An Acad Bras Cienc (2024) 96(Suppl. 1): e20231333 DOI 10.1590/0001-3765202420231333
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Efficiency of protein combinations in diets for *Rhamdia quelen*: growth, digestive and metabolic biochemistry and nutrient deposition

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Abstract: This study was conducted to determine the best combination of protein sources in diets for jundiá, based on growth, metabolism, and nutrient deposition. Five protein combinations were tested: casein + fish meal (control), casein + gelatin, casein + albumin, casein + albumin + fish meal, and albumin + fish meal, in diets containing 370 g Kg^{-1} of crude protein and 13.4 MJ Kg^{-1} of digestible energy. The fish (9.38 ± 0.12 g) were allocated in a water recirculation system at a density of 3.35 g L^{-1} per experimental unit and fed until apparent satiety for 40 days with the diets. The fish fed with the control diet had the highest final weight, specific growth rate, protein and feed efficiency ratio, protein retention, and best apparent feed conversion. On the other hand, fish that received casein + albumin and albumin + fish meal diets showed worse results in growth and body protein retention, low trypsin and chymotrypsin activity, and high intestinal amylase activity. Therefore, the combination referred to as control (casein + fish meal) conclusively provides the best rhythm for nutrient digestion and metabolism processes, enabling fish to reach greater growth and retention of body protein with low whole-fish fat content.

Key words: feed formulation, digestive enzymes, growth performance, proximate chemical composition, nutrient retention efficiency, silver catfish.

INTRODUCTION

Many studies on the nutritional requirements of fish have been carried out with semi-purified diets, in which the protein base consists of casein and gelatin because of the rapid availability of amino acids and digestibility of these sources (Lovell 1998, Meyer & Fracalossi 2004, Montes-Girao & Fracalossi 2006, Terjesen et al. 2006, Ahmed & Ahmad 2020). Milk casein is a phosphoprotein that is capable of binding and transporting calcium phosphate. Therefore, it is composed of different fractions, such as α-, β-, and κ-casein (heterogeneous proteins), and its hydrolysis releases amino acids and other non-protein substances (Bhat et al. 2016). Non-polar amino acids represent 32–42% of the casein constitution, but the presence of phosphate molecules, carbohydrates, and sulfur amino acids confers the soluble character of the protein. Its biological value is high because of the high concentration of lysine and other essential amino acids in its constitution, as well as its high digestibility (> 90%) (Pires et al. 2006, Mendes et al. 2009, Bhat et al. 2016).

In contrast, gelatin is a fibrous protein derived from collagen that is soluble, has a simple structure that releases amino acids only by hydrolysis, and is usually rich in glycine when processed in an alkaline medium or an

acid medium rich in alanine. It is a protein considered rich in proline, hydroxyproline, lysine, and hydroxylysine but deficient in tryptophan, cysteine, and methionine (Djagny et al. 2001, Roman & Sgarbieri 2007). Albumin has a simple structure and is classified as a globular protein. With 91% protein and a digestibility similar to that of casein (Mendes et al. 2009), it has high levels of lysine, methionine, and tryptophan (Linden & Lorient 1996, Bacila 2003).

Although these sources have a high protein content and digestibility, it has been observed in some studies that specimens of *Rhamdia quelen* fed diets based on casein, gelatin, and synthetic amino acids, fail to express maximum growth, especially in weight gain, specific growth rate, and feed conversion (Meyer & Fracalossi 2004, Montes-Girao & Fracalossi 2006, Moro et al. 2010). One explanation for this limitation is the rapid absorption of free amino acids, peptides, or hydrolyzed proteins by enterocytes; whereas when intact proteins are used, they must be degraded by proteases, and the absorption process can occur more slowly and gradually (Tonheim et al. 2005, Champe et al. 2009). Thus, the rapid availability of amino acids can saturate the intestinal transport mechanism (antagonistic absorption), resulting in an imbalance in the uptake and oxidation of these amino acids, negatively affecting protein retention (Boirie et al. 1997, Berge et al. 1999, Cahu et al. 1999, Aragão et al. 2004, Bodin et al. 2012).

The solution to this problem may lie in the use of combinations of protein sources, including conventional semi-purified proteins (casein, gelatin, and albumin) and alternative intact proteins (e.g., fish meal) in the diet. Although animals require different digestion and absorption times for these sources, synchronization and longer availability of amino acids can occur and benefit protein deposition (Ambardekar et al. 2009, Bodin et al. 2012).

In this sense, fish meal produced from by-products of the fish processing industry is rich in protein, minerals, and fat and is very palatable to fish (Feiden et al. 2005). Protein digestibility coefficients between 67 and 90% were observed depending on the proximate composition of the meal and the fish species evaluated (Sampaio et al. 2001, Godoy et al. 2016). In addition, fish meal produced from fish by-products constitutes an alternative and sustainable protein source for aquaculture production, with production rates similar to those of traditional whole fish meal (Sotolu 2009, Mo et al. 2018, Hua et al. 2019).

Therefore, due to the differences in the availability of amino acids from different protein sources, as well as the possible synchronism regarding the absorption of amino acids in the digestive tract and their biological effects on *Rhamdia quelen*, this study aimed to evaluate the best combination of protein sources (casein, gelatin, albumin, and fish meal) to be used in diets to study the nutritional requirements of species, evaluating growth, digestive and metabolic parameters, and body deposition of nutrients in fish.

