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DIETARY MANNAN-OLIGOSACCHARIDE INCREASES REACTIVE OXIGEN SPECIES PRODUCTION BUT DECREASES SERUM LYSOZYME IN HIGH LEVELS OF INCLUSION FOR NILE TILAPIA

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ABSTRACT

In the present experiment, the effects of mannan-oligosaccharide (MOS) on health and growth of Nile tilapia juveniles were investigated. In addition to the control treatment (without MOS),three levels of MOS were included in Nile tilapia diets (1, 8, and 15 g kg^{-1}), and hematology, reactive oxygen species (ROS) production, lysozyme and productive parameters were analyzed. Fish blood was sampled at day zero (basal sample) and after 60 days of trial, and the productive parameters were evaluated at the end of the experiment. MOS feeding decreased the feed consumption ($p = 0.0299$) in fish fed with 1 and 8 g kg^{-1}, but without any alteration in weight gain (WG) and feed conversion ratio (FCR). No changes were observed in the hematology due to MOS feeding after 60 days. However, the prebiotic caused changes in the innate immunity of fish, giving rise to ROS production in fish fed with 1 g kg^{-1} ($p<0.0001$) and decreasing the serum lysozyme activity of fish fed with 15 g kg^{-1} ($p<0.0001$). In conclusion, the authors recommend the inclusion of 1 g kg^{-1} for Nile tilapia juveniles feeding due to the positive effect in innate immune system.

Keywords: hematology; immunology; prebiotic; *Oreochromis niloticus*; productive parameters.

INCLUSÃO DE MANANOLIGOSACARÍDEO (MOS) NA DIETA AUMENTA PRODUÇÃO DE ESPÉCIES REATIVAS DE OXIGÊNIO (EROS) MAS DIMINUI A LISOZIMA SÉRICA DE TILÁPIAS DO NILO

RESUMO

No presente experimento, os efeitos do mananoligossacarídeo (MOS) na saúde e no crescimento de tilápias do Nilo foram investigados. Além do tratamento controle (sem adição de MOS), três níveis de MOS foram adicionados na dieta de tilápias do Nilo (1, 8 e 15 g kg^{-1}). Foram analisadas a hematologia, a produção de espécies reativas de oxigênio (EROs), a lisozima e parâmetros produtivos. Os peixes tiveram o sangue colhido no dia zero (amostragem basal) e depois de 60 dias de experimento, juntamente com a avaliação dos parâmetros produtivos. A alimentação com MOS diminuiu de forma significativa o consumo ($p = 0.0299$) em peixes alimentados com 1 e 8 g kg^{-1}, mas sem quaisquer alterações sobre o ganho de peso (GP) e conversão alimentar (CA). Não foram observadas mudanças nos parâmetros hematológicos devido à alimentação com MOS. No entanto, o prebiótico alterou sua resposta imune, aumentando a produção de EROs nos animais alimentados com 1 g kg^{-1} ($p<0,0001$) e diminuindo a atividade de lisozima sérica em peixes alimentados com 15 g kg^{-1} ($p<0,0001$). Em conclusão, os autores recomendam a inclusão de 1 g kg^{-1} de MOS na dieta para tilápias do Nilo juvenis devido ao efeito positivo sobre o sistema imune.

Palavras-chave: hematologia; imunologia; prebiótico; *Oreochromis niloticus*; parâmetros produtivos.

INTRODUCTION

In 2018, the world aquaculture produced 82 million tonnes of fish, the equivalent to USD 250 billion (FAO, 2020), and one of the major agents of such successful activity are the fish from the tilapia group. Reared in more than 130 countries (FAO, 2016) and with a world production of 5.5 million tonnes in 2018, the tilapia corresponded to 10% of all finfish globally produced in the same year (FAO, 2020). Moreover, tilapia culture has been considered strategic for the aquaculture development of several countries, such

as Brazil, the 8th largest world inland aquaculture producer (FAO, 2020), of wich 60% of its production is represent by Nile tilapia *Oreochromis niloticus* (IBGE, 2018). However, concomitantly with such expansion of the tilapia culture, the number and intensity of diseases outbreaks are raised due to the stress caused by the increase of stock densities, handling and the use of artificial feeds (Mauel et al., 2007; Mian et al., 2009; Iwama et al., 2011), leading to the use of antibiotics such as oxytetracycline and florfenicol.

