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Effect of dietary supplementation of inulin and *W. cibaria* on haemato-immunological parameters of hybrid surubim (*Pseudoplatystoma* sp)

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Abstract

The dietary supplementation of prebiotics, probiotics and symbiotic in hybrid surubins (a *Pseudoplatystoma corruscans* and *P. fasciatum* cross) was evaluated for the effects on their autochthonous intestinal microbiota and on haematological and immunological parameters. A total of 160 fish were divided into four treatment groups with four replicates each. The treatment groups were fed with the following diets for 15 days: control diet without supplementation; 0.5% inulin (prebiotic) supplementation; *Weissella cibaria* (CPQBA 001-10 DRM 02) (7.87 ± 0.2 log CFU g^{-1}) supplementation; or 0.5% inulin and *W. cibaria* supplementation (symbiotic group). The midgut intestines of the fish with the symbiotic diet supplementation had higher concentrations of lactic acid bacteria (7.07 ± 1.11 log CFU g^{-1}) and low levels of *Vibrio* spp (1.90 ± 0.60 log CFU g^{-1}) and *Pseudomonas* spp (2.23 ± 1.48 log CFU g^{-1}). In addition, increased erythrocytes and reduced circulating neutrophils were observed in this group. No differences in blood glucose, serum protein or lysozyme levels were detected between treatment groups. However, a higher concentration of total immunoglobulin was observed in fish fed with the probiotic and symbiotic diets. The addition of 0.5% inulin (prebiotic) thus *W. cibaria* (probiotic) to the diet of *Pseudoplatystoma* hybrid surubins reduce the number of pathogenic bacteria and stimulate the beneficial intestinal microbiota and may possibly alter their immune defence system.

KEY WORDS: fish, gut microbiota, immunology, prebiotic, probiotic, symbiotic

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Introduction

Catfish of the genus *Pseudoplatystoma* have great economic importance both in fishing and aquaculture (Barthem & Goulding 1997; Roubach *et al.* 2003; Crepaldi *et al.* 2006). In 2007, the aquaculture production of these catfish species in inland waters of Brazil was about 670 tons, resulting in about US $1 467 000 in revenue (FAO 2009).

The nutrition of cultured fish is critical to their health, especially in the maintenance of their immuno-competence and ability to resist diseases and this must be considered when developing production strategies of this and other native Brazilian species (Resende 2009; Merrifield *et al.* 2010).

According to Merrifield *et al.* (2010), the gastrointestinal microbial flora is directly related to the host's nutrition and health. Thus, a balance between pathogenic and beneficial microorganisms can have positive effects when incorporated into the animals' diets. Merrifield *et al.* (2009) described the microbial diversity of the allochthonous and autochthonous intestinal mucosa of rainbow trout (*Onchorhynchus mykiss*). *Pseudomonas* spp. and *Enterobacteriaceae* were found in 80% of the allochthonous microbiota and 60% of the autochthonous microbiota.

Prebiotic, non-digestible substances to the host that alter the balance of beneficial and pathogenic bacteria in the intestinal tract promoting the ability of some probiotic bacteria to compete in the gut and thus aid them to proliferate and exert their effects in the host gut. In resume, the prebiotic

give a competitive advantage to probiotics over the other bacteria (Fuller & Gibson 1997; Gibson *et al*. 2005; Gatesoupe 2008; Yousefian & Amiri 2009; Merrifield *et al*. 2010).

The use of lactic acid bacteria has been effective in several aquatic species including snook (*Centropomus* spp.), tilapia (*Oreochromis* spp.) and shrimp (Vieira *et al*. 2007) owing to their ability to colonize the digestive tract. This alters the natural balance of the intestinal microbiota to enhance the animals' immune systems (Carnevali *et al*. 2006; Vieira *et al*. 2007, 2008; Jatoba *et al*. 2008). However, many studies have demonstrated that non-endogenous probiotics can be just as effective, and in some cases better, than endogenous/indigenous bacteria. Although the host gut it is at least a logical place to screen for probiotics and thus autochthonous candidates have been the focus of many studies (Gatesoupe 2008; Merrifield *et al*. 2010).

One example of non-endogenous probiotics was analyzed in brown trout (*Salmo trutta*) by Balcazar *et al*. (2009). The treatment that received the probiotic strains (*Lactococcus lactis* and *Leuconostoc mesenteroides*) resulted in a higher survival rate after challenge, activation of phagocytic cells in the head kidney and a lower rate of *Aeromonas salmonicida* proliferation in the intestine. On the other hand, Ringo (2008) study the ability endogenous carnobacteria isolated from the intestine of different fishes to inhibit growth of fish pathogenic bacteria and encountered a great inhibition results *in vitro*.

In vitro screening of 199 microorganisms candidates for probiotic characteristics was performed by Cai *et al*. (1998) from the intestine of flounder (*Paralichthys olivaceus*). Highlighted strain in that study was *W. hellenica* DS-12 with probiotic characteristics. This species of bacteria are Gram-positive, irregular or coccoid rods, obligately heterofermentative, catalase negative organisms that produce D- or DL- isomers of lactic acid as a main end product fermentation (Collins *et al*. 1993; Jang *et al*. 2002).

