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Pirarucu By-Product Acid Silage Meal in Diets for Commercial Laying Hens

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ABSTRACT

This study aimed to evaluate the inclusion of pirarucu by-product acid silage meal in diets for laying hens on performance and egg quality. One hundred sixty eight Hissex White laying hens 73-wk-old were distributed in completely randomized design with seven treatments (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) and four replicates of six birds each. The experiment lasted 84 days divided into four periods of 21 days. Estimates of pirarucu by-product meal levels were determined by polynomial regression. Differences (p<0.05) were observed in all variables of performance, in egg weight, yolk height and yolk pigmentation, with pirarucu by-product meal inclusion in diets showed better results than control diet. The pirarucu by-product acid silage meal can be used as alternative food in diets for commercial laying hens. Up to the 2.5% inclusion level there wasn't negative effect in performance and egg quality.

INTRODUCTION

Currently, poultry production (egg and meat) have featured in the international_economic activity, being uniformed and without geographical frontier. However, one of the obstacles faced by this sector in some Brazilian regions is a great need of corn and soybean meal, the main ingredients used in the poultry feed production, which usually raises the production cost.

In this context, researches with alternative ingredients show potential for the inclusion or substitution of the conventional ingredients in poultry feed, aimed to lower the feed costs that represents 70% of production costs (Cruz *et al.*, 2016). Therefore, the by-products used for poultry feed can have lower investments, mainly because of the by-podructs being low cost ingredients, and that offer diets with higher nutritive composition (Enke *et al.*, 2010).

A product that's currently studied is the fish by-product in silage form, with its production offering great economic advantages, requiring a simple technology and independent of production scale (Hisano *et al.*, 2012). Furthermore, it has a low cost transformation of waste materials in products with high nutritional quality and minimizes problems with environmental pollution.

Silage may be defined as a pasty product with high nutritional value resulting from the conservation of the whole fish or its residual parts. It consists in acidifying the pH through the addition of organic acids (Borghesi *et al.*, 2008) that benefit the autolytic enzymatic reactions present in the muscle, raising the levels of oligopeptides and free amino acids in the final product (Honorato *et al.*, 2012).

Thus, this study aimed to evaluate the inclusion of pirarucu by-product acid silage meal in diets for laying hens on performance and egg quality.

MATERIAL AND METHODS

The experiment was developed in the Poultry Sector of the Department of Animal and Plant Production (DPAV), College of Agrarian Sciences (FCA), Federal University of Amazonas (UFAM), located in the Southern Sector of the University Campus, Manaus, state of Amazonas, Brazil.

The experimental procedures were conducted in accordance with the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and the experimental procedures were approved by the local Committee for Ethical Animal Use (CEUA - protocol n. 040/2015) of the Federal University of Amazonas, Manaus, Amazonas, Brazil.

The experimental period lasted 84 days, divided into four periods of 21 days. Before the start of the experiment, the birds were subjected to an adaptation period of seven days regarding the feed and facilities.

The experimental aviary used had dimensions of 17.0 m in length and 3.5 m in width, containing galvanized wire cages, trough feeders and nipple drinkers. 168 Hissex White hens with 73 weeks of age were used. The birds were weighed at the beginning of the experiment in order to standardize the experimental plots, presenting a mean weight of 1.53 ± 0.0025 kg. Egg collection was performed two times a day (8 a.m. and 3 p.m.), recording each daily occurrence (mortality, number of eggs, among other information). The temperature and relative humidity were recorded two times a day (9:00 a.m. and 3:00 p.m.) from a digital term hygrometer positioned above the bird's cage in the aviary, with mean results of 28.65±0.01 °C and 73.45%, respectively.

The experimental design was completely randomized consisting of seven treatments corresponding to the inclusion levels of pirarucu by-product acid silage meal (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) in the diet, with four replicates of six birds each. Throughout the experimental period 16 hours of light/day (12 natural hours + 4 artificial hours) were provided to the birds.