Alternatives to replace the use of antibiotics as prophylaxis and to avoid their side effects, such as toxicity and bacterial resistance, have been developed for several fish species, and one of the most prominent strategies is the use of prebiotics (Pohlenz and Gatlin, 2014; Song et al., 2014), such as fructooligossaccharides, arabinoxylan-oligosaccharide and the mannan-oligosaccharide (MOS). MOS, commonly isolated from *Saccharomyces cerevisiae*, is a glycoprotein able to modulate fish intestinal microbiota by promoting the colonization of beneficial bacterial, such as lactic acid bacteria (LAB), which are able to use mannose as substrate, and eliminating some pathogenic strains, such as *Aeromonas* sp. and *Pseudomonas* sp. (Gómez and Balcázar, 2008; Levy-Pereira et al., 2018). Moreover, the mannose present in MOS particles can be recognized by the innate immune system through the mannose binding lectin (MBL), immunomodulating several immune responses (Torrecillas et al., 2014). Dietary use of MOS has been reported enhancing innate immune parameters in fish, such as white blood cell numbers, phagocytosis, lysozyme and reactive oxygen species (ROS) (Song et al., 2014), and also increasing growth and survival in *Dicentrarchus labrax* (Torrecillas et al., 2007; 2011), *Oncorhynchus mykiss* (Staykov et al., 2007), *Labeo rohita* (Andrews et al., 2009) and *Piaractus mesopotamicus* (Soares et al., 2018). However, according to the consulted literature, the level of inclusion of MOS in fish diets vary due to the species and few are the studies presenting substantial results in health of Nile tilapia (Sado et al., 2008; Selim and Reda, 2015; Levy-Pereira et al., 2018).

Thus, the present study aimed to evaluate the effects of MOS on Nile tilapia ROS production, serum lysozyme, hematology and productivity parameters.

MATERIAL AND METHODS

Fish and feeds

The experiment was conducted in Centro de Aquicultura da UNESP (CAUNESP), in Jaboticabal, São Paulo state, Brazil (21°15′17”S, 48°19′20”W). Four experimental diets were prepared according to NRC (1977) for juvenile Nile tilapias (Table 1). Active MOS (Biorigin®, Lençóis Paulistas, Brazil) was added to the diets at zero (control), 1, 8, and 15 g kg^{-1}. In order to maintain the same levels of crude energy (3000 kcal kg^{-1}) and protein (30%), an inert material (Kaolin) was complementary added to the diets in 15 g kg^{-1} to control, 14 g kg^{-1} to the diet containing 1 g kg^{-1} of MOS, 7 g kg^{-1} to the diet containing 8 g kg^{-1} of MOS, and no kaolin in the diet containing 15 g kg^{-1} of MOS. All the ingredients were grounded (0.1 mm) and mixed three times. The resultant mixture was extruded into 1.0 mm pellets (Ex Micro, Exteec, Brazil), dried in forced ventilation chamber at 25°C and stored at -20°C. The centesimal composition of diet was bromatologically determined and is also presented in Table 1.

Masculinized Nile tilapia juvenile, GIFT strain, were bought from a commercial farm and fed with commercial diets (Crude Protein 32% and Digestible Energy 3000 kcal kg^{-1}) during 30 days, until the beginning of the acclimation period, characterizing the grow-out period. In the acclimation period, all fish were fed with the control diet during 30 days and, after this, they were weighed, measured and randomly distributed in 12 310-L fiberglass tanks with continuous aeration and water flow, totalizing 14 fish (101.42 ± 2.71 g) per tank (a total of 168 fish). During the grow-out and acclimation periods, the fish were fed four times per day (9:00 h, 12:00 h, 14:00 h and 16:00 h) and twice a day (9:00 h and 15:00 h), respectively, until apparent satiation.

