v. 3, n. 7, 2023

Journal of
Engineering
Research

USE OF FISH WASTE FOR THE PRODUCTION OF ANIMAL NUTRITIONAL SUPPLEMENT THROUGH THE ACID SILAGE PROCESS

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Abstract: The use of fish waste to obtain by-products with added value is one of the appropriate ways to reduce waste generation. The acid silage process can be used to reuse these residues, as it is economically viable and easy to prepare. The present work aimed to standardize the acid silage process for the production of animal nutritional supplement from fish residues, and to compare the nutritional parameters with those described in the legislation, in scientific articles and on labels of sachets of commercial products. The results obtained for the nutritional parameters were satisfactory, corresponding to 1.70% (m/m) in gray matter; 0.06% (w/w) in calcium; 35.72% (w/w) crude protein; 1.63% (m/m) in crude fiber, except for the ether extract (50.31% m/m) and 31.00% moisture (m/m). The significant difference (≅ 25%) in the ether extract content and (≅ 16%) in relation to the moisture content is probably due to the type of raw material used. However, they are in accordance with the limits established by Brazilian legislation for supplements classified as semi-moist or moist. Depending on the characteristics, the product can be used to supplement animal feed.
Keywords: Waste, reuse, animal nutritional supplement, acid silage.

INTRODUCTION

Accelerated population growth has resulted in serious environmental problems, caused, in most cases, by the generation of improperly discarded urban and agro-industrial waste. According to the Brazilian Association of Public Cleaning and Special Waste Companies (ABRELPE, 2017), around 78.4 million tons of urban waste were produced in Brazil in 2017. Among the various types of waste, agro-industrial waste stands out as raw material for the production of animal feed, adding nutritional value to the by-product (SILVA et al., 2019). During the stages of the fish farming production chain, from production to marketing, significant amounts of organic waste are generated, with 65% of this fraction being discarded in inappropriate places, compromising, mainly, the quality of river water (VALENTE et al. al., 2014).

The reuse of fish waste through acid silage, in order to obtain by-products with added value, is one of the ways to avoid environmental contamination and produce an additive that can be used for food enrichment. The production process is economically viable and easy to perform, generating a by-product rich in omegas 3 and 6, in addition to proteins and fats (OLSEN; TOPPE, 2017; PINTO et al., 2017). In acidic silage, the previously crushed residual mass liquefies by enzymatic action, originating a lipid layer that preserves enzymatic activity for a few months (PINTO et al., 2017).

Animal feed is a balanced food composed of nutrients of animal or vegetable origin, including grains and bran from by-products of industrial processes of grains and animal tissues or from by-products of the poultry, beef, pork, sheep and fish (FRANÇA et al., 2011). Fish residues have been widely used in the production of food for animal nutrition, whose effectiveness can be proven from the standardization of nutritional parameters with the minimum and maximum limits defined for nutrients (PESSOA, 2017).

Analyzes of nutritional parameters are relevant from the point of view of food security and aim to meet the specific needs of nutrients to supply animals in their different stages of life (FRANÇA et al., 2011).

The monitoring of nutrients in animal feed samples is conducted by physical-chemical methods that stand out for being fast and providing reliable results. The Weende method is widely used because it allows the characterization of the centesimal composition, from the determination of moisture, inorganic matter (ash), nitrogen compounds (crude protein), ether extract (fat) and non-nitrogen extracts (fibers) (ING et al., 2016; FERNANDES et al., 2017).

In Brazil, the responsibility for regulating feed for dogs and cats lies with the Ministry of Agriculture, Livestock and Supply, provided for in Decree No. 76,986 of January 6, 1976, which regulated Law No. of products intended for animal feed (CARCIOFI, 2006).

Normative Instruction No. 09 of 2003 establishes limits for the parameters that deal with the quality of animal feed (INMETRO, 2020).

