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Digestibility and physico-chemical characteristics of acid silage meal made of pirarucu waste in diets for commercial laying hens

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ABSTRACT. The objective of this study was to evaluate the effects of acid silage meal made of pirarucu waste in diets for commercial laying hens on apparent digestibility and energy metabolism. Seventy-two Hisex White hens with 71 weeks of age were assigned to a completely randomized with two treatments (control diet and diet with 3% pirarucu waste acid silage) with six replicates of six birds each. The ensiled biomass was light brown in color, showing acidified aroma; creamy consistency; 4.38 ± 0.11 pH; 84.16% dry matter; 40.06% crude protein; 26.82% ether extract; 9.31% mineral matter, 65.16 g kg⁻¹ calcium and 22.90 g kg⁻¹ phosphorus. Differences ($p > 0.05$) were detected in digestibility of crude protein, non-fiber carbohydrates (soluble carbohydrates), etherextract, mineral matter, metabolizable energy and metabolizable energy coefficient. Our results indicate that the acid silage meal made of pirarucu waste can be included up to 3% in diets for laying hens, showing satisfactory nutrient digestibility and potential to be used as an energy source.

Keywords: alternative food, fish waste, metabolizable energy, nutrients, silage.

Características físico-química e digestibilidade da farinha de silagem ácida de resíduo de pirarucu em rações de poedeiras comerciais leves

RESUMO. Objetivou-se com este estudo avaliar as características físico-químicas e nutricionais da farinha de silagem ácida de resíduos de pirarucu em rações para poedeiras comerciais leves sobre a digestibilidade aparente e metabolização energética. Foram utilizadas 72 poedeiras da linhagem Hisex White com 71 semanas de idade distribuídas em um delineamento inteiramente casualizado com seis repetições de seis aves cada. Os tratamentos foram constituídos pelo controle (sem inclusão do produto) e a ração experimental (com 3% de inclusão de silagem ácida). A biomassa ensilada apresentou coloração marrom claro; aroma levemente acidificado; consistência ligeiramente cremosa; pH de $4,38 \pm 0,11$; com valores de 84,16% de matéria seca; 40,06% de proteína bruta; 26,82% de extrato etéreo; 9,31% de matéria mineral, 65,16 g kg⁻¹ de cálcio e 22,90 g kg⁻¹ de fósforo. Foram observadas diferenças significativas na digestibilidade da proteína bruta, extrato não nitrogenado (carboidratos solúveis), extrato etéreo, matéria mineral, energia metabolizável e no coeficiente de metabolização da energia metabolizável. Os resultados do presente estudo indicaram que a silagem ácida produzida a partir da biomassa residual do pirarucu pode ser incluída na forma de farinha até 3% em rações para poedeiras leves, apresentando boa digestibilidade dos nutrientes e potencial para ser utilizada como fonte energética.

Palavras-chave: alimento alternativo, ensilado, energia metabolizável, nutrientes, resíduo de pescado.

Introduction

The Amazon is home to a large diversity of aquatic organisms, and this is the main source of income and subsistence of riverine communities living on the exploitation of fish resources along the Solimões-Amazonas River and its tributaries (Costa, Silva, Souza, Batalha & Hoshiba, 2013). Among the species most exploited and consumed by the regional population, the pirarucu *Arapaima gigas* (Schinz 1822), known as Amazon cod, is highly

valued in the market, especially when its meat is marketed dried and preserved with salt.

For processing pirarucu, in the form of frozen fillets or salty-dry, only fish fillets are used, while waste (carcasses, viscera, fins, scales and skins) are disposed in the environment. However, some of these by-products have the potential to be used as new products.

In this context, a prominent product in recent studies developed in Brazil for the use of waste from fish is silage. This is a viable alternative for the use of fillet waste (Costa, Portz, Hisano, Druzian & Ledo,

2009), as well as being a simple, low-cost operating technique used for the conversion of waste material into products of high nutritional quality, which can minimize problems with environmental pollution, as well as serve as an ingredient in feed formulation.

