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Growth and Hematology of Juvenile *Piaractus Mesopotamicus* Stocked At 10 Up To 40kg/m³ For Twenty-One Days

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Abstract

Pacu *Piaractus mesopotamicus*, a freshwater fish, has great potential for intensive commercial production, showing resistance to adverse conditions, such as high stocking density. Growth performance and hematological condition were evaluated in juvenile pacu stocked at high density. Juveniles were distributed into different stocking densities: 10, 20, 30 and 40kg of fish/m^3 (n=4) and fed with a commercial feed for 21 days. Growth data were collected at 0 and 21 days and blood samples every 7 days. Differences were not detected in performance of pacu under different stocking densities ($P > 0.05$). Feed conversion rate (3.9±0.5) and specific growth rate (4.0±0.1%/ day) were worse than normally observed at this stage of life, indicating an unsuitable condition. Hematocrit of juveniles stocked at 30 and 40kg/m^3 showed higher values than ones at 10kg/m^3. Thus, stocking density from 10 up to 40kg/m^3 did not affect growth and hematology of pacu after 21 days. However, the low growth rates indicate that all stocking densities could have determined an adverse condition to fish. Considering the high stocking density used in the present work and the intensive management without mortality, we suggest that pacu is very resistant and a promising species for fisheries.

Keywords: Teleost; Omnivorous; Performance; Red blood cells indices

Introduction

Intensive fish farming involves a great challenge for the producer, that is, the concentration of large populations of animals within confined areas that increases predisposition to bacterial and parasitic diseases [1,2]. This problem is especially critical in the early larval stages of teleost, since in this period they depend on maternal components, such as nutritional and immunological factors, for their survival. According to Falcon et al. [3] and Baldwin [4] (2010), handling, biometry, vaccination and stocking density in the intensive culture system induce changes in the physiological responses interfering with health, behavior status, adaptation and welfare of fish determining situations of constant stress. Falcon et al. [3] also point out that the change in temperature may be one of the main causes of losses in production due to the drop in resistance and greater susceptibility to diseases.

Stressful conditions determine the release of cortisol and the consequent depression of the immune system, making fish more susceptible to infectious diseases [5]. The understanding of the causative factors and inhibitors of stress make possible the development of strategies that attenuate this physiological condition.

The influence of stocking density in fish growth parameters has not had an explicit adverse trend in some fish species, showing contradictory results. While *S. senegalensis* exhibits satisfactory growth rate at high stocking density, survival and development were not affected in *Siganus rivulatus* and were compromised in *Oreochromis niloticus* [6-8]. Although the occurrence of chronic stress caused by high stocking densities can depress the immune system, dietary supplementation represents a valuable tool to neutralize the oxidative damages in freshwater fish [9,10]. The levels of lipids and some vitamins, such as vitamins E and C, and minerals in the diet also influence antioxidant defenses and the oxidative state of fish [11]. The main features of pacu that endorse it as a commercial species are related to its high adaptability to rearing in ponds and fish nurseries, resistance to pathogens and low demands on water quality, resulting in a species of high rusticity [12]. Thus, the objective of the present work was to evaluate the effects of high stocking density of 10 up to 40kg/m^3 for twenty-one days in the performance and hematological parameters of juvenile pacu.

Material and Methods

Experimental procedures

Plastic blue cages of 40 l were placed inside tank with continuous aeration and water recirculating system with controlled conditions (temperature 25.4±0.2 ºC; pH 7.9±0.2, dissolved oxygen 4.0±0.5mg l^{-1}; dissolved ammonia:<0.05mg l^{-1}), seeking to guarantee the same condition for all juveniles. Farm-raised, feed-conditioned juvenile pacu (120.2±7.5g and 14.6±0.4cm) were randomly distributed into 16 cages with four different stocking densities (10, 20, 30 and 40kg of fish per m^3, 4 cages per stocking density). Juveniles were hand-fed to apparent satiety twice a day (08h 30 and16h 30) with commercial diet (Agromix 3.3mm: 360g kg^{-1} of CP, 68g kg^{-1} of fat, 110g kg^{-1} of minerals and 35g kg^{-1} of fiber) for 21 days. Growth data was collected in the beginning and end of experiment period for performance evaluation and blood samples every 7 days (3 collections) for hematological and biochemistry analysis. Juvenile pacu were kept, maintained and treated according to accepted standards for the humane treatment of animals (ESALQ/USP ethics committee).

