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Mannanoligosaccharide (mos) and ß-glucan (ß-glu) in dietary supplementation for Nile tilapia juveniles dept in cages

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Abstract. This experiment was conducted at an aquicolous enterprise with the objective of evaluating the use of MOS and ß-GLU as dietary supplements in an experimental lot in order to follow the zootechnical performance, establishing a relationship with the hematological parameters, the morphological alterations of the intestine, and the enzymatic activity (protease, lipase and amylase), and the water quality of 3,000 tilapia juveniles kept in cages (Wt = 24 ± 0.26g). Nine cages (6.0 m^3) were used, with three treatments and three replications. 1: commercial feed without supplementation (control); 2: 0.1% per ton of MOS; 3: 0.03% per ton of purified β-GLU. The feed contained 36% of crude protein (CP) incorporated into the premix MOS and purified β-GLU (BIORIGIN®). The tilapia that had received the diet supplemented with β-GLU in a period of 90 days showed a favorable condition of the immune system, increase in the absorption surface of the front part of the intestine and consequently, growth in the activity of the digestive enzymes, denoting higher efficiency in the use of the nutrients in juveniles, providing satisfactory zootechnical performance in comparison with the other diets. This product may be used as a dietary supplement for this species when kept in cages.

Key words: enzymatic activity, hematology, intestine morphology, *Oreochromis niloticus*, zootechnical performance

Resumo. **Mananooligossacarídeo (MOS) e Beta Glucano na suplementação dietaria para juvenis de tilápia mantidos em tanques-rede.** Este experimento foi conduzido em uma empresa aquicola com o objetivo de avaliar o uso de MOS e ß-GLU como suplementos dietéticos em um lote experimental, a fim de acompanhar o desempenho zootécnico, estabelecendo uma relação com os parâmetros hematológicos, as alterações morfológicas do intestino, e a atividade enzimática (protease, lipase e amilase), e a qualidade da água de 3.000 juvenis de tilápia mantidos em gaiolas (Pi = 24 ± 0,26 g). Nove gaiolas (6,0 m^3) foram utilizados, com três tratamentos e três repetições. 1: ração comercial sem suplementação (controle); 2: 0,1% por tonelada de MOS; 3: 0,03% por tonelada de purificado β-Glu. A ração continha 36% de proteína bruta (PB), MOS e purificado de β-Glu (BIORIGIN ®) foram incorporados no premix. As tilápias que receberam a dieta suplementada β-Glu em um período de 90 dias mostraram uma condição favorável do sistema imunológico, aumento da superfície de absorção da parte da frente do intestino e, consequentemente, o crescimento, na actividade das enzimas digestivas, denotando uma maior eficiência na utilização dos nutrientes, proporcionando um desempenho satisfatório zootécnico em comparação com as outras dietas. Este produto pode ser utilizado como um suplemento dietético para esta espécie quando mantidos em gaiolas.

Palavras chave: atividade enzimática, hematologia, morfologia intestinal, *Oreochromis niloticus*, desempenho zootécnico

Introduction

Fishculture has showed significant advances in the aquicolous production lately, and among the species reared, we should pay special attention to the Nile tilapia, whose production corresponds to 6.7% of the global production of cultured fishes (FAO 2007, Ostrenski & Boeger 2008). The culture of Nile tilapia in cages stands out because it fulfills the needs for the maintenance of the fishes and ensures its efficiency in economic terms (Ayroza 2009). However, as in any culture system, the ration offered must be nutritionally balanced to satisfy the requirements of the species, which involves high costs (Kubitza 2003, Pezzato *et al*. 2004). Some problems related to inadequate management, which lead to situations of stress and the appearance of infirmities arise with the growth of this activity (Shrimpton *et al*. 2001). One of the alternatives to face those difficulties is the use of ingredients of microbial origin, such as the prebiotics (Li & Gaitlin 2004), especially the mannanoligosaccharides (MOS) and β-glucan (β-GLU) derived from the cell wall of yeast, *Saccharomyces cerevisiae.* They are substances used as an alternative to the growth promoters, keeping the beneficial balance of the intestinal microbiota in young animals or under imminent stress condition (Silva & Nörnberg 2003).

MOS and β-GLU act as stimulants of the defense mechanisms of the fishes. Studies demonstrate that mannose, when added to the diet, reduces the colonization of pathogenic bacteria in the animal's organism (Lima 2008). The glucans act increasing the activity of macrophages, the phagocytosis by neutrophils, monocytes and lymphocytes, and the production of immunoglobulins and lysozymes (Raa 1996, Sakai 1999, Falcon 2007). In fishes, the prebiotics act promoting immune responses against infectious agents, according to Volpatti *et al*. (1998), through the production of immune response mediator cells (Siwicki *et al*. 1993, Verlhac *et al*. 1996). These diagnoses and prognoses are determined as routine procedures, by means of hematological parameters (Ranzani-Paiva & Silva-Souza 2004). Nevertheless, results of investigations about the use of prebiotics in fishes are still limited and contradictory (Li & Gaitlin III 2004).

This study was conducted with Nile tilapia, *Oreochromis niloticus* juveniles kept in cages, with the objective of assessing the zootechnical performance with regard to the dietary supplementation of prebiotics, MOS and ß-GLU. This assessment was based on the variations of hematological parameters, morphological alterations of the intestine and changes in the activities of the digestive enzymes protease, lipase and amylase.

Material and methods

This experiment was carried out at the company *Piscicultura Escama Forte Ltda ME*, in the Zacarias city – SP/Brazil, located by the waters of the Nova Avanhandava Hydroelectric Powerplant Reservoir, on the Lower Tietê River (lat.179º 22' 48, 20"W; long. 69º 7' 54, 40'' N).