Prior to the beginning of the experiment, the tanks were divided in four groups, of which three of them were fed with the diets enriched with different concentrations of MOS and the fourth

Table 1. Formulation and centesimal composition of the experimental diets.

Experimental diets	Treatments (MOS)			
	Control	1 g kg^{-1}	8 g kg^{-1}	15 g kg^{-1}
Formulation (%)				
Fish meal	14.7	14.7	14.7	14.7
Soybean meal	35.6	35.6	35.6	35.6
Corn meal	19	19	19	19
Wheat meal	15.08	15.08	15.08	15.08
Rice meal	9	9	9	9
Soybean oil	2.3	2.3	2.3	2.3
Antioxidant	0.02	0.02	0.02	0.02
Dicalcium	1	1	1	1
Limestone	0.7	0.7	0.7	0.7
Minerals and Vitamins[1]	0.5	0.5	0.5	0.5
Antifungal	0.1	0.1	0.1	0.1
DL-methionine	0.5	0.5	0.5	0.5
Kaolin[2]	1.5	1.4	0.7	0
ActiveMOS®	0	0.1	0.8	1.5
Composition (%)				
Moisture	8.3	6.9	7.4	7.2
Fat	5.75	5.75	5.5	6.75
Crude protein	29.99	31.03	31.37	31.54
Crude fiber	4	4.4	4.4	5.6
Ash	8.5	10	10.5	8.5

MOS - mannan-oligosaccharide. [1]Minerals and vitamins: calcium, 10-30 g; phosphorus, 6000 mg; magnesium, 31.25 mg; zinc, 100 mg; copper, 25 mg; cobalt, 0.6 mg; iodine, 1.25 mg; selenium, 0.25 mg; conlin, 800 mg; folic acid, 5.4 mg; niacin, 112.5 mg; biotin, 0.58 mg; pantothenic acid, 36 mg; vitamin A, 9000 IU; vitamin B1, 20.25 mg; vitamin B12, 22.25 mg; vitamin B2, 20.25 mg; vitamin B6, 20.25 mg; vitamin C, 300 mg; vitamin D3, 3150 IU; vitamin E, 135 IU; vitamin K3, 9 mg; inositol 80, mg. [2]The inert ingredient kaolin was gradually substituted by the ActiveMOS® inclusion levels.

was fed with the control diet. During the experiment, every day, 100 g of feed was weighed and stored in plastic containers, one for each tank. During the experimental period, the fish were fed twice per day (at 9:00 h and 16:00 h), until the apparent satiation, and the total feed consumption was determined by the difference of weight of the containers. The experiment was carried out for 60 days.

The pH, dissolved oxygen, and temperature of water were measured weekly, using a multiparameter probe (Horiba U-52G, Japan). Throughout the experiment, the water parameters were maintained at pH of 7.63 ± 0.15, dissolved oxygen of 4.14 ± 1.04 mg L^{-1} and temperature of 28.48 ± 0.89°C, ranges considered acceptable for Nile tilapia rearing (Ibrahim and El Naggar, 2010) with no disease outbreaks recorded. All tanks were cleaned once a day, at 16:30 h.

Research on animals was conducted according to the institutional committee on animal use (no. 006792/12, Universidade Estadual Paulista "Julio de Mesquita Filho", Jaboticabal, SP, Brazil

Blood sampling, hematology, ROS production and serum lysozyme

After 60 days, five fish form each tank were randomly caught with a net (15 per treatment), anesthetized in clove oil solution (0.1 mL L^{-1} of water), and had the blood sampled through the caudal vessel using a non-heparinized syringe. Before the experiment (day zero), 15 fish were sampled and treated as basal group.

A 200-µL blood aliquot was transferred to a microtube (2 mL) containing 15 µL of anticoagulant (0.65% NaCl, sodium heparin 100 IU mL^{-1}). The remaining blood was transferred to 5 mL glass tubes and incubated for 45 min at room temperature to obtain the serum, which was collected using a micropipette and maintained at -20°C for subsequent analysis.

