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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.

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% de MOS. Las muestras de sangre fueron colectadas los días 42 y 63 del experimento. El recuento de glóbulos rojo (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*, manan 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 mannan oligosaccharide (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 *Oncorhynchus 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).

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

Vitamin and mineral supplementation per kg of feed (from Purina do Brasil Ind. Com. Ltd., SP, Brazil): Mg, 700 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 D_3, 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 B_{12}, 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).

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

$$\text{MCV (L)} = \frac{\text{Htc x 10}}{\text{RBC}}$$

$$\text{MCH (}\rho\text{g)} = \frac{\text{Hb x 10}}{\text{RBC}}$$

$$\text{MCHC } \left(\text{g dL}^{-1}\right) = \frac{\text{Hb x 10}}{\text{Htc}}$$

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®; 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:

$$\text{WBC } \left(\mu\text{L}^{-1}\right) = \frac{\text{leukocytes number in blood smear x RBC}}{\text{2000 erythrocytes counted in blood smear}}$$

$$\text{Thrombocytes } \left(\mu\text{L}^{-1}\right) = \frac{\text{thrombocytes number in blood smear x RBC}}{\text{2000 erythrocytes counted in blood smear}}$$

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 ± 1.7ºC) 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 ($\alpha = 0.05$). 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.

Variable	*P*-values		
	MOS levels	Time	Interaction MOS levels x Time
RBC (10^6 μL^{-1})	0.016	< 0.0001	0.285
Hemoglobin (g dL^{-1})	0.235	0.046	0.121
Hematocrit (%)	0.089	< 0.0001	0.277
MCV (*f*l)	0.119	< 0.0001	0.861
MCH (pg $cell^{-1}$)	0.522	0.050	0.857
MCHC (g dL^{-1})	0.547	0.0004	0.120
Total protein (g dL^{-1})	0.044	< 0.0001	0.773
Glucose (mg dL^{-1})	0.264	< 0.0001	0.380
Trombocytes (μL^{-1})	0.768	0.654	0.291
WBC (μL^{-1})	0.198	< 0.0001	0.520
Lymphocytes (μL^{-1})	0.069	< 0.0001	0.620
Monocytes (μL^{-1})	0.197	< 0.039	0.753
Neutrophils (μL^{-1})	0.001	< 0.0001	0.503
Eosinophils (μL^{-1})	0.097	< 0.0001	0.363
SGC (μL^{-1})	0.747	<0.0001	0.010

Table 3. Hematological parameters ($\mu \pm$ 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).

MOS* %	Hematological parameters		
	RBC x 10^6 (μL^{-1})	Total plasmatic protein (g dL^{-1})	Neutrophil number (μL^{-1})
0.0	1.75 ± 0.12^{ab}	5.85 ± 0.29^{ab}	606.4 ± 252.5^{b}
0.2	1.87 ± 0.25^{ab}	5.92 ± 0.15^{a}	749.5 ± 636.5^{b}
0.4	1.71 ± 0.09^{b}	5.83 ± 0.21^{ab}	601.0 ± 412.7^{b}
0.6	1.89 ± 0.20^{ab}	5.67 ± 0.28^{b}	909.7 ± 540.0^{ab}
0.8	1.96 ± 0.22^{a}	5.76 ± 0.20^{ab}	816.5 ± 434.1^{ab}
1.0	1.83 ± 0.39^{ab}	5.63 ± 0.20^{b}	1308.6 ± 786.7^{a}
1.5	1.92 ± 0.19^{ab}	$5.75 \pm 0{,}34^{ab}$	1057.4 ± 579.7^{ab}
2.0	1.85 ± 0.18^{ab}	$5.62 \pm 0{,}29^{b}$	831.1 ± 556.7^{ab}

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

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, pepti-deglycans, 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.

Variables	Experimental time (days)	
	42	63
RBC (10^6 μL^{-1})	1.99 ± 0.25^a	1.67 ± 0.11^b
Hemoglobin (g dL^{-1})	9.8 ± 1.7^a	9.0 ± 0.4^b
Hematocrit (%)	33.5 ± 1.6^a	36.0 ± 1.4^b
MCV (fL)	172.0 ± 23.6^a	218.3 ± 15.0^b
MCH (pg $cell^{-1}$)	50.1 ± 9.3^a	54.9 ± 4.9^b
MCHC (g dL^{-1})	29.6 ± 5.9^a	25.1 ± 1.1^b
Total protein (g dL^{-1})	5.5 ± 0.2^a	5.8 ± 0.2^b
Glucose (mg dL^{-1})	51.7 ± 7.2^a	75.5 ± 9.4^b
WBC (μL^{-1})	18657 ± 5221^a	7367 ± 2336^b
Lymphocytes (μL^{-1})	14988 ± 3840^a	5311 ± 2169^b
Monocytes (μL^{-1})	1566 ± 595^a	1198 ± 481^b
Neutrophils (μL^{-1})	817 ± 444^a	407 ± 154^b
Eosinophils (μL^{-1})	658 ± 311^a	242 ± 130^b
SGC (μL^{-1})	219 ± 202^a	59 ± 56^b
Trombocytes (μL^{-1})	43854 ± 8960^b	41139 ± 5412^b