Initially, 3,000 sexually reversed *Oreochromis niloticus* at juvenile phase, with mean initial weight of 24±0.26g (Wi), belonging to the Chitralada strain were randomly distributed in nine square-sectioned cages (C) with 4.0 m^2 of surface and 1.5 m of water column. The fishes were fed three times a day to satisfaction. The ration offered per cage was weighed before and at the end, in order to evaluate total consumption. The feed used was commercial feed (FRI-RIBE S.A®, Pitangueiras, São Paulo, Brazil), containing 36% of crude protein (CP), to which were incorporated through premix (BIORIGIN®, Lençóis Paulista, São Paulo, Brazil): mannanoligosaccharide, MOS (22.0% mannanoligosaccharide and 20.0% β-glucan) and beta glucan, ß-GLU (70.0% of purified $ß_{1-3}$ glucan and $ß_{1-6}$ glucan). The addition was performed by the company FRI-RIBE S.A®, without alterations to the ration formula (Table I). The period of ration distribution was 90 days (October to December, 2006). The treatments complied with the following experimental design: 1) commercial feed without supplementation (control); 2) addition of 0.1% of MOS per ton of feed; 3) addition of 0.03% of β-glucan per ton of feed. The physicochemical parameters of the water, temperature and dissolved oxygen, were monitored daily in each cage. The pH and the electrical conductivity were registered weekly.

During the experiment, three biometries were performed at regular intervals of 30 days. In each biometry, the fishes were anesthetized, according to the ethical principles of animal manipulation established by the Brazilian School of Animal Experimentation (COBEA, htpp://*www.cobea.org.br*). After being anesthetized, the tilapias were weighed and measured, for the determination of the following parameters: *1)* relationship weight-length ($K= Wf /Lf^b$), where Wf is the final weight and Lf is the final length, while b is obtained by the allometric equation of the relationship weight/length, $y=ax^b$; *2)* condition factor (K), where the coefficient "b" of the relationship Wf x Lf is defined as the allometry

coefficient; *3)* daily weight gain (*DWG = Wf-Wi/t*), where t = time in days); *3)* apparent feed-conversion (*AFC = TFC/WG*), where TFC = total feed consumption and WG = weight gain; *4)* specific growth rate (*SGR = ln Wf - ln Wi x 100 days*); *5)* protein efficiency ratio (*PER = WG x 100/ TFC % CP diet*); *6)* survival (S%), calculated by the percentual relationship between the number of fishes at the beginning and at the end of the experiment.

For each biometry two animals were collected from each cage, totalizing six fishes per treatment. The fishes were punched in the caudal vein to withdraw 1.0 mL of blood, using needles (0.70 x 25 mm), disposable syringes (5.0 mL) and siliconized and heparinized plastic tubes (2.0 mL). The blood samples were kept at 4ºC for 1 hour until processing. After the blood collection, the animals were killed by spinal cord puncture, eviscerated, and had their digestive tract removed completely, discarding the digestive content. The digestive tract was transferred to ice bath and kept at -20ºC for later removal of the intestine and analytical determinations.

The blood samples were transferred to microhematocrit tubes, centrifuged at 4,500 x g for 5 minutes and read in standardized cards as proposed by the *National Committee for Clinical Laboratory Standards* (NCCLS). The values of hematocrit are expressed in percentage (%). The concentrations of total plasma protein (TPP) were assessed through the plasma of each microhematocrit tube in a manual refractometer and expressed in g dL^{-1}. Hemoglobin (Hb) determinations were performed in duplicate by the cyanohemoglobin method, using the commercial Drabkin reactive and optical absorbance reading at 540 nm. In order to avoid interference, the reading of the hemoglobin reaction was carried out after the separation by centrifugation of the suspended nuclear material. The concentrations of Hb are expressed in g dL^{-1}. For the erythrocyte count (Er), performed manually in Neubauer chamber, the Natt-Herrick diluent was used. The leucocyte (LDC) and thrombocyte (TTC) differential counts were accomplished by means of slides stained with hematoxylin-eosin (HE), totalizing 200 cells, and for the total leucocyte count (TLC), 2,000 cells were evaluated. The hematimetric indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were assessed according to the methodology recommended by Thrall (2004).

Fragments of the front part of the intestine were fixed in Bouin solution for 8 hours, embedded in blocks of paraffin, cross-sectioned (5.0 µm), and stained with hematoxylin-eosin (HE). The sections were examined under an optical microscope, attached to an Olympus DP11-N system for image capture. The analyses determined: *1)* height of the villi (from the apex of the villi to the beginning of the muscle layer); *2)* total height of the villi (from the apex of the villi until the end), and *3)* thickness of the epithelium of the villi, using the *software* Image-Pro Plus®.

The remainder of the intestine was homogenized in buffer solution $NaHPO_4$ 0.01M/ Tris 0.01M pH 7.0 in glycerin 1:1(v:v) under ice bath, with a rotating homogenizer at 1,000 rpm for 1 minute. After being homogenized, it was centrifuged at 12000 x g for 10 minutes and the supernatant was used as source of enzymes. The activities of total protease were analysed using casein as substrate, and afterwards the content of free aminoacids in the supernatant was established (Walter 1984). The lipolytic activities were analysed according to Albro (1985), using the p-NO-phenyl meristate as substrate. In order to verify the amilohydrolytic activities, also analysed, a solution of 2% soluble starch was used as reaction substrate; the free glucose content was determined by the glucose oxidase method (Bernfeld 1995).

Statistical analysis

The experimental design comprised three treatments with three replications. The parameters studied were submitted to analysis of variance, accepting a level of significance of 5%. When the differences were significant, the Tukey test was applied, and the statistical package SAS (2005) was used for the comparison of the means. The comparison of the hematological variables was carried out through the non-parametric Kruskal-Wallis test. The harvesting periods were compared by the Friedman test, and the Dunn test was used for the multiple comparisons.

