fishes

MDPI

Article

Fish Viscera Silage: Production, Characterization, and Digestibility of Nutrients and Energy for Tambaqui Juveniles

Thiago Macedo Santana [1,*], Francisco de Matos Dantas [1], Driely Kathriny Monteiro Dos Santos [2], Juliana Tomomi Kojima [2], Yugo Moraes Pastrana [3], Rogério Souza De Jesus [3] and Ligia Uribe Gonçalves [3,*]

[1] Programa de Pós-graduação em Ciência Animal e Recursos Pesqueiros, Universidade Federal do Amazonas, Av. General Rodrigo Octavio Jordão Ramos, 1200, Manaus 69067-005, AM, Brazil
[2] Programa de Pós-graduação em Aquicultura, Universidade Nilton Lins, Av. Prof. Nilton Lins, 3259-Flores, Manaus 69058-030, AM, Brazil
[3] Instituto Nacional de Pesquisas da Amazônia, Av. André Araújo 2936, Manaus 69060-001, AM, Brazil
* Correspondence: zootecthiago@gmail.com (T.M.S.); ligia.goncalves@inpa.gov.br (L.U.G.)

Abstract: Fish viscera can be an important source of protein and energy for aquafeed, and its use contributes to circular aquaculture. The aim of this study was to produce acid and fermented silage from fish viscera to determine their nutritional value and the apparent digestibility coefficient of nutrients and energy for juvenile tambaqui (*Colossoma macropomum*). An acid silage and three fermented silages with different sources of carbohydrates (molasses, wheat bran, and cassava waste) were produced with 0.25% sorbic acid as an antifungal in the formulation. Silages presented an average of 55% dry matter, 62.9% lipids, and 12% crude protein. Leucine and lysine predominated as essential amino acids in the acid silage and fermented silages. Fish viscera silages presented EPA and DHA content from 5.4 to 17.8 and 1.7 to 8.9 mg.g^{-1} of lipids, respectively. The apparent digestibility coefficient (ADC) (indirect method) was above 82% for gross energy for all the formulations. The ADCs for protein were similar for the fermented silages, with a maximum 92% level for the fermented wheat bran silage. The bioconversion of fish viscera into silage makes it an energy ingredient for aquafeed that is well digested by tambaqui juveniles.

Keywords: fish waste; neotropical fish; protein hydrolysis

Citation: Santana, T.M.; Dantas, F.d.M.; Monteiro Dos Santos, D.K.; Kojima, J.T.; Pastrana, Y.M.; De Jesus, R.S.; Gonçalves, L.U. Fish Viscera Silage: Production, Characterization, and Digestibility of Nutrients and Energy for Tambaqui Juveniles. *Fishes* **2023**, *8*, 111. https://doi.org/10.3390/fishes8020111

Academic Editor: Shi-Jian Fu

Received: 23 December 2022
Revised: 16 January 2023
Accepted: 17 January 2023
Published: 14 February 2023

1. Introduction

World aquaculture produced 88 million tons of fish for consumption in 2020, of which 99.8% was destined for human consumption [1]. While processing fish, around 20–80% of waste is generated. Improper disposal of fish waste can damage natural resources, tourism, and local life with serious environmental, economic, and social impacts [2]. However, fish waste is a rich source of protein and essential amino acids, lipids, essential fatty acids, collagen, vitamins, and minerals [3]. When fish waste is correctly processed, it can become a coproduct with potential use as ingredients for aquafeed and pet food industries [4].

Fish viscera silage is a hydrolyzed product preserved by the addition of acids or by microbial fermentation induced by carbohydrates. The liquefaction of the product is the result of the action of the proteolytic enzymes naturally present in fish and available when the original raw material is milled and homogenized [3]. Acid silage is produced by the inclusion (average of 3%) of organic or inorganic acids or a blend of both acids in fish waste [5,6]. Fermented silage is produced by adding microorganisms from the lactic acid group (*Lactobacillus* sp. and *Lactococcus* sp. or yeasts of the genus *Hansenula* and *Saccharomyces*) that use the carbohydrate source added to fish waste. The lactic acid formation lowers the pH of silage and, consequently, inhibits the proliferation of pathogenic and deteriorating bacteria [4,7].

Silage provides partially hydrolyzed protein, peptides, free amino acids, unsaturated fatty acids, vitamins, and minerals [3]. Silage is an easy-to-produce, low-cost technology

that can convert fish waste into a feed ingredient for aquaculture [8]. The crude protein digestibility of *Katsuwonus pelamis* viscera silage is 88.1% for *Rhamdia quelen*, which is similar to that of fish meal (88.9%) [6].

Tambaqui, *Colossoma macropomum*, is the most produced native species in Brazilian fish farming. Tambaqui production has gone from 13 thousand tons in 2000 to 100.6 thousand tons in 2020 [9]. Tambaqui production is well established in other Latin American countries such as Bolivia, Colombia, Panama, Peru, the Dominican Republic, Venezuela, and Suriname. It is also produced in the United States, Mexico, China, Thailand, and the Philippines [10,11]. Tambaqui is preferably marketed eviscerated, and its viscera correspond to 10–15% of its body weight [12], which can be used for silage production. The aim of this study was to produce fermented and acid silages from tambaqui viscera, which were physicochemically characterized, and the apparent digestibility coefficient of nutrients and energy was determined for tambaqui juveniles.

2. Materials and Methods

The study was carried out at the Aquaculture Experimental Station of the Brazilian National Institute for Research in the Amazon, Manaus, Amazonas, Brazil. All the experimental procedures were conducted in accordance with the ethical principles adopted by the National Council for the Control of Animal Experimentation (CONCEA). They were approved by the Ethical Committee for the Use of Animals (CEUA) of INPA (protocol No. 0036/2020).

2.1. Production of Fish Viscera Silage

Viscera (liver, stomach, intestine, swim bladder, kidney, spleen, and gonads) were obtained after evisceration processing of tambaqui in a slaughterhouse located in the city of Manaus, Amazonas, Brazil. Fish viscera were collected and ground in a meat grinder (5 mm). One fish viscera sample was lyophilized and analyzed for proximate composition according to the methods standardized by AOAC [13], amino-acid composition (Item 2.2), and fatty-acid composition (Item 2.3). Four silage types were formulated, namely, acid silage (acid VS), fermented molasses silage (molasses VS), fermented wheat bran silage (wheat VS), and fermented cassava waste silage (cassava VS), in four replicates (plastic containers with a useful volume of 20 L). The acid silage was produced with a 3% (weight/volume) mixture of hydrochloric acid and citric acid at the 2:1 ratio. The fermented silages were produced with 15% carbohydrates (weight/weight) and 5% full-fat out-of-date yogurt (weight/volume). Sorbic acid was added at a rate of 0.25% (weight/weight) to all the silage formulations as an antifungal (Figure 1).

