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Growth and hematological and immunological responses of *Arapaima gigas* fed diets supplemented with immunostimulant based on *Saccharomyces cerevisiae* and subjected to handling stress

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ABSTRACT

Saccharomyces cerevisiae is rich in bioactive ingredients and nutrients such as essential amino acids, peptides, cell wall carbohydrates, nucleotides and B vitamins. Diets with this immunostimulant product may offer improves in growth performance and immune of farmed fish. This study evaluated the effects of a commercial immunostimulant based on *S. cerevisiae* on growth performance, hematology and immunity of *Arapaima gigas* subjected to handling stress after 30 days of supplementation (0, 4, 6 and 8 g kg^{-1}). Diet with 6 and 8 g kg^{-1} of immunostimulant increased final biomass, final length, body weight gain, daily weight gain, daily feed intake, specific growth and decreased the cholesterol levels. After handling stress, supplemented fish with 4, 6 and 8 g kg^{-1} of immunostimulant had increase in glucose levels and decrease in globulin levels, while supplemented fish with 4 and 6 g kg^{-1} had decrease in cholesterol, and supplemented fish with 4 and 8 g kg^{-1} in globulin levels. Protein levels decreased in supplemented fish with 4 g kg^{-1}, while hematocrit decreased in all treatments and lymphocytes number and burst respiratory of leukocytes decreased in supplemented fish with 8 g kg^{-1} and submitted to handling stress. For *A. gigas*, since 6 to 8 g kg^{-1} of immunostimulant has not negative impacts on the hematological and biochemical parameters, thus such concentrations may be used as a growth promoter in *A. gigas*.

1. Introduction

Modern aquaculture involves high stocking densities and rearing practices, such as handling, which can cause stress in fish and have negative effects on its welfare, health and growth performance (Biller-Takahashi et al., 2014; Batista et al., 2016; Hisano et al., 2018). Thus, previous studies have demonstrated a significant improvement of growth performance and resistance to diseases in fish supplemented with feed additives such as immunostimulants (Bricknell and Dalmo, 2005; Amin et al., 2015; Ramos et al., 2015).

The immunostimulants offer an alternative to probiotics, which have the use limited in many countries due to concern with farmed animals because they may show a greater risk of contracting gastrointestinal diseases (De Baets et al., 2009). Thus, feed additives based on *Saccharomyces cerevisiae* or its co-products have been investigated for improving the growth performance and immunity in *Labeo rohita* (Tewary and Patra, 2011), *Huso huso* (Hoseinifar et al., 2011), *Oreochromis niloticus* (Amin et al., 2015; Manoppo et al., 2015), *Piaractus mesopotamicus* (Biller-Takahashi et al., 2014; Hisano et al., 2018), *Rutilus frisii* (Rufchaie and Hoseinifar, 2014), *Solea senegalensis* (Batista et al., 2016), *Oncorhynchus mykiss* (Huyben et al., 2017) and *Brycon amazonicus* (Montoya et al., 2018). Many of these studies showed that the dietary supplementation with immunostimulants based on *S. cerevisiae* improved growth parameters and immune system of the fish.

Saccharomyces cerevisiae contain glycoproteins, glucan (β-1,3-d-glucan and β-1,6-d-glucan branching), and small quantity of chitin, giving a favorable energetic and protein support compared to other food supplements (Amin et al., 2015). Thus, there is a growing interest

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of researchers and fish farmers on use of *S. cerevisiae* in commercials and non-commercial diets to mitigate the negative impact of intensive cultivation on welfare, health, immunity and growth performance of farmed fish (Batista et al., 2016; Dias et al., 2019) Therefore, diets contained immunostimulant based on *S. cerevisiae* might be tested in *Arapaima gigas*, known as pirarucu, the giant fish from the Amazon.

Arapaima gigas is an excellent fish species for Amazonian aquaculture due to its very fast growth rates, high market price, reproduction in captivity, tolerance to poor water quality, high flesh quality and fillet yield (< 45 %), that surpasses other species (Cavero et al., 2003; Oliveira et al., 2012). However, diseases are a primary restriction in the increase of aquaculture production of *A. gigas*, mainly fingerling stage (Lima et al., 2017). Thus, the aim of study was to evaluate the effects of dietary supplementation with a commercial immunostimulant based on *S. cerevisiae* on the growth performance and physiological and innate immunity parameters in *A. gigas*.

