



## Growth and intestinal morphology of juvenile pacu *Piaractus mesopotamicus* (Holmberg 1887) fed dietary prebiotics (mannanoligosaccharides - MOS)

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### ABSTRACT

Intensification of aquaculture production systems exposes fish to numerous stressors, which may negatively affect their growth and limit profitability of aquaculture systems. This study determined effects of increasing levels of dietary mannanoligosaccharides on growth and intestine morphology of pacu. Fish (44.04 g) were randomly distributed into 32 tanks (500 L; 10 fishes per tank) and fed during 63 days with a commercial diet supplemented with 0.0; 0.2; 0.4; 0.6; 0.8; 1.0; 1.5 and 2.0% dietary mannanoligosaccharides. Growth parameters did not differ ( $P>0.05$ ) between fish fed control diet and mannanoligosaccharide supplemented diets. Intestinal villi perimeter was performed in fish fed control diet, 0.4 and 1.5% dietary mannanoligosaccharides and also showed no differences ( $P>0.05$ ) between treatments. Dietary supplementation of mannanoligosaccharides unclear did not have effects on pacu. Studies on the characterization of intestinal microbiota together with experiment that reproduce commercial fish production systems rearing conditions are necessary to determine the effective use of this dietary supplement for the species.

**Key words:** fish nutrition, histology, *Piaractus mesopotamicus*, prebiotics.

### INTRODUCTION

Intensification of aquaculture production systems expose fish to numerous stressors such as poor water quality, crowding, handling and transport which may negatively affect their growth and health, and thus limit revenue of aquaculture systems (Gatesoupe 1999, Plumb 1999, Sakai 1999). In addition, fish farmers are now obliged to conform to Best Management Practices (BPMs) regulations

(Boyd and Schmittou 1999, Boyd et al. 2005). This current setup favors the use of dietary prebiotics for management of farmed fish as an environmentally friendly practice. Attention to the use of these feed additives in fish farming is thus on the rise (Cuesta et al. 2002, Gatesoupe 1999, Kumari and Sahoo 2006, Sakai 1999). Nutrition plays an important role in the growth and health maintenance of fish (Merrifield et al. 2010), so the development of non-antibiotic and environmentally friendly feed supplements are key factors for fish growth and

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health management. In addition, because of the complex nature of aquatic culture systems, diversity of cultured species and pathogens, few antibiotics can be licensed for efficient and safe use (Qi et al. 2009). Therefore, there is an urgent need for the development of new alternatives to overcome the abuse of antibiotics.

Mannan oligosaccharides (MOS) are complex carbohydrates derived from yeast cell walls and present mannose as primary carbohydrate (Gouveia et al. 2006). This mannose, provides substrate for selective attachment of pathogenic intestinal bacteria, impairing bacterial adhesion to enterocytes, thus preventing infection of host cells, and leading to better gut health and integrity of gut villi (Gouveia et al. 2006).

Improved weight gain and survival rate have been reported for farm animals fed MOS supplemented diets (Newman and Newman 2001, Spring et al. 2000). Dietary MOS supplementation was studied in Mexico sturgeon *Acipenser oxyrinchus desotoi* (Pryor et al. 2003), Nile tilapia *Oreochromis niloticus* (Sado et al. 2008), rainbow trout *Oncorhynchus mykiss* (Staykov et al. 2007), European sea bass *Dicentrarchus labrax* (Torrecillas et al. 2007), channel catfish *Ictalurus punctatus* (Welker et al. 2007), tiger shrimp *Penaeus semisulcatus* (Genc et al. 2007), lobsters *Homarus gammarus* (Daniels et al. 2006, 2007), cobia *Rachycentron canadum* (Salze et al. 2008), and Atlantic salmon *Salmo salar* (Grisdale-Helland et al. 2008). Results can be deemed contradictory at best.

