Lat. Am. J. Aquat. Res., 43(3): 435-445, 2015
DOI: 10.3856/vol43-issue3-fulltext-5

Research Article

Growth and metabolic responses of whiteleg shrimp *Litopenaeus vannamei* and Nile tilapia *Oreochromis niloticus* in polyculture fed with potential probiotic microorganisms on different schedules

Juan P. Apún-Molina[1, 2], Apolinar Santamaría-Miranda[2], Antonio Luna-González[2] José C. Ibarra-Gámez[3], Vladimir Medina-Alcantar[4] & Ilie Racotta[5*]

[1]Programa de Doctorado en Ciencias en Biotecnología del Instituto Tecnológico de Sonora Ciudad Obregón, Sonora 85000, México

[2]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-IPN Unidad Sinaloa, Boulevard Juan de Dios Bátiz Paredes 250, Guasave, Sinaloa 81101, México

[3]Laboratorio de Sanidad Acuícola, Instituto Tecnológico de Sonora, Ciudad Obregón, Sonora 85000, México

[4]Universidad de Occidente, Campus Guasave, Sinaloa 81200, México

[5]Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California Sur 23096, México

Corresponding author: Ilie S. Racotta (iracotta@cibnor.mx)

ABSTRACT. Probiotics and co-culture of shrimp and tilapia are two strategies to improve yield and eco-efficiency of both species. However, only few studies have analyzed the combination of these two strategies. This study analyzes the effect of a mixture of potential probiotics supplied in the diet at different frequencies in a system of shrimp (10 m^{-2}) and tilapia (5 m^{-2}) in a trial lasting 84 days. The probiotics mixture was composed of four lactic acid bacteria and one yeast strain that were supplied either daily, every 5 days, or every 10 days in the diet and compared with a control without any supply of probiotics. At the end of the experiment, blood chemistry and hematology of shrimp and tilapia were analyzed as an index of physiological condition. Water quality did not differ between treatments. The final weight, feed conversion ratio, and yield of shrimp were significantly higher with daily supply of probiotics compared to shrimp that did not receive probiotics supply, with intermediate values for treatments with probiotics supply every 5 or 10 days. However, level of supplementation every 5 days could be considered as optimal because no significant differences with daily supply were observed for any variable, whereas the supply each 10 days resulted in a significantly lower yield. Significantly higher final weight, feed conversion ratio, and yield of tilapia occurred with daily supplements. Blood cholesterol in tilapia significantly decreased with increasing frequency of probiotics supplementation. These results indicate that probiotics supplements for shrimp and tilapia cultivated simultaneously improved yields and physiological condition.

Keywords: *Lipenaeus vannamei*, *Oreochromis niloticus*, shrimp, tilapia, probiotics, blood chemistry, growth.

Crecimiento y respuesta metabólica del camarón blanco *Litopenaeus vannamei* y tilapia del Nilo *Oreochromis niloticus* en policultivo alimentado con microorganismos probióticos potenciales en diferentes frecuencias

Los probióticos y el co-cultivo de camarón y tilapia son estrategias para mejorar el rendimiento y la eco-eficiencia de ambas especies. Sin embargo, pocos estudios han analizado la combinación de estas dos estrategias. Este artículo analiza el efecto de una mezcla de probióticos potenciales suministrados en la dieta, en diferentes frecuencias, en un cultivo de camarones (10 m^{-2}) y tilapia (5 m^{-2}) durante 84 días. La mezcla de probióticos, compuesta de cuatro bacterias ácido lácticas y una cepa de levadura, fue suministrada cada 24 h, cada 5 días y cada 10 días, en la dieta y fueron comparados con un grupo control sin probióticos. Al final del experimento, se analizaron los resultados de la química sanguínea y la hematología del camarón y la tilapia. No se observaron diferencias significativas en la calidad del agua entre los cuatro tratamientos. Los valores de peso final, factor de conversión alimenticia y rendimiento de camarón fueron significativamente mayores con el suministro diario de los probióticos en comparación a los camarones del grupo control, con valores intermedios para los tratamientos con probióticos suministrados cada 5 o 10 días. Sin embargo, el suplemento ofrecido cada 5 días podría

Corresponding editor: Ronaldo Cavalli

considerarse como óptimo, dado que no se observaron resultados significativamente diferentes al suministro cada 24 h, mientras que el suministro cada 10 días fue significativamente inferior. El peso final, conversión alimenticia y rendimiento de tilapia fueron significativamente mayores con el suplemento cada 24 h. El colesterol en la tilapia se redujo significativamente al aumentar la frecuencia del suplemento de probióticos. Estos resultados indican que los probióticos mejoran los rendimientos y la condición fisiológica de camarones y tilapias en co-cultivo.

Palabras clave: *Lipenaeus vannamei*, *Oreochromis niloticus*, camarón, tilapia, probióticos, química sanguínea, crecimiento.

INTRODUCTION

Shrimp farming is the most developed segment of the aquaculture industry in Mexico, with a total production of 109,815 ton in 2011 (CONAPESCA, 2012). Tilapia consumption in Mexico greatly increased in recent years, greatly exceeding national fisheries and aquaculture production, and therefore requires large imports from Asia. A national priority for tilapia aquaculture was recognized, estimating that production could increase 10-fold in the next 10 years, reaching 160,000 ton by 2020 (Panorama Acuícola Magazine, 2010). Shrimp polyculture in Mexico is not a common practice among farmers. It represents an important alternative to solving and/or minimizing some of the problems (environmental pollution, diseases, and decreasing prices), that shrimp aquaculture has encountered in the past two decades. The polyculture of marine shrimp and tilapia is a common practice in some countries, such as The Philippines, where tilapia is used to control particular bacterial diseases (Cruz *et al*., 2008). Although the most common practice is to grow tilapia in reservoirs, where the water is pumped to shrimp ponds, co-existence in the same pond was also tested, providing better results because a better plankton profile and reduced accumulation of waste was achieved (Cruz *et al*., 2008). Such integrated systems provide control of phytoplankton, less accumulation of organic matter, improves P and N conversion rate and decreases viruses load (Rodríguez-Grimón, 2003).

Probiotics in polyculture systems may serve as an alternative for preventing disease and producing higher growth rates. However, less is known about the effects of probiotics on integrated culture systems involving two or more productive species (Jatobá *et al*., 2011). On a single-species basis, improved yields from probiotics in fish or shrimp cultivation have been reported, based on positive effects included promotion of growth, stimulation of immune system, and reduced incidence of diseases (for reviews, see Balcázar *et al*., 2006; Welker & Lim, 2011). The particular microbial strains that we used in the present work, considered as potentially probiotic (PPB), increased resistance to environmental stress events in Nile tilapia *Oreochromis niloticus* (Apún-Molina *et al*., 2009), and increased survival to white spot syndrome virus (WSSV) exposure in whiteleg shrimp *Litopenaeus vannamei* (Peraza-Gómez *et al*., 2009).

