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Cultivation of Juvenile Fat Snook (*Centropomus parallelus* Poey, 1860) Fed Probiotic in Laboratory Conditions

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ABSTRACT

The objective of this study was to investigate the growth of juvenile fat snook (Centropomus parallelus) *in laboratory conditions when fed a diet supplemented with the probiotic* Lactobacillus plantarum. *Changes in the intestinal flora, haematological parameters and growth performance were assessed using 180 fishes (54.2 ± 13.4 g each). The fishes were subjected to two treatments divided into six cages: 1) feed supplemented with probiotic, and 2) feed without probiotic (control). The temperature, dissolved oxygen and salinity were maintained at 25 ± 1 ° C, 4.0 mg.L^{-1} and 33 ‰, respectively. After 10 weeks of culture, the fishes fed probiotic had reduced viable culturable heterotrophic bacteria and* Vibrio *spp. and increased lactic acid bacteria in the intestinal tract, as well as a higher number of thrombocytes, leukocytes and lymphocytes in the blood. No significant difference was observed in the growth, survival or body composition, but the hepatosomatic index was significantly higher in the fishes fed with probiotic and control.*

Key words: *Lactobacillus plantarum*, hematology, bacterial microbiota, body index

INTRODUCTION

The Brazilian coast has several marine fishes species with potential for aquaculture, including the Fat Snook (*C. paralellus*), because it is euryhaline and can be cultured in marine and estuarine environments, endure low temperatures (10 °C lethal temperature) and dissolved oxygen concentrations (1 mg.L^{-1}). This fish found throughout the Brazilian coast, was more abundant in the north and northeast (Cerqueira, 2004).

Fat snook are of great interest for the commercial and sport fishing and are used to restock the coastal lagoons (Cerqueira, 2004). Their production in large-scale is constrained by the difficulty of producing large quantities of juveniles due to the high mortality rates in this stage (Hjelm et al., 2004; Temple et al., 2004).

Bacterial diseases are commonly associated with aquaculture production. Some causative agents include bacteria from *Vibrios, Aeromonas, Pseudomonas, Streptococcus* and other genera

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(Austin and Austin, 2007). In marine fish farming, bacterial disease is recorded on eggs, larvae, juveniles and adults, and the development of diseases results from the interaction of pathogen, host and environment (Toranzo et al., 2005). Dixon (1991) correlated the bacterial diseases in the fishes with minor problems such as stress, temperature changes, salinity, water quality, parasites and chemotherapy treatments. To control the bacterial diseases, antibiotics are commonly used, but the inappropriate use of chemotherapy may lead to the selection of some resistant pathogenic bacterial strains (Vázquez et al., 2005), and also be a source of environmental pollution (Boyd and Massaut, 1999).

Probiotics may be a viable alternative to prevent the bacterial disease in aquaculture (Jatobá et al., 2008a). They can act in preventing the disease through reducing the bacterial load by competitive exclusion or production of inhibitory substances, and can also stimulate the host animal's immune system and produce additional digestive enzymes (Verschuere et al., 2000). Gatesoupe (1999) defined the probiotics for aquaculture as the microbial cells added in such a way that they entered the digestive tract of animals while still alive, with the aim of improving the health of the animal.

The use of *Lactobacillus* showed good results in fish culture. Souza (2007) observed a probiotic effect of *L. plantarum* and *Lactococcus* sp. *in vitro* and *in vivo* for fat snook, which enhanced the activity of an alkaline protease that could help the digestive activities of the fish. Jatobá et al. (2008a and 2008b) confirmed the probiotic effect of *L. plantarum* on Nile tilapia in fresh and brackish water.

The objective of this study was to assess the changes in intestinal flora, haematological parameters and growth performance of juvenile fat snook in laboratory conditions when fed a diet supplemented with probiotic (*L. plantarum*).

MATERIALS AND METHODS

The experiments used 180 fat snook (*Centropomus parallelus*) juveniles, weighing 54.2 ± 13.4 g and with a total length of 18.3 ± 1.6 cm. The probiotic bacteria used was *Lactobacillus plantarum* strain (CPQBA 227-08 DRM), which was molecularly identified in the *Centro Pluridisciplinar de Pesquisa Químicas, Biológicas e Agrárias da Universidade Estadual de Campinas*. This strain was isolated from the intestinal tract of tilapia, and approved by *in vitro* and *in vivo* tests (Jatobá et al., 2008a).Six cages (3.4 m^3, 2 m x 1.7 m x 1 m) were used in two fibreglass circular ponds (6 m radius) with a capacity of 50 m^3 and constant water renewal, four cages in one and two in the other. The experimental units were divided into two treatments with three replicates each, with a completely randomized design. The treatments were as follows: (1) fat shook fed a commercial diet supplemented with probiotics (*L. plantarum*), and, (2) fat shook fed a commercial diet without the probiotic, for 10 weeks.