Results and discussion

The Nile tilapias submitted to the treatment with MOS and β-GLU exhibited significantly higher mean values of weight and length than the control ones from 60 days of ration distribution onwards (Table II). Those alterations could also be observed by the morphological analysis of the digestive tract showing significant increase in the thickness and lenght of the villi (Fig. 2). A similar result was described with the same species and culture system, though fed with yeast (Medri *et al*. 1999). In that case, low values of growth and weight were reported, which was attributed to the population densification. Similar results to ours were observed by Pereira da Silva & Pezzato (2000). However, Nile

tilapia juveniles reared in earthen ponds and fed yeast as a vitamin supplement also presented a slight increase in the mean values of weight (Baccarin & Pezzato 2001). When fed lower content of protein (28.0% DP/3000 kcal DE/kg), but with addition of vitamin C (400 and 600 mg kg^{-1}) and ß-glucan (0.1-0.8%), that species presented a satisfactory performance even with shorter periods of time (six weeks) than the ones observed in the present study (Falcon 2007). Likewise, tilapia larvae showed promissing results with the MOS supplementation for a period of 21 days (Samrongpan *et al*. 2009). Nevertheless, *Colossoma macropomum* juveniles fed diets supplemented with brewery residue (barley) showed a reduction in the values of weight and length, in disagreement with what has been described for tilapias (Cruz 1997). Those physiological variations are worth mentioning, since the addition of prebiotics demands previous knowledge of the physiological responses of the species to the supplement.

Table I. Percent and chemical composition of the experimental rations during the experiment.

Ingredients (%)	**Control**[1]	**D – MOS**[2]	**D- GLU**[3]
Crushed corn	31.20	31.20	31.20
Wheat meal	5.00	5.00	5.00
Soybean meal	19.20	19.20	19.20
Broken rice	12.00	12.00	12.00
Corn gluten	7.46	7.46	7.46
Feather meal	5.00	5.00	5.00
Fish meal	5.00	5.00	5.00
Poultry guts meal	12.00	12.00	12.00
NaCL	0.24	0.24	0.22
Vixil lignosulfonate - agglutinant	1.00	1.00	1.00
Mineral and vitamin supplement [4]	0.50	0.50	0.50
Choline chloride %	0.50	0.50	0.50
DL Methionine	0.90	0.90	0.90
MOS	--	***0.1***	--
ß- glucan	--	--	***0.03***
Composition %			
Crude Protein	36.00	36.00	36.00
Ethereal extract	4.64	4.64	4.64
Crude Fiber	3.25	3.25	3.25
Mineral Matter	7.75	7.75	7.75
Calcium	1.55	1.55	1.55
Phosphorus	1.00	1.00	1.00
Non-nitrogenated extract [5]	50.19	50.19	50.19

[1] CONTROL – commercial feed without supplementation (control).

[2] D- MOS -1kg MOS per ton of ration;

[3] D- GLU- 300g purified ß- glucans per ton of ration;

[4] Vitamins and Minerals, amount kg/ration: 2,000.00 choline, 0.2143% sodium, 0.2531% chlorine, 0.7819% potassium, 0.1979% magnesium, 0.3231% sulfur, 268.88 mg of iron, 21.10 mg of copper, 21.40 mg manganese, 198.07 mg of zinc, 0.054 md cobalt, 4.84 mg iodine, 0.478 mg selenium, 14,056.67 UI vitamin A, 3,000.48 UI vitamin D3, 200 mg vitamin E, 6.00 mg vitamin K3, 8.01 mg vitamin B1, 10.93 mg vitamin B2, 10.89 mg vitamin B6, 23.21 mcg vitamin B12, 134.45 mg niacin, 27.56 mg pantothenic acid (B3), 1.30 mg folic acid, 0.256 biotin, 400 mg vitamin C.

[5] NNE = DM – (CP+MM+CF+EE), where DM = dry matter, CP = crude protein, MM = mineral matter, CF = crude fiber, EE = ethereal extract.