Silages were homogenized daily for 7 days. Then, pH and temperature were measured (TECNOPON, mPA-210, Piracicaba, São Paulo, Brazil). At the end of the seven storage days, silage samples were collected to analyze proximate [13], fatty-acid, and amino-acid composition.

2.2. Composition of Amino Acids

The nitrogen contents were analyzed in a nitrogen distiller (Model TE-036/1, Tecnal, Piracicaba, SP, BR) using the Kjeldahl method. For amino acids, 100 mg of sample was hydrolyzed with 6 M HCl at 110 °C for 24 h, followed by neutralization with 4 mL of 25% (weight/volume) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an amino-acid analyzer (1260 Infinity LCs (Agilent Technologies, Santa Clara, CA, USA). Tryptophan was determined by the colorimetric method of Spies [14] using a standard curve of pure tryptophan (Merck KGaA, Darmstadt, HE, Germany) and detected at 590 nm with a spectrophotometer (DU-640 UV/Vis—Beckman Coulter, Basking Ridge, NJ, USA). Cystine and methionine were analyzed as cysteic acid and methionine sulfone by oxidation with performic acid for 16 h at 0 °C and neutralization with hydrobromic acid prior to hydrolysis.

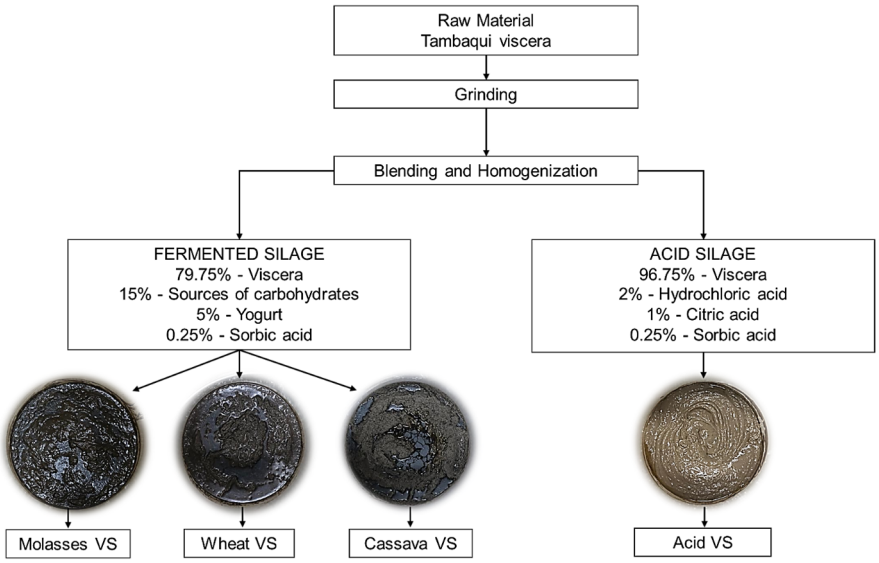

Figure 1. Flow chart of fermented and acid silage production. Molasses VS—fermented molasses silage; wheat VS—fermented wheat bran silage; cassava VS—fermented cassava waste silage; acid VS—acid silage.

2.3. Composition of Fatty Acids

Total lipid samples were determined using the method of Bligh and Dyer [15]. Fatty acid methyl esters (FAME) were prepared using the method proposed by Santos Júnior et al. [16] for all samples of fish viscera and silages. Methyl esters were separated by gas chromatography using a Thermo Scientific Trace Ultra Gas Chromatograph (Thermo Scientific, Waltham, MA, USA) fitted with a flame ionization detector (FID) and fused silica capillary column (100 m × 0.25 mm i.d., 0.25 µm cyanopropyl CP-7420 Select Fame). The operation parameters were as follows: detector temperature, 240 °C; injection port temperature, 230 °C; column temperature, 165 °C for 18 min, programmed to increase at 4 °C·min^{-1} up to 235 °C, with a final holding time of 14.5 min; carrier gas, hydrogen at 1.2 mL·min^{-1}; nitrogen was used as the make-up gas at 30 mL·min^{-1}; split injection at 1:80 ratio. For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma, St. Louis, MO, USA). Retention times and peak area percentages were automatically computed by the Software Chronquest 5.0. Quantification of fatty acids (mg·g^{-1} of total lipids) was performed using tricosanoic acid (23:0) methyl ester (Sigma-Aldrich, USA) as an internal standard [17]. Theoretical FID correction factor values were used to obtain concentration values of fatty acids in mg·g^{-1} of total lipids using the following equation: FAs = [(AX × WIS × CFX)/(AIS × CFAE × WX)], where FAs denote the fatty acids in mg·g^{-1} of total lipids, AX is the peak area (fatty acids), WIS is the standard weight (mg), CFX is the theoretical correction factor, AIS is the standard peak area (23:0), CFAE is the necessary conversion factor in order to express results in mg of fatty acid rather than as methyl ester, and WX is the sample weight (g).

2.4. Determination of the Apparent Digestibility Coefficient (ADC)

The determination of the apparent digestibility coefficient (ADC) was performed using the indirect method of decanting in a water column described by Cho [18] and Bureau [19]. A reference diet (RD; Table 1) and test diets (TDs) were formulated by replacing 30% of the RD with the experimental silages and 69.5% RD, using 0.5% chromium oxide III (Cr_2O_3) as a marker in the TDs [19].

Table 1. Formulation and proximate composition of the reference diet of the in vivo digestibility assay.

Ingredient (%)	Reference Diet	Molasses VS	Wheat VS	Cassava VS	Acid VS
Soybean meal	47.3	33.04	33.04	33.04	33.04
Corn meal	39.2	27.38	27.38	27.38	27.38
Wheat meal	6.3	4.40	4.40	4.40	4.40
Meat and bone meal	5.0	3.49	3.49	3.49	3.49
Molasses VS	-	30.0	-	-	-
Wheat VS	-	-	30.0	-	-
Cassava VS	-	-	-	30.0	-
Acid VS	-	-	-	-	30.0
Vitamin/mineral supplement [a]	1.0	0.70	0.70	0.70	0.70
Dicalcium phosphate	0.5	0.35	0.35	0.35	0.35
Salt	0.1	0.07	0.07	0.07	0.07
DL-Methionine	0.08	0.06	0.06	0.06	0.06
BHT	0.02	0.01	0.01	0.01	0.01
Chromium oxide III	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)					
Dry matter	96.10	96.63	96.97	96.83	96.97
Crude protein	31.90	28.43	29.07	28.90	29.10
Crude lipid	3.40	16.05	14.15	14.35	19.20
Crude fiber	4.16	3.72	4.54	3.61	7.75
Ash	8.05	7.05	7.00	6.95	6.95
Calcium	0.54	0.60	0.68	0.65	0.63
Phosphor	0.42	0.39	0.38	0.38	0.39
Gross Energy (kcal·100 g^{-1}) [b]	310.23	331.19	305.00	327.40	334.63