Probiotic characteristics of *Weissella* species associated with the use of prebiotic could enhance the effect on the immunological parameters of fish. On this context, an additive symbiotic for aquaculture is defined as a supplement that contains both a prebiotic and a probiotic working together to improve the 'friendly' gut microbiota of fish and should be considered as functional food. In fact, this optimizes the production of these species and ensure sustainable aquaculture without chemotherapy additives.

Gibson & Roberfroid (1995) defined the symbiotic as a mixture of prebiotic and probiotic that beneficially affects the host by improving the survival and attachment of live microbial dietary supplements to the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria. Hence improving the host 'welfare'.

An overview written by Ringo *et al*. (2010), a prebiotic commonly used in animal feeds include inulin, a type fructans composed of β-d-fructufuranoses attached by a β-2-1 linkages. This additive is not a natural fibre in fishes diets, although may have interesting applications in aquaculture to stimulate the 'good' gut microbiota, suppressing pathogens and enhancing immune responses in fishes. Although another work made by Olsen *et al*. (2001) showed that 15% inclusion of inulin resulted in intestinal damage, and this effect was justified by the authors to an accumulation of lamellar structures and large vacuoles, altering the organization of the microvillous in the hindgut of Arctic charr (*Salvelinus alpinus* L.) compared with the control diet that not received the inulin supplementation.

The purpose of this study was to evaluate the addition of prebiotic, probiotic and symbiotic supplements to the diet of a hybrid surubim to elucidate their effects on the autochthonous intestinal microbiota and haematological and immunological characteristics.

Material and methods

Biological materials

Weissella cibaria (CPQBA 001-10 DRM 02) strain was used as probiotic. This strain was isolated from the midgut of healthy hybrids surubins cultivated in Mato Grsso do Sul Brazil and had been selectionated both *in vitro* (antagonistic activity against bacterial pathogens by wheel disk assay method, biochemical characteristics, kinetics of microbial growth) and *in vivo* (colonization of the intestinal tract and haematological changes) to this fish (Mouriño, J.L.P., do Nascimento Vieira, F., Jatobá, A.B., da Silva, B.C., Jesus, G.F.A., Seiffert, W.Q. & Martins, M.L., unpublished data). This strain was molecularly identified at the State University of Campinas (UNICAMP) and the amplification of rRNA 16S gene was performed using PCR (p10f, 765f, 782r and p1100r) (Fig. 1). The strain was incubated at 35 °C for 48 h in Man–Rogosa–Sharpe broth (MRS) (Difco®, São Paulo, Brazil) for enumeration of lactic acid bacteria to a concentration of 10^9 CFU mL^{-1} to maintain its viability for diet pellet colonization.

A male pintado (*P. corruscans*) (Agassiz 1829) and a female cachara (*P. fasciatum*) (Linnaeus 1766) from the Company Mar e Terra Ltda. (Dourados, Mato Grosso do Sul, Brazil) were crossed to produce 160 hybrid fish (73.6 ± 19.5 g in weight and 23.7 ± 1.9 cm in length).

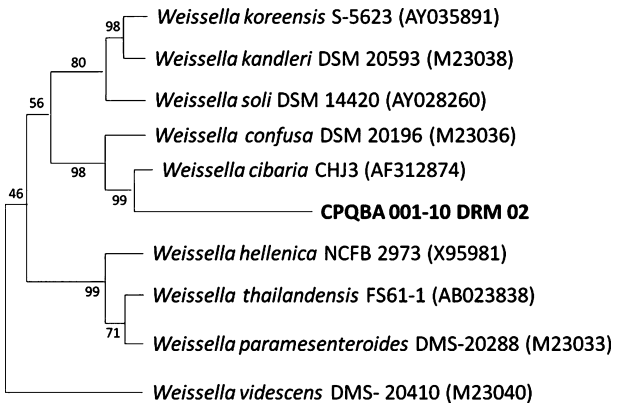

Figure 1 Phylogenetic tree showing the phylogenetic relationships among the sequences of 16S ribosomal RNA gene of CPQBA 001-10 DRM 02 and another's microorganisms presented in the related databases genbank and RDP.

Table 1 Components of the diet used in this study, Douramix® (Revolution Fingerlings)

Informed composition	Diet (g kg^{-1})
Calcium (max)	35.00
Ether extract (min)	120.00
Phosphorus (min)	20.00
Fibrous matter (max)	30.00
Mineral matter (max)	120.00
Crude protein (min)	400.00
Moisture (max)	120.00

Diet preparation

Three inulin (Orafti® HPX, Tienen, Belgium) concentrations were investigated with a commercial diet (Douramix®, Revolution Fingerlings, Dourados, Mato Grosso do Sul, Brazil) to determine the amount to be incorporated in the diet (Table 1). The commercial diet was previously milled and sampled to be included with 0.00%, 0.25%, 0.50% or 1.00% inulin. Each sample was homogenized for 15 min in a Y mixer (Artabras® model ARTMVY, Bastos, Brazil) with 20-kg capacity. Once homogenized, the mixture was pelleted and dried for 18 h at 45 °C by air circulation.

