# ACID SILAGE OF TUNA VISCERA: PRODUCTION, COMPOSITION, QUALITY AND DIGESTIBILITY

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## ABSTRACT

Fish waste processed in the form of silage may constitute an alternative to the use of fishmeal (FM). In this study the composition and quality of the acid silage produced from tuna viscera (TV) were characterized and the digestibility of the nutrients of this product was determined for jundia *Rhamdia quelen*, using ytrium oxide as an inert marker and a completely randomized design. At the end of thirty days, 61.74% of the crude protein of TV was solubilized. Acid silage from TV presented good nutritional composition (high protein, good amino acid profile and essential fatty acids) and good microbiological quality. Crude protein digestibility was similar (88.52%) for TV and FM, but dry matter digestibility was higher (P<0.05) for (TV) (92.20%). Tuna silage presented as a high nutritional quality and nutrient digestibility for jundiá juveniles, *R. quelen*. Therefore, this novel ingredient has potential as an alternative protein source in aquafeeds.

Keywords: Fish waste; hydrolysis; protein solubility; Rhamdia quelen

## SILAGEM ÁCIDA DE VÍSCERAS DE ATUM: PRODUÇÃO, COMPOSIÇÃO, QUALIDADE E DIGESTIBILIDADE

## **RESUMO**

Resíduos de pescado processados na forma de silagem podem se constituir em uma alternativa ao uso de farinha de peixe (FM). Neste estudo caracterizou-se a composição e qualidade da silagem ácida produzida a partir de vísceras de atum (TV) e determinou-se a digestibilidade dos nutrientes deste produto para jundiá *Rhamdia quelen*, utilizando-se o óxido de ítrio como marcador inerte em delineamento completamente casualizado. Ao final de trinta dias, 61.74% da proteína bruta da TV estava solubilizada. A silagem ácida de TV apresentou boa composição nutricional (alta proteína, bom perfil de aminoácidos e de ácidos graxos essenciais) e qualidade microbiológica satisfatória. A digestibilidade da proteína bruta foi similar (88.52%) para TV e FM, mas a da matéria seca foi maior (P<0.05) para a TV (92.20%). A silagem de atum apresentou-se como um ingrediente proteico de alta qualidade nutritiva e digestiva para juvenis de jundiá, *R. quelen*. Portanto, este novo ingrediente tem potencial como fonte alternativa de proteína para rações de espécies aquícolas.

Palavras-chave: resíduos de pescado; hidrólise; solubilidade proteica; Rhamdia quelen

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## INTRODUCTION

In fish farming, feed costs can vary from 30 to 60% and it may exceed 85% of the total production costs in intensive systems (SILVA and ANDERSON, 1995). Fishmeal is traditionally considered an important protein ingredient in aquaculture diets because it has high palatability and excellent source of amino acids, fatty acids, vitamins and minerals (VALLE et al., 2015), however the increase in its demand, implies an increase in its cost. Alternative protein-rich ingredients with potential to replace fishmeal are fish waste, which includes by-catch and residues from fish processing industries, not intended for human consumption, with potential environmental and/or health hazzard if discarded incorrectly. There are techniques for the conservation of fish residues that allow their processing and incorporation as an ingredient in animal feed such as acid, biological or enzymatic silage (ESPE et al., 1999).

The nutritional value of fish silage is in its high protein digestibility due to the high degree of protein hydrolysis and the presence of essential amino acids (OETTERER, 1994, JUNIOR and SALES, 2013). The degree of hydrolysis should be used as a chemical quality criterion for silage, because due to autolysis and rancification, the product quality may be impaired.

Fish silage can be produced from a variety of raw materials such as heads, bones, viscera, fins, skins, whole fish or a mixture of different parts of fish, which may influence the nutritional composition of the silages produced. Thus, the production of fish silage with a standard residue such as tuna viscera becomes interesting, so that the final nutritional composition does not vary greatly and the protein content may be relatively greater than the mineral matter or fat.