Recent studies indicate that, besides performance during culture, biochemical variables in hemolymph and tissues of shrimp are associated with overall physiological condition (Rosas *et al*., 2004). Protein, hemocyanin, and glucose levels in the hemolymph are associated with the nutritional status of shrimp. Acylglycerides and cholesterol can be used as indicators of food quality (Pascual *et al*., 2003) Protein levels are also related to the immune response (Perazzollo *et al*., 2002; Mercier *et al*., 2006), whereas glucose and lactate levels represents the most typical stress response (Racotta & Palacios, 1998; Racotta *et al*., 2002; Mercier *et al*., 2009). Even if some of the studies provided evidence and suggested that biochemical composition of hemolymph or blood can be used as indicators of physiological performance, there are no practical approaches ever been tested.

The purpose of this study was to determine the influence of probiotics supplied at different schedules on growth, survival, and associated hemolymph and blood biochemical composition of shrimp and tilapia, when fed probiotics at different intervals during joint culture.

MATERIALS AND METHODS

Selection of strains of microorganisms and supply trough feed

The probiotics mixture used in this work included four lactic acid bacteria strains (*Pediococcus parvulus* Lta2, *Pediococcus parvulus* Lta6, *Pediococcus parvulus* Lta8, *Pediococcus parvulus* Lta10) and the yeast (*Candida parapsilosis* Lta6) as potential probiotics (PPB). All of them were originally isolated from the intestine of apparently healthy Nile tilapia *O. niloticus* cultivated at Centro Interdisciplinario de Investigaciones para el Desarrollo Integral Regional-Instituto Politécnico Nacional (Apùn-Molina *et al*., 2009) and identified by Luna-González *et al.* (2013).

The mixture of PPB was sprayed on commercial tilapia and shrimp pellets (32% protein), both from

Purina® (Ciudad Obregón, Mexico), at a concentration of 500,000 CFU g^{-1} (100,000 CFU/strain). A feed additive, Dry Oil® (DO, Innovaciones Acuícolas S.A. de C.V., Culiacán, Mexico) was used as an adhesive and a feed attractant, following the manufacturer's instructions. Feed was dried at room temperature (26 ± 1°C) for 5 h and stored at 4°C for 10 days, with an initial concentration of bacteria in the feed of 500,000 CFU g^{-1} and a decrease to 400,000 by day 10 (Apún-Molina *et al*., 2009).

Experimental design

Tilapia *O. niloticus* specimens (initial weight 6.2 ± 0.08 g) were obtained from the Aquaculture Laboratory of CIIDIR-IPN (Guasave, Sinaloa, Mexico), and shrimp *L. vannamei* (initial weight 2.5 ± 0.06 g) were obtained from a commercial hatchery. Both species were acclimated to salinity of 5 and stocked at density of 10 shrimp m^{-2} and 5 fish m^{-2} in 1000 L tanks, supplied with aerated water. Based on the supply frequency of PPB, the bioassay was designed with four treatments in triplicate: D/1 (daily supply), D/5 (supplied every 5 days), D/10 (supplied every 10 days), and DX (control, without bacteria supplementation). Dry oil without PPB sprayed on DX treatment, as well as on D5 and D10 treatments on days not supplemented with PPB.

The experiment conducted for 84 days, with 30% water exchange weekly for the first 30 days and 50% for the rest of the experiment. Shrimp and fish fed at the rate of 3% of the body weight. Shrimp and tilapia were grown under natural photoperiod (average 12 h light: 12 h dark cycle), ambient temperature, and salinity of 5. During the experiment, pH, temperature, salinity, and dissolved oxygen were measured daily. Ammonia, nitrites, nitrates, and phosphates were analyzed twice monthly using the method described by Strickland & Parsons (1972).

Relative and absolute growth and feed conversion ratio were calculated with the following equations:

The absolute daily growth rate (AGR) estimated for each treatment as a function of $W_f - W_0$ over time (T) where: W_f and W_0 is the final and initial weight, respectively, and T is the number of days in the experimental period.

Specific growth rates (SGR) = $[(\ln W_f - \ln W_0) / T)] \times 100$, where T is the duration of the experiment, W_0 is the weight of the animals at the beginning of the experiment, and W_f is the final weight of the animals at the end of the trial. Feed conversion ratio (FCR) = $F / (W_f - W_0)$, where F is the weight of feed supplied to the fish and shrimp during the trial (Ziaei-Nejad, 2006).

Sampling and analyses of metabolic variables in shrimp

Metabolic response of shrimp (n = 15 per treatment, 5 per replicate) was determined in hemolymph. The hemolymph (200 µL) was sampled individually, from the ventral sinus at the base of the first abdominal segment with a 3 mL syringe rinsed with cooled 5% potassium oxalate in isotonic saline anticoagulant solution (Mercier *et al.*, 2006). Hemolymph, centrifuged at 800 g for 10 min at 4°C, and plasma separated and stored at -75°C for further analyses. All shrimp used in the analysis were in the intermolt or early premolt stages. Lactate, glucose, and triglyceride concentrations were analyzed from plasma, using commercial kits: lactate (PAP, Randox Laboratories, Antrim, UK), glucose (GOD-PAP, Roche Diagnostics, Basel, Switzerland), triglycerides (GPO-PAP, Randox Laboratories). Determinations were adapted to microplates using 20 µL samples and 200 µL enzyme chromogen reagent (Racotta & Palacios, 1998). Optical density recorded with a microplate reader. Concentrations calculated from a standard solution of substrates. Total proteins were determined according to Bradford (1976), using a protein dye reagent concentrate and hemocyanin by direct absorbance at 335 nm (Hagerman, 1983).

Sampling and analyses of metabolic and hematological variables of tilapia

Blood samples collected from all fish that survived were placed in tubes containing either lithium heparin for biochemical analysis or EDTA for hematologic analysis, and used for determining erythrocyte count; hematocrit and hemoglobin content (Dacie & Lewis, 1984). For biochemical analyses, plasma was obtained by centrifugation of blood at 800 g for 15 min, separated, and stored at -75°C for later analyses of triacylglycerides, glucose, total protein, and cholesterol, as described for shrimp samples.

Statistical analyses

A one-way analysis of variance (ANOVA) was used to examine differences between treatments. A Newman-Keuls HSD multiple comparison tests were used to determine significance among individual groups identifying these differences ($P < 0.05$).

RESULTS

Water quality

Water quality variables did not differ among treatments. Temperature decreased from 28.8°C in

August to 23.2°C in November. Salinity was maintained at 5, while pH ranged between 8.17 and 8.38, and dissolved oxygen from 7.21 to 8.43 mg L^{-1}. Levels of ammonia (0.0225 mg L^{-1}), nitrites (0.145 mg L^{-1}), nitrates (0.124 mg L^{-1}), and phosphates (0.156 mg L^{-1}) were not affected by the supply of potencial probiotics (PPB) (Table 1).