Growth performance

Juveniles were anesthetized with benzocaine (0.05g l^{-1}), measured and weighed at the beginning and end of the experimental period. The following parameters were evaluated:

I. Relative weight gain - RWG (%) = 100 x (final biomass weight - initial biomass weight)/initial biomass weight;

II. Feed conversion ratio – FCR = feed intake per cage (g) / WG (g);

III. Specific growth rate – SGR (% day^{-1}) = 100 x [(ln final biomass weight - ln initial biomass weight) days^{-1}].

Hematological and biochemistry parameters

Blood samples were collected by puncture of the caudal vessel using syringes previously moistened with disodium ethylenediaminetetraacetic acid (Na_2EDTA) solution (3%). Red blood cell indices (RBC) were evaluated following the procedures described by Ranzani-Paiva et al. [13].

Blood aliquots were diluted (1:200) in sodium formaldehyde solution and red cells counted in Neubauer's chamber under an optical microscope. The result was expressed as number of erythrocyte µl^{-1}. The hematocrit value was determined by the microhematocrit procedure and expressed as a percentage of red cells in relation to whole blood. The hemoglobin (Hb) was determined by the hemoglobin cyanide method (HiCN), a colorimetric endpoint reaction system and the results were expressed as g dl^{-1}. The red blood cell indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined according to Wintrobe [14] by the following calculations:

a. MCV = (Hematocrit x 10) erythrocyte^{-1}

b. MCH (pg) = (Hemoglobin x 10) erythrocyte^{-1}

c. MCHC (g dl^{-1}) = (Hemoglobin x 100) hematocrit^{-1}

Plasma glucose was determined by enzyme-colorimetric test using glucose oxidase, which catalyzes the oxidation of glucose into glycolic acid and hydrogen peroxide. Total protein was determined in samples of blood collected without anticoagulant by the biuret test.

Statistical analyses

Statistical analysis was performed using the SAS (v 9.1) program package (SAS Institute, Cary, NC, USA). The performance data was analyzed based on a completely randomized design, considering different stocking densities (10, 20 30 and 40Kg/m3) the main effects. The hematological variables were analyzed as a repeated measure-over-time design, considering stocking density, 10, 20, 30 and 40kg/m3 as main effects. The cage effect was considered random, and the other effects were considered fixed in the model. After the data was confirmed for normal distribution with the Shapiro-Wilk test, a variance analysis was performed using the PROC MIXED (SAS Institute Inc., 2008). If the F value was significant, the Tukey test was used for multiple comparisons between pairs of means at 5% of probability.

Results

Growth performance

Table 1: Growth performance (mean±standard error) of juvenile pacu, *Piaractus mesopotamicus*, under different stocking densities. SEM-standard error mean; n.s. – non-significant, P > 0.05.

Perfomance	Stocking Densities kg/m^3				SEM	P
	10	20	30	40		
Relative weight gain (%)	6.1	5.3	13.6	12.5	1.8	n.s.
Feed converstion ration	4.6±0.9	5.6±1.0	2.3±0.5	2.9±0.7	0.5	n.s.
Specific growth rate (%)	0.3±0.03	0.2±0.04	0.6±0.1	0.5±0.2	0.08	n.s.
Survival (%)	100	100	100	100		

The different stocking densities did not induce differences in growth performance of juvenile pacu (P>0.05), Table 1. Independent of stocking density, the overall mean of RWG, FCR and SGR were 9.6±1.8 %, 3.9±0.5 and 0.4±0.1%.

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Hematological parameters

Only hematocrit value was influenced by different stocking density (P<0.05), Table 2. Juvenile pacu maintained at 30 and 40kg/m^3 showed higher hematocrit value than juveniles maintained at 10kg/m^3. Effect of sampling time was observed for all variable analyzed (P<0.05), except for VCM.

Table 2: Hematological parameters of juvenile pacu, *Piaractus mesopotamicus*, under different stocking densities.

