Lat. Am. J. Aquat. Res., 46(3): 615-620, 2018
"II International Congress of *Macrobrachium*"
Fernando Vega-Villasante, Marcelo Ulises García-Guerrero, Carlos Alfonso Álvarez-González
Luis Héctor Hernández-Hernández & Saúl Rogelio Guerrero Galván (Guest Editors)
DOI: 10.3856/vol46-issue3-fulltext-17

Short Communication

Attractability and palatability of ingredients in longarm river prawn *Macrobrachium tenellum* feed

Cynthia Montoya-Martínez[1], Héctor Nolasco-Soria[2], Fernando Vega-Villasante[1]
Olimpia Carrillo-Farnés[3], Alfonso Álvarez-González[4] & Roberto Civera-Cerecedo[2]
[1]Laboratorio de Acuicultura Experimental, Centro de Investigaciones Costeras
Universidad de Guadalajara, Ciudad de México, México
[2]Programa de Acuacultura, Centro de Investigaciones Biológicas del Noroeste S.C.
La Paz, B.C.S., México
[3]Facultad de Biología, Universidad de La Habana, La Habana, Cuba
[4]Laboratorio de Acuicultura Tropical, División Académica de Ciencias Biológicas
Universidad Juárez Autónoma de Tabasco, México
Autor de correspondencia: Fernando Vega (fernandovega.villasante@gmail.com)

ABSTRACT. The present work evaluates the attractant and palatable potential of six ingredients of animal origin in longarm river prawn *Macrobrachium tenellum* juveniles in a Y type maze system. Ingredients were pelletized for the first bioassay and included in neutral gelatin (in wet base) in the second bioassay. The ingredient to evaluate was placed in one of the Y-maze arms, allowing the free movement of prawn for 15 min. On both bioassays, attractability was evaluated by quantifying the time required for the first prawn to enter the region where the feed was found and the total of prawns which entered that region. In the second bioassay, also evaluated the palatability quantifying the time for the first prawn to have contact with the ingredient, the total of prawns which had contact with it and the time they remained feeding. No significant differences were obtained between treatments in the first bioassay. Significant differences were found in the second bioassay showing that pork meal, fish meal, feather meal and shrimp meal have greater attractability due to the number of prawns attracted, results also show significant differences in palatability, where fishmeal, shrimp meal and pork meal stimulating a higher number of organisms and promoting a longer consumption time.

Keywords: *Macrobrachium tenellum*, chemoattraction, Y type maze, prawn, feeding, aquaculture.

The increase on the demand for aquaculture feeds is directly related with aquaculture growth worldwide. Among the most important issues feeding is among the most relevant factors since determines the productive and economic results of commercial culture (Montemayor-Leal *et al*., 2005). In relation with prawn feeding, feed is the most expensive issue due to the high cost of protein ingredients. It also has a costly manufacture procedure because of the stability required to maintain its nutritional value (Muñoz, 2004). To formulate diets that meet prawn nutritional requirements at low cost, it is necessary to perform studies about protein alternative sources with good attractability and palatability, this might improve its efficiency for culture purposes (Montemayor-Leal *et al*., 2005; Sacristán *et al*., 2014). Prawn are capable of detecting feed at certain distance through antennal receptors and once located by contact, it is tastesd with the sensitive receptors located in its first pair of legs and in the mouth, with feed acceptance or rejection as response (Suresh *et al*., 2011). A nutritionally balanced feed loses its nutritional value if the cultured species do not consume it soon, so attractability and palatability are essential for an efficient feeding in aquatic cultures (García-Galano *et al*., 2007; Jaime-Ceballos *et al*., 2007; Tantikitti, 2014).

The aim of this work is to evaluate the attractability and palatability of six different ingredients in *Macrobrachium tenellum* juveniles prawn.

The assays were carried out at the Laboratorio de Calidad de Agua y Acuicultura Experimental of the Universidad de Guadalajara in Puerto Vallarta, Jalisco, Mexico. Six ingredients were used: pork meal (meat and bones), prime poultry meal, hydrolyzed feather meal, turkey poultry meal, fish meal (sardine), shrimp meal (from Proteínas Marinas y Agropecuarias, S.A. de C.V.). The chemical composition is presented in Table 1. Meals were grinded and screened with a 250

Table 1. Proximal composition of ingredients.