[a] Vitamin and mineral supplement for fish, values per kg of feed: folic acid (250 mg), pantothenic acid (5000 mg), antioxidant (600 mg), biotin (125 mg), cobalt (25 mg), copper (2000 mg), iron (13,820 mg), iodine (100 mg), manganese (3750 mg), niacin (5000 mg), selenium (75 mg), vitamin A (1,000,000 UI), vitamin B1 (1250 mg), vitamin B12 (3750 mg), vitamin B2 (2500 mg), vitamin B6 (2485 mg), vitamin C (28,000 mg), vitamin D3 (500,000 UI), vitamin E (28,000 UI), vitamin K3 (500 mg), and zinc (17,500 mg). [b] Gross energy analyzed using IKA 2600 bomb calorimeter.

The in vivo digestibility assay for tambaqui juveniles was performed according to the methodology recommended by the NRC [20].

Juvenile tambaqui (82.3 ± 8.2 g) were distributed in 15 conical fiberglass tanks adapted for feces collection (170 L; n = 3; 10 fish·$tank^{-1}$) in three replicates for the RD and TDs (molasses VS, wheat VS, cassava VS, and acid VS) and acclimated to laboratory conditions for 10 days. The conical tanks formed part of an open system with water renewal from an artesian well and constant aeration. Fish were fed until apparent satiation three times a day (8:00 a.m., 12:00 p.m., and 4:00 p.m.), and its fecal material was collected once a day for 21 days. One hour after the last meal, tanks were cleaned, and a container (50 mL) was fitted to the lower conical tank end to collect faces [21]. At 7:00 a.m. the following day, feces were collected and stored in a freezer (−20 °C) for the later lyophilization and bromatological analysis [13] and to determine chromium III oxide content [22]. The standard curve was calculated from the nitro-perchloric digestion of the samples with known chromium III oxide concentrations. Chromium III oxide concentrations were measured using a direct reading spectrophotometer (DR6000, HACH Company, Loveland, CO, USA), adjusted to a wavelength of 350 nm. The ADCs of nutrients and energy (dry matter basis) of both RD and TDs were calculated according to the following equation:

$$\text{ADC (\%)} = 100 - [100 \times (\text{ChD})/(\text{ChF}) \times (\text{NF})/(\text{ND})], \quad (1)$$

where ChD is the percentage chromium(III) in the diet, ChF is the percentage chromium(III) in feces, NF is the percentage nutrients (or energy) in feces, and ND is the percentage nutrients (or energy) in the diet.

The ADCs of the nutrients and energy of silages (molasses VS, wheat VS, cassava VS, and acid VS) were calculated using the equation proposed by Bureau and Hua [23]:

$$ADCi\ (\%) = ADCtd + (ADCtd - ADCrd) \times [(r \times Nrd)/(i \times Ni)], \quad (2)$$

where ADCi is the apparent digestibility coefficient of the ingredient, ADCtd is the apparent digestibility coefficient of the TD nutrient, ADCrd is the apparent digestibility coefficient of the RD nutrient, r is the proportion of the RD in the TDs (0.65), i is the proportion of the test ingredient in the TDs (0.3), Nrd is the nutrient concentration in the RD (%), and Ni is the nutrient concentration in the test ingredient (%).

2.5. Statistical Analyses

Statistical analyses were performed using the GraphPad Prism software (version 6.0). The percentage data were previously transformed using square root arcsine. The assumptions of parametric statistics were verified by the tests of normality (Kolmogorov–Smirnov) and homogeneity of variances (Bartlett). A one-way analysis of variance (ANOVA) was used and when a statistical difference was detected, and the treatments were compared using the Tukey test. In all tests, significance was observed at $p < 0.05$, and all data are presented as the mean ± standard deviation.

3. Results

The pH of silages lowered on the storage day 1 and stabilized on days 3, 4, and 5 for cassava VS, wheat VS, and molasses VS, respectively (Figure 2). The acid silage had the lowest pH value on storage day 1, which stabilized from storage day 2.

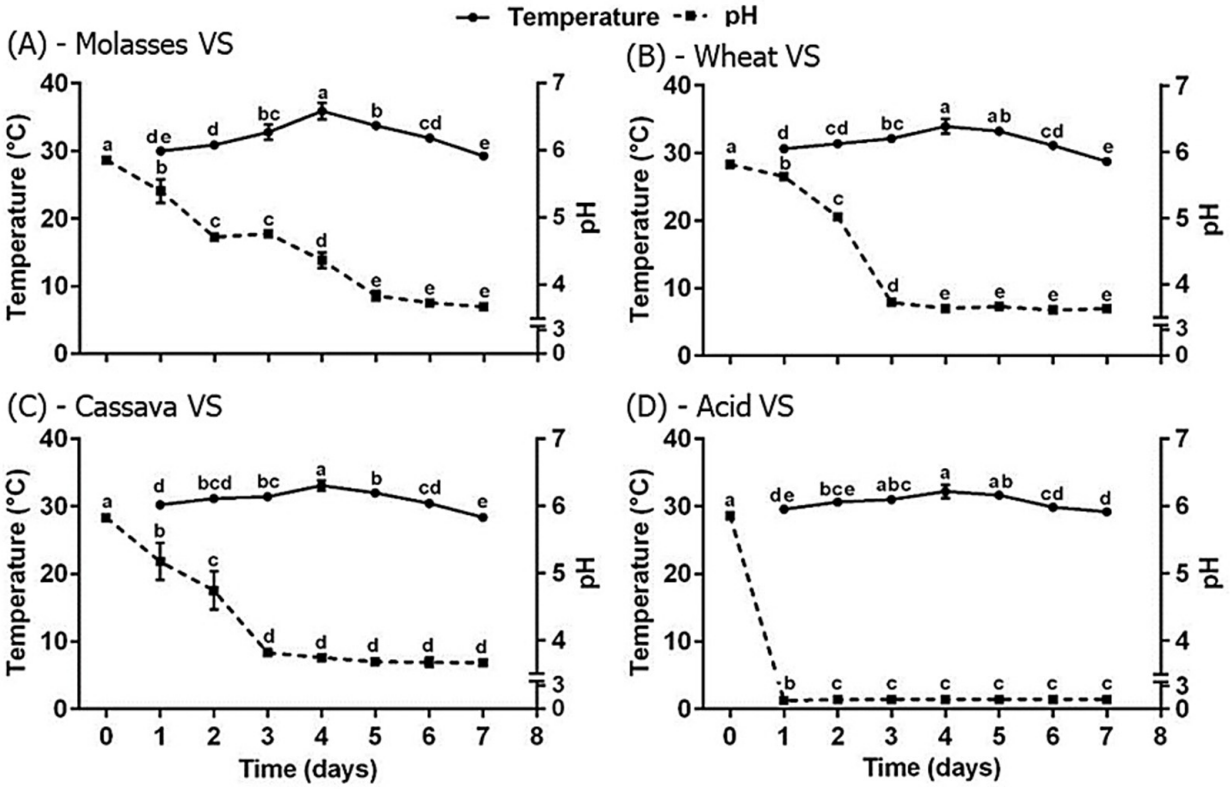

Figure 2. Temperature and pH of viscera silage stored for 7 days: (**A**) molasses VS; (**B**) wheat VS; (**C**) cassava VS; (**D**) acid VS.

