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# The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): A metabolic and endocrine approach

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ABSTRACT

The influence of stocking density and food deprivation on energy metabolism, stress processes and the pituitary endocrine system of silver catfish (*Rhamdia quelen*) was investigated after a period of 14 days, in which plasmatic and hepatic parameters and the mRNA expression of prolactin (PRL), growth hormone (GH) and somatolactin (SL) were assessed. The fish were subjected to four experimental conditions: (1) fed under high stocking density (32 kg/m$^3$, HSD); (2) fed under mean stocking density (16 kg/m$^3$, MSD); (3) fed under low stocking density (8 kg/m$^3$, LSD); and (4) food-deprived under low stocking density (8 kg/m$^3$, LSD-FD). After 14 days, plasma and liver samples were obtained to analyze the metabolite levels and enzymatic activities related to metabolism, and pituitary glands were obtained to analyze hormone expression (PRL, GH and SL). Liver weight and the hepato-somatic index (HSI) revealed that specimens maintained at HSD and/or MSD had higher hepatic stores, which were observed in the triglyceride and glycogen levels in this tissue, than animals submitted to the LSD and LSD-FD groups. Triglyceride levels in the plasma and liver revealed the consumption of fatty acid reserves in the fasting group. Enzymatic activities, such as glutamate dehydrogenase (GDH), phosphorylase (GPase), pyruvate kinase (PK), aspartate transaminase (AST) and glycerol-3-phosphate dehydrogenase (G3PDH), indicated an increase in gluconeogenic pathways in the HSD group and an increase in glycolitic metabolism in the LSD groups. The expression of PRL was not affected by stocking density and/or food deprivation and GH decreased with increased density and increased in fasting conditions. A negative effect of density and fasting was observed on the expression of SL. Overall, the data suggested that juvenile silver catfish reared at stocking densities of 16 to 32 kg/m$^3$ were better maintained than those maintained at the lowest density.



## 1. Introduction

Studies of stress in fish have often been conducted in the field of physiology (Arjona et al., 2010; Costas et al., 2011; Herrera et al., 2012; Ruane et al., 2002). In the environment, stress response can be observed as the ability of fish to mobilize energy reserves to avoid or overcome threatening situations. However, in fish culture systems, stress is constant and may affect productive development, thus harming fish health and increasing susceptibility to disease (Barcellos et al., 2011).

Primary stress is characterized by a significant increase in corticosteroid hormones (cortisol) and the concentration of catecholamines (epinephrine and norepinephrine) that stimulate the hydrolysis of glycogen in the liver, which increases blood glucose levels, decreases muscle protein and increases heart rate (Barton, 2002). These hormones induce secondary stress responses, which are characterized by a reduction in hepatic glycogen content and increased plasma glucose levels that provide glucose to tissues for homeostasis (Barton, 2002). For chronic stress, tertiary responses could affect whole-animal changes such as growth, reproductive efficiency, disease resistance and behavior (Barton, 2002; Wendelaar Bonga, 1997).

For cost-effective production, a balance between maximum profit and minimum incidence of physiological and behavioral disorders, many of them due to high stocking densities, although other variables such as the species and culture conditions may also influence these responses (Herrera et al., 2012). The technology of fish farming in cages

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has revealed a promising technique to reconcile the use of sustainable environment with high productivity resulting from the use of high stocking density (Brandão et al., 2004).

Previous studies have focused on evaluating the effects of stocking density on fish growth, behavior, metabolism and physiological and biochemical parameters (Herrera et al., 2009; Li et al., 2012; Montero et al., 1999; Sangiao-Alvarellos et al., 2005b). Stocking density is a stressor that activates stress responses in fish, which affect different metabolic enzymes related to lipid, carbohydrate and protein metabolism (Costas et al., 2008; Laiz-Carrión et al., 2012; Montero et al., 1999; Sangiao-Alvarellos et al., 2005a). Due to the diversity of stress response in fish (Barton, 2002), these effects appear to be species-specific and mainly dependent on the sensitivity of fish at high stocking density and the increase of social interactions at very low and/or very high stocking densities (Ellis et al., 2002; Montero et al., 1999; North et al., 2006). Consequently, inappropriate stocking densities may compromise the health conditions of farmed fish, thus affecting also the profitability of the aquaculture industry.

Food deprivation is also a common type of stress in fish that induces energy-releasing catabolic processes that compensate for reduced energy intake (Wunderink et al., 2012). During the initial stages of fasting, the maintenance of glycemia is directly related to the mobilization capacity of hepatic glycogen and depends on the subsequent activation of hepatic gluconeogenesis and subsequent reduction of the rate of glucose utilization (Navarro and Gutiérrez, 1995). Thus, fasting is frequently used to study whether a rhythm in glucose metabolism is independent of the effect of feeding since daily rhythms dependent on feeding should disappear in food-deprived animals (Polakof et al., 2007). In fish, the effects of fasting on daily changes of several plasma metabolites and hormones have been characterized in different species (Laiz-Carrión et al., 2012; Polakof et al., 2006; Sangiao-Alvarellos et al., 2005b). The metabolic responses to this situation vary depending on several factors such as age, size, and species displaying alterations in the carbohydrate, lipid, and protein metabolism in order to maintain homeostasis (Navarro and Gutiérrez, 1995).

