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Metabolical and hematological responses of silver catfish (*Rhamdia quelen*) to diet composition

Respostas metabólicas e hematológicas de jundiás (*Rhamdia quelen*) frente à composição da dieta

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ABSTRACT

This study investigated the metabolic and hematological responses of silver catfish fed diets composed of different protein sources for 90 days. Six different diets were formulated: MBY (meat and bone meal + yeast); SY (soybean meal + yeast); S (soybean meal); MBS (meat and bone meal + soybean meal); FY (fish meal + yeast); FS (fish meal + soybean meal). In the liver, the concentration of glucose, lactate and protein was lower in fish fed SY and S diets. Liver glycogen did not differ between diets. At the muscular level, the glycogen, glucose, lactate content was lower in the FS diet, while the protein decreased in the fish that received the soybean meal-based diet (S). On the other hand, higher values of protein, glucose and lactate were observed in the diet containing meat and bone meal + soybean meal (MBS). The silver catfish fed diets based on fish meal (FY and FS) had a higher protein content in the kidney. Hematological parameters were influenced by diets. The absence of animal protein sources in the diets resulted in lower values of hematocrit and hemoglobin in silver catfish blood. It is concluded that diets containing vegetable and animal protein sources improve the nutritional and health status of silver catfish.

Keywords: Glycogen; Vegetal protein; Hematocrit; *Rhamdia quelen*

RESUMO

Este estudo investigou as respostas metabólicas e hematológicas de jundiás alimentados com dietas compostas por diferentes fontes proteicas durante 90 dias. Foram formuladas seis dietas diferentes: MBY (farinha de carne e ossos + levedura); SY (farelo de soja + levedura); S (farelo de soja); MBS (farinha de carne e ossos + farelo de soja); FY (farinha de peixe + levedura); FS (farinha de peixe + farelo de soja). No fígado, a concentração de glicose, lactato e proteína foi menor nos peixes alimentados com as dietas SY e S. O glicogênio hepático não diferiu entre as dietas. No nível muscular, o conteúdo de glicogênio, glicose e lactato foi menor na dieta FS, enquanto a proteína diminuiu nos peixes que receberam a dieta à base de farelo de soja (S). Por outro lado, foram observados maiores valores de proteína, glicose e lactato na dieta contendo farinha de carne e ossos + farelo de soja (MBS). Jundiás alimentados com dietas à base de farinha de peixe (FY e FS) apresentaram maior teor de proteína no rim. Os parâmetros hematológicos foram influenciados pela dieta. A ausência de fontes de proteína animal nas dietas resultou em menores valores de hematócrito e hemoglobina no sangue dos peixes. Conclui-se que dietas contendo fontes de proteína vegetal e animal melhoram o estado nutricional e de saúde do jundiá.

Palavras-chave: Glicogênio; Proteína vegetal; Hematócrito; *Rhamdia quelen*.

INTRODUÇÃO

The silver catfish (*Rhamdia quelen*) is a native fish from South America, with potential for intensive production in captivity. Some nutritional studies have been carried out with this species (GOULART et al., 2015; LAZZARI et al., 2006, 2008; PIANESSO et al., 2015; RODRIGUES et al., 2012; SCHNEIDER et al., 2020). Silver catfish is an omnivorous fish that requires lower levels of protein in the diet compared to carnivorous species. This is due to the omnivorous ability to use non-protein energy, which makes it possible to increase the efficiency of muscle protein synthesis (GOMES et al., 2000; MEYER; FRACALOSSI, 2004).

Fish meal is the most used source of protein in animal feed. However, it is a high-cost ingredient, with variable composition and limited availability (HARDY, 2010). High protein content and a good amino acid profile are also found in fish, poultry, pork, insect and cattle slaughter residues. These raw materials can be used partially or completely to replace fish meal without affecting animal performance (ABIMORAD et al., 2014; EL-SAYED, 1999; MACUSI et al., 2023; PEZZATO et al., 2002; ROSSATO et al., 2013).