2. Materials and methods

2.1. Fish and experimental design

One hundred and forty-four *A. gigas* fingerlings were acquired in a commercial farming from the Rio Branco, Acre State (Brazil) and transported to the Laboratory of Aquaculture and Fisheries of Embrapa Amapá, in Amapá State (Brazil). Fish were acclimated in 1000 L tanks with aeration and continuous water flow and fed *ad libitum* four times day with a diet containing 45 % crude protein (Presence®, São Paulo, Brazil). The levels of dissolved oxygen (6.6 ± 0.3), temperature (29.3 ± 0.1) and pH (5.3 ± 0.3) were daily monitored using a multiparameter probe (Horiba Mod. U52, Kyoto, Japan).

After acclimatization period, fish (9.8 ± 1.4 g and 11.7 ± 0.5 cm) were distributed in 12 tanks of 100 L each using 3 replicates per treatment and 18 fish per tank. During 30 days, fish were fed with experimental diets, four times day (8:00, 11:00, 14:00 and 17:00), at 10 % of the biomass to satiation.

2.2. Preparation of experimental diets

The four experimental diets (45 % crude protein and 9% isolipidic) were formulated using extruded ration with 45 % crude protein (Presence®, São Paulo, Brazil) for carnivorous fish that were supplemented with Levabon® Aquagrow E (Biomin, Austria) at 0, 4, 6 or 8 g per kg, which are levels recommended by the manufacturer. Levabon® Aquagrow E is a commercial immunostimulant formulated from the standardized autolytic degradation and spray drying of *S. cerevisiae* cells. Each concentration of Levabon® Aquagrow E was diluted in 50 m L^{-1} of cereal alcohol and incorporated by spraying in the commercial diets, which were dried in the environment temperature for 24 h. The analyses of the chemical composition of the experiment diets (Table 1) were performed in triplicate, according to the Association of Official Analytical Chemists (AOAC, 1995) guidelines.

Table 1
Composition of diets with Levabon® Aquagrow E used in the supplementation of *Arapaima gigas*.

Parameters	Basal diet	Experimental diets
Crude Protein (%)	50.6 ± 0.9[a]	52.2 ± 2.6
Dry matter (%)	91.0 ± 0.03[a]	91.0 ± 0.2
Ether extract (%)	3.8 ± 0.2[a]	3.7 ± 0.2
Ashes (%)	14.5 ± 0.1[a]	14.1 ± 0.1
Phosphorus (%)	1.4 ± 0.04[a]	1.3 ± 0.07

Values express mean ± standard deviation. Composition of micronutrients in the basal diet:
Calcium = 2.0–3.0 % and Vitamin C: 1500 mg/kg, Crude fiber: 36 g/kg.

2.3. Growth parameters of the supplemented fish

After the feeding with the supplemented diets, 36 fish per treatment (3 replicates and 12 fish per replicate) were used to evaluate the growth performance parameters: Weight gain, daily weight gain, final biomass (kg/m^3), feed conversion ratio, daily feed intake (%BW/day), specific growth rate (see Dias et al., 2019) and relative condition factor (Le Cren, 1951). Fish were ten euthanized by medulla section for collect of organs for determination of hepatosomatic index (%) [Liver weight (g)/ body weight (g) x 100 and viscerosomatic index (%) [Viscera weight (g)/body weight (g) x 100].

2.4. Blood parameters and respiratory activity of leukocytes on supplemented fish

Prior to euthanasia, blood samples were immediately collected from 18 fish per treatment (3 replicates and 6 fish per replicate) by caudal vessel puncture using syringes containing sodium heparin (5000 UI mL^{-1}). The blood was divided into two aliquots: one first aliquot was used to determine the hematocrit using the microhematocrit method, total erythrocyte count in hemocytomer, and hemoglobin concentration by the cyanmethemoglobin method. With these data, the hematimetric indices of Wintrobe: mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined. Blood smears were also confectioned and stained panchromatically with the May Grünwald-Giemsa-Wright combination, for differential counting of leukocytes in up to 200 cells of interest (lymphocytes, monocytes, neutrophils and eosinophils), at each blood smear. The identification and classification of leukocytes followed the recommendations of Tavares-Dias et al. (2007). The blood smears were also used to determine the total number of leukocytes and thrombocytes (Ranzani-Paiva et al., 2013). Respiratory burst leukocytes was determined according to the methods previously described in literature (Sahoo et al., 2005; Biller-Takahashi et al., 2013). The absorbance readings were carried out in a spectrophotometer (Biospectro SP-220, Curitiba, Brazil).