Shrimp performance

The final weight and SGR of shrimp fed daily with PPB were significantly higher ($P < 0.05$) than in shrimp without PPB. However, we found similar results in shrimp fed with PPB every 5 and 10 days. Results showed that AGR of shrimp fed daily with PPB was significantly higher ($P < 0.05$) than shrimp without PPB and shrimp fed PPB every 10 days. FCR was significantly lower ($P < 0.05$) in shrimp fed daily with PPB, compared with shrimp without PPB and fed every 10 days. Survival was not significantly affected by the supply of PPB. Treatments D and D/5 had significantly higher ($P < 0.05$) final yields (kg ha^{-1}), compared to the DX and D/10 treatments (Table 2).

Plasma of shrimp fed with PPB every 5 days showed the highest concentration of glucose (Fig. 1a). No significant differences were observed between treatments for the concentration of triglycerides, total protein, and hemocyanin (Fig. 1).

Performance of tilapia

The final average weight, FCR, SGR, AGR, and yield were significantly higher ($P < 0.05$) in shrimp fed daily with PPB compared with shrimp without PPB and shrimp fed PPB every 5 and 10 days (Table 3). The supply of PPB did not significantly affected survival

No significant differences of hematological variables of tilapia were observed in relation to the dietary supply of PPB (Fig. 2). The highest concentration of cholesterol in tilapia blood occurred in tilapia that did not receive any PPB or were treated every 10 days as compared with tilapia without PPB ($P < 0.05$), with intermediate values for tilapia shrimp fed PPB every 5 days (Fig. 3a). The protein, glucose, and triglycerides concentration, in relation to the frequency of PPB dietary supply, were not significantly different (Fig. 3).

DISCUSSION

Performance of co-cultured shrimp and tilapia

Survival and growth rates were 87% and 0.185 g day^{-1}, respectively. Muangkeow *et al*. (2007) obtained similar results with survival ranging from 84.7-90.8%; whereas growth rate was between 0.21 and 0.24 g day^{-1}. After 8 weeks attaining a final weight of 13.4 to 16 g, when shrimp and tilapia were co-cultured in recirculation systems with shrimp and tilapia stocked in separate tanks, at different ratios of shrimp:tilapia (from 13:1 to 100:1, with a fixed initial density of shrimp of 40 m^{-2}). Under similar conditions as in the present study, in terms of shrimp and tilapia density in the same tanks (10 shrimp m^{-2} and 2 tilapia m^{-2} for 12 weeks), growth and survival rates were 0.1 g day^{-1} and 79.8%, respectively (Jatobá *et al*., 2011). In another study, also using a recirculation system, survival was 100% and daily growth rates ranged from 0.153 to 0.185 g day^{-1} at densities of shrimp: tilapia m^{-2} of 20:0 to 20:8, respectively (Hernández-Barraza *et al*., 2012). Another study obtained a lower yield, with 3-12 shrimp m^{-2} co-cultured with tilapia (2 tilapias m^{-2}). In this case, survival was ~40% and growth rate was from 0.07 to 0.13 g day^{-1}, with a final weight of 8.9-16.1 g after 124 days (Bessa-Junior *et al*., 2012).

In our study, survival of tilapia (86.7%) was similar to the 84.7-90.8% reported by Muangkeow *et al*. (2007), 70.6-83.1% depending on tilapia density (Thien *et al*., 2004), 97.7% (Jatobá *et al*., 2011), or 72-85%, depending on shrimp density (Bessa-Junior *et al*., 2012). The growth rates in this study were 0.8 g day^{-1} without PPB, which is lower than 1.3-1.5 g day^{-1} reported by Bessa-Junior *et al*. (2012), 1.9 g day^{-1} (Jatobá *et al*., 2011), 2.5-3.3 g day^{-1} (Thien *et al*., 2004), or 0.65-3.09 g day^{-1} (Muangkeow *et al*., 2007). It is unlikely that a higher tilapia–shrimp ratio could explain this low growth rate because higher ratios (1:1.5) gave better results (Bessa-Junior *et al*., 2012). In this study, the tilapia density was among the highest (5 tilapias m^{-2}) considering previous studies, for which the highest density was 4 tilapias m^{-2}, and corresponded to the poorest growth rate of 0.65 g day^{-1} (Muangkeow *et al*., 2007). Although Thien *et al*. (2004) reported that the same tilapia density of 4 m^2 grown with the giant tiger prawn *Penaeus monodon* yielded a growth rate of 2.5 g day^{-1}. A decrease in water temperature towards the end of the experiment could also explain the poor growth.

Although the most common practice is to rear tilapia in reservoirs from which water is pumped to shrimp ponds, co-existence in the same pond was also tested with even better results based on the assumption that a better plankton profile and that a reduction in waste accumulation are obtained (Cruz *et al*., 2008). Moreover, competition is minimal as each species uses particular niches: tilapia can filter feed on phyto-plankton and zooplankton in the upper water column, while shrimp spend most of the time in the pond bottom grazing on bacterial films on the bottom substrate and on settling detritus. Therefore, such integrated system will allow the control of phytoplankton growth, decrease the accumulation of organic matter, and reduce the prevalence of viruses (Rodríguez-Grimón, 2003).

Table 1. Effect of dietary supplementation of potential probiotics at different rates on levels nitrogenous and phosphorous compounds in water form a co-culture system in shrimp (10 m^{-2}) and tilapia (5 m^{-2}). Data reported as mean ± SEM of three replicates pooled from weekly determinations.

Concentration (mg L^{-1})	Treatment			
	Daily	Every 5 days	Every 10 days	Control
Ammonia	0.012 ± 0.006	0.022 ± 0.007	0.023 ± 0.003	0.033 ± 0.002
Nitrates	0.145 ± 0.057	0.155 ± 0.023	0.147 ± 0.032	0.133 ± 0.048
Nitrites	0.118 ± 0.032	0.138 ± 0.087	0.125 ± 0.042	0.115 ± 0.073
Phosphates	0.188 ± 0.037	0.175 ± 0.073	0.138 ± 0.045	0.123 ± 0.026

Table 2. Effect of dietary supplementation of potential probiotics at different rates on overall performance of shrimp *Litopenaeus vannamei* (10 m^{-2}) co-cultured with tilapia (5 m^{-2}). SGR: specific growth rate; AGR: absolute growth rate; FCR: feed conversion rate. Data reported as mean ± SEM of three replicates. Means not sharing the same superscripts are significantly different ($P < 0.05$). The yield values were extrapolated from kg m^{-2}.

