

# Growth and hematology of pacu subjected to sustained swimming and fed different protein levels

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**Abstract** – The objective of this work was to evaluate the effect of sustained swimming and dietary protein levels on growth and hematological responses of juvenile pacu (*Piaractus mesopotamicus*). A completely randomized design was used in a 3x2 factorial arrangement, with three levels of dietary protein (24, 28, and 32% crude protein), two rearing conditions (sustained swimming or motionless water), and 15 replicates. Fish were subjected to sustained swimming at the velocity of two body lengths per second ( $2 \text{ BL s}^{-1}$ ), for 45 days. The level of dietary protein and the swimming conditions affected the performance, growth, and hematological profile of pacu. Swimming conditions influenced nutritional factors, increasing daily weight gain, specific growth rate, number of erythrocytes, mean corpuscular volume, and mean corpuscular hemoglobin. Fish under sustained swimming and fed with 24% crude protein showed better growth performance, with higher specific growth rate ( $4.11 \pm 0.88$ ) and higher daily weight gain ( $2.19 \pm 0.47$  g per day). Sustained swimming can increase the productive performance of pacu and simultaneously reduce dietary protein levels.

**Index terms:** *Piaractus mesopotamicus*, erythropoiesis, exercise, growth rate, weight gain.

## Crescimento e hematologia de pacu submetido a natação sustentada e alimentado com diferentes níveis proteicos

**Resumo** – O objetivo deste trabalho foi avaliar o efeito da natação sustentada e de níveis de proteína na dieta sobre o crescimento e a resposta hematológica de juvenis de pacu (*Piaractus mesopotamicus*). Utilizou-se o delineamento inteiramente casualizado, em arranjo fatorial 3x2, com três níveis de proteína na dieta (24, 28 e 32% de proteína bruta), duas condições de criação (natação sustentada ou água imóvel) e 15 repetições. Os peixes foram submetidos à natação sustentada na velocidade de duas vezes o comprimento corporal por segundo ( $2 \text{ CC s}^{-1}$ ), por 45 dias. O nível de proteína da dieta e as condições de nado afetaram o desempenho, o crescimento e o perfil hematológico do pacu. As condições de nado influenciaram os fatores nutricionais, com aumento de ganho de peso diário, taxa de crescimento específico, número de eritrócitos, volume corpuscular médio e hemoglobina corpuscular média. Peixes sob natação sustentada e alimentados com 24% de proteína bruta apresentaram melhor desempenho de crescimento, com maior taxa de crescimento específico ( $4,11 \pm 0,88$ ) e maior ganho em peso diário ( $2,19 \pm 0,47$  g por dia). A natação sustentada pode aumentar o desempenho produtivo de pacus e ao mesmo tempo reduzir os níveis de proteína na dieta.

**Termos para indexação:** *Piaractus mesopotamicus*, eritropoiese, exercício, taxa de crescimento, ganho em peso.

### Introduction

Pacu (*Piaractus mesopotamicus*) is an omnivorous fish species with high growth rates, rusticity, and easy adaptation to artificial feeding. It lives in the Paraná, Paraguay, and Uruguay Rivers (Saint-Paul, 1986), and is a relevant fish species in Brazil.

Currently, a challenge for the fish farming industry is to reduce production costs by improving culture techniques and developing more feasible and efficient diets for different species. Protein, used for promoting

tissue growth and meeting energetic demands, is the most expensive diet component (Wilson, 2002). Efforts have been made towards reducing dietary protein content, especially animal protein, due to economic and environmental reasons (Tacon & Metian, 2008). In addition, several works have reported metabolic impairments in consequence of high protein levels (Singh et al., 2006; Portz & Furuya, 2012).

Different strategies have been proposed to decrease protein content in diets. One of them is protein sparing, in which a higher concentration of nonprotein

sources of energy is used to meet physiological demands (Bicudo et al., 2012). Subjecting fish to exercise can lead to the protein sparing effect, make carbohydrates and lipids more efficient in meeting energetic demands, and redirect protein to an anabolic process (Arbeláez-Rojas & Moraes, 2010; Felip et al., 2013). These adaptations result in faster growth, better feed conversion ratios, increased survival rates, and decreased aggressive behavior (Moraes et al., 2009; Hackbarth, 2010). Sustained swimming also acts on blood flow, blood capillary diameter, and heart beating frequency (Sandblom et al., 2005).