The highest silage temperature value ($p < 0.05$) was reached on storage day 4 (Figure 2). The temperature in the molasses VS was 30.0 ± 0.47 °C on day 1, increased to 35.9 ± 1.22 °C

on day 4, and decreased to 29.28 ± 0.10 °C on day 7. In wheat VS and cassava VS, the highest temperature was recorded on storage day 4 (33.9 °C and 33.1 °C respectively). The initial acid VS temperature was 29.6 ± 0.17 °C, with the highest value (32.2 ± 0.98 °C) on day 4, before stabilizing at 29.2 ± 0.39 °C.

At the end of the ensiling period, different degrees of fish viscera dissolution were observed. At the beginning of silage storage, all the silages had a dense to solid consistency. As the days went by, they gradually changed to a pasty consistency. On day 7, acid VS presented a liquid and well-dissolved consistency, and the molasses VS consistency was intermediate, between liquid and pasty. Wheat VS and cassava VS showed a pasty consistency.

Wheat VS had the highest dry matter content (62.4% ± 0.9%), while molasses VS had the lowest (45.8 ± 0.4%) (Table 2). Tambaqui viscera had a higher crude protein content (30.8%) compared to the other silages. The percentage of crude protein in acid VS did not differ ($p > 0.05$) from that of molasses VS and wheat VS, but was 3.5% higher ($p < 0.05$) than in cassava VS. Fermented silages molasses VS and wheat VS contained the highest crude protein content (13.1% and 11.9%). Tambaqui viscera contained a high lipid content (29.4%), as reflected in the high lipid content of silages (around 60%). Wheat VS and cassava VS (5.41% and 9.5%, respectively) showed crude fiber content from the wheat bran and cassava waste, respectively. The fermented silages had the lowest ash content (1.7–5.3%).

Table 2. Proximate composition of fish viscera and silages after 7 days of storage (% dry matter).

Composition	Fish Viscera	Molasses VS	Wheat VS	Cassava VS	Acid VS	*p*-Value
Dry matter	52.4 ± 1.7	45.8 ± 0.4 ^{c}	62.4 ± 0.9 ^{a}	56.1 ± 0.6 ^{b}	55.7 ± 0.2 ^{b}	<0.001
Crude protein	30.8 ± 0.4	13.1 ± 1.0 ^{a}	11.9 ± 0.5 ^{ab}	9.8 ± 1.5 ^{b}	13.3 ± 0.5 ^{a}	<0.001
Crude lipid	29.4 ± 0.6	59.3 ± 0.6 ^{b}	60.5 ± 0.6 ^{b}	61.9 ± 1.1 ^{ab}	69.9 ± 0.2 ^{a}	<0.001
Crude fiber	1.4 ± 0.2	1.2 ± 0.1 ^{b}	5.41 ± 0.7 ^{ab}	9.5 ± 0.4 ^{a}	0.1 ± 0.02 ^{c}	<0.001
Ash	18.9 ± 0.5	5.3 ± 0.9 ^{ab}	1.7 ± 0.2 ^{b}	4.4 ± 0.6 ^{ab}	10.3 ± 0.5 ^{a}	<0.001
Gross energy (kcal·100 g^{-1}) ^{i}	405.7 ± 4.3	608.5 ± 53.3 ^{b}	629.4 ± 105.1 ^{ab}	611.1 ± 121.0 ^{b}	686.6 ± 28.5 ^{a}	<0.001

Distinct lowercase letters on the same line indicate a statistical difference in fish viscera silages according to the Kruskal–Wallis and Dunn tests ($p < 0.05$, $n = 6$ each silage). ^{i} Gross energy analyzed using IKA 2600 bomb calorimeter.

All the essential amino acids for fish were present in tambaqui viscera and, consequently, in all the produced silages (Table 3). In the fermented silages, the essential amino acids present in a higher proportion, in decreasing order, were leucine, arginine, and lysine. The most frequently detected nonessential amino acid in all silages was glutamic acid. The contents of alanine and tryptophan amino acids did not differ ($p > 0.05$) among the produced silages.

The fatty acids present in the fermented silages were predominantly unsaturated fatty acids (Table 4). Acid VS presented the highest content of saturated fatty acids. Molasses VS showed the widest variation between the contents of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), with 20.1% higher MUFAs versus SFAs, unlike acid VS, which contained 6.2% more SFAs than MUFAs. Oleic (18:1n-9) and linoleic (18:2n-6) fatty acids showed the highest concentration between MUFAs and polyunsaturated fatty acids (PUFAs), respectively. Silages had a lower n-6:n-3 ratio compared to tambaqui viscera.

The ADC of viscera silages for juvenile tambaqui was above 84% for nutrients, 76% for calcium and phosphorus, and 82% for gross energy (Table 5). Wheat VS showed the highest ADC for crude protein (91.94%). All the silages presented a calcium ADC value of around 90%, except for molasses VS with an ADC of 76.45%. The ADCs of dry matter, lipids, non-nitrogen extract, gross energy, and phosphorus were not significantly influenced by silage formulation ($p > 0.05$).

Table 3. Amino acids of fish viscera and viscera silages after 7 days of storage (mg·g^{-1} crude protein).