Among plant protein sources, soybean meal and yeast (Saccharomyces cerevisiae) have nutritional characteristics that enhance their use as an alternative to protein ingredients of animal origin (COLDEBELLA; RADÜNZ NETO, 2002; LOSEKANN et al., 2008; MONTOYA-CAMACHO et al., 2019). However, the low palatability and rigid attraction of plant sources can decrease nutrient digestibility and affect amino acid availability for protein synthesis, as well as negative effects on fish performance and metabolism (DE LA HIGUERA et al., 1999; NRC, 1993).

The evaluation of the metabolic and hematological profile can indicate the nutritional and health status of fish submitted to experimental diets (DAL'BÓ et al., 2015; GOULART et al., 2015). The different ingredients may contain substances that can modify the normal metabolic-enzymatic state of the fish. Thus, the aim of this study was to evaluate the metabolic and hematological responses of silver catfish (*R. quelen*) fed diets containing protein sources.

MATERIAL AND METHODS

The study was carried out on the premises of the Pisciculture Laboratory of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. The fish were reared in a closed recirculating system equipped with biofilters and 18 tanks (280 L). The feeding trial was conducted during 90 days. They were used 30 fish (initial weight = 15.0±0.62 g; initial length = 11.98±0.35 cm) per tank from artificial spawning (São Carlos Fish Farming, Ibirubá, RS, Brazil). The initial stocking density was 1.6 g/L. Before the starting of the experiment (seven days), the fish were submitted to adaptation and profilatic treatment with sodium chloride (4 g/L) (MIRON et al., 2003).

Six diets composed by the mixture of different protein sources (Table 1): MBY - meat and bone meal + yeast; SY - soybean meal + yeast; S - soybean meal; MBS - meat and bone meal + soybean meal; FY - fish meal + yeast; FS - fish meal + soybean meal.

Fish fed twice a day (9:00 am and 5:00 pm), at apparent satiation. For diet manufacture, the ingredients were weighted, mixed, at completely homogenization. The following diets were moistened, pelleted in meat grinder and dried in the sun, reaching a maximum temperature of 58ºC, which was measured using a thermometer. After drying, the diets were ground and sieved to obtain beads of 1 mm in diameter. During the feeding trial, with the growth of fish the food was adjusted to size. In the 24 hours preceding the start of the experiment, all animals were fasted prior to and daily supply of food were removed all waste by siphoning.

Table 1 - Composition of experimental diets (g/kg)

Ingredient	Diets[1]					
	MBY	SY	S	MBS	FY	FS
Yeast (sugar cane)	280	332.7	-	-	259.2	-
Meat and bone meal	385.3	-	-	300	-	-
Fish meal	-	-	-	-	300.6	224.8
Soybean meal	-	332.7	685.3	320	-	300
Grannel corn	150	134.4	124.5	169.5	200	195
Wheat flour	80	100	100	80.3	150	180
Canola oil	74.5	70	60	100	60	70
Salt	10	10	10	10	10	10
Dicalcium phosphate	10	10	10	10	10	10
Vitamin/mineral mixture[2]	10	10	10	10	10	10
Antioxidant[3]	0.2	0.2	0.2	0.2	0.2	0.2
Nutrient	Composition (g/100 g)					
Crude protein[4]	34.67	32.14	33.03	34.45	32.92	31.92
Crude ash[4]	13.24	5.27	6.23	12.98	9.98	10.7
Crude lipid[4]	12.75	8.0	7.2	16.4	9.83	11.42
Crude fiber[4]	1.80	3.09	4.23	3.67	1.83	3.68
Moisture[4]	6.94	7.91	8.95	5.52	6.50	8.00
Calcium[4]	3.49	1.36	1.59	3.00	2.16	2.16
Phosphorus[4]	2.01	0.75	0.87	1.84	1.57	1.58
Digestible energy[5] (kJ/g)	14.30	13.28	12.92	15.18	13.67	13.59
Arginine[6]	1.79	1.84	2.54	2.15	1.70	2.11
Phenylalanine[6]	1.04	1.48	1.86	1.28	1.29	1.49
Histidine[6]	0.52	0.75	0.95	0.65	0.95	1.00
Isoleucine[6]	1.00	1.45	1.60	1.07	1.31	1.33
Lysine[6]	2.08	2.02	2.14	1.94	2.10	2.00
Leucine[6]	1.80	2.28	2.60	1.95	2.28	2.35
Methionine + cystine[6]	0.81	0.84	1.03	0.87	0.92	0.98
Threonine[6]	1.14	1.44	1.40	1.04	1.43	1.28
Tryptophan[6]	0.13	0.27	0.47	0.28	0.27	0.40
Valine[6]	1.32	1.61	1.72	1.31	1.59	1.55

