

# EFFECT OF PROTEIN SOURCE AND PROBIOTIC ON THE INTESTINAL TRACT OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Adolfo JATOBÁ<sup>1</sup>

Klayton Natan MORAES<sup>1</sup>

Jorge Pedro RODRIGUES-SOARES<sup>1</sup>

Gabriel Fernandes Alves JESUS<sup>2</sup>

Felipe Do Nascimento VIEIRA<sup>2</sup>

<sup>1</sup>Instituto Federal Catarinense – IFC, Laboratório de Aquicultura, Rodovia BR 280, km 27, CP 21, CEP 89245-000, Araquari, SC, Brasil. E-mail: jatobaadolfo@gmail.com (corresponding author).

<sup>2</sup>Universidade Federal de Santa Catarina – UFSC, Departamento de Aquicultura, Laboratório de Camarões Marinhos, Rua dos Coroas, 503, Florianópolis, SC, Brasil.

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## ABSTRACT

This study aimed to evaluate the viability of supplementing two diets for the shrimp *Litopenaeus vannamei* with *Lactobacillus plantarum*. One using fish meal as a protein source and another using soy protein concentrate, as well as the effect of these formulations on shrimp intestinal microbiota. To assay probiotic viability in the formulated diets, the number of CFU g<sup>-1</sup> was observed weekly over the course of four weeks. The viability of *Lactobacillus plantarum* in relation to the physical parameters of the diets, including stability, buoyancy, and expansion, was quantified. The effect of the diets on microbiota and intestinal tract morphology was determined by performing a 2x2 factorial experiment (two diets, with or without supplementation) in triplicate, totaling 12 experimental units, with five animals per unit, fed with 3.5% of biomass for 17 days. The concentration of lactic acid bacteria decreased over time, irrespective of protein source. The diet with fishmeal as a protein source, independent of probiotic supplementation, presented good stability and did not disintegrate after four hours. In contrast, the diet with soy protein concentrate, supplemented or not, disintegrated between 2.5 and 3 hours, presenting low stability. All diets presented 0% buoyancy. The expansion rate was higher in diets with soy protein concentrate, but without the influence probiotic supplementation or interaction between the factors. In the *in vivo* assay, both supplemented diets showed greater total heterotrophic bacteria count than without probiotic; however, no difference in count was noted in diets with different protein source. Lactic acid bacteria were only observed in the shrimp fed diets supplemented with probiotic. Histology of the intestinal tract showed that all intestines had intact, well-developed and well-organized cells, irrespective of diet. Thus, *L. plantarum*, when combined with different protein sources, produced similar effects on the structure and microbiota of the marine shrimp *Litopenaeus vannamei*.

**Key words:** *Litopenaeus vannamei*; nutrition; microbiota; lactic acid bacteria

## EFEITO DA FONTE DE PROTEÍNA E PROBIOTICO SOBRE TRATO INTESTINAL DO CAMARÃO BRANCO PACÍFICO *Litopenaeus vannamei*

### RESUMO

Este trabalho objetivou avaliar viabilidade da suplementação de duas dietas ao camarão *Litopenaeus vannamei* com *Lactobacillus plantarum*. Uma usando farinha de peixe como fonte proteica e outra usando concentrado de proteico de soja, bem como o efeito destas formulações na microbiota intestinal de camarão. Para testar a viabilidade probiótica nas dietas, o número de UFC g<sup>-1</sup> foi observado semanalmente ao longo de quatro semanas. A viabilidade de *Lactobacillus plantarum* em relação aos parâmetros físicos das dietas, incluindo estabilidade, fluabilidade e expansão, foram testados. O efeito das dietas na microbiota e na morfologia do trato intestinal foi determinado por meio de um experimento fatorial 2x2 (duas dietas, com ou sem suplementação), em triplicata, totalizando 12 unidades experimentais, com cinco animais por unidade, alimentados com 3,5% de biomassa por 17 dias. A concentração de bactérias do ácido láctico diminuiu ao longo do tempo, independentemente da fonte de proteína. A dieta com farinha de peixe como fonte proteica, independente da suplementação probiótica, apresentou boa estabilidade e não se desintegrou após quatro horas. Em contrapartida, dieta com concentrado proteico de soja, suplementado ou não, desintegrou-se entre 2,5 e 3 horas, apresentando baixa estabilidade. Todas as dietas apresentaram fluabilidade de 0%. A taxa de expansão foi maior nas dietas com concentrado proteico de soja, mas sem influência da suplementação probiótica ou interação entre os fatores. No ensaio *in vivo*, ambas as dietas suplementadas apresentaram maior contagem de bactérias heterotróficas totais; no entanto, nenhuma diferença na contagem foi observada em dietas com diferentes fontes de proteína. Bactérias ácido-lácticas só foram observadas em dietas suplementadas com probiótico. A histologia do trato intestinal mostrou que todos intestinos tinham células intactas, bem desenvolvidas e organizadas, independentemente da dieta. Assim, *L. plantarum*, quando combinado com diferentes fontes proteicas, produziu efeitos similares na estrutura e microbiota do camarão marinho *Litopenaeus vannamei*.