This work aimed to standardize the acidic silage employed in the production of animal nutritional supplement, using fish waste as a raw material, determine the centesimal composition and compare nutritional parameters with those instituted by legislation and those described in scientific articles and product sachet labels commercial. Tilapia and tambaqui residues found abundantly found in Rio Grande, located in the city of Uberaba (MG). The concentrations of sulfuric acid (H_2SO_4) and phosphoric (H_3PO_4) in the acid mixture were evaluated for the best efficiency of the acidic silage process. For nutritional assessment of the supplement, centesimal composition and calcium content was investigated.

METHODOLOGY

PRODUCTION OF ANIMAL NUTRITIONAL SUPPLEMENT

The raw material used for the production of animal nutritional supplement was acquired at a free fair in the city Uberaba (MG), which is part of the Rio Grande Basin and has fishing as one of the main sources of income of several families (Krüger, 2017). The production process involving acidic silage included gills, fins and viscera from tilapia and tambaqui waste. Initially, fish residues were mixed with 10.0 ml of distilled water and crushed with the aid of an industrial blender until a homogeneous appearance.

The 20 g mass of the crushed residue was mixed with 30.0 ml of distilled water and 1.50 ml of the acid mixture, containing concentrations of H2SO4 and H3PO4, in ratio 3: 1. After homogenization, the mixture was evenly distributed in 8 labeled plastic containers. Different concentrations of the acid mixture were evaluated in order to obtain the best development of the silage process (Figure 1). After homogenization, the pH was constantly measured until the ideal value (pH = 4.0) was obtained (HISANO, et al., 2012).

PHYSICOCHEMICAL ANALYZES OF NUTRITIONAL PARAMETERS OF ANIMAL NUTRITIONAL SUPPLEMENT

The physicochemical analyzes of nutritional nutritional parameters were conducted in triplicate, following the Weende methodology. The centesimal composition involved the analysis of proteins, fats, moisture and gross fiber, in addition to the calcium content (AOAC, 1995).

In the determination of moisture (ashes), previously clean and dry cadinhos were heavy, prior to the analysis. The 5.0 g mass of the sample was sent to the muffle (550 ° C) for 4 h until ashes.

For protein analysis, the Kjeldhal method was selected, which is based on the "digestion" of the nitrogen sample with concentrated H_2SO_4, in the presence of catalyst. The method involved the steps of digestion, distillation and title of the sample, being nitrogen converted into ammonium sulfate $[(NH_4)\ _2SO_4]$ by "acid digestion", and amoniacal nitrogen during alkaline distillation. In this method, ammoniacal nitrogen reacts with boric acid (H_3BO_3) and produces ammonium borato ($NH_4H_2bo_3$), which is quantified by titling

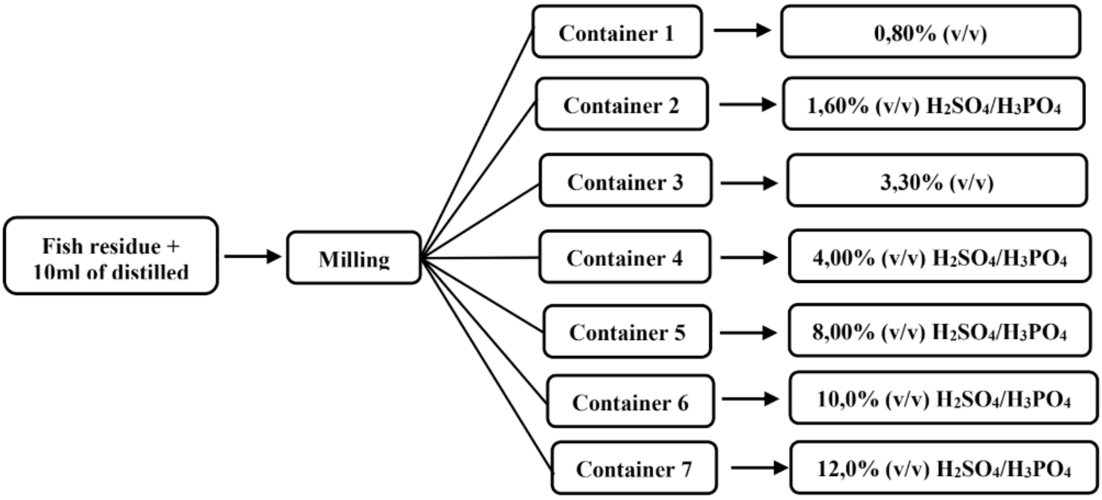

Figure 1: Methodology flowchart for the standardization of acid concentration in the acid mixture used in the silage process.