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

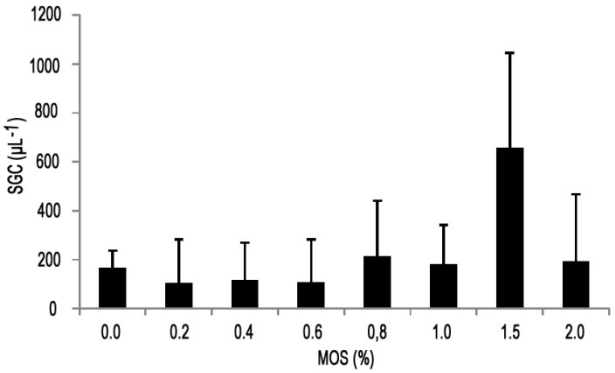

Figure 1. Special granulocytic cells (SGC) numbers (μ ± SD) of juvenile pacu *P. mesopotamicus* (three fish per treatment) fed graded levels of mannan oligosaccharides (MOS) at 42 days trial. Superscript symbol above column denote difference by *t* test (α = 0.05).

alues 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) and 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 immune-supression, 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 (benzocaine) 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* (Lupes *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 phosphatasis, 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 *Scorpis 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 (Railo *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

Abreu, J.S., L.S. Takahashi, M.A. Hoshiba & E.C. Urbinati. 2009. Biological indicators of stress in pacu (*Piaractus mesopotamicus*). Braz. J. Biol., 69: 415-421.

Ai, Q., K. Mai, L. Zhang, B. Tan, W. Zhang, W. Xu & H. Li. 2007. Effects of dietary β-1,3 glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea*. Fish Shellfish Immunol., 22: 394-402.

Afonso, A., S. Lousada, J. Silva, A.E. Ellis & M.T. Silva. 1998. Neutrophil and macrophage responses to inflammation in the peritoneal cavity of rainbow trout *Oncorhynchus mykiss*. A light and electron microscopic cytochemical study. Dis. Aquat. Organ., 34: 27-37.

Akrami, R., H. Chitaz, A. Hezarjarib & R. Ziaei. 2012. Effect of dietary mannan oligosaccharide (MOS) on growth performance and immune response of Gibel carp juveniles (*Carassius auratus gibelio*). J. Vet. Adv., 2(10): 507-513.

Barton, B.A. 2000. Stress. In: R.R. Stickney (ed.). Encyclopedia of aquaculture. Wiley, New York, pp. 892-898.

Blaxhall, P.C. & K.W. Daisley. 1973. Routine hematological methods for use with fish blood. J. Fish. Biol., 5: 771-781.

Carneiro, P.C.F., E.C. Urbinati & M.L. Martins. 2002. Transport with different benzocaine concentrations and its consequences on hematological parameters and gill parasite population of matrinxã *Brycon cephalus* (Günther, 1869) (Osteichthyes, Characidae). Acta Sci., 24(2): 555-560.

Castro, R., N. Couso, A. Obach & J. Lamas. 1999. Effect of different β-glucans on the respiratory burst of tuebot (*Psetta maxima*) and gilthead seabream (*Sparus aurata*) phagocytes. Fish Shellfish Immunol., 9: 529-541.

Couso, N., R. Castro, B. Magariños, A. Obach & J. Lamas. 2003. Effect of oral administration of glucans on the resistance of gilthead seabream to pasteurellosis. Aquaculture, 219: 99-109.

Dalmo, R.A., K. Ingebrigtsen & K. Bogwald. 1997. Non-specific defence mechanism in fish, with particular reference to the reticuloendothelial system (RES). J. Fish Dis., 20: 241-273.

Davis, E.L. & C.B. Schreck. 1997. The energetic response to handling stress in juvenile coho salmon. T. Am. Fish. Soc., 126: 248-258.

Davis, K.B., B.R. Griffin & W.L. Gray. 2002. Effect of handling stress on susceptibility of channel catfish *Ictalurus punctatus* to *Ichthyophthirius multifiliis* and channel catfish virus infection. Aquaculture, 214: 55-66.

