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Research Article

Effect of diets containing different types of sardine waste (*Sardinella* sp.) protein hydrolysate on the performance and intestinal morphometry of silver catfish juveniles (*Rhamdia quelen*)

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ABSTRACT. In this study two fractions of muscle hydrolysate were tested, soluble and insoluble (FSM and FIM), they were assessed individually and combined with each other (FSM+FIM). Also, two fractions of hydrolyzed viscera: soluble of natural and industrialized viscera (FSVN and FSVI) were tested on the performance and on the intestinal morphology of juvenile catfish (*Rhamdia quelen*). The experimental design was completely randomized with five treatments and five replicates. Juveniles were kept in aquariums with density of eight fish per tank and were cultured for 56 days. The results were analyzed using parametric variance analysis (ANOVA) and subjected to the Duncan test (5% significance level). The best results on final weight, weight gain, feed conversion rate, and protein efficiency ratio were obtained with the diet containing FSM+FIM, and the diet containing the FSVI. The latter was also the most consumed by the animals. The diet containing FIM was the one that provided the worst consumption results. The worst feed conversion rate was obtained for the diet containing FSM. Survival and body composition did not differ between treatments. The separation of the soluble and insoluble fractions is not necessary or even recommended when the goal is to use the muscle hydrolysate as the ingredient in feed for silver catfish juveniles. The best performance results obtained were with the combination of soluble and insoluble fractions of muscle hydrolysate, and with the industrial viscera soluble hydrolysate. The degree of hydrolysis has a direct effect on feed consumption.

Keywords: *Rhamdia quelen*, enzymatic hydrolysis, fish waste, animal feed, aquaculture.

Efecto de dietas que contienen diferentes tipos de residuos de sardina (*Sardinella* sp.) como hidrolizado de proteínas sobre el rendimiento y morfometría intestinal de juveniles de bagre (*Rhamdia quelen*)

RESUMEN. En este estudio se analizaron dos fracciones de hidrolizado muscular, soluble e insoluble (FSM y FIM), las cuales fueron evaluadas individualmente y combinadas (FSM+FIM). Además, dos fracciones de vísceras hidrolizadas: soluble de vísceras naturales e industrializadas (FSVN y FSVI) fueron probadas en el ren-

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dimiento y en la morfología intestinal de bagre juvenil (*Rhamdia quelen*). El diseño experimental fue completamente aleatorio con cinco tratamientos y cinco réplicas. Los juveniles se mantuvieron en acuarios con una densidad de ocho peces por estanque y se cultivaron durante 56 días. Los resultados se analizaron mediante análisis de varianza paramétrico (ANOVA) y se sometieron a la prueba de Duncan (nivel de significación 5%). Los mejores resultados de peso final, ganancia de peso, conversión alimenticia, y la tasa de eficiencia proteica, se obtuvieron con la dieta que contenía FSM+FIM, y la dieta que contenía el FSVI. Esta última fue también la más consumida por los animales. La dieta que contiene el FIM fue la que proporcionó los peores resultados de consumo. Se obtuvo el peor índice de conversión alimentaria con la dieta que contiene el FSM. La supervivencia y la composición corporal no difirieron entre tratamientos. La separación de las fracciones soluble e insoluble, no es necesaria ni recomendable cuando el objetivo es utilizar el hidrolizado muscular como ingrediente en la alimentación de los juveniles de bagre. Los mejores resultados de rendimiento obtenidos fueron con la combinación de las fracciones solubles e insolubles de hidrolizado muscular, y con el hidrolizado soluble de vísceras industrial. El grado de hidrólisis tiene un efecto directo sobre el consumo de alimento.

Palabras clave: *Rhamdia quelen*, hidrolizado enzimático, vísceras de pescado, alimentación animal, acuicultura.

INTRODUCTION

The importance of sustainability in industrial processes has been discussed in several countries with the objective to minimize environmental impacts, reduce wastes of reusable materials, and solve problems related to the poor management of natural resources, among which are fish (Hardesty *et al.*, 2015). In this context, the sardine (*Sardinella brasiliensis*) is a target species for having a global production of 98,315 ton in 2013 (FAO, 2015). The industrialization of sardines, however, generates elevated quantities of wastes. More specifically, the waste ranges from 35% to 47.8% of the total biomass due to a low carcass yield and comes from the eviscerated and splayed materials (Lee, 1963; Pessatti, 2001; Seibel & Soares, 2003).