The second blood aliquot was centrifuged at 75 G, for 5 min (Centrifuge Mod. 5424, Hamburg, Germany) for obtaining of plasma and to determine the glucose, albumin, total cholesterol and triglycerides levels using colorimetric kits (Biotécnica, MG, Brazil), for each propose, with readings in the spectrophotometer (Biospectro SP-220, Curitiba, Brazil)

2.5. Handling stress challenge in supplemented fish

After the feeding trial, 18 fish per treatment (3 replicates and 6 fish per replicate) were subjected to a handling stress. Fish were removed of tanks with a hand net and held in the air for 60 s, according method of Barton and Zitzow (1995) and Davis and Schreck (1997). After period of six hours of this handling stress, blood samples were collected for blood parameters and biochemical analyses as previously mentioned for the determination of hematocrit, hemoglobin concentration, and number of erythrocytes, thrombocytes, leukocytes, respiratory activity of leukocytes, plasma glucose, total proteins, albumin, cholesterol, triglycerides and globulins.

2.6. Statistical analyses

All data were evaluated on the assumptions of normality and homoscedasticity using Shapiro-Wilk and Bartlett, respectively. The data that showed a normal distribution were analyzed by variance (ANOVA - One Way), followed by the Tukey test, to compare the means, while the data that showed not a normal distribution were analyzed by Kruskal-Wallis, followed by the Dunn test to compare the medians (Zar, 2010).

Table 2
Corporal parameters and growth performance of *Arapaima gigas* supplemented with Levabon® Aquagrow E, during 30 days.

	Diets concentration			
Parameters	0 g kg^{-1}	4 g kg^{-1}	6 g kg^{-1}	8 g kg^{-1}
Initial length (cm)	11.7 ± 0.5[a]	11.5 ± 0.6[a]	11.5 ± 0.5[a]	11.7 ± 0.7[a]
Initial weight (g)	10.0 ± 1.2[a]	9.6 ± 1.4[a]	9.6 ± 1.2[a]	9.8 ± 1.4[a]
Final length (cm)	22.8 ± 1.4[a]	23.0 ± 1.4[a]	24.3 ± 1.0[b]	24.3 ± 0.8[b]
Final biomass (kg m^3)	9.7 ± 0.2[a]	10.1 ± 0.2[a,c]	10.9 ± 0.7[b,c]	11.3 ± 0.1[b]
Daily weight gain (g)	1.8 ± 0.4[a]	1.9 ± 0.4[a]	2.3 ± 0.3[b]	2.3 ± 0.3[b]
Body weight gain	54.5 ± 11.5[a]	57.9 ± 12.3[a]	65.5 ± 9.4[b]	65.8 ± 8.0[b]
Daily feed intake (g)	24.0 ± 0.001[a]	24.6 ± 0.003[a,b]	25.1 ± 0.1[a,b]	26.0 ± 0.001[b]
Feed conversion ratio	1.2 ± 0.1[a]	1.1. ± 0.05[a]	1.0 ± 0.02[a]	1.0 ± 0.01[a]
Specific growth rate	6.3 ± 0.8[a]	6.5 ± 0.8[a,b]	6.8 ± 0.3[b]	6.8 ± 0.3[b]
Daily feed intake (%BW/day)	1.4 ± 0.1[a]	1.2 ± 0.2[a]	1.3 ± 0.1[a]	1.3 ± 0.1[a]
Condition factor (Kn)	1.00 ± 0.02[a]	1.00 ± 0.01[a]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
HSI (%)	1.7 ± 0.3[a]	1.5 ± 0.2[a]	1.6 ± 0.4[a]	1.7 ± 0.3[a]
SVI (%)	9.2 ± 0.9[a]	8.5 ± 0.7[a]	8.7 ± 0.7[a]	8.9 ± 1.0[a]

Values express mean ± standard deviation. HSI: Hepatosomatic index, HSI: Viscerossomatic index SVI: Specific growth rate. Values followed by different letters on the same line, indicate differences between treatments by Tukey test ($p < 0.05$).