As for the use of silages made of fish waste in animal feed, these have already presented potential to provide economic benefits to poultry production systems, since their feeding represents 70% total production costs, where corn and soybeans are the main ingredients used as energetic and protein sources in feed formulations. In this sense, the use of these wastes generated by the fish industry to transform by-products into alternative foods, offering advantages of accessibility, especially in Brazilian regions that face barriers to grain logistics and raw materials with high costs (Cruz et al., 2016).

Based on this perspective, the use of silage as an alternative ingredient mainly aims at the productive and economic aspect, without impairing, above all, the performance and physiology of the animal. Thus, it is necessary to have a clear understanding of the protein and mineral composition, energy concentration, nutrient accessibility, and its use by the bird organism (Oliveira, Pinheiro, Fonseca & Oba, 2012).

In view of the above, this study evaluated the effects of chemical and nutritional characteristics of the acid silage meal made of pirarucu waste in diets for commercial laying hens on apparent digestibility and energy metabolism.

Material and methods

The experiment was conducted at the Fish Technology Laboratory, Technology and Innovation Coordination-COTI, National Institute for Amazonian Research (INPA), where the procedures for obtaining acid silage made of *Arapaima gigas* pirarucu waste were developed (Schinz, 1822) as well its physical and chemical characterization; and at the Poultry Sector, Department of Animal and Plant Production (DPAV), Faculty of Agricultural Sciences (FCA), Federal University of Amazonas (UFAM), located in the Southern Sector of the University Campus, Manaus, state of Amazonas, Brazil, where the digestibility assay was performed.

The raw material used came from the Mamirauá Sustainable Development Reserve, Amazonas-Brazil, and was acquired in February 2016 from a fish processing warehouse, consisting of the carcass (backbone and ribs). Such residues were transported in closed raffia bags to the COTI-INPA Fish Technology Laboratory.

The residues were fragmented in an electric crusher and divided into plastic buckets with a

capacity of 15 liters, where each received 10 kg crushed mass. Each 10 kg was added with a mixture of propionic and formic acids in the proportion 1: 1, in the ratio of 3% of the volume of the acid solution to the weight of the residue, under constant homogenization, following part of the methodology proposed by Borghesi, Portz, Oetterer and Cyrino (2008). The containers were kept at room temperature for three days.

For complete standardization, the ensiled material was stirred every 24 hours during the three days of storage to allow a greater contact of the acids with the residues and to guarantee the quality of the silage, from which samples were taken for pH determination. This measurement was carried out using a pHmeter OHAUS (Starter 3100), with results expressed in two decimal places.

Alterations in the product were accompanied by observations of the organoleptic characteristics, based on color, aroma and consistency. At the end of the three days, to reduce moisture, the ensiled material was placed on aluminum trays, taken to a forced ventilation oven, at 65°C for 72 hours. In this interval, the material was turned over to ensure uniform drying and obtaining a quality dry product. At the end of the drying period, the total dry product presented a yield of 50% in relation to the ensiled mass, and was ground to obtain the acid silage meal made of pirarucu waste.

The chemical composition (dry matter: DM, crude protein: PB, ether extract: EE, mineral matter: MM, potential of hydrogen: pH and total carbohydrates: TC), obtained by the equation: $100 - (\% \text{Humidity} + \% \text{CP} + \% \text{MM} + \% \text{EE})$ of acid silage meal and pirarucu waste were determined at the Laboratory of Chemistry and Physical Chemistry of Food, according to methodologies proposed by Silva and Queiroz (2012) and the minerals were digested in nitric-perchloric extract and the concentrations quantified by atomic absorption spectrophotometer (Ca) and by colorimetry (P) in the spectrophotometer using ammonium molybdate and ascorbic acid, according to the methodology described by Sarruge and Haag (1974) at the Thematic Laboratory of Soils and Plants of Chemistry of INPA.

The diets (Table 1) were formulated using the computational software Supercrac (2004) to meet the nutritional requirements of animals and according to the values of the ingredients provided by the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011), except for the composition of the chemical silage of pirarucu waste.