Ingredient	Protein (%)	Lipids (%)	Ash (%)
Pork meal	57	10	28
Poultry meal	66	16	14
Feather meal	77	3	1
Turkey meal	57	14	26
Fish meal	59	16	15
Shrimp meal	48	12	26

µm mesh. Two bioassays were carried out. Bioassay I: each ingredient was mixed with 4% of gelatin as agglutinant in a beater (KitchenAid, St Joseph, MI, USA). Water was added gradually until full homogenization was obtained. The resulting mix was pelletized with a meat grinder (Torrey®, Mod. M-12-FS, Monterrey, NL, México) using a sieve with perforations of 4.8 mm in diameter. The extruded pellets were dried in an oven at 40°C for 12 h. Then, feed were kept under refrigeration at 4°C. Bioassay II: for each ingredient, 15 g of the same meals were mixed with 5 g of gelatin dissolved in 80 mL of water. After that, they were homogenized with a manual blender; 5 mm were poured in Petri dishes (6 cm diameter) and then, refrigerated at 4ºC for its gelation.

M. tenellum juveniles of 0.5 to 1.0 g were used for the first bioassay and of 1.0 to 2.0 g for the second bioassay, captured in the artificial pond at Centro Universitario de la Costa, Universidad de Guadalajara, Puerto Vallarta. The longarm river prawns (200 juveniles) were distributed in eight glass (40 L) aquariums provided with cascade filters (Elite Hush®) and under controlled temperature conditions at 28°C (Sunny® heaters with thermostat). Prawns were acclimatized first during 20 days prior to the start of the bioassay during which they were fed with commercial shrimp pellets (Camaronina® Purina®, 35% protein, humidity 12%, fat 8%, crude fiber 5%, ash 10%, nitrogen free extract 30%). Feeding time and frequency were established *a priori* between 10:00-11:00 h AM as a single daily feeding *ad libitum*. Surplus food were daily removed.

Once acclimation period ended, only intermolt stage prawns were selected to carry out the bioassays in agreement with Reyes & Luján (2003) and in order to avoid any possible interference with this phenomenon on perception, as stated by Montemayor-Leal *et al*. (2005). Prawns were kept at 28ºC and no food was given for 24 h previous to the beginning of the trial in order to avoid any kind of interference that might affect results feeding preferences (Montemayor, 1995; Jaime-Ceballos *et al*., 2007).

The experimental device consisted of a Y type maze system (Fig. 1). This system has a retainer gate which could be inserted between the N zone and the R region.

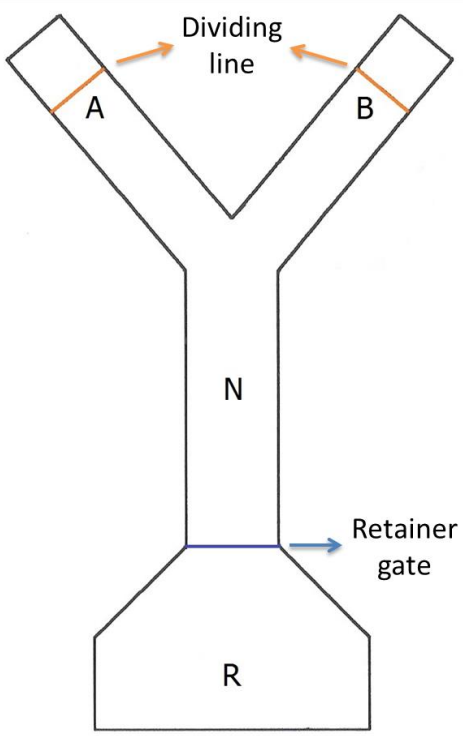

Figure 1. Y type maze system used in the evaluation of atractability by ingredient chemodetection.

A video camera (GoPro®) was placed in the front of the maze to record the prawns movements.

A total of 10 prawns were located in region R of the device for final acclimation. An hour later, the ingredient was randomly placed in the end of one of the arms of the device (A or B). The ingredient to evaluate in the first bioassay was placed at a ratio of 20% the prawns biomass inside an organdy bag. For the second bioassay, the gelled ingredient was placed without the Petri dish. Ten min after placing the ingredient, the retainer gate on R region was removed and video recording started for 15 min. To diminish the possible influence of feeds from zones A or B, four repetitions were made randomly changing the position of the ingredient. Observations were performed with the same feeding schedule and the tests were performed under twilight conditions (only enough light to distinguish and determine responses). Observations recordings were performed (through video recording analysis) and the time required for the first prawn to enter region A or B was measured from time zero (once the gate was removed) until all prawns enter to one of the zones (attractability) for both bioassays. Additionally, the time in which the first prawn had contact with the ingredient was quantified, as well as the time at which all prawns (adding the individual time of feed consumption), had contact with the ingredient. The

time they remained feeding for 15 min (palatability) were quantified only for the second bioassay.

