Latin American Journal of Aquatic Research

E-ISSN: 0718-560X

lajar@ucv.cl

Pontificia Universidad Católica de Valparaíso

Chile

Yuji-Sado, Ricardo; Raulino-Domanski, Fernanda; Franchi de Freitas, Patricia; Baioco-Sales, Francielli

Growth, immune status and intestinal morphology of Nile tilapia fed dietary prebiotics (mannan oligosaccharides - MOS)

Latin American Journal of Aquatic Research, vol. 43, núm. 5, noviembre, 2015, pp. 944-952

Pontificia Universidad Católica de Valparaíso

Valparaíso, Chile

Lat. Am. J. Aquat. Res., 43(5): 944-952, 2015
DOI: 10.3856/vol43-issue5-fulltext-14

Research Article

Growth, immune status and intestinal morphology of Nile tilapia fed dietary prebiotics (mannan oligosaccharides-MOS)

Ricardo Yuji-Sado[1], Fernanda Raulino-Domanski[2]
Patricia Franchi de Freitas[1] & Francielli Baioco-Sales[3]
[1]Universidade Tecnológica Federal do Paraná, 85660-000, Dois Vizinhos, PR, Brazil
[2]Bolsista Iniciação Científica CNPq, Universidade Tecnológica Federal do Paraná
85660-000, Dois Vizinhos, PR, Brazil
[3]Programa de Pós-Graduação em Zootecnia PPGZO
Universidade Tecnológica Federal do Paraná 85660-000, Dois Vizinhos, PR, Brazil
Corresponding author: Ricardo Yuji-Sado (ricardoysado@utfpr.edu.br)

ABSTRACT. Farmers must conform to Best Management Practices in fish production such as the development of non-antibiotic dietary supplements for fish growth and health management. We determined the effects of increasing levels of dietary mannan oligosaccharides on growth, immune system and intestine integrity of Nile tilapia. Fish (49.6 ± 10.8 g) were randomly distributed into 12 tanks (250 L; 20 fish per tank) and fed during 60 days with a practical diet supplemented with 0.0, 0.2, 0.4 and 0.6% dietary mannan oligosaccharides (n = 3). Fish growth and immune system were not affected ($P > 0.05$) by treatments. Fish fed 0.4% prebiotic supplementation presented increased ($P < 0.05$) intestinal fold height. Moreover, the intestine muscular layer thickness was increased in fish fed 0.4 and 0.6% dietary prebiotic. After 60 days, there were no effects on intestinal morphology. Studies regarding characterization of intestinal microbiota and experiment that reproduce commercial fish production systems hearing conditions are necessary to determine the effective use of this dietary supplement for the species.

Keywords: *Oreochromis niloticus*, fish, oligosaccharides, nutrition, immune system, aquaculture.

Crecimiento, estado inmunológico y morfología intestinal de la tilapia del Nilo alimentadas con prebióticos (mananoligosacáridos-MOS) en la dieta

RESUMEN. Los acuicultores deben cumplir con buenas prácticas de gestión en la producción de peces, como el uso de probióticos en la dieta, dado que las prácticas amigables com el medio ambiente merecen atención creciente. Se determinó los efectos de los crecientes niveles de mananoligosacáridos en la dieta sobre el crecimiento, sistema inmunológico y morfología intestinal de la tilapia del Nilo. Los peces (49,6 ± 10,8 g) fueron distribuidos al azar en 12 estanques (250 L; 20 peces por estanque) y alimentados durante 60 días con una dieta práctica suplementada con 0,0; 0,2; 0,4 y 0,6% de mananoligosacáridos (n = 3). El crecimiento de los peces y el sistema inmunológico no fueron afectados ($P > 0,05$) por los tratamientos. En los peces alimentados com probióticos durante 30 días se encontraron alteraciones histológicas. Los peces alimentados con 0,4% de suplementación probiótica presentaron incremento ($P < 0,05$) de la altura de las pliegues intestinales. Además, el espesor de la capa muscular del intestino se incrementó en los peces alimentados con 0,4 y 0,6% de probiótico dietético. Después de 60 días no se observaron efectos sobre la morfología intestinal. Se requiere efectuar estudios relativos a la caracterización de la microbiota intestinal y experimentos que reproducen las condiciones de cultivo en los sistemas de producción de peces, para determinar el uso eficaz de este suplemento dietético para esta especie.

Palabras clave: *Oreochromis niloticus*, peces, oligosacáridos, nutrición, sistema inmunológico, acuicultura.

INTRODUCTION

In animal production, including aquaculture, for decades antibiotics are usually used to prevent diseases outbreaks or/and as growth promoters in sub-therapeutic dosage that can select bacterial strains with resistance to these antibiotics (Antibiotic Multiple Resistance-AMR) and already described in Brazil in

Corresponding editor: Alvaro Bicudo

fish (Belém-Costa & Cyrino, 2006). Moreover, the growing concern in public health, fish farmers must conform to Best Management Practices (BMPs) in fish production for human consumption (Boyd *et al*., 2005).

Attention start being given to the use of mannan oligosaccharides (MOS) derived from yeast *Saccharomyces cerevisiae*. These molecules are easily isolated from yeast cell wall as well as incorporated into fish feed and do not cause environmental impact (Hisano *et al*., 2004).