There are no reports on effects of dietary MOS on growth and intestine morphology of neotropical, freshwater teleosts. The omnivorous Characin pacu *Piaractus mesopotamicus*, native from the rivers, floodplains, lakes and flooded forest of Parana, Paraguay and Uruguay river basins is widely used in South American fish farming industry (Jomori et al. 2005, Urbinati and Gonçalves 2005). To date, no studies are found regarding the effects of

dietary MOS supplementation for pacu. This study was set out to evaluate the effects of increasing levels of dietary MOS on the growth and intestinal morphology of pacu.

#### MATERIALS AND METHODS

Trials were set up in indoor, water recirculation system, with continuous aeration. Water quality parameters (pH  $7.67 \pm 0.28$ ; dissolved oxygen  $6.10 \pm 0.77 \text{ mg.L}^{-1}$ ; ammonia  $\leq 0.5 \text{ mg.L}^{-1}$ ; temperature  $28.7 \pm 1.76^\circ\text{C}$ ) remained within acceptable values for pacu (Urbinati and Gonçalves 2005). Juvenile pacus ( $44.04 \pm 5.27 \text{ g}$ ) were acclimatized to the experimental conditions for seven days, feeding on a 32% crude protein (CP) commercial feed. Then, the same commercial fish feed (Table I) was powdered, supplemented with 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0 of MOS (ActiveMOS - Biorigin<sup>®</sup>, Lençóis Paulista, SP, Brazil), granulated and stored under refrigeration ( $4^\circ\text{C}$ ).

**TABLE I**  
Chemical composition of basal, practical diet (dry matter basis).

Nutrient	Content (%)
Moisture	5.13
Crude protein	27.43
Crude fiber	5.48
Crude fat	9.69
Dry matter	94.87
Ash	14.72

Vitamin and mineral supplementation per kg of feed (from Purina do Brasil Ind. Com. Ltd., SP, Brazil): Mg, 700.0 mg; Fe, 100.0 mg; Cu, 15 mg; Zn, 200.0 mg; Mn, 30 mg; I, 1.0 mg; Se, 0.3 mg; vitamin A, 9,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E, 112.0 IU; vitamin K, 7.50 IU; folic acid, 7.50 mg; biotin, 0.6 mg; choline, 500.0 mg; niacin, 112.0 mg; calcium pantothenic, 37.0 mg; thiamin, 22.0 mg; riboflavin, 22.0 mg; pyridoxine, 22.0 mg; vitamin B<sub>12</sub>, 26.0 µg; vitamin C, 150.0 mg.

After acclimation, fishes, were randomly assigned to 500-L polyethylene tanks (10 fish per group), each tank representing a replication of the following treatments 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0% of MOS in the diet, arranged

in a completely randomized experimental design (n=4). Fish were then fed with the experimental diets until apparent satiation twice a day (0700h and 1600h) for 63 days. At the end of the trial fish were fasted for 24 h, anesthetized with alcoholic solution of benzocaine (50 mg.L<sup>-1</sup>) and sampled for biometrical and histological data.

Growth parameters of fish were evaluated according to Tacon (1990) as follows: Weight gain (WG=FW – IW); Feed conversion ratio (FCR=feed consumption ÷ weight gain); Daily feed consumption (FC=feed consumption ÷ t); Specific growth rate (SGR=100 x [(lnFW – lnIW) ÷ t]). Where: FW=final weight (g); IW=initial weight (g); t=experimental time (days).

The proximal intestine fragment of two specimens from each replicate of 0.0 (control), 0.4 and 1.5% MOS supplemented diets was taken for histological observations. Tissue samples were immediately washed with saline solution (0.6%) and fixed in a 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2) and submitted to dehydration through alcohol solutions series (30 to 100%). After dehydration process, tissues were pre-infiltrated in glycol metacrilate resin (JB-4, Polyscience Inc., Warrington, PA, USA) and 100% ethanol solution (1:1 proportion) for

four hours and transferred to 100% resin solution until inclusion in plastic resin in histomoulds. The histological sections (5 µm) were stained with haematoxylin and eosin (H & E) and documented photographically with a digital camera (Olympus DP71/12.5 megapixels, Japan) connected to a light microscope (Olympus BX51, Japan). The images were analyzed by using Image Pro Plus 6.1 software (Media Cybernetics Inc., Bethesda, MD, USA) for intestinal villi perimeter measures.