Protein hydrolyzate produced exclusively from sardine viscera (*Sardina pilchardus*), improved growth and survival of European sea bream (*Dicentrarchus labrax*) when included at 10% diet (KOTZAMANIS *et al.*, 2007). Similarly, the replacement of 50% of fishmeal by biological silage produced from fish viscera provided good growth performance of the catfish (*Heteropneus fossilis*) (MONDAL *et al.*, 2008). Addidtionaly, good digestibility of the silage produced from surubin viscera (*Pseudoplatystoma* sp.) was reported for Nile tilapia, with values of 83.52, 93.30 and 87.20% for dry matter, crude protein and ether extract digestibility, respectively (HISANO *et al.*, 2012).

In Brazil, the skipjack (*Katsuwonus pelamis*) is the main species of tuna caught for canning. In 2011, this species capture surpassed 800.000 t (FAO, 2013), representing more than 95% of the raw material canned by the Brazilian industry (GONÇALVES, 2011). Fish waste resulting from the canning process can be an important raw material for the production of protein feed ingredients.

The jundiá, Rhamdia quelen (Siluriformes: Heptapteridae), is a freshwater catfish, native to the American continent, present in watersheds that extend from southeastern Mexico to central Argentina (FUKUSHIMA and ZANIBONI FILHO, 2009). Jundiá presents good features for intensive farming in South Brazil, such as easy reproduction, good tolerance to handling and growth during the winter months (MEYER and FRACALOSSI, 2004). Its feeding habit is omnivore with carnivorous tendencies (FRACALOSSI et al., 2007), which makes it an interesting model for the study of alternative protein ingredients. Therefore, this study aimed to characterize the composition and quality of the tuna viscera acid silage (TV) and to determine its nutrient digestibility for jundiá.

#### MATERIAL E METHODS

Acid silage: preparation, composition, quality and protein solubility

Tuna residues (*K. pelamis*), consisting only of viscera (intestine, stomach, liver, pancreas, swimming bladder, kidney, spleen, gonads) were purchased from a fish processing company in the state of Santa Catarina, Brazil. The viscera were transported frozen in polyethylene containers for one hour to the Laboratory of Nutrition of Aquatic Species (LABNUTRI).

In the laboratory, the raw material remained at room temperature for thawing (30 min) and then it was crushed using an electric meat grinder. Sixty kg of crushed mass were weighed and packed in 200-L polyethylene container with lid. Subsequently, in order to avoid bacterial growth and lipid oxidation, 10% acetic acid (w/v) and 2%butyl hydroxy toluene (BHT) were added. The crushed mass was stirred once a day, using a wooden stick (1.5 m), during the first five days in order to homogenize and promote greater contact between the acid and the crushed mass (SEIBEL and SOUZA-SOARES, 2003). During the 30 days of the silage process, the temperature and pH were monitored daily with a mercury thermometer and a potentiometer. After 30 days, the silage was dried in a forced air circulation oven at 55°C until reaching approximately 15% humidity, for later use in the preparation of the experimental diets.

The analyses performed in the acid silage were: 1) proximate composition (A.O.A.C., 1999); 2) microbiology (BRASIL, 2013); 3) biogenic amines content by extraction with trichloroacetic acid (5%) and separation by HPLC (VALE and GLORIA, 1997); 4) concentration of toxic chemical elements such as chromium, cadmium, lead and mercury by mass spectrometry (FLAMENT *et al.*, 2002), and 5) fatty acid composition by lipid extraction (FOLCH *et al.*, 1957), followed by methylation (HARTMAN and LAGO, 1973) and gas chromatography.

During the ensiling process, the degree of protein solubilization was also monitored by the determination of soluble nitrogen. Before the addition of acetic acid, an aliquot of the ground raw material was sampled for centesimal analysis and determination of soluble protein. After the addition of the acid, silage samples were collected every two days to determine soluble nitrogen, which was determined by precipitating the protein from 1 g of sample with 10 mL of trichloroacetic acid (40%) and 10 mL of distilled water (HAARD *et al.*, 1985). After 30 min, the mixture was filtered using filter paper and the soluble nitrogen determined in the filtrate by the Kjeldahl method (N x 6.25).