As an alternative, economic value can be given to the fish residues from the agro-industry by producing hydrolyzed protein from the waste. Hydrolyzed protein from fish are defined as products from the hydrolysis reaction of peptide bonds in proteins that result in shorter peptides or amino acids easily absorbed for animals (Wisuthiphaet *et al.*, 2015). Enzymatic hydrolysis is currently the most used process, since the addition of enzymes to the raw material, the control of pH, temperature, and other variables contribute to the acceleration of the process (Wheaton & Lawson, 1985).

The enzymatic fish protein hydrolysate is an ingredient with great potential to for aquaculture (Oliva-Teles *et al.*, 1999). It is an excellent protein supplement consisted heavily of free amino acids and peptides with low molecular weight (Oliva-Teles *et al.*, 1999), and can also act as a growth promoter with the presence of bioactive peptides (Friedman, 1996). Furthermore, two distinct fractions (soluble and insoluble) of hydrolysates can be obtained through enzymatic hydrolysis, in addition to the oil fraction. The soluble fraction is composed of more hydrolyzed material such as peptides with a lower molecular weight, and the insoluble fraction has less or non-hydrolyzed material such as peptides with a higher molecular weight (Liaset & Espe, 2008). The insoluble fraction tends to be richer in lipids. The results from using the fractions separated as compared to united in a pre-stipulated concentration in animal feed, however, has not been sufficiently described (Liaset & Espe, 2008).

The silver catfish (*Rhamdia quelen*) is an omnivorous fish species native from South America, tolerant to low temperatures, and has a great potential for aquaculture. The nutritional requirements of silver catfish has been the focus of several studies (Meyer & Fracalossi, 2004; Copatti *et al.*, 2005; Coldebella *et al.*, 2010), but the rearing methodology and technology of the species has not been completely defined. Therefore, this study aimed to evaluate the effect of soluble and insoluble fractions of the protein hydrolysates from different sardine (*Sardinella* sp.) residues on the performance, organometric indices, body composition, and intestinal morphometry of silver catfish juveniles.

MATERIALS AND METHODS

The productive performance experiments were carried out at the Laboratory of the Fish Aquaculture Center of the Agro-veterinary Sciences at Santa Catarina State University, located in the city of Lages, SC, and was approved by the Ethics Committee of the Santa Catarina State University (protocol 1.14.13). Two types of sardines (*Sardinella* sp.) hydrolyzed muscle (soluble and insoluble fractions) were tested individually and combined together, and two types of sardines (*Sardinella* sp.) hydrolyzed viscera, which were the soluble fractions of natural and industrialized viscera. The raw materials used to produce the hydrolysates

were kindly provided by the company GDC Alimentos S.A. The experimental design was completely randomized into five treatments (type of hydrolysate) with five replicates per treatment.

Production of hydrolysates

The muscle protein hydrolysate was produced with clean carcasses (devoid of head, tail and viscera) of fish fresh and derived the soluble (FSM) and insoluble (FIM) fractions. The hydrolysates of the viscera were produced from two raw materials: a) Natural viscera (VN), composed by integral viscera manually removed from whole animals previously fresh frozen and maintained in cold chamber, and kindly provided in boxes by Gomes da Costa Alimentos S.A. (Itajaí, Santa Catarina, Brasil) and kept intact in a freezer at -20°C until processing. b) Industrial viscera (VI), which were collected directly from production lines from industry by suction machine. The samples collected by the industry were conditioning in 1 L plastic bottles, labeled, and kept in ice until arrival at the laboratory. The samples were fractionated in the same day to avoid cycles of freezing and thawing, and kept in a freezer at -20°C until processing. As the industrial visceras are extracted by suction machine, which uses water, this sample contains higher moisture content, in addition to already be, by the process, partially homogenized. Because of this, these samples tend to start the process of autolysis immediately if they are not kept on ice and processed or stored in a freezer in a few hours (for this work, always less than 2 h after collection).