Results were submitted to statistical analysis of variance (ANOVA). Means showing significant differences were compared by *t* test ( $\alpha=0.05$ ) (Steel and Torrie 1980).

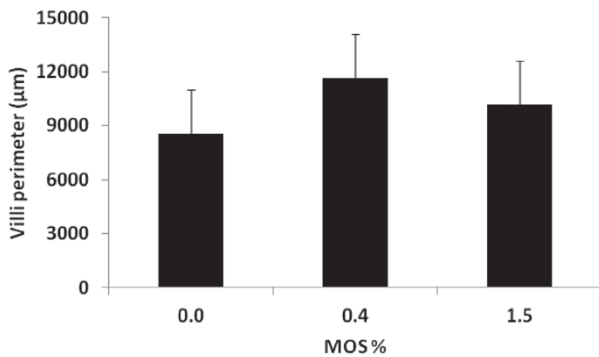
## RESULTS

Growth parameters of fish fed MOS-supplemented diets did not differ ( $P>0.05$ ) from that of fish fed control diet. Results are summarized in Table II. Dietary MOS supplementation (0.4 and 1.5%) also did not significantly affect total intestinal villi perimeter, although fish fed MOS-supplemented diets had higher absolute intestinal villi perimeter (11673.6 ± 2448 µm and 10173.4 ± 2439 µm for 0.4 and 1.5% MOS supplementation, respectively) in comparison to fish fed control diet (8586.6 ± 2428 µm) (Fig. 1).

**TABLE II**  
Means and standard deviation (SD) of individual weight gain (WG), feed conversion rate (FCR), feed consumption (FC), specific growth rate (SGR) and survival rate (SR) of pacu, (*P. mesopotamicus*) fed increasing levels of dietary mannanoligosaccharide (MOS).

MOS* %	Individual				
	WG g	FCR	FC g	SGR %	SR %
0.0	47.2 ± 8.2	2.0 ± 0.04	108.5 ± 16.5	1.0 ± 0.21	100
0.2	45.4 ± 3.7	2.1 ± 0.01	95.8 ± 7.3	1.1 ± 0.06	100
0.4	51.1 ± 13.2	2.1 ± 0.21	105.5 ± 17.6	1.2 ± 0.22	100
0.6	45.1 ± 6.8	2.0 ± 0.09	92.9 ± 9.9	1.1 ± 0.12	100
0.8	40.2 ± 3.6	2.1 ± 0.14	87.5 ± 2.9	1.0 ± 0.05	100
1.0	58.7 ± 6.3	2.0 ± 0.03	117.8 ± 13.1	1.3 ± 0.10	100
1.5	56.9 ± 3.4	2.0 ± 0.07	116.2 ± 4.5	1.3 ± 0.06	100
2.0	50.0 ± 8.1	1.9 ± 0.2	96.2 ± 17.4	1.2 ± 0.13	100
<i>ANOVA</i>	0.100	0.405	0.070	0.109	

\*Mannanoligosaccharide: ActiveMOS<sup>®</sup> (Biorigin, Lençóis Paulista, SP, Brazil).



**Figure 1** - Intestinal villi perimeter ( $\mu \pm$  SD) of juvenile pacu (*P. mesopotamicus*) fed control diet, 0.4 and 1.5% MOS supplemented diets for 63 days ( $P>0.05$ ).