## Digestibility assay

Juveniles of female jundiá were obtained at the Agricultural Research and Rural Extension Company (EPAGRI) in Caçador, SC. Fish handling followed guidelines approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Santa Catarina (UFSC) (protocol #PP00815). Fish were acclimated for two weeks to the experimental conditions, in a closed water recirculation system. After that, groups of five animals (272.58 ± 20.04 g) were distributed into nine 200-L cylinder-conical incubators. Water quality variables, temperature (27.34 ± 0.66 °C), dissolved oxygen concentration  $(6.20 \pm 0.57 \text{ mg L}^{-1})$ , salinity  $(1.80 \pm 0.47 \text{ g L}^{-1})$ , pH (6.68  $\pm$  0.32) and electrical conductivity (3.60  $\pm$ 0.89 mg L<sup>-1</sup>) were monitored daily with the aid of a multi-parameter water quality meter. Total ammonia, nitrite and nitrate were monitored once a week, using a colorimetric kit (Alfa Tecnoquímica, Florianópolis, SC), and did not exceed 0.40 mg L-1. Photoperiod was adjusted to 12 h. Water quality remained adequate for jundiá growth.

During 55 days, the experimental diets were offered, twice a day (7:00 a.m. and 4:00 p.m.), until apparent satiation, for three groups of fish. methodology Digestibility followed that adopted for channel catfish (KITAGIMA and FRACALOSSI, 2011). Daily, 1 h after the first feeding, feces were collected for four hours. Feces settled in 50-mL tubes coupled to the bottom of each tank. During the collection period, tubes remained immersed in ice to avoid microbial degradation of feces. Fecal collections were performed twice a day: in the morning (8:00 a.m. to 12:00 p.m.) and in the afternoon (1:00 p.m. to 5:00 p.m.). After collection, tubes were centrifuged (1150 x g) for 5 min, the supernatant was discarded and the fecal matter immediately frozen to evaluate apparent digestibility of dry matter and protein.

#### Experimental diets

To formulate the reference diet of the digestibility trial, we used the amino acid and gross energy requirements of jundiá (MEYER and FRACALOSSI, 2004; MONTES-GIRAO and FRACALOSSI, 2006). However, the requirement of another freshwater omnivore, the American catfish, *Ictalurus punctatus* (NRC, 2011), were used for the other nutrients (lipids, vitamins and minerals).

The amino acid profile of the protein-rich ingredients (fishmeal and tuna silage) was

analyzed for the correct formulation of the experimental diets. The aminogram was obtained by reverse-phase high performance liquid chromatography (HPLC), with UV detection at 240 nm and quantification by multilevel internal calibration using aminobutyric acid as an internal standard (HAGEN *et al.*, 1989).

The reference diet was prepared using semipurified ingredients such as casein, gelatin, cellulose and starch. Dry ingredients were weighed first, mixed (using a bread dough mixer), then the oils and water were added. The test diets consisted of 69.9% of the reference diet, 30% of silage or fishmeal as test ingredients and 0.1% of the yttrium oxide marker (Table 1). Diets were prepared by homogenizing the dry ingredients first and then adding the oils and water. In all diets the water was added not exceeding 11% of moisture. Diets were then pelleted (3 mm) and dried in a forced air circulation oven (55 °C) for 4 h. After drying, the diets were packed and stored under refrigeration (4 °C) until use.

