



ANIMAL SCIENCE

Growth performance, plasma and hepatic biochemistry of jundiá *Rhamdia quelen* fed dephytinized rice bran protein concentrate

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Abstract: A 45-day feeding assay was carried out to evaluate the effects of crescent levels of dephytinized rice bran protein concentrate (DRBPC) on growth performance, nutrient deposition, plasma and liver parameters of jundiá *Rhamdia quelen*. Five experimental diets were formulated with inclusion of 0 (control), 10, 15, 20, and 30% of DRBPC. In total 500 jundiás (initial body weight 6.28 ± 0.12 g) were allocated in 20 tanks (230 L) to give four groups for each treatment. Fish were fed to apparent satiation for 45 days. Weight gain and specific growth rate were evaluated by cubic regression analysis ($P < 0.05$) and displayed maximal growth on the inclusion level of 25% of DRBPC. The results indicated that fish fed DRBPC15 and DRBPC30 had lower body protein deposition and hepatosomatic index compared to CONTROL diet, respectively. No significant differences ($P > 0.05$) were assessed in plasma parameters. The alanine aminotransferase activity was higher in fish fed DRBPC30 compared to CONTROL group. The present study has demonstrated that DRBPC displayed significant nutritional quality for the jundiá. Thus, this new ingredient could be included as a protein source in fish for minimizing the use of fish meal.

Key words: hepatic metabolism, nutrient deposition, phytic acid, protein concentrate, rice bran.

INTRODUCTION

Protein is the most important component in aquaculture feed. The most protein source used in aquaculture is fish meal, for containing essential nutrients and due to balanced amino acid profile (Fernandez Gimenez et al. 2009, Nguyen et al. 2009).

On the other hand, the irregular production and the high price of fish meal have made it complicated to answer the growing production requirement of the aquatic feeds industry. This fact encourages the sector to explore the use of different protein sources in substitution of fish meal (Tusche et al. 2012).

For this reason, finding alternative protein sources to minimize the use of fish meal in fish diets has become a worldwide priority (Nguyen et al. 2009). Publications of the National Research Council (NRC 2011) emphasize the need to replace fish meal by plant-based products in fish feeds. Vegetable raw materials are the main options for a continuous feed supply with high-quality nutrients (Tusche et al. 2012).

Vegetal feedstuffs, such as defatted rice bran (DRB) obtained after the rice oil extraction, have been demonstrated to be a useful alternative for monogastric nutrition due to its high protein content (Palmegiano et al. 2006). However, the

presence of phytic phosphorus (Walter et al. 2008) causes a decrease on the mineral absorption and, consequently, on the the digestibility of ingredients (Rasid et al. 2017, Liu et al. 2014). The phytic acid extraction from rice bran can be used as an option for DRB utilization on fish feed. In this process, two different products are obtained: the phytate, used in the food industry, as an antioxidant substance (Walter et al. 2008), and the dephytinized and defatted rice bran (DDRBR), which it has been studied due to its better availability of nutrients and low quantities of complexed phosphorus (Ferreira et al. 2013). The inclusion of this ingredient in fish feed could be an alternative for using this residue generated by the industry and corroborating for solve the problems with the incorrect disposal in the environment. Aiming to improve the levels of protein from vegetable sources, methodologies for protein concentration have been used as an alternative for increasing the crude protein levels, balancing the amino acid profile, and reducing the antinutritional factors (Lovatto et al. 2017a).

Some plant protein concentrates have been investigated in diets for different fish species, such as pea protein concentrate for rainbow trout (Zhang et al. 2012) and gilthead sea bream (Sánchez-Lozano et al. 2011), soy protein concentrate for Atlantic cod (Hansen et al. 2007) and canola protein concentrate for rainbow trout (Collins et al. 2012). For our knowledge, up to now, there has been no research on the effects of the inclusion of this new ingredient on fish feeding.

In Brazil, there are some native fish species that can be used for fish farming. One of them is jundiá *Rhamdia quelen*, an omnivorous specie (Salhi et al. 2004, Meyer & Fracalossi 2004) that adapts easily to diets with high concentration of vegetable ingredients, which reduces production cost (Lovatto et al. 2014, Goulart et al. 2013). Then,

the present study aimed to evaluate the effects of crescent levels of DRBPC inclusion on the growth performance and metabolic responses of jundiá *Rhamdia quelen*.