MATERIALS AND METHODS

Experimental diets

The experimental diets were formulated to contain the following sources constituting the protein base: casein + gelatin (CASGE), casein + albumin (CASALB), casein + albumin + fish meal (CASALBFM), and albumin + fish meal (ALBFM). The casein + fish meal diet was considered the CONTROL diet (Corrêia et al. 2019). The experimental diets were formulated to meet the nutritional requirements of juvenile jundiá approximately 370 g kg^{-1} of crude protein (CP) and approximately 13.4 MJ kg^{-1} of digestible

energy, according to Meyer & Fracalossi (2004). Prior to use, all feed ingredients were analyzed for proximate composition: dry matter, crude protein, and ash, according to AOAC (1995). Fat was extracted and quantified according to the method described by Bligh & Dyer (1959), neutral detergent fiber was quantified according to the methods outlined by Van Soest et al. (1991), and amino acids were quantified using high performance liquid chromatography (casein, gelatin, albumin) or near-infrared reflectance spectroscopy (fish meal) (Mycotoxicological Analysis Laboratory – LAMIC/UFSM). The analysis of calcium and phosphorus followed the protocol proposed by Tedesco et al. (1995). The data obtained were used as the basis for feed formulation. To prepare the diets, the dry ingredients were mixed manually until completely homogenized, and soybean oil and water were added shortly afterwards. The diets were pelleted, oven-dried at 50 °C for 24 h, crushed, placed in plastic bags, and stored in a freezer (–18 °C) until the fish were fed. The formulations and proximate compositions of the experimental diets are presented in Table I.

Ethics statement, fish farming and feeding trial

This study was submitted and approved by the Ethics Committee on Animal Experimentation of Universidade Federal de Santa Maria under number 103/2011. The experiment was conducted in a recirculating water system at the Fish Farming Laboratory of the Department of Animal Science, UFSM, Brazil. The recirculating water system consisted of 15 polypropylene tanks (70 L capacity) fitted with individual inlets and outlets connected to a closed water recirculation system equipped with mechanical and biological filtering and a Clarifier TetraPond®UV sterilizer (GreenFreeTMUV-2 18 W). The experimental jundiá consisted of 375 juveniles with an average initial weight of 9.38 ± 0.12 g. The jundiá were distributed into polypropylene tanks (25 animals per unit). Each of the five experimental diets was tested in triplicate. Prior to the feeding trial, the fish were acclimated to the experimental conditions for 20 days. During the experimental period, water temperature was measured daily with a mercury bulb thermometer (26.55 ± 0.15 ºC) and dissolved oxygen with pulse oximetry (550A, YSI, Yellow Springs, Ohio, USA) (5.94 ± 0.04 mg L^{-1}). The pH (7.02 ± 0.05), total ammonia (0.19 ± 0.02 mg L^{-1}), nitrite (0.11 ± 0.05 mg L^{-1}), alkalinity (40.67 ± 9.70 mg L^{-1} of $CaCO_3$) and hardness (44.67 ± 1.77 mg L^{-1} of $CaCO_3$) were measured weekly by colorimetric kits (Alfa-Tecnoquímica). According to Baldisserotto & Silva (2004), these parameters were within the optimum range for culturing *R. quelen*. During the experimental period, which lasted 40 days, the fish were fed three times a day (9:00 AM, 1:00 PM, and 5:00 PM) until apparent satiation. Daily siphoning was performed to remove waste debris (8:00 AM and 4:00 PM).

Sample collection and assessed variables

At the end of the experimental period, the fish were fasted for 12 h and anesthetized with benzocaine (30 mg L^{-1}) (Corrêia et al. 2021) to collect growth data. Based on the total number, weight and length measurements of fish in each tank, as well as the analysis of feed consumption, the following data were collected (according Fracalossi et al. 2012): final weight (g), total length (cm), condition factor (CF) = weight/(total length)^3 × 100; specific growth rate (SGR): [(ln (final weight) – ln (initial weight))/days] × 100, where ln = Neperian logarithm; apparent feed conversion (AFC): feed intake/weight gain; feed efficiency (FER) = gain in weight/dry matter consumption; and protein efficiency rate (PER) = weight gain (g) / ingested protein (g).

Blood samples were obtained from nine animals per treatment via tail vein puncture,

Table I. Formulation of the experimental diets (g Kg^{-1} dry matter basis).