Aliquots of ~300 g of sample were homogenized in a blender with three volumes of water and incubated with the bacterial protease enzyme *Bacillus licheniformis* and *Bacillus amyloliquefaciens* (Protamex® Novozymes A/S) 1:500 E:F (enzyme:fish) at 50°C for 90 min, followed by the inactivation of the enzyme and pasteurization of the hydrolysates at 75-90°C for 15 min. The suspensions were mixed and subjected to Büchner filtration with a paper filter of 80 g (Unifil®) and vacuumed into a flask. The retained material was considered as the insoluble fraction and the filtrate, the soluble fraction. Both fractions were dried at 60°C in a dry air circulation, until the moisture be reduced to 50% (maximum acceptable for its use as ingredient in the preparation of animal feeds). Due to very high lipid content in the insoluble fraction samples of the viscera hydrolysate, this insoluble hydrolysate was not used.

The chemical analyses of the hydrolysates and the respective raw materials were performed according to the methods AOAC (2000). The moisture content was determined by infrared radiation and the lipid content by the Soxhlet method. The composition of the mineral matter was determined with a gravimetric analysis, with incineration at 650ºC for 2 h and the total protein determined by the Kjeldahl method. The soluble protein content was determined by the method described in Lowry *et al.* (1951), and a calibration curve was created using bovine albumin serum as the standard. The compositions of the hydrolysates are presented in Table 1.

The degree of hydrolysis (GH) was measured according Nielsen *et al.* (2001), with some modifications. Was exploited the reactivity of the o-phthaldialdehyde (OPA) with amino groups. The assays were carried out in microplates with transparent bottoms by adding 40 µL of sample and 260 µL of the OPA reagent. The absorbance readings were performed at 340 nm in the microplate reader model Genius, of the brand Tecan, and the result expressed as GH (%) by the equation:

$$GH\ (\%) = htot \times 100\ [\ (Serine\text{-}NH2 - \beta) / \alpha\ meqv / g\ protein]$$

The values of α, β and htot were previously determined by Adler-Nissen (1986) for fish: 1.00; 0.40 and 8.6, respectively.

The analyses of the amino acids were carried out by the laboratory CBO - Análises Laboratoriais®, which used the methodology of analysis by chromatography in HPLC, where the individualization of the monomers was initially conducted through the hydrolysis with HCl 6N in an oven at 110°C for 24 h. Then, the HPLC was used which had a precision better than ± 0.5%, following a spectrophotometric detector reader. The amino acid composition in the formulated diets was estimated from the data obtained about the amino acid composition of each hydrolysate.

Animals and setup

Juveniles of silver catfish (0.78 ± 0.23 g) were acclimated in the tank for at least 30 days, in 500 L containers equipped with an aeration and heating system. The animals received a commercial feed and the excretions and food remains were siphoned daily. The fish were then distributed in experimental aquariums with a used volume of 30 L in an initial density of eight fish per aquarium, where the fish were cultured for 56 days.

The experiment was carried out with a constant temperature (~24ºC) maintained by heaters, and each aquarium were equipped with an individual biological filter system. The feed was provided twice daily until apparent satiation. Each day, ~20% of the water was renewed, the food remnants were removed, and the tanks were examined for the presence of dead animals. Water quality was monitored periodically and the averages were: temperature 24.72 ± 0.91°C; oxygen 5.24 ± 0.41 mg L^{-1}; ammonia 0.01 ± 0.1 µ L^{-1} and pH

Table 1. Bromatological composition of the hydrolysates. CP: crude protein, GE: gross energy, EE: ether extract, MM: mineral matter.

		Humidity (%)	CP (%)	GE (%)	EE (%)	MM (%)
Muscle soluble fraction	FSM	93.28	85.5	5108.8	2.00	8.0
Muscle insoluble fraction	FIM	35.08	77.0	6097.7	20.3	3.3
Natural viscera soluble fraction	FSVN	81.50	77.0	5169.9	4.28	6.2
Industrial viscera soluble fraction	FSVI	90.40	61.4	4607.8	1.69	11.3

8.16 ± 0.25. The values remained within the parameters recommended for the production of silver catfish (Baldisserotto & Radünz, 2004).