Seventy-two, 71 week-old Hisex White hens were housed in 12 cages with 1.0m in length, 0.45 m in depth, 0.45 m in height, with internal lengthwise divisions of 0.50 m. Birds were weighed to

standardize the plots, presenting an average weight of 1.50 ± 0.0029 kg and distributed in a completely randomized experimental design, where the treatments consisted of a control diet (based on corn and soybean meal) and an experimental diet with 3% inclusion of acid silage meal of pirarucu waste in the diets, with six replicates of six birds each. The experimental period lasted for 12 days, considering seven days for adaptation of birds to the diets and facilities and another five days for collection of excreta and data according to methodology proposed by Rodrigues, Martinez, Freitas, Bertechini and Filho (2005) and Sakomura and Rostagno (2007).

Table 1. Composition of experimental diets containing pirarucu waste meal.

Ingredients	Experimental diets	
	Control	3.0%
Corn 7.88%	62.255	61.756
Soybean meal 46%	25.871	23.758
Acid silage	0.000	3.000
Calcitic limestone	9.239	8.806
Dicalcium phosphate	1.693	1.716
Vit. Min. Premix ¹	0.500	0.500
Salt	0.350	0.350
DL- Methionine 99	0.092	0.114
Total (Kg)	100.000	100.000
Calculated nutrients		
Metabolizable energy, kcal kg ⁻¹	2692.59	2629.18
Crude protein, %	17.000	17.000
Methionine + Cystine, %	0.627	0.620
Methionine, %	0.360	0.404
Calcim, %	4.000	4.000
Available phosphorus, %	0.400	0.400
Sodium, %	0.157	0.156

¹ Guaranteed levels per kilogram of the product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2 760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium Pantothenate 2,000 mg, Folic acid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg, Selenium 40 mg. Vehicle q.s.p. 1,000 g.

Nutrient digestibility of the diets was determined using the total collection of excreta. For this procedure, trays were used under the cage floor and covered with plastic, from which the excreta were collected twice a day, in the early morning (8:00 hours) and in the late afternoon (16:00 hours). Then, excreta were packaged in sealed bags identified according to the treatment and stored in a freezer.

At the end of the excreta collection period, samples were thawed at room temperature, homogenized per experimental unit and a composite sample was taken for drying in a forced ventilation oven at 55°C for 72 hours, and then ground. Together with the experimental diets, excreta samples were taken for analysis of dry matter, crude protein, ether extract and crude fiber and ash, according to the techniques described by Silva and Queiroz (2012).

After analysis, we calculated the coefficients of nutrient digestibility, values of metabolizable energy

and coefficients of apparent metabolization of crude energy of the diets, according to the equations described by Matterson, Potter, Stutz and Singen (1965) for food evaluation (Sakomura & Rostagno, 2007).

Data collected, except for pH, were tested by ANOVA, followed by Tukey's test for comparison of means ($p < 0.05$) using the Statistical Analysis System (SAS, 2008).

Results and discussion

The results of pH behavior over a 72-hour period for the acid silage and the fresh waste of pirarucu are listed in Table 2. The pH was monitored during the period when the biomass remained ensiled, initially at 6.61 ± 0.01 in the crushed waste, a reduction occurred by adding the acid mixture to the value of 4.22 ± 0.22 on the first day and 4.38 ± 0.11 on the last day. The final pH result obtained in the present study complies with the limit of 4.5 recommended by Benites and Souza-Soares (2010) to prevent the microbiological action in fish acid silage.

Table 2. Values of pH of pirarucu waste and the acid silage*.

Days/hours	Fresh waste	Acid silage
Start	6.61 ± 0.01	-
1st (24 hours)	-	4.22 ± 0.22
2nd (48 hours)	-	4.28 ± 0.19
3rd (72 hours)	-	4.38 ± 0.11

* Means of three replicates $\pm$ standard deviation.

The initial pH of the fresh waste is within the range (6.67 ± 0.01 to 6.81 ± 0.01) observed by Oliveira et al. (2014) during the 6-12 day ice storage period of pirarucu muscles, proving that the waste presented favorable conditions to be used in the preparation of acid silage.

With the final pH value of 4.38 ± 0.11 , no changes were found that could compromise the quality of the ensiled mass during the experimental period. As acid pH produces conditions to decrease or prevent the growth of undesirable bacteria that cause protein decomposition and putrefaction of the ensiled mass (Vieira et al., 2015).