	Treatments[a]				
Ingredients	**CONTROL**	**CASGE**	**CASALB**	**CASALBFM**	**ALBFM**
Casein[b]	223.50	348.00	100.00	150.00	-
Albumin:Dextrin (60:40)[c]	-	-	566.00	245.00	361.50
Commercial fish meal[d]	330.00	-	-	216.50	330.00
Gelatin[e]	-	97.50	-	-	-
L-lysine	-	0.32	-	-	-
DL-methionine	0.77	3.01	0.32	0.52	0.05
Maltodextrin[c]	25.00	267.20	63.40	187.50	117.00
Microcrystalline cellulose[b]	78.80	108.50	97.50	73.40	72.29
Coated vitamin C	0.50	0.50	0.50	0.50	0.500
Canola Oil	46.60	60.80	51.10	43.00	42.80
Cod liver Oil	20.00	20.00	20.00	20.00	20.00
Vitamins and minerals[f]	30.00	30.00	30.00	30.00	30.00
Calcitic limestone	-	14.00	11.00	1.00	-
Dicalcium phosphate	-	25.00	35.00	2.00	-
Iodized sodium chloride	5.00	5.00	5.00	5.00	5.00
Melbond®[g]	20.00	20.00	20.00	20.00	20.00
Butylated hydroxytoluene	0.20	0.20	0.20	0.20	0.20
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Chemical composition (g Kg^{-1} diet)					
Crude protein[h]	370.90	373.30	370.50	370.70	370.10
Lysine[i]	18.60	16.60	19.00	18.90	19.50
Methionine + Cystine[i]	13.70	13.70	13.70	13.70	13.70
Threonine[i]	14.60	13.60	14.50	14.40	13.70
Tryptophan[i]	3.70	3.80	6.70	4.90	4.90
Valine[i]	19.00	18.50	21.30	19.80	19.00
Isoleucine[i]	14.70	14.70	18.50	16.10	15.70
Leucine[i]	28.90	28.30	30.70	29.30	27.30
Phenylalanine[i]	12.60	9.80	12.50	12.70	13.20
Histidine[i]	8.70	9.80	12.50	10.10	9.70
Arginine[i]	15.20	15.80	18.30	16.90	20.00
Total essential amino acids	149.7	144.6	167.7	156.8	156.7
Digestible energy (MJ/kg^{-1})[j]	13.398	13.399	13.400	13.399	13.398
Ether extract[h]	95.10	88.80	84.30	86.50	93.00
NDF[k]	70.40	103.90	93.40	80.00	69.90
NDSC[l]	246.40	267.50	287.80	281.20	255.90

Table I. Continuation.

Mineral matter[h]	99.30	8.40	18.60	72.40	105.30
Calcium[i]	20.40	12.90	13.20	14.20	19.50
Phosphorus[i]	11.00	7.10	7.20	7.60	9.50
Ca/P ratio[i]	1.85	1.82	1.83	1.87	2.05

[a]Treatments: CONTROL: casein + fish meal; CASGE: casein + gelatin; CASALB: casein + albumin; CASALBFM: casein + albumin + fish meal; ALBFM: albumin + fish meal. [b]Synth®. [c]D.N.A. Design Nutrição Avançada. [d]Fish Waste Industry, Canoas/RS, Brazil. [e]APTI®. [f]Composition of vitamin and mineral mixture: (Mig Fish 1% inclusion/Mig Plus®): Folic acid: 300mg, Pantothenic acid: 3000mg, Glutamic acid: 1mg, Cobalt: 60mg, Copper: 1000mg, Choline: 102.120 mg, Iron: 5000mg, Biotin: 60 mcg, Iodine: 45mg, Manganese: 8000mg, Magnesium: 5%, Selenium: 60mg, Zinc: 14000mg, Vit.A: 1000UI, Vit. B1: 1500mg, Vit. B2: 1500mg, Vit. B6: 1500mg, Vit. B12: 2000mcg, Vit. C: 15000 mg, Vit. D: 240 UI, Vit. E: 10000 mg, Vit. K: 400 mg, Inositol 10000 mg, Niacin 9000 mg, antioxidant: 792 mg. [g]Calcium and magnesium lignosulfonate (binder and appetence factor) – Ligno Tech Brasil®. [h]Values based on the analysis of diets. [i]Calculation based on analysis of ingredients. [j]Digestible energy = [(CP × 5640 kcal/kg × 0.9) + (EE × 9510 kcal/kg × 0.85) + (NDSC × 4110 kcal/kg × 0.50)] (Jobling 1983). [k]NDF: neutral detergent fiber. [l]NDSC: neutral detergent-soluble carbohydrates = 100 - (moisture + crude protein + mineral matter + ether extract + neutral detergent fiber).

using heparinized syringes. These samples were centrifuged (1200 *g*/10 min) to obtain plasma for biochemical analysis. The levels of total circulating proteins (g dL^{-1}), albumin (g dL^{-1}), glucose (mg dL^{-1}), cholesterol (mg dL^{-1}), and triglycerides (mg dL^{-1}) in the plasma were determined using Doles® commercial kits. The concentration of free amino acids was determined according to the method described by Spies (1957).

After blood collection, the fish were euthanized by benzocaine overdose (250 mg L^{-1}) according to the American Veterinary Medical Association (AVMA 2013). The fish were dissected and the digestive tract and liver were sampled and weighed to determine the digestive somatic index (DSI): (weight of the digestive tract/weight of the whole fish) × 100 and the hepatosomatic index (HSI): (weight of the liver/weight of the whole fish) × 100. These tissues were stored at –20 °C until further enzymatic and metabolic analyses. Abdominal fat was removed and weighed to calculate the abdominal fat index (AFI): (weight of the abdominal fat/weight of the whole fish) × 100.

Analysis of digestive enzymes

The digestive tracts of the nine fish sampled from each treatment group were separated into the stomach and total intestine and weighed. Each section was ground (tissue/buffer ratio of 1:20) in a homogenizer (Turrax, MA 102, Marconi, Brazil). The homogenizing buffer solution contained 10 mM phosphate/20 mM Tris at pH 7.5 in 50% (v/v) glycerol. After centrifugation (1200 *g*/10 min), the supernatant was used as the enzyme source. Acid protease activity was measured in the stomach homogenate using casein as a substrate according to the methods described by Hidalgo et al. (1999). The assay was performed using 1.5% casein in 0.2 M KCl buffer at pH 1.8, as substrate, and the samples were incubated at 30 °C for 40 min. The reaction was terminated with 15% trichloroacetic acid solution, centrifuged for 10 min at 1000 *g*, and the optical density of the supernatant recorded at 280 nm.