Performance	Treatments groups			
	Daily	Every 5 days	Every 10 days	Control
Initial weight (g)	2.44 ± 0.10	2.55 ± 0.09	2.57 ± 0.11	2.69 ± 0.13
Final weight (g)	20.25 ± 0.30^{a}	18.75 ± 0.49^{ab}	18.55 ± 0.47^{ab}	17.41 ± 0.506^{b}
SGR (% day^{-1})	2.63 ± 0.061^{a}	2.37 ± 0.043^{ab}	2.35 ± 0.094^{ab}	2.27 ± 0.084^{b}
AGR (g day^{-1})	0.215 ± 0.004^{a}	0.193 ± 0.004^{ab}	0.189 ± 0.006^{b}	0.185 ± 0.011^{b}
FCR	1.19 ± 0.017^{a}	1.28 ± 0.032^{ab}	1.50 ± 0.053^{c}	1.42 ± 0.057^{bc}
Survival (%)	96.0 ± 5.8	95.6 ± 3.3	92.3 ± 6.7	92.0 ± 5.8
Yield (kg ha^{-1})	2025.3 ± 30.47^{a}	1875.7 ± 48.44^{a}	1676.7 ± 55-81^{b}	1667.6 ± 65.94^{b}

Effects of PPB on shrimp and tilapia performance

For FCR and final yield, there is a beneficial effect of PPB on growth of shrimp and tilapia. Although, the treatment with daily supply presented the best values of performance, we could consider that optimum rate of supplementation for shrimp was every 5 days, because there was no significant difference for any variable with supplementation on a daily basis. Supplementation every 10 days resulted in significantly lower yields. In contrast, for tilapia the daily supplementation was the optimum rate as final yield was significantly higher by 33% than the rate of 5 days. A stronger link between frequency of PPB supplementation and higher benefits for tilapia than for shrimp is likely tied to the origin of these PPB strains, which derive from tilapia digestive tract (Apún *et al*., 2009). The benefits of administering PPB in the diet on culture performance has been widely documented for shrimp (Ziaei-Nejad *et al*., 2006; Wang, 2007; Li *et al.*, 2008; Far *et al*., 2009) and tilapia (El-Haroun *et al*., 2006; Wang *et al*., 2008; Welker & Lim, 2011). Few studies have examined the benefits of probiotics when the two species are mutually cultured. Jatobá *et al.* (2011) did not find any improvement in shrimp yields, but did find an increase in final weight, feed efficiency, and final yield in tilapia, when fed with *Lactobacillus plantarum* at a rate of four times daily, which was more frequent than our D group. For the Malaysian prawn *Macrobrachium rosenbergii* co-cultured with tilapia, the addition of *Bacillus subtilis* did not result in any improvement for either species in terms of growth rate and feed conversion efficiency (Günther & Jiménez-Montealegre, 2004).

The mixture of PPB used in our study was previously characterized (Apún-Molina, 2007) and showed important benefits for tilapia growth (Apún-Molina *et al*., 2009) and reduction in mortality of shrimp exposed to WSSV (Peraza-Gómez *et al*., 2009, 2011) or *Vibrio sinaloensis* (Flores-Miranda *et al*. 2011). Our study highlights the beneficial effect of this particular PPB mixture on growth of jointly cultured tilapia and shrimp. Flores-Miranda *et al*. (2011) observed the importance of frequency of administration for which protection against *V. sinaloensis* and associated hemocyte count was effective with daily or every 3 days -supplementation, but not every 6 days.

Biochemical parameters in hemolymph of shrimp and blood of tilapia

It is recognized that the beneficial role of probiotics has several physiological bases at different levels. This includes digestive physiology, immune function, and stress response in shrimp (Rengpipat *et al*., 2000; Wang, 2007; Liu *et al*., 2010; Zokaeifar *et al*., 2012)

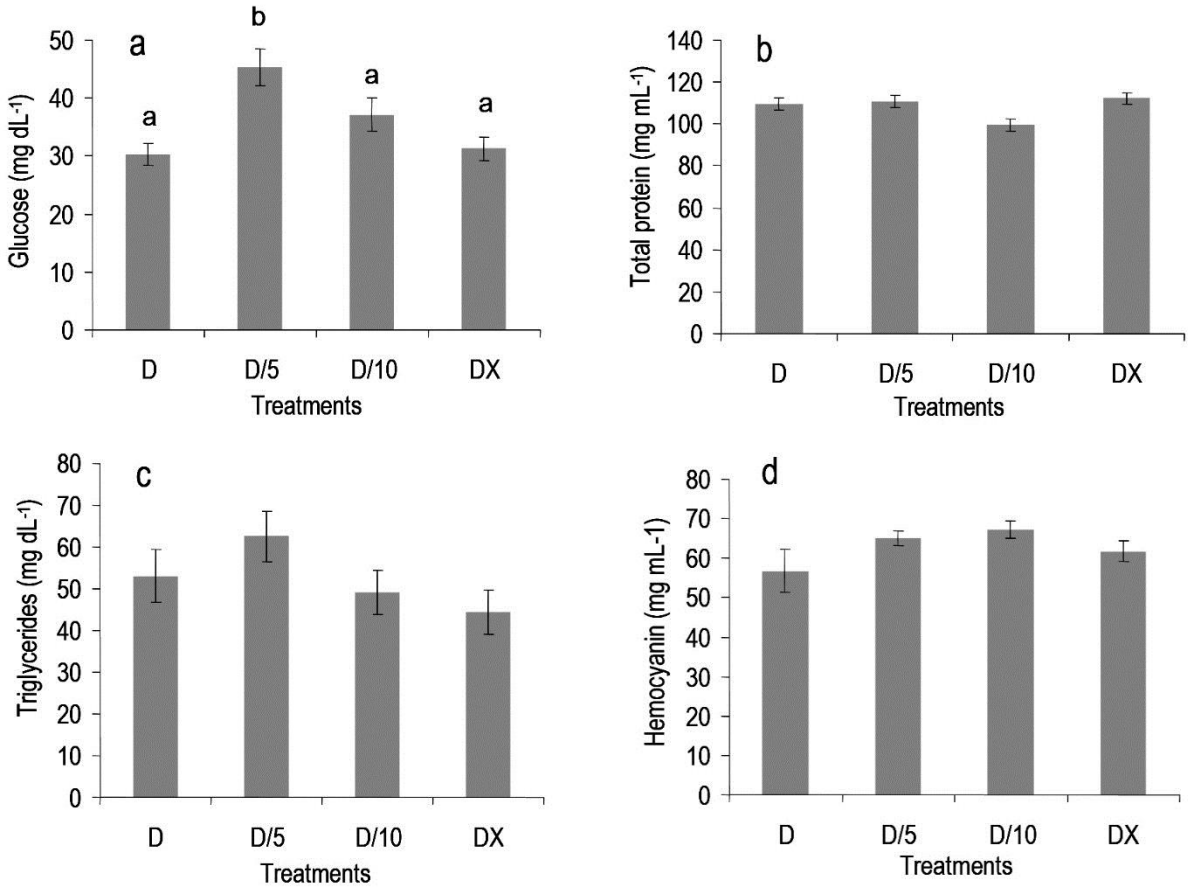

Figure 1. Biochemical variables in hemolymph in shrimps co-cultured with tilapia and fed with dietary supplementation of potential probiotics at different rates. a) Glucose, b) total protein, c) triglycerides, d) hemocyanin. D: Daily supply, D/5: every 5 days supply, D/10: every 10 days supply and DX: Control without any supply. Data reported as means ± SEM of 15 organisms. Means not sharing the same superscripts are significantly different ($P < 0.05$).