The effects of dietary MOS on growth and health parameters have been recently performed in aquatic animals such as European sea bass *Dicentrarchus labrax* (Torrecillas *et al*., 2007), rainbow trout *Oncorhynchus mykiss* (Staykov *et al*., 2007), Nile tilapia (Sado *et al*., 2008), channel catfish *Ictalurus punctatus* (Peterson *et al*., 2010), Atlantic salmon *Salmo salar* (Refstie *et al*., 2010) and marron *Cherax tenuimanus* (Sang *et al*., 2011) and promising results such as improved weight gain, serum lysozyme concentration and disease resistance were observed.

Fish immune system can recognizes non-self-molecules (*i.e.*, MOS prebiotic) through receptors that identify molecular patterns, which are characteristic of microbes (MAMPs-Microbe Associated Molecular Patterns) that stimulates fish leukocytes to produce lysozyme and others antimicrobial peptides (Song *et al*., 2014). Moreover, MOS provide mannose substrate upon which pathogenic gut bacteria selectively attach, impairing the adhesion to enterocytes, leading to better gut health and villi integrity and diet nutrients uptake (Ghosh & Mehla, 2012).

The effect of dietary MOS on fish intestinal morphology was described for several economic important fish species (Dimitroglou *et al*., 2010a, 2010b; Genc *et al*., 2007; Pryor *et al*., 2003; Salze *et al*., 2008; Zhou *et al*., 2010) including the Brazilian Neotropical characin fish pacu, *Piaractus mesopotamicus* (Sado *et al*., 2014a). However, the use of MOS as prebiotic in fish nutrition is still in infancy as well as for the one of the most important fish in aquaculture, the Nile tilapia. Therefore, this study was set out to determine the effects of increasing levels of dietary MOS supplementation on growth, immune system and intestinal morphology of juvenile Nile tilapia.

MATERIALS AND METHODS

Experimental design and animals

Trials were set up in water recirculation system, with continuous aeration and temperature control. Juvenile Nile tilapia (49.6 ± 10.8 g; 13.9 ± 7.1 cm) were randomly distributed into 250 L polyethylene circular tanks (20 fish per tank) in a totally randomized experimental design with four treatments, 0.0, 0.2, 0.4 and 0.6% MOS (YES-MOS®, YES - YesSinergy do Brasil Agroindustrial, Jaguariuna, Sao Paulo, Brazil) dietary supplementation (n = 3). Fish were acclimated to basal diet for 15 days prior experiment. Fish were fed with experimental diets for 60 days until apparent satiation (09:00 and 17:00h). Water quality parameters (pH 7.3 ± 0.4; dissolved oxygen 4.12 ± 0.52 mg L^{-1} and temperature 25.3 ± 1.2ºC) were monitored electronically on a daily basis.

Experimental diets

A commercial fish feed formulation (PEIXES 32®, Anhambi Alimentos Ltda., Itapejara do Oeste, Parana, Brazil) was used for the basal experimental diets composition (Table 1). Into this basal diet it was added the respective treatments (0.0, 0.2, 0.4 and 0.6% dietary MOS) and extruded. The extruded feeds were dried in a forced ventilation oven at 45ºC for 24 h; and pellets were packed in black plastic bags and stored under refrigeration until use.

Growth parameters

At 30 and 60 days trial fish were fasted for 24 h and sedated for biometrical procedures and growth parameters calculated as follows: weight gain (WG (g)

Table 1. Chemical composition of basal, practical diet (dry matter basis). *Anhambi Alimentos Ltda., Itapejara do Oeste, Parana, Brazil. Vitamin and mineral supplementation per kg of feed: calcium (min-max):14-34 g kg^{-1}, phosphorous (min) 10 g kg^{-1}, lysine 17 g kg^{-1}, metionin 6100 mg kg^{-1}, vitamin A (min) 15,000 UI kg^{-1}, vitamin D3 (min): 3,000 UI kg^{-1}, vitamin E (min): 180 mg kg^{-1}, vitamin K3 (min): 6.0 mg kg^{-1}, vitamin B1 (min): 18 mg kg^{-1}, vitamin B2 (min): 32 mg kg^{-1} vitamin B6 (min): 22 mg kg^{-1}, vitamin B12 (min): 40 mcg, vitamin C (min): 422 mg kg^{-1}, nicotinic acid 150 mg kg^{-1}, pantothenic acid 60 mg kg^{-1}, folic acid (min): 10 mg kg^{-1}, biotin (min): 1.50 mg kg^{-1}, inositol (min): 238 mg kg^{-1}, Fe (min): 65 mg kg^{-1}, Cu (min): 10.40 mg kg^{-1}, Zn (min): 130 mg kg^{-1}, Mg (min): 65 mg kg^{-1}, iodine (min): 1.30 mg kg^{-1}, Se (min): 0.40 mg kg^{-1}, cobalt (min): 0.35 mg kg^{-1}, Sodium 2400 mg kg^{-1}, choline 350 mg kg^{-1}, antioxidant 200 mg kg^{-1}, enzimatic aditive 125 mg kg^{-1}.