Experimental diets

Five isonitrogenous (39% crude protein) and isocaloric (about 4,450 kcal of gross energy kg^{-1}) diets were evaluated. Different types of sardine residue hydrolysate were used to provide about 50% of the protein in the diets (Table 2). The diets were also formulated using powdered meat meal and soybean meal as protein sources. Corn starch and fish oil were used as sources of energy. The ingredients were analyzed (Horwits, 1997) prior to the experiment to guarantee the best precision during the formulation. After mixing, the ingredients were finely ground in a knife mill with a sieve of 2 mm mesh diameter. The diets were pelleted (5 mm), separated by particle size, and stored in a freezer until the time of use.

Performance test

The fish were weighed at the beginning of the experimental period, and at 28 and 56 days. The following performance parameters were evaluated: final weight, weight gain, apparent individual feed consumption (CR = food consumed during the experimental period), apparent feed conversion rate (FC = feed intake/weight gain), protein efficiency rate [TEP = 100x (absolute weight gain/consumed crude protein)] and survival.

Organometric indices and body composition

A fish from each repetition was disposed for the immediate withdrawal of the liver and the viscera. The organs were weighed to calculate the hepatosomatic index (HSI), which is the ratio of the liver weight and the animal weight, and fat viscerossomatic index (IGVS), which is characterized as the ratio of the weight of visceral fat and the weight of the animal. A fish from each repetition was also sampled for the analysis of dry material body composition, protein, and ether extract, according to the methodology recommended by Horwits (1997).

Intestinal morphometry

To evaluate the intestinal morphology, the middle portion of the duodenum from two fish per replicate was removed. The samples were fixed in a formaldehyde buffer solution. Then, they were dehydrated in a series of increasing alcohol solutions, diaphonized in benzol and embedded in paraffin. Two slides were prepared from each sample and the sections were stained following the hematoxylin and eosin method (Behmer *et al.*, 2003). The slides were photographed using a digital camera coupled to a trinocular Opticam microscope (10x zoom). The morphometric measurements of the duodenum were carried out with the software ToupTek ToupView (version x64, 3.7.2270).

Statistical analysis

The results were analyzed using a parametric analysis of variance (ANOVA) and subjected to the Duncan test (5% significance). The normality of errors (Cramer von Mises) and homoscedasticity of variances (Levene test) were verified before all of the analyses.

RESULTS

Characterization of the hydrolysates

In the present study, the values of the GH up to 10% were considered of a low degree of hydrolysis; between 10 and 16% were average GH; between 16 and 40% were high GH, and above 40% were too high GH (Table 3). In general, the soluble fractions showed the greatest GH, while the insoluble fractions showed the lowest GH.

From the data obtained on the composition of amino acids contained in the hydrolysates (Table 4), and of the composition of the amino acids from the other protein sources used in the diets from previous studies, the amino acid composition was able to be estimated in the formulated diets (Table 5).

Performance test

The best results ($P < 0.05$) of the final weight, weight gain, feed conversion rate, and protein efficiency rate

Table 2. Composition of the experimental feed. FSM: muscle soluble fraction, FIM: muscle insoluble fraction, FSVN: natural viscera soluble fraction, FSVI: industrial viscera soluble fraction.

Ingredients (%)	FSM	FIM	FSM+FIM	FSVN	FSVI
Soybean meal	20.00	20.00	20.00	20.00	20.00
Corn	27.90	31.90	29.90	24.90	15.90
Muscle soluble fraction	24.00	0.00	12.00	0.00	0.00
Muscle insoluble fraction	0.00	26.00	13.00	0.00	0.00
Natural viscera soluble fraction	0.00	0.00	0.00	26.00	0.00
Ind. viscera soluble fraction	0.00	0.00	0.00	0.00	33.00
Meat meal	20.00	20.00	20.00	20.00	20.00
Fish oil	7.00	1.00	4.00	7.00	7.00
Premix*	1.10	1.10	1.10	1.10	1.10
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition (%)					
Dried material	92.85	92.38	92.62	93.11	94.01
Crude protein	41.21	41.05	41.13	40.92	41.34
Gross energy (kcal kg^{-1})	4482.20	4458.50	4448.22	4414.54	4428.10
Ether extract	11.14	10.18	10.66	11.68	10.80
Crude fiber	2.46	2.55	2.51	2.47	2.42
Mineral matter	7.60	6.59	7.10	7.32	9.46