Table 3. Effect of dietary supplementation of potential probiotics at different rates on overall performance of tilapia *Oreochromis niloticus* co-cultured whit shrimp. SGR: specific growth rate, AGR: absolute growth rate, FCR: feed conversion rate. Data reported as means ± SEM of three replicates. Means not sharing the same superscripts are significantly different ($P < 0.05$). The yield values were extrapolated from kg m^{-2}.

Performance	Treatments groups			
	Daily	Every 5 days	Every 10 days	Control
Initial weight (g)	6.25 ± 0.43	6.22 ± 0.34	6.12 ± 0.37	6.11 ± 0.38
Final weight (g)	115.6 ± 10.71^{a}	76.93 ± 9.15^{b}	79.90 ± 1.26^{b}	72.97 ± 1.065^{b}
SGR (% day^{-1})	3.44 ± 0.11^{a}	2.97 ± 0.12^{b}	2.99 ± 0.13^{b}	3.06 ± 0.06^{b}
AGR (g day^{-1})	1.30 ± 0.13^{a}	0.84 ± 0.11^{b}	0.88 ± 0.02^{b}	0.80 ± 0.02^{b}
FCR	1.22 ± 0.12^{a}	1.85 ± 0.19^{b}	1.88 ± 0.15^{b}	2.21 ± 0.15^{b}
Survival (%)	100 ± 0.0	100 ± 0.0	93.3 ± 6.66	86.7 ± 6.66
Yield (kg ha^{-1})	5780 ± 535^{a}	3846 ± 457^{b}	3731 ± 291^{b}	31603 ± 236^{b}

and fish (Carnevalli *et al*., 2006; Wang *et al*., 2008; Gonçalves *et al*., 2011; Welker & Lim, 2011); therefore, growth enhancement seems to be related to the synergistic result of multiple biological effects (Welker & Lim, 2011).

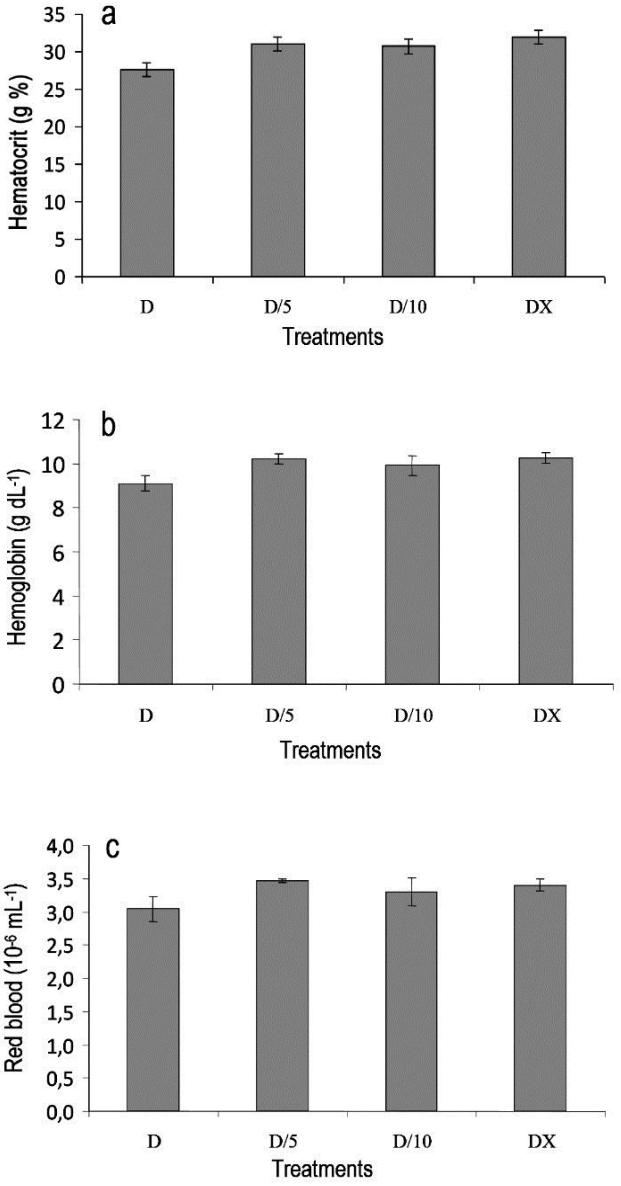

Figure 2. Hematological variables of tilapia co-cultured whith shrimp and fed with dietary supplementation of potential probiotics at different rates. a) Hematocrit percentage, b) hemoglobin, c) red blood cells (RBC). D: daily supply, D/5: every 5 days supply, D/10: every 10 days supply and DX: control without any supply. Data reported as means ± SEM of six organisms. Means not sharing the same superscripts are significantly different ($P < 0.05$).

Blood chemistry (such as plasma levels of glucose, proteins, cortisol, as well as humoral components of the immune system), and hematology (including cellular components of the immune system), of tilapia or other fish, are influenced by supplementation with probiotics. Here interpreted as higher immune response (Wang *et al*., 2008; Jatobá *et al.*, 2011), or capacity to cope with stress (Carnevalli *et al*., 2006; Gonçalves *et al*., 2011). In the present study, white blood cells, or other hematological variable related to immune function were not analyzed. Influence of PPB was not observed, regarding hematocrit, erythrocyte count, and hemoglobin content, as in a previous work with the use of *Lactobacillus plantarum* (Jatobá *et al*., 2011). The combination of *Bacillus subtilis* and *Lactobacillus acidophilus* increased hematocrit in tilapia (Aly *et al*., 2008), while supplementation with *Saccharomyces cerevisiae* decreased these variables (Goda *et al*., 2012). Protein content was not affected by the PPB, similar to the lack of effect by *Enteroccus faecium* on serum protein of tilapia (Wang *et al*., 2008), in contrast to the increase in plasma protean in rainbow trout *Oncorhynchus mykiss* following dietary supply of *Lactobacillus rhamnosus* (Panigrahi *et al*., 2010). If any influence of the mixture of PPB enhance the immune function an increase in protein levels is expected, as shown for different immunostimulants (Choudhury *et al*., 2005; Misra *et al*., 2006). We also expected an increase in blood protein levels, reflecting a better nutritional condition, as improved protein retention in tissues was observed following administration of commercial probiotics (El-Haroun *et al*., 2006). From an analysis of blood glucose, Gonçalves *et al*. (2011) concluded that supplementation of probiotics improve the capacity of tilapia to cope with crowding stress. Because no differences in blood glucose were obtained in our study, as well as in previous studies (Welker *et al*., 2007; Jatobá *et al*., 2011), it is not clear if the mixture of PPB could result in any benefit related to minimizing stress. While triglycerides levels were not affected, a pattern of decreasing levels of serum cholesterol with increasing frequency of PPB was observed. In contrast, circulating lipids increased in rainbow trout receiving probiotics, an effect potentially associated with fish health (Panigrahi *et al*., 2010). On the other hand, a beneficial effect of probiotics on reducing blood cholesterol is well known in humans (for review, see Lye *et al*. 2009) and chicken (Mansoub, 2010). Since these benefits are associated with hypertension in humans, it does not necessarily apply to fish. Therefore, the precise meaning of cholesterol levels as a beneficial or detrimental effect from probiotics in fish remains to be established.