Dimitroglou, A., D.L. Merrifield, P. Spring, J. Sweetman, R. Moate & S.J. Davies. 2010a. Effects of mannan oligosaccharides (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata).* Aquaculture, 300: 182-188.

Dimitroglou, A., S. Davis, J. Sweetman, P. Divanach & S. Chatzifotz. 2010b. Dietary supplementation of mannan oligosaccharide on white sea bream (*Diplodus sargus* L.) larvae: effects development, gut morphology and salinity tolerance. Aquacult. Res., 41: 245-251.

Dror, M., M.S. Sinyakov, E. Okun, M. Dym, B. Sredni & R.R. Avtalion. 2006. Experimental handling stress as infection-facilitating factor for the goldfish ulcerative disease. Vet. Immunol. Immunop., 109(3-5): 279-287.

Gangluy, S., K.C. Dora, S. Sarkar & S. Chowdhury. 2013. Supplementation of prebiotics in fish feed: a review. Rev. Fish. Biol. Fish., 23: 195-199.

Garcia, F., F. Pilarski, E.M. Onaka, F.R. Moraes & M.L. Martins. 2007. Hematology of *Piaractus mesopotamicus* fed diets supplemented with vitamins C and E, challenged by *Aeromonas hydrophila*. Aquaculture, 271: 39-46.

Gatesoupe, F.J. 1999. The use of probiotics in aquaculture. Aquaculture, 180: 147-165.

Goldenfarb, P.B., F.P. Bowyer, E. Hall & E. Brosius. 1971. Reproducibility in the hematology laboratory: the microhematocrit determination. Am. J. Clin. Pathol., 56: 35-39.

Gomes, L.C., C.A.R.M. Araújo-Lima, R. Roubach, A.R. Chippari, N.P. Lopes & E.C. Urbinati. 2003. Effect of fish density during transportation on stress and mortality of juvenile tambaqui *Colossoma macropomum*. J. World Aquacult. Soc., 34(1): 76-84.

Hisano, H., M.M. Barros & L.E. Pezzato. 2007. Levedura e zinco como pró-nutrientes em rações para tilápia do Nilo (*Oreochromis niloticus*): aspectos hematológicos. Bol. Inst. Pesca, 33(1): 35-42.

Imagawa, T., Y. Hashimoto, H. Kitagawa, Y. Kon, N. Kudo & M. Sugimura. 1989. Morphology of blood cells in carp (*Cyprinus carpio* L.). Japan. J. Vet. Sci., 51(6): 1163-1172.

Jeney, G. & Z. Jeney. 2002. Application of immunostimulants for modulation of the non-specific defense

mechanisms in sturgeon hybrid: *Acipenser ruthenus* x *A. baerii*. J. Appl. Ichthyol., 18: 416-419.

Jomori, R.K., D.J. Carneiro, M.I.E.G. Martins & M.C. Portella. 2005. Economic evaluation of *Piaractus mesopotamicus* juvenile production in different rearing systems. Aquaculture, 243: 175-183.

Kozinska, A., J. Antychowicz & P. Kostrzewa. 1999. Relationship between the trombocyte activity and the susceptibility of the carp (*Cyprinus carpio* L.) to *Aeromonas hydrophila* infection in different seasons. B. Vet. Inst. Pula., 43: 63-69.

Kumari, J. & P.K. Sahoo. 2006. Dietary β-1,3 glucan potentiates innate immunity and disease resistance of Asian catfish, *Clarias batrachus* (L.). J. Fish Dis., 29: 95-101.

Lupes, S.C., M.W. Davis, B.L. Olla & C.B. Schreck. 2006. Capture-related stressors impair immune system function in sablefish. T. Am. Fish. Soc., 135: 129-138.

Magnadóttir, B. 2006. Innate immunity of fish (overview). Fish Shellfish Immun., 20: 137-151.

Martins, M.L., D.T. Nomura, D.M.Y. Miyazaki, F. Pilarsky, K. Ribeiro, M.P. Castro & C.F.M. Campos. 2004. Physiological and hematological response of *Oreochromis niloticus* (Osteichthyes: Cichlidae) exposed to single and consecutive stress of capture. Acta Sci. Anim. Sci., 26: 449-456.

Merrifield, D.L., A. Dimitroglou, A. Foey, S.J. Davies, R.T.M. Baker, J. Bogwald, M. Castex & E. Ringo. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, 302: 1-18.