For hematology, 100 µL of heparinized blood was used. The hematocrit (Ht) was determined by the microhematocrit method (Goldenfarb et al., 1971), the hemoglobin (Hb) by the cyanmethemoglobin method (Collier, 1944) and the red blood cells (RBC) were counted in a Neubauer chamber. The hematimetric equations of Wintrobe (1934) were used to determine mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Blood smears were prepared with a drop of blood without anticoagulant, air-dried, stained with May Grünwald-Gyemsa-Writh (Tavares-Dias and de Moraes, 2003) and used for the determination of the total counts of thrombocytes and leukocytes and differential leukocytes count (Hrubec and Smith, 2000).

The reactive oxygen species (ROS) production was measured using the NBT assay according to Anderson and Siwicki (1995) modified by Biller-Takahashi et al. (2013). For this, 100 µL of heparinized blood was added to 100 µL of phosphate-buffered-saline (pH 8.4) containing 2% of nitrobluetetrazolium (NBT – Sigma, USA) in plastic tubes of 2 mL. After 30 minutes of incubation in the dark, 50 µL of this solution was added to 1 mL of N, N-dimethylformamide (Sigma, USA), shaken, and centrifuged at 700 g for 10 minutes. The optical density of the supernatant was measured in a spectrophotometer (540 nm).

Serum Lysozyme was then determined according to Abreu et al. (2009) with modifications. Hen egg white lysozyme (1 mg mL^{-1}) was used as standard. A calibration curve was made using a lysozyme working solution (1 ηg μL^{-1}) which was obtained by diluting the stock solution a hundred times with sodium phosphate buffer (PBS, NaH_2PO_4; 0.05 M; pH 6.2). After this, the fish serum had its complement inactivated by heat (water-bath at 56ºC for 30 minutes). 60 µL of serum was diluted in 40 µL of phosphate buffer solution in 96-well plates and the samples were incubated for 2 minutes in the spectrophotometer (Beckman DU-70S) at 26ºC. After this, 100 µL of PBS solution containing 1 µg μL^{-1} of *Micrococcus lysodeikticus* were added to the serum solution, completing a volume of 200 µL. A blank sample (200 µL of PBS) was used. The optical density (OD) was then measured after 0.5 and 10 min at 450 nm in spectrophotometer and the reduction of OD was divided by the sample volume and compared with the calibration curve to determine its lysozyme concentration in µg mL^{-1}.

Productive parameters

The fish were measured and weighed at day zero and 60. All the productive parameters were calculated according to the expressions shown below:

$$\text{Weight Gain (WG, g)} = \text{Final Weight} - \text{Initial Weight} \quad (1)$$

$$\text{Feed Conversion Ratio (FCR)} = \text{Feed Consumption} - \text{WG} \quad (2)$$

$$\text{Survival (\%)} = \text{Final Number} / \text{Initial Number} * 100 \quad (3)$$

Statistics

All data are presented as mean ± standard error of the mean. The statistical procedures were performed using the software R V 3.4.0. All data were checked for homoscedasticity and normality with Levene's test and Cramer-Von Mises' test, respectively. The hematological parameters were submitted to Kruskal-Wallis and the means were compared with Tukey's and Kramer's test (α = 5%). The NBT assay, serum lysozyme and productive parameters were submitted to ANOVA and the means were compared using Tukey's multiple range test (α = 5%).

RESULTS

The hematological parameters are expressed in Table 2. No significant differences were observed in Ht, MCH, thrombocytes, leukocytes, neutrophils, lymphocytes and basophils in all the experiment ($p>0.05$). No significant differences were observed among treatments or in relation to the control group at the end of the experiment, however, fish from the basal sample presented lower Hb ($p<0.0001$), MCHC ($p = 0.0008$), RBC ($p<0.0001$) and monocytes ($p<0.0001$) and higher MCV ($p<0.0001$) and immature leukocytes number ($p<0.0001$) then the other groups.

Table 2. Hematology of Nile tilapia juvenile before and after 60 days of mannan-oligosaccharide (MOS) feeding. Values are means ± standard error of the mean. The hematological parameters were submitted to Kruskal-Wallis and means followed by different letters showed significant difference according to the Tukey's and Kramer's test (α = 5%).