Levels of guarantee (according to the manufacturer*)	
Nutrient	Content (g kg^{-1})
Moisture (max)	120
Crude protein (min)	320
Crude fat (min)	40
Crude fiber (max)	60
Ash (max)	130

= FW-IW); feed consumption (FC); feed conversion rate (FCR = FC/WG); specific growth rate (SGR (% day^{-1}) = 100 x [(*ln*FW-*ln*IW) / t]; feed efficiency (FE = WG/FC); daily feed intake index (DFI = 100 x {FC/ [(FW-IW)/2]} /t) and condition factor (CF = WG/ [total length]^3). Where: FW = final weight (g); IW = initial weight (g); t = experimental time (days); *ln*FW = natural logarithm of final weight; *ln*IW = natural logarithm of initial weight.

Growth parameters at 30 days trial was calculated taken in account the biomass of 20 animals and for 60 days, 18 fishes, since two fishes from each replicate was euthanized for histological procedures at 30 days of experiment.

Histological procedures

Histological procedures were performed at 30 and 60 days trial. A snippet of the proximal intestine (3.0 cm from pyloric sphincter) of two specimens from each treatment replicate was sampled. Tissue samples were washed with saline solution (0.6%) and fixed for 24 h in Alfac solution. After 24 h, fixed samples were stored in a 70% alcohol solution, dehydrated in ethanol, diaphanized in xilol and blocked in histological paraffin. Histological sections (5 µm) were stained with haematoxylin and eosin (H & E) and documented photographically with a digital camera (DCM 130E/1.3 megapixels, CMOS Software Scopephoto, China) connected to a light microscope (EDUTEC 502 AC, Brazil). The images were analyzed by using BEL Eurisko Software (BEL-Engineering, Italy) for intestinal fold height and muscular layer thickness measures.

Immunological parameters

Immunological analyses were performed at 30 and 60 days trial. Four fish from each tank were anesthetized in benzocaine solution (1:10,000) and blood samples were drawn from caudal vessel using sterilized syringes and separated into two 1.5 mL microtubes, one containing EDTA for leukocyte respiratory burst and the other with no anticoagulant for serum lysozyme and total protein concentration.

Blood samples with EDTA were used for leukocyte respiratory burst by NBT (Nitroblue tetrazolium) colorimetric assay. To this, 100 µL of blood was added to 100 µL of 0.2% NBT solution (Sigma, St Louis, MO, USA), homogenized and incubated for 30 min at 25ºC. After the incubation, 50 µL of this suspension was added to 1.0 mL of N, N-dimethylformamide (DMF, Sigma, St Louis, MO, USA) and centrifuged (755 *g*) for 5 min. The absorbance of the supernatant was determined using a spectrophotometer at 540 nm.

Lysozyme concentrations (LC) were determined using fish serum from blood without EDTA based on the lyses of *Micrococcus lysodeikticus* microorganism by reduction of optical density during bacterial cell wall lyses. Prior to fish serum analysis, it was determined the calibration curve by quantification the difference of optical density (ΔOD) (0.5 to 5 min) of different concentrations of standard lysozyme (L 6876, Sigma, St Louis, MO, USA) according to Abreu *et al*. (2009).

Serum samples were submitted to heat (56ºC for 30 min) to inactivate complement system proteins and certify that lysis of *M. lysodeikticus* had occurred solely by lysozyme action. After this, 150 µL of fish serum and 150 µL sodium phosphate buffer was dispensed into glass cuvette and incubated at 26ºC for 2 min in the spectrophotometer and 300 µL of *M. lysodeikticus* suspension (0.2 mg mL^{-1} sodium phosphate buffer) was added to complete 600 µL final volumes. Difference between the initial and final optical density (Δ OD) was measured between 0.5 and 5 min in spectrophotometer at 450 nm. The equation of lysozyme calibration curve was used to determine the serum lysozyme levels (µg mL^{-1}).

Total serum protein concentrations were determined using a portable refractometer (Biobrix 301/Protein 0.0-12 g dL^{-1}) after blood sample without EDTA centrifugation and serum collection.

Statistical analysis

Significant effects of dietary MOS levels for 30 and 60 days were determined by one-way analysis of variance (ANOVA), at 5% probability. Brown and Forsythe and Shapiro-Wilk test, respectively validated the assumption of homogeneity of variance (homocedasticity) and normalit-y. Means of statistically difference were compared using Tukey's test ($\alpha = 0.05$) (Steel & Torrie, 1980).

This research was approved by the Ethics Committee on Animal Use (CEUA) of UTFPR (protocol Nº2014-001).

RESULTS

Dietary MOS supplementation for 30 and 60 days to juvenile Nile tilapia did not influenced ($P > 0.05$) growth parameters (Table 2).

Histological analysis carried on this study revealed increase ($P < 0.05$) in intestinal fold height (Fig. 1) and muscular layer thickness (Fig. 2) between fish fed control diet and MOS supplemented diets for 30 days.

Intestinal fold height was increased ($P < 0.05$) in fish fed dietary MOS when compared to fish fed control diet. Moreover, fish fed 0.4% dietary MOS presented

Table 2. Growth parameters (μ ± SD) of Nile tilapia *O. niloticus* fed increasing levels of dietary mannan oligosaccharide (MOS) for 30 and 60 days. *Mannan oligosaccharide-YES-MOS® (YES-YesSinergy do Brasil Agroindustrial, Jaguariuna, São Paulo, Brazil). WG: weight gain, FC: feed consumption, FRC: feed conversion rate, SGR: specific growth rate, FE: feed efficiency, DFI: daily feed intake index, CF: condition factor.