MATERIALS AND METHODS

Obtention of DRBPC

The DRBPC was obtained from the dephytinized and deffated rice bran provided by Indústria Gaúcha de Alimentos Ltda. (INGAL) (Santa Maria, RS, Brazil). The protein concentration methodology was developed at the Fish Farming Laboratory, Department of Animal Science of the Federal University of Santa Maria (Santa Maria, RS, Brazil). The company supplied the wet residue and, first, the ingredient was dried in a forced-air-drying oven at 50°C for 24 hours, and ground in a laboratory mill. The obtention of DRBPC was based on a chemical-enzymatic process.

Initially, the raw material was dispersed in aqueous medium at a ratio 1:10 (w/v) and the sample was mixed for 3 minutes using a magnetic stirrer. Protein solubilization occurred by increasing the pH of the aqueous solution to 11.0 with 1N NaOH. The sample was remained under agitation and heating (60°C for 15 minutes). Subsequently, the pH was reduced to 6.0 with 1N HCl 7.5% (v/w) and α amylase enzyme was added, and the sample was incubated for 60 min. Afterwards, the pH was adjusted to 4.5 with 1 N HCl with constant stirring for 15 minutes. Subsequently, the sample was filtered a 106 μ m sieve and the fraction retained in the sieve was discarded. The liquid fraction was centrifuged and dried in a forced-air-drying oven at 50°C for 24 hours (Loureiro et al. 2019). The proximate composition of the protein sources used in the present study (fish meal and DRBPC) is presented in Table I.

Table I. Proximate composition and amino acid profiles of FM and DRBPC.

| Content (% dry matter) | FM ¹ | DRBPC ² |
|---|-----------------|--------------------|
| Dry matter | 95.3 | 93.62 |
| Crude protein | 58.39 | 26.80 |
| Ash | 25.58 | 6.29 |
| Fat | 10.55 | 6.12 |
| Amino acid profile ³ (g AA 100 g protein ⁻¹) | | |
| Lysine | 3.12 | 1.14 |
| Arginine | 4.00 | 2.33 |
| Threonine | 2.36 | 1.28 |
| Tyrosine | 1.35 | 0.92 |
| Valine | 2.44 | 1.64 |
| Methionine | 1.37 | 0.51 |
| Cysteine | 0.19 | 0.25 |
| Isoleucine | 1.75 | 1.00 |
| Leucine | 3.43 | 2.12 |
| Phenylalanine | 1.88 | 1.25 |
| Histidine | 0.96 | 0.57 |
| Aspartic acid | 2.58 | 1.90 |
| Glutamic acid | 6.25 | 3.81 |
| Serine | 2.23 | 1.42 |
| Proline | 4.64 | 1.50 |
| Glycine | 6.44 | 1.56 |
| Alanine | 4.21 | 1.84 |
| Digestible energy ⁴ (MJ kg ⁻¹) | 16.00 | 8.88 |

¹Waste tilapia flour obtained from agro-industrial cooperative of Fish Farming (COPISCES – Cooperativa Agroindustrial de Piscicultura), Toledo, Paraná State, Brazil
²DRBPC was developed at the Laboratory of Fish Farming of the Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul State, Brazil

³Analyzed at the Protein Sources Laboratory (Laboratório de Fontes Proteicas (LaFoP) of the State University of Campinas (UNICAMP), Campinas, São Paulo State, Brazil

⁴Digestible energy: calculated digestible energy: [(Crude protein×5.65×0.85) + (Fat×9.4×0.9) + (Carbohydrates×4.15×0.7)] (adapted from Meyer & Fracalossi 2004)

Diet preparation

Five isonitrogenic (370 g kg⁻¹ crude protein) and isoenergetic (13.4 MJ kg⁻¹) experimental diets were formulated with crescent levels of inclusion of DRBPC: 0% (CONTROL), 10% (DRBPC10), 15% (DRBPC15), 20% (DRBPC20), and 30% (DRBPC30) (Table II).