The combination of organic acids, such as formic and propionic acids, in the proportion of (1: 1) or (3: 1) with 3% of the volume by weight, has been recommended in the preparation of acid silage, since they maintain the bacteriostatic properties at higher pH, not requiring the need for neutralization, due to the stabilizing power of the biomass pH (Borghesi, Portz, Oetterer and Cyrino, 2008); Maia Junior & Sales, 2013). In the present study, no neutralization of the silage before drying for inclusion in the feed

was performed, because the pH was close to the recommended limit for microbiological prevention.

Through the visual method, during ensiling, it was observed that the color of the residual mass changed with the addition of the mixture of formic and propionic acids, passing from the slightly pinkish color, typical of the fresh fish, to light gray. The contact of the acid with the biomass causes reactions that lead to the release of nutrients present (Tanuja, Prafulla, Kumar, Moharana & Sujit, 2014), inhibiting the growth of pathogenic and spoilage microorganisms (Venturoso et al., 2016), thus blocking the bad odor (Vieira et al., 2015). This caused the peculiar aroma of the pirarucu to disappear giving place to the acid smell, which with the passing of days became mild, indicating the volatilization of the added acids, making the product and work environment free of unwanted insects.

Through stirring, a procedure that was repeated every 24 hours, it was observed the formation of liquids on the mass, the liquid formed was incorporated to biomass by means of the same process, to allow the maximum contact of the acids with the residual fragments, giving a creamy consistency. This result is in agreement with the visual observations made by Vasconcelos, Mesquita, and Albuquerque (2011), who reported the beginning of liquefaction of the homogeneous mass within 24 hours and its increase until the end of the experiment.

The formation of liquids in fish silage is attributed to the continuous protein hydrolysis through the action of the proteolytic enzymes, naturally present in the fish muscle (Ribeiro, Ribeiro, Castro & Medeiros, 2015), which are accelerated through the addition of acids and reach higher activity with pH values between 2 and 4 (Tomczak-Wandzel & Mędrzycka, 2013). In general, the liquefaction rate is dependent on the type of raw material, its freshness, enzymatic activity, physiological state of the fish and other factors (Al-Abri et al., 2014). In the present study, the formation of liquid was somewhat accentuated due to the structure of the wastes that were not ground and only fragmented, the time the material passed through the silage process also contributed with this factor.

During the 72-hour storage period, the ensiled biomass acquired light brown color, with a slightly acidified aroma and a slightly creamy, slightly liquid consistency, corroborating with characteristics observed by Vasconcelos, Mesquita and Albuquerque (2011) in a study on the physical and chemical patterns and yield of acid silage of Nile tilapia (*Oreochromis niloticus*).

The final aroma, slightly acidified, was decharacterized during the drying and milling of the

product for inclusion in the feed, presenting a characteristic fish odor. This is related to the use of acids, because they are less corrosive and easier to handle (Borghesi, Ferraz de Arruda & Oetterer, 2007).

The chemical composition of the acid silage of pirarucu waste and the fresh waste of pirarucu are presented in Table 3. The chemical composition of the acid silage showed significant differences ($p > 0.05$) from the fresh waste of pirarucu, except for calcium and phosphorus.

Table 3. Chemical composition of the waste and the acid silage of pirarucu.

Chemical composition	Waste	Acid silage	P-Value	CV (%)
Dry matter, %	31.42 ^b	84.16 ^a	0.01*	0.65
Crude protein, %	15.71 ^b	40.05 ^a	0.01*	3.04
Ether extract, %	6.46 ^b	26.81 ^a	0.01*	1.13
Mineral matter, %	3.42 ^b	9.31 ^a	0.01*	5.93
Total carbohydrates %	5.82 ^b	7.97 ^a	0.01*	1.63
Calcium, g Kg ⁻¹	66.32	65.16	0.58 ^{ns}	3.70
Phosphorus, g Kg ⁻¹	27.09	22.90	0.34 ^{ns}	4.54
Gross energy, Kcal kg ⁻¹	4582.45 ^b	5771.49 ^a	0.01*	5.69