**Palavras-chave:** *Litopenaeus vannamei*; nutrição; microbiota; bactérias ácido-lácticas.

## INTRODUCTION

Feed represents the highest cost of production in shrimp farming, and protein sources are the most expensive ingredients (SOOKYING *et al.*, 2013; JATOBÁ *et al.*, 2014). Among the protein sources used to make diets for marine shrimps, fishmeal (FM) is highlighted because of its amino acid composition, vitamins, minerals and palatability (SOOKYING *et al.*, 2013; CHAVEZ *et al.*, 2016). However, FM is not considered a renewable or sustainable ingredient, owing to the practice of extractive industrial fishing (ZHAO *et al.*, 2017). This calls for the development of alternative protein sources for shrimp farming with similar nutritional value, but lower cost, thus allowing for greater profit margin (CHIU *et al.*, 2016; SUN *et al.*, 2016).

Several studies have sought alternative ways of complete or partial replacement of fishmeal with protein sources of plant origin to reduce production costs (SOOKYING *et al.*, 2013; SOARES *et al.*, 2015; BARBIERI *et al.*, 2016; CHAVEZ *et al.*, 2016; GAMBOA-DELGADO *et al.*, 2016; SUN *et al.*, 2016; Jatobá *et al.*, 2018). Among these alternatives, soy protein concentrate (SPC) has been suggested by its ample supply and good nutritional profile for marine shrimp (BANSEMER *et al.*, 2015; SOARES *et al.*, 2015; CHÁVEZ *et al.*, 2016).

In addition to nutritional challenges facing shrimp farmers, disease is a predominant problem, especially those of bacterial or viral origin that arise from inadequate management and low-quality feed (MOSS *et al.*, 2012; ZOKAEIFAR *et al.*, 2012). To minimize the losses caused by these diseases, new technologies that reduce

environmental impacts and production costs need to be evaluated (CORREIA *et al.*, 2014; WANG *et al.*, 2016).

The use of preventive measures, such as supplementation with probiotics capable of increasing the immunocompetence of aquatic organisms, have proved to be an efficient alternative (LUIS-VILLASENOR *et al.*, 2013; MERRIFIELD *et al.*, 2014), in particular the use of lactic acid bacteria (LAB) with satisfactory results, both *in vitro* (VIEIRA *et al.*, 2013) and *in vivo* (VIEIRA *et al.*, 2007, 2008; JATOBÁ *et al.*, 2008, 2011, 2017, 2018). However, little is known about the synergistic effects of the different feed ingredients used in combination with probiotics.

Therefore, this study aimed to evaluate the viability of supplementing two diets of the shrimp *Litopenaeus vannamei* with the lactic acid bacterium *Lactobacillus plantarum*, one using fishmeal as a protein source and another using soy protein concentrate, as well as the effect of these formulations on the shrimp intestinal microbiota.

## METHODS

The research was carried out at the Laboratório de Aquicultura do Instituto Federal Catarinense, campus Araquari.

### Formulation and preparation of diets

Two iso-energetic diets were formulated (Table 1) based on nutritional requirements for marine shrimp (NRC, 2011).