Results from the experiment were analyzed first with Kolmogorov-Smirnov test to determine its normality and then a one-way variance analysis was applied (ANOVA). When significant differences were found a Tukey analysis of multiple variance comparison was applied. All tests were performed with the SigmaPlot version 11.0 statistical (Systat Software, Inc. Chicago, IL, USA).

No statistically significant differences were found at all in the first bioassay ($P > 0.05$).

The experimental conditions used in the second bioassay are shown (Fig. 2). The attractive power of the ingredients on prawns response had no statistically significant differences. However, the statistical analysis showed significant differences ($P < 0.05$) between the number of prawns which entered the area where the test ingredient was located for the 15 min of the bioassay, suggesting that the fish meal (18 ± 4.90), pork meal (16.25 ± 1.71), feather meal (15.40 ± 4.12) and shrimp meal (13.00 ± 6.8) attracted statistically more prawns than the turkey meal (6.75 ± 1.26).

Palatability results are shown in Fig. 3. During the time required for the first prawn to have contact with the feed no statistically significant differences were found among treatments. Results show significant differences in ingredient palatability (intake time, in min). Turkey meal (6.25 ± 2.75) and poultry meal (6.75 ± 2.99) showed lower palatability (those less inciting feeding) while fish meal, shrimp meal and pork meal presented the highest palatability since those attracted significantly more prawns (19.50 ± 4.20, 14.75 ± 7.04, 14.25 ± 2.50, respectively) and they also promoting a longer consumption time (min) (87.61 ± 28.47, 101.29 ± 67.44, 56.24 ± 30.05 respectively).

Nevertheless, the progress in the study of nutrition and feeding of the *Macrobrachium* prawns are done mostly with *M. rosenbergii* species as observed in previous research (Harpaz *et al*., 1987; Mendoza *et al*., 1997; Felix & Sudharsan, 2004). However, research in the topic is scarce.

Responses to feeding effectors (term suggested by Smith *et al*. (2005), for chemoattractants, starters and stimulators) either natural, purified or synthetic compounds have been widely studied on marine shrimp because of its relevance in the understanding of feeding behavior of these crustaceans (Huang *et al*., 2005; Sánchez *et al*., 2005; Smith *et al*., 2005; Nunes *et al*., 2006; Ali *et al*., 2007; Grey *et al*., 2009). Chemoreceptors in crustaceans in general are sensitive to low molecular weight water soluble chemicals such as: aminoacids, ammonia quaternary compounds, nucleotides and biogenic amines (Lee & Meyers, 1996; Nunes *et al*., 2006). It is known that ingredients of aquatic animal origin (such as soluble meals of mollusks and crustaceans), are rich in these compounds and therefore, they act as excellent attractants (Smith *et al*., 2005; Ali *et al*., 2007). In the other hand, non-aquatic animal sub-product meals such as: poultry sub-products meals and blood meal show lower levels of those compounds. Because of this, the attractability and palatability of non-aquatic animal products is lower; however few studies are designed to demonstrate such observations (Suresh *et al*., 2011). This work is the first to show the efficiency of commercially available protein ingredients as feed effectors in *Macrobrachium* prawns.

In this context, Nunes *et al*. (2006) using the Y type maze system for *Penaeus vannamei* shrimps, demonstrated that blood meal was among the less stimulating ingredients and that meat and bone meal were similar to the fish solubles but inferior to fish and squid meals. This does not agree with present results, since pork meal did not show any significant differences regarding fish meal, in terms of attractability and palatability.

In the other hand, Suresh *et al.* (2011) conclude that attractability and palatability evaluations are consistent with the biochemical profile of the ingredients, finding that for *P. stylirostris*, poultry sub-products meals were the most attractant, and hydrolyzed feather meal caused the lowest response; while the most palatable ingredient was krill meal followed by the squid liver meal, both poultry sub-product meals, fish hydrolysate and hydrolyzed feather meal; which is contrary to our results where the hydrolyzed feather meal had a better response and the turkey meal and poultry meal had the lowest attractability and palatability responses.

