Lat. Am. J. Aquat. Res., 44(1): 121-128, 2016
DOI: 10.3856/vol44-issue1-fulltext-12

Research Article

The effects of *Yucca schidigera* and *Quillaja saponaria* on growth performance and enzymes activities of juvenile shrimp *Litopenaeus vannamei* cultured in low-salinity water

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ABSTRACT. The inclusion of *Yucca schidigera* and *Quillaja saponaria* extracts (NTF) in aquatic organisms display a positive response on production and organism's physiology. Fifteen tanks (140 L) with low-salinity water (S = 5) were stocked with 10 juvenile shrimp (*Litopenaeus vannamei*, 2.6 g of mean weight) feeding with 0, 0.25, 0.5, 1.0 and 2.0 g kg^{-1} of NTF of basal diet (triplicate treatment). The shrimp were cultured in a close recirculation system (control condition) and fed *ad libitum* daily for 40 days. General growth parameters (body weight, growth, body length, feed conversion rate, survival) and hepatopancreatic digestive enzyme activities (alkaline protease, alkaline phosphatase, α-amylase, leucine aminopeptidase, and lipase) were evaluated after 40 days of shrimp culture. The final mean body weight, individual mean body, weight gain, and feed conversion ratio from shrimp feeding with 1.0 and 2.0 g kg^{-1} of NTF have a significant ($P < 0.05$) result compared to other treatments. The highest values of alkaline protease, lipase, and α-amylase were detected in shrimp feeding with 0.5 g kg^{-1} of NTF, where a high level of leucine aminopeptidase and alkaline phosphatase were detected with 0.25 g kg^{-1} of NTF treatment. However, any significant differences in enzyme activities were detected between the control group and treatments. The increase effect in shrimp growth and any decrease effect in enzyme activity detected in present study suggest that NTF shows potential as a feed additive for shrimp cultured at low-salinity.
Keywords: *Litopenaeus vannamei*, growth, enzyme activity, low-salinity, aquaculture.

Los efectos de *Yucca schidigera* y *Quillaja saponaria* sobre el crecimiento y actividad enzimática de camarones juveniles de *Litopenaeus vannamei* cultivados a baja salinidad

RESUMEN. El uso de extractos de *Yucca schidigera* y *Quillaja saponaria* (NTF) en organismos acuáticos ha mostrado una respuesta positivas en la producción y fisiología de los mismos. Quince estanques (140 L) con agua a baja salinidad (S = 5) fueron preparados con 10 camarones juveniles (*Litopenaeus vannamei*, 2,6 g de peso promedio), alimentados con 0; 0,25; 0,5; 1,0 y 2,0 g de NTF kg^{-1} en la dieta base (tratamientos por triplicado). Los camarones fueron cultivados en un sistema cerrado de recirculación (condiciones controladas) y alimentados *ad libitum* diariamente por 40 días. En los organismos se evaluaron los parámetros generales de crecimiento (peso y longitud del cuerpo, crecimiento, conversión alimenticia, supervivencia, etc.) y la actividad de enzimas digestivas hepatopancreáticas (proteasa y fosfatasa alcalina, α-amilasa, leucina aminopeptidasa y lipasas). El peso promedio final e individual, ganancia de peso, y conversión alimenticia fue superior en los camarones alimentados con 1,0 y 2,0 g kg^{-1} de NTF con valores significativamente positivos ($P < 0,05$), comparado con los otros tratamientos. El valor más alto de proteasa alcalina, lipasa y α-amilasa fue detectada en camarones alimentados con 0,5 g kg^{-1} de NTF. Un valor alto de leucina aminopeptidasa y fosfatasa alcalina se detectó en el tratamiento con 0,25 g kg^{-1} de NTF. Sin embargo, no se observó diferencia significativa entre tratamientos. El efecto positivo en el crecimiento y carencia de efectos negativos en la actividad enzimática

mostrada en el presente trabajo sugieren que el NTF puede ser empleado potencialmente como aditivo alimenticio en camarones cultivados a baja salinidad.

