DIETARY MANNANOLIGOSACCHARIDE INFLUENCED FEED CONSUMPTION AND GUT MORPHOLOGY OF NILE TILAPIA RAISED IN NET-CAGE SYSTEMS*

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ABSTRACT

This work was set out to determine the effects of increasing levels of dietary mannanoligosaccharides (MOS) on growth and intestine morphology of Nile tilapia. Individuals (12.3 ± 0.3 g) were randomly distributed into 16 net-cages (250 L; 20 fishes per net cage) at Salto Caxias Hydroelectric reservoir in Jacutinga river, Paraná, Brazil and fed during 60 days with a commercial diet supplemented with 0.0; 0.2; 0.4 and 0.8% dietary mannanoligosaccharides (n = 4). Fish feed consumption showed quadratic correlation with dietary levels of prebiotic at 30 and 60 days. Intestinal villi height were increased in fishes fed with 0.4% dietary MOS for 30 days. However, after 60 days, fishes fed with control diet showed significant increase in villi height. On the other hand, none of the levels of mannanoligosaccharides had been tested shown efficiency on growth parameters. In conclusion, the prebiotic had improved the vilosity height of the gut epithelium, even though, no effects were observed in the growth parameters of the Nile tilapia.

Keywords: aquaculture; fish nutrition; histology; prebiotic

MANANOLIGOSSACARÍDEO DIETÉTICO INFLUENCIOU O CONSUMO DE RAÇÃO E MORFOLOGIA INTESTINAL DE TILÁPIAS DO NILO CRIADAS EM SISTEMAS DE TANQUE-REDE

RESUMO

O objetivo deste trabalho foi determinar os efeitos de níveis crescentes de mananoligossacarídeos (MOS) sobre o crescimento da tilápia do Nilo e sua morfologia intestinal. Os peixes (12,3 ± 0,3 g) foram distribuídos aleatoriamente em 16 tanques-rede (250 L; 20 peixes por tanque-rede) no rio Jacutinga, reservatório da hidrelétrica de Salto Caxias, Paraná, e alimentados durante 60 dias com dieta comercial suplementada com 0,0; 0,2; 0,4 e 0,8% de mananoligossacarídeos (n = 4). O consumo de ração apresentou correlação quadrática com os níveis dietéticos do prebiótico aos 30 e 60 dias. A altura das vilosidades intestinais foi maior nos peixes alimentados com 0,4% de prebiótico na dieta por 30 dias, porém, após 60 dias, os peixes alimentados com a dieta controle apresentaram maior altura das vilosidades. Nenhum dos níveis de mananoligossacarídeos avaliados mostrou-se eficiente sobre os parâmetros de crescimento. De fato, MOS atuou no crescimento das vilosidades intestinais. Porém, não foi efetivo com relação aos parâmetros de desempenho zootécnico em tilápia do Nilo.

Palavras chave: aquicultura; histologia; nutrição de peixes; prebiótico

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INTRODUCTION

For decades, antibiotics have often been used to prevent diseases outbreaks and as growth promoters in animal production applying sub therapeutic dosages. This use can select bacterial strains with resistance to these antibiotics (Antibiotic Multiple Resistance - AMR) as already described in Brazilian fishes (BELÉM-COSTA and CYRINO, 2006). Moreover, the growing concern about the indiscriminate use of antibiotics became a public health problem. Fish farmers must adjust to the Best Management Practices (BMPs) in fish production for human consumption (QUEIROZ *et al*., 2005).

Prebiotics are used as a functional growth promoter and stimulates the growth of benefic bacteria in the gut. Mannanoligosaccharides (MOS) is derived from the yeast *Saccharomyces cerevisae*, that provide mannose substrate upon which pathogenic gut bacteria selectively attach, impairing the adhesion to enterocytes, leading to better gut health and villi integrity (GHOSH and MEHLA, 2012). These molecules are easily isolated from the yeast's cell wall, processed into fish feed and do not cause environmental impact (HISANO *et al*., 2004).

Prebiotic concept, according to GILSON *et al*. (2004) is based at least on three aspects: (1) resistance to digestion, (2) fermentation by the large intestinal microbiota, acting as a selective substance on the microbiota that has favorable influence on the immune system, and (3) competition with pathogenic organisms, neutralizing and expelling them from the gut. This association could promote healthy effects in the organism.