The fact of finding no statistical differences from the first bioassay results with pelletized ingredients could be because the chemoattractant efficiency is related to its diffusion coefficient and its water solubility as stated by Lee & Meyers (1996). Those authors stated that this could be affected by the use of the organdy bag as an ingredient pellet container, reducing the lixiviation. Unfortunately, the ingredients could not be placed freely due to the observations from previous assays (Montoya-Martínez *et al.*, in press) in where agonistic behavior among *M. tenellum* prawns causes some prawns to take the feed and move away with it, taking the stimulation source to other parts in the system.

According to our results in two bioassays regarding ingredient detection time, it was observed that prawns wander around in the maze regardless the location of the ingredient. This was previously observed by Sacristán *et al*. (2014) who, considering feeding habits,

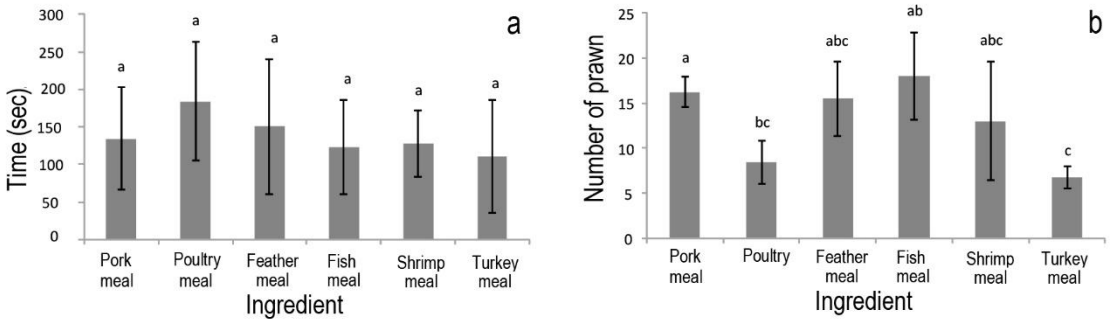

Figure 2. Attractability phase in the second bioassay. a) Time required for the first prawn to enter A or B region, b) total of prawns which entered the area where the ingredient was present. Different letters on the bars, indicate significant differences ($P < 0.05$).

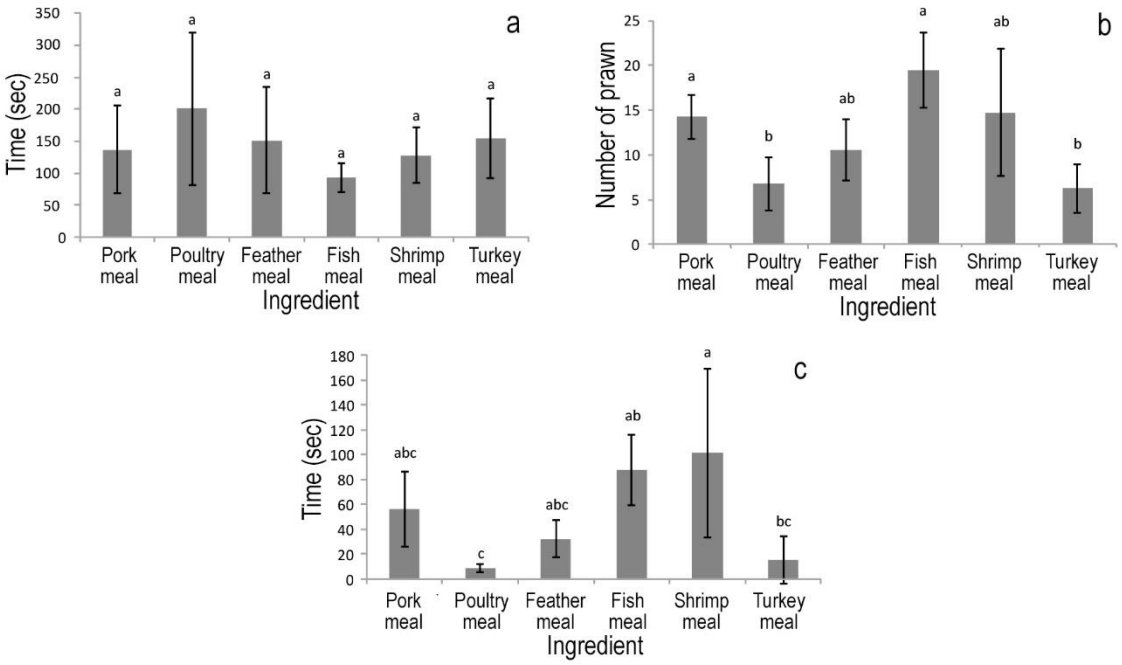

Figure 3. Palatability phase in the second bioassay. a) Time for the first prawn to have contact with the ingredient, b) total of prawns which have contact with the ingredient, c) time prawns feed (for 15 min). Different letters on the bars indicate significant differences ($P < 0.05$).

