN "LA") presentó mayor contenido de proteína (1.90%) y contenido de lisina de 0.37%. Los resultados evidenciaron que el ligante acuícola con atractante de atún presentó características superiores tanto organolépticas como nutricionales que influyen directamente en alimentación del camarón blanco (*Litopenaeus vannamei*)

Palabras clave: Camarón, ligante acuícola, *Litopenaeus vannamei*, pellets.

1 Introduction

The aquaculture started in Ecuador at the end of the 1960's, when some local farmers from El Oro province, noticed that there were shrimp growing in coastal ponds (Lopez-Alvarado *et al*., 2016). The shrimp is the second non-oil product of economic importance; the aquaculture has an average annual growth rate of 8.6% (Rivera *et al*., 2018). This increment in shrimp farms also represented greater foreign exchange and source of labor in the country, however some diseases such as: the emergence of white spot syndrome virus (WSSV) infection, seagull and Taura that presented a decline in the shrimp production that affected socially and economically the country (Brock, 1997; Rivera *et al*., 2018). For all these reasons the shrimp industrial field has development different methods to decrease the increment of animal stress, together with bacterial,

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viral or parasite infections and also improve the diet formulation (Martinez-Cordova *et al*., 2003).

During diet formulation the selection of dietary ingredients establishes upper and lower limits of each, to create a mixture that is palatable, nutritious, inexpensive that is common called "pellet" (Hardy and Barrows, 2002). In the formulation of the aquaculture feeds is necessary the used of binders to maintain the valuable dietary nutrients (Meyers *et al*., 1972; Partridge and Southgate, 1999), the most common of the binders are: agar, gelatine, carrageenan and purified binding agent carboxymethylcellulose (CMC) (Ruscoe *et al*., 2005).

The binders affect the pellet stability in three ways: by reduction of void spaces, resulting in a more compact and durable pellet by acting as adhesives, sticking particles together; and by exerting a chemical action on the ingredients and altering the nature of the feed resulting in a more durable pellet (DeSilva and Anderson, 1995; Palma *et al*., 2008). The selection of the best binder depends on many factors such as: length of time for which pellet stability is required, cost of binder, type of processing equipment available, and ingredients available to make the shrimp diet (Dominy and Lim 1991; Arredondo-Figueroa *et al*., 2003). Nowadays, the binders are used to decrease the leaching of medication applied to balanced food, medication such as antibiotics, vitamins, organic acids. Binder like gluten mixture in the diet can be used to obtain highest values of apparent digestibility of protein (ADP) and apparent dry matter digestibility (ADMD) (Arguello-Guevara and Molina-Poveda, 2013). However, there are not investigations of the use of the binders like attractant in the feeding shrimp.

The purpose of this study was to determinate the consumption of pellets using binder with tuna attractant in the feeding of white shrimp (*Litopenaeus vannamei*) in comparison with other commercial binder and only pellets.

2 Materials and methods

2.1 Composition of the binder

The aquaculture binder with tuna attractant (AQUAPEGA ATUN "LA") was made using the following composition: pectin solution 20%, xhantan gum 20%, guar gum 20%, chemistry hydrolyzed of tuna subproducts 20% and enzymatic hydrolyzed of tuna subproducts 20%. All the ingredients of the binder were heated at 90 ºC and mixed with constant agitation (800 RPM) until the total uniformity. The binder was realized at the Research and Development Laboratory of Ecuahidrolizados Industry, and the experimentation in the aquaculture pool was realized at the Shrimp Farm "La Chorrera".

2.2 Preparation of the mixtures

Mixture 1 (M1): The pellets were mixed with the binder with tuna attractant previously made. The formulation was 200 mL of tuna binder (AQUAPEGA ATUN "LA") on 2 L of water for 25 kg of pellets. Mixture 2 (M2): The pellets were mixed with a commercial binder "LB" (composition not presented). The specification was 200 mL of commercial binder on 2 L of water for 20 kg of pellets. Mixture 3 (M3): Only pellets.

2.3 Microbiological parameters and chemical composition of the aquaculture binders

The microbiological parameters evaluated were: aerobic mesophilic bacteria, *vibrio* spp., yeasts and molds (AOAC, 2005). The humidity, ash, fat and protein (N $\times$ 6.25) contents were determined using (Valencia del Toro *et al*., 2018; Valenzuela-Cobos, 2018; Valenzuela-Cobos *et al*., 2019).