The experimental diets were prepared according to the crude protein and amino acid requirements for jundiá established by Meyer & Fracalossi (2004) and Montes-Girao & Fracalossi (2006), respectively. The ingredients were ground, weighed, and mixed manually until complete homogenization. Water was added and diets were extruded in an EX-MICRO laboratory micro extruder (Loureiro et al. 2019). The extruded (4 mm) was dried in a forced-air-drying (50°C for 24 hours) and stored at -18°C until used (Loureiro et al. 2019). Proximate composition, formulation, and amino acid profiles of diets are shown in Table II.

Fish and feeding

The trial was conducted at the Laboratory of Fish Farming, Department of Animal Science of the Federal University of Santa Maria (UFSM) (Santa Maria, RS, Brazil), after approved by the UFSM's Ethics Committee on Animal Trials under number 1586211015.

A total of 500 jundiá with initial average weight of 6.28 ± 0.12 g, were randomly distributed into 20 polypropylene tanks (230 L) at a density of 25 fish per tank (four tanks per treatment). Seven days before the beginning of the experimental period, jundiá were adapted to the recirculation system and the experimental diets. For 45 days, jundiá were fed to apparent satiation three times a day at 9:00 a.m, 1:00 p.m. and 5:00 p.m. (Lovatto et al. 2017b). Twice daily, siphoning were performed for removal of waste debris.

Table II. Ingredients, proximate composition, and essential amino acid content of the experimental diets (% dry matter).

| Contents | Experimental diets | | | | |
|---|--------------------|---------|---------|---------|---------|
| | CONTROL | DRBPC10 | DRBPC15 | DRBPC20 | DRBPC30 |
| Ingredients (g kg ⁻¹) | | | | | |
| DRBPC | 0 | 100 | 150 | 200 | 300 |
| FM | 400 | 354 | 331 | 308 | 262 |
| SPC ^a | 222.7 | 222.7 | 222.7 | 222.7 | 222.7 |
| Starch | 196.6 | 186 | 181 | 175.4 | 120 |
| Soy oil | 30 | 30 | 30 | 30 | 46.5 |
| Mix vitamin/mineral ^b | 30 | 30 | 30 | 30 | 30 |
| Dicalcium phosphate | 10 | 10 | 10 | 10 | 6 |
| Monosodium glutamate | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| BHT ^c | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Limestone calcitic | 10 | 10 | 10 | 10 | 10 |
| Inert ^d | 97.9 | 54.5 | 32.5 | 11.1 | - |
| Proximate composition (g kg ⁻¹) | | | | | |
| Crude protein ^e | 370.1 | 370.1 | 370.1 | 370.1 | 370 |
| Fat | 77.6 | 78.8 | 79.5 | 79.9 | 79.6 |
| Dry matter ^e | 959.4 | 963 | 961.2 | 960.2 | 957.9 |
| Digestible energy (MJ kg ⁻¹) ^f | 13.4 | 13.4 | 13.4 | 13.4 | 13.4 |
| Ash ^e | 253.9 | 207 | 186.1 | 164.3 | 138.4 |
| NDF ^{e,g} | 202 | 249 | 267.9 | 282.7 | 299.3 |
| NDSC ^h | 57.6 | 64.4 | 57.6 | 63.2 | 70.6 |
| Calcium ⁱ | 8.1 | 8.1 | 8 | 8 | 7.4 |
| Total phosphorus ⁱ | 7.2 | 7.1 | 7.1 | 7.1 | 6.3 |
| Amino acid ⁱ (% dry matter) | | | | | |
| Lysine | 2.67 | 2.64 | 2.62 | 2.61 | 2.58 |
| Arginine | 3.24 | 3.29 | 3.32 | 3.34 | 3.39 |
| Threonine | 1.85 | 1.87 | 1.88 | 1.89 | 1.91 |
| Tyrosine | 1.37 | 1.40 | 1.41 | 1.43 | 1.46 |
| Valine | 2.04 | 2.10 | 2.12 | 2.15 | 2.2 |

Table II. Continuation.