3. Results

At the end of 30 day of fed, the fish supplemented with 6 and 8 g kg^{-1} of Levabon® Aquagrow E showed increase ($p < 0.05$) in final biomass, final length, body weight gain, daily weight gain and specific growth compared to control fish. The remaining growth parameters did not show significant differences between the dietary treatments (Table 2).

Dietary supplementation of 4 and 8 g Levabon® Aquagrow E kg^{-1} diet decreased ($p < 0.05$) the glucose levels compared to control group and supplemented fish with 6 g, while 4, 6 and 8 g Levabon® Aquagrow E kg^{-1} diet decreased ($p < 0.05$) cholesterol levels. Dietary supplementation of 4 g increased ($p < 0.05$) the triglycerides levels compared to supplemented fish with 6 and 8 g kg^{-1}. Supplemented fish with 4 g had a decreases ($p < 0.05$) in hemoglobin concentration when compared to supplemented fish with 8 g, and in the erythrocytes number when compared to fed fish with 6 g kg^{-1} of Levabon® Aquagrow. The MCHC was higher ($p < 0.05$) in supplemented fish with 6 and 8 g than in control and fed fish with 4 g kg^{-1}. Moreover, neutrophils number decreased ($p < 0.05$) in fish supplemented with 4 g of Levabon® Aquagrow E kg^{-1} diet in comparison to other treatments. The respiratory burst of leukocytes increased ($p < 0.05$) and number of lymphocytes decreased ($p < 0.05$) in fish supplemented with 8 g kg^{-1} of Levabon® Aquagrow E when compared to other experimental groups. However, in all treatments the number of monocytes decreased ($p < 0.05$) when compared to control group (Table 3).

The handling stress did not cause fish mortality, regardless of the dietary treatment. After the handling stress, there was increase ($p < 0.05$) in plasma glucose levels and decrease ($p < 0.05$) ($p < 0.05$) in globulin levels in supplement fish with 4 and 8 g Levabon® Aquagrow E kg^{-1} diet when compared to controls and supplemented fish with 6 g kg^{-1}. Dietary supplementation with 4 and 6 g Levabon® Aquagrow E kg^{-1} caused a decreases ($p < 0.05$) in cholesterol levels compared to controls and supplemented fish with 8 g kg^{-1}. Dietary supplementation with 8 g caused decrease ($p < 0.05$) in triglycerides and albumin levels and respiratory burst, and increase ($p < 0.05$) in MCV, MCHC and lymphocytes number when compared to other groups. However, number of leukocytes, monocytes and neutrophils number increased ($p < 0.05$) in supplemented fish with 6 g Levabon® Aquagrow E kg^{-1} diet when compared to other groups. In supplemented fish with 4 g kg^{-1}, the plasma protein levels decresed ($p < 0.05$) when compared to other groups, while hemoglobin concentration increased ($p < 0.05$) when compared to control group and supplemented fish with 8 g of Levabon® Aquagrow E (Table 4).

4. Discussion

Increasing the survival and growth rates is one of the most

Table 3
Blood and immunological parameters of *Arapaima gigas* supplemented in the diet with Levabon® Aquagrow E, during 30 days.