**Table 1.** Formula of the experimental diets for marine shrimp (*L. vannamei*) with different protein source.

Ingredients (g.Kg <sup>-1</sup> )	Replacement levels	
	0%	100%
<sup>1</sup> Fishmeal (590 g.kg <sup>-1</sup> CP)	209	0
<sup>2</sup> Soy protein concentrate	0	172
Soy meal (450 g.kg <sup>-1</sup> CP)	350	350
Broken rice	80	80
Wheat flour	250	250
Soy lecithin	15	15
Fish oil	06	25
Soybean oil	20	20
Potassium chloride	15	09
Sodium chloride	14	15
Magnesium sulfate	08	08
Vitamin-C monophosphate	03	03
Kaolin	08	31
Phosphate monocalcium (calcium phosphate 20)	07	07
<sup>3</sup> Premix	15	15

<sup>1</sup>Nicoluzzi Rações Ltda. (Penha, SC, Brazil); <sup>2</sup>IMCOPA - Importação, Exportação e Indústria de Óleos S.A. (Araucária, PR, Brazil), proximate composition, 630.7g.Kg<sup>-1</sup> crude protein, 13.8 g.Kg<sup>-1</sup> crude lipid, 46.6 g.Kg<sup>-1</sup> crude fiber, 67.9 g.Kg<sup>-1</sup> moisture, 63.2 g.Kg<sup>-1</sup> ash, 177.8 g.Kg<sup>-1</sup> non-nitrogen extract, 13.8 g.Kg<sup>-1</sup> extract by acid hydrolysis, 4,426.0 cal.g<sup>-1</sup>; <sup>1</sup> Amino acid profile: Aspartic acid, 6.67 g.Kg<sup>-1</sup>; Glutamic acid, 100.3 g.Kg<sup>-1</sup>; Serine, 26.5 g.Kg<sup>-1</sup>; Glycine, 19.0 g.Kg<sup>-1</sup>; Histidine, 16.8 g.Kg<sup>-1</sup>; Arginine, 36.9 g.Kg<sup>-1</sup>; Threonine, 17.4 g.Kg<sup>-1</sup>; Proline, 27.3 g.Kg<sup>-1</sup>; Tyrosine, 16.9 g.Kg<sup>-1</sup>; Valine, 27.3 g.Kg<sup>-1</sup>; Methionine, 7.1 g.Kg<sup>-1</sup>; Methionine + Cystine, 13.7 g.Kg<sup>-1</sup>; Isoleucine, 28.2 g.Kg<sup>-1</sup>; Leucine, 49.9 g.Kg<sup>-1</sup>; Phenylalanine, 30.4 g.Kg<sup>-1</sup> and Lysine 39.2 g.Kg<sup>-1</sup>. <sup>3</sup>Guarantee levels per kg - vitamin A, 1,250,000 UI; vitamin D3, 350,000 UI; vitamin E, 25,000 UI; vitamin K3, 500 mg; vitamin B1, 5,000 mg; vitamin B2, 4,000 mg; vitamin B6, 10 mg; nicotinic acid, 15,000 mg; pantothenic acid, 10,000 mg; biotin, 150 mg; folic acid, 1,250 mg; vitamin C, 25,000 mg; choline, 50,000 mg; inositol, 20,000 mg; iron 2,000 mg; copper, 3,500 mg; copper chelate, 15,000 mg; zinc, 10,500 mg; zinc chelate, 4,500 mg; manganese, 4,000 mg; selenic acid, 15 mg; selenium chelate, 15 mg; iodine, 150 mg; cobalt, 30 mg.

Diets were produced at the Instituto de Ciências do Mar (LABOMAR), Universidade Federal do Ceará (UFC), Eusébio, Ceará, Brazil. The dry ingredients of the diet were previously ground and sieved (500  $\mu$ L). Subsequently, the micro-ingredients were homogenized in a Y-mixer for 10 min and were added to the macroingredients to be homogenized in a food mixer for 10 min. Soon after, the oils and soy lecithin were added, and then 40% of hot water. The resulting mixture was pelletized through a meat grinder and dried for approximately 18 h at 40 °C.