| | | | | | |
|-----------------------|------|------|------|------|------|
| Methionine + cysteine | 1.23 | 1.24 | 1.24 | 1.24 | 1.25 |
| Isoleucine | 1.75 | 1.77 | 1.78 | 1.79 | 1.81 |
| Leucine | 3.13 | 3.19 | 3.21 | 3.24 | 3.29 |
| Phenylalanine | 1.93 | 1.97 | 1.99 | 2.00 | 2.04 |
| Histidine | 0.97 | 0.98 | 0.99 | 1.00 | 1.01 |

^aSoybean protein concentrate (60% crude protein)

^bVitamin and mineral mixture (per kg of diet): folic acid, 997.50 mg; pantothenic acid, 9975.00 mg; biotin, 159.60 mg; cobalt, 39.90 mg; copper, 2800.00 mg; etoquin, 24.78 g; iron, 19.62 g; iodine, 120.00 mg; manganese, 5200.00 mg; niacin, 19.95 g; selenium, 119.70 mg; vitamin A, 1995000 UI; vitamin B1, 4987.50 mg; vitamin B12, 5985.00 mg; vitamin B2, 4987.50g; vitamin B6, 4987.50 mg; vitamin C, 70.00 g; vitamin D3, 198000.05 UI; vitamin E, 19950.00 UI; vitamin K, 997.50 mg; zinc, 28.00 g

^cButyl-hydroxy-toluene (antioxidant)

^dFine sand washed

^eAnalyzed – Laboratory of Fish Farming, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul State, Brazil

^fDigestible energy: calculated digestible energy: [(Crude protein × 5.65 × 0.85) + (Fat × 9.4 × 0.9) + (Carbohydrates × 4.15 × 0.7)] (adapted from Meyer & Fracalossi 2004)

^gNeutral detergent fiber (Van Soest et al. 1991)

^hNDSC (Neutral detergent soluble carbohydrate) = 1000 – (Crude protein + Ash + Fat + Neutral detergent fiber + moisture)

ⁱCalculated by analyzing the ingredients

Water quality

During the experimental period, the physical and chemical parameters of water were monitored. Daily, temperature ($23.63 \pm 1.76^\circ \text{C}$) was monitored with mercury bulb thermometer. Weekly, were determined dissolved oxygen ($6.47 \pm 0.29 \text{ ppm}$), pH (7.5 ± 0.23), total ammonia ($0.08 \pm 0.04 \text{ mg L}^{-1}$) nitrite ($0.02 \pm 0.01 \text{ mg L}^{-1}$) alkalinity ($43.50 \pm 5.79 \text{ mg L}^{-1} \text{ CaCO}_3$) and hardness ($44.17 \pm 11.14 \text{ mg L}^{-1} \text{ CaCO}_3$) of water recirculation system, by the colorimetric method using the Alfacit® colorimetric kit. The measured parameters were in agreement with the optimal levels for cultivation of jundiá (Baldisserotto & Silva 2004).

Sample collection and analytical methods

At the end of the trial (45-day), fish were in fasted for 24 hours and anesthetized with benzocaine (100 mg L^{-1}) according to the American Veterinary Medical Association (AVMA 2013). Afterwards, fish were weighed (g) and measured (cm) individually to estimate the following parameters:

Weight gain (WG, %) = (final weight - initial weight × 100 / initial weight

Specific growth rate (SGR, %/day) = $100 \times [\ln(\text{final body weight}) - \ln(\text{initial body weight})] / \text{days of experiment}$

Feed conversion rate (FCR) = total feed consumption (g) / (final fish weight (g) – initial fish weight (g))

Survival rate (SR, %) = $100 \times \text{final fish number} / \text{initial fish number}$

Three fish were randomly selected from each tank (12 animals per experimental diet) and euthanized by overdose of benzocaine (250 mg L^{-1}) in accordance with the American Veterinary Medical Association (AVMA 2013) for determining nutrient retention:

Protein efficiency ratio (PER) = body weight gain (g) / protein intake (g).

Body protein deposition BPD (g) = [final weight × (% final body protein / 100)] – [initial weight × (% initial body protein / 100)].

Body fat deposition BFD (g) = [final weight × (% final body fat / 100)] – [initial weight × (% initial body fat / 100)].