	Table 1. Proximal	composition of	the experimental	diets (expressed	in dry matter).
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Ingradiants (g. kgl)	Analyzed nutritional composition		experimental diets
ingreatents (g kg <sup>1</sup> )	Reference	Fishmeal	Silage
Fishmeal <sup>1</sup>	0.00	300.00	0.00
Silage <sup>2</sup>	0.00	0.00	300.00
Caseine	327.60	229.32	229.32
Gelatine	50.00	35.00	35.00
Starch	390.20	273.14	273.14
Cellulose	80.00	56.00	56.00
Soy oil	20.00	14.00	14.00
Cod liver oil	50.90	35.63	35.63
Vitamin and micro mineral premix <sup>3</sup>	10.00	7.00	7.00
Macro mineral premix <sup>4</sup>	37.10	25.97	25.97
Dicalcium phosphate	33.60	23.52	23.52
Yttrium oxide	1.00	0.70	0.70
Butyl-hydroxy-toluene	0.50	0.35	0.35
Nutritional composition (g kg <sup>-1</sup> )			
Dry matter	946.80	933.50	819.10
Crude protein	368.60	479.60	463.50
Mineral matter	164.40	156.60	157.10
Ether extract	90.50	72.90	60.10
Gross energy (kcal kg <sup>-1</sup> )	4,349	4,276	4,381
Crude fiber	21.20	20.70	16.10
Yttrium oxide	0.09	0.09	0.09

<sup>1</sup> Salmon processing wastemeal (Pesqueira Pacific Star S.A., Chile). Composition (g kg<sup>-1</sup>, wet basis): dry matter =  $870.03 \pm 0.81$ ; crude protein =  $648.90 \pm 0.62$ ; ether extract =  $104.00 \pm 0.37$ ; mineral matter =  $113.40 \pm 0.78$ ; crude energy =  $4,874 \pm 0.38$ . <sup>2</sup>Acid silage of tuna viscera (intestine, stomach, liver, pancreas, swimming bladder, kidney, spleen, gonads) (Gomes da Costa (Itajaí, SC, Brazil). Composition (g kg<sup>-1</sup>, wet basis): dry matter =  $227.80 \pm 0.26$ ; crude protein =  $669.00 \pm 0.97$ ; ethereal extract =  $112.10 \pm 0.40$ ; ashes =  $122.30 \pm 0.23$ ; crude energy, kcal kg<sup>-1</sup> =  $5,351 \pm 0.89$ . <sup>3</sup>Nutron Alimentos (Toledo, PR, Brazil). Product composition kg<sup>-1</sup>: 250 mg folic acid; 5,000 mg pantothenic acid; 0.6 g antioxidant; 125 mg biotin; 25 mg cobalt; 2,000 mg copper; 75,000 mg coline; 13,820 mg iron; 100 mg iodine; 3,750 mg manganese; 5,000 mg niacin; 75 mg selenium; 1,000,000 UI vitamin (vit.) A; 1,250 mg vit. B<sub>1</sub>; 3,750 mg vit. B<sub>12</sub>; 2,500 mg vit. B<sub>2</sub>; 1,785 mg vit. B<sub>6</sub>; 42,000 mg vit. C; 500,000 UI vit. D<sub>3</sub>; 20,000 UI vit. E; 35,000 mg vit. K; 17,500 mg zinc. <sup>4</sup> Composition (g kg<sup>-1</sup> product): dicalcium phosphate = 130; potassium chloride = 120; sodium chloride = 130; magnesium sulfate = 620. The apparent digestibility coefficients (CDA) of protein and dry matter were determined using the equation proposed by CHO and SLINGER (1979) for the reference diet and the equation proposed by BUREAU and HUA (2006) for the tested ingredients:

 $CDA_{diet}$  (%) = 100 - [100 x (% $M_{diet}$ /% $M_{feces}$ ) x (% $Nutrient_{feces}$ /% $Nutrient_{diet}$ )];

where:  $CDA_{diet}$  = apparent digestibility coefficient of the diet; %M = inert tracer concentration (% in dry matter) and% N = nutrient content (% in dry matter).

$$\begin{split} &CDA_{test ingredient} (\%) = CDA_{test ingredient} + [(CDA_{test diet} - CDA_{reference diet}) \times (0.7 \times D_{reference diet}) / (0.3 \times D_{ingredient})]; \end{split}$$

where: D <sub>reference diet</sub> = % Nutrient in reference diet, D <sub>ingredient</sub> =% of nutrient in test ingredient.