The feed was prepared according to Jatobá (2008a). The culture of *L. plantarum* in MRS medium (De Man, Rogosa and Sharpe, 1960) was sprayed on commercial extruded diet (with 11.1% moisture, 18.6% ash, 10.5% total fat, 7.8% acid soluble fibre and 55.3% total protein) and incubated at 35 ° C for 24 h (Ramírez et al., 2006). The diet of the control treatment was sprayed only with sterile MRS culture medium. Five ten-fold serial dilutions were cultured in modified MRS Agar culture medium (Ramírez et al., 2006) to quantify the cells in the diet. This resulted in a count of $1x10^7$ colony-forming units (CFU) of *L. plantarum* per gram.

The feed rate of 1.5% of biomass per day, adjusted after five weeks through biometrics, were used in 30% of the fishes in each experimental unit to do the biometrics. The feeding frequency was twice daily (8:00 and 14:00 h). At the end of the biometrics experiments, after a day of fasting, all the fishes were evaluated for growth and weight. The temperature, dissolved oxygen and salinity were maintained at 25 ± 1 ° C, 4.0 mgL^{-1} and 33 %, respectively. To measure the water quality parameters (total ammonia, nitrite, phosphate and silicate), daily water samples from each experimental unit were collected using a plastic bottle (250 mL). The assays were carried out with a spectrophotometer 2K SL microprocessor. The daily renewal rate in the water tanks was 100% a day.

The total dissolved ammonia was measured by the Solorzano (1969) method as modified by Strickland and Parsons (1972). The total dissolved phosphate was measured by the method of Murphy and Riley (1962) and silica by the second method of Mullin and Riley (1955). The methods used for the determination of phosphate and silicon were as described by Aminot and Chaussepied (1983).

Water samples were collected every two weeks from two fibreglass circular ponds to measure the pH, presence of lactic acid bacteria, orthophosphate and silicate. All the analysis were done in triplicate.

After 10 weeks, survival, specific growth rate (SGR = {100 x [(natural logarithm of final weight - natural logarithm of initial weight)/Number of days]), feed conversion rate and condition factor (CF = body weight/Standard Length^3 x 100) were measured. Liver, gonads, viscera and peritoneal fat were removed from five fishes of each experimental unit to calculate the following indices: hepatosomatic = (liver weight/total weight) x 100, gonadosomatic = (gonad weight/total weight) x 100, viscerosomatic = (viscera weight/weight) x 100 and liposomal = (peritoneal fat weight/total weight) x 100. The analysis of final body composition (the five dissected fishes of each replicate) were carried out as detailed by the Association of Official Analytical Chemists (AOAC, 1999). The dry matter was obtained by drying at 105 °C (gravimetric method), mineral matter (ash) by incineration in a muffle for five hours, fat by ether extraction after acid hydrolysis and the crude protein by acid digestion. The samples were homogenized prior to analysis.

At the end of the experiment, the intestinal tracts of a group of three fishes per experimental unit were dissected for the microbiological evaluations. After 24 h without feed, the gut tracts were macerated and serially diluted ten-fold in sterile 0.65 % saline solution. Samples of each dilution were cultured on tryptone soy agar (TSA), thiosulfate citrate bile sucrose (TCBS) agar and MRS, and incubated for 48 h at 30 °C for viable culturable heterotrophic bacterial counts, *Vibrionaceas* and lactic acid bacteria, respectively. Five fishes per experimental unit (15 per treatment) were anaesthetized with benzocaine (50 mg.L^{-1}) and approximately 1.0 mL of blood was drawn from the caudal vein of each fish for the preparation of blood smears in duplicate. Blood slides were stained with Giemsa/May-Grunwald stain (Rosenfeld, 1947) for differential leukocyte count and total count of thrombocytes and leukocytes by the indirect method (Martins et al., 2004). A blood aliquot was used for the determination of the hematocrit (Goldenfarb et al., 1971). The remainder was stored in glass containers on ice for total erythrocyte count in a hemocytometer. Only the microbiological data were transformed to $\log_{(x+1)}$. All data were evaluated by t-test at 5% level of significance.

RESULTS

The pH, total ammonia (NH_4^+, NH_3), toxic ammonia (N - NH_3), orthophosphate (P - PO_4) and silicate (SiO_3) were 7.6 ± 0.1, 0.49 ± 0.23 mg.L^{-1}, 0, 01 ± 0.01 mg.L^{-1}, 0.01 ± 0.03 mg.L^{-1} and 0.39 ± 0.11 mg.L^{-1}, respectively. There were no significant differences in the growth, survival, feed conversion rate and specific growth rate between treatments (Table 1). Among the body indices, only the hepatosomatic index showed a significant difference (Table 2). The body composition of the fishes did not differ between the treatments (Table 2). The hematocrit percentage, erythrocytes, monocytes and neutrophils did not change between the treatments (Table 3), but fat snook fed the probiotic diet showed a higher number of thrombocytes, total leukocytes and circulating lymphocytes (Table 3).