The pellets were sprinkled with an inoculum of *W. cibaria* in a proportion of 100 mL kg^{-1} at a concentration of 10^9 CFU mL^{-1}. After that, the diets were homogenized again for 15 min in the same Y mixer to be dried in an oven with circulating air at 35 °C for 24 h, and three 1-g samples of each diet were macerated with 1 mL of sterile 0.65% saline solution and one-tenth serially diluted nine times. The 10^{-5} to 10^{-9} dilutions were plated on MRS agar medium (Difco®) at 35 °C for 48 h. The diets supplemented with 0, 0.25%, 0.5% and 1% inulin were prepared in quadruplicate and stored in plastic bags at room temperature (23 °C) in a room with a dehumidifier. The samples were collected to quantify the amount of lactic acid bacteria and determine the concentration of inulin that results in an increase of their concentration.

Experimental design

The 160 fish were evenly distributed in 16 100-L circular tanks with aeration and heating systems (26 ± 0.5 °C). Each tank had a biological filtration system independent of the others to maintain fresh water quality. The water was kept clear by replacing 30% of the water daily and removing the sediment from the tank bottoms. Throughout the test, the water characteristics were as follows: dissolved oxygen, 8.5 ± 0.5 mg L^{-1}; total ammonia, 1.0 ± 0.5 mg L^{-1}; nitrite, 0.25 ± 0.2 mg L^{-1}; and pH 7.0 ± 0.3.

The fish were indiscriminately distributed in the tanks to ensure four random replicates for each treatment. They were acclimated for five days and subsequently treated for 15 days by feeding four times a day with one of the following diets: control without supplementation; 0.5% inulin (prebiotic) supplementation, *W. cibaria* (probiotic) supplementation; and 0.5% inulin plus *W. cibaria* (symbiotic) supplementation.

All experimental diets used were prepared as described, and groups inoculated with *W. cibaria* reached a concentration of 10^7 CFU g^{-1}.

Haemato-immunological parameters

Following treatment, five fish from each experimental unit were anaesthetized with benzocaine (1 g per 10 L of water) and blood was collected from the caudal vessel with two 3-mL syringes (21 G), one with the anticoagulant EDTA and another without. The blood collected with EDTA was used for haematological analyses and serum was obtained from the blood without the anticoagulant. Blood without an anticoagulant was pooled from five fish of each experimental unit and stored for 1 h at 25 °C for coagulation. It was then centrifuged at 1400 ***g*** for 10 min, and the serum was stored at −20 °C.

The glycemic index was determined using a serum aliquot in spectrophotometer at 505 nm according to the manufacturer's recommendations (Biotechnical Glucose Test Kit, São Paulo, Brazil).

The serum lysozyme activity was determined by a method adapted from a previous study (Sankaran & Gurnani 1972). A suspension of lyophilized *Micrococcus lysodeikticus* (Sigma-Aldrich, St. Louis, MO, USA) was diluted in PBS buffer (0.04 M Na_2HPO_4, pH 6.2) to a concentration of

0.50 mg mL^{-1} immediately before use. Serum aliquots of 30 μL were seeded in triplicate in 96-well flat bottom microplates, and 200 μL of *M. lysodeikticus* cell suspension was added to each well. The initial absorbance and the absorbance after 20 min of incubation at 35 °C were measured at 550 nm, and the rate of decrease in absorbance was converted to lysozyme concentration (μg mL^{-1}) using a standard curve from hen egg white lysozyme (Sigma-Aldrich).

The concentration of total immunoglobulin was measured according to the method described by Amar *et al.* (2000). Briefly, 100 mL of serum was mixed with 100 mL of 12% polyethylene glycol (PEG, 10 000 MW; Sigma Chemical, St. Louis, MO, USA). Samples were incubated for 2 h to precipitate the immunoglobulin molecules, which were then removed by centrifugation at 5000 ***g*** for 10 min (4 °C). The supernatant was removed and the amount of total protein was measured (Bradford & Williams 1976) using bovine serum albumin to construct a standard curve. The concentration of total immunoglobulin (Ig) is expressed in mg mL^{-1} and was calculated as follows:

$$\text{Total Ig}\,(\text{mg mL}^{-1}) = (\text{total serum protein} - \text{total protein treated with PEG}) \times \text{vol}^{-1}$$

Duplicates of blood samples collected with the anticoagulant were stained with May Grunwald/Giemsa (Rosenfeld 1947) for differential leucocyte, total thrombocyte and total leucocyte counts by blood smears, and an established procedure was used to determine the haematocrit (Goldenfarb *et al.* 1971). The total numbers of thrombocytes and leucocytes were determined from blood by the indirect method described by Martins *et al.* (2004). The remaining material was stored in glass vials on ice to quantify the total number of red blood cells using a haemocytometer.

Intestinal microbiota

After anaesthetization with 0.05% of benzocaine, the midgut intestinal tracts of fish sampled from each tank starved for 24 h were excised aseptically with forceps and scissors to be washed twice to remove faecal and digest material from the tissue with sterile saline solution (SSE 0.65%), and then homogenized in sterile saline (0.65%) in a grail and serially diluted 1 : 10. Then, 10^{0} to 10^{-6} dilutions were plated on tryptic soy agar (TSA) culture medium (total heterotrophic bacteria), MRS agar (lactic acid bacteria), Cetrimide Agar (*Pseudomonas*) and thiosulfate citrate bile salts sucrose (TCBS) agar (*Vibrio*) and incubated at 30 °C. The number of colony forming units (CFUs) was measured after 24 h of incubation on TSA, Cetrimide Agar or TCBS agar or 48 h on MRS Agar. Gram staining was performed using colonies grown on MRS and the strains with similar characteristics to *Weisella* were phenotypically characterized using the API 50CH Kit (API, Biomerieux, Hazelwood, MO, USA); lactic acid consuming bacteria were identified by the fermentation of 49 carbohydrates. The results of the kit were interpreted according to the APIWEB (API, Biomerieux) software.