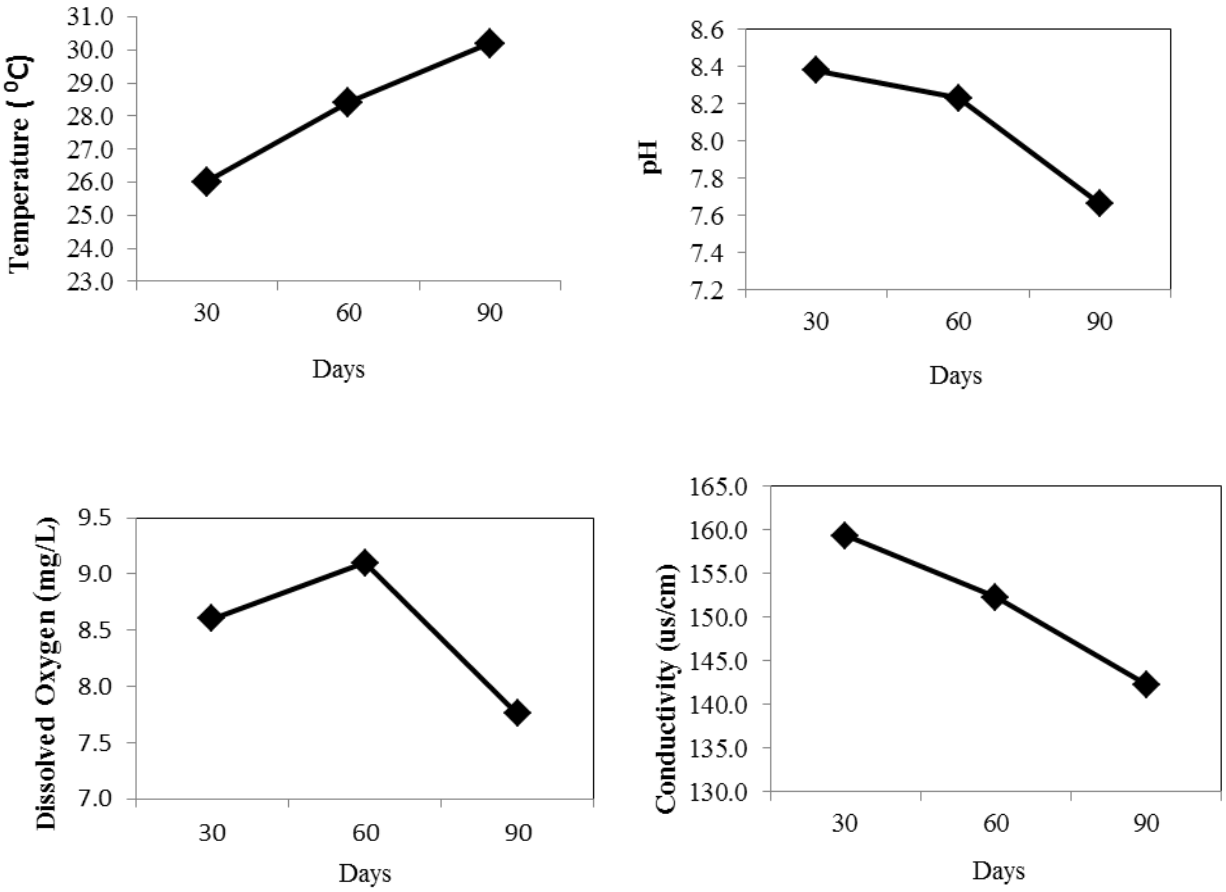

Figure 1. Physicochemical parameters of the water during the 90 days of experiment

The increase in the values of weight gain (WG) in fishes might be related to a probable glucan degradation by the glucanase, promoting the transference of more proteins (protein saver effect) for the growth (Lopéz *et al.* 2003). However, there is no evidence that the fishes are able to digest the ß-glucan, or of its action on the value of WG (Ai *et al.* 2007), as observed in shrimp, *Penaeus monodon* (Wigglesworth & Griffith 1994). Weight gain by addition of glucans to the ration was not reported only in studies with tilapia, but also with "yellow croaker", *Pseudosciaena crocea* fed diets added with lower values than 0.09% of glucan, for 56 days, despite the fact that there were no significant differences in the values of DWG (Ai *et al.* 2007). Higher values of WG were described for rainbow trout fed yeast (Rumsey 1991). Another important parameter for the evaluation of the growth is the condition factor, considered a corporal index that is able to reflect interactions between the fishes and the environment, which represents an indication of the well-being of the species (Tavares-Dias *et al*. 2008). In the present study, an isometric growth with b was observed, ranging between 3.108 (control), 3.173 (MOS) and 3.016 (ß-GLU) in the equation of the relationship weight-length. According to Landell (2007), studying Nile tilapias reared in 18.0 m^3 cages in a system divided into culture phases from 30 to 870g, they presented the same pattern of growth, with lower b, ranging between 2.9077 and 3.0344. Thus, in the species studied, the probable effect of the supplementation of β-glucan in the diet might be related to the increase in the immune response and the well-being of the fish.

The values of AFC, which oscillated between 1.5 and 2.6, did not present significant variations between the treatments and harvestings during the experimental period. However, the values of S, TFC, SGR, and PER showed significant differences only between the harvestings, when there was an increase in the consumption of food throughout the development, and also a situation of invariability of the SGR values in the treatments with prebiotics and a decrease in the PER values in the three treatments (Table II). Similar results were described by Falcon (2007), who did not observe

differences in the AFC values for tilapias fed the glucan supplement and levels of vitamin C. Constant values of AFC suggest that the increase in DWG must not be directly correlated with digestive mechanisms, but with other types of physiological alterations, able to modify, for example, values of metabolic rates, and even promote more specific biochemical responses in the cells, directing the proteins towards the growth of muscle mass. Despite staying the same between the treatments, an increase in the values of DWG was observed in each treatment over the harvestings. *Dentex dentex* (Hidalgo *et al.* 2006) and "snapper" *Pagrus auratus*-Sparidae (Cook *et al.* 2003) juveniles fed prebiotic did not show significant differences in the values of SGR. Furthermore, rainbow trout, *Oncorhynchus mykiss* (Rumsey 1991) and tilapia juveniles (Baccarin & Pezzato 2001) showed significant decrease in efficiency when fed yeast.

In the present study, the mean values of hematocrit and hemoglobin in Nile tilapia juveniles did not show significant variations between the treatments (Table III). However, over the harvestings the concentration of Hb showed a general tendency of reduction, more pronounced in the second harvesting. Considering that the variations of Hb were general, the reduction in its value could be attributed to external factors, such as environmental or other types, without accrediting them to the treatments. These results are in accordance with the ones obtained by Tavares Dias & Moraes (2004), but are in disagreement with the ones obtained with *Ictalurus punctatus* fed glucan, mannanoligosaccharide and yeast, which presented increase in the values of hematocrit when they received β-glucan at the concentration of 1,000 mg per kg of ration for four weeks (Welker *et al.* 2007). Throughout the experimental period, a reduction of the number of erythrocytes was observed, which was not observed in the fishes that had received complement with b-glucan. That reduction might be attributed to the loss of red blood cells due to hemorrhages, observed in the control fishes and also in the ones treated with MOS. The values of MCHC reflected, as expected, on the variations of total Hb. Nevertheless, the constant values of Ht together with reduction in TTC might be originated from an increase in MCV. It is important to notice that this variable exhibited higher values in the fishes treated with b-glucan. Hybrids of sturgeons, *Acipenser ruthenus x A. baerii* also presented reduction in the number of erythrocytes (TEC) and a gradual increase, around 40%, of the values of MCV, due to the increase in the levels of immunostimulants offered in the diet (Jeney & Jeney 2002). The situation observed in the present study suggests slight macrocytic anemia, whose connection with the treatments is yet to be confirmed. Invariable values of TPP may indicate the presence of an ionic balance in the blood, as well as suggesting nutritional regularity and insuring that the blood losses observed must have been minor, without blood volume alterations. The decrease in TPP, together with the presence of prebiotics, is described in pacu, *Piaractus mesopotamicus* fed diets supplemented with ≥ 0.3% glucan (Schorer 2008). However, tilapias treated with different levels of yeast and derivatives did not show changes in TPP (Hisano *et al.* 2006).