Variables	Stocking Densities (kg/m^3)				Samplig Time (days)			SEM	Probabilty		
	10	20	30	40	7	14	21		SD	ST	SDxST
Serum total protein (g dL^{-1})	5.1	5.4	5.2	6.1	2.1c	6.2b	8.1a	0.4	n.s.	*	n.s.
Plasm glucose (mg dL^{-1})	112	112	112	113	150a	93c	95b	4	n.s.	*	n.s.
Hemoglobin (g dL^{-1})	7.7	7.7	7.9	8.3	8.4a	7.9b	7.6c	0.1	n.s.	*	n.s.
Hematocrit (%)	28b	29ab	30a	31a	27b	30a	31a	0.4	*	*	n.s.
Erytrocytes (106µl^{-1})	1.9	1.9	2	2	1.9b	2.1a	2a	0.03	n.s.	*	n.s.
MVC	145	149	149	151	146	147	152	1	n.s.	n.s.	n.s.
MCH	41	41	39	41	47a	38b	37b	1	n.s.	*	n.s.
MCHC	29	28	26	27	32a	26b	25b	1	n.s.	*	n.s.

SEM- standard error mean; SD – stocking density effect; ST – sampling time effect; SDxST – interaction between SD and ST. n.s. – non significant, P>0.05; * significant, P<0.05 using Tukey's test.

Discussion

Intensive culture is a cost-effective production system; however, high stocking density can trigger chronic stress in fish, since there is competition for food and swimming movements are limited. Considering performance and animal health, the ideal capacity of stocking needs to be adequately determined, since each species presents different responses in the same stocking density. In the present work, pacu had unaffected growth parameters under different stocking densities. In *Siganus rivulatus,* stocking density at 5kg/m^3 also did not affect growth of juveniles [6]. In *Oreochromis niloticus*, in turn, the stocking density at 13kg (m^3)$^{-1}$ decreased development and survival [8]. Bittencourt et al. [15] observed that stocking densities at 5.6; 8.5; 11.36kg/m^3 do not influence erythrocyte parameters, glucose level or carcass yields of *Piaractus mesopotamicus*; however, density of 5.6kg/m^3 results in higher final weight. Di Maggio et al. [16] suggest that densities of up to 0.5 fish l^{-1} (4.70kg/m^3 appear to be conducive to the culture of juvenile Orthopristis chrysoptera. The above authors worked with densities much lower than in the present study. Merola & Souza [17] however, suggest that standing crop values of 45-50kg/m^3 might be approximating a critical level for growth of Piaractus mesopotamicus. Sadhu et al. [18] in turn, observed that fish stocked at 35m^3 had significant decrease in growth and final mean weight compared to ones stocked at 14m^3. Although parameters were not influenced by different stocking density, the present juveniles showed FCR and SGR (3.9±0.5 and 0.4±0.1% day^{-1}, respectively) worse that normally observed for the species at this stage of life and much lower than expected for the period, indicating an unsuitable condition for juveniles. Machado-Neto et al. [19] observed better rates of relative weight gain and feed conversion (1246% and 1.2, respectively) in juvenile pacu with initial weight of 8.5±0.7g fed twice daily for 60 days with diet containing 320g kg^{-1} of crude protein and 21g MJ^{-1} of energy. Bicudo et al. [20], in turn, observed a FCR and SGR (% day^{-1}) around 1.05 and 2.2, respectively, after 70 experimental days when dietary protein requirement of pacu juveniles are met. Tesser et al. [21], in turn, restricting the supply of diets to 2.5% of biomass per day, observed in pacu juveniles (initial weight of 4.3±0.1g) a FCR and SGR (% day^{-1}) of 0.60 and 3.53, respectively.

Considering hematological parameters, only hematocrit value was influenced by different stocking density, with juvenile pacu maintained at 30 and 40kg/m3 showing higher hematocrit value than juveniles maintained at 10kg/m3. Hemoconcentration caused by stress raises hematocrit values in several freshwater species [22]. The elevated hematocrit values will aid in blood oxygenation and overcoming stress [23]. Urbinati et al. [1] evaluating loading and transport stress of juvenile matrinxã (*Brycon cephalus*, Characidae) at various densities, observed higher hematocrit values after loading the fish. The authors suggest that higher hematocrit values after loading is due to swelling of red blood cells.

Thus, in the present work, considering the experimental conditions used in this work, the stocking density from 10 up to 40kg/m^3 did not affect growth and hematology of juvenile pacu, *Piaractus mesopotamicus,* for 21 days. However, the low growth rates indicate that all stocking densities could have determined an adverse condition to fish. Considering the high stocking density used in the present work and the intensive management, collecting blood every week, without mortality, we suggest that *P. mesopotamicus* is very resistant and a promising species for fisheries.

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