Previous studies show an immunostimulatory effect of the same mixture of PPB on shrimp, based on several humoral and cellular variables, such as total hemocyte count, phenoloxydase and lysosomal enzyme activity, which would explain enhanced resistance to pathogens (Peraza-Gómez *et al*., 2011; Flores-Miranda *et al*., 2011).

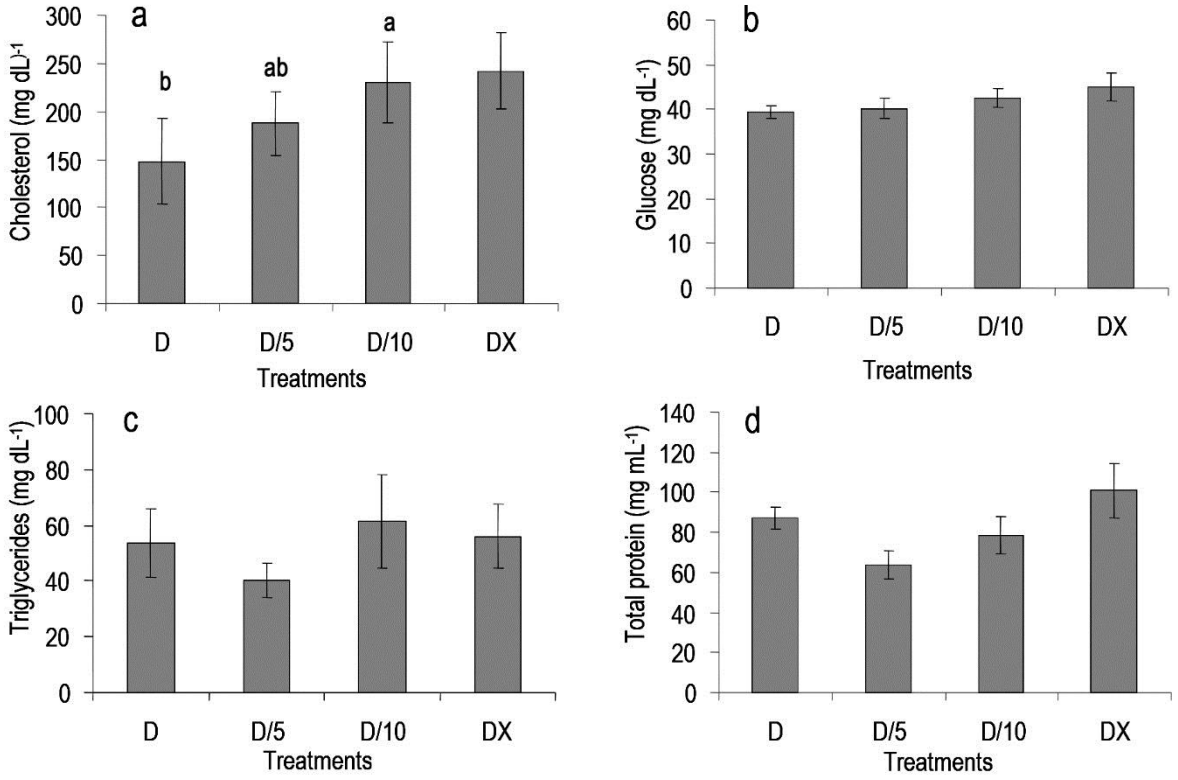

Figure 3. Biochemical variables in blood of tilapia blood co-cultured with shrimp and fed with dietary supplementation of potential probiotic bacteria at different rates. a) Cholesterol, b) glucose, c) triglycerides, d) total protein. D: daily supply, D/5: every 5 days supply, D/10: every 10 days supply and DX: control without any supply. Data reported as means ± SEM of 6 organisms. Means not sharing the same superscripts are significantly different ($P < 0.05$).

However, hemolymph chemistry in shrimp has not been analyzed in relation to supplementation with probiotics. Protein levels could be related to immune function (Perazzolo *et al*., 2002; Mercier *et al*., 2006) and nutritional status (Pascual *et al*., 2003; Rosas *et al*., 2004). Therefore, as reported for tilapia (Choudhury *et al*., 2005; Misra *et al*., 2006), we expected increased protein levels in shrimp hemolymph following PPB that would explain not only increased immuno-competence, but also a better nutritional status related to enhanced growth. It is recognized that, in addition to digestion and absorption of nutrients, energy partitioning within the individual (nutrient use) is associated with increased growth (Welker & Lim, 2011). However, protein content in hemolymph was not affected by PPB supplementation; therefore, it cannot be considered a good indicator of a better immunological and nutritional status under the trial conditions of our study. A similar interpretation could be considered for hemocyanin, which is also considered an indicator of nutritional condition (Pascual *et al*., 2003) and immune function because hemocyanin has antimicrobial proprieties (Destoumieux-Garzon *et al*., 2001). Glucose levels in shrimp hemolymph is considered a good indicator of short and long-term stress (Mercier *et al*., 2009) and also as an indicator of nutritional condition (Pascual *et al*., 2003). Therefore, an increase in glucose for the trial group receiving PPB every 5 days could indicate that shrimp from this group were stressed or had better nutritional condition. Since the shrimp were not subject to any stress challenge, the first assumption is not likely to explain the results, unless joint culture induced long-term stress (high total density and high ratio of tilapia to shrimp). Short-term stress from handling during sampling is another possibility, based on the high levels of glucose (45 mg dL^{-1}), commonly observed in stressed shrimp (Racotta & Palacios, 1998; Mercier *et al*., 2009), whereas shrimp from this group respond more abruptly or that uncontrolled stress occurred only in this group. Considering glucose as an indicator of nutritional condition, it is difficult to explain why shrimp form this group were in better condition than shrimp with daily supply, especially because no other indicator reinforced this possibility. Since triglycerides are also related to the type of feed, particularly to lipid content (Rosas *et*

al., 2004) and no effect of PPB was observed, it is assumed that no influence of PPB on assimilation of lipids from feed or lipid metabolism occurred.