Trypsin, chymotrypsin, amylase, and lipase activities in intestinal homogenates were determined. Trypsin activity was assayed with TAME (α-ρ-toluenesulfonyl-L-argininemethyl ester hydrochloride) as the substrate, and the extracts were incubated (25 °C) in 2 mL buffer (0.2 M Tris/0.01 $CaCl_2$) at pH 8.1 for 2 min. Chymotrypsin activity was assayed with BTEE (benzoyl L-tyrosine ethyl ester) as the substrate, and the extracts were incubated in 2 mL buffer (0.1 M Tris/0.1 $CaCl_2$) at pH 7.8 for 2

min. The activity of enzymes was recorded in a spectrophotometer (Biospectro®, SP220), at 247 and 256 nm, respectively, following the methodology described by Hummel (1959).

Amylase activity was determined using the modified Bernfeld protocol (1955). The enzyme assay was performed in 0.2M phosphate-citrate buffer, pH 7.0, 0.5% NaCl with a starch concentration of 2.5%. The reaction was stopped by adding Ba(OH)2 0.3N and ZnSO4 5%. The amount of starch hydrolyzed by the enzyme was determined using the methodology described by Park & Johnson (1949). The absorbance was recorded at 660 nm.

Lipase activity was measured according to the method described by Gawlicka et al. (2000). The reaction was incubated with 0.4 mM p-nitrophenyl myristate in 24 nM ammonium bicarbonate (pH 7.8) with 0.5% Triton X-100 at 30 °C for 30 min. The reaction was stopped with 10 mM NaOH and the optical density was followed at 405 nm. All samples were assayed in duplicate, and the readings were normalized using blank solutions. The protein content of the crude extracts was determined using the Bradford (1976) method, with bovine serum albumin as the standard.

Liver parameters

The liver was divided into 50 mg samples. Liver glycogen levels were determined according to the protocol described by Bidinotto et al. (1997) after the addition of potassium hydroxide (KOH) 6 N and ethanol 96° for the hydrolysis and precipitation of glycogen. For the protein analysis, the tissue was heated at 100 °C with KOH and centrifuged at 1000 *g* for 10 min, and the supernatant was used to estimate the total protein level according to the Bradford method (1976). Other tissue samples were homogenized by adding 10% trichloroacetic acid using a motor-driven Teflon pestle and centrifuged (1000 *g*/10 min) for protein flocculation. The completely deprotonated supernatant was used to determine soluble sugar (Park & Johnson 1949) and ammonia concentrations (Verdouw et al. 1978). To measure amino acids, liver samples were mechanically disrupted by adding 1 mL of phosphate buffer (20 mM, pH 7.5), and the homogenate was centrifuged at 1000 *g* for 10 min. Neutral supernatant extracts were used for colorimetric amino acid determination according to the method by Spies (1957) and to measure the concentration of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using colorimetric kits (Doles®).

Chemical analysis

For the analysis of proximate body composition, an initial sample of six fish and nine additional animals per treatment were obtained at the end of feeding. We used the standard methods prescribed by AOAC (1995) for moisture concentration determination (heating at 60 °C for 24 h and then at 105 °C for 12 h), mineral matter (heating at 550 °C for 4 h), and crude protein using the micro-Kjeldahl method (N × 6.25). The fat content was determined using the method described by Bligh & Dyer (1959).

Protein retention was calculated according to the following equation (Ma et al. 2016): PRE (%) = 100 × (Wt × Wtp – W0 × W0p) / (Wf × Wfp), where Wt = final weight (g), W0 = initial weight (g), Wtp = final body protein, W0p = initial body protein, Wf = amount of feed intake, and Wfp = crude protein in the diet.

Statistical analysis

Initially, the data were analyzed for outlier identification using the mean ± (2 × SD) criterion. The experimental design was completely randomized, with five treatments and three replicates. The data were subjected to the Shapiro–Wilk normality test and analysis of

variance (ANOVA), and the means were compared using Tukey's test (P < 0.05). Statistical analyses were performed using the Statistical Analysis System SAS® software version 8.2.

RESULTS

Growth performance and digestive indices

Based on the results represented in Table II, it is observed that the fish that received the CONTROL diet (casein + fish meal) presented significantly (P<0.05) the best performance results according to the following parameters: FW, TL, CF, SGR, AFC, PER and FER. However, the lowest growth performance (P<0.05) was observed in jundiás that received the CASALB (casein + albumin) diet. Fish fed diets containing casein + albumin + fishmeal (CASALBFM) and casein + gelatin (CASGE) as protein sources showed similar growth responses for the parameters FW, TL, SGR, AFC and FER.

Regarding digestive indexes, no significant differences (P>0.05) were observed for the variable somatic digestive index (DSI). The fish fed the control diet showed a lower hepatosomatic index (HSI) compared to the animals that received the CASGE diet, while the jundiás from the other experimental groups showed no differences between them for this variable. Regarding the abdominal fat index (AFI), animals fed the ALBFM diet had the highest index compared to fish fed the control diet.

Activity of digestive enzymes

Analysis of digestive enzymes of jundiás fed with different combinations of protein sources

Table II. Growth performance and digestive indexes of jundiá juveniles fed with different combinations of protein sources, after 40 experimental days.