	Diets composition			
Parameters	0 g kg^{-1}	4 g /kg^{-1}	6 g kg^{-1}	8 g kg^{-1}
Glucose (mg dL^{-1})	72.3 ± 7.7[a]	41.9 ± 13.4[b]	33.5 ± 17.2[b]	101.4 ± 27.7[a]
Total cholesterol (mg dL^{-1})	175.8 ± 41.6[a]	110.3 ± 32.6[b]	111.8 ± 35.4[b]	125.6 ± 32.3[b]
Triglycerides (mg dL^{-1})	90.6 ± 51.3[ab]	64.4 ± 31.1[b]	103.9 ± 19.0[ac]	120.2 ± 48.8[ac]
Albumin (g dL^{-1})	1.2 ± 0.3[a]	1.2 ± 0.6[a]	1.0 ± 0.5[a]	1.1 ± 0.4[a]
Hematocrit (%)	33.4 ± 2.5[a]	30.2 ± 3.0[a]	31.3 ± 7.4[a]	31.5 ± 4.2[a]
Hemoglobin (g dL^{-1})	8.8 ± 33.0[ab]	7.8 ± 0.7[b]	8.9 ± 1.8[ab]	9.4 ± 1.5[a]
Erythrocytes (x 10^6 μL^{-1})	1.86 ± 0.30[ab]	1.49 ± 0.40[b]	2.06 ± 0.70[a]	1.59 ± 0.70[ab]
MCV (fL^{-1})	184.6 ± 34.1[a]	224.7 ± 87.9[a]	162.7 ± 51.3[a]	247.9 ± 88.8[a]
MCHC (g dL^{-1})	26.4 ± 5.0[a]	26.0 ± 2.0[a]	29.2 ± 5.8[b]	29.5 ± 3.4[b]
Respiratory burst (OD)	0.22 ± 0.07[a]	0.22 ± 0.06[a]	0.19 ± 0.05[a]	0.39 ± 0.12[b]
Thrombocytes (x 10^3 μL^{-1})	27.3 ± 10.6[ab]	20.8 ± 6.8[a]	32.9 ± 12.5[b]	26.1 ± 12.6[ab]
Leukocytes (x 10^3 μL^{-1})	188.6 ± 34.1[ab]	151.1 ± 45.5[a]	210.2 ± 69.5[b]	162.6 ± 67.3[ab]
Lymphocytes (x 10^3 μL^{-1})	95.3 ± 20.1[a]	90.8 ± 27.7[a]	113.0 ± 34.3[a]	87.1 ± 38.7[b]
Monocytes (x 10^3 μL^{-1})	13.6 ± 4.5[a]	4.8 ± 2.5[b]	6.3 ± 4.3[b]	4.5 ± 2.8[b]
Neutrophils (x 10^3 μL^{-1})	78.7 ± 13.9[a]	54.3 ± 18.2[b]	89.5 ± 33.9[a]	69.4 ± 27.8[a]
Eosinophils (x 10^3 μL^{-1})	1.0 ± 1.0[a]	1.0 ± 1.3[a]	1.4 ± 1.6[a]	1.4 ± 1.7[a]

Values express mean ± standard deviation. MCV Mean corpuscular hemoglobin concentration, MCHC: Mean corpuscular hemoglobin concentration. Values followed by different letters on the same line, indicate differences between treatments by Dunn's test ($p < 0.05$).

Table 4
Blood and immunological parameters of *Arapaima gigas* submitted to handling stress challenge, after 30 days of supplementation with Levabon® Aquagrow E in the diet.