Statistical analysis

Data from *in vivo* experiments were evaluated for homogeneity of variance by Bartlett's test. The observed heterogeneity of variance was transformed by log $(x + 1)$ for microbiological counts and blood counts, arcsine for survival data or square root for other data. Subsequently, the data were evaluated by analysis of variance with a 5% significance level. If significant differences were detected, Tukey's mean separation test was used ($P < 0.05$) (Zar 1996).

Results

Supplementation with 0.5% and 1% inulin resulted in higher levels of lactic acid bacterial counts compared to the no and 0.25% inulin groups, 7.87 ± 0.2, 7.77 ± 0.4, 6.43 ± 0.2 and 6.35 ± 0.2 Log CFU g^{-1}. However, the 0.5% and 1% inulin groups did not differ in the amounts observed. The lower effective concentration (0.5%) was used in our study to reduce costs in incorporating this functional ingredient into the animals' diets. For the intestinal bacterial concentrations in fish supplemented with *W. cibaria* and the symbiotic combination of 0.5% inulin and *W. cibaria*, the bacterial counts for *Vibrio spp.* were lower than in groups that received only prebiotics and the control group, although the concentrations of total heterotrophic bacteria did not differ. A significant reduction in the concentration of *Pseudomonas* was observed with the symbiotic combination. As these strains were not identified, we cannot affirm safely their pathogenic action to this hybrid catfish (Table 2).

In addition, a direct influence on the concentration of lactic acid bacteria was observed with the prebiotic and probiotic diets. The counts of lactic acid bacteria of the intestinal tract in groups that received only the probiotic (4.87 ± 0.30 Log CFU g^{-1}) or the prebiotic (4.68 ± 0.61 Log CFU g^{-1}) were similar to one another but different from the control group (1.54 ± 0.06 Log CFU g^{-1}). The group treated with the symbiotic combination had a concentration of 7.07 ± 1.11 log CFU g^{-1}, differing from the other groups

Table 2 Bacterial counts of the intestinal tract of surubins fed with a control diet, a diet supplemented with 0.5% inulin, a diet supplemented with *Weissella cibaria* or a symbiotic diet (0.5% inulin + *Weissella cibaria*)

	Bacterial counts log (CFU gr^{-1})			
Treatment	Total bacteria	*Vibrio*	Lactic acid	*Pseudomonas*
Control diet	7.46 ± 1.17[a]	4.64 ± 1.06[b]	1.54 ± 0.06[a]	4.86 ± 0.23[a]
0.5% inulin	7.62 ± 0.97[a]	4.15 ± 0.36[b]	4.87 ± 0.30[b]	3.03 ± 0.20[ab]
W. cibaria	5.91 ± 0.81[a]	0.75 ± 0.90[a]	4.68 ± 0.61[b]	3.68 ± 0.42[ab]
W. cibaria + 0.5% inulin	6.21 ± 0.36[a]	1.90 ± 0.60[a]	7.07 ± 1.11[c]	2.23 ± 1.48[b]

Means ± SD followed by the same letters do not differ by Tukey's test at 5% probability.

Table 3 Haematological parameters in surubins fed with a control diet or diets supplemented with the following: 0.5% inulin; *Weissella cibaria*; or a symbiotic combination (0.5% inulin + *Weissella cibaria*)

	Treatments			
Blood cells	Control diet	0.5% inulin	*W. cibaria*	*W. cibaria* + .5% inulin
Erythrocytes ($\times 10^6$ mL^{-1})	1.76 ± 0.03[a]	1.88 ± 0.05[a]	1.77 ± 0.09[a]	1.99 ± 0.02[b]
Thrombocytes ($\times 10^3$ mL^{-1})	17.0 ± 0.38[a]	24.5 ± 0.36[a]	19.0 ± 0.28[a]	20.8 ± 0.19[a]
Leucocytes ($\times 10^3$ mL^{-1})	39.4 ± 0.39[a]	46.3 ± 0.95[a]	53.7 ± 1.56[a]	51.4 ± 0.98[a]
Lymphocytes ($\times 10^3$ mL^{-1})	35.5 ± 0.32[a]	46.2 ± 0.82[a]	51.1 ± 1.57[a]	52.1 ± 0.67[a]
Monocytes ($\times 10^3$ mL^{-1})	0.94 ± 1.95[a]	0.76 ± 2.71[a]	0.53 ± 0.71[a]	0.75 ± 2.48[a]
Eosinophils ($\times 10^3$ mL^{-1})	0.19 ± 0.62[a]	0.34 ± 1.62[a]	0.28 ± 0.94[a]	0.29 ± 1.88[a]
Basophils ($\times 10^3$ mL^{-1})	0.66 ± 1.19[a]	0.54 ± 1.49[a]	0.66 ± 2.71[a]	0.47 ± 1.61[a]
Neutrophils ($\times 10^3$ mL^{-1})	1.66 ± 3.91[b]	1.45 ± 1.40[b]	0.89 ± 2.22[a]	0.88 ± 0.49[a]
Haematocrit (%)	21.11 ± 3.47[a]	21.08 ± 1.73[a]	20.21 ± 4.52[a]	18.38 ± 1.54[a]

Means ± SD followed by the same letters do not differ by Tukey's test at 5% probability.