### Incorporation of *Lactobacillus plantarum* in the feed

*Lactobacillus plantarum* is a probiotic strain isolated from healthy shrimp. It was selected and approved by *in vitro* tests against pathogens and identified as *Lactobacillus plantarum* at the Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), Universidade Estadual de Campinas, with accession number CPQBA 007-07 DRM01 (VIEIRA *et al.*, 2007, 2008, 2013).

Experimental diets were inoculated with 100 mL Kg<sup>-1</sup> of probiotic, while a sterile medium was inserted in control diets, according to the protocol established by JATOBA *et al.* (2011).

### Experimental design

After producing diets with different protein sources and incorporation of probiotic, a two-factor experimental design (2x2), including factor 1, protein source, FM or SPC, and factor 2, diet supplemented or not with probiotic, was established and resulted in four treatments.

### Assay of physical and microbiological parameters of diets

Three 1.0 g samples of each treatment were submerged in a beaker with 1 L salinized water (3% NaCl) to evaluate the stability and buoyancy of the diets. The assay was carried out during four hours at 30-minute intervals. The floating pellets were collected and counted to estimate buoyancy (adapted from ABCC, 2005).

The pellet expansion rate was determined by measuring the initial and final diameter (digital caliper), as samples of the stability and buoyancy test. After four hours, the pellets were removed from the water, and the final diameter was measured.

All physical tests of the diets occurred weekly over the course of four weeks.

After 24 hours of dietary supplementation, 1.0 g samples were macerated and serially diluted (factor 1:10) in sterile saline solution (SSE) 0.65%. Samples of each dilution were seeded in MRS agar medium and incubated for 48 h at 30 °C for LAB count. This procedure was repeated weekly for four weeks.

### In vivo assay

Sixty Pacific white shrimp (average weight of 6.85  $\pm$  0.04 g) were distributed in 12 experimental units (22 L), five in each (stocking density of 227 shrimps per m<sup>3</sup>), equipped with canister filter and constant temperature (28.1 - 29.2 °C). During the experiment, dissolved oxygen, temperature and pH were measured

two times per day. The experimental units were divided into four treatments with three replicas. Shrimp were fed three times a day (8:30, 1:30 and 5:00 p.m.), offering 3.5% of biomass, a schedule maintained throughout the 17-day experiment.

Following 12h of starvation at the end of the experimental period, guts of three shrimp from each tank were removed and pooled for microbiological and histological analysis. The pooled shrimp guts were homogenized and serially diluted 1:10 in 0.65% of NaCl sterile saline. Samples from each dilution were cultured in PCA (Plate Count Agar), TCBS (thiosulfate citrate bile salts sucrose) agar, cetrimide agar, and MRS agar media and incubated for 48h at 30 °C for viable culturable heterotrophic bacterial counts, including *Vibrio* spp., *Pseudomonas* spp., and LAB, respectively.

The cephalothorax and the last abdominal segments of two different shrimp used in the microbiological evaluation were sectioned with a razor, and the abdominal portion was fixed in standard marine Davidson solution for 24-48 h and then transferred to alcohol solution 70%. Afterwards, the samples were submitted to the standard histology procedure with dehydration, diaphanization and paraffin inclusion.

Paraffin blocks 5- $\mu$ m in thickness were cut with a microtome for subsequent staining with Harris Haematoxylin and Eosin (HOWARD *et al.*, 2004; KIM *et al.*, 2006) and observed in a Differential Interference Contrast (DIC) AxioImager A2 (Zeiss).

At the end of the experiment, the apparent feed efficiency, protein efficiency ratio, final body weight, weekly gain and survival were assessed.

### Statistical analysis

Data were first subjected to Bartlett's analysis to verify the homogeneity of variance, and microbiological data were log(x + 1) transformed. All data were subjected to bifactorial (two-way) ANOVA, and significant differences among treatments were analyzed using the Student-Newman-Keuls (SNK) test. All tests were conducted at a 5% level of significance (ZAR, 2010).

## RESULTS

### Physical and microbiological parameters of diets

The diet with FM as a protein source, independent of probiotic supplementation, did not disintegrate during the evaluated time (4 hours), whereas the diet with SPC disintegrated between 2.5 and 3 hours, supplemented or not. All diets showed 0% buoyancy, an essential characteristic for any benthic aquatic organism.