The effects of dietary MOS on growth and health parameters have been recently evaluated in aquatic animals, such as the 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) and Atlantic salmon *Salmo salar* (REFSTIE *et al*., 2010). However, there is just a few reports regarding the effects of dietary MOS on fish gut morphology, as described for the sturgeon *Acipenser oxyrinchusde sotoi* (PRYOR *et al*., 2003), hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus* (GENC *et al*., 2007), cobia *Rachycentron canadum* (SALZE *et al*., 2008), red drum *Scianops ocellatus* (ZHOU *et al*., 2010), gilthead sea bream *Sparus aurata* (DIMITROGLOU *et al*., 2010a) and white sea bream *Diplodus sargus* (DIMITROGLOU *et al*., 2010b).

The use of MOS as prebiotic in fish nutrition is still incipient in respect to its mechanism and influence of growth parameters. The knowledge on its effects is restricted to very few species and, until now, there is a lack of information about the specificity in the prebiotic action. This matter has already been treated in other publications, such as CARVALHO *et al*. (2011) and SADO *et al.* (2008) for *O. niloticus*; DIMITROGLOU *et al.* (2010b) for *D. sargus;* MANSOUR *et al.* (2012) for *S. aurata*; HERNÁNDEZ *et al*. (2012) for *Rhamdia quelen*; SADO *et al*. (2014a, b) with *Piaractus mesopotamicus* and GRISDALE-HELLAND *et al.* (2008) for *S. salar*. These authors reported no effects in weight gain, neither in immunity.

On the other hand, several authors found out that dietary prebiotic (MOS) improved the local vilosity absorption surface in fish species such as for *D. labrax* (TORRECILLAS *et al*., 2007; 2011), *O. mykiss* (STAYKOV *et al.,* 2007), *Carassius auratus gibelio* (AKRAMI *et al.,* 2012), *Sciaenops ocellatus* (ZHOU *et al*., 2010), *Channa striata* (TALPUR *et al.,* 2014) and *S. aurata* (GÜLTEPE *et al*., 2011).

The purpose of this work was to determine the effects of increasing levels of dietary MOS supplementation on growth and gut morphology in juveniles of Nile tilapia raised on net cage under commercial production conditions.

MATERIAL AND METHODS

The experiment was set up at Salto Caxias Hydroelectric reservoir in Jacutinga River, located in the municipality of Boa Vista da Aparecida, Paraná, Brazil, between January and March 2012. Nile tilapia juveniles were randomly distributed into sixteen 250 L-net cages (20 fishes per cage; 12.3 ± 0.3 g) in an experiment designed with four treatments: 0.0; 0.2; 0.4 and 0.8% MOS dietary supplementation, with four replicates for each treatment (n = 4). Fishes were suited to basal diet for 15 days prior to experiment. Afterwards, they

were fed with the experimental diet, until apparent satiation, four times a day (08h00m; 11h30m; 14h30m and 18h00m), for 60 days. Parameters of water quality (pH 7.99 ± 0.69; dissolved oxygen 6.56 ± 0.55 g L^{-1}; conductivity 6.8 ± 0.14 µS cm^{-1} and temperature 30.4 ± 0.75 ºC) were monitored electronically on a daily basis.

A commercial fish feed formulation (Anhambi Alimentos, Itapejara do Oeste, Parana, Brazil) was used for the basal experimental diets composition (Table 1) containing 32% crude protein. The different levels (0.0; 0.2; 0.4 and 0.8%) of MOS (YES-MOS®, YES – YesSinergy do Brasil Agroindustrial, Jaguariuna, Sao Paulo, Brazil) were added to this basal diet and extruded. Then, the extruded feed was dried out in a forced ventilation oven at 45 ºC for 24 hours; the dried pellets were packed in black plastic bags and stored under refrigeration until use.

Table 1. Chemical composition of basal, practical diet (dry matter basis)[1]. max = maximum; min = minimum.

Nutrient	Content (g kg^{-1})
Moisture (max)	120
Crude protein (min)	320
Crude fat (min)	40
Crude fiber (max)	60
Ash (max)	130

[1] *Levels of guarantee according to manufacturer - Anhambi Alimentos, Ltda. Itapejara do Oeste, Paraná, 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}.*

Upon 30 and 60 days, trial fishes were fasted for 24 hours and sedated for biometrical procedures and growth parameters calculated according to SADO *et al.* (2010) as follows:

- Weight Gain (WG):

$$WG = FW - IW;$$

- Feed Consumption (FC);

- Feed Conversion Ratio (FCR):

$$FCR = \frac{\text{food consumption}}{\text{weight gain}};$$

- Specific Growth Rate (SGR):

$$SGR = 100 \times \frac{(\ln FW - \ln IW)}{t};$$

where: FW = final weight (g); IW = initial weight (g); t = experimental time (days).