30 days					
MOS* %	0.0	0.2	0.4	0.6	*P*-values
WG (g)	797.5 ± 103.7	965.1 ± 113.7	921.0 ± 54.2	905.1 ± 51.6	0.128
FC (g)	1161.6 ± 128.3	1118.8 ± 122.6	1270.9 ± 11.3	1261.6 ± 29.4	0.182
FCR	1.45 ± 0.04	1.17 ± 0.24	1.38 ± 0.06	1.39 ± 0.10	0.163
SGR (% day^{-1})	1.93 ± 0.23	2.40 ± 0.26	2.18 ± 0.12	2.07 ± 0.10	0.096
FE	0.68 ± 0.02	0.87 ± 0.21	0.72 ± 0.03	0.71 ± 0.05	0.224
DFI (%)	1.66 ± 0,13	1.60 ± 0.23	1.75 ± 0.04	1.70 ± 0.11	0.642
CF	1.03 ± 0.20	0.95 ± 0.15	0.86 ± 0.08	0.85 ± 0,19	0.549
60 days					
MOS* %	0.0	0.2	0.4	0.6	*P*-values
WG (g)	1562.6 ± 59.1	1548.0 ± 40.5	1566.2 ± 71.2	1637.9 ± 144.5	0.618
FC (g)	2363.5 ±161.6	2385.1 ± 249.6	2139.3 ± 216.6	2400.6 ± 165.4	0.399
FCR	1.51 ± 0.05	1.53 ± 0.13	1.36 ± 0.16	1.46 ± 0.10	0.399
SGR (% day^{-1})	3.11 ± 0.13	3.30 ± 0.14	3.15 ± 0.06	3.13 ± 0.20	0.440
FE	0.66 ± 0.02	0.65 ± 0.05	0.73 ± 0.09	0.68 ± 0.04	0.384
DFI (%)	2.55 ± 0.16	2.71 ± 0.20	2.34 ± 0.29	2.49 ± 0.06	0.229
CF	1.03 ± 0.14	1.07 ± 0.14	1.03 ± 0.15	1.04 ± 0.01	0.982

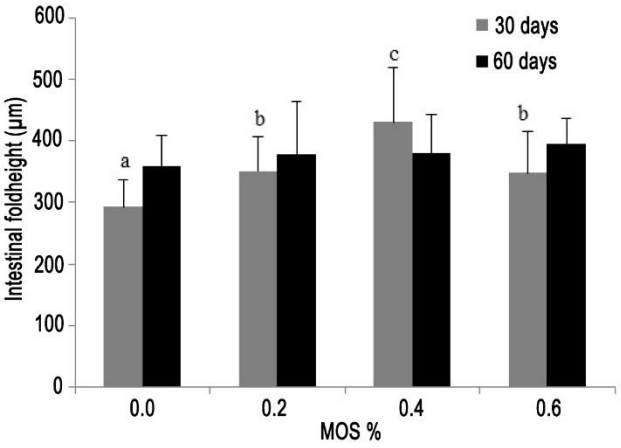

Figure 1. Intestinal fold height of Nile tilapia *O. niloticus* fed increasing levels of dietary mannan oligosaccharides (MOS) for 30 and 60 days trial. Different letters above same color columns indicate differences by Tukey test (α = 0.05).

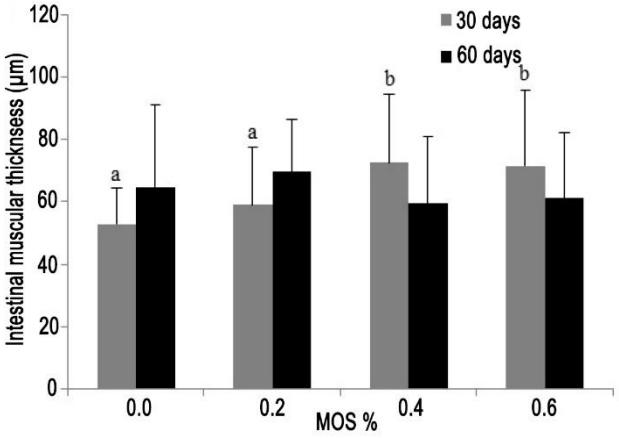

Figure 2. Intestinal muscular thickness of Nile tilapia *O. niloticus* fed increasing levels of dietary mannan oligosaccharides (MOS) for 30 and 60 days trial. Different letters above same color columns indicate differences by Tukey test (α = 0.05).

the highest ($P < 0.05$) intestinal fold height (430.27 ± 89.72 μm) compared to others treatments: control (292.81 ± 45.11 μm), 0.2% (351.31 ± 56.00 μm) and 0.6% (348.23 ± 37.69 μm). Fish fed 0.4 and 0.6% dietary MOS had significant increasing in muscular layer thickness (72.5 ± 21.95 μm and 71.44 ± 24.48 μm, respectively). After 60 days trial there were no effects ($P > 0.05$) on gut morphology.

Fish immunological parameters also did not influenced by dietary MOS (Table 3). Serum lysozyme concentrations (LC) were determined based on calibration curve equation (LC = 7150.7(Δ OD) + 50.793; $R^2 = 0.972$).