CV – Coefficient of variation; * Means followed by different lowercase letters in the same row are significantly different by Tukey's test at 5% significance ($p < 0.05$); ns – non-significant. The crude protein content found in the present study was higher than the 38.12% verified by Ferraz de Arruda, Borghesi, Portz, Cyrino, and Oetterer (2009), using the treatment of sulfuric and formic acids (3: 1) in silage produced with fresh waste and similar to 40.62±0.12, 40.45±0.06 and 40.38±0.06% crude protein reported by Ramasubburayan et al. (2013) using 2; 2.5 and 3% formic acid in the preparation of acid fish silage. According to the aforementioned authors, the reduction of crude protein in silage made with acids is related to the continuous protein hydrolysis.

The chemical composition of the fresh pirarucu waste found in the present study showed only results for crude protein below the results obtained by Oliveira, Jesus, Batista and Lessi (2014), who reported 17.56±0.12% CP; 0.62±0.02% EE; 0.87±0.06% MM and 1.44% total carbohydrates for the dorsal part and 16.10±0.37% CP; 2.49±0.03% EE; 0.84±0.05% and 2.69% carbohydrates in the ventral part of pirarucu muscle from fish farming.

This difference in chemical composition between the acid silage and the fresh waste of pirarucu is related to the addition of the mixture of formic and propionic acids in the preparation. Acids contribute for enzymes to hydrolyze the proteins into peptides and amino acids and the lipids present in the residues (Tomczak-Wandzel & Mędrzycka, 2013). This accelerated breakdown by the incorporation of acids, in addition to reducing pH, also breaks bones and cartilages, preventing the growth of deteriorating bacteria (Goddard & Perret, 2005).

The mineral matter content (9.31%) found in the present study was higher than those observed by Boscolo et al. (2010) in the order of 6.57, 5.91 and 5.77 at 7, 91 and 201 days of storage of acid silage made of tilapia waste, in which the authors state that the amount of mineral matter in fish silage is due to

its residual constitution, where the minerals are more concentrated in these residues.

The values of ether extract and gross energy of 26.82% and 5771.49, respectively, observed in the acid silage of pirarucu waste are associated with the ventral part, referring to the ribs, which concentrate the greater content of fat of fish and contributed to the greater release of fat during the ensiling period. Tanuja et al. (2014) obtained $39.19 \pm 0.53\%$ ether extract in acid silage made of carp offal waste. This value presented by the authors demonstrates that as well as the ventral part, the offal also constitute an important site for deposition of lipids in fish.

The analyzed minerals, calcium and phosphorus, found for acid silage are due to the use of the parts of the bone structure of pirarucu, such as the ribs and vertebrae, which can influence the results. The calcium and phosphorus contents obtained in the present study were well above those found by Hisano, Ishikawa and Portz (2012), whose values were 0.06% calcium and 0.27% phosphorus and those obtained by Dale and Valenzuela (2016) in the order of 1.01% calcium and 1.08% phosphorus in the mineral composition of dried salmon acid silage.

The chemical composition of fish silage may vary according to the raw material used in the production, such as: fish species, portion within the same species, breeding system, stage of fish development, sex, part analyzed and type of acids used for hydrolysis (Borghesi et al., 2007; Oliveira et al., 2014).

The observed results for apparent digestibility coefficients of nutrients in the diets are shown in Table 4. Significant differences ($p < 0.05$) were detected in the digestibility coefficients of the non-nitrogen extract, ether extract and mineral matter. It was observed that laying hens fed diets containing 3% acid silage meal showed better use of ether extract and mineral matter in comparison to control diet, and these results could be related to the higher content of these components in the acid silage meal of pirarucu waste.

Table 4. Apparent digestibility coefficients of the control diet and the experimental diet containing 3% acid silage meal of pirarucu waste for laying hens.

Digestibility coefficients (%)	Experimental diets		P Valor	CV (%)
	0.0%	3.0%		
Dry matter	70.62	70.87	0.89 ^{ns}	2.94
Crude protein	55.48 ^a	35.91 ^b	0.01*	9.87
Crude fiber	4.09	4.63	0.32 ^{ns}	13.45
Non-nitrogen extract	83.61 ^b	88.67 ^a	0.01*	1.20
Ether extract	67.59 ^b	74.36 ^a	0.02*	3.51
Mineral matter	14.92 ^b	32.22 ^a	0.03*	20.54

CV – Coefficient of variation; * Means followed by different lowercase letters in the same row are significantly different by Tukey's test at 5% significance ($p < 0.05$); ns – non-significant.