propose the hypothesis that freshwater decapod *Cherax quadricarinatus* find its feed mainly due to the time they invest in roaming the environment since detection of chemical signals are strongly affected by flow dynamics. Therefore, feed detection should also be studied in water flow systems. Hereafter and considering that feeding habits in *M. tenellum* are carried out in hydrographic basins with slow currents (Espinosa-Chaurand *et al.*, 2011), these flows must affect chemical signal detection abilities. However, Pittet *et al.* (1996) notes that the main disadvantage of aquariums with water flow is that chemical stimulation can start the movement, but the unidirectional current perpetuates it in such a way, that the animal can respond mainly to rheotaxis. In this context, Montemayor (1995) observed that *M. rosenbergii* prawns reacted to the rheostatic stimulation of water flow and for that reason, a water flow system was not used in present work.

Even when most assays on crustaceans feeding behavior have been performed with groups of organisms due to the experimental convenience, conditions would be closer to the culture conditions. Some possible disadvantages are the fact that a specimen would respond to the stimulation from the movement of other members of the group or might react intimidated by other more active or aggressive members of the group, as observed in present work, due to the territorial behavior for these species (Harpaz *et al.*, 1987). This behavior may cause a bias when counting

the feeding time due to the agonistic behavior, which dissuades some individuals from approaching the feed. In this sense, Lee & Meyers (1996) highlights some of the methodological limitations of feed chemoreception, suggesting that is required to make several bioassays in different conditions and aquariums to avoid an erroneous interpretation and improve the opportunity for the development of useful alimentary stimulants. Therefore it is necessary, as Pittet *et al.* (1996) states, perform a hierarchical test protocol, consisting in a method of sequential process, from a quick selection of a big number of materials as potential stimulants through more discriminating procedures that might help to evaluate chemotaxis. Then, a final evaluation of the more powerful chemostimulants with laboratory feeding assays is required in order to obtain a better assessment of compounds or mixes as feeding attractants.

In this study, it was found that pork meal and feather meal have remarkable feeding stimulator properties for prawns, only slightly lower than fish meal and shrimp meal in terms of attractability and palatability, therefore they should be considered for its replacement. Finally, is necessary to state that is important to carry out experiments with the ingredients that may produce strong attractant responses in order to find more effective feeding stimulators and find their optimum inclusions in the diet. It is also necessary to carry out feeding trials with a wide variety of ingredients that are compatible with those attractants, favoring the design of a balanced feed that is cheap, easy to handle and promptly consumed.

ACNOWLEDGEMENTS

We thank the National Council for Science and Technology of Mexico by the doctoral scholarship to the first author of this paper and to Proteínas Marinas y Agropecuarias, S.A. de C.V. for donating the animal meals used in this research.

REFERENCES

Ali, S.A., C. Gopal & J.V. Ramana. 2007. Attractant and growth promoting properties of some feed materials and chemicals incorporated in the diets for *Penaeus monodon* (Fabricius). Indian J. Fish., 54(1): 67-73.

Espinosa-Chaurand, L.D., M.A. Vargas-Ceballos, M. Guzmán-Arroyo, H. Nolasco-Soria, O. Carrillo-Farnés, O. Chong-Carrillo & F. Vega-Villasante. 2011. Biología y cultivo de *Macrobrachium tenellum*: estado del arte. Hidrobiológica, 21(2): 98-117.

Felix, N. & M. Sudharsan. 2004. Effect of glycine betaine, a feed attractant affecting growth and feed conversion of juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquacult. Nutr., 10(3): 193-197.

García-Galano, T., H. Villarreal-Colmenares & J.L. Fenucci. 2007. Manual de ingredientes proteicos y aditivos empleados en la formulación de alimentos balanceados para camarones peneidos. Subprograma II "Acuacultura" red temática. II. C Proyecto II-8, Editorial Universitaria de Mar del Plata, Mar del Plata, 264 pp.

Grey, M., I. Forster, W. Dominy, H. Ako & A.F. Giesen. 2009. Validation of a feeding stimulant bioassay using fish hydrolysates for the Pacific white shrimp, *Litopenaeus vannamei*. J. World Aquacult. Soc., 40(4): 547-555.