Table 3. Immunological parameters (μ ± SD) of Nile tilapia *O. niloticus* fed increasing levels of dietary mannan oligosaccharide (MOS) for 30 and 60 days. *Mannan oligosaccharide-YES-MOS® (YES-YesSinergy do Brasil Agroindustrial, Jaguariuna, São Paulo, Brazil). Lys: serum lysozyme concentrations, Burst: leukocyte respiratory burst activity, Prot: serum total protein concentration, DO: optical density.

30 days					
MOS* %	0.0	0.2	0.4	0.6	*P*-values
Lys (μg mL^{-1})	1.50 ± 0.16	1.50 ± 0.08	1.03 ± 0.38	1.35 ± 0.04	0.245
Burst (DO)	0.268 ± 0.01	0.269 ± 0.02	0.272 ± 0.02	0.268 ± 0.02	0.986
Prot (g dL^{-1})	5.38 ± 0.42	5.78 ± 0.43	5.47 ± 0.52	5.72 ± 0.26	0.196
60 days					
MOS* %	0.0	0.2	0.4	0.6	*P*-values
Lys (μg mL^{-1})	1.73 ± 0.18	1.67 ± 0.13	1.41 ± 0.21	1.49 ± 0.11	0.171
Burst (DO)	0.304 ± 0.09	0.346 ± 0.08	0.307 ± 0.07	0.332 ± 0.07	0.651
Prot (g dL^{-1})	5.21 ± 1.37	5.85 ± 0.45	5.88 ± 1.06	5.81 ± 1.00	0.497

DISCUSSION

Prebiotics in aquaculture are used to enhance fish growth and disease resistance, improving economic viability and sustainability of fish farming (Ringø *et al*., 2010). Several studies have shown that dietary prebiotics enhances growth and health of aquatic animals (Sakai, 1999; Bricknell & Dalmo, 2005; Mazlum *et al*., 2011).

Increased growth parameters in fish fed dietary MOS was observed in rainbow trout (Staykov *et al*., 2007), European sea bass (Torrecillas *et al*., 2007, 2011) and gilthead sea bream *Sparus aurata* (Gültepe *et al*., 2011). However, dietary MOS in fish nutrition are still controversial since some studies did not observe improvements on growth parameters.

As herein observed, increasing levels of dietary MOS did not affect fish growth and similar results was observed for Nile tilapia supplemented for 45 days with 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0% (Sado *et al*., 2008) and 0.0, 1.0, 2.0 and 3.0% MOS for 53 days trial (Schwarz *et al*., 2010) as well as for pacu fed 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0% dietary MOS for 63 days (Sado *et al*., 2014a).

In the same way, several authors observed no effects on growth in another fish species such as for Gulf of Mexico sturgeon *Acipenser oxyrinchus desotoi* fed 0.3% dietary MOS (Pryor *et al*., 2003), Atlantic salmon *Salmo salar* fed 1.0% dietary MOS (Grisdale-Helland *et al*., 2008), gilthead sea bream *Sparus aurata* fed 0.2 and 0.4% dietary MOS (Dimitroglou *et al*., 2010a) and giant sturgeon *Huso huso* fed 0.2 and 0.4% dietary MOS (Mansour *et al*., 2012).

The variability in results found in literature may be explained by the complex carbohydrate structure present in the cell wall of yeast, different strains and fermentation conditions, processing methods can all alter their function (Newman, 2007). Moreover, depending on MOS concentration, administration period, fish growing stage, rearing conditions, feed formulation and extrusion procedures different results can be presented (Pryor *et al.*, 2003; Carvalho *et al.*, 2011; Peterson *et al.*, 2012; Torrecillas *et al.*, 2014).

Prebiotics are defined as short-chain carbohydrates that modulate the composition and metabolism of the microorganisms of the intestinal tract in a beneficial way (Macfarlane *et al*., 2006). They are non-digestible fibers by enzymes, acids and salts produced by the animals' digestion process, acting as a substrate, stimulating the growth of beneficial bacteria in the gastrointestinal system, which produce acids, which decrease the concentration of bacteria and other pathogenic microorganisms and protect the intestinal mucosa (Song *et al*., 2014). This brings benefits to the host with improvements in growth, digestion of nutrients, immunity and resistance to disease (Burr & Gatlin 2005).

Dietary MOS can enhance gut health by eliciting better intestinal development and increase nutrient absorption area and well documented in fish such as cobia (Salze *et al*., 2008), red drum (Zhou *et al*., 2010), gilthead sea bream (Dimitroglou *et al*., 2010a), white sea bream (Dimitroglou *et al*., 2010b) and Nile tilapia (Hisano *et al*., 2006; Carvalho *et al*., 2011), corroborating the results observed in this trial.

Mannan oligosaccharides provide mannose substrate upon which pathogenic gut bacteria selectively attach. The inhibition of pathogenic bacteria adhesion to enterocyte prevents colonies formation and infection of host cells, increasing gut health, regularity, height and integrity of the gut tissue and consequent better utilization and absorption of nutrients (Pryor *et al.*, 2003; Heidarieh *et al*., 2013).