Histological procedures were carried out in 30 and 60 days trial. A snippet of the proximal intestine of two specimens from each replicate of the 0.0, 0.2, 0.4 and 0.8% MOS supplementation treatments was sampled for histological observations. Tissue samples were immediately washed with saline solution (0.6%) and fixed for eight hours in a Bouin solution (5.0 mL formaldehyde 5% + 25 mL glacial acetic acid 5% + 75 mL saturated aqueous solution of picric acid 5%). Then, fixed samples were washed in a 70% alcohol solution (three times for 15 minutes) and submitted to dehydration through an alcohol solution series (80 to 100%). After dehydration process, tissues were diaphanized in xilol, embedded in histological paraffin and 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 villi height measures.

Significant effects of dietary MOS levels were determined by one-way analysis of variance (ANOVA), at 5% probability. A polynomial regression analysis was applied for fish growth significant results. Intestinal morphometric results were compared using Tukey's test ($\alpha = 0.05$).

RESULTS

Increasing levels of dietary MOS did not affect (p>0.05) some growth parameters of the Nile tilapia such as weight gain (WG), feed conversion rate (FCR) and specific growth rate (SGR) (Table 2).

Table 2. Means and standard deviation of individual weight gain (WG), specific growth rate (SGR) and feed conversion rate (FCR) of Nile tilapia *Oreochromis niloticus* fed with increasing levels of dietary mannanoligosaccharide (MOS) for 30 and 60 days.

MOS*	0.0%	0.2%	0.4%	0.8%	*p* values
30 days					
WG (g)	16.50 ± 0.61	16.74 ± 2.32	18.54 ± 0.88	18.68 ± 1.36	0.1031
SGR (% day^{-1})	2.77 ± 0.24	2.94 ± 0.17	2.91 ± 0.24	3.00 ± 0.17	0.5129
FCR	1.18 ± 0.05	1.29 ± 0.18	1.26 ± 0.11	1.26 ± 0.12	0.6305
60 days					
WG (g)	69.31 ± 1.11	70.84 ± 5.75	76.74 ± 3.98	75.36 ± 1.14	0.0664
SGR (% day^{-1})	3.10 ± 0.04	3.15 ± 0.10	3.25 ± 0.12	3.24 ± 0.10	0.1496
FCR	1.49 ± 0.08	1.68 ± 0.18	1.72 ± 0.08	1.61 ± 0.07	0.0761

**Mannanoligosaccharide - YES-MOS® (YES – YesSinergy do Brasil Agroindustrial, Jaguariúna, São Paulo, Brazil).*

However, fish feed consumption (FC) seems to increase until 0.4% MOS supplementation level, as from that point it became stable ($y = -9.834x^2 + 13.254x + 19.41$; $p < 0.05$) with increasing levels of dietary MOS (Figure 1) at 30 day trial. When the first derivative from the equation was equaled to zero, the highest feed consumption could be observed when fish are fed 0.67% dietary MOS.

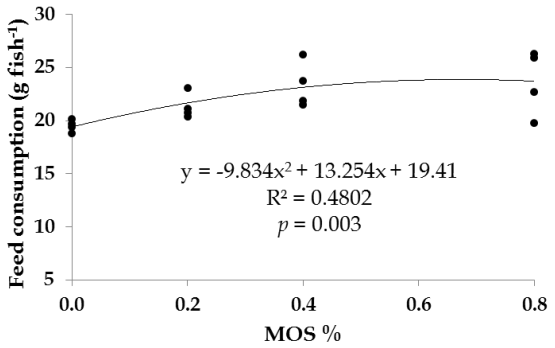

Figure 1. Relationship between feed consumption of Nile tilapia *Oreochromis niloticus* and increasing levels of dietary mannanoligosaccharides (MOS) at 30 days trial.