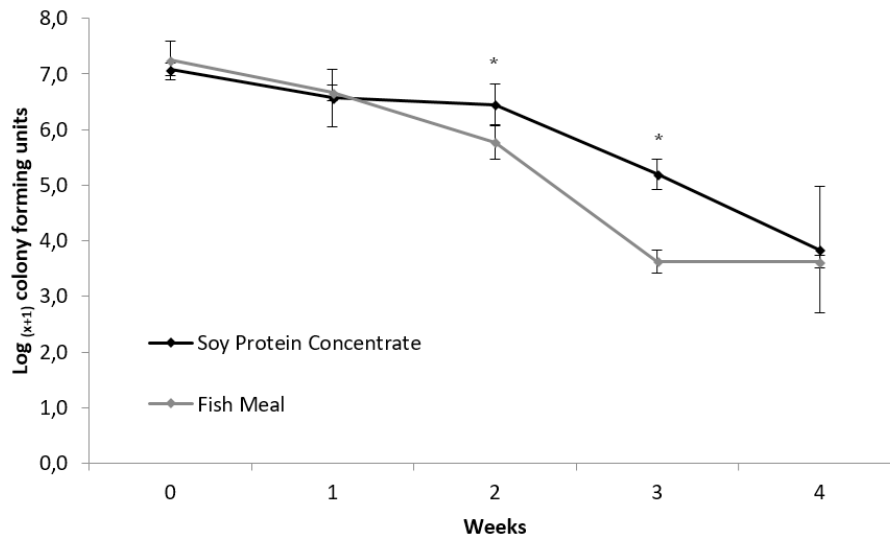
The expansion rate of the diets differed in the ingredient factor. Diets elaborated with SPC expanded more than those formulated with FM; however, probiotic supplementation did not influence this process (Table 2).

In the second and third week, the SPC and supplemented diet had higher concentrations of LAB, whereas at weeks 0, 1 and 5, no differences were observed between the supplemented diets (Figure 1). LAB was not observed in diets without probiotic supplementation.

**Table 2.** Expansion rate (%) in diets formulated with different protein sources supplemented with probiotic over time.

Treatment		Weeks				
F1	F2	0	1	2	3	4
Fishmeal	Control	25.0±0.0 <sup>Aa</sup>	26.7±2.9 <sup>Aa</sup>	25.0±0.0 <sup>Aa</sup>	25.0±0.0 <sup>Aa</sup>	25.0±0.0 <sup>Aa</sup>
	Probiotic	25.0±0.0 <sup>Aa</sup>	25.0±2.9 <sup>Aa</sup>	25.0±0.0 <sup>Aa</sup>	25.0±0.0 <sup>Aa</sup>	25.0±0.0 <sup>Aa</sup>
Soy protein concentrate	Control	53.3±0.0 <sup>Ba</sup>	51.7±0.0 <sup>Ba</sup>	55.0±0.0 <sup>Ba</sup>	40.9±2.6 <sup>Ba</sup>	47.6±0.0 <sup>Ba</sup>
	Probiotic	55.0±2.9 <sup>Ba</sup>	55.0±2.9 <sup>Ba</sup>	55.0±0.0 <sup>Ba</sup>	42.4±0.0 <sup>Ba</sup>	47.6±0.0 <sup>Ba</sup>
		Significance (p)				
Ingredient (F1)		0.00020	0.00029	0.00000	0.00132	0.00049
Supplementation (F2)		0.88020	0.76831	0.998371	0.71398	0.81243
F1xF2		0.25520	0.26832	0.26984	0.24465	0.25012

Different uppercase letters indicate significant differences between ingredients, and different lowercase letters indicate significant differences between probiotic and control diet in the bifactorial (two-way) ANOVA and SNK tests.

**Figure 1.** LAB count in diets formulated with different protein sources supplemented with probiotic over time. \*Indicates significant differences between diets.**Table 3.** Bacteriological count of the intestinal tract of *L. vannamei* fed with different protein sources, with and without probiotic supplementation.