#### Statistical analysis

Digestibility data showed normality and homoscedasticity and were submitted to t-Student test to determine the differences between apparent digestibility coefficients of dry matter and crude protein. The level of significance adopted was 5%. Statistical analyzes were performed using the STATISTICA program, version 7.0 (StatSoft, Inc., 2004).

#### RESULTS

## Proximal composition of tuna viscera silage

The protein hydrolysis period was 30 days, with an average temperature of  $28.0 \pm 1.30$  °C; pH 4.18 ± 0.12, within the standard for acid silage production. Both the raw material and the silage produced presented crude protein values suitable

for use as a protein ingredient in fish diets (Table 2). During the silage process, the contents of dry matter, ether extract and ashes registered a slight reduction but crude protein remained unchanged.

**Table 2**. Proximal composition, expressed as dry matter, of the raw material and acid silage produced after 30 days. Means and standard deviation of three replicates.

Fraction (g kg <sup>-1</sup> )	Raw material <sup>1</sup>	Silage
Raw material	$244.60\pm0.05$	$227.80 \pm 0.26$
Crude protein	$669.43\pm0.92$	$669.40 \pm 0.97$
Ether extract	$129.50 \pm 0.33$	$112.10 \pm 0.40$
Mineral matter	$128.10\pm0.19$	$122.30 \pm 0.23$

<sup>&</sup>lt;sup>1</sup>*Tuna viscera (intestine, stomach, liver, pancreas, swimming bladder, kidney, spleen, gonads) acquired at Gomes da Costa (Itajaí, SC, Brazil).* 

#### Silage microbiological quality

Microbiological analyzes of the raw material and silage at 30 days showed absence of contaminating microbiological agents (Table 3).

#### Contamination of silage by toxic chemical elements

The silage presented the following profile of toxic chemical elements: chromium  $2.60 \pm 0.02$  mg kg<sup>-1</sup>, cadmium  $4.25 \pm 0.03$  mg kg<sup>-1</sup>, lead  $0.25 \pm 0.01$  mg kg<sup>-1</sup>, and mercury  $0.53 \pm 0.08$  mg kg<sup>-1</sup>.

## Amino acid profile of silage and fishmeal

The silage presented a profile of essential and non-essential amino acids numerically superior to that of fishmeal for all amino acids, except for glycine (Table 4).

Table 3. Microbiological analysis of the raw material (tuna viscera) and silage.

Analysis	Raw material	Silage
<i>Staphylococcus</i> coagulase positive <sup>1</sup> (CFU g <sup>-1</sup> )	< 1.0 x10	< 1.0 x10
Thermotolerant coliforms <sup>2</sup> (45°C) (CFU g <sup>-1</sup> )	< 1.0 x10	< 1.0 x10
Salmonella spp.	Ausência em 25 g	Ausência em 25 g

<sup>1</sup>Analysis performed in triplicate; <sup>2</sup>Analysis performed in duplicate; <sup>3</sup>CFU = Colony-forming units.

## Concentration of biogenic amines

During the production process of acid silage from tuna viscera, the concentration of biogenic

amines increased (Table 5), with the exception of putrescine and spermidine, whose concentration decreased.

	Aminoacids (g 100-1)	Silage	Fishmeal
	Histidine	2.11	1.24
	Arginine	8.04	3.71
	Threonine	5.02	2.29
	Valine	1.87	0.99
Essentials	Methionine	2.89	1.47
	Lysine	3.70	3.32
	Isoleucine	4.24	2.36
	Leucine	6.12	3.75
	Phenylalanine	4.08	2.55
	Alanine	5.30	4.40
Non essentials	Proline	3.62	3.42
	Tyrosine	5.75	5.05
	Serine	5.12	3.40
	Glyicine	5.08	6.20
	Aspartic acid	7.10	5.72
	Glutamic acid	14.60	10.70
	Cistine	3.64	1.87

**Table 4.** Aminogram of the protein ingredients used in the manufacture of the diets for the digestibility experiment.

Table 5. Concentration of biogenic amines in the raw material (tuna viscera) and silage.