[1]Diets: MBY: meat and bone meal + yeast; SY: soybean meal + yeast; S: soybean meal; MBS: meat and bone meal + soybean meal; FY: fish meal + yeast; FS: fish meal + soybean meal.
[2]Mixture composition (kg/product): Vit. A: 6000000 UI; Vit. B1: 1400 mg; Vit. B2: 3375 mg; Vit. B6: 4830 mg; Vit. B12: 5000 µg; Vit. C: 25000 mg; Vit. D3: 530000 UI; Vit. E: 22500 mg; Vit. K3: 5000 mg; Zinc: 40000 mg. Folic acid: 400 mg; Nicotinic acid: 14000 mg; Cobalt: 1500 mg; Pantothenic acid: 8000 mg; Copper: 15000 mg; Colin: 1500 mg; Iron: 50000 mg; Iodine: 700 mg; Manganese: 23000 mg; Selenium: 250 mg;

[3]Etoxiquin (32% etoxiquin; 18% propyl-galate; 50% inert).
[4]Analyzed values.
[5]DE = [(23.6kJ/g x %Protein x 0.9) + (39.8kJ/g x %Lipids x 0.85) + (17.2 kJ/g x %Cho x 0.5)]/100.
[6] Calculated values (Ajinomoto Biolatina Ind. e Com. Ltda., Brazil).

Every day there was some physical-chemical parameters of water: temperature, dissolved oxygen, pH, total ammonia, nitrite and alkalinity. To measure the temperature used the thermometer bulb with mercury for oxygen, a pulse oximetry; for pH, a digital pH meter, and for other analysis, a colorimetric kit (Alfa-Tecnoquímica, Florianópolis, SC, Brazil). The water quality parameters in the rearing system were: temperature (26.85±1.02ºC), dissolved oxygen (5.53±0.62 ppm), pH (7.30±0.26), total ammonia (0.50±0.22 ppm), nitrite (0.06±0.01 ppm) and alkalinity (48.96±13.40 mg $CaCO_3$/L). These values are within the range considered adequate for the creation of the species (GOMES et al., 2000).

After 90 days of experiment, nine animals per treatment were sacrificed by medullary puncture to collect biological material (liver, muscle and kidney). The collected tissues were stored and frozen in eppendorfs (-20ºC) for further analysis of metabolic intermediates. Glycogen in liver, muscle and kidney was determined according to Bidinotto; Moraes; Souza (1997) after addition of potassium hydroxide (KOH) hydrolysis and ethanol precipitation of the protein and glycogen. For protein analysis, tissues (liver, muscle and kidney) were heated to 100°C with KOH, centrifuged at 3000 rpm for 10 min and the supernatant used to estimate the total protein seconds Lowry et al. (1951). For determination of lactate and glucose, liver and muscle samples were first homogenized in trichloroacetic acid (TCA) 10% (1:20 dilution) using a Potter-type apparatus Elvejhem and centrifuged at 3000 rpm for 10 min. The supernatant, free of protein, was used for determination of lactate as Harrower; Harmon (1972) and glucose according to Park; Johnson (1949).