From the present results, analyzing general biochemical indicators of nutritional and health status, higher performance of shrimp and tilapia with dietary supplementation of probiotics was not clearly related to such variables measured in the present work, and therefore should be analyzed on the basis of more specific indicators of digestive physiology, immune response capacity and energy balance.

ACKNOWLEDGEMENTS

We are grateful to Adolfo Ramírez "Cuate Machado farm" for shrimp supply. Ira Fogel of CIBNOR provided editorial services. This research was supported by grants from SIP-IPN 20100176 and SEP-CONACYT 101733. Juan Pablo Apún Molina is a recipient of a CONACYT fellowship N-22906. Juan Pablo Apún Molina, Apolinar Santamaría Miranda and Antonio Luna González received support from COFAA and EDI of the Instituto Politécnico Nacional.

REFERENCES

Aly, S.M., Y.A. Ahmed, A.A. Ghareeb & M.F. Mohamed. 2008. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunol., 25: 128-136.

Apún-Molina, J.P. 2007. Efecto de bacterias con potencial probiótico en el crecimiento y supervivencia de la tilapia *Oreochromis niloticus* (Linnaeus, 1758), cultivada en el laboratorio. M.Sc. Thesis, CIIDIR-IPN, Unidad Sinaloa, Sinaloa, 60 pp.

Apún-Molina, J.P., A. Santamaría-Miranda, A. Luna-González, S.F. Martínez-Díaz & M. Rojas-Contreras. 2009. Effect of potential probiotic bacteria on growth and survival of tilapia *Oreochromis niloticus* L., cultured in the laboratory under high density and suboptimum temperature. Aquacult. Res., 40: 887-894.

Balcázar, J.L., I.D. Blas, I. Ruiz-Z, D. Cunningham, D. Vendrell & J.L. Muzquiz. 2006. The role of probiotics in aquaculture. Vet. Microbiol., 114: 173-186.

Bessa-Junior, A.P., C.M.S.B. Borges-Azevedo, F.S. Thé Pontes & G.G. Henry-Silva. 2012. Polyculture of Nile tilapia and shrimp at different stocking densities. R. Bras. Zootec., 41: 1561-1569.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-253.

Carnevalli, O., L. Vivo, R. Sulpizio, G.I. Gioacchini, I. Olivotto, S. Silvi & A. Cresci. 2006. Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquaculture, 258: 430-438.

Choudhury, D., A.K. Pal, N.P. Sahu, S. Kumar, S.S. Das & S.C. Mukherjee. 2005. Dietary yeast RNA supplementation reduces mortality by *Aeromonas hydrophila* in rohu (*Labeo rohita* L.) juveniles. Fish Shellfish Immunol., 19(2005): 281-291.

Cruz, P.S., M.N. Andalecio, R.B. Bolivar & K. Fitzsimmons. 2008. Tilapia shrimp polyculture in Negos Island, Philipines: a review. J. World Aquacult. Soc., 39: 713-725.

Dacie, J.V. & S.M. Lewis. 1984. Practical hematology. Churchill Livingstone, New York, 32 pp.

Destoumieux-Garzon, D., D. Saulnier, J. Garnier, C. Jouffrey, P. Bulet & E. Bachère. 2001. Crustacean immunity. Antifungal peptides are generated from the C terminus of shrimp hemocyanin in response to microbial challenge. J. Biol. Chem., 276: 47070-47077.

El-Haroun, E.R., A.S. Goda & K. Chowdhury. 2006. Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquacult. Res., 37(14): 1473-1480.

Far, H.Z., C.R.B. Saad, H.M. Daud, S.A. Harmin & S. Shakibazadeh. 2009. Effect of *Bacillus subtilis* on the growth and survival rate of shrimp *Litopenaeus vannamei*. Afr. J. Biotechnol., 8: 3369-3376.

Flores-Miranda, M.C., A. Luna-González, A.I. Campa-Córdova, H.A. González-Ocampo, J.A. Fierro-Coronado & B.O. Partida-Arangure. 2011. Microbial immunostimulants reduce mortality in whiteleg shrimp *Litopenaeus vannamei* challenged with *Vibrio sinaloensis* strains. Aquaculture, 320: 51-55.

Goda, A.M.A., H.A. Mabrouk, M.A. Wafa & T.M. El-Afifi. 2012. Effect of using baker's yeast and exogenous digestive enzymes as growth promoters on growth, feed utilization and hematological indices of Nile tilapia, *Oreochromis niloticus* fingerlings. J. Agr. Sci. Tech., B2: 15-28.

Gonçalves, A.T., M. Maita, K. Futami, M. Endo & T. Katagiri. 2011. Effects of a probiotic bacterial *Lactobacillus rhamnosus* dietary supplement on the crowding stress response of juvenile Nile tilapia *Oreochromis niloticus*. Fish. Sci., 77: 633-642.

Günther, J. & R. Jiménez-Montealegre. 2004. Efecto del probiótico *Bacillus subtilis* sobre el crecimiento y alimentación de tilapia (*Oreochromis niloticus*) y

langostino *Macrobrachium rosenbergii* en laboratorio. Rev. Biol. Trop., 52: 937-943.

Hagerman, L. 1983. Haemocyanin concentration of juvenile lobsters *Homarus gammarus* in relation to moulting cycle and feeding conditions. Mar. Biol., 77: 11-17.

Hernández-Barraza, C., J. Loredo, J. Adame & K. Fitzsimmons. 2012. Effect of Nile tilapia *Oreochromis niloticus* on the growth performance of Pacific white shrimp *Litopenaeus vannamei* in a sequential polyculture system. Lat. Am. J. Aquat. Res., 40(4): 936-942.

Jatobá, A., F.N. Vieira, C.C. Buglione-Neto, J.L.P. Mouriño, B.C. Silva, W.Q. Seiffert & E.R. Andreatta. 2011. Diet supplemented with probiotic for Nile tilapia in polyculture system with marine shrimp. Fish Physiol. Biochem., 37: 725-732.

Li, J., B. Tan, K. Mai, Q. Ai, W. Zhang, Z. Liufu & W. Xu. 2008. Immune responses and resistance against *Vibrio parahaemolyticus* induced by probiotic bacterium *Arthobacter* XE- in Pacific white shrimp, *Litopenaeus vannamei*. J. World Aquacult. Soc., 39: 477-489.

Liu, K.-F., C.H. Chiu, Y.-L. Shiu, W. Cheng & C.H. Liu. 2010. Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp *Litopenaeus vannamei* larvae. Fish Shellfish Immunol., 28: 837-844.

Luna-González, A., D. Quiñónez-Zúñiga, J.A. Fierro-Coronado, H.A. González-Ocampo, Á.I. Campa-Córdova, M. Flores-Miranda & V. Peraza-Gómez. 2013. Effect of *Pediococcus parvulus* and *Candida parapsilosis* on growth and survival of tilapia, *Oreochromis niloticus* and *Oreochromis* sp. Afr. J. Microbiol. Res., 7: 2976-2982.