The gut tracts of the fishes fed the diet supplemented with probiotic had reduced viable culturable heterotrophic bacteria and *Vibrio* spp. as well as increased acid lactic bacteria (Fig. 1).

Table 1 - Survival, final weight and length, specific growth rate, feed rate and condition factor (mean ± standard deviation) of fat snook (*Centropomus parallelus*) fed a probiotic (*Lactobacillus plantarum*) and control diet.

Indices	**Treatment**	
	Control	**Probiotic**
Survival (%)	90,00 ± 8,82	91,11 ± 5,09
Final weight (g)	71,02 ± 18,72	74,12 ± 16,20
Final length (cm)	19,45 ± 1,66	19,63 ± 1,32
Specific growth rate (%.day^{-1})	0,33 ± 0,00	0,37 ± 0,05
Feed rate	0,89 ± 0,19	1,11 ± 0,08
Condition factor	0,97 ± 0,04	0,98 ± 0,04

Different letters indicate significant differences ($p < 0.05$) in t-test between treatments.

Table 2 - Biochemical composition and body indices (mean ± standard deviation) of fat snook (*Centropomus parallelus*) fed a probiotic (*Lactobacillus plantarum*) and control diet.

Body índices (%)	**Treatment**	
	Control	**Probiotic**
Hepatossomatic índex	1,15 ± 0,20 [a]	1,35 ± 0,25 [b]
Gonadossomatic índex	0,11 ± 0,06	0,10 ± 0,04
Viscerossomatic índex	2,24 ± 0,29	2,11 ± 0,32
Lipossomal index	3,86 ± 1,40	4,13 ± 1,68
Body biochemical composition (%)		
Moisture	67,26 ± 1,02	67,28 ± 1,80
*Ash	16,73 ± 1,73	17,52 ± 1,64
*Total fat	19,49 ± 3,20	20,95 ± 6,09
*Total protein	61,34 ± 0,71	59,02 ± 2,03

*Dry matter; different letters indicate significant differences ($p < 0.05$) in t-test between treatments.

Table 3 - Haematological parameters (mean ± standard deviation) of fat snook (*Centropomus parallelus*) fed a probiotic (*Lactobacillus plantarum*) and control diet.

Hematological parameters	**Treatments**	
	Control	**Probiotic**
Hematocrit (%)	33,9 ± 5,1	34,2 ± 5,0
Erythrocyte ($x10^6$ μL^{-1})	2,2 ± 0,4	2,9 ± 0,1
Thrombocyte ($x10^3$ μL^{-1})	6,8 ± 1,7 [a]	12,6 ± 1,3 [b]
Leukocyte ($x10^3$ μL^{-1})	39,3 ± 4,9 [a]	55,0 ± 9,0 [b]
Lymphocyte ($x10^3$ μL^{-1})	35,5 ± 5,6 [a]	50,3 ± 5,6 [b]
Neutrophil ($x10^3$ μL^{-1})	2,3 ± 2,6	2,4 ± 1,7
Monocyte ($x10^3$ μL^{-1})	1,4 ± 0,5	2,3 ± 0,5

Different letters indicate significant differences ($p < 0.05$) in t-test between treatments.

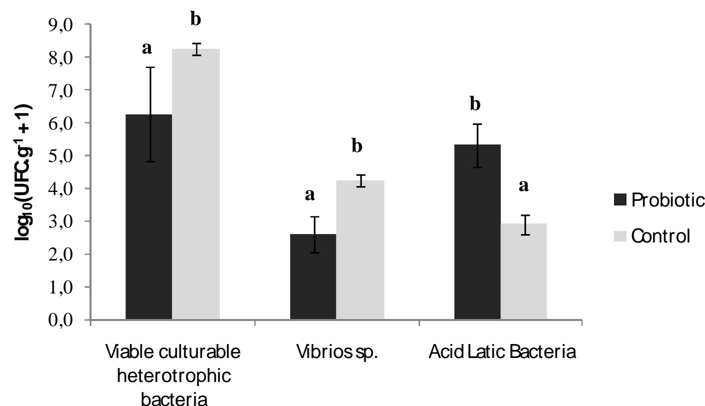

Figure 1 - Bacterial counts in the gut of fat snook (mean ± standard deviation) of fat snook (*Centropomus parallelus*) fed a probiotic (*Lactobacillus plantarum*) and control diet. Different letters indicate significant differences ($p < 0.05$) in t-test between treatments log ($ufc.g^{-1} + 1$).