Variable	Treatments					
	CONTROL	CASGE	CASALB	CASALBFM	ALBFM	P
IW (g)	9.87±0.11^{a}	9.83±0.29^{a}	9.74±0.14^{a}	9.89±0.03^{a}	9.8±0.04^{a}	0.9637
FW (g)	35.35±2.13^{a}	27.45±1.27^{b}	19.50±0.24^{c}	28.08±0.66^{b}	23.10±0.45^{bc}	<0.0001
TL (cm)	14.95±0.27^{a}	13.88±0.16^{bc}	12.59±0.04^{d}	14.25±0.14^{ab}	13.37±0.12^{c}	<0.0001
CF	1.05±0.005^{a}	1.02±0.009^{a}	0.97±0.004^{b}	0.96±0.01^{b}	0.96±0.01^{b}	0.0003
SGR (%/day)	3.19±0.14^{a}	2.56±0.11^{b}	1.71±0.03^{d}	2.62±0.05^{b}	2.13±0.04^{c}	<0.0001
AFC	1.02±0.02^{d}	1.27±0.01^{c}	2.12±0.07^{a}	1.39±0.02^{c}	1.77±0.05^{b}	<0.0001
PER	2.63±0.05^{a}	2.1±0.03^{b}	1.27±0.04^{e}	1.94±0.03^{c}	1.52±0.04^{d}	<0.0001
FER	0.98±0.02^{a}	0.78±0.01^{b}	0.47±0.01^{d}	0.72±0.01^{b}	0.56±0.01^{c}	<0.0001
DSI (%)	2.87±0.06	2.75±0.14	2.89±0.25	2.78±0.05	3.09±0.17	0.6
HSI (%)	2.3±0.13^{b}	3.11±0.20^{a}	2.41±0.27^{ab}	2.61±0.14^{ab}	2.51±0.17^{ab}	0.03
AFI (%)	2.19±0.24^{b}	2.89±0.29^{ab}	3.32±0.19^{ab}	2.58±0.26^{ab}	3.61±0.39^{a}	0.01

Values (mean ± standard error of mean, n=3 for growth parameters and n=9 for digestive indexes) with different letters in rows represent a significant difference by Tukey's test (P<0.05). Variables: IW: initial weight; FW: final weight; TL: total length; CF: condition factor; SGR: specific growth rate; AFC: apparent feed conversion expressed as dry matter intake; PER: protein efficiency ratio of dry matter intake; FER: feed efficiency ratio with dry matter intake; DSI: digestive somatic index; HSI: hepatosomatic index; AFI: abdominal fat index. Treatments: CONTROL: casein + fish meal; CASGE: casein + gelatin; CASALB: casein + albumin; CASALBFM: casein + albumin + fish meal; ALBFM: albumin + fish meal.

are presented in Table III. Greater acid protease activity (P<0.05) was observed in the fish fed the CASGE diet. The lowest activity (P<0.05) of this enzyme was observed in juvenile jundiá fed the CONTROL diet. For alkaline proteases, greater enzymatic actions (P<0.05) of trypsin and chymotrypsin were observed in fish that consumed the CASALBFM diet. The diet that resulted in lower action (P<0.05) of the trypsin enzyme in jundiás was CASALB, similarly, lower activity of the chymotrypsin enzyme was observed in fish from this group, in addition to the CASGE and ALBFM treatments. Higher amylase activity was observed in juveniles treated with the CASALB, CASALBFM, and ALBFM diets, and less amylase activity was observed in those fed the CONTROL diet. Finally, lipase activity was higher in fish that consumed the CONTROL and ALBFM diets and lower in those fed the CASALBFM diet.

Plasma and liver parameters

There was no change (P>0.05) in the concentration of proteins and amino acids in the plasma of fish fed the different diets (Table IV). However, the concentration of albumin was higher (P<0.05) in fish fed the ALBFM diet than in those fed the other treatments (Table IV). Glycemia was higher (P<0.05) in the fish fed the CONTROL diet than in those fed the other treatments. The concentration of triglycerides was higher (P<0.05) in fish that consumed the CASALBFM and ALBFM diets and lower in fish that consumed the CASGE diet. Fish fed the ALBFM diet showed a higher (P<0.05) plasma cholesterol content. Feeding with the CASGE diet resulted in lower (P<0.05) levels of total cholesterol in jundiá juveniles (Table IV).

In the liver, no differences (P>0.05) were observed in the concentrations of protein, ammonia, glycogen, glucose, and ALT activity in fish fed the different diets. Amino acid content was higher (P<0.05) in fish from the CONTROL and CASALB treatments than in those fed the CASALBFM and ALBFM diets (Table IV). Higher AST activity (P<0.05) was observed in fish fed the CASGE, CONTROL, and ALBFM diets and lower in those fed the CASALBFM diet.

Body composition and nutrient deposition

The body composition of the fish at the end of the experiment revealed a higher percentage (P<0.05) of ash in juvenile jundiá that received the CONTROL, CASALBFM, and ALBFM treatments,

Table III. Digestive enzymes and biochemical parameters of jundiá juvenile fed different combinations of protein sources, after 40 experimental days.