There are studies on sustained swimming and animal nutrition in tropical freshwater fish (Hackbarth, 2010; Arbeláez-Rojas et al., 2011). However, few of them have shown beneficial effects of it on fish growth and metabolism. In general, each of these variables is studied independently, but it is reasonable to assume that the association of an ideal swimming activity and a well-balanced diet will result in more satisfactory responses on fish performance than sustained swimming or diet alone. One of the recurring concerns in the commercial breeding of fish is the reduction of dietary crude protein (CP) levels, since it would reduce production costs and the undesired effects of nitrogen excretion.

The objective of this work was to evaluate the effect of sustained swimming and dietary protein levels on growth performance and on hematological responses of juvenile pacu.

## Materials and Methods

The experiment was carried out from February to April 2009 at the Universidade Federal de São Carlos, SP, Brazil (22°1'4"S, 47°23'57"W, at 860 m altitude). A completely randomized design was used, in a 3x2 factorial arrangement, with three levels of dietary protein (24, 28, and 32% CP), two rearing conditions (sustained swimming or motionless water) and replicates. Each fish was considered as an experimental unit.

Juvenile pacu were obtained from the São Geraldo commercial fish farm, located in Sertãozinho, SP, Brazil. The fish were held in 2,000 L tanks for one month in order to be acclimatized to the experimental conditions. After this period, 90 fish were anaesthetized with 40 mg L<sup>-1</sup> of eugenol (Inoue et al., 2003) and individually tagged with a microchip, implanted in the abdomen. After seven days of recovery, fish

were anaesthetized, weighted (23.9±4.7 g), gauged (10.6±0.77 cm), and randomly distributed into six 200 L circular fiber tanks. Twenty-four hours after the biometry, the exercise protocol was performed in three tanks at the swimming velocity of two body lengths per second (BL s<sup>-1</sup>), in accordance with Moraes et al. (2009). The water velocity was generated by a NXDP4 pump (Grundfos do Brasil, Ltda., São Bernardo do Campo, SP, Brazil) as previously reported (Arbeláez-Rojas et al., 2011), and the flow was regulated every other day during the entire experimental period (45 days). Water speed was checked with a 2030 series mechanical flow meter (General Oceanics Inc., Miami, FL, USA). The following water parameters were checked daily: dissolved oxygen (6.01±0.39 mg L<sup>-1</sup>), temperature (27.9±1.8°C), and ammonia (0.05±0.001 mg L<sup>-1</sup>). Besides first biometry, two other biometric evaluations were made on the 22<sup>nd</sup> and 45<sup>th</sup> days of the experimental period.

Three experimental diets were formulated (Table 1), containing three levels of crude protein (24, 28, and

**Table 1.** Formulation and chemical composition of the experimental diets.

Ingredient (g kg <sup>-1</sup> )	Dietary protein levels (g kg <sup>-1</sup> )		
	240	280	320
Corn	240	240	240
Grinded rice	50	50	50
Soybean meal	145	110	101
Fishmeal	260	330	410
Soybean oil	130	120	116
Mineral and vitamin supplement <sup>(1)</sup>	20	20	20
Carboxymethyl cellulose <sup>(2)</sup>	155	123	68
Chemical composition (g kg <sup>-1</sup> )			
Dry matter	907	906	906
Crude protein (CP)	241	281	320
Digestible protein	210	240	273
Total lipid (TL)	157	151	151
Carbohydrate (CHO) <sup>(3)</sup>	257	250.3	250.3
Crude fiber	157	125	72
Mineral matter	76	79	94
Gross energy (MJ kg <sup>-1</sup> )	16.47	16.76	17.68
CHO: TL ratio	1.63	1.65	1.65
CHO: CP ratio	1.06	0.89	0.78

<sup>(1)</sup>Rovimix (Hoffmann-La Roche, Ltda., Basel, Switzerland): vitamin A, 5,000,000 IU; vitamin D3, 200,000 IU; vitamin E, 5,000 IU; vitamin K3, 1,000 mg; vitamin B1 (thiamine), 1,500 mg; vitamin B2 (riboflavin), 1,500 mg; vitamin B6 (pyridoxine), 1,500 mg; vitamin B12, 4,000 mg; vitamin C, 15,000 mg; folic acid, 500 mg; pantothenic acid, 4,000 mg; BHT, 12.25 g; biotin, 50 mg; inositol, 1,000 mg; nicotinamide, 7,000 mg; choline, 40 g; cobalt, 10 mg; copper, 500 mg; iron, 5,000 mg; iodine, 50 mg; manganese, 1,500 mg; selenium, 10 mg; zinc, 5,000 mg; vehicle q.s., 1,000 g.