Prolactin (PRL), growth hormone (GH) and somatolactin (SL) belong to an identical hormone family because of their structural similarities but have different functions related to diverse physiological processes (Manzon, 2002; Pérez-Sánchez and Le Bail, 1999; Vega-Rubin de Celis et al., 2004). PRL is essential for acclimation to hyposmotic environments and is also related to processes such as reproduction, stress and metabolism (Laiz-Carrión et al., 2009; Mancera and McCormick, 2007). In addition, GH regulates growth and intermediary metabolism and has osmoregulatory effects in some species (Mancera and McCormick, 2007; Sakamoto and McCormick, 2006). SL is related to different physiological processes, including stress response, reproduction, acid–base regulation, growth and reproduction (Fukamachi and Meyer, 2007; Vega-Rubin de Celis et al., 2004).

The silver catfish (*Rhamdia quelen*) is a suitable species for aquaculture in south Brazil because of its elevated growth rate, good carcass yield and easy reproductive handling in the subtropical climate (Baldisserotto, 2009; de Amorin et al., 2009). This species has been intensively cultured and has even been used as a model to improve the management of several fish in this family (Barcellos et al., 2010). Previous studies verified that stocking density affects its growth (Barcellos et al., 2004; Piaia and Baldisserotto, 2000), but no analysis regarding the biochemical and physiological changes induced by this parameters was performed; this knowledge might be useful to silver catfish production.

Several studies have analyzed the effects of simple stressors on fish energy metabolism (Barcellos et al., 2010, 2011; Polakof et al., 2006; Sangiao-Alvarellos et al., 2005a). Nevertheless, this study was designed to investigate the effects of different stocking densities and feeding regimes on biochemical and physiological parameters in the plasma and liver and the expression of pituitary hormones (PRL, GH and SL) in silver catfish.

## 2. Materials and methods

### 2.1. Experimental procedures

Silver catfish (173.20 ± 8.33 g and 27.17 ± 0.55 cm) were acquired from the Fish Culture Laboratory at the Federal University de Santa Maria (southern Brazil) and transferred to the Fish Physiology Laboratory. Prior to the experiment, the fish were maintained for 10 days in eight 250-L tanks with an equal number of fish in each (n = 16 per tank) with continuously aerated, running water under a natural photoperiod (12 h light and 12 h dark) and fed with commercial pellets (Supra, 32% crude protein, Alisul Alimentos S.A., Carazinho, Brazil) once daily at 1% body mass. The following water quality was used throughout the acclimation and experimental periods: temperature, 22.0 ± 0.7 °C; pH, 7.0 ± 0.5; dissolved oxygen levels, 8.0 ± 0.2 mg/L; nitrite, 0.08 ± 0.01 mg/L; alkalinity, 37.0 ± 3.2 mg/L $CaCO_3$ and total ammonia nitrogen, 0.009 ± 0.001 mg/L.

After the acclimation period, the fish were transferred to 250-L tanks with continuously aerated and running water, and adjusted to ~180 L to maintain the experimental stocking density required. The specimens (n = 128) were randomly assigned to four experimental groups, in duplicate, under the following experimental conditions: (1) fed fish under high stocking density with thirty two fish per tank (32 kg/$m^3$, HSD); (2) fed fish under mean stocking density with sixteen fish per tank (16 kg/$m^3$, MSD); (3) fed fish under low stocking density with eight fish per tank (8 kg/$m^3$, LSD); and (4) food-deprived fish under low stocking density also with eight fish per tank (8 kg/$m^3$, LSD-FD). The number of replicates (two) in each condition was chosen based on previous studies carried out in other fish species submitted to different stocking densities and food deprivation (Laiz-Carrión et al., 2012; Sangiao-Alvarellos et al., 2005b).

The fish were fed as described above, except for the starved fish, which were food-deprived for the entire experiment. After 14 days from the start of the experiment, eight fish from each treatment (four from each tank) were anesthetized with 50 mg/L of eugenol for 3 min, and were sampled. Length and weight were measured and then blood was collected from the caudal vein using heparinized syringes, and plasma was obtained by centrifugation of the blood (3 min, 10 000 g, 4 °C) and stored at −80 °C until analysis. The fish were then euthanized by spinal sectioning and the pituitary gland and liver were removed. The liver was weighed separately for the hepato-somatic index (HSI) and was immediately frozen in liquid nitrogen. The tissues were stored at −80 °C for subsequent analyses. The methodology of this experiment was approved by the Ethics Committee on Animal Experimentation at UFSM under registration number 24/2007 for the use of laboratory animals.

### 2.2. Plasma physiological and biochemical assays

Plasma osmolality was measured using a vapor pressure osmometer (Fiske, 110 Osmometer, Norwood Massachusetts, USA). Glucose, triglycerides and lactate levels were measured using commercial kits from Spinreact (glucose-HK, ref. 1001200; lactate, ref. 10013300; TAG, ref. 10013110). Total proteins were determined using bicinchoninic acid and a commercial thermo kit (Pierce BCA protein assay kit, ref. 23225, Thermo Scientific, USA) using bovine serum albumin as the standard. The total α-amino acid levels were assessed colorimetrically using the ninhydrin method described by Moore (1968) and adapted to microplates with L-alanine (Sigma, ref. A-7469) as the standard. All assays were performed using a Bio-Tek PowerWave 340 Microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) using KCjunior Data Analysis Software for Microsoft Windows XP.

The plasma cortisol levels (expressed in ng/mL) were measured (in duplicate) by indirect enzyme immunoassay (ELISA) as previously described in Rodríguez et al. (2000) for testosterone. The steroids were extracted as described by Baldisserotto et al. (2014). The standards