The acid silage was produced and dehydrated in the Fish Technology Laboratory of National Researches Institute of the Amazon (INPA). The pirarucu residues (ribs and vestebras) were crushed in an electric crusher machine. Formic and propionic acids were mixed in 1: 1 proportion of 3% acid solution volume of total weight, with constant homogenization to acidify the medium, lower the pH to inhibit the proliferation of microorganisms and to promote protein hydrolysis according to Borghesi *et al.* (2008).

The recipients were kept at ambient temperature for three days, and scrambled each 24 hours to promote the incorporation of the acids into the ensiled mass. In the end of 72 hours, to reduce humidity, the ensiled material was dried in laboratory stove (65 °C for 72 hours).

The chemical composition was determined in the Laboratory of Chemistry and Physical- Chemistry of Food of INPA, with methodology by Silva and Queiroz (2002). The minerals were digested in nitric-perchloric extract and the concentrations quantified by atomic absorption spectrophotometer (Ca) and colorimetry (P) according to Sarruge and Haag (1974) in the Thematic Laboratory of Soils and Plants Chemistry of INPA. After the determination of the chemical composition (Table 1), the product was packed and sent to the Poultry Sector of FCA/UFAM, being crushed to obtain the pirarucu by-product acid silage meal.

Table 1 – Chemical composition of pirarucu by-product acid silage meal.

Chemical composition	Pirarucu by-product acid silage meal*
Dry matter, %	84.16±0.08
Crude protein, %	40.05±0.83
Ether extract, %	26.81±0.13
Mineral matter, %	9.31±0.22
Total carbohydrates, %	7.99±0.74
Calcium, g/kg	65.16±1.49
Phosphorus, g/kg	22.90±2.30
Metabolizable energy, kcal/kg	3,253.01**

*Averages withdrawal of three replicates ±standard deviation.

**Determined by the method for apparent metabolizable energy calculation according to Rostagno *et al.* (2011).

The experimental diets were formulated according to the nutritional requirements of laying hens according to Rostagno *et al.* (2011), also using the composition obtained for pirarucu by-product acid silage meal (Table 2).

For animal performance, we evaluated in each period of feed intake (g/bird/day), the egg production (%), egg mass (g), feed conversion (kg feed per kg egg produced - kg/kg) and feed conversion (kg feed per dozen eggs produced - kg/dz). In the last two days of each period, four eggs from each plot were taken at random to evaluate egg quality, in which we analyzed egg weight (g), albumen weight (%), yolk weight (%), albumen height (mm), yolk height (mm), shell thickness (μm) and specific gravity (g/cm^3). Before evaluation, the eggs were stored for one hour in order to equalize the temperature to room temperature.

Table 2 – Diets composition containing pirarucu by-product acid silage meal (PBASM).

Ingredients	Pirarucu by-product acid silage meal levels						
	0.0%	0.5%	1.0%	1.5%	2.0%	2.5%	3.0%
Corn (7.88%)	62.255	62.170	62.087	62.005	61.922	61.839	61.756
Soybean meal (46%)	25.871	25.527	25.173	24.819	24.466	24.112	23.758
PBASM	0.000	0.500	1.000	1.500	2.000	2.500	3.000
Limestone	9.239	9.167	9.095	9.023	8.950	8.878	8.806
Dicalcium phosphate	1.693	1.696	1.700	1.704	1.708	1.712	1.716
Vit. min. supplement [1]	0.500	0.500	0.500	0.500	0.500	0.500	0.500
DL-methionine (99%)	0.092	0.089	0.094	0.099	0.104	0.109	0.113
Salt	0.350	0.350	0.350	0.350	0.350	0.350	0.350
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient	Nutritional levels						
M.E., kcal^{-1}/kg	2,692.6	2,686.8	2,671.3	2,660.8	2,650.2	2,639.7	2,629.2
Crude protein, %	17.000	17.000	17.000	17.000	17.000	17.000	17.000
Methionine + Cystine, %	0.627	0.620	0.620	0.620	0.620	0.620	0.620
Methionine, %	0.360	0.361	0.369	0.378	0.387	0.395	0.404
Calcium, %	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Available phosphorus, %	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Sodium, %	0.156	0.156	0.156	0.156	0.156	0.156	0.156

[1] Guaranteedlevels per kilogramoftheproduct: 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, CalciumPantothenate 2,000 mg, Folicacid 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.