Treatments		(log <sub>x+1</sub> ) CFUs g <sup>-1</sup> of intestinal tract		
F1 (Ingredient)	F2 (Supplementation)	Heterotrophic Total Bacteria	Lactic acid bacteria	Vibrio spp.
Fishmeal	Control	8.8 ± 0.1 <sup>Aa</sup>	ND	5.9 ± 0.8 <sup>Aa</sup>
	Probiotic	7.6 ± 0.5 <sup>Ab</sup>	5.5 ± 0.4 <sup>A</sup>	5.9 ± 0.1 <sup>Aa</sup>
Soy protein concentrate	Control	8.4 ± 0.4 <sup>Aa</sup>	ND	6.8 ± 0.6 <sup>Aa</sup>
	Probiotic	7.5 ± 0.3 <sup>Ab</sup>	5.4 ± 0.8 <sup>A</sup>	6.3 ± 0.3 <sup>Aa</sup>
Factor		Significance		
Ingredient		0.36584	0.23619	0.06677
Supplementation		0.00120	-	0.47356
Ingredient x Supplementation		0.47489	-	0.44998

Not detectable (ND); Different uppercase letters indicate significant differences between ingredients, and different lowercase letters indicate significant differences between probiotic and control diet in bifactorial (two-way) ANOVA and the SNK tests.

### In vivo assay

*Lactobacillus plantarum* changed the microbiota of the intestinal tract of shrimp fed with the probiotic diets, irrespective of protein source, whereas LAB was not detected in shrimp fed with control diets, and total heterotrophic bacteria decreased in shrimp fed with the probiotic diet. Neither protein source nor probiotic interfered with the bacterial count of *Vibrio* spp. (Table 3).

The qualitative analysis of the histological sections of the middle portion of the intestinal tract of the shrimp presented structures similar to those described by BELL and LIGHTNER (1988). In the histological sections of the intestinal tract, no

qualitative differences between the treatments were recorded (Figure 2). The intestinal epithelium presented intact, developed, organized and well-defined cells, as well as absence of vacuoles and intercellular spaces (Figure 2).

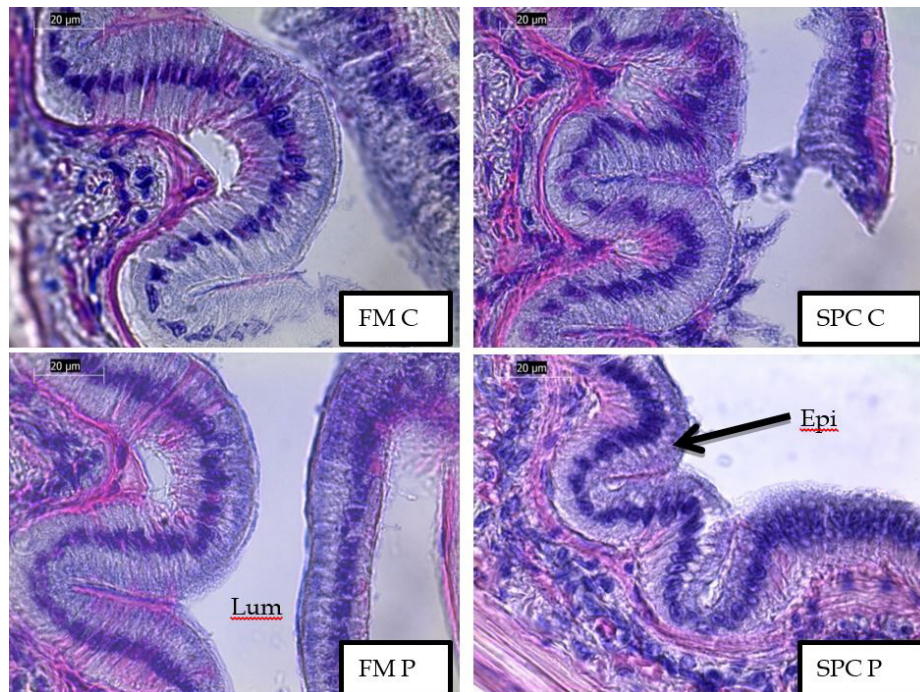
The dissolved oxygen remained above 4.5 mg L<sup>-1</sup> and pH at 7.9 throughout the experiment, in all treatments, not diverging among them.