In the same way, after 60 days the FC also increased until 0.4% MOS supplementation level, as from that point it became stable ($y = -114.72x^2 + 114.96x + 103.02$; p<0.05) (Figure 2). When the first derivative from the equation was equaled to zero, the highest feed consumption could be observed when fish are fed 0.5% dietary MOS and start to decrease.

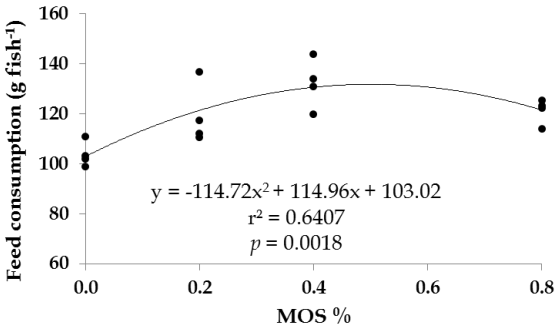

Figure 2. Relationship between feed consumption of Nile tilapia *Oreochromis niloticus* and increasing levels of dietary mannanoligosaccharides (MOS) at 60 days trial.

Histological analysis carried on this study revealed differences (p<0.05) in intestinal villi height between fishes fed with control diet and the ones with MOS supplemented diets for 30 and 60 days. Fish fed with 0.4% dietary MOS for 30 days showed significantly increased villi height (436.984 ± 66.82 μm) when compared to fish fed with control diet (401.011 ± 70.73 μm) and other supplementation levels (Figure 3).

However, when fishes were fed for 60 days, increased villi height was observed in fishes fed with control diet (436.300 ± 87.02 μm) when

compared to fish fed with MOS supplemented diets ($p < 0.05$) (Figure 4).

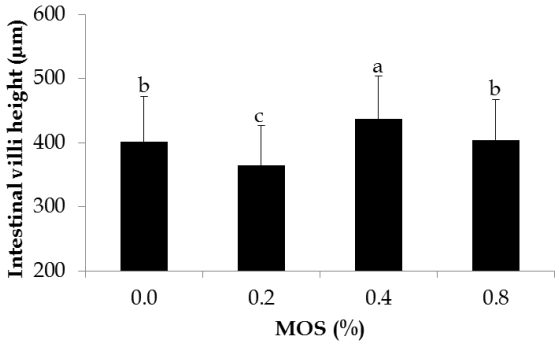

Figure 3. Intestinal villi height of Nile tilapia *Oreochromis niloticus* fed increasing levels of dietary mannanoligosaccharides (MOS) at 30 days trial. Different letters above columns indicate differences by Tukey test (α = 0.05).

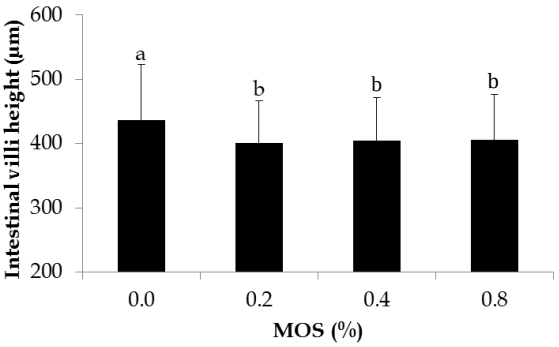

Figure 4. Intestinal villi height of Nile tilapia *Oreochromis niloticus* fed increasing levels of dietary mannanoligosaccharides (MOS) at 60 days trial. Different letters above columns indicate differences by Tukey test (α = 0.05).

DISCUSSION

The main goals of using prebiotics in aquaculture are enhancing fish growth and disease resistance, improving economic viability and sustainability of farming operations (MERRIFIELD *et al*., 2010; RINGØ *et al*., 2010). Increased growth parameters in fishes fed with dietary MOS was observed in the rainbow trout (STAYKOV *et al*., 2007), European sea bass (TORRECILLAS *et al*., 2007; 2011) and gilthead sea bream *S. aurata* (GÜLTEPE *et al*., 2011).

Dietary prebiotic such as MOS in fish nutrition is still controversial since some studies did not observe improvement on growth parameters. As herein observed for Nile tilapia fed increasing levels of dietary MOS.