Concentration % (v/v) H_2SO_4/H_3PO_4 in the acid mixture (3: 1)	pH
0,8	6,33
1,6	6,12
3,3	5,57
4,0	5,00
8,0	4,51
10,0	3,98
12,0	3,55

Volume of the added acid mixture: 1,50 mL

Table 1: Concentration and pH values after mass homogenization.

Source: of the authors, 2020

Figure 2: Sample of the dry animal nutritional supplement.

Journal of Engineering Research ISSN 2764-1317 DOI 10.22533/at.ed.317372315036

(ZENEBON, 2008).

Previously the distillation and title steps, damp sample masses corresponding to 0.20 and 0.40 g were added in triplicate in 12 distinct Kjeldahl test tubes and then mixed with 1.50 g from the catalyst and 5.0 ml of concentrated H_2SO_4. Pipes 1 and 2, which contained dry and mass samples were taken to the digestor block, whose temperature was gradually increased by 50 ° C, and maintained for 30 min, until 350 ° C, and a homogeneous solution to obtain a homogeneous solution and translucent.

The lipid extraction method by acid hydrolysis used for fat determination used ethyl ether as a solvent. Acid hydrolysis allowed lipids to be separated from carbohydrates and proteins for further gravimetric analysis (AOAC, 1995).

Sample masses corresponding to 3.0 g were distributed in 6 test tubes in order: tubes 1 and 2 (dry sample), 3, 4 and 5 (humid sample) and tube 6 (analytical white).

The method selected for the determination of gross fiber was based on the presence of the insoluble organic waste present in the sample and, later, in the "acid digestion" and "alkaline" (CUNHA, 2011).

Calcium content analysis involved the complexation volume method, in which the 5.0 g mass of the animal nutritional supplement was added to porcelain cadinhos and sent to the muffle (450ºC), for 4h (AOAC, 1995; Zenebon, 2008). After the sample reached the room temperature, it was washed with distilled water and 1.0 ml of solution 1.0 mol L^{-1} HNO3 (nitric acid). The resulting solution was transferred to a 10.0 ml volumetric flask, avolution with deionized water.

RESULTS AND DISCUSSION

DETERMINATION OF THE IDEAL PH FOR THE ACIDIC SILAGE STEP

Immediately after complete homogenization of the grind mass with the acid mixture, the pH was measured and the respective values can be observed in Table 1.

As acidic silage develops better at pH near 4.0 (HISANO et al., 2012), it can be inferred that the addition of 1.50 ml of the acid mixture of 10.0% (V/V) H2SO4/ H3PO4, in proportion 3: 1, efficiently conducted the process.

After two days of the beginning of the acidic silage process, a significant increase in the pH value was observed, requiring the daily increase of 1.0 ml of previously selected acid mixture (10% V/V H_2SO_4/H_3PO_4), for 3 consecutive days, until the process was completed. The total volume of 3.5 ml of the acid mixture was required.

After drying, the nutritional supplement had dark brown color (Figure 2) and odor very close to that found in commercial canine feed.

COMPARISON OF THE NUTRITIONAL PARAMETERS OF THE ANIMAL NUTRITIONAL SUPPLEMENT

Quantitative analyzes for the determination of centesimal composition and calcium quantification (ZENEBON, 2008) were performed in the nutritional supplement, after the acidic silage process has completed.

The results presented in Table 2 were compared to those presented in scientific articles in the literature.