Contradictory results was observed for hybrid tilapia (*O. niloticus* x *O. aureus*) (Genc *et al*., 2007) and pacu (Sado *et al*., 2014a) fed increasing levels of dietary MOS for 60 days and did not show improved fold height. In addition, Pryor *et al.* (2003) also did not find any significant difference in intestinal morphology of sturgeons fed 0.3% MOS supplementation for 28 days and similar results were reported by Torrecillas *et al.* (2007) for European sea bass fed diets containing 0.2 and 0.4% MOS for 48 days. Feeding 0.2 and 0.4% dietary MOS to gilthead sea bream also did not result in differences in gross intestinal and liver histology (Dimitroglou *et al*., 2010a).

Although ultrastructural analysis were not performed, the increased fold height herein observed in fish fed dietary MOS for 30 days that did not reflect better growth could be explained by the impossibility to observe integrity of intestinal brush border and microvilli by optical microscopy (Sado *et al*., 2014a).

Ultrastructural analysis of anterior intestine of cobia larvae fed rotifers enriched with 0.2% MOS showed increased microvilli height (Salze *et al*., 2008) as well as for gilthead sea bream fed 0.2 and 0.4% dietary MOS (Dimitroglou *et al.*, 2010a) and red drum fed 1% dietary prebiotics such as MOS, FOS (fructooligosaccharides) and GOS (galactooligosaccharides) (Zhou *et al*., 2010).

However, in both cases, in spite the fact that ultrastructural analysis showed increased density of microvilli structures and length that could improve the potential of nutrient absorption, dietary MOS did not influence the species' growth rate and feed utilization. Moreover, white sea bream larvae fed artemia enriched with 0.2% MOS also showed improved intestinal microvilli surface (about 12%) and length (Dimitroglou *et al*., 2010b), but no effects on performance of fish were reported.

Fish digestive system shows high phenotypic plasticity in response to diet composition, and it is more evident in omnivorous fishes (Gonçalves *et al.*, 2011). Oligosaccharides such as MOS can increase mucus secretion by enterocytes that improves digest's viscosity (Torrecillas *et al*., 2011). Therefore, the increase in digest's viscosity could stimulate intestine's muscular layer development to move the alimentary bolus through digestive tract as herein observed.

The innate immune system of fish can recognize non-self substances through protein recognition receptors that identify molecular patterns, which are characteristics of microbes (polysaccharides, lipopolysaccharide, peptidoglycans, bacterial DNA, and double-stranded viral RNA) and not ordinarily found on the surface of multicellular organisms, called Pathogen Associated Molecular Patterns-PAMPs (Rauta *et al*., 2014; Song *et al*., 2014).

Mannan oligosaccharides are compounds isolated from cell wall of yeast *Saccharomyces cerevisiae*. Thus, it can stimulate fish immune system such as antibody production, leukocyte bactericidal, lysozyme and complement activity, as observed for rainbow trout (Staykov *et al.*, 2007) and European seabass (Torrecillas *et al.*, 2007). In the same way, *Labeo rohita* fish fed prebiotic for 28 and 42 days (Misra *et al*., 2006a) or intraperitoneal injection (Misra *et al*., 2006b) showed increase in lysozyme concentrations, demonstrating that time and via of administration can influence the prebiotic effect.

Lysozyme is a molecule, primarily produced by leukocytes for protection against microbial infection, preventing bacteria invasion and infection (Saurabh & Sahoo, 2008). After phagocytosis initiates by leukocytes, increase in molecular oxygen consumption occurs, known as respiratory burst. In this process, the phagocytes produce reactive oxygen species that contribute for microorganism destruction (Biller-Takahashi; Urbinati, 2014).

However, in this trial it was observed no effect of dietary MOS on fish immune status. This results can be explained by the fact of the present work was performed under ideal controlled laboratory conditions and fishes were not challenged by biological and/or ambient stressor that reproduces intensive fish production system hearing conditions. In fact, when fish is exposed to biological challenge, the potential effect of dietary prebiotic on fish immune system can be expressed as observed for snakehead (*Channa striata*) fingerlings fed dietary prebiotics (MOS and glucans) and probiotics after challenge with *Aeromonas hydrophila* (Talpur *et al*., 2014).

Dietary MOS did not influence serum total protein concentrations. However, rainbow trout fed dietary prebiotic for seven days showed increase in total plasmatic protein (Siwicki *et al*., 1994) as well as for *L. rohita* (Misra *et al*., 2006a, 2006b). In addition, pacu fed 0.2% dietary MOS also showed increased total plasmatic protein (Sado *et al*., 2014b).

Total plasmatic protein represents several blood peptides such as lysozyme, immunoglobulins and albumin as well as complement factors (Misra *et al*., 2006a). Thus, the result regarding total plasmatic protein herein observed corroborate the absent of significant effect of treatments on serum lysozyme concentration.

This study shows the potential and functionality of prebiotics compounds such as mannan oligosaccharides as dietary supplement for Nile tilapia to modulate gut morphology. However, further researches must focus experiments that reproduce commercial fish pro-

duction systems hearing conditions are necessary to determine the effective use of this dietary supplement for the specie.

ACKNOWLEDGMENTS

To Yes Sinergy (Jaguaiuna, SP, Brazil) for providing the tested feed supplement-Yes-MOS®, Anhambi Alimentos Ltda. (Itapejara do Oeste, PR, Brazil) for providing the practical diet formulation and CNPq (Proc. 474153/2011-8) for financial support.