Parameters	Treatments (MOS)					
	Basal	Control	1 g kg^{-1}	8 g kg^{-1}	15 g kg^{-1}	p – value
Ht (%)	28.93±1.03	29.93±1.54	26.20±0.89	28.56±1.47	29.75±1.76	*0.2487*
Hb (g dL^{-1})	5.31±0.32^{b}	6.96±0.21^{a}	6.66±0.18^{a}	6.86±0.21^{a}	7.53±0.22^{a}	*< 0.0001*
MCV (fL)	167.66±7.40^{a}	126.47±10.23^{b}	111.36±4.72^{b}	110.26±6.80^{b}	117.84±8.94^{b}	*< 0.0001*
MCH (pg)	30.69±1.99	29.48±1.77	28.26±0.94	26.64±1.33	29.83±1.64	*0.2848*
MCHC (g dL^{-1})	18.66±3.87^{b}	24.12±3.87^{a}	25.66±3.87^{a}	24.70±3.87^{a}	26.20±3.87^{a}	*0.0008*
RBC (10^{6} µL^{-1})	1.761±0.074^{b}	2.452±0.131^{a}	2.381±0.079^{a}	2.643±0.111^{a}	2.421±0.053^{a}	*< 0.0001*
Thrombocytes (10^{3} µL^{-1})	2.459±0.257	3.248±0.391	3.341±0.611	4.354±0.444	3.995±0.615	*0.0602*
Leukocytes (10^{3} µL^{-1})	5.530±0.443	4.382±0.238	5.645±0.472	5.17±0.413	6.286±0.643	*0.1716*
Neutrophils (10^{3} µL^{-1})	0.096±0.022	0.099±0.021	0.084±0.022	0.125 ±0.033	0.054±0.025	*0.2161*
Monocytes (10^{3} µL^{-1})	0.109±0.025^{b}	0.43±0.047^{a}	0.537±0.078^{a}	0.462±0.062^{a}	0.447±0.084^{a}	*< 0.0001*
Lymphocytes (10^{3} µL^{-1})	5.194±0.453	3.843±0.207	4.995±0.441	4.564±0.421	5.749±0.592	*0.119*
Basophils (10^{3} µL^{-1})	0.000±0.000	0.012±0.017	0.011±0.023	0.024±0.039	0.025±0.037	*0.0865*
Immature (10^{3} µL^{-1})	0.130±0.036^{a}	0.011±0.006^{b}	0.018±0.014^{b}	0.002±0.002^{b}	0.010±0.006^{b}	*< 0.0001*

Ht = Hematocrit, Hb = hemoglobin, MCV = mean corpuscular volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, RBC = Red Blood Cells.

All fish fed with MOS presented higher ROS production than fish from basal group ($p<0.0001$). However, only fish fed with 1 g kg^{-1} presented a ROS production statistically higher than control ($p<0.0001$), with no significant difference among control fish and fish fed with 8 and 15 g kg^{-1} (Figure 1).

All fish presented higher serum lysozyme at day 60 when compared to fish from basal sample. However, the group fed with 15 g kg^{-1} presented a significant decrease on this parameter in comparison to control ($p<0.0001$, Figure 2).

Fish fed with 1 and 8 g kg^{-1} of MOS presented a lower feed consumption than control fish and fish fed with 15 g kg^{-1}, but this last group did not differ significantly from the others ($p = 0.0299$). Despite the decrease in consumption, no significant changes were observed in FCR, final weight, weight gain and survival ($p>0.05$, Table 3)