Misra, C.K., B.K. Das, S.C. Mukherjee & P. Pattnaik. 2006a. Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. Aquaculture, 255: 82-94.

Misra, C.K., B.K. Das, S.C. Mukherjee & P. Pattnaik. 2006b. Effect of multiple injections of β-glucan on no-specific immune response and disease resistance in *Labeo rohita* fingerlings. Fish Shellfish Immunol., 20: 305-319.

Natt, M.P. & C.A. Herrick. 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Sci., 31: 735-738.

Newman, K. 2007. Form follows function in picking MOS product. Feedstuffs, 79(4): 1-2.

Nikinmaa, M. & W.H. Huestis. 1984. Adrenergic swelling of nucleated erythrocytes: cellular mechanism in a bird, domestic goose, and two teleost, striped bass and rainbow trout. J. Exp. Biol., 113: 215-224.

Pankhurst, N.W. & M. Dedual. 1994. Effects of capture and recovery on plasma levels of cortisol, lactate, gonadal steroids in a natural population of rainbow trout. J. Fish Biol., 45: 1013-1025.

Pankhurst, N.W., R.M.G. Wells & J.F. Carracher. 1992. Effects of stress on plasma cortisol levels and blood viscosity in blue mao mao, *Scorpis violaceus* (Hutton), a marine teleost. Comp. Biochem. Physiol., 101A(2): 335-339.

Peterson, B.C., T.C. Bramble & B.B. Manning. 2010. Effects of Bio-MOS® on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. J. World Aquacult. Soc., 42(1): 149-155.

Pickering, A.D., T.G. Pottinger & P. Christie. 1982. Recovery of the trout, *Salmo trutta* L., from acute handling stress: A time-course study. J. Fish Biol., 24: 731-740.

Pryor, G.S., J.B. Royes, F.A. Chapman & R.D. Miles. 2003. Mannan oligosaccharides in fish nutrition: effects of dietary supplementation on growth and gastrointestinal villi structure in Gulf of Mexico sturgeon. N.A. J. Aquacult., 65: 106-111.

Pulsford, A.L., S. Lemaire-Gony, M. Tomlinson, N. Collingwood & P.J. Glynn. 1994. Effects of acute stress on the immune system of the dab, *Limanda limanda*. Comp. Biochem. Physiol., 109C(2): 129-139.

Ranzani-Paiva, M.J.T., F.A. Salles, J.C. Eiras, A.C. Eiras, C.M. Ishikawa & A.C. Alexandrino. 1998/1999. Análises hematológicas de curimbatá (*Prochilodus scrofa*), pacu (*Piaractus mesopotamicus*) e tambaqui (*Colossoma macropomum*) das estações de piscicultura do Instituto de Pesca, Estado de São Paulo. Bol. Inst. Pesca, 25: 77-83.

Railo, E., A. Nikinmaa & A. Soivo. 1985. Effects of sampling on blood parameters in the rainbow trout, *Salmo gardneri* Richardson. J. Fish Biol., 26: 725-732.

Refstie, S., G. Baeverfjord, R.R. Seim & O. Elvebo. 2010. Effects of dietary yeast cell wall β-glucan and MOS on performance, gut health and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. Aquaculture, 305: 109-116.

Ringo, E., R.E. Olsen, T.O. Gifstad, R.A. Dalmo, H. Amlund, G.I. Hemre & A.M. Bakke. 2010. Prebiotics in aquaculture: a review. Aquacult. Nutr., 16: 117-136.

Rosenfeld, G. 1947. Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só

corante de emprego rápido. Mem. Inst. Butantã, 20: 329-334.

Sado, R.Y., A.J.A. Bicudo & J.E.P. Cyrino. 2008. Feeding dietary mannan oligosaccharides to juvenile Nile tilapia, *Oreochromis niloticus* have no effect on hematological parameters and showed decreased feed consumption. J. World Aquacult. Soc., 39(6): 821-826.

Sado, R.Y., A.J.A. Bicudo & J.E.P. Cyrino. 2010. Dietary levamisol influenced hematological parameters of juvenile pacu *Piaractus mesopotamicus* (Holmberg 1887). J. World Aquacult. Soc., 41(S1): 66-75.

Sakai, M. 1999. Current research status of fish immunostimulants. Aquaculture, 172: 63-92.

Sang, H.M. & R. Fotedar. 2010. Effects of mannan oligosaccharide dietary supplementation on performances of the tropical spiny lobster juvenile (*Panulirus ornatus*). Fish Shellfish Immunol., 28: 483-489.

Sealey, W.M., F.T. Barrows, A. Hang, K.A. Johansen, K. Overturf & S.E. LaPatra. 2008. Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*. Anim. Feed Sci. Tech., 141: 115-128.