Biogenic amines (mg 100 g-1)	Raw Matter	Silage
Putrescine	12.90	9.71
Cadaverine a	57.69	77.97
Histamine	0.74	1.30
Tyramine	28.47	36.59
Agmatine	88.33	90.38
Spermidine	88.08	76.56
Phenylethynamine	1.53	2.03
Tryptamine	nd1	nd

<sup>1</sup> Not detected; detection limit 0.4 mg kg<sup>-1</sup>.

## Composition of silage fatty acids

The fatty acid composition of the silage produced showed that fatty acid concentrations of the n-3 series were higher than those of the n-6 series.

## *Fatty acid composition (% lipid fraction) of silage from tuna viscera*

The fatty acid composition of the produced tuna silage showed that the content of the n-3 fatty acid series, Eicosapentanoid and Docosahexanenoic, were  $6.07 \pm 0.05\%$  and  $21.72 \pm 0.70\%$ , respectively.

The n-6 polyunsaturated fatty acids, Linoleic and Arachidonic were  $3.19 \pm 0.1\%$  and  $3.04 \pm 0.73\%$ , respectively.

### Protein solubility of silage

The protein hydrolysis period for obtaining the silage was 30 days, with the mean temperature of  $28 \pm 1.30$  °C and mean pH of  $4.18 \pm 0.12$ , within the standard for the production of acid silage. The soluble protein increased until the  $23^{rd}$  day, reaching a plateau from there until day  $30^{th}$ . During the silage process, the acid hydrolysis increased the protein solubility from 32.38% to 41.33%, corresponding to 48.37% and 61.74% of the crude protein, respectively. The final product was in a pasty liquid form, due to the continuous protein hydrolysis, potentiated by the enzymes present in the viscera. Digestibility assay

Protein digestibility was similar between silage and fishmeal protein ingredients. As for dry matter, silage was more digestible than fishmeal (P<0.05) (Table 6).

**Table 6.** Apparent protein and dry matter digestibility coefficients of tuna viscera silage and fish residue meal for jundiá (*Rhamdia quelen*).

Test ingradiant	Apparent digestibility coefficient (%)		
rest ingreatent	Protein	Dry Matter	
Silage	$88.12\pm0.58^{\rm a}$	$92.20 \pm 3.50^{a}$	
Fishmeal	$88.92 \pm 1.98^{a}$	$83.84 \pm 2.80^{b}$	

 $^{a,b}$  Means followed by the same letter in the same column do not differ from one another by the t-student test (P>0.05).

## DISCUSSION

Crude protein constituted the largest fraction of the silage produced, since we used a standardized residue (viscera), devoid of bones, fins or heads. According to SILVA et al. (2013), the presence of heads and fins in the raw material contribute to a decrease in protein and an increase in mineral content. When compared to other silages of fish residues (ABIMORAD et al., 2009; ARRUDA et al., 2009; SILVA et al., 2013), where the mineral matter ranged from 17.3 to 43.60%, our acid silage of tuna viscera presented low content of mineral matter. This is a positive aspect, considering that excess mineral matter is undesirable in fish diets (SILVA et al., 2013). However, silage high humidity should be considered when preparing large quantities of fish feeds commercially.

Microbiological analyzes did not indicate significant growth of microorganisms in the raw material nor in the silage produced, which shows the good quality of the tuna viscera used. The absence of microorganisms in the silage also reflects the important role of acetic acid in preventing the proliferation of microorganisms during the process.

