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Research Article

Hematology of juvenile pacu, *Piaractus mesopotamicus* (Holmberg, 1887) fed graded levels of mannan oligosaccharides (MOS)

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ABSTRACT. Intensification of aquaculture production systems exposes fish to numerous stressors, which may negatively affect their health. This study determined the effects of increasing levels of dietary mannan oligosaccharides (ActiveMOS®-Biorigin) on biochemical and hematological parameters of juvenile pacu, *Piaractus mesopotamicus*. Fish (44.04 ± 5.27 g) were randomly distributed into 24 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% MOS. Blood samples were collected at 42 and 63 trial. Red blood cell count (RBC) and total plasmatic protein were affected by dietary MOS levels (*P* < 0.05). Fish fed 1.0% dietary MOS presented higher neutrophils numbers when compared to fish fed control diet and fish fed 1.5% MOS for 42 days presented significant higher granulocytic cell numbers. During trial fish presented increased (*P* < 0.05) hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and plasmatic glucose concentrations and decreased (*P* < 0.05) RBC, hemoglobin concentration, mean corpuscular hemoglobin concentration, white blood cell and differential leukocyte count. Dietary MOS levels did not present prebiotic effects for pacu and did not minimize stress effects on hematological and biochemical parameters for the species.

Keywords: *Piaractus mesopotamicus*, mannan oligosaccharide, hematology, nutrition, prebiotic, fish.

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Hematología de juveniles de pacu, *Piaractus mesopotamicus* (Holmberg, 1887) alimentado con raciones suplidas con manan oligosacáridos (MOS)

RESUMEN. La intensificación de los sistemas de producción acuícola expone a los peces a numerosos factores estresantes que pueden afectar negativamente su salud. Este estudio determinó el efecto de niveles crecientes de manan oligosacáridos (ActiveMOS®-Biorigin) en la dieta respecto a parámetros bioquímicos y hematológicos de juveniles de pacu, *Piaractus mesopotamicus*. Los peces (44.04 ± 5.27 g) fueron distribuidos al azar en 24 tanques (500 L; 10 peces por tanque) y alimentados durante 63 días con una dieta comercial suplementada con 0,0; 0,2; 0,4; 0,6; 0,8; 1,0; 1,5 y 2,0% MOS. Las muestras de sangre fueron colectadas los días 42 y 63 del experimento. El recuento de glóbulos rojos (RBC) y la concentración de proteínas plasmáticas totales fueron afectados por los niveles de MOS en la dieta (*P* < 0.05). Peces alimentados con dieta suplementada con 1,0% MOS presentaron mayor número de neutrófilos en comparación a peces alimentados con la dieta control y peces suplementados con 1,5% de MOS presentaron alto número de células granulocíticas (SGC) a los 42 días del experimento. Durante el ensayo, los valores de hematocrito, volumen corpuscular promedio y hemoglobina corpuscular media incrementaron (*P* < 0,05) y las concentraciones de glucosa plasmática, hemoglobina, concentración promedio de hemoglobina, glóbulos blancos, RBC y el recuento diferencial de los leucocitos disminuyeron (*P* < 0,05). Los niveles dietéticos de MOS no presentaron efectos prebióticos en pacus y no minimizaron los efectos del estrés sobre los parámetros hematológicos y bioquímicos de la especie.

Palabras clave: *Piaractus mesopotamicus*, mannan oligosacáridos, hematología, nutrición, prebiótico, peces.

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INTRODUCTION

Intensification of aquaculture production systems expose fish to numerous stressors, such as poor water quality, crowding and handling, which may affect negatively their immune status and health (Davis et al., 2002). Impaired immune system of fish lead to increased disease susceptibility and limited economic performance of aquaculture systems (Gatesoupe, 1999; Sakai, 1999). Reduction or, preferably, complete banning antibiotics from the production cycle, favoring the use of immunostimulants for health management of farmed fish as environmentally friendly practice, deserve increasing attention of researchers and fish farmers alike (Kumari & Sahoo, 2006; Merrifield et al., 2010). Dietary prebiotics supplementation has been used in aquaculture in an attempt to increase growth, health, and stress resistance of farmed fish (Ringo et al., 2010; Gangluy et al., 2013).