Palabras clave: *Litopenaeus vannamei*, crecimiento, actividad enzimática, baja salinidad, acuicultura.

Corresponding editor: Jesús Ponce-Palafox

INTRODUCTION

The shrimp culture activities in the world display a high economic importance in the international market, which have been generating significant advances in the culture of several shrimp species (Pérez-Rostro & Ibarra, 2003). This enhancement in shrimp culture is mainly due to the improved knowledge about genetics, health, nutrition, physiology, and reproduction of the cultured organisms (Martín *et al.*, 2006).

The relevance of shrimp industry, its technology, and a better understanding of the shrimp's physiology may help to develop a small industry of shrimp in low-salinity water (Pérez-Castañeda *et al.*, 2015). Currently, different countries display evidence of those farms that work at a commercial scale. The postlarvae are cultured in greenhouses at low densities for a better control of the environmental conditions and where they are adapted daily to low-salinity water (2-10). Poste-riorly, early juvenile shrimp continue growing up until they reach a harvest size to be transferred to grow-out in ponds with low-salinity water (Araneda *et al.*, 2013; Pérez-Castañeda *et al.*, 2015).

A regular stock of adequate shrimp feed (quality and quantity) and the correct supplying process are elements that will sustain the development of this industry. Both are important aspects to be considered in shrimp culture, which can represent a significant reduction on the production cost in semi-intensive and intensive systems of shrimp farms. The use of additives in the shrimp feed as antioxidants, amino acids, immunostimulants, pigments, plant extracts, vitamins, etc. have enhanced the shrimp growth, survival, stress and feed conversion ratio (Lyle-Fritch *et al.*, 2006; Venkatramalingam *et al.*, 2007). Also, a better understanding of the enzymes of the shrimp hepatopancreas (amylases, proteinases, lipases, etc.) and changes generated by diets, help to get a better shrimp production. At present, researches about the application of natural growth promotors as feed additive are carried out. Yucca (*Yucca schidigera*) and Soapbark (*Quillaja saponaria*) are used as feed additive in animal production with results on growth and health of livestock species (Qi-hui *et al.*, 2015). Those products are sources of polysaccharides, polyphenols, and steroidal saponin extracts. Few studies have been conducted on the use in shrimp production and other aquatic animal feed (Francis *et al.*, 2002; Qi-hui *et al.*, 2015). A result was observed in shrimp growth (*L. vannamei*) when those products were incorporated in the diet (Casillas-Hernández *et al.*, 2009). In addition, *Y. schidigera* and *Q. saponaria* induce progressive effects on growth performance and haematological parameters, and decrease total ammonia-nitrogen excretion in *Pangasianodon hypophthalmus* (Güroy *et al.*, 2014). Martínez *et al.* (2008) evaluated the effect of yucca extract in diets of *L. vannamei* (0, 1, 2 and 3 g kg^{-1}) where a significant result was found in shrimp growth at 2 g kg^{-1} of diet of yucca extract (*Y. schidigera*) compared to control diet. Also, a lower total ammonia-nitrogen result in water of shrimp production was found in diet with yucca extract.

The objective of the present work was to evaluate the effects of several inclusion levels of commercial product with *Y. schidigera* and *Q. saponaria* on the growth parameters of Pacific white shrimp *L. vannamei* cultured at low-salinity water (S = 5).

MATERIALS AND METHODS

Experimental diet

Nutrafito plus (NTF)^{TM} is a mixture of *Yucca schidigera* and *Quillaja saponaria*, which is a source of polyphenols and steroidal saponin (3% of active product). This is a commercial feed additive for aquafeeds (Desert King International Company, San Diego, CA, USA).