Table IV shows that the fishes treated with ß-glucan presented a slight increase in the number of lymphocytes. A similar response was observed with tilapias fed diets supplemented with vitamin C and b-glucan (Falcon 2007). Rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with 1.0% of β-glucan and submitted to transportation stress presented lymphocytosis, neutropenia and monocytopenia. However, lower values of supplementation do not seem to interfere in the production of defense cells after stress (Jeney *et al.* 1997). Immature forms of erythrocytes associated to hemoparasites were often observed in the present study, specifically in anemic tilapias from the control and MOS treatments. Such observations coincided with the lowest percentages of survival described in those two groups. At the thrombocyte, neutrophil and monocyte differential counts, significant differences were not observed between the treatments. However, the number of neutrophils and monocytes in the fishes from the control group and treated with MOS remained higher when compared to the ones that had received ß-glucan. This condition is probably caused by the population densification, which normally leads those animals to stress.

The structural analysis of the front part of the intestine (Fig. 2; Table V) shows significant differences in the values of villi height, total height of the villi and thickness of the epithelium of the villi in the treatments MOS and β-glucan. Nile tilapia fed Flavofeed® displayed similar responses to the ones observed in this study when the height of the villi was evaluated according to Frabegat (2006). Rainbow trout, *Oncorhynchus mykiss* fed diets supplemented with Bio-mos exhibited increase in the height and thickness of the villi, according to Staykov *et al*. (2007). Burrel *et al*. (2001), studying Atlantic salmon described positive structural responses found in the intestine of fishes fed diets supplemented with nucleotides.

Table II. Means ($\overline{x}$) and respective standard deviation (s) of the parameters: final weight and length, condition factor (K), feed consumption, daily weight gain (DWG), apparent feed-conversion (AFC) and survival, according to the ration and period collected.

Parameters	Harvestings	Ration ($\overline{x}$ ± s)		
		Control	MOS	β-glucan
Final weight (g)	1	55.33 ± 2.31 cA	61.33 ± 4.16 cA	67.67 ± 4.51 cA
	2	110.33 ± 7.09 bB	128.00 ± 4.36 bAB	136.67 ± 6.66 bA
	3	179.00 ± 28.05 aB	201.33 ± 18.15 aAB	219.67 ± 6.66 aA
	$\overline{x}$	114.88	130.22	141.33
Final length (cm)	1	14.37 ± 0.31 cA	14.90 ± 0.46 cA	14.63 ± 0.15 cA
	2	17.93 ± 0.40 bB	18.00 ± 0.00 bAB	18.80 ± 0.17 bA
	3	20.90 ± 0.53 aB	21.73 ± 0.46 aA	21.93 ± 0.55 aA
	$\overline{x}$	17.73	18.21	18.45
Condition factor (K)	1	1.404 ± 0.142 aB	1.162 ± 0.057 aC	2.069 ± 0.129 aA
	2	1.400 ± 0.029 aB	1.331 ± 0.045 aB	1.961 ± 0.042 aA
	3	1.407 ± 0.149 aB	1.149 ± 0.043 aC	1.986 ± 0.147 aA
	$\overline{x}$	1.403	1.214	2.005
Daily weight gain (DWG, $gday^{-1}$)	1	1.74 ± 0.13 bA	2.07 ± 0.23 bA	2.43 ± 0.25 bA
	2	2.39 ± 0.34 abA	2.90 ± 0.31 abA	3.00 ± 0.47 bA
	3	3.27 ± 1.13 aA	3.49 ± 0.69 aA	3.95 ± 0.38 aA
	$\overline{x}$	2.46	2.82	3.12
Apparent feed conversion (AFC)	1	2.59 ± 1.11 aA	1.88 ± 1.17 aA	1.52 ± 0.16 aA
	2	2.24 ± 0.89 aA	1.59 ± 0.36 aA	1.48 ± 0.51 aA
	3	1.88 ± 1.90 aA	1.70 ± 0.76 aA	1.10 ± 0.41 aA
	$\overline{x}$	2.23	1.72	1.36
Survival (S,%)	1	69.6 ± 10.3 aA	73.5 ± 2.4 aA	75.2 ± 2.1 aA
	2	68.6 ± 0.8 bA	72.7 ± 0.3 bA	73.0 ± 0.3 aA
	3	63.8 ± 2.7 abA	68.8 ± 2.7 abA	71.0 ± 1.8 aA
	$\overline{x}$	67.3	71.6	73.0
Feed consumption (FC,kg)	1	38.95 ± 1.42 cA	39.62 ± 1.65 cA	33.08 ± 8.84 cA
	2	127.72± 8.44 bA	128.88 ± 0.00 bA	124.26 ± 0.00 bA
	3	174.54 ± 8.70 aA	189.02 ± 0.00 aA	165.67 ± 35.87 aA
	$\overline{x}$	113.73	119.17	107.67
Specific growth Rate (SGR)	1	4.63±0.229 bA	5.20±0.372 aA	9.52±0.372 aA
	2	6.62±0.278 bA	7.24±0.215 aA	10.25±0.147 aA
	3	9.57±0.747 aA	7.54±0.144 aA	10.54±0.435 aA
	$\overline{x}$	6.94	6.66	10.10
Protein efficiency ratio (PER)	1	40.31±4.24 aA	47.03±14.13 aA	68.26±3.33 aA
	2	27.68±5.15 bA	33.05±5.56 bA	35.48±3.52 bA
	3	22.74±6.98 bA	22.63±9.85 bA	30.62±4.50 bA
	$\overline{x}$	30.24	34.23	44.78