Regarding the gray content, a standard deviation of 7.5% compared to other supplements reported in scientific articles was observed, and the largest discrepancy was found after the analysis of Borghesi (2004) and Espíndola Filho (2001). According to Borghesi, ash contents are directly related to the strictest parts of fish, such as scales, heads, spine and bones, however, in this work were used gills and fins, excluding the other parts

Nutritional parameters (%)	Animal supplement produced (%)	*a	*b	*c	*d	*e	*f	Standard deviation (%)
Gray matter	1,70*	-	8,26	2,10	4,17	17,73	17,90	7,50
Calcium	0,06*	0,06	2,49	-	-	8,03	-	3,80
Moisture	31,0*	21,7	67,8	72,1	21,6	25,20	10,0	24,3
Gross protein	35,7*	25,7	50,5	20,1	59,2	54,20	36,3	14,8
Ethereal extract	50,3*	42,4	13,2	2,10	18,4	12,40	21,8	17,3
Gross fiber	1,63*	-	-	-	-	-	1,40	0,20

* value corresponding to the average triplicate analysis

* Hisano et al., 2012; *B Vasconcelos et al., 2011; *C Santos et al., 2015; *D Arruda et al., 2006; *and Borghesi, 2004; *F Espíndola Filho, 2001

Table 2: Comparison of nutritional values of animal nutritional supplement produced by acidic silage and those of literature.

Source: Authors, 2020

Nutritional Parameters (%)	Limits established by law for adult animals [INMETRO, 2020]									Animal supplement produced (%)
	Limits	Dry supplement		Semi-moist supplement		moist supplement		Liquid Supplement		
		Dogs	Cats	Dogs	Cats	Dogs	Cats	Dogs	Cats	
Gray matter	Maximum	12,0	12,0	10,0	10,0	2,5	2,5	0,7	0,7	1,70*
Calcium	Maximum	2,5	2,5	2,0	2,0	0,5	0,5	2,0	2,0	0,06*
Phosphor	Minimum	0,1	0,3	0,08	0,2	0,02	0,02	0,02	0,02	-
Humidity	Maximum	12,0	12,0	30,0	30,0	84,0	84,0	95,0	95,0	31,0*
Crude protein	Minimum	7,0	24,0	5,6	19,0	1,3	4,4	0,4	1,4	35,7*
Ethereal extract	Minimum	4,0	7,0	3,2	5,6	0,7	1,3	0,3	0,4	50,3*
Crude fiber	Maximum	26,0	16,0	21,0	13,0	5,0	3,0	1,5	1,0	1,6*

* value corresponding to the mean of triplicate analyzes

Table 3: Comparative between the nutritional values of the adult animal nutritional supplement and the limits established by the legislation.

Source: of the authors, 2020

of the fish residue were used, thus obtaining a product with low gray content.

The calcium content was compatible with the results of Hisano research; Ishikawa; Portz (2012) and destruction of the other works evaluated, probably due to the lowest proportion of bones and scales used as raw material.

Since the volume of acid added to biomass is a determining factor in moisture variation, the animal nutritional supplement produced had values similar to those found by Borghesi, differing from Vasconcelos; Mosque; Albuquerque (2011) and Santos et al., In (2015). Borghesi added to the biomass, the concentration 3.0% (V/V) in propionic acid and formic, whose values were close to those selected in this work (3.5% V/V of the H_2SO_4/H_3PO_4 acid mixture).

In 2011, Vasconcelos; Mosque; Albuquerque added 7% (V/V) H_3CCOOH (glacial acetic acid) and $C_6H_8O_7$ (citric acid) to biomass for the silage process. Santos et al. (2015) employed a distinct methodology for the acidic silage step and observed significant moisture levels in the product.

Following the verification of the gross protein content, similarities were observed in the values obtained by Espíndola Filho (2001), distinguishing from the research conducted by Arruda (2006). This may have occurred due to the difference between the types of acids used in silage, considering that the greater the force of acid, the greater the degradation of gross protein. The type of raw material employed is also a likely factor responsible for changing the protein content.

Regarding the ethereal extract, the result that came closest to the content obtained in this work was the one presented by Hisano; Ishikawa; Portz (2012), having distinguished from the amounts reported by Borghesi (2004). Prior to the determination of this parameter, Borghesi extracted part of the supernatant lipid portion by centrifugation, after 3 days from the beginning of nutritional supplement production. Although Hisano; Ishikawa; Portz (2012) used different types of fish, the accumulation of visceral fat, responsible for increasing the lipid content was similar to that of Tambaqui, used as a raw material in this work.