A commercial shrimp diet (40% protein, 8% lipid, Rangen Inc., USA) was ground to a powder and used as basal diet. Five experimental diets containing the basal diet with different levels of NFT inclusion were elaborated (0, 0.25, 0.5, 1.0 and 2.0 g kg^{-1} of NTF basal diet). Experimental diets were manufactured in the laboratory at the University of Arizona, Tucson, Arizona, USA. The corresponding NTF level was diluted in distilled water at room temperature using a magnetic stirrer, where 7 g kg^{-1} of grenetin was added as agglutinant. The solution was mixed (20 min) with basal diet in a Hobart food mixer. Each diet was then passed through a meat grinder (3 mm of diameter), where a spaghetti-like strings was obtained. After pelleting, the experimental diets were dried in an drying cabinet using an air blower at 80°C overnight

until the moisture level was <10%, broken up (6 mm length), and stored at 4°C until be used. The control diet was manufacture similar to experimental diet but without Nutrafito plus (NTF)^{TM}.

The diet stability in low-salinity water (S = 5) was done according to Obaldo *et al.* (2002) protocol, where 5 g of each diet was exposed to water (2 h). The pellets were removed, dried (80°C, previous description) and weighed. The stability rate was calculated using the following formula: weight of initial pellet-weight of final pellet remaining divided by weight of initial pellet multiplied by 100.

Shrimp and experimental protocol

Shrimp postlarvae (*L. vannamei*) were obtained from a private company (Shrimp Improvement Systems, FL, USA). Those organisms were transported in seawater (Temp. = 27°C, pH = 7.8-8.2, and S = 32) to University of Arizona, Tucson, Arizona, USA. Upon arrival, they were acclimated at low-salinity water in fiberglass tank where, changes in salinity were performed at a rate of 1 per hour (Laramore *et al.*, 2001; McGraw *et al.*, 2002). The shrimp postlarvae were cultured to juvenile age, feed with a commercial shrimp diet, provided with continues air supply (≥5 mg L^{-1}), and a 30% of water exchange. The low-salinity water was prepared with freshwater + commercial available artificial seawater (Crystal Sea Marine Mix, Baltimore, USA).

The experiment was carried out in a greenhouse at the Environmental Research Laboratory, University of Arizona, where shrimp (2.6 ± 0.5 g mean weight, 7.3 ± 0.3 cm mean body length) were selected and randomly distributed into fifteen fiberglass tanks (140 L, 10 ind $tank^{-1}$) with a recirculating system with low-salinity water (S = 5), biofilter, continues air supply (≥5 mg L^{-1}), and a daily water exchange (>200%).

The shrimp were fed for 40 days using the control or corresponding experimental diets. Three replicates were assigned to each of the five diets (n = 5). Daily, the shrimp were fed *ad libitum* for 2 h (only one time for day, 18:00) at a rate of 6-10% of body weight using a feed tray (20 cm of diameter). The daily feed ration was adjusted according to feed consumption. The diet excess was removed after feeding period (2 h), dried at 80°C, weighted for daily feed ration, and stored at -20°C until be used for calculating the feed conversion ratio. Fecal, molt exoskeletons and dead shrimps were removed every day before the feeding period.

At the end of the experiment (40 days), the shrimp were counted, sized and weighed to determine general growth parameters as: 1) IBW (g ind^{-1}): initial mean body weight; 2) FBW (g ind^{-1}): final mean body weight; 3) IGW (g ind^{-1}): individual mean body weight gain: (FBW-IBW); 4) WG (weight gain, %): 100 x (FBW-IBW)/IBW; 5) IBL (g ind^{-1}): initial mean body length; 6) FBL (cm ind^{-1}): final mean body length; 7) ILG (cm ind^{-1}): individual mean body length gain (FBL-IBL); 8) LG (length gain, %): 100 x (FBL-IBL)/IBL; 9) SGR (specific growth rate, $\%day^{-1}$): 100 x [ln(FBW)-ln(IBW)]/days; 10) FCR (feed conversion ratio): total feed consumed / total weight gain; and 11) survival (%): 100 x (final shrimp number)/(initial shrimp number). The shrimp size was measured using the length from rostrum to telson.

Water quality parameters such as temperature, pH, and dissolved oxygen (oximeter, YSI) were measured daily. Ammonia, nitrite, and nitrate were monitored once every 10 d with a test kit (Hach model DR/890).