Crude protein content was determined by the micro-Kjeldahl method (method 960.52) using the N X 6.25 factor (AOAC 2000) and fat

was measured according to Bligh & Dyer (1959) method.

Blood sampling and analysis

At the end of the experimental period, after 24 hours of fasting, three fish per tank (12 fish per experimental diet) were used for analysis of plasma parameters. Blood samples were collected by puncture in the tail vein with prefilled heparin syringes and placed in refrigerated micro centrifuge tubes for plasma separation by centrifugation (1000 x g for 10 minutes). Plasma was stored and refrigerated (-20°C) for total proteins, glucose, and albumin analyzes. The quantification was carried out using a colorimetric commercial kit (Doles®) (Doles Reagents and Laboratory Equipment Ltda., Goiânia, GO, Brazil). The free amino acid level was determined by Spies (1957) method.

Hepatic biochemistry analysis

After the blood sample collection, fish were euthanized by overdose of benzocaine (250 mg L⁻¹) (AVMA 2013). Subsequently, animals were eviscerated and the liver was removed to calculate the hepatosomatic index:

$$(\text{HSI } \%) = (\text{weight of the liver} / \text{weight of the whole fish}) \times 100$$

Then, the liver samples were frozen at -20°C for analyses of biochemical parameters. Liver glycogen levels were determined according to Bidinotto et al. (1997). The samples were weighed (50 mg) and KOH and ethanol (1 and 3 mL, respectively) were added for glycogen hydrolysis and precipitation. For protein analysis, the samples were heated at 100°C with KOH and centrifuged (1000 x g for 10 minutes). Supernatant was used to determine the total protein level according to the method described by Bradford (1976), using bovine serum albumin as standard. For hepatic ammonia analysis, tissue samples were homogenized by adding 10% trichloroacetic

acid and centrifuged (1000 x g for 10 minutes) for protein flocculation. Hepatic ammonia was measured according to the method described by Verdouw et al. (1978), based on the ammonia reaction with phenol and hypochlorite forming a blue-colored indophenol compound. For the free amino acid content, samples (50 mg) were homogenized by adding 1 mL of a phosphate buffer (20 mM, pH 7.5) and centrifuged (1000 x g for 10 minutes). Then, the supernatant extract was used to determine amino acid concentration by colorimetry (Spies 1957), using 1.5% ninhydrin solution in isopropyl alcohol as the color reagent. Activity of the alanine aminotransferase (ALAT) enzyme was measured using colorimetric procedures following the protocols described in the kits (Doles®).

Statistical analysis

Initially, data were checked for outlier existence. The experimental design was completely randomized with five treatments and four replications. Data were analyzed using one-way ANOVA using the Dunnett test for comparing means among treatments and control group. The SGR and WG results were analyzed by cubic regression analysis. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Growth, nutrient deposition, and hepatosomatic index

During the trial, no mortalities were observed (SR = 100%). At the end of the experimental period, WG and SGR parameters (Figure 1) were fitted for cubic regression ($P = 0.003$ and $P = 0.004$, respectively). Compared to the control group, there was a reduction of about 20% and 16.66% in the weight gain of fish fed diets containing 10% and 15% of DRBPC, respectively. Regarding the specific growth rate, there was a reduction