Attention start being given to the use of these products in fish and shellfish culture, especially the mannooligosaccharide (MOS) derived from yeast Saccharomyces cerevisiae cell wall (Sang & Fotedar, 2010). Studies on the effect of dietary MOS supplementation on fish hematology usually involve immunological parameters and have been carried out for Mexican Gulf sturgeon Acipenser oxyrinchus desotoi (Pryor et al., 2003), rainbow trout Oncorhyncus mykiss (Staykov et al., 2007), red drum Sciaenops ocellatus (Zhou et al., 2010), Atlantic salmon Salmo salar (Refstie et al., 2010), channel catfish Ictalurus punctatus (Peterson et al., 2010), gilthead seabream Sparus aurata (Dimitroglou et al., 2010a), white seabream Diplodus argus (Dimitroglou et al., 2010b) and Gibel carp Carassius auratus gibelio (Akrami et al., 2012). Results obtained in the above works are contradictory.

The presence, number and proportion of circulating blood cells reflect the moment physiological status of fishes and studies on the effects of dietary MOS on hematology are still scarce, especially regarding pacu Piaractus mesopotamicus. For instance, the effect of dietary MOS supplementation on blood cells and biochemical profile were performed in Nile tilapia (Sado et al., 2008), channel catfish (Welker et al., 2007; Zhu et al., 2012) and kutum Rutilus frisii kutum (Yousefian et al., 2012). Pacu is one of the most economically important and produced characin in Brazil and intensification of the species’ production systems is the common trend (Jomori et al., 2005). In addition little is known on the effects of MOS on hematology of south American neotropical teleosts. Therefore, this trial was set up to evaluate effects of time and dose administration of dietary MOS upon hematological parameters of juvenile pacu.

MATERIALS AND METHODS

Experimental design and animals

Trial was set up in a closed water recirculation system, with supplemental aeration and emergency oxygenation systems. Water quality parameters, such as pH, dissolved oxygen, ammonia, conductivity and temperature were measured daily during trial. A 12 h light: 12 h dark photoperiod was maintained. Juvenile pacu (10.62 ± 0.64 cm; 44.04 ± 5.27 g) obtained from commercial hatchery were randomly assigned to 24, 500 L polyethylene tanks (10 fish per tank) and acclimatized to the experimental conditions for seven days. Trial was conducted during 63 days with eight treatments (0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0% of dietary MOS) and three replicates for each treatment (n = 3).

Experimental diets

A commercial fish feed (Table 1) was powdered and supplemented with 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0% of MOS (ActiveMOS-Biorigin®, Lençóis Paulista, SP, Brazil), granulated and stored under refrigeration (4ºC).

Table 1. Chemical composition of basal diet (dry matter basis).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Contents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.13</td>
</tr>
<tr>
<td>Crude protein</td>
<td>27.43</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>5.48</td>
</tr>
<tr>
<td>Crude fat</td>
<td>9.69</td>
</tr>
<tr>
<td>Dry matter</td>
<td>94.87</td>
</tr>
<tr>
<td>Ash</td>
<td>14.72</td>
</tr>
</tbody>
</table>

Vitamin and mineral supplementation per kg of feed (from Purina do Brasil Ind. Com. Ltd., SP, Brazil): Mg, 700 mg; Fe, 100 mg; Cu, 15 mg; Zn, 200 mg; Mn, 30 mg; I, 1 mg; Se, 0.3 mg; vitamin A, 9,000 IU; vitamin D3, 3,000 IU; vitamin E, 112.0 IU; vitamin K, 7.50 IU; folic acid, 7.50 mg; biotin, 0.6 mg; choline, 500 mg; niacin, 112 mg; calcium pantothenic, 37 mg; thiamin, 22 mg; riboflavin, 22 mg; pyridoxine, 22 mg; vitamin B12, 26 µg; vitamin C, 150 mg.

Routine procedures

Fish were fed with experimental diets to apparent satiation twice daily (08:00 h and 17:00 h). At 42 and 63 days trial, three fish from each tank were anaesthetized with alcoholic (ethanol) solution of benzocaine (1:10000), and sampled for evaluation of hematological parameters.