Amino Acids	Fish Viscera	Molasses VS	Wheat VS	Cassava VS	Acid VS	*p*-Value
Essential						
Arginine	9.4 ± 0.3	2.0 ± 0.0 ^{b}	3.3 ± 0.1 ^{b}	2.0 ± 0.1 ^{b}	8.9 ± 0.1 ^{a}	<0.001
Histidine	2.9 ± 0.16	1.9 ± 0.0 ^{b}	2.3 ± 0.0 ^{b}	1.9 ± 0.0 ^{b}	3.1 ± 0.0 ^{a}	<0.001
Isoleucine	5.3 ± 0.1	3.4 ± 0.0 ^{b}	3.7 ± 0.0 ^{b}	3.8 ± 0.0 ^{b}	5.0 ± 0.1 ^{a}	0.002
Leucine	10.5 ± 0.2	5.6 ± 0.1 ^{b}	7.4 ± 0.0 ^{b}	7.0 ± 0.1 ^{b}	10.2 ± 0.1 ^{a}	<0.001
Lysine	8.7 ± 0.1	5.2 ± 0.0 ^{b}	3.7 ± 0.0 ^{c}	3.6 ± 0.1 ^{c}	8.9 ± 0.1 ^{a}	<0.001
Methionine	3.4 ± 0.1	1.8 ± 0.0 ^{b}	1.9 ± 0.0 ^{b}	1.9 ± 0.0 ^{b}	3.2 ± 0.0 ^{a}	<0.001
Phenylalanine	5.3 ± 0.2	2.7 ± 0.0 ^{c}	3.8 ± 0.0 ^{b}	3.4 ± 0.0 ^{bc}	5.4 ± 0.1 ^{a}	<0.001
Threonine	5.8 ± 0.2	3.2 ± 0.0 ^{c}	4.2 ± 0.0 ^{b}	3.8 ± 0.0 ^{bc}	5.7 ± 0.1 ^{a}	<0.001
Tryptophan	1.2 ± 0.1	1.0 ± 0.0	1.2 ± 0.0	1.3 ± 0.1	1.3 ± 0.0	ns
Valine	6.7 ± 0.2	4.3 ± 0.0 ^{b}	5.1 ± 0.0 ^{b}	4.8 ± 0.1 ^{b}	6.7 ± 0.1 ^{a}	0.001
Nonessential						
Aspartic acid	11.3 ± 0.2	4.2 ± 0.0 ^{c}	7.3 ± 0.1 ^{b}	5.6 ± 0.1 ^{bc}	10.9 ± 0.1 ^{a}	<0.001
Alanine	16.4 ± 0.1	7.6 ± 0.0	8.7 ± 0.1	8.1 ± 0.1	9.7 ± 0.1	ns
Cystine	10.2 ± 0.4	0.0 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	1.2 ± 0.0 ^{a}	<0.001
Glycine	0.9 ± 0.1	6.0 ± 0.0 ^{b}	11.3 ± 0.2 ^{a}	9.3 ± 0.3 ^{ab}	13.6 ± 0.1 ^{a}	0.006
Glutamic acid	14.2 ± 0.7	9.1 ± 0.0 ^{c}	13.6 ± 0.1 ^{a}	10.4 ± 0.1 ^{bc}	15.3 ± 0.2 ^{a}	0.001
Serine	5.4 ± 0.2	3.1 ± 0.0 ^{bc}	3.8 ± 0.0 ^{b}	2.9 ± 0.0 ^{c}	5.3 ± 0.1 ^{a}	<0.001
Proline	9.2 ± 0.4	4.4 ± 0.0 ^{b}	7.8 ± 0.1 ^{a}	6.3 ± 0.1 ^{ab}	8.7 ± 0.1 ^{a}	0.004
Taurine	1.7 ± 0.0	1.4 ± 0.0 ^{b}	1.2 ± 0.0 ^{b}	1.3 ± 0.0 ^{b}	2.2 ± 0.0 ^{a}	<0.001
Tyrosine	4.1 ± 0.2	1.0 ± 0.0 ^{b}	0.8 ± 0.0 ^{b}	0.9 ± 0.0 ^{b}	4.0 ± 0.0 ^{a}	<0.001
Crude protein (%)	30.8	13.1	11.9	9.8	13.3	

Distinct lowercase letters on the same line indicate a statistical difference in fish viscera silages according to one-way ANOVA and Tukey's tests ($p < 0.05$, $n = 3$ each silage); ns = nonsignificant.

Table 4. Fatty acids of fish viscera and viscera silages after 7 days of storage (mg·g^{-1} lipid).

Fatty Acids	Fish Viscera	Molasses VS	Wheat VS	Cassava VS	Acid VS	*p*-Value
Lauric (12:0)	nd	5.2 ± 0.3	4.0 ± 2.6	2.7 ± 1.8	6.5 ± 1.9	ns
Myristic (14:0)	13.7 ± 0.0	15.8 ± 2.2	12.9 ± 1.9	14.7 ± 1.3	15.2 ± 1.8	ns
Palmitic (16:0)	309.6 ± 0.1	239.4 ± 6.0	254.8 ± 0.5	257.3 ± 28.1	271.7 ± 15.8	ns
Heptadecanoic acid (17:0)	1.2 ± 0.0	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.2	1.6 ± 0.2	ns
Stearic acid (18:0)	162.8 ± 0.1	124.0 ± 4.8	140.6 ± 17.4	121.6 ± 11.5	132.3 ± 4.3	ns
Arachidic acid (20:0)	3.1 ± 0.0	4.8 ± 1.4	4.4 ± 0.7	4.6 ± 0.5	4.8 ± 0.6	ns
Heneicosanoic acid (21:0)	3.2 ± 0.0	6.1 ± 0.2	3.6 ± 0.5	4.2 ± 0.4	4.3 ± 0.5	ns
∑SFAs	493.6 ± 0.2	396.9 ± 1.6	421.9 ± 15.1	406.6 ± 34.8	436.4 ± 18.3	ns
Palmitoleic acid (16:1n-7)	27.3 ± 0.0	33.9 ± 3.3	32.5 ± 4.3	36.4 ± 1.3	3.15 ± 0.1	ns
Palmitoleic acid (16:1n-9)	3.5 ± 0.0	5.0 ± 0.7	7.7 ± 2.7	6.1 ± 1.5	0.48 ± 0.0	ns
Vaccenic acid (18:1n-7)	16.4 ± 0.0	23.1 ± 4.6	19.3 ± 0.8	21.5 ± 1.3	1.76 ± 0.2	ns
Oleic acid (18:1n-9)	380.5 ± 0.0	434.7 ± 2.9	406.7 ± 24.4	425.2 ± 37.0	403.9 ± 2.9	ns
∑MUFAs	427.7 ± 0.1	496.7 ± 2.0	466.2 ± 17.5	489.2 ± 17.0	409.3 ± 2.93	ns
Linoleic acid (18:2n-6)	58.4 ± 0.1	72.9 ± 1.6	70.6 ± 3.9	72.4 ± 2.5	67.6 ± 2.9	ns
Linolenic acid (18:3n-3)	15.5 ± 0.0	17.5 ± 2.9	19.6 ± 5.6	16.7 ± 4.5	18.9 ± 7.6	ns
Arachidonic acid (20:4n-6)	3.0 ± 0.0	5.2 ± 1.1	4.1 ± 0.8	6.2 ± 3.1	4.2 ± 0.8	ns
Eicosapentaenoic acid (20:5n-3)	1.1 ± 0.1	5.4 ± 6.4	17.8 ± 15.8	8.2 ± 1.4	10.0 ± 1.28	ns
Docosahexaenoic acid (22:6 n-3)	0.5 ± 0.0	1.7 ± 2.5	6.7 ± 5.5	1.7 ± 2.0	8.9 ± 1.18	ns
∑PUFAs	78.3 ± 0.22	102. 7 ± 3.8	118.8 ± 23.2	105.2 ± 3.8	109.6 ± 15.6	ns
∑n-6	61.4 ± 0.08	78.1 ± 1.3	74.7 ± 4.5	78.6 ± 3.9	71.8 ± 3.6	ns
∑n-3	17.1 ± 0.08	24.6 ± 3.3	55.9 ± 11.3	27.3 ± 2.5	46.7 ± 6.5	ns
n-6:n-3	3.6 ± 0.4	3.2 ± 0.4	1.4 ± 0.4	2.9 ± 0.4	1.6 ± 0.3	ns

SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids. Data from fish viscera silages were analyzed by one-way ANOVA ($p > 0.05$, $n = 3$ each silage); nd = not determined, ns = nonsignificant.

Table 5. Apparent digestibility coefficient of nutrients and energy of viscera silages for tambaqui juveniles (% dry matter).

Composition	Molasses VS	Wheat VS	Cassava VS	Acid VS	*p*-Value
Dry matter	79.46 ± 2.7	86.88 ± 6.6	78.77 ± 3.2	82.97 ± 5.6	ns
Crude protein	88.32 ± 0.6 ^{ab}	91.94 ± 2.9 ^{a}	86.91 ± 0.3 ^{ab}	84.24 ± 4.4 ^{b}	0.040
Crude lipid	94.96 ± 1.8	94.60 ± 1.2	94.10 ± 0.8	93.57 ± 0.1	ns
Gross energy	85.06 ± 2.8	84.20 ± 8.7	82.64 ± 1.2	87.15 ± 6.9	ns
Calcium	76.45 ± 2.0 ^{b}	90.98 ± 1.6 ^{a}	90.58 ± 7.2 ^{a}	89.11 ± 4.6 ^{a}	0.039
Phosphorus	81.20 ± 11.4	80.69 ± 8.8	76.95 ± 11.5	80.09 ± 4.9	ns

Distinct lowercase letters on the same line indicate a statistical difference according to one-way ANOVA and Tukey's tests ($p < 0.05$, $n = 4$ each silage); ns = nonsignificant.

4. Discussion

The silage pH values remained below 4.5, which is a safe pH value for silage conservation because it prevents the proliferation of pathogenic and spoilage microorganisms [24]. The acid VS pH, because of the inclusion of hydrochloric acid and citric acid, remained close to 1, which is considered a very acidic ingredient to be included in aquafeeds. However, pH neutralization can lead to the accumulation of mineral salts [25], which can be a limiting factor for including acid silage in fish diets [26]. The slowly lowering pH of the fermented silages is related to gradual lactic acid production by microorganisms from yogurt [27].

The ideal silage storage temperature varies from 25 °C to 35 °C. Values beyond this range can reduce or inhibit lactic acid-producing bacteria activity [28]. The studied silages presented an average temperature of 32.8 °C, which was influenced by the high ambient temperature (33.2 ± 2.93 °C) of the Amazon region of Brazil where the experiment was carried out, as well as by the intense microbial activity of the silage process [29]. Lactic acid-producing bacteria, mainly of the *Lactobacillus* genus, use carbohydrate sources to produce lactic acid, antibacterial compounds (bacteriocins), and energy [4]. These compounds promote silage conservation by inhibiting the growth of pathogenic microorganisms, and by allowing the action of the proteolytic enzymes present in fish viscera, which collaborate in the hydrolysis and dissociation of fish waste [3].

The difference in proximal composition was related to variation in the carbohydrate sources used in the formulation of each fermented silage. The dry matter content of wheat VS was the highest because wheat bran has greater water absorption power, unlike sugarcane molasses that are easily diluted in water because 60% of its solids are composed of glucose, fructose, sucrose, and minerals. These molasses compounds are substrates for lactic acid-producing bacteria growth, which promotes a bigger bacterial production and, consequently, greater hydrolysis to generate a more liquefied product [4].

The lowering crude protein content of silage vs. that of the fish viscera could be due to the proportion of ingredients in the silage formula, the composition of the added carbohydrate sources, or loss by protein nitrogen being released in the form of volatile ammonia (NH^3) that results from the protein hydrolysis process [30,31]. The high fat content of the fish viscera was reflected in the silages. Tambaqui accumulates visceral fat, which can represent up to 70% of its abdominal cavity [32]. The tambaqui viscera silages produced in this study can be characterized as an energy ingredient for aquafeed due to low protein content (<20%) and high fat content (around 60%) in dry matter.

The silages in our study presented low ash content because we used only viscera in the silage formulation. The ash content of silages produced with whole fish waste (bones, heads, fins, scales, and viscera) ranges from 11.9% to 21.5% [33]. Although fish viscera silages have a lower protein content compared to fish meal (54% to 61%), fish meal contains fivefold more ash than tambaqui viscera silages. High ash content is not desirable in the fish diet because some mineral needs can be supplied directly through aquaculture system water [6].

Fish viscera silage is an interesting ingredient for aquafeeds because essential amino acid content improves the dietary nutritional value by consequently improving the feed

efficiency of aquatic organisms [20]. Generally, diets for omnivorous fish are formulated with ingredients of plant origin because this involves lower costs. However, some plant origin ingredients are deficient in amino acid, such as lysine and methionine [34]. Therefore, the inclusion of a low fish viscera silage content to vegetable ingredient mixes may suffice to meet the nutritional requirements in feed formulations for omnivorous fish.

The high glutamic acid concentration present in silages can influence the animal immune system by promoting the synthesis of cytokines, immunomodulatory substances needed to induce lymphocyte proliferation [35]. The low pH values of silages did not promote tryptophan stability, especially when not bound to protein, which resulted in low tryptophan content in the experimental silages [5].

Silages showed a high lipid content (above 59%), and tambaqui juveniles presented an ADC for lipids above 93%. The ADC of lipids from silages was similar to that of fish oil (92%) and higher than that of the corn (85.8%) and soybean (85.1%) oils evaluated for tambaqui juveniles by Buzollo et al. [35]. The tambaqui viscera silage presented a similar ADC of gross energy to those of salmon meal (81.1%), poultry byproduct meal (83.8%) and defatted black soldier fly larvae (86.6%), and it was higher than that of feather meal (77.2%) and tilapia processing waste meal (70.2%) when evaluated for tambaqui juveniles [35,36].