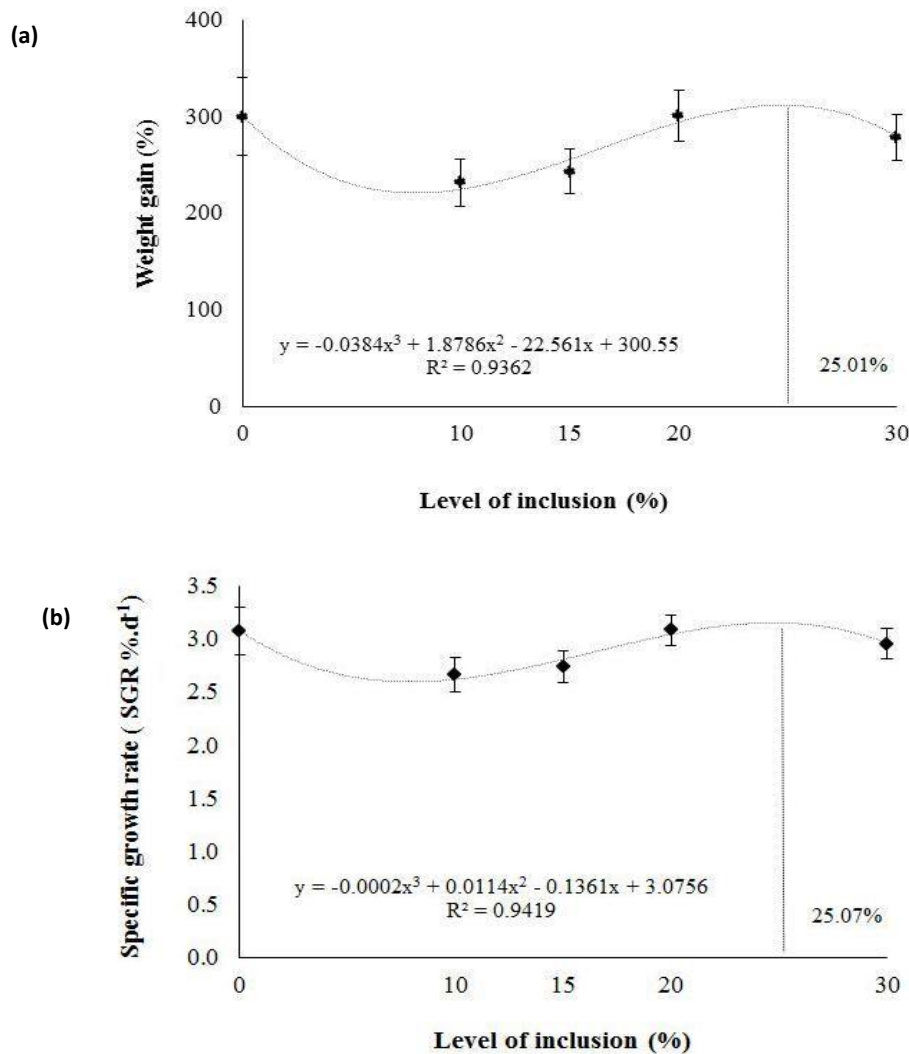


Figure 1. Cubic regression analysis for weight gain (a) and specific growth rate (b) of jundiá (initial average weight, 6.28 ± 0.12 g) fed for 45 days diets formulated with crescent levels of DRBPC.

of 14.82% and 11.68% of the jundiás of the treatments DRBPC10 and DRBPC15, respectively. Jundiá fed DRBPC15 presented lower ($P < 0.05$) BPD. Fish fed DRBPC30 showed smaller HSI and PER compared to fish fed CONTROL diet. No significant differences ($P > 0.05$) were found for FCR and BFD parameters compared to CONTROL group (Table III).

Plasmatic biochemistry

The effects on the plasma parameters of jundiá fed crescent levels of DRBPC are shown in Table

IV. After the 45-day feed trial, no statistical differences ($P > 0.05$) were observed for all plasmatic parameters evaluated – proteins, glucose, albumin, and free amino acid.

Hepatic metabolism

There were no significant differences ($P > 0.05$) for the hepatic parameters evaluated - free amino acid, total protein, glycogen, and ammonia. However, ALAT activity was significantly higher ($P < 0.05$) in fish fed DRBPC30 compared to CONTROL group (Table V).

Table III. Zootechnical indexes and nutrient retention of jundiá fed for 45 days diets formulated with crescent levels of DRBPC.