*Folic acid-2,400 mg, nicotinic acid-48 g, pantothenic acid-24 g, biotine-96 mg, vit. A-2,400,000 UI, vit. D3-400,000 UI, vit. E-24,000UI, vit. B1-9,600 mg, vit. B2-9,600 mg, vit. B6-9,600 mg, vit. B12-9,600 mg, vit K3-4,800 mg, vit. C-96 g, iron-100 g, manganese-40 g, zinc-6,000 mg, cobalt-20 mg, iodine-200 mg, selenium-200 mg antioxidant-19.6 g.

Table 3. Degree of hydrolysis of the protein hydrolysates from the sardine waste.

	GH (%)
Muscle soluble fraction	20.10
Muscle insoluble fraction	9.97
Natural viscera soluble fraction	17.20
Industrial viscera soluble fraction	54.00

were obtained from the diet FSM+FIM, and with the FSVI diet (Table 6). The FSVI diet was also the most consumed ($P < 0.05$) by the animals. The FIM diet provided the worst ($P < 0.05$) results of consumption. The worst feed conversion rate was obtained for the FSM and FSVN diet. The survival did not differ ($P < 0.05$) between treatments.

Organometric indices and body composition

There was no difference ($P > 0.05$) between the fish that received the different diets for the organometric indices and body composition (Table 7).

Intestinal morphometry

For the intestinal morphometry, the FSM+FIM diet increased ($P < 0.05$) the height of the villi as compared to the FSVN and FSVI diets (Table 8).

Table 4. Amino acid composition (% dry material) from the hydrolysates. FSM: muscle soluble fraction, FIM: muscle insoluble fraction, FSVN: natural viscera soluble fraction, FSVI: industrial viscera soluble fraction.

Amino acids	FIM	FSM	FSVN	FSVI
Aspartic acid	7.29	4.02	1.57	5.52
Glutamic acid	9.06	8.04	2.59	10.10
Serine	2.83	1.79	1.03	2.19
Glycine	3.56	4.76	1.57	5.73
Histidine	2.08	4.61	0.86	2.60
Taurine	0.02	1.49	0.49	1.98
Arginine	4.05	2.83	0.22	0.52
Threonine	3.07	1.64	0.92	3.65
Alanine	3.85	3.72	1.30	5.31
Proline	2.80	2.53	1.08	3.96
Tyrosine	2.71	0.74	0.54	0.63
Valine	3.65	1.79	1.19	4.69
Methionine	2.37	1.19	0.59	2.08
Cysteine	1.43	0.45	0.32	1.15
Isoleucine	3.57	1.19	0.92	3.23
Leucine	5.62	3.42	1.84	6.35
Phenylalanine	3.20	1.19	0.86	2.60
Lysine	5.87	4.61	1.46	5.31
Tryptophan	0.42	0.15	0.22	0.63

Table 5. Calculated composition of amine acids (% of PB of the diet) of the experimental diets. FSM: muscle soluble fraction, FIM: muscle insoluble fraction, FSVN: natural viscera soluble fraction, FSVI: Industrial viscera soluble fraction. *Silver catfish amino acid requirements (Meyer & Fracalossi, 2005).