<sup>(2)</sup>Obtained from Rhoster Indústria e Comércio, Ltda. All ingredients were grinded in a 0.5 mm pore sieve. <sup>(3)</sup>Carbohydrate = dry matter - (crude protein - total lipid + crude fiber + mineral matter).

32%), one level of total lipids (15%), and one of carbohydrate (25%). The concentration of total lipids and carbohydrate was based on previous studies developed in the laboratory and posteriorly published by Hackbarth (2010). Fish were fed three times a day to apparent satiation, during the entire experimental period.

At the end of the experimental span, eight fish from each tank were randomly sampled, and 1 mL of blood was withdrawn from the caudal vein with a heparinized plastic syringe. Hematocrit (Ht), hemoglobin concentration (Hb) (Drabkin, 1948), number of red blood cells (RBC) (Lima et al., 1969), mean corpuscular volume, and mean corpuscular hemoglobin were determined. Growth performance and feed utilization were determined and calculated as follows: daily weight gain (DWG; g per day) = (mean final weight - mean initial weight)/[mean initial weight x time (days)]; feed conversion ratio (FCR) = feed intake (g)/weight gain (g); and specific growth ratio (SGR) = 100 x (ln final weight - ln initial weight)/time (days).

Data were analyzed by two-way analysis of variance, and means were compared by Tukey's test, at 5% probability. Data were analyzed using the SAS software, v.8 (SAS Institute, Cary, NC, USA).

## Results and Discussion

Both dietary protein levels and sustained swimming affected growth performance and the hematological profile of pacu. Fish fed with 24 and 32% CP showed the highest DWG, whereas the highest SGR was observed in fish fed with 24% CP (Table 2). This

**Table 2.** Growth performance of pacu (*Piaractus mesopotamicus*) fed with three levels of dietary protein and subjected to sustained swimming<sup>(1)</sup>.

Factor	Growth performance		
	DWG (g per day)	FCR	SGR
Crude protein level			
240 g kg <sup>-1</sup>	2.19±0.47A	0.93±0.03	4.11±0.88A
280 g kg <sup>-1</sup>	1.88±0.25B	0.92±0.04	3.02±0.41B
320 g kg <sup>-1</sup>	2.02±0.46A	0.83±0.10	2.83±0.65B
Rearing condition			
Sustained swimming <sup>(2)</sup>	2.21±0.45A	0.99±0.09	3.61±0.99A
Motionless water	1.90±0.35B	0.80±0.10	3.09±0.71B

<sup>(1)</sup>Means±SD followed by equal letters do not differ by Tukey's test, at 5% probability. <sup>(2)</sup>Swimming velocity of 2 body length per second. DWG, daily weight gain; FCR, food conversion ratio; SGR, specific growth rate.

indicates that the 24% CP group – in which the protein anabolism was more noticeable – had a faster growth. According to several studies, the best CP values for pacu are between 26 and 27% (Abimorad et al., 2007; Bicudo et al., 2009), which supports the result obtained here. Considering that lower dietary protein reflects both lower diet costs and nitrogen wasting, the diet containing 24% CP is a sound alternative to feed pacu subjected to swimming at 2 BL s<sup>-1</sup>.

The distinct values (Table 1) of gross energy (GE) and crude fiber (CF) did not change FCR (Table 2), showing that these conditions do not interfere in food intake of exercised pacu. Although high concentration of dietary fiber reduces food intake, increasing bolus size (Montagne et al., 2003) and satiation (Hansen & Storebakken, 2007), the fiber used as a diet component (carboxymethyl cellulose) did not affect FCR in all groups. However, diets with more than 11% of microfine cellulose reduce the growth of pacu (Rodrigues et al., 2010). It is possible that swimming attenuated the effect of high crude fiber concentration, since all fish exhibited good FCR values.