Variables	Treatments					
	CONTROL	CASGE	CASALB	CASALBFM	ALBFM	P
Acid protease	0.36 ± 0.01^{b}	0.48 ± 0.05^{a}	0.38 ± 0.02^{ab}	0.40 ± 0.02^{ab}	0.39 ± 0.02^{ab}	0.03
Trypsin	15.44 ± 0.76^{abc}	16.18 ± 1.17^{ab}	12.57 ± 0.65^{c}	17.33 ± 0.96^{a}	13.47 ± 0.58^{bc}	0.002
Chymotrypsin	11.67 ± 0.60^{ab}	10.54 ± 0.36^{b}	10.18 ± 0.35^{b}	12.65 ± 0.27^{a}	10.67 ± 0.46^{b}	0.001
Amylase	0.96 ± 0.13^{b}	1.71 ± 0.13^{ab}	1.87 ± 0.19^{a}	1.94 ± 0.30^{a}	1.83 ± 0.2^{a}	0.007
Lipase	18.38 ± 2.15^{a}	16.32 ± 1.08^{ab}	16.80 ± 1.2^{ab}	11.21 ± 1.83^{b}	21.07 ± 0.96^{a}	0.001

Values (mean ± standard error of mean, n=9) with different letters in rows represent a significant difference by Tukey's test (P<0.05). Variables: Acid protease: μg tyrosine min^{-1} mg $protein^{-1}$; trypsin: μmol TAME min^{-1} mg $protein^{-1}$; chymotrypsin: mmol BTEE min^{-1} mg $protein^{-1}$; amylase: μmol glucose min^{-1} mg $protein^{-1}$; lipase: μmol p-nitrophenil myristate min^{-1} mg $protein^{-1}$. Treatments: CONTROL: casein + fish meal; CASGE: casein + gelatin; CASALB: casein + albumin; CASALBFM: casein + albumin + fish meal; ALBFM: albumin + fish meal.

whereas the fish in the CASGE treatment had lower ash content in the carcass (Table V). The percentage of fat was higher (P<0.05) in the fish that received the CASALB diet and lower in the fish from the CONTROL and CASALBFM treatments. The values of moisture and protein in the fish carcasses did not change (P>0.05) according to the treatments (Table V).

Regarding protein retention, the results followed the same response as the growth data shown by the fish, in decreasing order: CONTROL > CASGE = CASALBFM > ALBFM > CASALB (Table V).

DISCUSSION

The CONTROL diet (casein + fish meal) provided superior growth results in jundiá juveniles. These results may be attributed to the different digestion times of these protein sources because casein (semi-purified protein) is more quickly digested, while fish meal (intact protein) is more slowly digested, which results in synchronization and a steadier pace of the availability of amino acids that optimize performance (Ambardekar et al. 2009). A similar response was observed by Cahu et al. (2004)

Table IV. Biochemical parameters of jundiá juvenile fed different combinations of protein sources, after 40 experimental days.

Variables	Treatments					
	CONTROL	CASGE	CASALB	CASALBFM	ALBFM	P
Plasma						
Total protein	2.98±0.13	3.10±0.07	2.76±0.13	2.79±0.18	3.30±0.15	0.07
Amino acids	3.68±0.07	3.72±0.06	3.82±0.08	3.98±0.13	3.90±0.13	0.23
Albumin	0.57±0.03^{b}	0.54±0.03^{b}	0.54±0.04^{b}	0.52±0.03^{b}	0.68±0.02^{a}	0.013
Glucose	56.44±4.41^{a}	39.39±3.45^{b}	34.10±2.74^{b}	41.68±3.48^{b}	31.68±1.03^{b}	<0.0001
Triglycerides	0.53±0.04^{ab}	0.46±0.05^{b}	0.54±0.04^{ab}	0.72±0.06^{a}	0.69±0.04^{a}	0.0061
Cholesterol	111.46±3.86^{ab}	65.36±5.13^{d}	85.05±8.18^{cd}	95.44±3.91^{bc}	129.35±6.48^{a}	<0.0001
Liver						
Total protein	33.7±2.06	33.75±1.93	32.00±1.28	29.36±0.60	32.92±2.01	0.39
Amino acids	64.62±2.58^{a}	55.11±2.98^{ab}	63.03±1.94^{a}	48.12±2.20^{b}	46.19±2.06^{b}	<0.0001
Ammonia	6.22±0.12	6.29±0.12	6.76±0.17	6.02±0.26	6.18±0.13	0.08
Glycogen	4.27±0.12	3.57±0.15	3.99±0.13	4.62±0.39	4.65±0.46	0.09
Glucose	58.64±7.42	59.78±5.65	64.52±3.81	54.44±5.15	66.39±4.16	0.56
AST	563.52±46.54^{a}	570.78±44.15^{a}	458.40±33.93^{ab}	288.14±24.84^{b}	515.41±56.81^{a}	0.0002
ALT	8.13±0.58	7.95±0.43	7.23±0.18	7.04±0.16	7.65±0.64	0.48

Values (mean ± standard error of mean, n=9) with different letters in rows represent a significant difference by Tukey's test (P<0.05). Variables: Plasma: Total protein: g dL^{-1}; amino acids: mmol dL^{-1}; albumin: g dL^{-1}; glucose: mg dL^{-1}; triglycerides: mg dL^{-1}; cholesterol: mg dL^{-1}. Liver: Total protein: mg protein g tissue^{-1}; amino acids: µmol g tissue^{-1}; ammonia: µmol g tissue^{-1}; glycogen: µmol glucose g tissue^{-1}; glucose: µmol glucose g tissue^{-1}; AST: aspartate aminotransferase: UI mg tissue^{-1}; ALT: alanine aminotransferase: UI mg tissue^{-1}. $^{(b)}$Treatments: CONTROL: casein + fish meal; CASGE: casein + gelatin; CASALB: casein + albumin; CASALBFM: casein + albumin + fish meal; ALBFM: albumin + fish meal.