Means followed by different letters, small letters in the column and capital letters in the line, are different according to the Tukey test ($p < 0.05$)

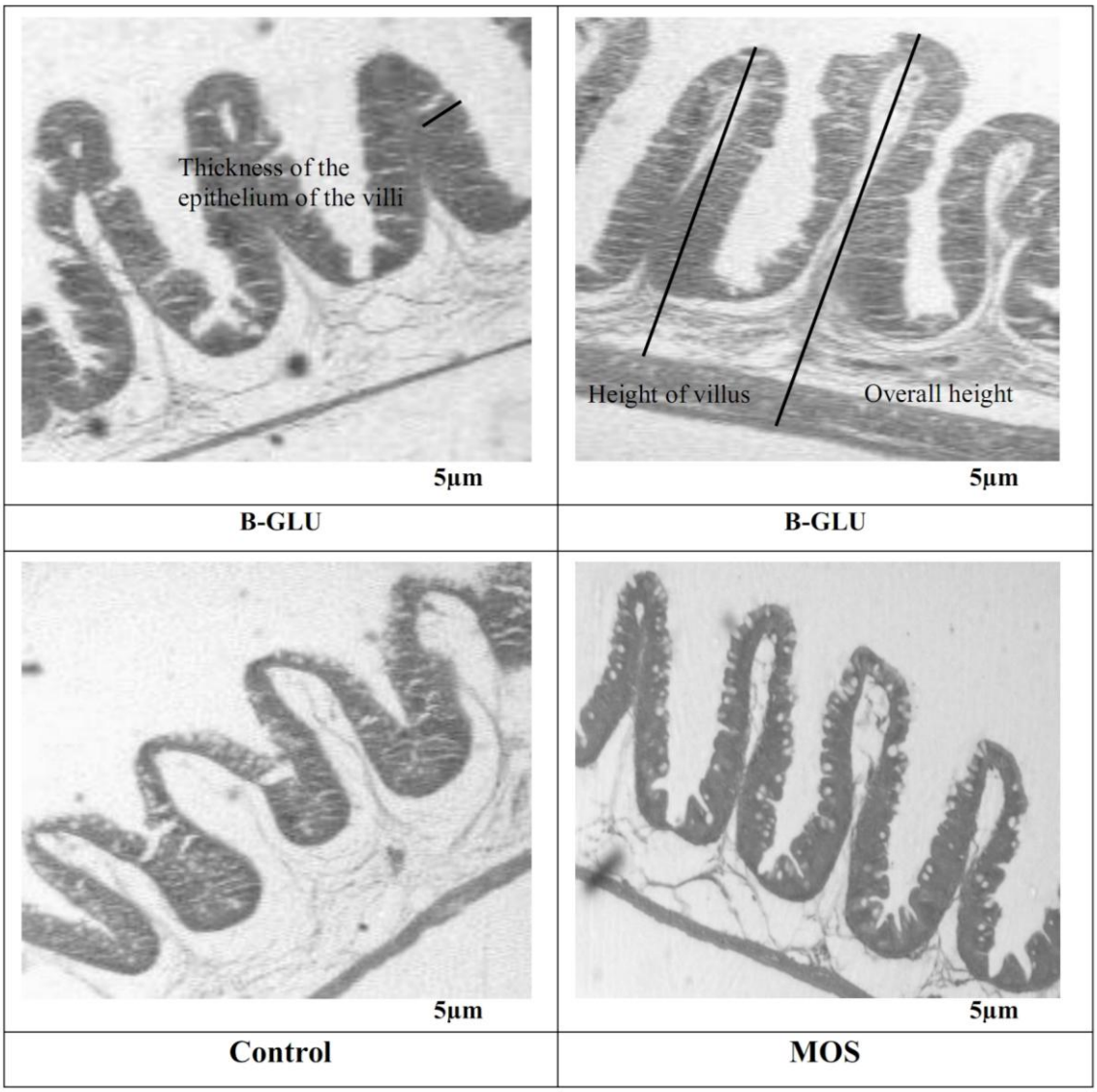

Figure 2. Villi of the intestine of Nile tilapia fed diets supplemented with prebiotics

According to Hisano *et al.* (2006), those intestinal morphological responses may have occurred due to a higher content of mannanoligosaccharide and nucleotides included in the diet, which might have influenced the intestinal tract and its microbiota. Besides, responses with regard to the height of the villi were
also reported with rainbow trout, *O. mykiss* fed soybean protein (Escaffre *et al.* De 2007), a vegetal nutrient that is rich in structural polysaccharides. The alterations observed in this study exhibited a beneficial aspect to the morphological characteristics of the intestinal tract, promoting the increase in the area of absorption of the mucosa of Nile tilapias fed diets supplemented with MOS. Similar alterations were reported for non- ruminant animals fed diets supplemented with prebiotics (Silva & Nörnberg 2003).

In fig. 3A, fishes fed β-glucan presented inhibition of the inespecific proteolytic activity. However, the presence of MOS in the diet permitted the preservation of that activity. It was possible to observe that the activities of amylase and lipase were not altered by the addition of MOS or β-glucan (Figs. 3B and 3C). Throughout the time, the addition of MOS in the diet generated a tendency towards increase, while in the diet supplemented with β-glucan there was reduction of the amylase activity. It was possible to verify that the lipase activity increased throughout the harvestings in tilapias that had received β-glucan in their diet. According to Almeida (2006), this feeding behavior in fishes is considered an adaptation to the variation in diet composition. The ability of fishes to process food is fundamental and depends on some characteristics such as the enzymatic profile of the alimentary canal

of the species (Fagbenro *et al.* De 2000). There is a consensus on the proportional variation of amylase, lipase and protease secretion due to the variation of the content or level of its substrates in the diet (Lhoste *et al.* De 1994). The same was described for mullet (*Mugil platanus)* by Galvão *et al.* (1997), and jundia (*Rhamdia quelen*) by Lundstedt *et al.* (2002).

