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NONRUMINANT NUTRITION

Apparent digestibility of fish waste oil in diets for laying hens

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ABSTRACT. This study aimed to assess the impact of incorporating fish waste oil into laying hens' diets on apparent nutrient digestibility and apparent metabolizable energy. A total of 72 Hisex White laying hens (25 weeks old) were employed in a completely randomized design, with treatments consisting of a control diet and an experimental diet (containing 3.5% fish oil), each with six replicates of six birds. The collected data underwent polynomial regression analysis at a 5% significance level. No significant differences ($p > 0.05$) were observed in the digestibility of dry matter, crude protein, mineral matter, crude fiber, and non-nitrogenous extract between the reference diet and the experimental diet containing fish waste oil. However, there were significant differences ($p < 0.05$) in the digestibility of ethereal extract (fat). Despite this, values for apparent metabolizable energy and apparent metabolizable energy corrected by nitrogen balance did not exhibit significant differences ($p > 0.05$) between the reference and experimental diets. In conclusion, the incorporation of 3.5% fish waste oil in laying hens' diets led to satisfactory nutrient digestibility and enhanced fat digestibility. Importantly, this inclusion did not adversely impact energy metabolism.

Keywords: alternative food; fish by-product; metabolizable energy; nutrients use.

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Introduction

Feedstuffs of animal origin are important nutrient sources for animal diets, where fish byproducts are one of the most significant alternatives used. These fish byproducts represent up to 60% of the total that is produced and/or captured per year by aquaculture (Arvanitoyannis & Kassaveti, 2008; Silva, Cruz, Rufino, Miller, & Flor, 2017), and their use may be an interesting alternative to poultry production due to their low cost, nutritional content, and positive impact on the environment (Enke, Tabeleão, Rocha, Rutz, & Soares, 2010; Mousavi, Mohammadi, Khodadadi, & Keysami, 2013; Cruz & Rufino, 2017; Shabani, Boldaji, Dastar, Ghoorchi, & Zerehdaran, 2018).

The use of fish by-products in poultry diets is not innovative, especially because these feedstuffs offer advantages to regions that face problems related to logistics and the high cost of grains (Cruz et al., 2016; Cruz & Rufino, 2017). The use of fish by-products in poultry diets is a viable alternative due to its simple acquisition process and rich nutritional content (Costa et al., 2009a; Batalha et al., 2017). The inclusion of certain types of fish oil in hens' diets may result in a significant increase in yolk omega-3 fatty acids, producing the so-called "enriched eggs" a popular trend in the worldwide market, especially in developed countries (Dong, Liu, & Tong, 2018; Kralik et al., 2020; Kralik, Kralik, Košević, Galović, & Samardžić, 2023).

Nutritionally, oils and fats are used as additives in poultry diets to increase energy content, improve palatability, and facilitate the digestion and absorption of non-lipid nutrients (Nogueira, Cruz, Tanaka, Rufino, &Santana, 2014). Fats and oils contain fat-soluble vitamins that provide essential fatty acids, enhance the palatability of feed, and reduce friction in feed mills. It is important to mention that the major component of fats and oils is fatty acids, where their metabolism depends primarily on the fatty acid profile (Nogueira et al., 2014; Skřivan et al., 2018).

Previous studies utilizing oils and fats in hens' diets have indicated that the key factors influencing the metabolizable energy value are related to the concentration of triglycerides in the dietary composition, the length of their carbon chains, the number of double bonds, the presence or absence of ester bonds, and the composition of free fatty acids (Gjorgovska & Filev, 2011; Dalle Zotte, Andrighetto, Giaccone, Marchesini, 2015; Ravindran, Tancharoenrat, Zaefarian, & Ravindran, 2016; Promila, Sihag, Shunthwal, Verma, & Baloda, 2017; Kralik, Kralik, & Hanžek, 2020; Kralik et al., 2021; Kralik et al., 2023). These authors also noted that the age and health of the gastrointestinal tract are other crucial factors influencing the metabolizable energy value of oils and fats for hens.