Table 4 Immunological parameters of surubins fed with a control diet or diets supplemented with the following: 0.5% inulin; *Weissella cibaria;* or a symbiotic combination (0.5% inulin + *W. cibaria*)

Treatments	Glucose (mg dL^{-1})	Protein (mg mL^{-1})	Total Ig (mg mL^{-1})	Lysozyme (μg mL^{-1})
Control	62.27 ± 2.45[a]	17.64 ± 2.06[a]	1.80 ± 0.58[a]	7.78 ± 0.03[a]
0.5% inulin	68.39 ± 4.08[a]	14.60 ± 2.08[a]	4.17 ± 0.84[ab]	7.53 ± 0.81[a]
W. cibaria	67.49 ± 4.44[a]	13.44 ± 0.73[a]	7.43 ± 1.44[b]	7.78 ± 0.57[a]
W. cibaria + 0.5% inulin	62.35 ± 4.13[a]	16.09 ± 1.50[a]	7.17 ± 2.47[b]	8.95 ± 0.59[a]

Means ± SD followed by the same letters do not differ by Tukey's test at 5% probability.

(Table 2). The results of API 50CH Kit confirmed the identification of *W. cibaria* in the midgut of the fishes that received this bacteria in the diets. Fish fed with a symbiotic diet had the largest total number of erythrocytes ($P < 0.05$) compared to the others, and fish fed with probiotic and symbiotic diets had a lower number of circulating neutrophils compared to the other treatments (Table 3). Other haematological parameters were not influenced by the treatments.

Glucose concentration, protein levels and lysozyme activity did not differ between treatments. However, the concentration of total immunoglobulin was significantly higher in fish fed with probiotic and symbiotic diets compared to the control group (Table 4).

Discussion

According to Bowden (2008), advances in understanding environmental impact or changes can identify areas of immune function and water quality parameters that can influence in the immune system of fish. In this context, research on prebiotics, probiotics and symbiotics on animal health are of the utmost importance for the understanding and modification of the intestinal tract of aquatic organisms (Merrifield *et al*. 2010; Gibson *et al*. 2005; Nayak 2010).

Because the intestinal tracts of animals are an environment rich in nutrients and have different typical intestinal bacterial population profiles, functional ingredients or food additives can modify their bacterial flora. The inulin used as a prebiotic in this study is from fructan-based ingredients and is not fully digestible. It can contribute to the growth of the animals increasing the lactic acid bacterial population in the intestinal tract (Van Loo 2007; Pompei *et al*. 2008).

Rurangwa *et al*. (2009) cultured *Lactobacillus plantarum* and *L. delbrueckii* for 48 h in the presence of beta glucans, xylo-oligosaccharides, arabinoxylo-oligosaccharides, inulin, oligofructose and glucose, and they observed increased

growth for all conditions except those that contained glucose. In this case, the fermentation of carbohydrates by these microorganisms was dominated by the production of acids, such as short chain fatty acids.

The observation that increased lactic acid bacteria were observed in the intestinal tract of surubim fed a diet containing inulin is in agreement with Burr *et al*. (2008), in which the effects of beer yeast, fructo-oligosaccharides and Gro-Biotic-A on the intestinal microbial flora of red drum were studied.

Supplementation with *W. cibaria* influenced the immune system of fish in this study, evidenced by the increased total immunoglobulin concentration in the blood serum. This finding is concordant with several studies of the use of lactic acid bacteria as probiotics for fish showing that the bacteria can abound in the intestinal tract of freshwater fish and stimulate their immune system (Gatesoupe 2008; Jatoba *et al*. 2008; Nikoskelainen *et al*. 2003; Panigrahi *et al*. 2007, 2005, 2004).

The processes of agglutination and inhibition of pathogens consist in two important mechanisms to fish immune response. They are composed by molecules present in blood serum named lectins and immunoglobulins (Ig). Lectins are proteins capable of binding to certain sugars, mainly mannose found in the wall of Gram-negative or Gram-positive bacteria, but do not have as much specificity as the Ig's. However, molecules with higher capacity binder are the Ig, which have a more specific action between antigens and is produced by B lymphocytes. As for example, Silva *et al*. 2009 and Swain *et al*. 2007 reported increased agglutination titre in the blood serum of Nile tilapia and Indian carp (*Labeo rohita*) after vaccination with multivalent antigens.

According to Beelen *et al*. (1998, 2004), monoclonal antibodies and the haematological parameters in surubim hybrid have been described to obtain bases for comparison and standardization of techniques for further studies and their potential applicability. The present data are still limited for this fish which could lead to environmental interactions, disease outbreaks or even the use of immunostimulants and/or probiotic.