| Growth indexes | Experimental diets | | | | |
|----------------------|--------------------|-------------|--------------|-------------|--------------|
| | CONTROL | DRBPC10 | DRBPC15 | DRBPC20 | DRBPC30 |
| SR (%) ^a | 100 | 100 | 100 | 100 | 100 |
| FCR ^b | 1.36 ± 0.06 | 1.36 ± 0.02 | 1.43 ± 0.09 | 1.37 ± 0.11 | 1.43 ± 0.13 |
| PER (g) ^b | 1.26 ± 0.51 | 1.20 ± 0.14 | 1.15 ± 0.41 | 1.26 ± 0.69 | 1.10 ± 0.79* |
| BPD (g) ^b | 2.30 ± 0.22 | 2.17 ± 0.17 | 1.68 ± 0.37* | 2.18 ± 0.46 | 2.40 ± 0.40 |
| BFD (g) ^b | 1.39 ± 0.28 | 1.19 ± 0.19 | 1.08 ± 0.15 | 1.58 ± 0.32 | 1.16 ± 0.29 |
| HSI (%) ^b | 2.19 ± 0.06 | 2.18 ± 0.09 | 2.02 ± 0.22 | 1.94 ± 0.20 | 1.88 ± 0.12* |

Values are expressed as means ± standard deviation (n=100)^a; (n =12)^b. Values in the same row with asterisks (*) differ significantly from the control by Dunnett's test (P < 0.05).

Table IV. Plasma parameters of jundiá fed for 45 days diets formulated with crescent levels of DRBPC.

| Contents | Experimental diets | | | | |
|----------------------------------|--------------------|--------------|---------------|---------------|---------------|
| | CONTROL | DRBPC10 | DRBPC15 | DRBPC20 | DRBPC30 |
| Free AA (mmol dL ⁻¹) | 2.84 ± 0.53 | 2.42 ± 0.29 | 2.61 ± 0.32 | 2.78 ± 0.44 | 2.96 ± 0.48 |
| Protein (g dL ⁻¹) | 3.35 ± 0.18 | 3.36 ± 0.61 | 3.68 ± 0.22 | 3.68 ± 0.13 | 3.85 ± 0.41 |
| Albumin (g dL ⁻¹) | 0.65 ± 0.18 | 0.78 ± 0.37 | 0.68 ± 0.49 | 0.56 ± 0.16 | 0.52 ± 0.12 |
| Glucose (mg dL ⁻¹) | 46.41 ± 10.96 | 39.06 ± 5.66 | 59.59 ± 15.93 | 58.53 ± 13.23 | 49.86 ± 10.28 |

Values are expressed as means ± standard deviation (n =12).

Table V. Hepatic parameters of jundiá fed for 45 days diets formulated with crescent levels of DRBPC.

| Contents | Experimental diets | | | | |
|----------------------------------|--------------------|--------------|--------------|--------------|---------------|
| | CONTROL | DRBPC10 | DRBPC15 | DRBPC20 | DRBPC30 |
| Free AA (μmol g ⁻¹) | 119.2 ± 1.55 | 118.5 ± 2.11 | 123.6 ± 2.51 | 135.8 ± 3.26 | 138.9 ± 2.82 |
| Protein (mg g ⁻¹) | 68.1 ± 1.86 | 72.9 ± 2.63 | 69.4 ± 3.33 | 75.2 ± 3.64 | 59.2 ± 1.46 |
| ALAT (UI mg ⁻¹) | 35.61 ± 5.81 | 35.82 ± 4.79 | 36.47 ± 3.92 | 39.75 ± 4.88 | 41.39 ± 3.01* |
| Glycogen (μmol g ⁻¹) | 5.72 ± 2.05 | 6.78 ± 0.83 | 6.34 ± 1.03 | 6.45 ± 2.14 | 6.23 ± 1.61 |
| Ammonia (mol g ⁻¹) | 6.35 ± 1.69 | 6.29 ± 2.06 | 7.07 ± 1.61 | 7.34 ± 1.15 | 7.60 ± 1.15 |

Values are expressed as means ± standard deviation (n =12). Values in the same row with asterisk (*) differ significantly from the control by Dunnett's test (P < 0.05).

DISCUSSION

The use of vegetable protein sources reduced the fish growth performance, with increasing the levels of fish meal substitution by whole soybean meal and corn gluten meal (Mundheim et al. 2004), concentrated rapeseed protein isolate (Nagel et al. 2012), soybean protein concentrate (Deng et al. 2006), corn gluten meal and wheat gluten meal (Pratoomyot et al. 2010), soybean meal (Ye et al. 2011), and rice protein concentrate (Güroy et al. 2013).