The eggs were weighed on an electronic scale to the nearest 0.01 g. The egg mass was obtained by calculating the quotient between egg weight and egg production multiplied by 100. The whole eggs, immediately after weighing, were placed in wire baskets and immersed in plastic buckets containing different levels of sodium chloride (NaCl), from the lowest to the highest concentration, with density variations from 1.075 to 1.100 g/cm^3, with 0.005-interval. The eggs were removed as they floated to the surface and their respective values were recorded.

For the analysis of the albumen and yolk weight, a manual separator of albumen and yolk was used. The albumen and the yolk were placed in plastic cups, tared-weight in an analytical balance, and weighed. To calculate the albumen and yolk weight, they were placed on a flat glass plate to determine their respective values. The criterion for measuring albumen and yolk height is to take measure at the medial region between the outer border of the albumen and the yolk. For the measurement of the height, an electronic caliper was used; the values are expressed in millimeters.

The egg shell weight was obtained after they were washed, dried at room temperature for 48 hours and then weighed in grams. Dry shells were used to determine the shell thickness, which were measured using a micrometer. The readings were performed in three regions of the shell: basal, meridional and apical, and the values were recorded. From the values obtained in the three regions, the average of the eggshell thickness, in micrometer, was calculated. The egg yolk color was evaluated with the Roche colorimetric fan with a score of 1 to 15. Haugh unit was performed using the egg weight and albumen height values and the results obtained by the formula $UH = 100 \times \log (H + 7.57 - 1.7 \times W^{0.37})$, where H = albumen height (mm); and W = egg weight (g).

Data collected were tested by analysis of variance with the GLM procedure of the Statistical Analysis System-SAS (2008) software and subjected to the polynomial regression analysis at the 5% level of significance.

RESULTS AND DISCUSSION

Differences ($p<0.05$) were observed in feed intake, egg production, feed conversion (kg/kg and kg/dz) and egg mass results of laying hens fed with diets containing pirarucu by-product acid silage meal (Table 3).

Feed intake showed quadratic effect ($Y = - 0.3122x^2 + 0.4704x + 110.77$, $R^2 = 0.85$) with great intake of 111.14g/bird/day in inclusion level of 0.75% of pirarucu by-product acid silage meal. These results desagreed of Enke *et al.* (2010) that working with fish by-product acid silage meal, added to defatted rice meal in the diets of Japanese quail (*Coturnixcoturnix japonica*) didn't observe differences among levels tested.

The low intake observed in the higher levels can be related to the reduction of the palatability from the growing inclusion of pirarucu by-product acid silage

Table 3 – Feed intake (FI), egg production (EP), egg mass (EM) and feed conversion (FC, kg/kg e kg/dz) of commercial laying hens fed diets containing pirarucu by-product acid silage meal.