### DISCUSSION

Several studies have shown that dietary prebiotics enhance growth and health (Burrells et al. 2001, Couso et al. 2003, Sakai 1999). Mannan oligosaccharides are a feature in farm animal nutrition (Ghosh and Mehla 2012, Newman and Newman 2001, Spring et al. 2000, Yalçinkaya et al. 2008). In aquatic animals, dietary MOS has only been recently used in an attempt to improve fish growth. Positive results on weight gain and immune response to dietary MOS were observed in rainbow trout, *O. mykiss* (Staykov et al. 2007), common carp *Cyprinus carpio* (Staykov et al. 2005), European sea bass *Dicentrarchus labrax* (Torrecillas et al. 2007), turbot *Scophthalmus maximus* (Li et al. 2008), tiger shrimp *P. semisulcatus* (Genc et al. 2007) and European lobster *H. gammarus* (Daniels et al. 2006, 2007) and crayfish *Astacus leptodactylus* (Mazlum et al. 2011). MOS are indigestible gluco-mannoproteins, providing mannose substrate upon which pathogenic gut bacteria selectively attach. Thereby, the inhibition of bacteria adhesion to enterocytes, prevents the formation of mixed colonies, the entrapment of nutrients for bacterial growth and the infection of host cells. This leads to better gut health by increasing regularity, height and integrity of the gut villi and a consequent better utilization and absorption of nutrients (Gouveia et al. 2006, Li et al. 2008, Pryor et al. 2003). However, the effects of dietary prebiotics in fish nutrition are still inconclusive.

Dietary MOS did not affect growth of pacu; identical results were recorded by Pryor et al. (2003) for Gulf of Mexico sturgeon fed 0.3% dietary MOS, Grisdale-Helland et al. (2008) for Atlantic salmon *S. salar* fed 1.0% dietary MOS, and by Dimitroglou et al. (2010a) for gilthead seabream *Sparus aurata* fed 0.2 and 0.4% dietary MOS. Growth of channel catfish *Ictalurus punctatus* fed 0.2% dietary MOS for six weeks did not differ from fish fed a control diet, although supplemented fish presented improved resistance when challenged by *Edwardsiella ictaluri* (Peterson et al. 2010). Nile tilapia fed 0.2, 0.4, 0.6; 0.8 and 1.0% dietary MOS for 45 days not only did not experience any improvement on growth parameters, but also had a negative correlation between dietary MOS supplementation and feed consumption (Sado et al. 2008).

Dietary MOS can enhance gut health by eliciting better intestinal villi development and increasing nutrient absorption area. Effects of dietary prebiotics on gut villi absorption area are well documented in poultry, swine and fish. Turkey fed MOS supplemented diets showed increased intestinal villi height and absorption area (Juskiewicz et al. 2002); however, sows and piglets fed dietary MOS at 0.1% supplementation for 77 days did not have significantly different villi height (Chiquieri et al. 2007).

Ultrastructural analysis of anterior intestine of Cobia larvae fed rotifers enriched with 0.2% MOS showed increased villi height (Salze et al. 2008). Similar observations were recorded for gilthead sea bream fed 0.2 and 0.4% dietary MOS (Dimitroglou et al. 2010a) and red drum *Scianops ocellatus* fed diets supplemented with 1% dietary prebiotics such as MOS, FOS and GOS (Zhou et al. 2010). However, in both cases, in spite of the fact that the ultrastructural analysis showed increased density of microvilli structures and length that could improve the potential of nutrient capture and absorption, dietary MOS did not influence the species' growth rate and feed utilization. White sea bream *Diplodus sargus* larvae

fed artemia enriched with 0.2% MOS also showed improved intestinal villi surface (about 12%) and length (Dimitroglou et al. 2010b), but no effects on performance of fish were reported by either authors.

Histological analysis carried out in this study revealed no differences ( $P>0.05$ ) in intestinal villi perimeter between fish fed control diet and 0.4 and 1.5% MOS-supplemented diets. Pryor et al. (2003) did not find any significant difference in intestinal morphology of sturgeons fed 0.3% MOS supplementation for 28 days; similar results were reported by Torrecillas et al. (2007) for European seabass fed diets containing 0.2 and 0.4% MOS for 48 days. Feeding 0.2 and 0.4% dietary MOS to gilthead seabream did not result in differences in gross intestinal and liver histology (Dimitroglou et al. 2010a) and Genc et al. (2007) also did not report effects of dietary MOS (1.5, 3.0 and 4.0 g.kg<sup>-1</sup> diet) on hepatopancreas histology of tiger shrimp.