Blood samples were drawn from the caudal vein using 3.0 mL sterilized syringes and 10% EDTA-
coated needles. Red blood cells (RBC) count was performed in Neubauer chamber using the Natt & Herrick (1952) diluent; hematocrit (Htc) evaluation followed the microhematocrit method of Goldenfarb et al. (1971); hemoglobin concentration (Hb) was performed following the cyanometahemoglobin method (Blaxhall & Daisley, 1973).

Hematometric indexes calculated were mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Wintrobe, 1934), as follows:

\[
MCV (L) = \frac{\text{Htc} \times 10}{\text{RBC}}
\]

\[
MCH (\mu g) = \frac{\text{Hb} \times 10}{\text{RBC}}
\]

\[
MCHC (\text{g dL}^{-1}) = \frac{\text{Hb} \times 10}{\text{Htc}}
\]

Total plasma protein concentrations were determined using a portable refractometer (WZ-301/Protein 0.0-12 g dL \(^{-1}\)) after blood centrifugation and plasma collection (Sado et al., 2008). Plasma glucose was determined by the enzymatic method using a standard kit (GLICOSE GOD-PAP Liquid Stable Mono Reagente, LABORLAB\textsuperscript{8}; Guarulhos, São Paulo, Brazil).

Blood smears from individual fish were stained with May-Grünwald-Giemsa (Rosenfeld, 1947) and examined under light microscopy using an oil immersion objective for differential leukocyte, white blood cell (WBC) and thrombocyte counts. White blood cell (WBC) and thrombocyte count were performed by indirect method (García et al., 2007; Sado et al., 2010) as follow:

\[
\text{WBC (µL}^{-1}) = \frac{\text{leukocytes number in blood smear x RBC}}{2000 \text{ erythrocytes counted in blood smear}}
\]

\[
\text{Thrombocytes (µL}^{-1}) = \frac{\text{thrombocytes number in blood smear x RBC}}{2000 \text{ erythrocytes counted in blood smear}}
\]

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

**RESULTS**

Water quality parameters such as pH (7.67 ± 0.28), dissolved oxygen (6.10 ± 0.77 mg L \(^{-1}\)), ammonia (≤ 0.5 mg L \(^{-1}\)), conductivity (-48.86 ± 8.4 mV) and temperature (28.7 ± 1.7°C) remained within acceptable values for the specie (Urbinati & Gonçalves, 2005).

Dietary MOS supplementation influenced some hematological parameters of pacu (Table 2). Total RBC and plasmatic protein were influenced \((P < 0.05)\) by MOS inclusion levels, but did not differ \((P > 0.05)\) from control diet. Fish fed diet containing 1.0% MOS showed higher neutrophil numbers \((P < 0.05)\) than fish fed with the control diet (Table 3). During trial, hematological and biochemical variables were significantly affected by experimental time, with the exception of thrombocyte number (Table 2). Values of hematocrit, MCV, MHC and plasma glucose concentration increased \((P < 0.05)\) along the feeding period (42 to 63 days). On the other hand, RBC, hemoglobin concentration, MCHC, WBC, and differential leukocyte count (lymphocytes, monocytes, neutrophils, eosinophils and specially granulocytic cells) decrease significantly along the feeding period (Table 4). Moreover, the number of special granulocytic cells (SGC) showed significant interaction, and fish fed 1.5% MOS supplementation for 42 days presented increased \((P < 0.05)\) SGC number (Fig. 1).

**DISCUSSION**

Dietary prebiotics supplementation aims to increase disease resistance and growth of farmed fish. However, contradictory results found in literature shows that the mode of action is still unclear regarding time, dose and form of administration, since it can cause negative effects to fish. In addition, the presence, number and proportion of circulating blood cells reflect the physiological status of fishes (Yousefian et al., 2012).

Research regarding fish hematology and dietary MOS supplementation are still scarce. For instance, Welker et al. (2007) did not register differences on hematological parameters of channel catfish fed 2.0 g MOS kg \(^{-1}\) diet, neither did Sado et al. (2008) for Nile tilapia fed increasing levels of dietary MOS. In the same way, 2% dietary yeast also did not affect hematological parameters in Nile tilapia (Hisano et al., 2007).