Table III. Means ($\overline{x}$) and standard deviation (s) of the hematological parameters: Ht (hematocrit), Hb (total hemoglobin), TPP (total protein), erythrocyte, MCHC (mean corpuscular hemoglobin concentration), MCV (mean corpuscular volume) according to the rations and periods of harvesting.

Parameters	Harvestings	Ration ($\overline{x}$ ± s)		
		Control	MOS	β-glucan
Ht (%)	1	32.00 ± 2.76 aA	34.33 ± 6.68 aA	36.00 ± 6.81 aA
	2	31.40 ± 3.05 aA	34.33 ± 4.08 aA	32.33 ± 2.94 aA
	3	33.33 ± 2.80 aA	34.00 ±4.34 aA	34.33 ± 2.66 aA
	$\overline{x}$	32.24	34.22	34.22
Hb ($g\ dL^{-1}$)	1	7.22 ± 0.44 aA	7.42 ± 0.77 aA	6.96 ± 0.72 aA
	2	5.41 ± 0.26 cA	5.89 ± 0.63 cA	5.68 ± 0.60 bA
	3	6.47 ± 0.47 bA	6.62 ± 0.81 bA	6.93 ± 0.36 aA
	$\overline{x}$	6.36	6.64	6.49
TPP	1	3.70 ± 0.33 aA	3.93 ± 0.74 aA	4.72 ± 0.75 aA
	2	3.54 ± 0.17 aA	4.03 ± 0.44 aA	3.92 ± 0.33 aA
	3	3.70 ± 0.40 aB	4.32 ± 0.43 aA	4.25 ± 0.67 aAB
	$\overline{x}$	3.64	4.09	4.29
MCHC ($g\ dL^{-1}$)	1	22.39 ± 1.95 aA	22.42 ± 3.52 aA	19.59 ± 1.65 abA
	2	17.30 ± 1.07 bA	17.21 ± 0.82 cA	18.62 ± 1.72 bA
	3	19.46 ± 1.02 bA	19.48 ± 0.49 bA	20.24 ± 0.76 aA
	$\overline{x}$	19.71	19.70	19.48
MCV (fL)	1	1.63 ± 0.28 bB	1.71 ± 0.34 bAB	2.07 ± 0.24 bA
	2	2.02 ± 0.52 aA	2.15 ± 0.37 aA	1.85 ± 0.27 bA
	3	2.02 ± 0.17 aB	2.20 ± 0.24 aAB	2.51 ± 0.35 aA
	$\overline{x}$	1.89	2.02	2.14
TTC (10^4 µL)	1	39.2 ± 15.2 aA	32.2 ± 10.5aA	19.3 ± 6.2bA
	2	22.2 ± 10.6aA	16.8 ± 5.8aA	19.0 ± 2.4aA
	3	15.7 ± 7.4aA	15.7 ± 4.4aA	13.0 ± 5.3aA
	$\overline{x}$	25.7	21.56	17.1
Er (10^4 µL)	1	202.33± 32.23 aA	202.00 ± 27.57 aA	173.50 ± 22.56 aA
	2	157.20± 35.53 bA	161.33 ± 25.38 bA	178.50 ± 34.45 aA
	3	166.67± 24.04 bA	138.67 ± 18.78 bA	156.67 ± 28.32 aA
	$\overline{x}$	181.1	167.33	169.69

Means followed by different letters, small letters in the column and capital letters in the line, are different according to the Tukey test ($p < 0.05$)

Table IV. Means ($\overline{x}$), standard deviation (s) and median (Md) of the parameters of the differential count: neutrophil, monocyte and lymphocyte in %, according to the rations and periods of harvesting.

Parameters	Harvestings	Ration ($\overline{x}$ ± s)		
		Control	MOS	β-glucan
Neutrophils	1	32.1 ± 8.8 aA	23.0 ± 7.9 aA	26.9 ± 4.3 aA
	2	25.0 ± 12.6 aA	22.9 ± 13.8 aA	13.6 ± 7.6 bA
	3	24.6 ± 5.6 bA	14.6 ± 1.0 aB	13.5 ± 1.5 bB
	$\overline{x}$	27.23	20.16	18.0
Monocytes	1	6.2 ± 3.2 aA	1.7 ± 1.2 aB	1.7 ± 1.4 aB
	2	5.6 ± 1.7 aA	5.6 ± 5.4 aA	4.5 ± 1.6 aA
	3	7.4 ± 5.2 aA	2.0 ± 1.6 aB	1.9 ± 0.8 aB
	$\overline{x}$	6.4	3.1	2.7
Lymphocytes	1	61.0 ± 6.2 aB	74.5 ± 8.4 aA	69.6 ± 2.7 bAB
	2	69.0 ± 13.7 aA	70.2 ± 17.7 aA	82.0 ± 9.2 aA
	3	66.1 ± 3.5 aB	80.7 ± 2.8 aA	82.7 ± 2.0 aA
	$\overline{x}$	65.33	75.13	78.1

Means followed by different letters, small letters in the column and capital letters in the line, are different according to the Tukey test ($p < 0.05$)

Table V. Means ($\overline{x}$) and standard deviation (s) of the height of the villi, height of the walls and thickness of the epithelium, according to the rations and periods of harvesting.