At the end of the 90 days of the experiment, for hematological analysis, nine fish were randomly kept from each treatment, where blood was collected from the caudal vessels in syringes (1.5 mL/fish). After this procedure, the blood was packed in small recipients and storage in ice, later taken for analysis. After the blood collection, fish were killed by severing the spinal cord. Before each sampling the animals were fasted 24 hours. The blood collected by puncturing the tail vein using ethylenediaminetetraacetic acid (EDTA) as anticoagulant was analyzed for eritograma (hematocrit, hemoglobin) in electronic cell counter Pentra 120. All the procedures of fish manipulations were in agreement with the ethics and animal welfare norms.

A completely randomized design was used, with six treatments and three replicates. The data were submitted to the normality test, after which the ANOVA was used. Means, when significant ($p<0.05$), were compared using Duncan's test. Values are expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Diets with insufficient amounts of protein or inadequate amino acid composition cause reduced growth and decreased feed efficiency due to mobilization of proteins in some tissues in order to maintain vital functions (HU et al., 2021; LIM et al., 2011; SCHNEIDER et al., 2020). In this study, a combination of different protein sources was performed in order to seek an adequate amino acid profile for the growth phase of the silver catfish, also seeking to analyze the metabolic and hematological behavior of the fish after consumption of the diets. Based on the performance data, it was concluded that the MBS and FS diets provided better growth for silver catfish in the rearing phase (LAZZARI et al., 2006, 2008).

Relating the performance of the animals with the variables of hepatic metabolism makes it possible to have a better understanding of the use of nutrients, since the liver acts by receiving and distributing nutrients from the digestive process (GOULART et al., 2015; LUNDSTEDT; MELO; MORAES, 2004). Glycogen is a reserve form of carbohydrate broken down into glucose for energy (HALVER; HARDY, 2002). In the present study, the glycogen concentration was not influenced by the diets (Table 2).

Table 2 - Biochemical changes in liver in *Rhamdia quelen* fed with different protein sources for 90 days

	Diets*					
	MBY	**SY**	**S**	**MBS**	**FY**	**FS**
Glycogen	4.25±2.2	4.20±0.31	3.72±0.34	3.55±0.21	4.38±0.34	3.37±0.44
Glucose	18.5±0.98^{bc}	16.4±0.85^{c}	19.9±1.2^{abc}	20.7±0.58^{ab}	22.2±0.91^{a}	18 ±0.97^{c}
Lactate	5.21±0.52^{ab}	4.22±0.53^{bc}	3.80±0.35^{c}	5.94±0.43^{a}	5.59±0.39^{a}	5.96±0.34^{a}
Protein	146.9±6.7^{a}	125.1±7.8^{b}	119.8±4.9^{b}	146.9±8.4^{a}	145.1±9.1^{a}	140.7±4.4^{ab}

*MBY: meat and bone meal + yeast; SY: soybean meal + yeast; S: soybean meal; MBS: meat and bone meal + soybean meal; FY: fish meal + yeast; FS: fish meal + soybean meal. Glycogen (µmol/g tissue); Glucose (mg/g tissue); Lactate (µmol/g tissue); Protein (mg/g tissue).
Values are mean ± SE (n=9). Different letters show significant differences between diets (p < 0.05).

Possibly, fish used similar amounts of this metabolite to keep glucose stored. In addition, energy production can come from amino acids, this occurs when there is an imbalance of nutrients in the diets (LUNDSTEDT; MELO; MORAES, 2004). Although the amino acid concentration was not evaluated, in this study, the highest levels of protein and glucose were observed in fish fed with the FY, MBY and MBS diets. Thus, it is possible to suggest that these diets provided sufficient energy intake, since there was a decrease in these metabolites (glucose, protein and lactate) in fish that received other diets, such as SY and S.

At the muscular level, the amount of glycogen was higher in fish fed with the SY, S and FY diets, that is, there was more energy stored (Table 3). Glucose and lactate levels were also higher on the MBS and SY diets compared to the FS diet. This may indicate excess energy and/or not using nutrients. Furthermore, fish fed soybean meal (S) had lower muscle protein content compared to the MBS diet. According to silver catfish zootechnical data Lazzari et al. (2006; 2008), these changes in the metabolic level in response to the MBS and FS diets may be occurring to keep the metabolism active, maximizing the use of nutrients for growth, since that these diets provided the best performance responses of silver catfish.