2.4 Amino acids determination

- Reagents: In this research was used pure reference compounds and diethyl ethoxymethylenemalonate (DEEMM) were purchased from Sigma and Fluka. HPLC grade acetonitrile and methanol were obtained from Honeywell. The ultrapure water was obtained using a Milli-Q purification system. Sodium azide was purchased from Sangon and the solutions of amino acids were prepared with 0.1 M HCl.
- Derivatization: The derivatization was determined using the methodology of (Alaiz *et al*., 1992; Gómez-Alonso *et al*., 2007; Ya-Qin *et al*., 2014). The reacting mixture included 430 μL of 1 M borate buffer (pH 9.0), 2500 μL methanol, 350 μL sample, 10 μL internal standard (2-aminoadipic acid, 1.00 g/L) and 9 μL DEEMM. The derivatization reaction was carried out in a screw-cap test tube over 30 min in ultrasound bath. The mixture was then heated at 80 ºC for 3 h to allow complete degradation of excess DEEMM and other byproducts.

- HPLC analysis: The amino acids were performed according the methodology of (Ya-Qin *et al*., 2014).

2.5 *Consumption of the mixtures by the shrimps*

In the feeders were put 200 g of pellets with 160 mL of aquaculture binder with tuna attractant (M1), 200 g of pellets with 160 mL of commercial binder (M2), and only 200 g of pellets (M3). Tests were performed for 10 consecutive days, two hours after of the first feeding and were put in alternating feeders during the experimentation.

2.6 *Statistical analysis*

In all experiments, a completely randomized design and the results were examined using one-way analysis of variance (ANOVA) to determine the significance of individual differences at $p < 0.05$ level, of the microbiological parameters and chemical composition of the aquaculture binders, and also the consumption of the mixtures by the shrimps in the aquaculture pool, when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. The analyses were carried out using statistical software (Statgraphic ver. 16).

3 Results and discussion

3.1 *Microbiological and chemical properties of the aquaculture binders*

The microbiological parameters of the aquaculture binders are indicated in the Table 1. The aquaculture binder with tuna attractant (AQUAPEGA ATUN "LA") presented aerobic mesophilic values of 1.20×10^3 UFC/g, yeasts and molds values $< 1.00 \times 10^3$ UP/g, whereas vibrio was not detected. On the other hand, the commercial binder (LB) presented aerobic mesophilic values of 1.90×10^3 UFC/g, yeasts and molds values $< 1.00 \times 10^3$ UP/g, while vibrio was not detected.

The chemical composition values of the binders are indicated in the Table 2. The highest content of ash (2.07%) was presented by the commercial binder (LB). The highest humidity content (94.57%) was showed by the binder (AQUAPEGA ATUN "LA"), the two aquaculture binders presented similar values of fat content between 0.11 and 0.19%. The aquaculture binder with tuna attractant (AQUAPEGA ATUN "LA") presented the highest content of protein (1.90%) in relation with the commercial binder (LB).

The effects of protein levels on growth and survival do not present relation with dietary lipid levels (Kanazawa *et al*., 1985). The dietary crude protein requirement of penaeid shrimp is an important nutritional consideration because protein is often the major limiting nutrient for growth (Kureshy and Davis, 2002; Ezquerra-Brauer *et al*., 2003). The content protein is related with the species and size of shrimp, feeding management and culture conditions (Lim and Dominy, 1990). The aquaculture binder with tuna attractant (AQUAPEGA ATUN "LA") due to its protein content can be used in the feeding of white shrimp (*Litopenaeus vannamei*).

Table 1. Microbiological parameters of the aquaculture binders

Aquaculture Binder	**Aerobic mesophilic (UFC/g)**	***Vibrio* spp. (25/g)**	**Yeasts and molds(UP/g)**
AQUAPEGA ATUN "LA"	1.20×10^{3b}	Nd	$< 1.00 \times 10^{3a}$
COMMERCIAL BINDER "LB"	1.90×10^{3a}	Nd	$< 1.00 \times 10^{3a}$

*Different letters in each column indicated significant difference among the presence of aerobic mesophilic, yeasts and molds of the aquaculture binders at level $p < 0.05$, according to Duncan's test, n = 3.
Nd= Not detected.

Table 2. Chemical composition of the aquaculture binders.