Parameters	Diets concentration			
	0 g kg^{-1} (n = 18)	4 g /kg^{-1} (n = 18)	6 g kg^{-1} (n = 18)	8 g kg^{-1} (n = 18)
Glucose (mg dL^{-1})	36.2 ± 7.3^{a}	73.2 ± 12.2^{b}	62.0 ± 21.9^{a}	112.7 ± 24.3^{c}
Total proteins (g dL^{-1})	3.2 ± 0.9^{a}	1.7 ± 0.4^{b}	3.4 ± 0.5^{a}	2.6 ± 0.6^{a}
Total cholesterol (mg dL^{-1})	137.2 ± 26.5^{a}	89.5 ± 36.5^{b}	87.7 ± 36.4^{b}	121.1 ± 31.7^{ac}
Triglycerides (mg dL^{-1})	125.6 ± 36.7^{a}	123.4 ± 15.6^{a}	116.7 ± 44.4^{ab}	100.0 ± 21.9^{b}
Albumin (g dL^{-1})	0.8 ± 0.3^{a}	0.8 ± 0.3^{a}	1.1 ± 0.3^{a}	1.8 ± 0.3^{b}
Globulin (g dL^{-1})	2.5 ± 0.9^{a}	0.9 ± 0.5^{b}	2.3 ± 0.4^{a}	0.8 ± 0.6^{b}
Hematocrit (%)	34.1 ± 3.1^{a}	29.7 ± 3.3^{b}	27.0 ± 4.1^{b}	30.4 ± 2.3^{b}
Hemoglobin (g dL^{-1})	8.3 ± 1.1^{a}	9.9 ± 1.1^{b}	9.2 ± 4.3^{ab}	9. 0 ± 1.1^{a}
Erythrocytes (x 10^{6} μL^{-1})	1.76 ± 0.26^{ab}	1.98 ± 0.48^{b}	1.81 ± 0.50^{b}	1.45 ± 0.37^{a}
MCV (fL^{-1})	197.6 ± 32.5^{b}	160.1 ± 51.1^{a}	56.1 ± 7.2^{a}	213.3 ± 59.3^{b}
MCHC (g dL^{-1})	24.6 ± 3.6^{a}	33.6 ± 2.9^{b}	33.3 ± 9.1^{b}	29.1 ± 3.6^{ab}
Respiratory burst (OD)	0.26 ± 0.06^{a}	0.25 ± 0.05^{a}	0.20 ± 0.04^{ab}	0.18 ± 0.04^{b}
Thrombocytes (x 10^{3} μL^{-1})	37.7 ± 12.5^{a}	29.7 ± 14.2^{a}	72.1 ± 28.7^{a}	25.2 ± 13.4^{a}
Leukocytes (x 10^{3} μL^{-1})	179.6 ± 27.2^{a}	200.6 ± 48.8^{ab}	478.6 ± 117.8^{b}	146.6 ± 38.2^{a}
Lymphocytes (x 10^{3} μL^{-1})	124.5 ± 18.1^{a}	124.9 ± 28.8^{a}	215.4 ± 58.6^{a}	80.2 ± 18.2^{b}
Monocytes (x 10^{3} μL^{-1})	6.3 ± 3.2^{a}	6.3 ± 3.5^{a}	23.9 ± 6.3^{b}	7.0 ± 3.6^{a}
Neutrophils (x 10^{3} μL^{-1})	47.8 ± 14.7^{a}	68.3 ± 25.0^{a}	236.9 ± 46.0^{b}	58.3 ± 20.7^{a}
Eosinophils (x 10^{3} μL^{-1})	0.9 ± 0.9^{a}	1.0 ± 0.6^{a}	2.3 ± 1.2^{a}	1.0 ± 1.2^{a}

Values express mean ± standard deviation. MCV Mean corpuscular hemoglobin concentration, MCHC: Mean corpuscular hemoglobin concentration. Values followed by different letters on the same line, indicate differences between treatments by Dunn's test ($p < 0.05$).

important issues that fish farmers face, especially in the initial stages of the fish's life cycle as the *A. gigas*, which suffer high mortality due to diseases (Lima et al., 2017; Dias et al., 2019). Thus, dietary products based on *S. cerevisiae* has been recommended for supplementation in fish due to its high proteins content, immunostimulant effects, and growth improvements (Hoseinifar etal., 2011; Tewary and Patra, 2011; Amin et al., 2015; Manoppo et al., 2015; Huyben et al., 2017; Dias et al., 2019). In current study, the experimental diets with Levabon® Aquagrow E had only a slight increase in crude protein levels when compared to basal diet, since *S. cerevisiae* contain a small quantity of chitin that favors to increase protein in diet (Amin et al., 2015). Hence, *A. gigas* supplemented with 6 and 8 g kg^{-1} of Levabon® Aquagrow E increased the final biomass, final length, body weight gain, daily weight gain, and specific growth, but the daily feed intake increased only in supplemented fish with 8 g kg^{-1}. *Solea senegalensis* supplemented with diets containing 4 g kg^{-1} Levabon® Aquagrow E had increased dietary protein utilization, feed conversion, and longer intestinal villus (Batista et al., 2016). Similar findings on the improvements of growth performance due to dietary supplementation with *S. cerevisiae* were also reported for L. *rohita* (Tewary and Patra, 2011), *H. huso* (Hoseinifar et al., 2011) and *O. niloticus* (Amin et al., 2015). The improvement on growth performance with such dietary supplementation has been attributed to enhances in health status of the fish, increment provision of nucleotides and digestibility, stimulation of gastric development and/or enzymatic secretion (Tewary and Patra, 2011; Hoseinifar et al., 2011). In contrast, low dietary supplementation with *S. cerevisiae* (9×10^{5} CFU/g) in *Leporinus macrocephalus* had no improvements in the growth performance of fish (Lima et al., 2015). In addition, different results can be also associated with differences in diet composition and immunostimulant used in supplementation of diets in fish. Since dietary supplementation with immunostimulants has emerged as an indispensable tool to improve farmed fish growth and health for increasing, the search for new immunostimulants continues, for improving more and more the intensive fish farming.