Siwick, A.K., D.P. Anderson & G.L. Rumsey. 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Vet. Immunol. Immunop., 41: 125-139.

Staykov, Y., P. Spring, S. Denev & J. Sweetman. 2007. Effect of mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquacult. Int., 15: 153-161.

Steel, R.G.D. & J.H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York, 569 pp.

Stoskopf, M.K. 1993. Fish medicine. W.B. Saunders Company, Philadelphia, 882 pp.

Tavares-Dias, M. & M.I. Mataqueiro. 2004. Características hematológicas, bioquímicas e biométricas de *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae) oriundos de cultivo intensivo. Acta Sci. Biol. Sci., 26(2): 157-162.

Tavares-Dias, M., R.A. Tenani, L.D. Gioli & C.D. Faustino. 1999b. Características hematológicas de teleósteos brasileiros. II. Parâmetros sangüíneos do *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae) em policultivo intensivo. Rev. Bras. Zool., 16: 423-431.

Tavares-Dias, M., E.F.S. Sandrim, F.R. Moraes & P.C.F. Carneiro. 2001. Physiological responses of "tambaqui" *Colossoma macropomum* (Characidae) to acute stress. Bol. Inst. Pesca., 27(1): 43-48.

Tavares-Dias, M., F.R. Bozzo, E.F.S. Sandrin, E. Campos-Filho & F.R. Mores. 2004. Células sangüíneas, eletrólitos séricos, relação hepato e esplenossomática de carpa-comum, *Cyprinus carpio* (Cyprinidae) na primeira maturação gonadal. Acta Sci. Biol. Sci., 26(1): 73-80.

Tavares-Dias, M., S.H.C. Schalch, M.L. Martins, E.D. Silva, F.R. Moraes & D. Perrecin. 1999a. Hematologia de teleósteos brasileiros com infecção parasitária. I. Variáveis do *Leporinus macrocephalus* Garavelo e Britski, 1988 (Anostomidae) e *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes, Characidae). Acta Sci., 21(2): 337-342.

Urbinati, E.C. & P.F.C. Carneiro. 2001. Metabolic and hormonal responses of matrinxã, *Brycon cephalus* (Teleost: Characidae) to transport stress under influence benzocaína. J. Aquacult. Trop., 16(1): 75-85.

Urbinati, E.C. & F.D. Gonçalves. 2005. Pacu (*Piaractus mesopotamicus*). In: B. Baldisserotto & L.C. Gomes. (eds.). Espécies nativas para a piscicultura no Brasil. Editora da UFSM, Santa Maria, 10: 225-255.

Veiga, M.L., M.I. Egami, M.J.T. Ranzani-Paiva & E.L. Rodriguez. 2002. Aspectos morfológicos y citoquímicos de las células sanguíneas de *Salminus maxilosus* Valenciennes, 1840 (Characiformes, Characidae). Rev. Chil. Anat., 18(2): 245-250.

Wang, S.H. & J.C. Chen. 2005. The protective effect of chitin and chitosan against *Vibrio alginolyticus* in white shrimp *Litopanaeus vannamei*. Fish Shellfish Immunol., 19: 191-204.

Welker, T.L., C. Lim, M. Yildirim-Aksoy, R. Shelby & P.H. Klesius. 2007. Immune response and resistance to stress and *Edwardsiella ictaluri*, challenge in channel catfish, *Ictalurus punctatus* fed diets containing commercial whole-cell yeast or yeast subcomponents. J. World Aquacult. Soc., 38(1): 24-35.

Wells, R.M. & N.W. Pankhurst. 1999. Evaluation of simple instrument for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. J. World Aquacult. Soc., 30(2): 276-283.

Wintrobe, M.M. 1934. Variations on the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Haematol., 51: 32-49.

Yousefian, M., M. Hedayatifard, S. Fahimi, M. Shikholeslami, M. Irani, C. Amirinia & S.E. Mousavi. 2012. Effect of prebiotic supplementation on growth performance and serum biochemical

parameters of kutum (*Rutillus frisii kutum*) fries. Asian J. Anim. Vet. Adv., 7: 684-692.

Zhou, Q.C., J.A. Buentello & D.M. Gatlin. 2010. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture, 309: 253-257.

Zuh, H., H. Liu, J. Yan, R. Wang & L. Liu. 2012. Effect of yeast polysaccharide on some hematologic parameter and gut morphology in channel catfish (*Ictalurus puntactus*). Fish Physiol. Biochem., 38: 1441-1447.

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