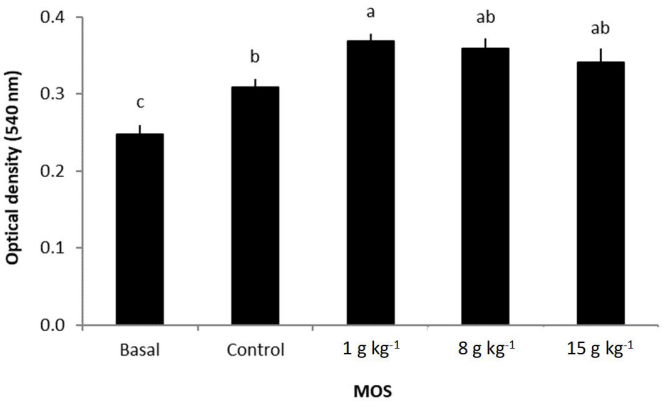

Figure 1. Reactive oxygen species (ROS) production of Nile tilapia juvenile before and after 60 days of mannan-oligosaccharide (MOS) feeding. Values are means ± standard error of the mean. ROS data was submitted to ANOVA and means followed by different letters showed significant difference according to the Tukey`s test (α = 5%).

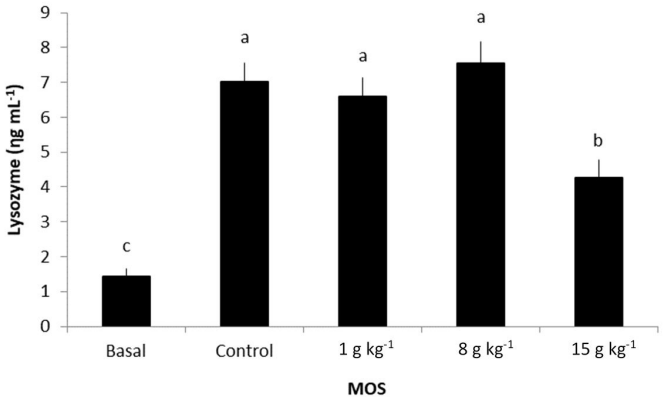

Figure 2. Serum lysozyme of Nile tilapia juvenile before and after 60 days of mannan oligosaccharide (MOS) feeding. Values are means ± standard error of the mean. Serum lysozyme data was submitted to ANOVA and means followed by different letters showed significant difference according to the Tukey`s test (α = 5%).

Table 3. Growth parameters of Nile tilapia juvenile after 60 days of mannan oligosaccharide (MOS) feeding. Values are means ± standard error of the mean. Productive parameters data was submitted to ANOVA and means followed by different letters showed significant difference according to Tukey's test (α = 5%).

Parameter	Treatments (MOS)				
	Control	1 g kg^{-1}	8 g kg^{-1}	15 g kg^{-1}	p - value
Initial weight (g)	104.5±1.4	99.4±0.8	100.±1.4	101.2±1.0	*0.0796*
Final Weight (g)	220.8±9.3	221.9±9.1	222.9±5.4	204.3±3.2	*0.2910*
Weight Gain (g)	116.2±8.2	122.4±8.6	122.5±6.8	103.1±3.3	*0.2520*
Consumption (g)	3572.3± 6.7[a]	3249.0±117.5[b]	3259.6±68.8[b]	3479.8±27.4[ab]	*0.0299*
Feed Conversion Ratio	1.34±0.06	1.15±0.11	1.18±0.12	1.31±0.05	*0.4400*
Survival (%)	93.3±1.9	94.4±2.9	93.3±1.9	98.8±1.1	*0.1910*

DISCUSSION

The investigation of hematological parameters is widely employed as a complementary method for evaluation of fish health. In this study, no changes were observed in hematological parameters due to the MOS feeding, which corroborates with the observed in *Huso huso* (Mansour et al., 2012), *O. niloticus* (Sado et al., 2008) and *Channa striata* (Talpur et al., 2014). However, in contrast, Levy-Pereira et al. (2018), feeding *O. niloticus* with the same levels of MOS of the present experiment during 45 days, observed increases in the number of leukocytes, monocytes and lymphocytes, and a decrease in neutrophils. In the present study, the differences in hematology came from the fish growth, since in 60 days, the final weight reached twofold the initial weight. Differences in hematology due to fish size were also reported in hybrid striped bass *Morone chrysops* × *Morone saxatilis* (Hrubec et al., 2001) and *Labrisomus philippii* (Guzmán and González, 2012), and are attributed to the increasing necessity of oxygen and energy by the body tissues and the maturation of the immune system.