Lye, H.S., C.Y. Kuan, J.A. Ewe, W.Y Fung & M.T. Liong. 2009. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, rennin, and phytoestrogens. Int. J. Mol. Sci., 10: 3755-3775.

Mansoub, N.H. 2010. Effect of probiotic bacteria utilization on serum cholesterol and triglycerides contents and performance of broiler chickens. Global Vet., 5(3): 184-186.

Mercier, L., E. Palacios, A.I. Campa-Cordova, D. Tovar-Ramirez, R. Hernández-Herrera & I.S. Racotta. 2006. Metabolic and immune responses in Pacific whiteleg shrimp *Litopenaeus vannamei* exposed to a repeated handling stress. Aquaculture, 258: 633-640.

Mercier, L., G. Yepiz-Plascencia, A. Muhlia-Almazán, R. Civera, M.F. Quiñones-Arreola & E. Palacios. 2009. Effect of diets containing different levels of highly unsaturated fatty acids on physiological and immune responses in Pacific whiteleg shrimp *Litopenaeus vannamei* (Boone) exposed to handling stress. Aquacult. Res., 40: 1849-1863.

Misra, C.K., B.K. Das, S.C Mukherjee & P. Pattnaik. 2006. Effect of multiple injections of b-glucan on non-specific immune response and disease resistance in *Labeo rohita* fingerlings. Fish Shellfish Immunol., 20: 305-319.

Muangkeow, B., K. Ikejima, S. Powtongsook & Y. Yi. 2007. Effects of white shrimp, *Litopenaeus vannamei* (Boone), and Nile tilapia *Oreochromis niloticus* L., stocking density on growth, nutrient conversion rate and economic return in integrated closed recirculation system. Aquaculture, 269: 363-376.

Pascual, C., G. Gaxiola & C. Rosas. 2003. Blood metabolites and hemocyanin of the white shrimp, *Litopenaeus vannamei*: the effect of culture conditions and a comparison with other crustacean species. Mar. Biol., 142: 735-745.

Panorama Acuícola Magazine. 2010. Rentabilidad y perspectivas del cultivo de tilapia en México. Panorama Acuícola Magazine, Directorio de Proveedores, pp. 4-7.

Panigrahi, A., V. Kiron, S. Satoh & T. Watanabe. 2010. Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum). Fish Physiol. Biochem., 36: 969-977.

Peraza-Gómez, V., A. Luna-González, A.I. Campa-Córdova, M. López-Meyer, J.A. Fierro-Coronado & P. Alvarez-Ruiz. 2009. Probiotic microorganisms and antiviral plants reduce mortality and prevalence of WSSV in shrimp *Litopenaeus vannamei* cultured under laboratory conditions. Aquacult. Res., 40: 1481-1489.

Peraza-Gómez, V., A. Luna-González, A.I. Campa-Córdova, J.A. Fierro-Coronado, H.A. González-Ocampo & J.C. Sainz-Hernández. 2011. Dietary microorganism and plant effects on the survival and immune response of *Litopenaeus vannamei* challenged with the white spot syndrome. Aquacult. Res., 42: 559-570.

Perazzollo, L.M., R. Gargioni, P. Ogliari & M.A. Barracco. 2002. Evaluation of some hemato-immunological parameters in the shrimp *Farfantepenaeus paulensis* submitted to environmental and physiological stress. Aquaculture, 214: 19-33.

Racotta, I.S. & E. Palacios. 1998. Hemolymph metabolic variables in response to experimental manipulation stress and serotonin injection in *Penaeus vannamei*. J. World Aquacult. Soc., 29: 351-356.

Racotta, I.S. & R. Hernández-Herrera. 2000. Metabolic responses of the white shrimp, *Penaeus vannamei*, to ambient ammonia. Comp. Biochem. Phys. A, 125: 437-443.

Racotta, I.S., E. Palacios & L. Méndez. 2002. Metabolic responses to short and long-term exposure to hypoxia in white shrimp *Penaeus vannamei*. Mar. Fresh. Behav. Phys., 35: 269-275.

Rengpipat, S., S. Rukpratanporn, S. Piyatiratitivorakul & P. Menasaveta. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquaculture, 191: 271-288.

Rodríguez-Grimón, R.O. 2003 La tilapia y su efecto en la prevalencia del virus de la mancha blanca (WSSV) en poblaciones de camarón, Master Tesis Ciencias, Escuela Superior Politécnica del Litoral, Guayaquil, 72 pp.

Rosas, C., E.L. Cooper, C. Pascual, R. Brito, R. Gelabert, T. Moreno, G. Miranda & A. Sánchez. 2004. Indicators of physiological and immunological status of *Litopenaeus setiferus* wild populations Crustacea Penaeidae. Mar. Biol., 145: 401-413.

Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (CONAPESCA). 2012. Anuario Estadístico de Acuicultura y Pesca 2011, Mazatlán, 23 pp.

Strickland, J. & T.R. Parsons. 1972 A practical handbook of sea water analysis. Bull. Fish. Res. Bd. Can., 167: 1-130.

Thien, C.P., Y. Yi & K. Fitzsimmons. 2004. Effects of adding shrimp *Penaeus monodon* into intensive culture ponds of Nile tilapia *Oreochromis niloticus* at different densities. In: R. Bolivar, G. Mair & K. Fitzsimmons (eds.). New dimensions in farmed tilapia. Proceedings of ISTA, Manila, Philippines, Bureau of Fisheries and Aquatic Resources. pp. 790-805.

Wang, Y.B. 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture, 269: 259-264.

Wang, Y.B., Z.Q. Tian, J.T. Yao & W.F. Li. 2008. Effect of probiotics, *Enteroccus faecium*, on tilapia *Oreochromis niloticus* growth performance and immune response. Aquaculture, 277: 203-207.

Welker, T., C. Lim, M. Yildirim-Aksoy & P. Klesius. 2007. Growth, immune function, disease and stress resistance of juvenile Nile tilapia *Oreochromis niloticus* fed graded levels of bovine lactoferrin. Aquaculture, 262: 156-162.

Welker, T. & C. Lim. 2011. Use of probiotics in diets of tilapia. J. Aquacult. Res. Develop., S1: 014. doi:10.4172/2155-9546.S1-014.

Ziaei-Nejad, S., M. Habibi-Rezaei, G. Azari-Takami, D.L. Lovett, A.R. Mirvaghefi & M. Shakouri. 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture, 252: 516-524.

Zokaeifar, H., J.L. Balcázar, C.R. Saad, M.S. Kamarudin, K. Sijam, A. Arshad & N. Nejat. 2012. Effects on *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol., 33: 683-689.

Received: 12 September 2013; Accepted: 12 November 2014