Zootechnical variables did not diverge among treatments, regardless of the factor evaluated, supplementation or protein source (Table 4) and the survival rate of 100% were recorded in all treatments.

**Table 4.** Zootechnical variables (mean  $\pm$  standard deviation) during 17 days of *L. vannamei* fed with different protein sources, with and without probiotic supplementation.

Treatments		Zootechnical variables			
F1	F2	FW (g)	WG (g.week <sup>-1</sup> )	AFE	PER
Fishmeal	Control	10.01 $\pm$ 0.49 <sup>Aa</sup>	1.30 $\pm$ 0.13 <sup>Aa</sup>	0.46 $\pm$ 0.08 <sup>Aa</sup>	1.49 $\pm$ 0.23 <sup>Aa</sup>
	Probiotic	10.20 $\pm$ 0.45 <sup>Aa</sup>	1.38 $\pm$ 0.11 <sup>Aa</sup>	0.47 $\pm$ 0.07 <sup>Aa</sup>	1.54 $\pm$ 0.20 <sup>Aa</sup>
Soy protein concentrate	Control	10.08 $\pm$ 0.36 <sup>Aa</sup>	1.33 $\pm$ 0.10 <sup>Aa</sup>	0.45 $\pm$ 0.11 <sup>Aa</sup>	1.53 $\pm$ 0.30 <sup>Aa</sup>
	Probiotic	10.12 $\pm$ 0.28 <sup>Aa</sup>	1.35 $\pm$ 0.09 <sup>Aa</sup>	0.47 $\pm$ 0.06 <sup>Aa</sup>	1.50 $\pm$ 0.18 <sup>Aa</sup>
		Significance (p)			
Ingredient (F1)		0.33704	0.536056	0.448149	0.396534
Supplementation (F2)		0.285698	0.300567	0.386671	0.376378
F1xF2		0.310095	0.302341	0.234346	0.421923

AFE (Apparent feed efficiency); PER (Protein efficiency ratio); FW (Final weight); WG (Weekly gain); Different uppercase letters indicate significant differences between ingredients, and different lowercase letters indicate significant differences between probiotic and control diet in bifactorial (two-way) ANOVA and the SNK tests.



**Figure 2.** Histology of the intestinal tract of marine shrimp fed different diets. Epi: intestinal epithelium, Lum: intestinal lumen, FM: Fishmeal, SPC: Soy protein concentrate, P: Probiotic supplementation, C: Control.

## DISCUSSION

The ABCC (2005) suggests a minimum disintegration time of 3.0 to 3.5 hours for marine shrimp diets. In the present study, the diet with SPC disintegrated between 2.5 and 3 hours, supplemented or not, which demonstrated lower stability when compared to the diet with FM, independent of probiotic supplementation. Few studies evaluate the rate of pellet expansion. However, different rates of diet expansion may be related to the potential for agglutination of the ingredients used as protein source (LIM and DOMINY, 1990).

To be nutritionally useful, a probiotic microorganism must colonize and stay alive in the digestive tract of its host (GATESOUBE, 1999). Therefore, it is necessary to offer the probiotic microorganism in concentrations higher than those normally found in host intestinal tract, and/or even in concentrations higher than other bacterial genera in the host intestinal tract. In the present study, the decrease of LAB agrees with the finding of NAVIN CHANDRAN *et al.* (2014) who observed a decrease in the amount of probiotics over time. Bearing in mind 105 CFU per g of diet, the minimum concentration of LAB offered in probiotic diets, SPC was able to sustain this concentration for one week more than FM. This could be explained by the fact that soybean has a large amount of proteins and fibers, as well as inulin and oligosaccharides with prebiotic potential, all of which increase the viability of microorganisms (FUCHS *et al.*, 2005).