Variables	Pirarucu by-product acid silage meal levels (%)							P Valor	Effect	CV, %
	0.0	0.5	1.0	1.5	2.0	2.5	3.0			
FI, g/bird/day	110.42	110.84	110.81	110.52	109.24	109.89	104.78	0.01	Q	0.46
EP, %	89.98	93.90	88.09	83.93	89.53	89.38	66.86	0.01	Q	5.14
EM, g	70.50	69.45	68.98	68.34	68.57	67.66	52.42	0.01	Q	4.43
FC, kg/kg	1.56	1.60	1.62	1.63	1.59	1.58	2.01	0.01	Q	5.06
FC, kg/dz	2.92	2.81	3.00	3.14	2.91	2.93	3.74	0.01	Q	5.63

CV – Coefficient of variation. P Valor – Coefficient of Probability. Q – Quadratic effect.

meal in diets for laying hens and the specific composition of the fish species used. Similar results were obtained by Al-Marzooqi *et al.* (2010), that observed a decrease in intake from inclusion of sardine acid silage in diets for broilers, and Rahman & Koh (2015), who also observed a decrease in feed intake of broilers fed diets with shrimp meal treated with formic acid.

Another factor for the decrease in feed intake of laying hens fed with great inclusion levels can be related to the energy content in the by-product used, once excess fat in the diet leads to satiety by the animal and causes changes in the feed intake because it's an essential control factor.

According Carioca *et al.* (2010), the more energy in food, less will be consumed. And this decrease in intake, physiologically, can be caused by the presence of lipid in the duodenum that stimulates the release of hormone cholecystokinin (CCK), that causes the enterogastric reflex, decreasing passage rate through the digestive system, with consequent absorption of nutrients (Brunelli *et al.*, 2010; Pinheiro *et al.*, 2012; Torres and Drehe, 2015). However, the modern laying hens have the ability to adjust feed intake from food energy content (Gunawardana *et al.*, 2008; Ribeiro *et al.*, 2014).

It's important to mention that studies carried out to evaluate broilers fed diets with fish residue silage have obtained different results, such as increase in feed intake of 90-128g/bird at the 15% of salmon acid silage inclusion (Valenzuela *et al.*, 2015) and inclusion of 30% of this by-product in diets of broilers don't influenced the feed intake (Ramirez-Ramirez *et al.*, 2016).

The egg production showed quadratic effects from increased levels of pirarucu by-product acid silage meal ($Y = -1.0045x^2 + 1.5881x + 93.892$, $R^2 = 0.72$) with great production index (94.51%) obtained with the inclusion level of 0.79%. Consequently, when evaluating the egg mass, a quadratic effect was observed ($Y = -0.7946x^2 + 0.7612x + 70.611$, $R^2 = 0.74$) obtaining great result (70.79g) in 0.47% of pirarucu by-product acid silage meal inclusion.

From these results, it was also observed that hens fed lower levels of this by-product showed better production and egg mass, whereas the increase of levels caused a physiological break point with replied on feed intake and, consequently, other performance variables.

According Pérez-Bonilla *et al.* (2012), changes in diets composition, such as the inclusion of products of animal origin, may cause an increase in body weight gain and decrease in performance of hens.

The feed conversion (kg/kg) showed quadratic effect ($Y = 0.0203x^2 - 0.0753x + 1.7239$, $R^2 = 0.71$) with great conversion of 1.65 kg/kg in 1.85% of inclusion, corroborated with results of feed conversion (kg/dz), that also showed quadratic effect ($Y = 0.0361x^2 - 0.1156x + 3.3289$, $R^2 = 0.78$), with better conversion (3.23 kg/dz) in inclusion level of 1.60%. The feed conversions (kg/kg and kg/dz) showed worst results from inclusion of highest pirarucu by-product acid silage meal levels, with a direct relationship with feed intake results. These results agree with Hanna *et al.* (2013) that studying increasing levels of copaiba oil as an energetic alternative food in diets for laying hens observed a decrease on performance in most high inclusion levels.

In egg weight (Table 4) a quadratic effect was observed ($Y = -0.5817x^2 + 3.1783x + 60.226$, $R^2 = 0.78$) with better egg weight (64.56g) in 2.73% of pirarucu by-product acid silage meal inclusion in the diets. These results corroborated with Jonsson *et al.* (2011), who also observed differences to hens fed diets with 3.5% of clam meal.