Table 3 – Biochemical changes in muscle in *Rhamdia quelen* fed with different protein sources for 90 days

	Diets*					
	MBY	**SY**	**S**	**MBS**	**FY**	**FS**
Glycogen	1.44±0.11[ab]	1.70±0.13[a]	1.71±0.09[a]	1.55±0.14[ab]	1.75±0.08[a]	1.24±0.05[b]
Glucose	0.49±0.028[ab]	0.50±0.06[a]	0.45±0.022[ab]	0.49±0.025[a]	0.45±0.036[ab]	0.38±0.016[b]
Lactate	27.41±3.23[a]	27.67±1.16[a]	24.18±1.96[ab]	26.94±1.01[a]	22.67±0.68[ab]	21.88±0.51[b]
Protein	110.92±2.04[bc]	117.52±5.25[bc]	109.61±4.98[c]	137.17±1.62[a]	126.65±4.18[ab]	124.13±7.43[ab]

*MBY: meat and bone meal + yeast; SY: soybean meal + yeast; S: soybean meal; MBS: meat and bone meal + soybean meal; FY: fish meal + yeast; FS: fish meal + soybean meal. Glycogen (µmol/g tissue); Glucose (mg/g tissue); Lactate (µmol/g tissue); Protein (mg/g tissue).
Values are mean ± SE (n=9). Different letters show significant differences between diets ($p < 0.05$).

The kidney is an important organ for the immune system of fish (HU et al., 2021). In this study, after 90 days of feeding, fish fed the S, MBS and FS diets had a lower amount of glycogen in the kidney compared to the MBY, SY and FY diets (Table 4). In addition, the SY diet has a lower protein content. Soybean meal present in the following diets (SY; S; MBS and FS) can cause digestive problems in fish depending on the level of inclusion and its relationship with other ingredients (HARDY, 2010).

The protein quality of the ingredients and the digestibility of the amino acids, as well as the presence of antinutrients are factors that can limit the growth of the fish (FRANCIS; MAKKAR; BECKER, 2001; JIANG et al., 2019). Thus, an assessment of metabolic parameters in fish serves as an indicator of nutritional status, and is also an important contribution to the nutritional management of these animals (GOULART et al., 2015).

Table 4 - Biochemical changes in kidney in *Rhamdia quelen* fed with different protein sources for 90 days

	Diets*					
	MBY	**SY**	**S**	**MBS**	**FY**	**FS**
Glycogen	1.72±0.12[a]	1.72±0.15[a]	1.24±0.08[b]	1.38±0.08[b]	1.75±0.04[a]	1.31±0.11[b]
Protein	102.26±3.27[ab]	95.81±3.93[b]	106.39±3.23[ab]	103.84±3.20[ab]	108.79±6.73[a]	110.92±2.58[a]

*MBY: meat and bone meal + yeast; SY: soybean meal + yeast; S: soybean meal; MBS: meat and bone meal + soybean meal; FY: fish meal + yeast; FS: fish meal + soybean meal. Glycogen (μmol/g tissue); (mg/g tissue).
Values are mean ± SE (n=9). Different letters show significant differences between diets ($p < 0.05$).

Blood allows answers about the health status of fish submitted to different diets. In the present study, the diet based on soybean meal (S) resulted in statistically lower values of hemoglobin, RBC, hematocrit, MCV and MCH in fish (Table 5). Likewise, a decrease in hematocrit and hemoglobin was observed in pufferfish (*Takifugu rubripes*) with 30% inclusion of soybean meal in the diet (LIM et al., 2011). The presence of antinutritional factors affects the hematological values and causes a worsening of the physiological health conditions of the fish (LIM et al., 2011; MARASCA et al., 2020). In addition, decreased values of all blood parameters may indicate that fish suffer from anemia (NRC, 1993; WITESKA et al., 2022). In summary, the inclusion of 68.5% of soybean meal negatively influenced the hematological parameters, resulting in lower growth and worse health status in silver catfish. However, the inclusion of hydrolyzed soybean meal in the diet for the species in question did not cause changes in blood parameters (UCZAY et al., 2019).