REFERENCES

Abreu, J.S., C.M. Marzocchi-Machado, A.C. Urbaczek, L.M. Fonseca & E.C. Urbinati. 2009. Leukocytes respiratory burst and lysozyme level in pacu (*Piaractus mesopotamicus* Holmberg, 1887). Braz. J. Biol., 69(4): 1133-1139.

Belém-Costa, A. & J.E.P. Cyrino. 2006. Antibiotic resistence of *Aeromonas hydrophila* isolated from *Piaractus mesopotamicus* (Holmberg, 1887) and *Oreochromis niloticus* (Linnaeus, 1758). Sci. Agric., 63(3): 281-284.

Biller-Takahashi, J.D. & E.C Urbinati. 2014. Fish immunology. The modification and manipulation of the innate immune system: Brazilian studies. An. Acad. Bras. Ciênc., 86(3): 1483-1495.

Boyd, C.E., A.A. McNevin, J. Clay & H.M. Johnson. 2005. Certification issues for some common aquaculture species. Rev. Fish. Sci., 13: 231-279.

Bricknell, I. & R.A. Dalmo. 2005. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol., 19: 457-472.

Burr, G. & D. Gatlin. 2005. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. J. World Aquacult. Soc., 36(4): 425-436.

Carvalho, J.V., A.D. Lira, C.S.P. Costa, E.L.T. Moreira, L.F.B. Pinto, R.D. Abreu & R.C.B. Albinati. 2011. Desempenho zootécnico e morfometria intestinal de alevinos de tilápia-do-Nilo alimentados com *Bacillus subtilis* ou mananoligossacarídeo. Braz. J. Anim. Health Prod., 12(1): 176-187.

Dimitroglou, A., D.L. Merrifield, P. Spring, J. Sweetman, R. Moate & S.J. Davies. 2010a. Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilization, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). Aquaculture, 300: 182-188.

Dimitroglou, A., S.J. Davies, J. Sweetman, D. Pascal & S. Chatzifotis. 2010b. Dietary supplementation of mannanoligosaccharide on white sea bream (*Diplodus sargus* L.) larvae: effects on development, gut morphology and salinity tolerance. Aquacult. Res., 41: 245-251.

Genc, M.A., E. Yilmaz, E. Genc & M. Aktas. 2007. Effects of dietary mannan oligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus x O. aureus*). Isr. J. Aquacult., 59: 10-16.

Ghosh, S. & R.K. Mehla. 2012. Influence of dietary supplementation of prebiotics (mannanoligosaccharide) on the performance of crossbred calves. Trop. Anim. Health Prod., 44(3): 617-622.

Gonçalves, L.U., A.P.O. Rodrigues, G.V. Moro, E. Cargnin-Ferreira & J.E.P. Cyrino. 2011. Morfologia e fisiologia do sistema digestório de peixes. In: D.M. Fracalossi & J.E.P. Cyrino (eds.). Nutriaqua: nutrição e alimentação de espécies de interesse para aquicultura brasileira. Aquabio, Florianopólis, pp. 9-36.

Grisdale-Helland, B., S.J. Helland & D.M. Gatlin. 2008. The effects of dietary supplementation with mannanoligosaccharide fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture, 283: 163-167.

Gültepe, N., S. Salnur, B. Hossu & Q. Hisar. 2011. Dietary supplementation with mannanoligo saccharides (MOS) from Bio-Mos enhances growth parameters and digestive capacity of gilthead sea bream (*Sparus aurata*). Aquacult. Nutr., 17: 482-487.

Heidarieh, M., A.R. Mirvaghefi, M. Akbari, N. Sheikhzadeh, Z. Kamyabi-Moghaddam, H. Askari & A.A. Shahbazfar. 2013. Evaluations of Hilyses™, fermented *Saccharomyces cerevisiae*, on rainbow trout (*Oncorhynchus mykiss*) growth performance, enzymatic activities and gastrointestinal structure. Aquacult. Nutr., 19: 343-348.

Hisano, H., L.E. Pezzato, M.M. Barros, E.S. Freire, G.S. Gonçalves & J.E.C. Ferrari. 2004. Zinc and spray dried alcohol yeast as pronutrient for Nile tilapia fingerlings (*Oreochromis niloticus* L.). Acta Sci. Anim. Sci., 26(2): 171-179.

Hisano, H., M.D.P. Silva, M.M. Barros & L.E. Pezzato. 2006. Levedura íntegra e derivados do seu processamento em rações para tilápia do Nilo: aspectos hematológicos e histológicos. Acta Sci. Biol. Sci., 28(4): 311-318.

Macfarlane, S., G.T. Macfarlane & J.H. Cummings. 2006. Review article: prebiotics in the gastrointestinal tract. Aliment. Pharmacol. Ther., 24: 701-714.

Mansour, M.R., R. Akrami, S.H. Ghobadi, H. Amani Denji, N. Ezatrahimi & A. Gharaei. 2012. Effect of dietary mannan oligosaccharide (MOS) on growth

performance, survival, body composition, and some hematological parameters in giant sturgeon juvenile (*Huso huso* Linnaeus, 1754). Fish Physiol. Biochem., 38: 829-835.

Mazlum, Y., E. Yilmaz, M.A. Genc & O. Guner. 2011. A preliminary study on the use of mannan oligosaccharides (MOS) in fresh water crayfish, *Astacus leptodactylus* Eschscholtz, 1823 juvenile diets. Aquacult. Int., 19: 111-119.