Table V. Proximate composition and protein retention of jundiá juvenile fed different combinations of protein sources, after 40 experimental days.

Variables	Treatments					
	CONTROL	CASGE	CASALB	CASALBFM	ALBFM	P
Moisture (%)	72.15±0.57	72.17±0.38	71.25±0.31	72.46±0.34	71.06±0.32	0.06
Ash (%)	2.89±0.09^{a}	2.46±0.06^{b}	2.62±0.10^{ab}	2.89±0.04^{a}	2.9±0.03^{a}	0.0002
Protein (%)	14.85±0.10	14.76±0.11	14.56±0.12	14.82±0.07	14.69±0.14	0.422
Fat (%)	9.46±0.55^{b}	10.93±0.25^{ab}	11.82±0.38^{a}	10.09±0.34^{b}	10.96±0.28^{ab}	0.0017
PRE (%)	41.13±1.29^{a}	32.57±0.89^{b}	19.63±0.53^{d}	29.75±0.70^{b}	23.91±0.51^{c}	<0.0001

Values (mean ± standard error of mean, n=9) with different letters in rows represent a significant difference by Tukey's test (P<0.05). Variables: PRE: protein retention. Treatments: CONTROL: casein + fish meal; CASGE: casein + gelatin; CASALB: casein + albumin; CASALBFM: casein + albumin + fish meal; ALBFM: albumin + fish meal.

for sea bass (*Dicentrarchus labrax*) fed intact protein (fish meal) or different ratios of intact and hydrolyzed proteins. The authors noted a greater final weight of post-larvae fed diets consisting of intact protein only (74% fish meal) or diets with reduced inclusion of hydrolyzed protein (14% hydrolyzed fish meal + 62% fish meal) than post-larvae fed diets with a greater hydrolyzed protein content (46% hydrolyzed fish meal + 30% fish meal). Corroborating these results, Carvalho et al. (1997) detected greater growth in common carp (*Cyprinus carpio*) fed a diet containing a mixture of hydrolyzed and intact proteins (fish hydrolysate and casein) than in those fed a diet consisting of hydrolyzed protein only (hydrolysate of cod, fish, meat, soybean, lactoalbumin, casein [N-Z Amine AS, Sigma N4517], or casein [N-Z Amine A, Sigma C0626]). Excess protein hydrolysate may have disturbed the absorption dynamics of amino acids in these two cases and impaired protein synthesis and the consequent growth of animals.

Another issue was the lower performance of fish fed CASALB and ALBFM diets. An explanation is that the use of semi-purified protein sources (albumin and casein) generates rapid availability of amino acids, saturates the intestinal transport mechanisms (antagonistic absorption), and causes an imbalance in the uptake and oxidation of amino acids, which negatively affects protein retention (Berge et al. 1999, Cahu et al. 1999, Aragão et al. 2004). Conversely, in the fish treated with the ALBFM diet, the digestion time of the protein sources may have been vastly different, that is, faster for albumin and slower for fish meal; thus, the peaks of amino acid absorption show temporal differences and are asynchronous, which reduces protein synthesis (Ambardekar & Reigh 2007). In these cases, amino acids are deaminated and catabolized and the carbon skeletons are converted into fat rather than used for building up proteins (Nelson & Cox 2019). These facts may explain the higher rate of abdominal fat in the animals from the ALBFM treatment group than in the CONTROL group. Similarly, protein synthesis may have been partially compromised in fish fed the CASGE diet. Protein synthesis is hampered in the absence of specific amino acids or at an asynchronous pace of their availability, leading to the catabolism of amino acids and their conversion into energy (Ambardekar & Reigh 2007). The higher hepatosomatic index in fish from the CASGE treatment group indicates metabolic overload or even fat deposition in this organ (Nelson & Cox 2019).

The presence and availability of digestive enzymes in adequate quantities throughout the gastrointestinal tract of fish is an important factor in the digestive process. Digestive and

absorptive capacities also depend on the time that the nutrients are in contact under the action of enzymes (Moraes & Almeida 2014). Protein digestion is initiated in the stomach by the action of acid proteases and continues in the intestine by the complementary action of the alkaline proteases trypsin and chymotrypsin, in addition to collagenases and pancreatic elastases. In the present study, higher acid protease activity was observed in fish fed the CASGE diet. This is likely due to protein denaturation, which may occur during the industrial production of gelatin and casein, given the change in pH, because the denatured protein is more sensitive to enzymatic hydrolysis. Alkali treatment also causes the destruction of essential amino acids, racemization, and crosslinking between the peptide chains, which could prevent the activity of acid proteases and the absorption and use of amino acids (Araújo 2011).

In fish in which low activities of trypsin and chymotrypsin were found (CASALB and ALBFM treatments), there was a loss in the digestive process of proteins, and consequently, in the growth of the fish. This seemed to occur more effectively as the inclusion of albumin in the diets increased. According to Martos et al. (2010), in an in vitro analysis, egg white albumin was resistant to pepsin action when the enzyme/substrate ratio was similar to the physiological situation (1:20) and at pH values above 2.0. The pH values between 1.2 to 2.0 showed a greater effect on albumin digestion when a high enzyme:protein ratio was used. This study also revealed that the presence of bile salts increased albumin proteolysis, which was conditioned by a mixture of pancreatic enzymes. In contrast, fish fed the CASALBFM diet, which had a lower inclusion of albumin, exhibited greater action of intestinal proteases, which probably contributed to the animals showing greater growth in relation to other diets in which this source of protein was included. The presence of 40% dextrin in the composition of albumin may be related to these effects as well as to the higher amylase activity exhibited by fish in these three treatments. In addition, the lower activity of amylase in fish that consumed the CONTROL diet was possibly linked to the lower inclusion of maltodextrin in this treatment. In other studies, jundiá juveniles fed different combinations of protein and carbohydrate sources did not show any changes in the activities of amylase and acid protease enzymes (Corrêia et al. 2019, 2021). However, when fed diets based on casein, corn starch, gelatin, casein, and maltodextrin, fish exhibited higher trypsin and chymotrypsin activity (Corrêia et al. 2021).