Silage can be a source of MUFAs in aquafeed formulations. MUFAs contribute to fish health when constantly ingested by promoting the reduction of oxidized LDL in plasma, preventing the transport of cholesterol to tissues and inhibiting body fat accumulation [37]. Silages showed higher levels of PUFAs and n-3 series fatty acids than fish viscera. The presence of highly unsaturated fatty acids, such as EPA and DHA in silage, can contribute to enrich diets for omnivorous fish, which generally have no or low HUFA content because they consist mostly of ingredients of plant origin or terrestrial animal byproducts [32].

Fish viscera silages showed high digestibility for tambaqui juveniles. The hydrolysis of silage proteins into short-chain peptides and free amino acids makes it more bioavailable for digestion and absorption by fish [38]. The ADC values of crude protein from fish viscera silages are similar to the ADCs of Peruvian fish meal and corn gluten for tambaqui [35].

The difference in calcium ADC was due to the distinct carbohydrate sources used in silage formulation. Calcium forms part of the composition of bones and scales, is used by several enzymes in fish metabolism, and participates in the functioning of cell membranes, blood clotting, neural transmission, and muscle contraction [20]. Tambaqui viscera silages can be sources of dietary calcium and phosphorus to replace inorganic supplements in aquafeed.

5. Conclusions

Acid and fermented tambaqui viscera silages can be characterized as an energy ingredient for aquafeed due to the low protein (<20%) and high fat (around 60%) contents in dry matter. Fish viscera silage is an easy-to-produce technology that can convert fish waste into a feed ingredient for aquaculture. All the silages were well digested by juvenile tambaqui. It is necessary to evaluate the maximum level at which tambaqui viscera silages can be included in aquafeeds.

Author Contributions: Conceptualization, L.U.G., T.M.S. and F.d.M.D.; methodology, T.M.S. and L.U.G.; validation, J.T.K., F.d.M.D. and T.M.S.; formal analysis, L.U.G. and Y.M.P.; investigation, all the authors; resources, L.U.G.; data curation, T.M.S., D.K.M.D.S., J.T.K., Y.M.P. and L.U.G.; writing—original draft preparation, T.M.S.; writing—review and editing, D.K.M.D.S. and L.U.G.; visualization, L.U.G. and R.S.D.J.; supervision, L.U.G. and R.S.D.J.; project administration, L.U.G. and T.M.S.; funding acquisition, L.U.G. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to thank the Amazonas State Research Foundation (FAPEAM), who granted the research funds (004/2018 - AMAZONAS ESTRATÉGICO/FAPEAM and POSGRAD-FAPEAM 2021/2022 - Resolução 008/2021). T.M.S. received a doctoral scholarship from Coordination for the Improvement of Higher Education Personnel (CAPES). L.U.G. is a research fellow from the Brazil's National Council for Scientific and Technological Development (CNPq) (Process 312492/2021-9).

Institutional Review Board Statement: The procedures of this study were in compliance with the Ethical Committee of Research on the Use of Animals of the INPA (no. 0036/2020).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data of the study can be requested from the corresponding authors upon reasonable request.

Acknowledgments: The authors would like to thank the assistance from the staff of the National Institute of Amazonian Research (INPA) and all students of the Gigas Project at the INPA.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO (Food and Agriculture Organization of the United Nations). Fishery and Aquaculture Country Profiles—Mexico. In *Country Profile Fact Sheets*; Fisheries and Aquaculture Division: Rome, Italy, 2022; Available online: https://www.fao.org/fishery/en/facp/mex?lang=en (accessed on 8 November 2022).
2. Mozumder, M.M.H.; Uddin, M.M.; Schneider, P.; Raiyan, H.I.; Trisha, M.G.A.; Tahsin, T.H.; Newase, S. Sustainable Utilization of Fishery Waste in Bangladesh—A Qualitative Study for a Circular Bioeconomy Initiative. *Fishes* **2022**, *7*, 84. [CrossRef]
3. Toppe, J.; Olsen, R.L.; Peñarubia, O.R.; James, D.G. *Production and Utilization of Fish Silage. A Manual on How to Turn Fish Waste into Profit and a Valuable Feed Ingredient or Fertilizer*; FAO: Rome, Italy, 2018; Available online: www.fao.org/3/i9606en/I9606EN.pdf (accessed on 7 November 2022).
4. Kuley, E.; Özyurt, G.; Özogul, I.; Boga, M.; Akyol, I.; Rocha, J.M.; Özogul, F. The Role of Selected Lactic Acid Bacteria on Organic Acid Accumulation during Wet and Spray-Dried Fish-Based Silages. Contributions to the Winning Combination of Microbial Food Safety and Environmental Sustainability. *Microorganisms* **2020**, *8*, 172. [CrossRef] [PubMed]
5. Vidotti, R.M.; Viegas, E.M.M.; Carneiro, D.J. Amino acid composition of processed fish silage using different raw materials. *Anim. Feed Sci. Technol.* **2003**, *105*, 199–204. [CrossRef]
6. Banze, J.F.; da Silva, M.F.O.; Enke, D.B.S.; Fracalossi, D.M. Acid silage of tuna viscera: Production, composition, quality and digestibility. *Bol. Inst. Pesca* **2017**, *44*, 24–34. [CrossRef]
7. Mayta-Apaza, A.C.; García-Cano, I.; Dabrowski, K.; Jiménez-Flores, R. Bacterial Diversity Analysis and Evaluation Proteins Hydrolysis During the Acid Whey and Fish Waste Fermentation. *Microorganisms* **2021**, *9*, 100. [CrossRef] [PubMed]
8. Afreen, M.; Ucak, I. Fish processing wastes used as feed ingredient for animal feed and aquaculture feed. *Surv. Fish. Sci.* **2020**, *6*, 55–64. [CrossRef]
9. Instituto Brasileiro de Geografia e Estatística (IBGE). 2021. Available online: https://sidra.ibge.gov.br/Tabela/3940 (accessed on 29 November 2022).
10. Woynárovich, A.; Van Anrooy, R. *Field Guide to the Culture of Tambaqui (Colossoma macropomum, Cuvier, 1816)*; FAO Fisheries and Aquaculture Technical Paper; Food and Agriculture Organization of the United Nations: Rome, Italy, 2019; p. I-121.
11. Hilsdorf, A.W.S.; Hallerman, E.; Valladão, G.M.R.; Zaminhan-Hassemer, M.; Hashimoto, D.T.; Dairiki, J.K.; Takahashi, L.S.; Albergaria, F.C.; Gomes, M.E.D.S.; Venturieri, R.L.L.; et al. The farming and husbandry of *Colossoma macropomum*: From Amazonian waters to sustainable production. *Rev. Aquac.* **2022**, *14*, 993–1027. [CrossRef]
12. Marcos, R.; Filho, R.A.C.; De Abreu, J.S.; Seraphim, G.N.; Silva, A.C.; Fornari, D.C.; Ribeiro, R.P.; Ferreira, Y.A.; Gama, K.F.; Povh, J.A. Weight and body yield of selectively bred tambaqui (*Colossoma macropomum*) farmed in different environments. *An. Acad. Bras. Ciênc.* **2020**, *92*, e20180675. [CrossRef]
13. AOAC. *Official Methods of Analysis of the AOAC International*, 18th ed.; Association of Official Analytical Chemists: Rockville, MD, USA, 2005; Volume 2.
14. Spies, J.R. Determination of tryptophan in proteins. *Anal. Chem.* **1967**, *39*, 1412–1416. [CrossRef]
15. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef]
16. Júnior, O.O.S.; Montanher, P.F.; Bonafe, E.G.; Prado, I.N.D.; Maruyama, S.A.; Matsushita, M.; Visentainer, J.V. A Simple, Fast and Efficient Method for Transesterification of Fatty Acids in Foods Assisted by Ultrasound Energy. *J. Braz. Chem. Soc.* **2014**, *25*, 1712–1719. [CrossRef]
17. Joseph, J.D.; Ackman, R.G. Capillary Column Gas Chromatographic Method for Analysis of Encapsulated Fish Oils and Fish Oil Ethyl Esters: Collaborative Study. *J. AOAC Int.* **1992**, *75*, 488–506. [CrossRef]
18. Cho, C.Y. Fish nutrition, feeds, and feeding: With special emphasis on salmonid aquaculture. *Food Rev. Int.* **1990**, *6*, 333–357. [CrossRef]
19. Bureau, D.; Harris, A.; Cho, C. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **1999**, *180*, 345–358. [CrossRef]
20. National Research Council. *Nutrient Requirements of Fish and Shrimp*; National Academies Press: Washington, DC, USA, 2011.
21. Tibbetts, S.M.; Milley, J.E.; Lall, S.P. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). *Aquaculture* **2006**, *261*, 1314–1327. [CrossRef]