In the present study, hens used for the digestibility analysis were 71 weeks old, which may have directly influenced the digestibility of nutrients. According to Arruda, Melo, Oliveira, Souza and Oliveira (2012), older birds have a fully developed digestive system and a higher production of digestive enzymes, which allows greater permanence and absorption of nutrients contained in food.

On other hand, hens fed diets containing 3% inclusion of the acid silage of pirarucu waste had lower digestibility of crude protein ($p < 0.05$) than those fed the reference diet. This result may be attributed to the small fragments of peptides and free amino acids that, in the hydrolysates, are more readily absorbed than the amino acids of intact proteins, making them readily available for energy production, rather than being intended for protein synthesis (Hernández et al., 2013).

These results disagree with those obtained by Al-Marzooqi, Al-Farsi, Kadim, Mahgoub and Goddard (2010), who verified a higher amino acid digestibility coefficient in sardine silage made with hydrochloric acid, concluding that the proteins in the hydrolyzed form are easily used in protein synthesis by birds.

No difference ($p > 0.05$) was detected for the dry matter and crude fiber digestibility between the experimental diet containing 3% inclusion of acid silage from pirarucu waste and the reference diet, since the laying hens exhibited similar performance.

Several studies have demonstrated the benefits of silage prepared with the addition of organic acids in the diet, such as Widjastuti, Lengkey, Wiradimadja and Herianti (2011) who observed better results in birds fed diets containing 4% silage made with the addition of 3% formic and propionic acids (1: 1), and Rahman and Koh (2016) concluded that shrimp meal treated with 3% formic acid provided better digestibility of nutrients in broiler diets.

The results of apparent metabolizable energy and apparent metabolizable energy coefficient of diets are presented in Table 5. Values of apparent metabolizable energy coefficients and apparent metabolizable energy metabolism coefficients showed significant differences ($p < 0.05$) for laying hens fed diets containing 3% acid silage in the diet, due to the greater use of energy sources in the diet.

In a study carried out to obtain the values of apparent metabolizable energy, Oliveira et al. (2014) found energetic values ranging from 3804 kcal kg⁻¹ to 3842 kcal kg⁻¹ in diets elaborated with silage of fish ensiled with different sources of carbohydrates

in diets for broilers. In the present study, the apparent metabolizable energy of the feed containing acid silage flour made of pirarucu waste was 3253.01 kcal kg⁻¹, that is, close to the values reported in the literature.

Table 5. Apparent metabolizable energy (aME) and apparent metabolization coefficient of gross energy (CMAEB) of control diet and experimental diet (containing 3% acid silage meal made of pirarucu waste-FSARP) for commercial laying hens.

Coeficientes de Digestibilidade (%)	Experimental diets		P-Value (%)	CV (%)
	Reference diet	Diet with 3% FSARP		
aME ¹	2921.18 ^b	3253.01 ^a	0.01*	0.79
CMAEB ²	78.93 ^b	81.14 ^a	0.01*	0.23

CV – Coefficient of variation; *Means followed by different lowercase letters in the same row are significantly different by Tukey's test at 5% significance ($p < 0.05$); ns – non-significant. ¹Apparent Metabolizable Energy; ² Apparent metabolization coefficient of gross energy

Nevertheless, the difference in energy values between the studies may be related to variations in the chemical composition of the food (Calderano et al., 2010), and its better use by birds. In this way, the nutritional evaluation of alternative foods with poultry intended for egg production represents an investigative action of great technical and scientific value, since in many cases it is not fully used due to the scarcity of regional diversity (Melo et al., 2015).

Conclusion

The results of the present study indicated that the acid silage produced from the residual biomass of pirarucu can be included as a meal up to 3% in the diet for commercial laying hens, presenting satisfactory nutrient digestibility and potential to be used as an energy source.

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