DISCUSSION

Gildberg et al. (1995), using *Lactobacillus* sp. in juvenile Atlantic salmon (*Salmo salar*) and Hidalgo et al. (2006), using *Bacillus cereus* and *B. toyoi* in Dentex (*Dentex dentex*) did not show improved body indices, similar to what was seen in this work. Rengpipat et al. (2008) reported a beneficial effect on the growth and survival of Asian juvenile snook (*Lates calcarifer*) using *Lactobacillus* sp. Carnevali et al. (2006), using *L. delbrueckii delbrueckii* isolated from the European sea bass (*Dicentrarchus labrax*), reported an increase in weight gain in the fishes fed on a diet with probiotics; this result was related to the specificity between the probiotic bacteria and host fish, as well as increased expression of the gene for the growth.

The probiotic bacteria used in this study were assessed in a polyculture system with tilapia (*Oreochromis niloticus*) and marine shrimp (*Litopenaeus vannamei*). Following 12 weeks of growth, the feed efficiency, productivity and final weight increased 13.6 %, 7.5 % and 7.1 %, respectively in tilapia fed the probiotic diet (Jatobá, 2008a). The absence of differences in the indices evaluated in this work could be related to the diet offered or the lack of specificity between the bacteria and host (fish).

After 10 weeks, fishes gained an average of 20 g in both the treatments (0.5 g per week). Moreover, there was no difference in specific growth rate between the treatments (Table 1). Both were less than the results reported by Souza-Filho and Cerqueira, (2003). This suggested that the diet used did not meet the nutritional requirements for fat snook and/or laboratory conditions did not favour the fish growth. Laidley et al. (1988) found that any change in the hepatosomatic index could result from fat accumulation, a metabolism disorder or an increase in gluconeogenesis induced by a possible stress. The increase in the hepatosomatic index in the fishes treated with probiotic diet might be related to the lack of specificity between the probiotic (*L. plantarum* isolated from Nile tilapia) and host (fat snook), which might have caused some change in the fish metabolism. The lipid accumulation might be associated with lipase production from lactic acid bacteria, even in small quantities (Fryer et al., 1976). Dentex juveniles (*Dentex dentex*) fed a wet diet supplemented with *B. toyoi* and *B. cereus* in different concentrations showed a difference between the probiotic and control treatments in body composition (Hidalgo et. al., 2006). In contrast, the body composition of the fat snook did not differ between the treatments in this work (Table 3).

Ramírez et al. (2006) and Gatesoupe (2008) related the reduction of gut tract bacteria with a probable mechanism of inhibition by competitive exclusion for the space and nutrients or by the change of microbial metabolism in the gut tract. In tilapia, this probiotic colonized easily in the laboratory and field, reducing viable culturable heterotrophic bacteria, *Vibrio* spp. and *Pseudomonas* spp and increasing the number of viable lactic acid bacteria in the gut tract (Jatobá et al., 2008a and 2008b). Carnevali et al. (2006) observed an increase in lactic acid bacteria in the gut tract of sea bass fed a diet supplemented with probiotic (*L. delbrueckii delbrueckii*).

The presence of lactic acid bacteria was not detected in the water; this could be explained by the high rate of daily renewal (100% of the volume of water per day) and the possibility that the environment condition (water culture) might be inadequate to maintain live *L. plantarum*.

The hematocrit values were near those observed by Ranzani-Paiva et al. (2008) in fat snook inoculated with *Saccharomyces cerevisiae*. Welker et al. (2007), Aly et al. (2008) and Jatobá et al. (2008b) also reported no difference in the hematocrit between the probiotic and control treatments for tilapia fed a diet with probiotic. The thrombocytes in some species have roles in haemostasis, the defence mechanism, clotting and inflammation, and can also participate in the phagocytic activity during infection (Tavares-Dias, 2003). The high levels of thrombocytes suggest a better immune system in the fish treated with probiotics. The probiotic may have induced a higher production or release of lymphocytes and thrombocytes, which may be interesting, as these cells have important functions in the fish immune system (Secombes, 1996).

Changes in the numbers of neutrophils and monocytes in circulation are common in the fishes treated with probiotics when subjected to experimental infection (Aly et al., 2008; Kumar et al., 2008; Jatobá et al., 2008b). Having the same number of these cells (n and m) suggested that the probiotic did not activate the immune system, only made it more immunocompetent. This result was interesting, because the probiotic did not activate the immune system as an immunostimulant, and so did not waste the energy. This result suggested an improvement in response to infection, as was observed for Nile tilapia (Jatobá et al., 2008b).

CONCLUSION

The supplementation of *L. plantarum* in the diet of juvenile fat snook improved the bacterial microbiota in the gut tract and increased the number of thrombocytes, leukocytes and lymphocytes circulating in these fishes. The *L. plantarum* did not affect the growth, survival, feed conversion or body composition of fat snook fed with the probiotic diet.

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