Biogenic amines are formed by the decarboxylation of amino acids (for example: histidine in histamine, lysine in cadaverine, arginine in putrescine, tyrosine in tyramine) (RICQUE-MARIE *et al.*, 1998). A number of factors may affect biogenic amine concentrations, both abiotic (post-capture management, refrigeration

system and temperature) and biotic (genetics, sex, physiological state and tissue type of the residue) (SILVA *et al.*, 2013). During the silage production process of this study, the concentration of biogenic amines increased, with the exception of putrescine and spermidine. This increase may be related to the ambient temperature ( $28.0 \pm 1.3 \text{ °C}$ ), which was high during the ensiling process. However, the levels of putrescine, cadaverine and histamine found in this study are lower than those found in other studies (COWEY and CHO, 1992; MENDOZA *et al.*, 1997; FAIRGRIEVE *et al.*, 1994). Studies monitoring the concentration of biogenic amines in silage production for feeding fish are scarce.

In the natural aquatic environment, fish are subject to contamination by heavy metals. The amount of heavy metals in fish varies depending where fish are raised but fish viscera can concentrate these metals (GONÇALVES, 2011). The levels of heavy metals found in fish silage, except for lead and mercury, are above the maximum tolerated by ANVISA in predatory fish (chromium 0.10 mg kg-1, cadmium 1.0 mg kg-1, lead 2.0 mg kg-1 and mercury 1.0 mg kg-1) for human consumption (BRASIL, 1998). This is a negative aspect regarding the use of silage of tuna viscera, which would probably be attenuated with the removal of liver from raw material, since toxic substances ingested tend to accumulate in this organ for detoxification (GONCALVES, 2011). Specific studies on the toxicity of the metals present in the silage of fish tuna viscera are required.

Protein solubilization by enzymatic action is high, especially when the raw material of ensilage is constituted by viscera (RAA and GILDBERG, 1982), as is the case of the present study. However, partially hydrolyzed silage has a higher nutritional value than fully-hydrolyzed silage (STONE et al., 1989; VIANA et al., 1996). Studies report the manipulation of protein hydrolysis of silages so that the degree of protein solubilization is monitored. In this process, after the mass is liquefied, the acid hydrolysis process is interrupted (MENDOZA et al., 2001; LIANG et al., 2006; DELCROIX et al., 2014; VALLE et al., 2015) and the enzymes are inactivated by increasing the temperature. Thus, it is ensured that the proteins are no longer hydrolyzed and that the integrity of the peptides and free amino acids produced is maintained. In the present study, although there was no temperature control, protein hydrolysis was not complete in thirty days. This means that the conditions adopted in the present study were adequate for ensiling the tuna viscera.

Fish readily accepted the experimental diets, suggesting that the inclusion of tuna viscera silage did not affect diet palatability. The apparent digestion coefficient (ADC) of the tuna viscera silage protein was similar to the ADC of the fish waste meal protein. Lower ADCs protein values (84.08 and 85.11%) were reported for Indian carp (Labeo rohita), when fed fish residue silages, produced with formic and sulfuric acids, respectively (HOSSAIN et al., 1997). Similarly, the protein ADCs of biological silage of tilapia residue varied from 79.4 to 87.2% for African catfish (Clarias gariepinus) (FAGBENRO and JAUNCEY, 1995). The higher values of protein digestibility found in this study may be related to the raw material used for silage production. Tuna viscera constitutes a standardized residue, different from that used in other studies, whose raw material included heads, bones and other fish components, which can generate silages with varied nutritional composition, influencing nutrient digestibility. However, the protein digestibility of the acid silage of tuna viscera was lower than that found for acid fish silage (ADC = 96.7%), when fed to Nile tilapia (PIMENTA et al., 2008), which may be related to the long digestive tract of tilapia, favorable to the best utilization of nutrients.

On the other hand, the high dry matter digestibility of the silage when compared to that

recorded for fishmeal is probably due to the acid hydrolysis. High apparent digestibility of dry matter (95.5%) of acid silage for Nile tilapia was also reported by PIMENTA *et al.* (2008).

## CONCLUSION

The acid silage of tuna viscera has good nutritional composition (high protein, good amino acid and essential fatty acid profile), good microbiological quality and a protein solubility of 61.74%. Tuna viscera silage also has high protein and dry matter digestibilities for jundiá juveniles. Therefore, this novel ingredient has potential as an alternative protein source in aquafeeds.

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