Hens fed the highest levels of pirarucu by-product acid silage meal showed lighter eggs. Wall *et al.* (2010), affirms that changes in egg weight from diets containing increase levels of fish meal or fish oil are possibly associated with dietary omega-3 and changes caused in birds lipid metabolism.

In this context, the yolk height showed quadratic effect ($Y = -0.1306x^2 + 0.6193x + 19.178$, $R^2 = 0.77$) with better results (19.91 mm) in inclusion level of

Table 4 – Egg weight (EW), albumen percentage (PERA), yolk percentage (PERY), shell percentage (PERS), albumen height (AH), yolk height (YH), shell thickness (ST), specific gravity (SG), Haugh unit (HU) and yolk pigmentation (YP) of eggs from commercial laying hens fed diets containing pirarucu by-product acid silage meal.

Variables	Pirarucu by-product acid silage meal levels (%)							*p* Valor	Effect	CV, %
	0.0	0.5	1.0	1.5	2.0	2.5	3.0			
EW, g	63.85	62.23	61.67	61.21	62.77	63.19	50.01	0.01	Q	4.31
PERA, %	54.59	54.66	56.72	53.69	53.63	56.02	52.91	0.07	ns	3.28
PERY, %	26.42	27.05	27.18	27.88	27.89	27.87	26.85	0.31	ns	3.80
PERS, %	9.43	9.55	9.60	10.01	9.20	9.39	9.73	0.09	ns	3.77
AH, mm	7.73	7.21	7.68	7.86	7.50	7.77	7.49	0.29	ns	5.04
YH, mm	17.72	17.43	18.06	18.77	19.42	18.67	17.59	0.05	Q	5.15
ST, µm	33.77	35.90	35.17	35.35	35.09	35.02	36.26	0.32	ns	4.00
SG, g/mL	1084.92	1102.26	1084.22	1087.11	1083.05	1083.91	1133.54	0.43	ns	3.34
HU	85.55	84.15	86.90	87.63	85.49	86.92	85.87	0.43	ns	2.68
YP	4.79	5.23	5.17	5.36	5.29	5.26	4.73	0.01	Q	5.04

CV – Coefficient of variation. P Valor – Coefficient of Probability. Q – Quadratic effect. ns – non significant.

2.37%. According studies testing inclusion levels of fish meal and oil in diets for hens and quails performed by Al-Daraji *et al.* (2011), Alemayehu *et al.* (2015) and Amao *et al.* (2010), observed significant increase in yolk height. Cedro *et al.* (2010) and Sherr *et al.* (2014) affirm that these results can have relationship to omega-3 and omega-6 content and other lipid components present in fish residues, that through the process of acid ensilage (Costa *et al.*, 2012) can contribute to the increase in yolk height.

A quadratic effect was observed in yolk pigmentation ($Y = -0.0618x^2 + 0.194x + 5.3817$ $R^2 = 0.87$), showing better pigmentation (5.53) in inclusion level of 1.56%. Wall *et al.* (2010), observed that hens fed diets with 3.5% of clam meal inclusion showed significant influence on yolk pigmentation. These results may be attributed to pigments called carotenoids present naturally in corn (Brunelli *et al.*, 2012) that associated with lipid profile present in pirarucu by-product acid silage meal can contribute to the increase the yolk pigmentation.

According to Lopes *et al.* (2011) the carotenoids are liposoluble substances transferred by the same mechanisms from lipids to ovarian follicles, where stored as fats or lipoproteins in yolks. But, the yolk pigmentation will depend on the type of ingredients used in the feed composition and the pigments necessary for color development. Saleh (2013) observed that fish oil inclusion contributed to the darkening of yolk at 5% in diets for hens.

CONCLUSIONS

The pirarucu by-product acid silage meal can be used as alternative food in diets for commercial laying hens. Up to 2.5% inclusion levels there wasn't a negative effect in performance and egg quality.

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