The purpose of using prebiotic in aquaculture is to enhance fish growth and increase disease resistance, improving economic viability of farming operations (Gatlin et al. 2008, Ringø et al. 2010). However, conflicting results demonstrated that the mode of action of these substances is still unclear, regarding time, dose and methods of administration, since time-dose response can cause negative effects.

Olsen et al. (2001), for instance, reported that brook trout *Salvelinus alpinus* fed diets containing 150 g inulin per kg presented damaged enterocytes and that feeding dietary inulin at 0.5 and 1.0% to gilthead sea bream *Sparus aurata* for seven days resulted in impaired leukocyte phagocytosis and respiratory burst (Cerezuela et al. 2008). Hybrid surubim *Pseudoplatystoma* sp. fed 0.5 and 1.0% dietary inulin showed no effect on pathogenic bacteria population numbers when compared to fish fed control diet (Mouriño et al. 2012). In addition, Reza et al. (2009) feeding 1 to 3% dietary inulin to juvenile beluga, *Huso huso* for eight weeks observed impaired growth parameters compared to unsupplemented fish. Finally, European lobsters fed

diets supplemented with 200 ppm MOS presented elevated mortality and impaired morphological development at juvenile phase (Daniels et al. 2006).

The use of prebiotics as mannanoligosaccharides to improve growth and health status in fish still needs further research for better explanation of contradictory results. The complex carbohydrate structure in the cell wall of yeast, the different strains, fermentation conditions and processing methods can all alter their function (Newman 2007) as well as different ingredients used in diet formulation can widely vary among different fish species (Yousefian and Amiri 2009). Moreover, depending on MOS concentration, administration period, hearing condition and population status (age, sex, gonadal maturation) (Pryor et al. 2003) different results can be obtained.

The present study was performed in controlled laboratory hearing conditions. Thus, the higher water microorganisms concentrations and the ambient variation, normally observed in intensive fish production that continuously challenge fishes, were not reproduced. Therefore, experiment condition can be an additional relevant factor for contradictory results found in literature and in the present study.

## CONCLUSION

Dietary MOS supplementation did not positively affect growth and intestinal morphology of pacu. Results recorded to date are nothing but contradictory, thus studies regarding pacu's gut microbiota characterization and experiment that reproduce commercial fish production systems hearing conditions are necessary to determine the mode of action and the most effective use of this supplement as prebiotic for the species.

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### RESUMO

A intensificação dos sistemas de produção em aquicultura expõe os peixes a inúmeros estressores, os quais afetam negativamente seu crescimento e limitam a rentabilidade dos sistemas de aquicultura. Este estudo determinou o efeito de níveis crescentes de mananoligossacarídeos dietéticos sobre o crescimento e morfologia intestinal do pacu. Os peixes (44,04 g) foram aleatoriamente distribuídos em 32 tanques (500 L; 10 peixes por tanque) e alimentados por 63 dias com uma dieta comercial suplementada com 0,0; 0,2; 0,4; 0,6; 0,8; 1,0; 1,5 e 2,0% de mananoligossacarídeo dietético. Os parâmetros de crescimento não diferiram ( $P>0,05$ ) entre os peixes alimentados com a dieta controle e as dietas suplementadas com mananoligossacarídeo. O perímetro das vilosidades intestinais foi realizado nos peixes alimentados com a dieta controle, 0,4 e 1,5% de mananoligossacarídeos dietéticos e também não apresentaram diferenças ( $P>0,05$ ) entre os tratamentos. A suplementação dietética de mananoligossacarídeos não teve efeito no pacu. Estudos relacionados à caracterização da microbiota intestinal e experimento que reproduz sistemas comerciais de produção de peixes são necessários para determinar o uso efetivo deste suplemento dietético para a espécie.

**Palavras-chave:** nutrição de peixes, histologia, *Piaractus mesopotamicus*, prebióticos.

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