Fungi and yeast cell wall fragments does influence hematological and biochemical variables of fish (Jeney & Jeney, 2002; Wang & Chen, 2005; Misra et al., 2006a, 2006b). Dietary MOS affected \((P < 0.05)\) RBC of juvenile pacu, but results did not differ from fish fed the non-supplemented and were within normal values for this species (Ranzani-Paiva et al., 1998/1999; Tavares-Dias et al., 1999a, 1999b). In the same way, total plasmatic protein also showed
Table 2. Analysis of variance (ANOVA) for hematological parameters of juvenile pacu *P. mesopotamicus* fed graded levels of mannan oligosaccharides (MOS). *P*-values for MOS levels, sample collection period (42 and 63 days trial) and interaction (α = 0.05). RBC: red blood count, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood count, SGC: special granulocytic cell.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MOS levels</th>
<th>Time</th>
<th>Interaction MOS levels x Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10⁶ µL⁻¹)</td>
<td>0.016</td>
<td>&lt; 0.0001</td>
<td>0.285</td>
</tr>
<tr>
<td>Hemoglobin (g dL⁻¹)</td>
<td>0.235</td>
<td>0.046</td>
<td>0.121</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>0.089</td>
<td>&lt; 0.0001</td>
<td>0.277</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>0.119</td>
<td>&lt; 0.0001</td>
<td>0.861</td>
</tr>
<tr>
<td>MCH (µg cell⁻¹)</td>
<td>0.522</td>
<td>0.050</td>
<td>0.857</td>
</tr>
<tr>
<td>MCHC (g dL⁻¹)</td>
<td>0.547</td>
<td>0.0004</td>
<td>0.120</td>
</tr>
<tr>
<td>Total protein (g dL⁻¹)</td>
<td>0.040</td>
<td>&lt; 0.0001</td>
<td>0.773</td>
</tr>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>0.264</td>
<td>&lt; 0.0001</td>
<td>0.380</td>
</tr>
<tr>
<td>Trombocytes (µL⁻¹)</td>
<td>0.768</td>
<td>0.654</td>
<td>0.291</td>
</tr>
<tr>
<td>WBC (µL⁻¹)</td>
<td>0.198</td>
<td>&lt; 0.0001</td>
<td>0.520</td>
</tr>
<tr>
<td>Lymphocytes (µL⁻¹)</td>
<td>0.069</td>
<td>&lt; 0.0001</td>
<td>0.620</td>
</tr>
<tr>
<td>Monocytes (µL⁻¹)</td>
<td>0.197</td>
<td>&lt; 0.039</td>
<td>0.753</td>
</tr>
<tr>
<td>Neutrophils (µL⁻¹)</td>
<td>0.001</td>
<td>&lt; 0.0001</td>
<td>0.503</td>
</tr>
<tr>
<td>Eosinophils (µL⁻¹)</td>
<td>0.097</td>
<td>&lt; 0.0001</td>
<td>0.363</td>
</tr>
<tr>
<td>SGC (µL⁻¹)</td>
<td>0.747</td>
<td>&lt; 0.0001</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Table 3. Hematological parameters (µ ± SD) of juvenile pacu *P. mesopotamicus* fed graded levels of mannan oligosaccharides. SD: standard deviation. RBC: red blood count, *Mannan oligosaccharides – ActiveMOS®* (Biorigin, Lençóis Paulista, SP, Brazil).