In the same way, the growth increased the immunological parameters of fish, but in this case, both ROS production and serum lysozyme were affected by the MOS feeding. Phagocytes such as neutrophils and monocytes, under certain stimuli, produces H_2O_2 and O_2^-, known as ROS, in order to destroy engulfed particles or microorganisms (Babior, 1984; Hoidal, 2001; Quinn and Gauss, 2004). In this experiment, although no changes observed in the phagocytes number, the MOS feeding gave rise to the ROS production of all fish fed with the prebiotic, with a significant increase in fish fed with 1 g kg^{-1} in relation to the control group. This effect could have occurred due to two motives. The first is the ability of innate immune system to recognize mannose by mannose binding lectins, a soluble receptor able to activate the complement system which can lead to increase in ROS production for phagocytosis (Nakao et al., 2011). The second motive could be the increase the possible increase of lactic acid bacteria in fish gut, which can modulate the production of ROS and of several other substances related to host defense (Levy-Pereira et al., 2018). Our results contrast with Grisdale-Helland et al. (2008), that observed a decrease in ROS production feeding *Salmo salar* with 10 g kg^{-1} of MOS during 16 weeks, and with the usual idea that MOS is an exclusive anti-inflammatory compost as shown in humans (Bland et al., 2004; Radman et al., 2006).

Lysozyme is an important protein for fish innate immune response, responsible for the peptidoglycan hydrolysis present in the cell wall of gram-positive bacteria, activating the complement system and stimulating the phagocytic process (Ellis, 1999; Magnadóttir, 2006; Saurabh and Sahoo, 2008). In this experiment, it is possible to observe an increase in serum lysozyme of all fish after 60 days of experiment, with a negative effect of MOS feeding at 15 g kg^{-1}, what corroborates with findings of Grisdale-Helland et al. (2008). As well as in hematology, most of the difference in lysozyme between days 0 and 60 can be attributed to fish growth and maturation of the immune system (Guzmán and González, 2012). However, the decrease in lysozyme of fish fed with 15 g kg^{-1} of MOS was unexpected and contrasts with the observed in *Oncorhynchus mykiss* fed with 2 g kg^{-1} reared in net-cages (42 days) and reaceways (90 days) (Staykov et al., 2007) and in *Carassus aurata gibelio* fed with 1.5, 3.0 and 4.5 g kg^{-1} of MOS during 60 days (Akrami et al., 2012), that observed increases in the same parameter.

In the present experiment, no significant differences were observed in growth parameters due to MOS feeding. Similar results were observed by Staykov et al. (2007), with enhancements in final body weight and FCR of *Oncorhynchus mykiss* reared in net-cages and raceways, fed with 2g kg^{-1}. In the present experiment, it was possible to observe a significant decrease in feed consumption in the groups fed with 1 and 8 g kg^{-1} of MOS when compared to control, but not in the group fed with 15 g kg^{-1}.

Decreases in feed consumption was also observed in *Huso huso* fed with 4 g kg^{-1} of MOS, but not with 2g kg^{-1} (Mansour et al. 2012), and in *Piaractus mesopotamicus* fed with similar levels of MOS as those used in the present experiment (Sado et al., 2008). Olsen et al. (2001), feeding *Salvelinus alpinus* with inulin, also an oligosaccharide, observed an accumulation of this indigestible carbohydrate in the cell membrane of the enterocytes, which may explain the decrease in consumption observed in the present experiment. Moreover, although it was possible to observe a positive trend in FCR of fish fed with 1 and 8 g kg^{-1} of MOS, no significant differences were observed in this parameter, and this trend probably occurred due to the decrease of feed consumption.

CONCLUSION

The results of the present experiment suggest that the use of MOS in Nile tilapia feeding exerts beneficial effects on innate immunity. The authors recommend the inclusion of 1 g kg^{-1} of MOS due to the better immunological profile. However, in doses higher than the recommended, it could suppress some immune responses.

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