Misra, C.K., B.K. Das, S.C. Mukherjee & P. Pattnaik. 2006a. Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. Aquaculture, 255: 82-94.

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

Newman, K. 2007. Form follows function in picking MOS product. Feedstuffs, 79: 1-2.

Peterson, B.C., N.J. Booth, F.T. Barrows & B.B. Manning. 2012. Improved survival in channel catfish fed mannanoligosaccharides in an extruded diet. Open J. Anim. Sci., 2: 57-61.

Peterson, B.C., T.C. Bramble & B.B. Manning. 2010. Effects of Bio-Mos® on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. J. World Aquacult. Soc., 41(1): 149-155.

Pryor, G.S., J.B. Royes, F.A. Chapman & R.D. Miles. 2003. Mannan oligosaccharides in fish nutrition: effects of dietary supplementation on growth and gastrointestinal villi structure in Gulf of Mexico sturgeon. N. Am. J. Aquacult., 65: 106-111.

Rauta, P.R., M. Samanta, H.R. Dash, B. Nayak & S. Das. 2014. Toll-like receptors (TLRs) in aquatic animals: signaling pathways, expressions and immune responses. Immunol. Lett., 158: 14-24.

Refstie, S., G. Baeverfjord, R. Seim & O. Elvebo. 2010. Effects of dietary yeast cell wall β-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sun flower and soy bean meal. Aquaculture, 305: 109-116.

Ringø, E., R.E. Olsen, T.O. Gifstad, R.A. Dalmo, H. Amlund, G.I. Hemre & A.M. Bakke. 2010. Prebiotics in aquaculture: a review. Aquacult. Nutr., 16: 117-136.

Sado, R.Y., A.J.A. Bicudo & J.E.P. Cyrino. 2008. Feeding dietary mannan oligosaccharides to juvenile Nile tilapia, *Oreochromis niloticus* have no effect on hematological parameters and showed decreased feed consumption. J. World Aquacult. Soc., 39(6): 821-826.

Sado, R.Y., A.J.A. Bicudo & J.E.P. Cyrino. 2014a. Growth and intestinal morphology of juvenile pacu *Piaractus mesopotamicus* (Holmberg, 1887) fed dietary prebiotics (mannanoligosaccharides-MOS). An. Acad. Bras. Ciênc., 86(3): 1517-1523.

Sado, R.Y., A.J.A. Bicudo & J.E.P. Cyrino. 2014b. Hematology of juvenile pacu, *Piaractus mesopotamicus* (Holmberg, 1887) fed graded levels of mannan oligosaccharides (MOS). Lat. Am. J. Aquat. Res., 42(1): 30-39.

Sakai, M. 1999. Current research status of fish immunostimulants. Aquaculture, 172: 63-92.

Salze, G., E. Mclean, M.H. Schwarz & S.R. Craig. 2008. Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. Aquaculture, 274: 148-152.

Sang, H.M., R. Fotedar & K. Filer. 2011. Effects of dietary mannan oligosaccharide on survival, growth, physiological condition, and immunological responses of marron, *Cherax tenuimanus* (Smith 1912). J. World Aquacult. Soc., 42(2): 230-241.

Saurabh, S. & P.K. Sahoo. 2008. Lysozyme: an important defence molecule of fish innate immune system. Aquac. Res., 39: 223-239.

Schwarz, K.K., W.M. Furuya, M.R.M. Natali, M. Michelato & M.C. Gualdezi. 2010. Mananoligossacarídeo em dietas para juvenis de tilápias do Nilo. Acta Sci. Anim. Sci., 32(2): 197-203.

Siwick, A.K., D.P. Anderson & G.L. Rumsey. 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Vet. Immunol. Immunopathol., 41: 125-139.

Song, S.K., B.R. Beck, D. Kim, J. Park, J. Kim, H.D. Kim & E. Ringø. 2014. Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol., 10: 40-48.

Staykov, Y., P. Spring, S. Denev & J. Sweetman. 2007. Effect of mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquacult. Int., 15: 153-161.

Steel, R.G.D. & J.H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York, 569 pp.

Talpur, A.D., M.B. Munir, A. Mary & R. Hashim. 2014. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance agains *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. Aquaculture, 426-427: 14-20.

Torrecillas, S., A. Makol, R.J. Caballero, D. Montero, L. Robaina, F. Real, J. Sweetman, L. Tort & M.S. Izquierdo. 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrar-*

chus labrax) fed mannan oligosaccharides. Fish Shellfish Immunol., 23: 969-981.

Torrecillas, S., A. Makol, M.J. Caballero, D. Montero, R. Ginés, S. Sweetman & M. Izquierdo. 2011. Improved feed utilization, intestine mucus production and immune parameters in sea bass (*Dicentrarchus labrax*) feed mannan oligosaccharides (MOS). Aquacult. Nutr., 17: 223-233.

Torrecillas, S., D. Montero & M. Izquierdo. 2014. Improved health and growth of fish fed mannan oligosaccharides: potential mode of action. Fish Shellfish Immunol., 36: 525-544.

Zhou, Q.C., J.A. Buentello & D.M. Gatlin. 2010. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture, 309: 253-257.

Received: 22 December 2014; Accepted: 22 September 2015