The combination of protein sources in the diets (MBY, MBS, FY and FS) resulted in higher hemoglobin levels and hematocrit (Table 5). A similar result was observed in tilapia (*Oreochromis niloticus*) that received diets containing up to 24% of replacement of soybean meal protein by meat and bone meal (inclusion of 11.5%) (ABIMORAD et al., 2014). Animal flours are rich sources of iron, and this mineral is part of the hemoglobin composition. Although these flours have variation in their composition, these flours have greater palatability (NRC, 1993; PEZZATO et al., 2002). However, silver catfish has omnivorous eating habits with a carnivorous tendency, which highlights the importance of these sources in their diet in relation to other omnivorous fish (RODRIGUES et al., 2012).

Table 5 - Hematological parameters in *Rhamdia quelen* fed different protein sources for 90 days

	Diets*					
	MBY	**SY**	**S**	**MBS**	**FY**	**FS**
HB	8.98±0.35[a]	7.12±1.02[b]	6.92±0.65[b]	8.42±0.43[a]	9.36±1.32[a]	8.74±0.80[a]
HT	28.68±1.11[a]	24.38±3.88[bc]	23.26±2.03[c]	27.62±1.07[ab]	30.16±3.70[a]	27.66±1.73[ab]
RBC	1.79±0.11[bc]	1.64±0.18[c]	1.69±0.19[bc]	1.73±0.07[bc]	2.07±0.22[a]	1.89±0.12[ab]
MCV	160.55±6.20[a]	148.02±9.04[abc]	139.38±8.59[c]	159.38±6.85[ab]	145.42±5.63[bc]	146.14±4.42[bc]
MCH	50.40±1.88[a]	43.34±1.58[cd]	41.24±3.93[d]	48.58±0.76[ab]	45.22±2.26[c]	46.22±2.19[bc]
MCHC	31.40±0.53[ab]	29.34±1.49[b]	29.74±2.12[ab]	30.54±1.72[ab]	31.08±1.08[ab]	31.60±0.91[a]

*MBY: meat and bone meal + yeast; SY: soybean meal + yeast; S: soybean meal; MBS: meat and bone meal + soybean meal; FY: fish meal + yeast; FS: fish meal + soybean meal. HB: (Hemoglobin. g/dL) HT: (Hematocrit. %); RBC: (Red Blood Cells. $10^6/mm^3$) MCV: (Mean Corpuscular Volume. fL); MCV (Mean Corpuscular Haemoglobin. pg); MCHC: (Mean Corpuscular Haemoglobin Concentration. g/dL).
Values are mean ± SE (n=9). Different letters show significant differences between diets ($p < 0.05$).

Elevated values of erythrocyte indices (VCM, HCM and CHCM) may be due to the release of the splenic erythrocyte reserve, highly correlated with oxygen supply (LAY; BALDWIN, 1999; WITESKA et al., 2022). In this study, the hematological parameters are similar to those found in other studies (DAL'BÓ et al., 2015; MARASCA et al., 2020; UCZAY et al., 2019), with the exception of fish fed a soybean meal diet (S). We noticed a difference in the amino acid profile between the experimental diets. However, diets combined with protein sources of animal origin and soybean meal showed a better amino acid profile estimated for the species (MEYER; FRACALOSSI, 2004). It should be noted that amino acid supplementation is a promising method to increase the nutritional value of diets in which fish meal is replaced by vegetable protein sources (JIANG et al., 2019; LIM et al., 2011; SCHNEIDER et al., 2020).

CONCLUSION

In conclusion, the nutritional and health status of silver catfish improved with diets containing soybean meal combined with proteins of animal origin – meat and bone meal (MBS) and fish meal (FS). Soybean meal (S) based diet worsens metabolism and causes anemia in fish.

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