22. Furukawa, A.; Tsukahara, H. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bull. Jpn. Soc. Sci. Fish.* **1966**, *32*, 502–508. [CrossRef]
23. Bureau, D.; Hua, K. Letter to the Editor of Aquaculture. *Aquaculture* **2006**, *2*, 103–105. [CrossRef]
24. Panicz, R.; Eljasik, P.; Troszok, A.; Sobczak, M.; Lisiecki, S.; Nędzarek, A.; Sadowski, J. Safe management of *Cyprinid herpesvirus* 3-induced mortalities of common carp (*Cyprinus carpio*) by silaging process. *Aquac. Rep.* **2022**, *24*, 101116. [CrossRef]
25. Arason, S. Production of fish silage. In *Fisheries Processing*; Springer: Boston, MA, USA, 1994; pp. 244–272.
26. Millamena, O.M. Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*. *Aquaculture* **2002**, *204*, 75–84. [CrossRef]
27. Ozyurt, G.; Gokdogan, S.; Şimşek, A.; Yuvka, I.; Ergüven, M.; Kuley Boga, E. Fatty acid composition and biogenic amines in acidified and fermented fish silage: A comparison study. *Arch. Anim. Nutr.* **2016**, *70*, 72–86. [CrossRef]
28. Zahar, M.; Benkerroum, N.; Guerouali, A.; Laraki, Y.; El Yakoubi, K. Effect of temperature, anaerobiosis, stirring and salt addition on natural fermentation silage of sardine and sardine wastes in sugarcane molasses. *Bioresour. Technol.* **2002**, *82*, 171–176. [CrossRef] [PubMed]
29. Lindgren, S.; Pleje, M. Silage fermentation of fish or fish waste products with lactic acid bacteria. *J. Sci. Food Agric.* **1983**, *34*, 1057–1067. [CrossRef]
30. Land, M.V.; Vanderperren, E.; Raes, K. The effect of raw material combination on the nutritional composition and stability of four types of autolyzed fish silage. *Anim. Feed. Sci. Technol.* **2017**, *234*, 284–294. [CrossRef]
31. Zieliński, M.; Rusanowska, P.; Zielińska, M.; Dudek, M.; Nowicka, A.; Purwin, C.; Fijałkowska, M.; Dębowski, M. Influence of preparation of *Sida hermaphrodita* silages on its conversion to methane. *Renew. Energy* **2021**, *163*, 437–444. [CrossRef]
32. Guimarães, I.G.; Martins, G.P. Nutritional requirement of two Amazonian aquacultured fish species, *Colossoma macropomum* (Cuvier, 1816) and *Piaractus brachypomus* (Cuvier, 1818): A mini review. *J. Appl. Ichthyol.* **2015**, *31*, 57–66. [CrossRef]
33. Shabani, A.; Boldaji, F.; Dastar, B.; Ghoorchi, T.; Zerehdaran, S.; Ashayerizadeh, A. Evaluation of increasing concentrations of fish waste silage in diets on growth performance, gastrointestinal microbial population, and intestinal morphology of broiler chickens. *Anim. Feed. Sci. Technol.* **2021**, *275*, 114874. [CrossRef]
34. Miao, S.; Chang, E.; Han, B.; Zhang, X.; Liu, X.; Zhou, Z.; Zhou, Y. Dietary tryptophan requirement of northern snakehead, *Channa argus* (Cantor, 1842). *Aquaculture* **2021**, *542*, 736904. [CrossRef]
35. Wu, G. Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. *J. Anim. Sci. Biotechnol.* **2014**, *5*, 34. [CrossRef]
36. dos Santos, D.K.M.; Santana, T.M.; Dantas, F.D.M.; Farias, A.B.d.S.; Epifânio, C.M.F.; Prestes, A.G.; da Fonseca, F.A.L.; Parisi, G.; Viegas, E.M.M.; Gonçalves, L.U. Defatted black soldier fly larvae meal as a dietary ingredient for tambaqui (*Colossoma macropomum*): Digestibility, growth performance, haematological parameters, and carcass composition. *Aquac. Res.* **2022**, *53*, 6762–6770. [CrossRef]
37. Goosen, N.J.; de Wet, L.F.; Görgens, J.F. Rainbow trout silage as immune stimulant and feed ingredient in diets for Mozambique tilapia (*Oreochromis mossambicus*). *Aquac. Res.* **2016**, *47*, 329–340. [CrossRef]
38. Islam, J.; Peñarubia, O. Seafood Waste Management Status in Bangladesh and Potential for Silage Production. *Sustainability* **2021**, *13*, 2372. [CrossRef]