Enzymes activities

Shrimp hepatopancreas (HP) were removed after 24-h of starvation, frozen immediately in liquid nitrogen, lyophilized, and stored at 5ºC. Pools of three hepatopancreas per replicate were homogenized 1:10 w/v in ice-cold 50 mM Tris-HCl buffer, pH 7.5 (35 mg mL^{-1}), centrifuged at 16,000 *g* for 15 min at 5°C, and their cold HP supernatant (HPs) used for evaluation of enzymatic activity a microplate spectrometer. Protein level of HPs pool samples were evaluated by Bradford method with bovine serum albumin (1 mg mL^{-1}) as the standard protein.

Alkaline protease activity of HPs samples was evaluated using casein substrate (0.5%) in 50 mM Tris/HCl buffer, pH 9.0. The mixture was incubated at 37°C for 30 min and reaction was stooped with trichloroacetic acid (TCA) at 20%. The samples were placed for 60 min at 4°C, and their absorbance evaluated at 280 nm. One unit of enzyme activity was defined as 1 µg of tyrosine min^{-1} at a coefficient molar extinction (CME) of 0.005 mL μg^{-1} cm^{-1} (Martínez *et al.*, 1999; Alvarez-González *et al.*, 2006; Perera *et al.*, 2008).

Leucine aminopeptidase of HPs samples were determined using leucine p-nitroanilide as substrate (0.1 mM in DMSO) diluted in sodium phosphate buffer (50 mM, pH 7.2). The mixture was incubated at 25°C for 30 min and reactions were stopped with acetic acid (30%). The samples were placed for 60 min at 4°C, and their absorbance evaluated at 410 nm. One unit of enzyme activity was defined as 1 µg of nitroanilide min^{-1} at a CME of 8.2 mL μmol^{-1} cm^{-1} (Cuenca-Soria *et al.*, 2014)

Lipase activity of HPs samples were determined using β-naphtyl caprylate (0.1 mM in DMSO) as substrate. The mixture (30 µL 100 mM sodium taurocholate, 570 µL 50 mM Tris·HCl at pH 8, 6 µL

enzyme extract, and 6 µL 200 mM β-naphthyl caprylate) was incubated at 25°C for 30 min. Then 6 µL of 100 mM fast blue BB dissolved in DMSO was added. The reaction was stooped with TCA at 0.72 N. The samples were placed for 60 min at 4°C, and their absorbance evaluated at 540 nm. One unit of activity was defined as 1 µg of naphtol min^{-1} at a CME of 0.02 mL μg^{-1} cm^{-1} (Alvarez-González *et al.*, 2006; Rivera-Pérez *et al.*, 2010).

The α-amylase activity of HPs samples was carried using soluble starch (1%) as substrate in Tris-HCl buffer 50 mM pH 7.5. The mixture was incubated at 25°C for 30 min. For revelation, sodium carbonate (2N) and DNS reactive were added. The reaction was stopped by boiling for 15 min, and their absorbance evaluated at 550 nm. One unit of activity was defined as the amount of enzyme able to produce 1 µg of maltose min^{-1} (Martínez *et al.*, 1999; Alvarez-González *et al.*, 2006).

Alkaline phosphatase activity of HPs samples were determined using 4-nitrophenyl phosphate in acid citrate buffer (pH 5.5) as substrate (w/w). The mixture was incubated at 25°C for 30 min, and reaction was stooped with 0.05 N NaOH. The samples were placed for 60 min at 4°C, and their absorbance evaluated at 405 nm. One unit of activity was defined as 1 µg of nitrophenyl released min^{-1} at a CME of 0.0185 mL μg^{-1} cm^{-1} (Alvarez-González *et al.*, 2006).

Specific activity of extracts was determined according next equations: Units per mL = [(Δabs x reaction final volume (mL)] / (CME x min) x extract volume (mL), and Units per mg of protein = units per mL/mg of soluble protein.

Statistical analysis

Results were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple range tests ($P < 0.05$). Digestive enzymes activities between treatments were analyzed with Kruskal-Wallis test. Those results were performed using the Statistical software (version 7.0).