Buentello *et al*. (2010) studied the effects of fish food supplemented with fructo-oligosaccharides, mannan-oligosaccharides, transgalacto-oligosaccharides and GroBiotic-A on weight gain, feed efficiency and specific immunity of the red drum (*Sciaenops ocellatus*). Unlike this study, Buentello *et al*. observed higher serum lysozyme activity levels in animals with diets containing 1% prebiotic, and Panigrahi *et al*. (2009) observed increased lysozyme activity in rainbow trout (*Oncorhynchus mykiss*) fed *L. rhamnosus*. However, Panigrahi *et al*. (2009) observed higher values of total immunoglobulin in animals supplemented with *L. rhamnosus*, in agreement with the observations of this study. When analysing the effect of *Enterococcus faecium* ZJ4 on the growth and immune response of tilapia, Wang *et al*. (2008) observed that fish supplemented with the bacteria showed no significant difference in the concentrations of total serum protein and immunoglobulin after 40 days. However, they observed a higher final weight and daily weight gain of tilapia supplemented with *E. faecium* than those fed with the control diet.

Similar to our results for some parameters, when assessing the *in vitro* innate immune response in the Gilthead seabream (*Sparus aurata*), Cerezuela *et al*. (2008) found no influence on head kidney leucocytes measured by peroxidase release, phagocytosis, respiratory burst or natural cytotoxic activity. Moreover, *in vivo* supplementation with inulin inhibited phagocytosis and the respiratory burst of leucocytes.

Food additives and supplements are a promising alternative to antibiotics, and prebiotics have much potential to maintain the health of cultured fish (Merrifield *et al*. 2010). The administration of symbiotics in this assay did alter the microbiota profiles of the intestinal tract possibly by increasing the diversity and stimulating specifically the growth of lactic acid bacteria. Moreover, it was comproved that molecular studies of the intestinal microbiota could be performed to answer some questions about the way of action of prebiotics and probiotics in relation to the autochthonous microbiota on the fish immune system of the intestinal tract (GALT) (Dimitroglou *et al*. 2011).

Li & Gatlin (2005) studied *Morone chrysops* and *M. saxatilis* hybrids exposed to *Mycobacterium marinum* in regard to their growth when fed with a diet supplemented with the prebiotic GroBiotic®-A. They found that fish supplemented with 2% of commercial prebiotics had a higher survival rate (80%) compared to those supplemented with 1–2% beer yeast and 2% GroBiotic®-A (72–73%) after 21 days of treatment with a chronic infection. This topic is important and should be studied to further elucidate the effect of supplementing food with probiotics and prebiotics.

For this work, considerations have to be performed, because the use of lactic acid bacteria treated as probiotics considered these genera of bacteria non-pathogenic for the host. However, some works describing diseases in aquaculture assign it to the distinct bacteria belonging to these genera like *Streptococcus*, *Lactococcus*, *Vagococcus* and *Carnobacterium* (Ringo & Gatesoupe 1998). It must be emphasized that the combination between probiotic bacteria and prebiotic on the bacterial population in fish intestinal

tract should be evaluated by another parameters like complement system (NBT), phagocytosis and molecular techniques.

In conclusion, the haematological and immunological parameters of *Pseudoplatystoma* hybrids showed a positive response to the supplementation of *W. cibaria* when supplemented for 15 days via diet. Combined use of prebiotic and probiotic will allow beneficial manipulation of the intestinal microbiota and haemato-immunological parameters.

References

Agassiz, L. (1829) Selecta genera et species piscium quos in itinere per Brasiliam. Monachii, typis C. Wolf, p. 138.

Amar, C.E., Kiron, V., Satoh, S., Okamoto, N. & Watanabe, T. (2000) Effect of dietary β-carotene on the immune response of rainbow trout *Oncorhynchus mykiss*. *Fish. Sci*., **66,** 1068–1075.

Balcazar, J.L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I. & Muzquiz, J.L. (2009) Effect of *Lactococcus lactis* CLFP 100 and *Leuconostoc mesenteroides* CLFP 196 on *Aeromonas salmonicida* infection in brown trout (*Salmo trutta*). *J. Mol. Microbiol. Biotechnol*., **17,** 153–157.

Barthem, R.B. & Goulding, M. (1997) Os bagres balizadores: ecologia, migração e conservação de peixes amazônicos. SCM/CNPq/Ipaam, Amazonas.

Beelen, R., Heijeden, T.V.D., Booms, R., Verdegem, M. & Pavanelli, G.C. (1998) Blood values of young Brazilian catfish *Pseudoplatystoma corruscans* (Agassiz, 1829). *Acta Sci*., **20,** 147–150.

Beelen, R., Boyd, B., Garavello, J.C., Pavanelli, G.C. & Ainsworth, A.J. (2004) Generation, characterisation and applicability of monoclonal antibody to hybrid surubim catfish *Pseudoplatystoma corruscans* (Agassiz) × *Pseudoplatystoma fasciatum* (Linnaeus) immunoglobulin. *Comp. Clin. Pathol*., **12,** 191–198.

Bowden, T.J. (2008) Modulation of the immune system of fish by their environment. *Fish Shellfish Immunol*., **25,** 373–383.