Similar results were also observed for the same species 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 a 53-day trial (SCHWARZ *et al*., 2010). In the same way, several authors observed no effects on growth in other fish species, such as for the Gulf of Mexico sturgeon, fed with 0.3% dietary MOS (PRYOR *et al*., 2003), Atlantic salmon with 1.0% dietary MOS (GRISDALE-HELLAND *et al*., 2008), gilthead sea bream with 0.2 and 0.4% dietary MOS (DIMITROGLOU *et al*., 2010a) and giant sturgeon *Huso huso* with 0.2 and 0.4% dietary MOS (MANSOUR *et al*., 2012).

In the present study, the number of mucous cells in the intestine was not determined. Oligosaccharide digestion causes increase of mucus secretion by enterocytes that improves viscosity and better nutrient digestion and uptake (TORRECILLAS *et al*., 2011). However, excessive mucus production can also decrease digestion process by preventing enzyme-substrate interaction and satiation, which induces elevate feeding behavior (SILVA and NÖRNBERG, 2003) and could explain elevated feed consumption in fish fed dietary prebiotic, herein observed.

In opposition to our results, Nile tilapia fed with 0.2, 0.4, 0.6; 0.8 and 1.0% dietary MOS for 45 days had a negative correlation between dietary MOS supplementation and feed consumption (SADO *et al*., 2008). Indigestible carbohydrates could accumulate in the cytoplasmatic membrane of enterocytes causing destructive effects on microvillus organization as observed in the Artic charr *Salvelinus alpinus* fed with dietary inulin (OLSEN *et al*., 2001), another source of indigestible carbohydrate and could explain decreased feed consumption after 60 days trial in fish feed 0.8% dietary MOS.

Intestinal morphology influences the physiology and metabolism of nutrient absorption. Additionally, several other factors, such as nutritional components, stress and diseases, can affect gut integrity (HEIDARIEH *et al*., 2013).

Mannanoligosaccharides are indigestible oligosaccharides, providing mannose substrate

upon which pathogenic gut bacteria selectively attach. The inhibition of pathogenic bacteria adhesion to enterocyte prevents the formation of colonies, the entrapment of nutrients for bacterial growth and infection of host cells, leading to better gut health by increasing regularity, height and integrity of the gut villi and consequent better utilization and absorption of nutrients (PRYOR *et al*., 2003). This fact is well documented in other fish species, such as the 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).

However, in this trial, when the Nile tilapia was fed with dietary MOS for 60 days, the control group showed increased villi height. In the same way, several authors also did not find any improvement in villi height in fish fed with increasing levels of dietary MOS, such as the hybrid tilapia (*O. niloticus* x *O. aureus*) (GENC *et al*., 2007), sturgeons (PRYOR *et al*., 2003), European seabass (TORRECILLAS *et al*., 2007), gilthead seabream (DIMITROGLOU *et al*., 2010a) and pacu (SADO *et al.,* 2014a).

In fact, conflicting results demonstrated that the mode of action of prebiotics in fish nutrition is still unclear, regarding time, dose and methods of administration, since time-dose response can cause negative effects, as observed in artic charr (OLSEN *et al*., 2001), gilthead seabream (CEREZUELA *et al*., 2008) and hybrid surubim *Pseudoplatystoma* sp. (MOURIÑO *et al*., 2012).

Although ultraestructural analysis were not performed, the increased villi height observed in fish fed with dietary MOS for 30 days, that did not reflect better growth, could be explained by the impossibility to observe integrity of intestinal microvilli by optical microscopy.

Ultraestructural analysis of anterior intestine of cobia larvae fed with rotifers enriched with 0.2% MOS showed increased microvilli height (SALZE *et al*., 2008). Similar observations were recorded for gilthead sea bream fed with 0.2 and 0.4% dietary MOS (DIMITROGLOU *et al*., 2010a) and red drum fed with diets supplemented with 1% dietary prebiotics such as mannan, fructo and galactooligosccharides (ZHOU *et al*., 2010).

However, in both cases, despite the fact that ultraestructural 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. The white sea bream larvae fed with *Artemia* sp. 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 both authors.

CONCLUSIONS

This study corroborates the functionality of prebiotics compounds such as mannanoligosaccharides as dietary supplement for Nile tilapia to modulate gut morphology and food consumption to achieve maximum nutrient absorption and consequently, increase fish growth. However, further research for better explanation of contradictory results are warranted, since the complex carbohydrate structure in the cell wall of yeast, different strains and fermentation conditions, processing methods can alter their function and depending on MOS concentration, administration period and population status (age, sex, gonadal maturation), rearing conditions, feed formulation and extrusion procedures, different results can be presented.

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