RESULTS

Shrimp diet and bioassay

The manufacture pellets with experimental ingredient (0, 0.25, 0.5, 1.0 and 2.0 g kg^{-1} of NTF of basal diet) display an uniform texture, color, 6 mm of length size, and 3 mm of diameter. The pellets stability ranged from 84.42 ± 0.06 to 85.94 ± 0.05% at low-salinity water (S = 5), where a significant ($P < 0.05$) lower stability was observed with 0.5 and 1.0 g kg^{-1} of NTF of basal diet (T2 and T3) compared to others diets 0.25 and 2.0 g kg^{-1} of NTF of basal diet (T1 and T4). The higher stability value was detected with T1. Water quality parameters show low variation, the water temperature during shrimp experiment ranged from 24.3 to 27.5°C, dissolved oxygen from 5.7 to 6.3 mg L^{-1}, and pH from 7.1 to 7.4. Total ammonia ranged from 0.12 to 0.34 mg L^{-1}, nitrite from 0.25 to 0.33 mg L^{-1}, and nitrate from 5.13 to 19.23 mg L^{-1}. Salinity was 5 along shrimp experiment (40 days).

The general growth parameters (means ± SD) of juvenile shrimp (*L. vannamei*) are detected in Table 1, where a not significant difference was found in IBW and IBL ($P > 0.05$) for all treatment. Production results showed a significant increase ($P < 0.05$) for FBW, IWG, WG, and FCR in shrimp treatments with 1.0 and 2.0 g kg^{-1} of NTF of basal diet (T3 and T4, Table 1) compared to others experimental treatments (CD, T1, and T2), where a best result was observed in the higher NTF incorporation level. Other general growth parameters show a not significant difference among treatments ($P > 0.05$). However, treatments 3 and 4 display the best values. The survival of juvenile shrimp cultured in low-salinity water fluctuated from 96 to 100% (Table 1) without significant difference between treatments ($P > 0.05$).

Digestive enzymes activities

The activity levels of the hepatopancreas digestive enzymes of juvenile shrimp (*L. vannamei*) cultured in low-salinity water are observed in Figure 1. A clear variation in the activity of all enzymes was observed. The alkaline protease show an activity of 142.9-188.7 U mg $protein^{-1}$, lipase of 191.8-383 U mg $protein^{-1}$, and α-amylase of 44.7-84.1 U mg $protein^{-1}$. The treatment 2 displays the higher values and treatments 3 and 4 the lowest (Fig. 1). In all cases, not significant differences ($P > 0.05$) were detected between treatments. Both, lipase and α-amylase display a similar activity (Fig 1). The level of alkaline phosphatase displayed values of 0.31-0.55 U mg $protein^{-1}$ and leucine aminopeptidase 0.15-0.29 U mg $protein^{-1}$ (Fig. 1), where higher levels are detected in treatment 2. No significant values were detected for the rest of the digestive enzymes ($P > 0.05$).

DISCUSSION

The shrimp aquaculture has been a very significant alternative to increase the shrimp production for human consumption. The opportunity to increase the development of shrimp aquaculture is evident (Pérez-Castañeda *et al.*, 2015). *L. vannamei* show a high tolerance to a wide range of salinity from 1 to 50 (Ponce-Palafox *et al.*, 1997). Recently, some shrimp farms grow *L. vannamei* in low-salinity water conditions (2-15) (Laramore *et al.*, 2001), where *L. vannamei* is a species with similar growth from low to marine salinity (Rosas *et al.*, 2001; Ortega-Salas &

Table 1. Growth parameters (means ± SD) of juvenile shrimp *L. vannamei* cultured in low-salinity and feeding with *Yucca schidigera* and *Quillaja saponaria* (40 days). Values within the same row with different letters are significantly different ($P < 0.05$). Initial mean body weight (IBW), final mean body weight (FBW), individual weight gain (IWG), weight gain (WG), initial mean body length (IBL), final mean body length (FBL), individual length gain (ILG), length gain (LG), specific growth rate (SGR), feed conversion ratio (FCR), and survival. (a) Letters in same line indicate significantly different subsets as defined by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple range tests ($P < 0.05$).