Amino acid	FSM	FIM	FSM+ FIM	FSVN	FSVI	Silver catfish*
Lysine	4.98	6.04	5.51	3.22	6.46	5.80
Methionine	1.31	2.13	1.72	0.99	2.23	2.13
Met + Cys	2.10	3.59	2.85	1.72	3.64	3.11
Threonine	2.48	3.51	3.00	2.10	4.35	3.00
Tryptophan	0.49	0.68	0.59	0.54	0.89	0.27
Arginine	4.87	5.83	5.35	3.36	3.53	3.72
Valine	7.82	9.15	8.49	7.56	10.40	2.65
Isoleucine	2.26	3.86	3.06	2.15	4.07	2.54
Leucine	9.95	11.63	10.79	9.12	12.75	5.03
Histidine	3.65	2.31	2.98	1.51	2.98	1.31
Phenylalanine	2.57	3.94	3.26	2.42	3.86	2.69
Phe + Tyr	4.20	6.88	5.54	3.94	5.47	4.79

Table 6. Performance of the silver catfish juveniles fed with different types of hydrolysates after 56 days of the experiment. FSM: Muscle soluble fraction, FIM: muscle insoluble fraction, FSVN: natural viscera soluble fraction, FSVI: industrial viscera soluble fraction. WG: weight gain, FCR: feed conversion rate, PER: protein efficiency rate. Means followed by different letters differ among each other by the Duncan test ($P < 0.05$). CV: coefficient of variation.

	FSM	FIM	FSM+FIM	FSVN	FSVI	CV (%)
Final weight (g)	1.95 ± 0.37^{b}	1.95 ± 0.19^{b}	2.79 ± 0.46^{a}	2.12 ± 0.46^{b}	3.23 ± 0.76^{a}	28.47
WG (g)	1.16 ± 0.21^{b}	1.17 ± 0.29^{b}	2.03 ± 0.28^{a}	1.36 ± 0.35^{b}	2.47 ± 0.60^{a}	38.71
Consumption (g)	2.63 ± 0.16^{b}	2.09 ± 0.27^{c}	2.31 ± 0.11^{bc}	2.53 ± 0.45^{bc}	3.28 ± 0.43^{a}	19.49
FCR	2.31 ± 0.34^{c}	1.85 ± 0.36^{b}	1.16 ± 0.16^{a}	1.94 ± 0.44^{bc}	1.37 ± 0.21^{a}	29.70
PER	2.98 ± 0.54^{b}	2.99 ± 0.74^{b}	5.19 ± 0.73^{a}	3.48 ± 0.90^{b}	6.33 ± 1.55^{a}	38.71
Survivor (%)	97.5 ± 5.59^{a}	95.0 ± 11.1^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	8.28

DISCUSSION

The provision of the soluble and insoluble fractions of the muscle hydrolysate in a combined form improved the growth and the feed conversion rate of the fish. The combination of the two fractions enhances the nutritional value of the diet, since the fractionation by filtration separates the amino acids and the peptides among the soluble and insoluble fractions, as well as separating the nutrients derived from the raw material. The soluble fraction of the fish hydrolysates is generally rich in taurine, potassium, magnesium, vitamin B complex, and biogenic amines, while the insoluble fraction has a higher concentration of almost all of the amino acids with the exception of taurine (Liaset & Espe, 2008). Furthermore, the combination of the different types of peptides and amino acids improves the absorption of the dietary protein due to less competition for the absorption pathways. More specifically, when a pair of amino acids or peptides depends on the same transporter to be absorbed, this transporter becomes more easily saturated and thus limits the absorption of the nutrients (Baldisserotto, 2009).

Other authors have demonstrated that hydrolysates should not be the only source of protein, and that the best results for performance are obtained with a combination of intact proteins and different types of hydrolysates (Carvalho *et al.*, 1997). The mixture of intact proteins of fishmeal with hydrolysates from different sources was effective to increase the growth of the Atlantic salmon (Refstie *et al.*, 2004) and the Japanese flounder (Zheng *et al.*, 2012). Aksnes *et al.* (2006, 2006b), which affirmed that the partial replacement of the intact vegetal protein with fish protein hydrolysates improves the growth and the feed efficiency of rainbow trout and Atlantic cod and supported the hypothesis that some of the small peptides have an essential role for biological development.