The use of LAB is also advantageous in conserving food since it reduces the presence of bacteria with pathogenic potential capable of deteriorating the quality of the diet (HOOVER and STEENSON, 2014; O'BRYAN *et al.*, 2015), thus guaranteeing longer storage time, irrespective of protein source. According to VIEIRA *et al.* (2008), *L. plantarum*, the same strain used in the present study, remained at high concentrations for four days before the supply of probiotic was exhausted, however, the probiotic effects were maintained for two days, suggesting its continuous use. However, the authors did not consider the possibility of using prebiotics or other ingredients that might favor the colonization of these probiotic bacteria in the diet, with a view to increasing the viability and the residence time of these microorganisms. In the present study, shrimp fed with the diet supplemented with probiotic presented a reduction in the concentration of total heterotrophic bacteria, corroborating similar findings in other studies (VIEIRA *et al.*, 2008; JATOBÁ *et al.*, 2011, 2017, 2018; BUGLIONE-NETO *et al.*, 2013), most likely owing to the production of extracellular compounds, such as organic acids and bacteriocins (FULLER, 2012).

Neither protein source nor probiotic interfered with the count of *Vibrio* spp. This result diverged from that observed by VIEIRA *et al.* (2008, 2016) who observed a reduction in the vibriaceae population in the intestinal tract of *L. vannamei* fed with the same probiotic strain. It is common to report the decrease of *Vibrio* spp. in the intestinal tract of aquatic animals fed with diets supplemented with lactic acid bacteria (VIEIRA *et al.*, 2008; JATOBÁ *et al.*, 2008, 2011, 2018; ZHANG *et al.*, 2011; STANDEN *et al.*, 2015; HAI, 2015; VIEIRA *et al.*, 2016). However,

the short experimental time, only 17 days, may have contributed to the absence of inhibition of the *Vibrio* spp. in this trial.

The absence of changes in shrimp epithelium suggests that neither protein sources damaged the tissue. JATOBÁ *et al.* (2017) observed that *L. vannamei* fed diets with SPC and FM, the same sources as those used in this experiment, presented the same food efficiency, demonstrating that shrimp have the ability to digest both protein sources. In the histology of the intestinal tract of Japanese eel (*Anguilla japonica*) fed diets supplemented with *L. plantarum* at concentrations below 107 CFU per g of diet, no differences were observed relative to animals fed with control diet (LEE *et al.*, 2017). The same work shows that concentrations higher than 108 CFU per g of feed could modulate the intestinal microbiota of the Japanese eel (*A. japonica*). This result corroborates the results of other studies, demonstrating the need to offer probiotics consistently and in high concentrations (HAI, 2015; JATOBÁ *et al.*, 2018).

Both factors, protein source and probiotic, did not influence the growth performance, unlike the observed by JATOBÁ *et al.* (2017) which observed a decreased of apparent feed efficiency, protein efficiency ratio and weekly gain for *L. vannamei* reared in biofloc, during 40 days, fed with same diets (without supplementation), the data of these authors were higher than observed in this study, probably due to the presence of the biofloc. During *In vivo* assay water variables values were considered adequate for the species, according to (BOYD and GAUTIER, 2000), suggesting that all changes are related to treatments.

The use of this probiotic has already shown beneficial effects on shrimp farms under outdoors conditions (VIEIRA *et al.*, 2016; GAINZA *et al.*, 2018), however in this study the probiotic did not show benefits in the zootechnical variables, this fact may be related to the duration of the experiment that was designed to evaluate changes in the microbiota.

The *L. plantarum* strain used in the present study has already demonstrated its probiotic potential to shrimp (*L. vannamei*), increasing survival after experimental infections against *Vibrio* spp. in post-larvae (BUGLIONE *et al.*, 2008) and juveniles (VIEIRA *et al.*, 2008, 2010), as well as shrimp reared on commercial farms (VIEIRA *et al.*, 2016), increasing the digestibility of diets (BUGLIONE-NETO *et al.*, 2013) and improving food conversion (VIEIRA *et al.*, 2016), justifying its application regardless of the protein source used.

## CONCLUSION

The inclusion of *L. plantarum* did not interfere with the physical properties of the two dietary formulations. The protein source did not interfere with the viability of *L. plantarum* in diets, but soy protein concentrate kept it in higher concentrations for longer. *L. plantarum* combined with different protein sources, fishmeal or soy protein concentrate, produced similar effects on the histology and microbiota of the marine shrimp (*Litopenaeus vannamei*).

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