It is not common to measure fiber content in silage, however the result found resembles the work of Espíndola Filho, et al. (2001).

All measured nutritional values have resembled some work in the literature, proving the efficiency of the methods used and the viability of supplementing as a nutritional source for animals. The differences found are mainly the difference in the raw material used.

Table 3 presents the comparison between the nutritional limits determined by Normative Instruction No. 09 of 2003, of the Ministry of Agriculture, and the values found in this work.

The analysis of all samples revealed that the nutritional parameters of the supplement produced are among the limits established by legislation and can be classified as semi -ism or humid food. Excess moisture in a food may favor the proliferation of harmful microorganisms (INMETRO, 2020).

The animal supplement produced by acidic silage did not fit the maximum allowed limit for gray and gross fiber. Gray matter is critical to animal nutrition, and is characterized by its remaining inorganic content after complete removal of the organic matrix (Menezes, 2016). A high gray content content can characterize food tampering (Cosmo, et al., 2017), however, gross fiber is necessary for the intestinal health of dogs and cats. Excess gross fiber can compromise digestion and absorption of proteins and minerals, leading to malnutrition (INMETRO, 2020).

Comparison of the nutritional parameters of the animal supplement produced with those described in commercial product sachet labels was performed in order to verify adequacy with some products marketed for dog feeding (Figure 3).

The results presented in figure 3 indicated that the standard deviation for calcium content (≅ 0.80%) and gross fiber (≅ 0.90%) of feed and supplements were low, indicating that the supplement produced is in accordance with values of commercial products.

The gray matter of the supplement produced (≅ 1.70%) resembled that of the meat flavored sachet (≅ 3.0%) and the gross protein content (≅ 35.72%) approached the feed for flavored puppies chicken (≅ 28.0%).

The humidity is between the values obtained in the labels of the sachet (humid food) and the feed (dry food), being closer to the bifinho (semi-discreted food). The ethereal extract content did not resemble any food or supplement, however, is in accordance with the limits established by the legislation (Table 3).

Figure 4 presents the results of the supplement comparison produced, with products of different brands, types of feed and food supplements for cats.

Note low values for the standard deviation of calcium content (≅ 0.60%) and gross fiber (≅ 0.60%), indicating that the supplement produced by acidic silage falls into the values of commercial products (Figure 4).

The gray matter content was close to that found in Tuna -flavored cat sachets.

The value related to the moisture content was not agreement with any food or supplement analyzed, however, it is in accordance with the values presented in the labels of commercial products. Regarding gross protein, the content resembled commercial feed.

The ethereal extract content did not fit any food or supplement, but corroborates the minimum value stipulated by the legislation (Table 3).

FINAL CONSIDERATIONS

The proposed acidic silage procedure was effective and enabled the production of several samples with similar characteristics. The silage developed properly after selecting the concentration and volume of the acid mixture.

The values found for the parameters analyzed in the animal nutritional supplement are in accordance with those instituted by legislation and found in scientific articles in the literature that used acidic silage for supplement production, as well as the information described in the labels of commercial products. The divergences of moisture values can be explained by the difference in supplement production methods and the type of raw material used.

Animal supplementation produced may be effective as to the nutritional values required in the feeding of cats and dogs.

The ethereal extract content did not fit any type of animal feed or supplement. Probably the type of raw material used had a significant influence on this parameter.

Despite the differences found, when compared to commercial samples, the values of the nutritional parameters are within the limits specified by the legislation. The animal supplement of fish produced by acidic silage fell into the classification of semi -ism or humid food, which can be considered as a snack, used to complement animal feed.

For the improvement of the supplement produced, it is recommended to reduce the ethereal extract content of the samples and new studies to evaluate microbiological properties (presence of fungi and bacteria). People are recommended so that the product gets the appropriate format for commercialization.