Study of blood parameters is an important tool that can be used as an effective and sensitive index to monitor the physiological and pathological changes in farmed fish (Barton and Zitzow, 1995; Davis and Schreck, 1997; Hoseinifar et al., 2011; Tewary and Patra, 2011; Ranzani-Paiva et al., 2013; Dias et al., 2019). In *A. gigas* supplemented with 4 and 6 g kg^{-1} of Levabon® Aquagrow E, the plasma glucose levels decreased, as well as the levels of cholesterol in fish supplemented with 4, 6 and 8 g kg^{-1}. However, hemoglobin levels decreased in fish supplemented with 4 g kg^{-1} of Levabon® Aquagrow E, in comparison to fish supplemented with 8 g kg^{-1}, while the MCHC increased in fish supplemented with 6 and 8 g kg^{-1} when compared to controls and fish supplemented with 4 g kg^{-1}. Therefore, such hematological and biochemical changes in *A. gigas* supplemented with Levabon® Aquagrow E indicated influence in the metabolic processes. Similarly, in *A. gigas* fed with MycosorbA$^{+®}$, a dietary product based on *S. cerevisiae,* there was a decreases in glucose and triglyceride levels, while the total cholesterol levels increased in fish supplemented with 4 g kg^{-1} of this product in the diet (Hoshino et al., 2017). In contrast, Hoseinifar et al. (2011) did not find any changes in the erythrocytic (erythrocytes, hematocrit, and hemoglobin) and metabolic parameters (cholesterol and glucose) of *H. huso* supplemented with 10 and 20 g kg^{-1} of *S. cerevisiae* in diet. For *A. gigas*, the dietary supplementation with Aquate Fish™, a commercial immunostimulant that content also *S. cerevisiae*, did not influence the number of erythrocytes, hemoglobin, plasma glucose, globulins and triglycerides levels (Dias et al., 2019).

The immunostimulants are compounds that modulate the immune system of supplemented fish, increasing its resistance (Bricknell and Dalmo, 2005) to diseases and stress. In fish, dietary intake of immunostimulant based on lipopolysaccharides of yeasts cell-wall has advantages, and it is a useful method in large-scale fish culture; and hence, has been evaluated in several studies (Hoseinifar et al., 2011; Tewary and Patra, 2011; Rufchaie and Hoseinifar, 2014; Amin et al., 2015; Manoppo et al., 2015; Huyben et al., 2017; Hoshino et al., 2017). *Arapaima gigas* supplemented with 4 g kg^{-1} of Levabon® Aquagrow E showed a decreases in lymphocytes and monocytes that could compromise the immunity of the fish. In contrast, for *P. mesopotamicus* supplemented with β-glucan (Biller-Takahashi et al., 2014) and for *O. niloticus* supplemented with *S. cerevisiae* (Manoppo et al., 2015), the total number of leukocytes increased with the concentrations of immunostimulants. For *A. gigas*, dietary supplementation with Aquate Fish™ did not influence in total number of leukocytes, lymphocytes and monocytes (Dias et al., 2019).

Respiratory burst of leukocytes, an indicator of innate immunity of fish, when increases it express a good inflammatory response and a high rate of phagocytosis, followed by an intense oxygen consumption (Biller-Takahashi et al., 2013; Rebl and Goldammer, 2018; Dias et al., 2019), which can improve the fish resistance to infections and stress (Tewary and Patra, 2011; Huyben et al., 2017). Hence, as in *A. gigas*

supplemented with 8 g kg^{-1} of Levabon® Aquagrow E, the respiratory burst of leukocytes showed increase, this indicate a improve in immune system. Similarly, the respiratory burst of leukocytes increased in *L. rohita* supplemented with high concentrations (50 − 100 g kg^{-1}) of *S. cerevisiae* (Tewary and Patra, 2011). However, in *A. gigas* fed with concentrations of MycosorbA$^{+®}$, there were no benefits on respiratory burst of leukocytes of supplemented fish (Hoshino et al., 2017). Therefore, high concentrations of *S. cerevisiae* can improve the innate immunity of fish in fish farming, and possible contribute to an improvement in production and productivity.