However, in the present study, the highest levels of inclusion of DRBPC (20 and 30%) did not cause adverse effects on growth performance (Figure 1), survival (Table III), and plasma parameters of jundiá (Table IV). For WG and SGR, the cubic regression analysis indicated that the level of inclusion of DRBPC in the diet for maximum growth was 25.01% and 25.07%, respectively (Figure 1). SGR was higher in fish fed DRBPC20 and DRBPC30 than those fed lower contents of inclusion. These results do not agree with other studies, which replaced fish meal by vegetable sources, in different species of fish, such as gilthead sea bream (*Sparus aurata*; Sánchez-Lozano et al. 2011), black seabream (*Acanthopagrus schlegelii*; Zhou et al. 2011), European seabass (*Dicentrarchus labrax*; Güroy et al. 2013), turbot (*Psetta maxima*; Bonaldo et al. 2011), and Atlantic salmon (*Salmo salar* L.; Pratoomyot et al. 2010).

In the present study, DRBPC30 caused a significant decrease on PER and HSI (Table III). On the other hand, the ALAT activity was significantly increased in this group (Table V) compared to CONTROL. These results can be attributed to lower digestibility of DRBPC regarding fish meal (data not show). In addition, it is suggested that the decrease in the hepatosomal index was due to a physiological adaptation of the animal to maintain body

weight, since the increase in ALAT assumes that these animals are in gluconeogenesis. Day & Gonzalez (2000) reported that the reduction of PER can be associated with a higher proportion of protein used in the catabolic processes (energy production) when compared to anabolic (protein synthesis), caused by higher replacement levels of animal protein by vegetable protein. Albrektsen et al. (2006) also noted a decrease on the HSI as the inclusion of vegetable protein sources in the experimental diets increased. Similar results for PER and HSI have also been reported in juvenile black seabream (*Acanthopagrus schlegelii*; Sun et al. 2015), juvenile turbot (*Psetta maxima* L; Nagel et al. 2012), turbot (*Scophthalmus maximus*; Xu et al. 2016), Japanese flounder (*Paralichthys olivaceus*; Ye et al. 2011), and rainbow trout (*Oncorhynchus mykiss*; Aksnes et al. 2006) fed different vegetable protein sources. Higher ALAT activity in fish fed DRBPC30 (Table V) suggests increased protein catabolism and hepatic gluconeogenic activity (Metón et al. 1999, Lovatto et al. 2015). Increased ALAT activity may indicate unexpected amino acid deficiencies in the diet, resulting in the use of proteins (oxidation of excess AA) to obtain energy, affecting protein synthesis (Melo et al. 2006). Fournier et al. (2004) observed an increase on the ALAT activity and the ammonia excretion correlated with the reduction of PER in juvenile turbot (*Psetta maximum*) fed crescent levels of vegetable protein sources (wheat gluten, lupine, corn gluten bran) as partial fish meal replacement.

In the present study, similar behavior was found as the DRBPC30 yielded greater ALAT activity, lower PER, and tendency to increase liver ammonia (7.60 ± 1.15 mol g⁻¹ tissue) compared to CONTROL diet (6.35 ± 1.69 mol g⁻¹ tissue). The PER and ammonia behavior was also reported in other studies, which included vegetable protein

sources as partial replacement for fish meal (Fournier et al. 2003, Gómez-Requeni et al. 2004).

Among the studied nutrient deposition parameters, there was a lower concentration BPD of fish fed DRBPC15 compared to CONTROL group (Table III). WG and SGR (Figure 1) followed the same trend and had lower values than the ones found with the CONTROL diet. The inclusion of vegetable protein sources in the diet might have caused a reduction in dietary energy available for protein synthesis, leading to slower growth and nutrients deposition (Gaber 2006). However, this behavior was not observed in other levels (10, 20, and 30%) of DRBPC inclusion compared to CONTROL diet.

The DRBPC improved the nutritional quality when aiming to reduce the use of fish meal. Based on the parameters evaluated in the present study (growth performance, plasma parameters, and hepatic metabolism) the inclusion of DRBPC in the diet is feasible up to the highest level tested (30%). According to the results obtained from the regression model tested for variables WG and SGR, we suggest that inclusion levels of 20 to 25% of DRBPC can be included in the diet of jundiás.

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