<table>
<thead>
<tr>
<th>MOS* %</th>
<th>RBC x 10⁶ (µL⁻¹)</th>
<th>Total plasmatic protein (g dL⁻¹)</th>
<th>Neutrophil number (µL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.75 ± 0.12ab</td>
<td>5.85 ± 0.29ab</td>
<td>606.4 ± 252.5b</td>
</tr>
<tr>
<td>0.2</td>
<td>1.87 ± 0.25ab</td>
<td>5.92 ± 0.15a</td>
<td>749.5 ± 636.5b</td>
</tr>
<tr>
<td>0.4</td>
<td>1.71 ± 0.09b</td>
<td>5.83 ± 0.21ab</td>
<td>601.0 ± 412.7b</td>
</tr>
<tr>
<td>0.6</td>
<td>1.89 ± 0.20ab</td>
<td>5.67 ± 0.28b</td>
<td>909.7 ± 540.0ab</td>
</tr>
<tr>
<td>0.8</td>
<td>1.96 ± 0.22a</td>
<td>5.76 ± 0.20ab</td>
<td>816.5 ± 434.1ab</td>
</tr>
<tr>
<td>1.0</td>
<td>1.83 ± 0.39ab</td>
<td>5.63 ± 0.20b</td>
<td>1308.6 ± 786.7a</td>
</tr>
<tr>
<td>1.5</td>
<td>1.92 ± 0.19ab</td>
<td>5.75 ± 0.34ab</td>
<td>1057.4 ± 579.7ab</td>
</tr>
<tr>
<td>2.0</td>
<td>1.85 ± 0.18ab</td>
<td>5.62 ± 0.29b</td>
<td>831.1 ± 556.7ab</td>
</tr>
</tbody>
</table>

Different superscripts to values at same columns denote differences by *t* test (α = 0.05).

significant effect by dietary MOS levels. Increase in total plasmatic protein was observed in rainbow trout fed prebiotics for seven days (Siwick *et al.*, 1994), *Labeo rohita* fed for 28 and 42 days (Misra *et al.*, 2006a) or intraperitoneal injection (Misra *et al.*, 2006b) and kutum fries fed 3% dietary commercial prebiotic for 56 days (Yousefian *et al.*, 2012) indicating better immunological status in fish, since lysozyme and complement factors represents a fraction of total plasmatic protein and its values were elevated in fish fed or prebiotic injected.

Fish immune system can recognizes foreign substances (*i.e.*, prebiotics) through receptors that identify molecular patterns, which are characteristic of microbes (polysaccharides, lipopoly-saccharide, peptidoglycans, bacterial DNA and double stranded viral RNA), and not ordinarily found on the surface of multicellular organisms (Magnadóttir, 2006), that stimulates fish leukocytes to produce lysozyme and other antimicrobial peptides.

However, immunological analyses were not carried out in the present trial that could explain increased
Table 4. Hematological parameters (µ ± SD) of juvenile pacu *P. mesopotamicus* fed graded levels of mannan oligosaccharides at 42 and 63 trial. SD: standard deviation. RBC: red blood count, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood count, SGC: special granulocytic cell.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Experimental time (days)</th>
<th>42</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10⁶ µL⁻¹)</td>
<td></td>
<td>1.99 ± 0.25ᵃ</td>
<td>1.67 ± 0.11ᵇ</td>
</tr>
<tr>
<td>Hemoglobin (g dL⁻¹)</td>
<td></td>
<td>9.8 ± 1.7ᵃ</td>
<td>9.0 ± 0.4ᵇ</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td>33.5 ± 1.6ᵃ</td>
<td>36.0 ± 1.4ᵇ</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td></td>
<td>172.0 ± 23.6ᵃ</td>
<td>218.3 ± 15.0ᵇ</td>
</tr>
<tr>
<td>MCH (pg cell⁻¹)</td>
<td></td>
<td>50.1 ± 9.3ᵃ</td>
<td>54.9 ± 4.9ᵇ</td>
</tr>
<tr>
<td>MCHC (g dL⁻¹)</td>
<td></td>
<td>29.6 ± 5.9ᵃ</td>
<td>25.1 ± 1.1ᵇ</td>
</tr>
<tr>
<td>Total protein (g dL⁻¹)</td>
<td></td>
<td>5.5 ± 0.2ᵃ</td>
<td>5.8 ± 0.2ᵇ</td>
</tr>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td></td>
<td>51.7 ± 7.2ᵃ</td>
<td>75.5 ± 9.4ᵇ</td>
</tr>
<tr>
<td>WBC (µL⁻¹)</td>
<td></td>
<td>18657 ± 5221ᵃ</td>
<td>7367 ± 2336ᵇ</td>
</tr>
<tr>
<td>Lymphocytes (µL⁻¹)</td>
<td></td>
<td>14988 ± 3840ᵃ</td>
<td>5311 ± 2169ᵇ</td>
</tr>
<tr>
<td>Monocytes (µL⁻¹)</td>
<td></td>
<td>1566 ± 595ᵃ</td>
<td>1198 ± 481ᵇ</td>
</tr>
<tr>
<td>Neutrophils (µL⁻¹)</td>
<td></td>
<td>817 ± 444ᵃ</td>
<td>407 ± 154ᵇ</td>
</tr>
<tr>
<td>Eosinophils (µL⁻¹)</td>
<td></td>
<td>658 ± 311ᵇ</td>
<td>242 ± 130ᵇ</td>
</tr>
<tr>
<td>SGC (µL⁻¹)</td>
<td></td>
<td>219 ± 202ᵇ</td>
<td>59 ± 56ᵇ</td>
</tr>
<tr>
<td>Trombocytes (µL⁻¹)</td>
<td></td>
<td>43854 ± 8960ᵇ</td>
<td>41139 ± 5412ᵇ</td>
</tr>
</tbody>
</table>