General growth parameters	Shrimp treatment (g kg^{-1} of NTF basal diet)				
	Control (0)	T1 (0.25)	T2 (0.5)	T3 (1.0)	T4 (2.0)
IBW (g ind^{-1})	2.67 ± 0.17	2.56 ± 0.12	2.60 ± 0.09	2.61 ± 0.15	2.68 ± 0.08
FBW (g ind^{-1})	11.64 ± 0.81	12.04 ± 1.15	11.78 ± 0.50	13.63 ± 0.18[a]	13.46 ± 0.34[a]
IWG (g ind^{-1})	8.97 ± 0.97	9.48 ± 1.21	9.18 ± 0.53	11.02 ± 0.33[a]	10.78 ± 0.29[a]
WG (%)	338.95 ± 58.96	371.80 ± 57.82	354.20 ± 28.88	424.29 ± 36.31[a]	402.35 ± 10.01[a]
IBL (cm ind^{-1})	7.41 ± 0.17	7.31 ± 0.11	7.32 ± 0.09	7.33 ± 0.09	7.42 ± 0.11
FBL (cm ind^{-1})	12.03 ± 0.35	12.16 ± 0.42	12.14 ± 0.22	12.32 ± 0.05	12.24 ± 0.17
ILG (cm ind^{-1})	4.63 ± 0.50	4.85 ± 0.46	4.82 ± 0.21	4.99 ± 0.13	4.82 ± 0.09
LG (%)	62.58 ± 8.17	66.36 ± 6.84	65.86 ± 3.02	68.07 ± 2.54	64.93 ± 1.09
SGR (% day^{-1})	3.68 ± 0.33	3.87 ± 0.32	3.78 ± 0.16	4.14 ± 0.18[a]	4.03 ± 0.05[b]
FCR	1.53 ± 0.06	1.31 ± 0.07	1.31 ± 0.05	1.10 ± 0.07[a]	1.22 ± 0.04[a]
Survival (%)	96.60 ± 0.58	96.60 ± 0.58	96.60 ± 0.58	100 ± 0.00	100 ± 0.00

Rendón, 2013; Pérez-Castañeda *et al.*, 2015). Unfortunately low information is detected about the culture effect on general growth parameters, physiological responses, and digestive enzyme activity from shrimp hepatopancreas.

All ingredients used in shrimp diet affect the stability of water quality and this factor shows an important effect on general growth parameters of shrimp. The shrimp feed requires a minimum of an hour of stability into the water and during this period the pellets should maintain their integrity with a minimum of nutrient leaching (Subramanyam, 1994), as shrimp are benthic feeders and slow to recognize the food source. Leaching of aquaculture diets may result in the loss of nutrients, increase water contamination, and economic losses (Cruz *et al.*, 2002). Cruz-Suárez *et al.*, (2008) report losses of dry matter near to 15-17% when different additives have been used in shrimp diets and without significant effect on shrimp growth. The shrimp diet was elaborated with NFT inclusion of 0, 0.25, 0.5, 1.0 and 2.0 g kg^{-1} of NTF basal diet. All experimental diets in this study display stability from 85-86% did not showed significant difference ($P > 0.05$) in diets with 0 and 2.0 g kg^{-1} of NTF basal diet. Those suggest that inclusion of the NFT additive in the shrimp diet did not reduce significantly this feed parameter (stability) when shrimp diet is used in low-salinity water. However, the use of this type of water for the stability test probably increases the loss of matter of shrimp diet and this is an important characteristic for future diet formulation.