Harpaz, S., D. Kahan, R. Galun & I. Moore. 1987. Responses of freshwater prawn, *Macrobrachium rosenbergii*, to chemical attractants. J. Chem. Ecol., 13(9): 1957-1965.

Huang, G., S. Dong & F. Wang. 2005. Effects of different diets on the dietary attractability and selectivity of Chinese shrimp, *Fenneropenaeus chinensis*. J. Ocean Univ. China, 4(1): 56-60.

Jaime-Ceballos, B., R. Civera-Cerecedo, H. Villarreal, J. Galindo-López & L. Pérez-Jar. 2007. Uso de la harina de *Spirulina platensis* como atrayente en el alimento para el camarón *Litopenaeus schmitti*. Hidrobiológica, 17(2): 113-117.

Lee, P.G. & S.P. Meyers. 1996. Chemoattraction and feeding stimulation in crustaceans. Aquacult. Nutr., 2(3): 157-164.

Mendoza, R., J. Montemayor & J. Verde. 1997. Biogenic amines and pheromones as feed attractants for the freshwater prawn *Macrobrachium rosenbergii*. Aquacult. Nutr., 3(3): 167-173.

Montemayor, J. 1995. Uso de feromonas y aminas biogénicas como atractantes en alimento para langostinos, *Macrobrachium rosenbergii*. Tesis Doctoral, Universidad Autónoma de Nuevo León, Nuevo León, 94 pp.

Montemayor-Leal, J., R. Mendoza-Alfaro, C. Aguilera-González & G. Rodríguez-Almaraz. 2005. Moléculas sintéticas y extractos animales y vegetales como atractantes alimenticios para el camarón blanco *Litopenaeus vannamei*. Rev. AquaTIC, 22: 1-10.

Montoya-Martínez, C.E., C.D. Carrillo-Pérez, H. Nolasco-Soria, A. Álvarez-González, O. Carrillo-Farnés, R. Civera-Cerecedo & F. Vega-Villasante. 2018. Evaluation of different maze systems for the determination of feed attractability for longarm river prawn *Macrobrachium tenellum*. Lat. Am. J. Aquat. Res., in press.

Muñoz, L.O. 2004. Comparación entre extruído y pelletizado en alimentos de camarones. In: L.E. Cruz-Suárez, D. Ricque-Marie, M.G. Nieto-López, D.

Villareal, U. Sholz & M. González (eds.). Avances en Nutrición Acuícola. VII. Universidad Autónoma de Nuevo León, Nuevo León, pp. 397-417.

Nunes, A.J., M.V. Sá, F.F. Andriola-Neto & D. Lemos. 2006. Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 260(1): 244-254.

Pittet, A.O., J.C. Ellis & P.G. Lee. 1996. Methodology for the identification and quantitative measurement of chemical stimulants for penaeid shrimp. Aquacult. Nutr., 2(3): 175-182.

Reyes, W.E. & H. Lujan. 2003. Estados y subestados del ciclo de muda del camarón de río (*Cryphiops caementarius* Molina, 1872) (Crustacea: Decapoda: Palaemonidae). [http://biblioteca.uns.edu.pe/saladocentes/archivoz/publicacionez/civa_2003_estadios_muda.pdf]. Reviewed: 6 June 2017.

Sacristán, H.J., H. Nolasco-Soria & L.S.L. Greco. 2014. Effect of attractant stimuli, starvation period and food availability on digestive enzymes in the redclaw crayfish *Cherax quadricarinatus* (Parastacidae). Aquat. Biol., 23(1): 87-99.

Received: 1 April 2017; Accepted: 30 April 2018

Sanchez, D.R., J.M. Fox, A.L. Lawrence, F.L. Castille & B. Dunsford. 2005. A methodology for evaluation of dietary feeding stimulants for the Pacific white shrimp, *Litopenaeus vannamei*. J. World Aquacult. Soc., 36(1): 14-23.

Smith, D.M., S.J. Tabrett, M.C. Barclay & S.J. Irvin. 2005. The efficacy of ingredients included in shrimp feeds to stimulate intake. Aquacult. Nutr., 11(4): 263-272.

Suresh, A.V., K.P. Kumaraguru-Vasagam & S. Nates. 2011. Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry byproduct meal. Aquaculture, 319(1): 132-140.

Tantikitti, C. 2014. Feed palatability and the alternative protein sources in shrimp feed. Songklanakarin J. Sci. Technol., 36(1): 51-55.