Different superscripts to values at the same line denote differences by *t* test (α = 0.05).

Figure 1. Special granulocytic cells (SGC) numbers (µ ± SD) of juvenile pacu *P. mesopotamicus* (three fish per treatment) fed graded levels of mannan oligosaccharides (MOS) at 42 days trial. Superscript symbol above column denote difference by *t* test (α = 0.05).

Values found in fish fed supplemented with 0.2% MOS, although it was not different (*P* > 0.05) from fish fed control diet.

In accordance to our results, Welker *et al.* (2007) and Sado *et al.* (2008) did not observed any differences in WBC for channel catfish respectively Nile tilapia and elevated leukocyte number in fish circulation and its rapidly turnover possibly confers better protection against several pathogenic microorganisms that considerably appears in higher concentrations in aquatic environment as compared to terrestrial environments. In this trial, the number of neutrophils were higher in fish fed 1.0% dietary MOS. This leukocyte type as well as the monocytes show phagocytic activity against microorganisms and foreign substances, as demonstrated for rainbow trout by Afonso *et al.* (1998). The use of hematological parameters and absolute hematimetric index and its variables to evaluate MOS effects are scarce. In fish, ectothermic animals, the influence of environmental and individual factors on hematological parameters can be noticeable.

During trial, blood analysis showed a profile similar to that observed in stressed fish in intensive hearing condition. Negative effects, like immunosupression, was described *in vivo* for gilthead seabream (Couso *et al.*, 2003) and *P. crocea* (Ai *et al.*, 2007) fed prebiotics and *in vitro* for *Psetta maxima* and *S. aurata* (Castro *et al.*, 1999). Decrease in total leukocyte numbers and also in differential leukocyte count was observed (Table 4). The possibility of MOS toxic effects was discarded, because differences (*P* > 0.05) between fish fed control diet and fish fed dietary prebiotics were not observed as opposed to that observed by Sealey *et al.* (2008) in fish fed glucans that presented impaired immunity. Even the use of anesthetic (benzoicaine) to minimize stress from sampling collection can stress fishes, a fact observed by Carneiro *et al.* (2002) using benzocaine in an
attempt to reduce transport stress in matrinxã, *Brycon cephalus*.

Hematocrit represents the percentage of red cells in blood and its increased values during trial can be related to stressor conditions (Barton, 2000) in association to immunological and biochemical parameters, such as plasma glucose levels (Abreu et al., 2009). Elevated plasmatic glucose was also observed after stress capture in pacu (Abreu et al., 2009), rainbow-trout (Wells & Pankhurst, 1999), Nile tilapia (Martins et al., 2004), goldfish *C. auratus* (Dror et al., 2006), sablefish *Anoploma fimbria* (Lopes et al., 2006). Fish (*L. rohita*) fed glucan supplemented diets and submitted to consecutive blood sampling, as well as the same in the present experiment did not show any differences in blood glucose concentration during trial (Misra et al., 2006a, 2006b) and this results are attributed to adequate managing during sampling and biometrics and not to prebiotic supplementation.