Previous studies about the use of *Y. schidigera* and *Q. saponaria* as feed additives for fish and shrimp organisms from aquaculture industry are limited (Castillo-Vargasmachuca *et al.*, 2015; Qi-Hui *et al.*, 2015). In the present study, the NTF supplementation in shrimp diet presented a significant increase ($P < 0.05$) of the FBW, IWG, and WG, also a decrease of the FCR in shrimp, where 1.0 and 2.0 g kg^{-1} of NTF of basal diet show the best values (Table 1). Other studies show similar effect. For example, Valle *et al.* (2006) shows an increase of 23% in growth and 26% in production, with the use of *Q. saponaria* in *L. vannamei* diets (1.5 g of Hibotec®/kg of diet). Similarly, effects of inclusion of *Y. schidigera* extract in the diet of *L. vannamei* were evaluated by Martínez *et al.* (2008) where 2 g kg^{-1} of diet of yucca extracts significant increases the shrimp growth compared to control diets. Also, the FCR show a 1.6 value (3 g kg^{-1} of diet of yucca extract) compared to control group (2.32 values). In this study, the *Y. schidigera* and *Q. saponaria* use in shrimp diet display a low FCR (1.1-1.31) in all treatments compared to control diet (1.53) (Table 1). Present research show an effect of *Yucca schidigera* and *Q. saponaria* on general growth parameters (FBW, IWG, WG, FCR, etc.) of *L. vannamei* cultured at low-salinity. The increase of growth and reduction of FCR in response to *Y. schidigera* and *Q. saponaria* supplementation (1-2 g kg^{-1} of extract doses) in the diet may be related to increased protein synthesis, promotion of nutrient absorption in epithelial cells of digestive tract that help in the nutrient absorption, cell membrane per-

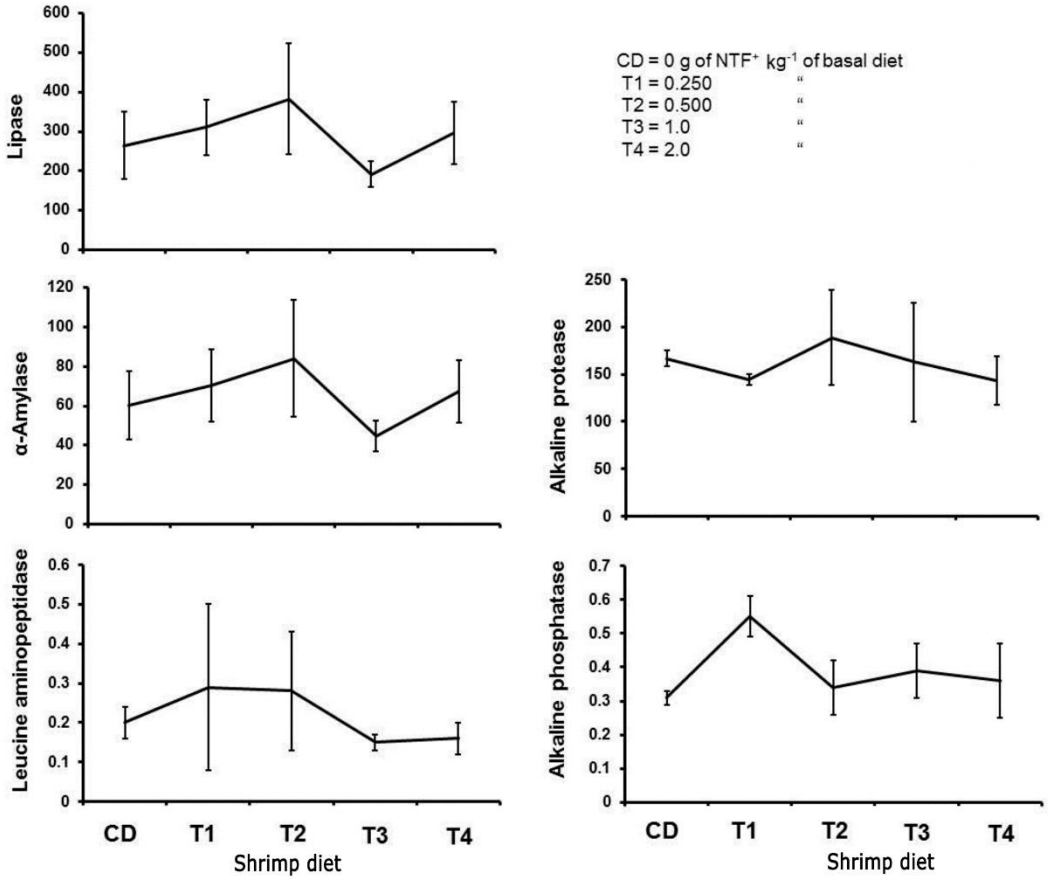

Figure 1. Juvenile shrimp (*L. vannamei*) hepatopancreas enzyme activity (means ± SD), cultured in low-salinity and feeding with *Y. schidigera* and *Q. saponaria* (40 days).