Bradford, M.M. & Williams, W.L. (1976) New, rapid, sensitive method for protein determination. *Fed. Proc*., **35,** 274–274.

Buentello, J.A., Neill, W.H. & Gatlin, D.M. (2010) Effects of dietary prebiotics on the growth, feed efficiency and non-specific immunity of juvenile red drum *Sciaenops ocellatus* fed soybean-based diets. *Aquac. Res*., **41,** 411–418.

Burr, G., Hume, M., Ricke, S., Nisbet, D. & Gatlin, D. 3rd (2008) A preliminary *in vitro* assessment of GroBiotic-A, brewer's yeast and fructooligosaccharide as prebiotics for the red drum *Sciaenops ocellatus*. *J. Environ. Sci. Health B*, **43,** 253–260.

Cai, Y.M., Benno, Y., Nakase, T. & Oh, T.K. (1998) Specific probiotic characterization of *Weissella hellenica* DS-12 isolated from flounder intestine. *J. Gen. Appl. Microbiol*., **44,** 311–316.

Carnevali, O., de Vivo, L., Sulpizio, R., Olivotto, I., Silvi, S. & Cresci, A. (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.

Cerezuela, R., Cuesta, A., Meseguer, J. & Esteban, M.A. (2008) Effects of inulin on gilthead seabream (*Sparus aurata* L.) innate immune parameters. *Fish Shellfish Immunol*., **24,** 663–668.

Collins, M.D., Samelis, J., Metaxopoulos, J. & Wallbanks, S. (1993) Taxonomic studies on some *Leuconostoc*-like organisms from fermented sausages - description of a new Genus *Weissella* for the *Leuconostoc paramesenteroides* Group of Species. *J. Appl. Bacteriol*., **75,** 595–603.

Crepaldi, D.V., Faria, P.M.C., Teixeira, E.A. *et al.* (2006) The Brazilian catfish in Brazil aquaculture. *Rev. Bras. Reprod. Anim*., **30,** 150–158.

Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C., Guroy, D. & Davies, S.J. (2011) Microbial manipulations to improve fish health and production – a Mediterranean perspective. *Fish Shellfish Immunology*, **30,** 1–16.

FAO (2009) FIGIS – Fisheries and aquaculture information and statistics service.

Fuller, R. & Gibson, G.R. (1997) Modification of the intestinal microflora using probiotics and prebiotics. *Scand. J. Gastroenterol. Suppl*., **222,** 28–31.

Gatesoupe, F.J. (2008) Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. *J. Mol. Microbiol. Biotechnol*., **14,** 107–114.

Gibson, G.R. & Roberfroid, M.B. (1995) Dietary modulation of the human colonic microbiota – introducing the concept of prebiotics. *J. Nutr*., **125,** 1401–1412.

Gibson, G.R., McCartney, A.L. & Rastall, R.A. (2005) Prebiotics and resistance to gastrointestinal infections. *Br. J. Nutr*., **93**(Suppl. 1)**,** S31–S34.

Goldenfarb, P.B., Bowyer, F.P., Hall, E. & Brosius, E. (1971) Reproductibility in the hematology laboratory: the microhematocrit determination. *Am. J. Clin. Pathol*., **56,** 35–39.

Jang, J., Kim, B., Lee, J., Kim, J., Jeong, G. & Han, H.G. (2002) Identification of *Weissella* species by the genus-specific amplified ribosomal DNA restriction analysis. *FEMS Microbiol. Lett*., **212,** 29–34.

Jatoba, A., Vieira, F.D., Neto, C.B., Silva, B.C., Mourino, J.L.P., Jeronimo, G.T., Dotta, G. & Martins, M.L. (2008) Lactic-acid bacteria isolated from the intestinal tract of Nile tilapia utilized as probiotic. *Pesq. Agrop. Bras*., **43,** 1201–1207.

Li, P. & Gatlin, D.M. (2005) Evaluation of the prebiotic GroBiotic (R)-A and brewers yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops* × *M-saxatilis*) challenged *in situ* with *Mycobacterium marinum*. *Aquaculture*, **248,** 197–205.

Martins, M.L., Pilarsky, F., Onaka, E.M., Nomura, D.T., Fenerick, J. Jr, Ribeiro, K., Myiazaki, D.M.Y., Castro, M.P. & Malheiros, E.B. (2004) Hematologia e resposta inflamatória aguda em *Oreochromis niloticus* (Osteichthyes: Cichlidae) submetida aos estímulos único e consecutivo de estresse de captura. *Bol. Inst. Pesca*., **30,** 71–80.

Merrifield, D.L., Burnard, D., Bradley, G., Davies, S.J. & Baker, R.T.M. (2009) Microbial community diversity associated with the intestinal mucosa of farmed rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquacult. Res*., **40,** 1064–1072.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bogwald, J., Castex, M. & Ringo, E. (2010) The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, **302,** 1–18.

Nayak, S.K. (2010) Probiotics and immunity: a fish perspective. *Fish Shellfish Immunol*., **29,** 2–14.

Nikoskelainen, S., Ouwehand, A.C., Bylund, G., Salminen, S. & Lilius, E.M. (2003) Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). *Fish Shellfish Immunol*., **15,** 443–452.