During trial, decreased WBC was observed, as well as for lymphocytes, neutrophils, monocytes, eosinophils and SGC. Decrease in leukocytes number can be harmful to fish, since granulocytes and mononuclear phagocytes have an important role in innate immune system and can, for instance, impaired responses to pathogens (Dalmo et al., 1997). Handling and sampling stress can ruled out as a causative agent of the variation in leukocyte numbers in the present study. Reduced lymphocyte numbers were described in stressed fishes (Pulsford et al., 1994; Martins et al., 2004) however, as opposed to our results, these authors observed an increase in neutrophils and leukocyte numbers. In fact, the contradictory results regarding neutrophil and leukocyte numbers occurs because the response depends on fish species, stress intensity, duration (Pickering et al., 1982; Tavares-Dias et al., 2001), nutritional condition (Pickering et al., 1982) and stressor (Davis & Schreck, 1997).

The special granulocytic cell increased in fish fed 1.5% MOS for 42 days. This leukocyte type have been named differently by several authors as type II granulocyte (Imagawa et al., 1989), type II neutrophil (Veiga et al., 2002), special granulocytic cell (Ranzani-Paiva et al., 1998/1999; Tavares-Dias et al., 1999b; Sado et al., 2008, 2010) or PAS-positive granular leukocyte (Tavares-Dias & Mataqueiro, 2004; Tavares-Dias et al., 2004). Its function is still unclear, but the presence of cytoplasmatic glycogen is related to energy source for phagocytosis, and positive reaction to myeloperoxidase are indicative of bactericidal and immunomodulatory properties (Veiga et al., 2002). Functions of fish eosinophils also are still unclear; presence of high number of eosinophils can be related to fish species or parasite infestation (Ranzani-Paiva et al., 1998/1999; Tavares-Dias & Mataqueiro, 2004).

Fish thrombocytes have homeostatic functions like mammal’s platelets, and also can participate in morbidity process (Stoskopf, 1993) and disease resistance (Kozinska et al., 1999). The presence of this cell in inflammatory exudates, substances involved in phagocytosis as acid phosphatase, glycogen and adherence capacity on *Aeromonas hydrophila* (Kozinska et al., 1999) show its effective participation in defense mechanisms.

Hematological parameters vary to great extent in stressful situations and depend on fish species, as reported for some neotropical fish such as tambaqui, *Colossoma macropomum* (Gomes et al., 2003) and pacu (Abreu et al., 2009). Hematological alterations by stressing factors can cause hemoconcentration or hemodilution.

During trial, it was observed hemodilution, since RBC and hemoglobin concentration decreased. This process occurs in stress conditions as a result of osmorregulatory disfunction (Urbinati & Carneiro, 2001) and it was also observed in *Scorpius violaceus* fish in hearing condition (Pankhurst et al., 1992), tambaquis *C. macropomum* (Tavares-Dias et al., 2001), and pacus *P. mesopotamicus* (Abreu et al., 2009). The mean corpuscular volume (MCV) represents the red blood cell volume, and it increased values during trail, suggest an hydration process, since the adrenalin, an catecholamine released in stress situation, causes *in vitro* erytrocyte tumefaction (Nikinmaa & Huestis, 1984) as consequence of intracellular sodium and chloride retention and water gain by osmosis process and increasing red blood cell volume (Raino et al., 1985). Decreased values of MCHC during trial were a consequence of decreased values in hemoglobin concentrations. Plasmatic protein can be related to some stress conditions (Pankhurst & Dedual, 1994), however, Wells & Pankhurst (1999) did not define any relationship between plasmatic protein and stress capture and density in rainbow trout.

The use of prebiotics as mannan oligosaccharides and its effects in fish hematology still needs further research for better explanation of contradictory results. According to Newman (2007) the complex carbohydrate structure in the cell wall of yeast, different strains and fermentation conditions, processing methods can all alter their function. In addition, depending on MOS concentration, administration period and population status (age, sex, gonadal maturation) (Pryor et al., 2003) different results can be obtained. Finally, fishes are ectothermic
animals and the influence of environmental and individual factors on hematological parameters can be noticeable. Notwithstanding, dietary mannan oligosaccharides did not presented positive effects on fish hematology since it is not capable to reduce the effects of handling and hearing stress in juvenile pacu.

REFERENCES


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