meability to amino acid and other nutrient as suggest Francis *et al.* (2005), Citarasu (2010), Güroy *et al.* (2014) and Qi-Hui *et al.* (2015). Best uses of feed diet generate a reduction of organic matter discharge from shrimp excretion with a probable decrease of ammonia, nitrate and nitrite levels in culture water. Similar effects were observed for Qi-Hui *et al.* (2015) and Castillo-Vargasmachuca *et al.* (2015) were use *Y. schidigera* extract on water quality and survival of *Lutjanus peru* and *L. vannamei*.

The shrimp hepatopancreas is the principal digestive gland and is a sensitive indicator for shrimp metabolism, where digestive enzyme activity plays an important role in nutritional physiology and shrimp growth. A clear variation in the activity of all enzymes from juvenile shrimp (*L. vannamei*) cultured in low-salinity water (S = 5) was observed, where treatment 2 display the higher values of alkaline protease, lipase, and α-amylase compared to other treatments (Fig. 1). However, the addition of *Y. schidigera* and *Q. saponaria* (Nutrafito plus^{TM}) in shrimp diet not affect, significantly, the hepatopancreatic enzyme activity ($P > 0.05$) (Fig. 1). Qi-Hui *et al.* (2015) reported a significantly increase of hepatopancreatic protease activity ($P < 0.05$) without effect on lipase and amylase when use *Y. schidigera* in *L. vannamei* diet (0.2 and 0.3%). The values of alkaline protease activity detected in present study (142.9-188.7 U mg protein^{-1}) were similar to that observed for Qi-Hui *et al.* (2015) (136.4-151.1 U mg^{-1}). Gamboa-Delgado *et al.* (2003) reported lipase value in shrimp juvenile of *L. vannamei* (10-12 g) near to 100 U mg protein^{-1}, which is low value to detect in present document (191.8-383 U mg protein^{-1}) for same shrimp weight. A suggested mechanism of action of saponins from plants as *Y. schidigera* and *Q. saponaria* include helping of intestinal absorption of dietary amino acid and fatty acid obtained after enzymatic digestion.

The increase of general growth parameter and FCR reduction reported in present document suggest that *Y. schidigera* and *Q. saponaria* supplementation in shrimp diet may affect those parameters. Probably, those are associated to increase the protein synthesis, promotion of nutrient absorption in epithelial cells of

digestive tract, or cell membrane permeability to amino acid and other nutrients as suggest Francis *et al*. (2005), Citarasu (2010), Güroy *et al.* (2014) and Qi-Hui *et al.* (2015). Also, may related with the low-salinity water which affect the enzyme activity as compensatory alternative to energy loss for osmoregulation, or could be associated to a possible strategy to take advantage of the capacity of obtaining energy from amino acids metabolism as suggest by Li *et al.* (2008) that report a higher enzyme activity from hepatopancreas of *L. vannamei* cultured at compared to other treatments (salinity of 17 and 32).

Further studies about the use of *Y. schidigera* and *Quillaja saponaria* on shrimp production (pilot or commercial scale) are recommended to determine the viability of those products, and their properties on growth, immunostimulation, and intestinal gut cells of *L. vannamei*.

ACKNOWLEDGMENTS

The PhD grant was funded by the Programa de Mejoramiento del Profesorado (SEP-Promep) Universidad Autónoma de Tamaulipas. Also, the University of Arizona supported this work with technical assistance and installation.

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Received: 11 July 2015; Accepted: 29 November 2015

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