Aquaculture Binder	Humidity (%)	Ash (%)	Fat (%)	Protein (%)
AQUAPEGA ATUN "LA"	94.57±0.87[a]	1.18±0.08[b]	0.11±0.01[a]	1.90±0.26[a]
COMMERCIAL BINDER "LB"	93.10±0.53[b]	2.07±0.14[a]	0.19±0.02[a]	1.42±0.18[b]

*Different letters in each column indicated significant difference among the chemical composition of the aquaculture binders at level $p < 0.05$, according to Duncan's test, n = 3.

3.2 Amino acids composition of the aquaculture binder with tuna attractant

Table 3 indicates the amino acids composition of the aquaculture binder with tuna attractant (AQUAPEGA ATUN "LA"). In the research were detected 9 amino acids after pre-column derivatization with DEEMM within 40 min. The selected conditions were a compromise between the chromatographic separation of all peaks and the need for a rapid analytical method. The wavelength of 280 nm selected for quantifying for all the compounds displayed good separation at this wavelength and the intensity of signals were strong enough for quantification.

Table 3. Amino acids composition of the aquaculture binder with tuna attractant AQUAPEGA ATUN "LA".

Amino acids	%
Proline	0.21
Cysteine	0.14
Tyrosine	0.16
Valine	0.24
Methionine	0.15
Lysine	0.37
Isoleucine	0.20
Leucine	0.29
Phenylalanine	0.14

The amino acid with more presence in the aquaculture binder with tuna attractant was the lysine with values of 0.37%. The lysine requirement for *L. vannamei* is 1.64% of dry diet (3.95% of the dietary protein) (Xie *et al*., 2012). Lysine is the one of the principal amino acids necessary in the diet of the shrimps (Akiyama *et al*., 1991). Survival of the shrimp increased with increasing levels of amino acids (methionine, lysine and arginine), and therefore leading to the conclusion that a reduction in any essential amino acid in the diet has a direct consequence on shrimp survival and growth (Palma *et al*., 2013). The content of amino acids in the binder are the directly related with the content of protein.

3.3 Attractability of the mixtures with different aquaculture binders

The consumption by the shrimps using the two kinds of aquaculture binders mixing with pellets and only pellets are indicated in the Table 4.

The mixture of aquaculture binder with tuna attractant with pellets (M1) presented a consumption of 83.00% by the shrimps, while using the mixture of commercial binder with pellets (M2) presented a consumption of 62.00% by the shrimps, and only using pellets (M3) the consumption was of 79.00% by the shrimps.

Table 4. Consumption of the mixtures by the shrimps and organoleptic properties of the binders.

Mixture	Consumption (%)	Color	Consistency
M1	83.00±0.37[a]	Coffee	Homogeneus
M2	62.00±0.75[c]	Coffee	Homogeneus
M3	79.00±1.08[b]	Pale brown	Solid

*M1: The pellets were mixed with the binder with tuna attractant (AQUAPEGA ATUN "LA") , M2: The pellets were mixed with a commercial binder "LB" and M3: Only pellets.

*Different letters in each column indicated significant difference among the consumption of the mixtures by the shrimps at level $p < 0.05$, according to Duncan's test, n = 10.

The aquaculture binder with tuna attractant presented a superior attractability in comparison with the other binder, the characteristic of the binder (AQUAPEGA ATUN "LA") can be used to mix with others ingredients that are necessary for growth of the shrimp such as: antibiotics, vitamins and organic acids. These ingredients are used to control the bacterial infections, improve shrimp reproductive performance and egg hatching rate (Wouters *et al*., 1999; Wouters *et al*., 2001; Defoirdt *et al*., 2011). The attractability and palatability assessments in shrimp were fairly consistent with the biochemical profile (Suresh *et al*., 2011). The mixture that used the aquaculture binder with tuna attractant (AQUAPEGA ATUN "LA") presented the highest consumption by the white shrimp (*Litopenaeus vannamei*).

Conclusions

The tuna attractant binder (AQUAPEGA ATUN "LA") showed the highest content of protein in comparison with the other binder, the amino acid with more presence in the aquaculture binder with tuna attractant was the lysine.

The aquaculture binder with tuna attractant mixed with pellets (M1) presented a higher consumption by the shrimp in comparison with a commercial binder (M2) and with only pellets (M3).

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