The diet containing the soluble fraction of the industrial viscera in high concentration provided good performance results, similar to those obtained from the

Table 7. Organometric index and body composition of the silver catfish juveniles fed with different types of hydrolysates after 56 days of the experiment. FSM: muscle soluble fraction, FIM: muscle insoluble fraction, FSVN: natural viscera soluble fraction, FSVI: industrial viscera soluble fraction, HI: hepatosomatic index, FVI: fat viscerosomatic index. Means followed by different letters differ among each other ($P < 0.05$). CV: coefficient of variation.

	FSM	FIM	FSM+FIM	FSVN	FSVI	CV(%)
IHS	0.81±0.10[a]	0.70±0.29[a]	0.91±0.24[a]	0.83±0.90[a]	0.92±0.23[a]	14.61
IGVS	0.29±0.16[a]	0.26±0.09[a]	0.19±0.05[a]	0.14±0.09[a]	0.17±0.04[a]	31.04
Dry material	22.80±3.51[a]	22.15±0.90[a]	19.97±2.13[a]	20.66±1.33[a]	19.94±0.70[a]	5.04
Protein	11.81±0.89[a]	12.52±0.50[a]	12.57±0.96[a]	12.75±0.74[a]	12.84±0.91[a]	3.44
Fat	1.40±0.46[a]	1.35±0.1a[a]	1.85±1.01[a]	2.49±0.34[a]	2.19±0.65[a]	16.90

Table 8. Intestinal morphometry of the catfish juveniles fed with diets containing different types of hydrolysates after 56 days of the experiment. Means followed by different letters differ among each other by the Duncan test ($P < 0.05$). CV: coefficient of variation.

Villi (μm)	FSM	FIM	FSM+FIM	FSVN	FSVI	CV(%)
Villi height	412.0±143.7[ab]	441.1±99.0[ab]	494.6±129.0[a]	344.8±49.4[b]	305.5±70.7[b]	25.7
Villi width	65.26±10.35[a]	76.15±16.15[a]	72.75±11.56[a]	73.87±12.45[a]	73.29±7.64[a]	16.3

administration of the soluble and insoluble fractions of the muscle combined. This improvement in the performance can be attributed to an improved palatability and to a better balance of the amino acids, as will be discussed below in the text. The hydrolysate of the industrial viscera has a high degree of hydrolysis (54%) (Table 3) and was incorporated in the most consumed diet (Table 6). The high palatability of the diet with a high degree of hydrolysis has been suggested to promote growth, determined also by the protein hydrolysates (Refstie *et al.*, 2004; Hevroy *et al.*, 2005). A higher degree of hydrolysis creates a greater proportion of the soluble proteins with low molecular weight. This may have favored the detection by the gustatory system of the fish, which is highly sensitive to the soluble substances dissolved in water (Marui & Caprio, 1992; Halver & Hardy, 2002) and thus generating a greater feed consumption (Hevroy *et al.*, 2005).

In relation to the balance of the amino acids, the treatment that used the soluble fraction of the industrial viscera was shown to meet the amino acid requirements of the silver catfish (Table 5). A sample of the industrial viscera was removed from the fish by suction and sent to a container in the exterior of the industry. The viscera was fragmented and exposed to the environmental temperature, which may have contributed to an increased release of amino acids to the soluble fraction since there were more conditions (suction, with tissue rupture; time and temperature) for the action of endogenous enzymes present in digestive tract of the sardines (Baldisserotto, 2009). According to Klompong *et al.* (2009), the composition of amino acids and peptides of a hydrolysate varies according to the enzymes and the conditions of the hydrolysis process, such as the temperature and the time of hydrolysis.

The soluble fraction of the muscle and the soluble fraction of the natural viscera provided a smaller weight gain and worse feed conversion rate for the fish, even with a consumption equivalent to the other treatments. The positive effect of the soluble fractions of the hydrolysates on the fish performance has already been described (Espe *et al.*, 1999; Hevroy *et al.*, 2005; Aksnes *et al.*, 2006a, 2006b). On the other hand, Gossen *et al.* (2014) reports that elevated levels (>15%) of soluble hydrolysates incorporated in the diet decrease the stability of the feed and can compromise the results of the feed conversion rate. In the present study, the diet containing 24% of soluble hydrolysates of muscle had the worst feed conversion rate. The present study showed, however, that the high incorporation (33%) of the soluble hydrolysate of the viscera with a high degree of hydrolysis (>50%) did not prevent good results of feed conversion rate from being obtained.