Independently of the dietary protein level, sustained swimming at 2 BL s<sup>-1</sup> resulted in the best growth performance. Fish held under sustained swimming presented higher DWG and SGR (Table 2), probably as consequence of a more efficient mobilization, due to the exercise, of carbohydrates and lipids, in order to meet energetic demand. At the same time, exercise could have redirected protein to anabolic pathways. Similar results were reported for matrinxã (*Brycon amazonicus*) kept under sustained swimming for 72 days at 1 BL s<sup>-1</sup>. An improvement in FCR was observed, increasing growth and leading to greater weight gain (Hackbarth & Moraes, 2006). In another work with the same species, with sustained swimming span extended to 90 days at 1–1.5 BL s<sup>-1</sup>, Arbeláez-Rojas & Moraes (2010) observed a 20% increase in growth and weight gain. Sustained swimming at velocities between 1 and 2 BL s<sup>-1</sup> improves pacu mean weight, mean length, FCR, and protein efficiency ratio, especially at 2 BL s<sup>-1</sup> (Hackbarth, 2010). Therefore, sustained swimming can be considered a stimulator of growth and of carbohydrate and lipid utilization in order to meet the increased metabolic expenditures (Moraes et al., 2009).

Other studies on sustained swimming have shown that it stimulates fish growth and weight gain, bringing

advantages over the usually stationary system (Hackbarth & Moraes, 2006; Arbeláez-Rojas & Moraes, 2010; Arbeláez-Rojas et al., 2011). Moreover, fish farming under moderate water streams facilitates food-pellet intake and improves their distribution, which reduces the potential aggressiveness of fish when searching for food. Therefore, moderate-velocity water have shown several advantages over lentic waters (Arbeláez-Rojas et al., 2011).

The dietary protein levels (Table 3) can explain the alterations observed in RBC (in the 32% CP group), in Ht and MCV (24 and 28% CP groups), and in MCH (24% CP group). Hematological alterations reflect the improvement of nutrient and oxygen transport, and of higher tissue oxygen uptake. Changes in MCV should be related to osmoregulatory status, cardiac dynamics, and blood flow, which change due to exercise. Matrinxã subjected to sustained swimming at 1 BL s<sup>-1</sup> did not show alteration in hemoglobin, MCV, and MCHC levels (Hackbarth & Moraes, 2006); whereas under sustained swimming at 1.5 BL s<sup>-1</sup>, Arbeláez-Rojas & Moraes (2010) reported increased contents of hemoglobin, Ht, and RBC. In both works, the authors concluded that exercise increases metabolic demand, which, in turn, caused a series of hematological adaptations. Hackbarth (2010) reported increased levels of hematocrit, hemoglobin, VCM, and HCM in pacu exposed to 2 BL s<sup>-1</sup> and attributed these alterations to exercise.

In the present study, RBC and MCH parameters were also altered by exercise (Table 3). The increased RBC observed in fish fed with 32% CP may be explained by one of the following factors: 1, RBC release into the blood stream from a spleen contraction, where a large number of these cells is stored; or 2, a higher RBC value followed by lower Ht, MCV, and MCH,

suggesting the presence of young erythrocytes from erythropoiesis. Studies with other species have also shown interference of CP on hematological variables. Camargo et al. (2005), for example, observed increased RBC, Hb concentration, and Ht values in silver catfish (*Rhamdia quelen*) fed with 50% CP. Abdel-Tawwab et al. (2010) also found changes in RBC, Hb, and Ht in response to dietary protein, when studying Nile tilapia (*Oreochromis niloticus*). However, specific mechanisms in fish involving erythropoiesis and dietary CP still remain to be elucidated.

The lower number of erythrocytes observed in fish under sustained swimming was probably offset by the larger volume of these cells. The number of RBC decreased about 13.8%, but an equivalent reduction of hematocrit was not observed. This means that the erythrocyte volume swelled, which is typical of the enhanced oxygen demand provided by aerobic exercise. The increases in MCV and MCH resulted from metabolic adaptation to deal with higher oxygen uptake and transport. The same responses were observed in matrinxã subjected to intermittent sustained swimming at 12x12 hours vs. rest (Fabrizzini et al., 2013). It is reasonable to infer that the hematological responses found in the present study may be typical of the species, and that they may also depend on external factors, such as swimming speed and exercise type (Hackbarth & Moraes, 2006; Moraes et al., 2009; Arbeláez-Rojas & Moraes, 2010; Fabrizzini et al., 2013). Furthermore, the observed hematological changes may indicate metabolic adaptation in order to meet oxygen demand.