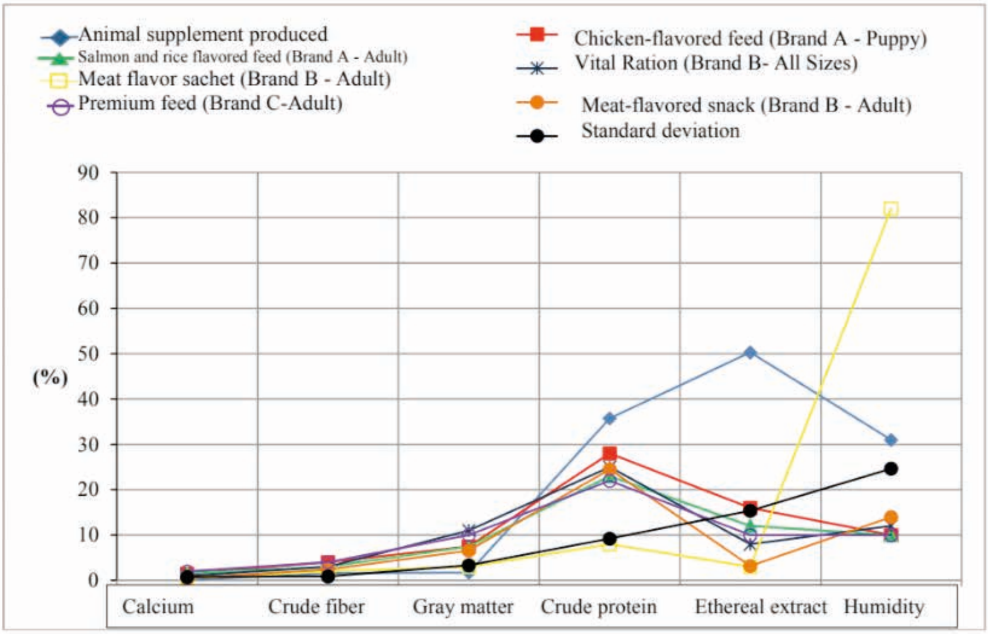

Figure 3: Comparison of the nutritional values of supplement produced with complete and special dog foods for dogs.

Source: of the authors, 2020

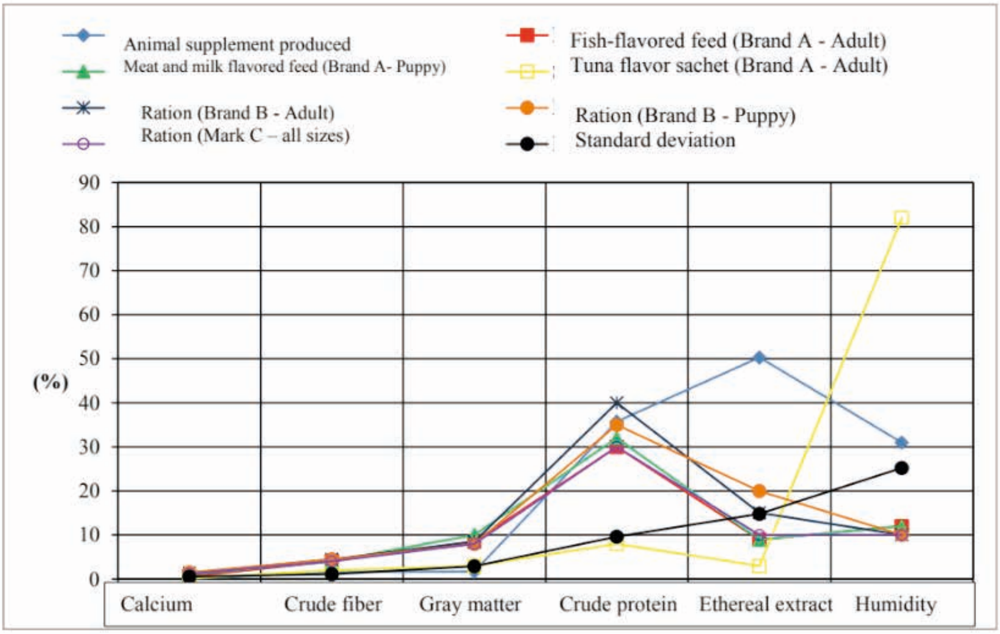

Figure 4: Comparison of nutritional values of animal supplement produced with complete and special commercial cat foods.

Source: from the authors, 2020

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