Olsen, R.E., Myklebust, R., Kryvi, H., Mayhew, T.M. & Ringo, E. (2001) Damaging effect of dietary inulin on intestinal enterocytes in Arctic charr (*Salvelinus alpinus* L.). *Aquac. Res*., **32,** 931–934.

Panigrahi, A., Kiron, V., Kobayashi, T., Puangkaew, J., Satoh, S. & Sugita, H. (2004) Immune responses in rainbow trout *Oncorhyn-*

chus mykiss induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. *Vet. Immunol. Immunopathol.*, **102,** 379–388.

Panigrahi, A., Kiron, V., Puangkaew, J., Kobayashi, T., Satoh, S. & Sugita, H. (2005) The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, **243,** 241–254.

Panigrahi, P., Braileanu, G.T., Chen, H. & Stine, O.C. (2007) Probiotic bacteria change *Escherichia coli*-induced gene expression in cultured colonocytes: Implications in intestinal pathophysiology. *World J. Gastroenterol.*, **13,** 6370–6378.

Panigrahi, A., Azad, I.S., Das, B.K., Dandpat, J., Das, G., Behera, S. & Mishra, S.S. (2009) Probiotic induced immunomodulation: investigation into the cellular and molecular mechanism involved. *Res. J. Biotechnol.*, **4,** 7–13.

Pompei, A., Cordisco, L., Raimondi, S., Amaretti, A., Pagnoni, U.M., Matteuzzi, D. & Rossi, M. (2008) *In vitro* comparison of the prebiotic effects of two inulin-type fructans. *Anaerobe*, **14,** 280–286.

Resende, E.K.D. (2009) Research in network in aquaculture: technological basis for sustainable development of aquaculture in Brazil. *Aquabrasil. Braz. J. Anim. Sci.*, **38,** 52–57.

Ringo, E. (2008) The ability of carnobacteria isolated from fish intestine to inhibit growth of fish pathogenic bacteria: a screening study. *Aquacult. Res.*, **39,** 171–180.

Ringo, E. & Gatesoupe, J. (1998) Lactic acid bacteria in fish: a review. *Aquaculture*, **160,** 177–203.

Ringo, E., Olsen, R.E., Gifstad, T.O., Dalmo, R.A., Amlund, H., Hemre, G.I. & Bakke, A.M. (2010) Prebiotics in aquaculture: a review. *Aquac. Nutr.*, **16,** 117–136.

Rosenfeld, G. (1947) Pancromic stain for haematology and clinical cytology. A new combination of the components May-Grünwald and Giemsa in just one formula for rapid staining. *Mem. Inst. Butantan*, **20,** 329–334.

Roubach, R., Correia, E.S., Zaiden, S.F., Martino, R.C. & Cavalli, R.O. (2003) Aquaculture in Brazil. *World Aquac.*, **34,** 28–34.

Rurangwa, E., Laranja, J.L., Van Houdt, R. *et al.* (2009) Selected nondigestible carbohydrates and prebiotics support the growth of probiotic fish bacteria mono-cultures *in vitro*. *J. Appl. Microbiol.*, **106,** 932–940.

Sankaran, K. & Gurnani, S. (1972) Variation in catalytic activity of lysozyme in fishes. *Indian J. Biochem. Biophys.*, **9,** 162.

Silva, B.C., Martins, M.L., Jatoba, A., Neto, C.C.B., Vieira, F.N., Pereira, G.V., Jeronimo, G.T., Seiffert, W.Q. & Mourino, J.L.P. (2009) Hematological and immunological responses of Nile tilapia after polyvalent vaccine administration by different routes. *Pesq. Vet. Bras.*, **29,** 874–880.

Swain, P., Behura, A., Dash, S. & Nayak, S.K. (2007) Serum antibody response of Indian major carp, *Labeo rohit*a to three species of pathogenic bacteria; *Aeromonas hydrophila*, *Edwardsiella tarda* and *Pseudomonas fluorescens*. *Vet. Immunol. Immunopathol.*, **117,** 137–141.

Van Loo, J. (2007) How chicory fructans contribute to zootechnical performance and well-being in livestock and companion animals. *J. Nutr.*, **137,** 2594S–2597S.

Vieira, F.N., Pedrotti, F.S., Buglione, C.C., Mouriño, J.L.P., Beltrame, E., Martins, M.L., Ramires, C. & Vinatea, L.A. (2007) Lactic-acid bacteria increase the survival of marine shrimp, *Litopenaeus vannamei,* after infection with *Vibrio harveyi*. *Braz. J. Ocean.*, **4,** 251–255.

Vieira, F.N., Buglione, C.C., Mouriño, J.L.P., Jatobá, A., Ramires, C., Martins, M.L., Barracco, M.A.A.M. & Vinatea, L.A. (2008) Time-related action of *Lactobacillus plantarum* in the bacterial microbiota of shrimp digestive tract and its action as inmunostimulation. *Pesq. Agropec. Bras.*, **43,** 763–769.

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

Yousefian, M. & Amiri, M.S. (2009) A review of the use of prebiotic in aquaculture for fish and shrimp. *Afr. J. Biotechnol.*, **8,** 7313–7318.

Zar, J.H. (1996) *Biostatistical analysis*. Prentice Hall, Upper Saddle River, NJ.