Since the current systems of intensive cultivation of *A. gigas* can cause stress and make the fingerlings more prone to diseases, resulting in economic losses mainly due to mortality due to diseases (Lima et al., 2017), are needs more efforts to increase the its production. Thus, to mitigate this negative effect, immunostimulants based on *S. cerevisiae* can be a promising practice for use in the routine of fish farming of *A. gigas*. This study showed that Levabon® Aquagrow E had significant effects on survival of *A. gigas* fingerlings after handling stress. Similar results were reported for *O. mykiss* supplemented with *S. cerevisiae* and submitted to stress by cannulation (Huyben et al., 2017) and *L. macrocephalus* submitted to stress by capture (Lima et al., 2015).

Hematological studies are also useful in the investigation of the extent of blood damage to fish. These parameters act as efficient and insightful index to examine physiological and metabolic changes occurring in fish, acting as nonspecific biomarkers of stress (Barton and Zitzow, 1995; Davis and Schreck, 1997; Ranzani-Paiva et al., 2013; Lima et al., 2015; Rebl and Goldammer, 2018). The health status of farmed fish may be evaluated thus with the help of some biochemical blood parameters, for example, glucose and total proteins levels. Levels of glucose are considered indicators of sympathetic activation during stress situations, and total protein level is an important indicator of the nutrition status of fish (Barton and Zitzow, 1995; Davis and Schreck, 1997; Hoseinifar et al., 2011; Lima et al., 2015; Rebl and Goldammer, 2018). After 6 h of handling stress, there was an increase in the levels of plasma glucose and total cholesterol in supplemented fish with 4 to 8 g kg^{-1} of Levabon® Aquagrow E, however, MCV increased and hematocrit decreased in *A. gigas* fed with 8 g kg^{-1} of Levabon® Aquagrow E. Therefore, after the stress occurred a higher release of glucose into the bloodstream due to a higher rate of oxygen absorption by the gills, the increased ventilation rate, the branchial flow stimulation, and high capacity of oxygen diffusion (Huyben et al., 2017). In addition, in response to stress, cortisol levels increase the metabolic capacity of the liver to meet the increased energy demand (Rebl and Goldammer, 2018; Montoya et al., 2018), however, we do not analyses cortisol levels in *A. gigas*.

In *A. gigas*, after of handling stress, the levels of total protein decreased in supplemented fish with 4 g kg^{-1} of Levabon® Aquagrow E, as well as the levels of albumin and respiratory burst of leukocytes and number of lymphocytes in fish that received 8 g kg^{-1} of supplement. Therefore, with decreases in number of lymphocytes the production immunoglobulins and immune system of *A. gigas* can be compromised (Dias et al., 2019), prejuding the defense againt pathogens after stress. However, in *A. gigas* supplemented with Aquate Fish™ and challenged with handling stress and inoculation com *Aeromonas hydrophilla* was reported increase in respiratory burst of leukocytes (Dias et al., 2019). Bricknell and Dalmo (2005) suggest that the fingerling stage is the best period to immuestimulation in fish, since in this life stage the fish have more potentially mature cells and insertion of immunostimulants molecules enhance the immune system positively.

5. Conclusions

This study providing growth, physiological and immune parameters for *A. gigas* supplemented with Levabon® Aquagrow E, an immunostimulant available on the market. Results indicate that diets containing 6 to 8 g kg^{-1} of this dietary immunostimulant may be used to promote growth in *A. gigas* fingerlings and improved its some immunity features. Therefore, the use of this dietary additive may increase the survival of *A. gigas* fingerlings in fish farming due to improve in immune to stress, and until possibly might to diseases resistance, which need be yet investigated.

6. Ethical disclosures

This study was conducted in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on the Use of Animals (CEUA/UFAC: 08/2014).

7. CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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