The solubility of the hydrolysate is not the only explanation for the various changes observed, since the diets containing the soluble hydrolysates of the muscle and natural viscera were deficient in some essential amino acids (lysine, methionine and threonine) for catfish (Meyer & Fracalossi, 2005). This observation can be explained by the existence of five hydrophobic amino acids (isoleucine, valine, leucine, phenylalanine and methionine), which constitute the regions in the hydrophobic domains of proteins and ultimately limit the access for the enzyme (Chothia, 1974, 1975). These

regions may not be hydrolyzed because enzymatic hydrolysis depends on the physical interactions between the substrate and the enzyme, thus the amino acids present in these domains are not released (Chothia, 1974, 1975).

However, the insoluble fraction is constituted of peptides with a higher molecular weight, which are retained during the filtration process and have a lesser degree of hydrolysis. This may be the determining factor for the low performance result. Fish protein hydrolysates of a high molecular weight cannot protect themselves from the aqueous environment, which leaves hydrophobic amino acids exposed that present a bitter taste (Hall & Ahmad, 1992). Thus, although it affected the consumption and growth, the hydrolysate insoluble fraction is noteworthy for providing a sufficient balance of amino acids to meet the requirements of silver catfish (Table 5).

In the present study, the body composition of the silver catfish juveniles was not affected by the use of different types of sardine hydrolysates in the diets, even in the body composition was not affected from the diets that did not provide an optimum performance, indicating that there were no metabolic alterations sufficient to influence the muscle and fat deposition. Oliva-Teles *et al.* (1999) and Bui *et al.* (2014) also found no differences in the chemical composition of the animal carcasses that received the fish protein hydrolysates in the feed.

The villus height was higher in the fish fed with the diets containing the combination of soluble and insoluble fractions of the hydrolyzate muscle in relation to those receiving the viscera hydrolyzates. However, the performance of fish fed on this diet was not better compared to those fed the diet containing industrial viscera Protein hydrolysates are rich in bioactive peptides, which may possess antimicrobial properties (Ahn *et al.*, 2012), immune modulators (Kotzamanis *et al.*, 2007), inhibit the angiotensin converting enzyme (Ace) inhibitors (Nakajima *et al.*, 2009), act as growth factors (Soares, 2013), and promote prebiotic (Gonçalves, 2011) and antioxidant activities (You *et al.*, 2010; Kumar *et al.*, 2011). Peptides with an antioxidant capacity can sequester oxygen radicals, acting as chelators of pro-oxidant metal ions and as inhibitors of lipid peroxidation in feeding systems (You *et al.*, 2010). These beneficial properties of the hydrolysate suggest that there was a protective effect on the intestinal epithelium, which would account for the increase in the size of the microvilli. The protein hydrolysate produced from *Merluccius productus* were observed to give a protective effect to the epithelial tissue against damage from indomethacin in rats (Marchbank *et al.*, 2009). However, best results productive performances with the use of feed additives are normally only observed when the animal is exposed to stressors (Li & Gatlin III, 2005). That would explain the similar results found between diet containing the combination of soluble and insoluble fraction of muscle hydrolysate and the diet containing the industrial viscera soluble fraction.

The use of protein hydrolysates can be an alternative to the problems associated with the environmental issues, in addition to providing gains in performance and health for the cultivated fish. The use of enzymatic hydrolysis can maximize the use of the wastes from the fish industry as an ingredient in diets and reduce the environmental liabilities. Therefore, more studies are necessary to find an ideal hydrolysis process that provides a good level of bioactive peptides and thus, adds value to the wastes generated by the fishing industry.

CONCLUSIONS

The separation of the soluble and insoluble fractions is not necessary or even recommended when the goal is to use the muscle hydrolysate as the ingredient in feed for silver catfish juveniles. The best performance results obtained were with the combination of soluble and insoluble fractions of muscle hydrolysate, and with the industrial viscera soluble hydrolysate. The degree of hydrolysis has a direct effect on feed consumption.

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