Parameters	Harvestings	Ration ($\overline{x}$ ± s)		
		Control	MOS	β-glucan
Height of the villi (µm)	1	32.02 ± 2.11 bA	33.17 ± 7.06 cA	36.20 ± 7.19 bA
	2	57.38 ± 7.36 aB	61.71 ± 6.56 bAB	64.90 ± 14.48 aA
	3	61.87 ± 3.67 aB	69.02 ± 11.27 aA	69.15 ± 11.41 aA
	$\overline{x}$	50.42	54.63	56.74
Total height of the villi (µm)	1	33.20 ± 2.21 bA	34.73 ± 7.19 bA	40.18 ± 7.66 bA
	2	61.30 ± 8.19 aB	71.38 ± 6.97 aA	71.09 ± 16.52 aA
	3	66.71 ± 4.40 aB	76.30 ± 13.99 aA	74.12 ± 10.99 aAB
	$\overline{x}$	53.73	60.80	61.79
Thickness of epithelium of villi (µm)	1	1.33 ± 0.23 cB	1.67 ± 0.43 bB	2.78 ± 0.59 cA
	2	3.43 ± 0.64 bB	6.93 ± 1.26 aA	6.35 ± 1.29 bA
	3	7.67 ± 1.30 aA	6.48 ± 1.18 aB	8.41 ± 0.92 aA
	$\overline{x}$	4.14	5.02	5.84

Means followed by different letters, small letters in the column and cspitsl letters in the line, are different according to the Tukey test ($p < 0.05$)

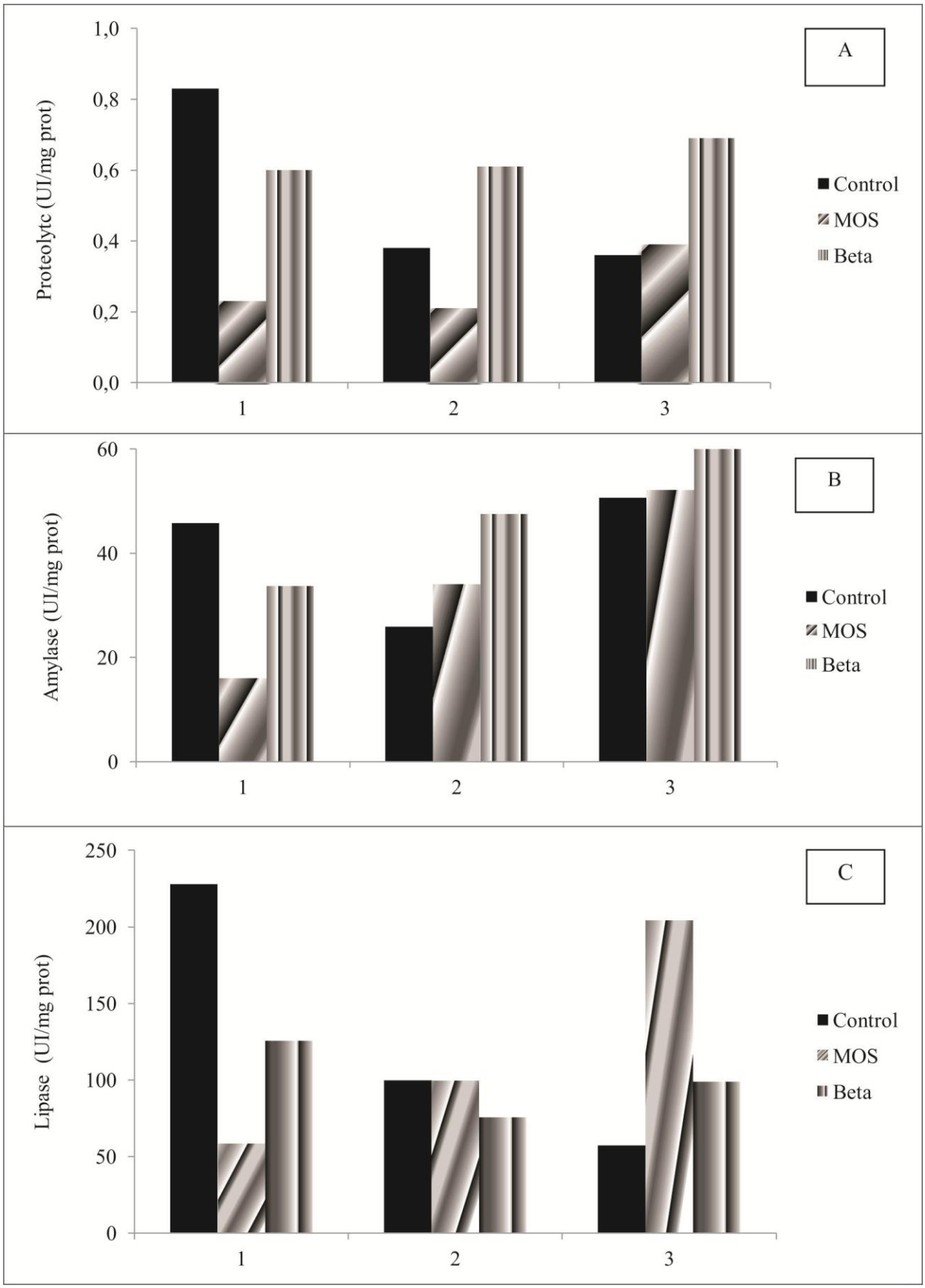

Figure 3. Inespecific activity of the digestive enzymes in the gastrointestinal tract (A = proteolytic; B= amylase; C = lipase) of Nile tilapia fed diets containing prebiotics for 90 days.

Conclusion

The product β-glucan used at the proportion of 0.03% per ton of ration in a period of 90 days increased the surface of absorption in the intestine, as well as the enzymatic activity, which could involve higher efficiency in the use of the nutrients. Thus, beneficial alterations of the hematological parameters may be inferred, leading to a satisfactory zootechnical performance of the Nile tilapia juveniles when compared to the other diets, suggesting that this product may be used as a food supplement for the species, when kept in cages.

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