Pacu fed with 24% CP and subjected to sustained swimming reached higher DWG and SGR than non-exercised fish (Table 4). Sustained swimming stimulated the use of other nutrients for energy demands, redirecting protein to an anabolic process. According to

**Table 3.** Hematological parameters of pacu (*Piaractus mesopotamicus*) subjected to sustained swimming and fed three levels of crude protein<sup>(1)</sup>.

Factor	Hematological parameter				
	RBC (10 <sup>6</sup> mm <sup>-3</sup> )	Hematocrit (%)	Hemoglobin (g%)	MCV (μ <sup>3</sup> )	MCH (μg)
Crude protein level					
240 g kg <sup>-1</sup>	1.68±0.33B	31.15±2.58AB	7.95±0.80	191.04±37.08A	48.43±8.66A
280 g kg <sup>-1</sup>	1.90±0.28B	31.34±1.19A	8.31±0.42	168.15±22.00A	44.69±6.70AB
320 g kg <sup>-1</sup>	2.28±0.47A	29.15±3.22B	8.74±1.33	134.30±39.49B	39.68±9.07B
Rearing condition					
Sustained swimming <sup>(2)</sup>	1.81±0.41B	30.81±2.27	8.48±1.10	178.31±39.26A	48.29±7.99A
Motionless water	2.10±0.42A	30.29±2.92	8.19±0.79	150.69±36.60B	40.25±7.81B

<sup>(1)</sup>Means±SD followed by equal letters do not differ by Tukey's test, at 5% probability. <sup>(2)</sup>Swimming velocity of 2 body length per second. RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

Richards et al. (2002), sustained swimming accelerates body growth through stimulation of protein synthesis and hypertrophy of muscle fibers. Therefore, in pacu exercised at 2 BL s<sup>-1</sup>, 24% CP is enough to promote higher growth values. Considering that the present study was conducted with specimens of the same brood, it may be inferred that the treatments are the main responsible for the observed effects. Similar responses have been reported in matrinxã fed with 28% CP and subjected to sustained swimming (Arbeláez-Rojas et al., 2011).

Sustained swimming and lower CP dietary levels in pacu interacted through the blood parameters RBC, MCV, and MCH (Table 4). Fish fed with 24% CP under sustained swimming showed reduction of RBC when compared with sedentary ones. However, an increase of MCV and MCH was observed in fish fed with 24 and 28% CP under sustained swimming. Low dietary protein levels associated with sustained swimming seem to reduce RBC and to increase erythrocyte volume and hemoglobin concentration. There is an inverse correlation between cell size and fish ability to endure sustained swimming. Therefore, pacu adapted to the sustained swimming condition showed reduced erythrocyte size and produced new RBC to meet the physiological demands.

Higher growth rates, ideal FCR, and protein sparing effect make sustained swimming at 2 BL s<sup>-1</sup> an excellent support for pacu farming.

**Table 4.** Interaction between sustained swimming and dietary protein in pacu (*Piaractus mesopotamicus*) for growth and hematological parameters<sup>(1)</sup>.

Rearing condition	Dietary protein levels (g kg <sup>-1</sup> )		
	240	280	320
	Daily weight gain (g per day)		
Sustained swimming <sup>(2)</sup>	2.47±0.45A	1.90±0.17	2.19±0.45
Motionless water	1.97±0.37B	1.86±0.29	1.87±0.42
	Specific growth rate		
Sustained swimming	3.73±0.45	3.06±0.27	3.07±0.64
Motionless water	3.18±0.42	3.00±0.47	2.62±0.59
	Red blood cells (10 <sup>6</sup> mm <sup>-3</sup> )		
Sustained swimming	1.51±0.25B	1.74±0.22B	2.18±0.47
Motionless water	1.86±0.32A	2.06±0.27A	2.38±0.49
	Mean corpuscular volume (μ <sup>3</sup> )		
Sustained swimming	213.22±25.34A	179.40±20.4A	142.33±37.12
Motionless water	168.87±43.35B	156.90±18.4B	126.27±42.22

<sup>(1)</sup>Means±SD followed by equal letters do not differ by Tukey's test, at 5% probability. <sup>(2)</sup>Swimming velocity of 2 body length per second.

## Conclusions

1. Sustained swimming and lower crude protein levels improve growth performance of juvenile pacu (*Piaractus mesopotamicus*) and favor hematologic adaptations in order to maintain the biological demands from the exercise.

2. It is possible to reduce the level of dietary protein in pacu farming if the fish are kept under sustained swimming at two body length per second.

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