Pancreatic lipases present in the anterior intestine and pyloric ceca are involved in the digestion of acylglycerols, phospholipids, cholesterol, and other lipids in fish. In this process, lipase activity is aided by bile salts and colipases (Moraes & Almeida 2014). According to Maldonado-Othón et al. (2020), the degree of unsaturation influences digestibility and the action of lipases on fatty acids. The order of digestibility varies as follows: highly unsaturated fatty acids > polyunsaturated fatty acids > monounsaturated fatty acids > saturated fatty acids. The higher content of fish meal, which contains saturated fatty acids in greater proportion than vegetable oils, in the CONTROL and ALBFM treatments may be one of the factors that led to higher lipase activity in fish fed these diets. In addition, other factors that influence the secretion, concentration, activity of lipases, and digestibility of lipids are size, developmental stage and species of fish, food status, and type and content of lipids in the diet (Morais et al. 2007).

The evaluation of biochemical parameters related to protein and energy metabolism in the plasma and liver of fish is an important tool

for understanding the dietary use of nutrients in the diet (Corrêia et al. 2021). In the present study, plasma glucose levels were considerably higher in fish fed a CONTROL diet. The plasma cholesterol level and the concentration of amino acids in the liver were also elevated in fish from this treatment group. The composition of the diet (association of protein and energy sources) combined with the action of digestive enzymes is reflected in the high availability of amino acids and energy precursors, with positive effects on growth and nutrient deposition in the carcasses of the fish. On the other hand, the ALBFM diet provided fish with low glycemia and high concentrations of plasma triglycerides, cholesterol, and albumin. Fish exhibited lower concentrations of amino acids and high AST activity in the liver. This metabolic situation is related to the digestive process of protein sources and inadequate use of amino acids from the diet. It is possible that asynchrony in the availability of essential amino acids for protein synthesis led to the catabolism of these molecules and was reflected in the indices of plasma and abdominal fat, nutrient deposition, and reduced fish growth. The greater circulation of lipid molecules (triglycerides, fatty acids, and cholesterol) in the body of fish may be the main cause of the increase in serum albumin, since albumin is responsible for the transport of fatty acids in the blood (Nelson & Cox 2019).

In fish fed the CASGE and CASALB diets, dietary and body amino acids may have been diverted to hepatic gluconeogenesis to produce glucose, as this energy metabolite was reduced in the animals' plasma and the activity of the AST enzyme was increased. Considering that this enzyme is involved in the catabolism of amino acids, an increase in its activity may indicate an excess or deficiency in amino acids, which may lead to the use of proteins for energy production, thus reducing protein synthesis (Champe et al. 2009, Nelson & Cox 2019). This process seems to have occurred more intensely in fish from the CASALB treatment, strongly reflecting their zootechnical performance and nutrient deposition (high carcass fat content and lower protein retention among the evaluated treatments). In another study carried out with jundiá juveniles, Corrêia et al. (2021) observed that diets based on gelatin and/or casein as protein sources resulted in lower growth, retention of protein and blood glucose in fish, and increased activity of hepatic aminotransferases.

In relation to composition and nutrient deposition, although the fish body protein concentration did not vary between treatments, protein deposition reflected the response observed for fish zootechnical performance, corroborating the best dietary use of the casein + fish meal combination, intermediate use of casein + gelatin and casein + albumin + fish meal combinations, and inferior use of diets containing albumin + fish meal and casein + albumin. The high ash content in the fish carcasses fed the CONTROL, CASALBFM, and ALBFM diets was explained by the inclusion of fish meal because it had a high mineral matter content.

CONCLUSIONS

The present research demonstrates that the diet containing the combination of protein sources casein + fish meal allows for better growth performance and protein deposition without negatively affecting the animals' metabolism, digestive enzymes and body composition. Therefore, it is suggested that this combination be used in mixed diets for nutritional studies of juvenile jundiás.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for the doctoral scholarship granted to Viviani Corrêia; to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the research productivity scholarship granted to João Radünz Neto (Protocol no. 304412/2008-2 and to Leila Picolli da Silva (Protocol no. 306596/2018-0) and to the Mycotoxicological Analysis Laboratory for collaboration with the amino acids evaluation of ingredients (LAMIC/UFSM).

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How to cite

CORRÊIA V, PRETTO A, PEDRON FA, FERRIGOLO FRG, VEIVERBERG CA, BATTISTI EK, FREITAS ST, LOUREIRO BB, SILVA LP & RADÜNZ NETO J. 2024. Efficiency of protein combinations in diets for *Rhamdia quelen*: growth, digestive and metabolic biochemistry and nutrient deposition. An Acad Bras Cienc 96: e20231333. DOI 10.1590/0001-3765202420231333.

Manuscript received on December 19, 2023; accepted for publication on May 4, 2024

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