The use of fish oil in poultry diets also reduces the synthesis of fatty acids, causing the birds to accumulate more energy for meat or egg production and increasing the level of long-chain n-3 FAs in these products (Ravindran et al., 2016; Alagawany et al., 2019; Brelaz et al., 2019; Attia et al., 2020; Tompkins et al., 2022). However, this oil may also introduce some organoleptic issues that could negatively affect the acceptability of poultry products due to the distinct odor and flavor of fish that can be transferred (Alagawany et al., 2019; Brelaz et al., 2019; Thanabalan & Kiarie, 2021; Berkhoff et al., 2020). Therefore, the objective of this study was to evaluate the inclusion of fish waste oil in laying hens' diets on nutrient digestibility and energy metabolism.

Material and methods

The study was conducted at the Poultry Sector facilities, Faculty of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas State, Brazil. The experimental procedures were approved by the Ethics Committee for the Use of Animals (protocol number 012/2017) at the Federal University of Amazonas, Manaus, Amazonas, Brazil.

The experimental period lasted for 12 days, including seven days for bird adaptation to the diets and facilities, and an additional five days for the collection of excreta following the methods proposed by Sakomura and Rostagno (2016). Seventy-two Hisex White hens, 25 weeks old, were housed in 12 cages (1.0 x 0.45 x 0.45 m). Birds were weighed to standardize the plots, with an average weight of 1.50±0.0029 kg.

The experimental design was completely randomized, with the treatments being the control diet (based on corn and soybean meal) and an experimental diet using 3.5% fish waste oil, with six replicates of six birds each. The fish waste oil was obtained from the fish processing plant RIOMAR© in Itacoatiara town, Amazonas State, Brazil.

The product was derived from the pressing of freshwater fish waste (head, bony structures, fins, tissue, and visceral residue) at an industrial level. The composition and fatty acid profile (Table 1) of fish waste oil were determined at CBO Laboratories© (Campinas, São Paulo, Brazil). The experimental diets were formulated according to the nutritional requirements for laying hens provided by Rostagno et al. (2017), using the obtained composition of fish waste oil (Table 2).

The digestibility of nutrients was determined using the method of total collection of excreta. Initially, trays were placed under the cage floor, and the excreta were collected in the morning (8:00 a.m.) and afternoon (4:00 p.m.). The collected excreta were packaged in bags, labeled according to the treatment, and stored in a refrigerator at 4°C. At the end of the collection period, the samples were thawed at room temperature, homogenized based on the collection day per treatment, dried in a forced ventilation oven at 55°C for 72 hours, and ground.

Table 1. Composition and fatty acids profile of fish waste oil.

Components	Composition
Palmitic acid (C16:0), %	29.01
Stearic acid (C18:0), %	9.62
Oleic acid (C18:1n9c), %	18.48
Linoleic acid (C18:2n6c)	4.39
Alpha Linolenic acid LNA (C18:3n3)	4.51
Arachidonic acid AA (C20:4n6)	3.09
Eicosapentaenoic acid 5,8,11,14,17- EPA (C20:5n3)	3.26
Docosahexaenoic acid DHA(C22:6n3)	4.20
Omega 3, %	12.79
Omega 6, %	8.57
Omega 9, %	20.37
Monounsaturated fat, %	28.92
Polyunsaturated fat, %	21.91
Unsaturated fat, %	50.83
Saturated fat, %	48.23
Humidity and volatiles, %	0.19
Ethereal extract, %	99.06
Acidity, %	13.38
Peroxide index, meq kg^{1}	4.36
Iodine index, meq kg^{1}	85.37

Table 2. Composition of the experimental diets.

Feedstuffs	Fish waste oil levels, %	
	0	3.5
Corn (7.88%)	65.0529	52.1422
Soybean meal (46%)	23.2198	22.0501
Wheatmeal	0.0000	10.0000
Fish waste oil	0.0000	3.5000
Limestone	8.8070	9.5122
Dicalcium phosphate	1.9812	1.8568
Vit. min. supplement [1]	0.5000	0.5000
DL-methionine (99%)	0.3500	0.3500
Salt	0.0891	0.0887
Total	100.0000	100.0000
Nutrient	Nutritional levels	
M.E., kcal.kg^{-1}	2,727.300	2,750.500
Crude protein, %	16.000	16.000
Methionine + Cystine, %	3.900	3.900
Methionine, %	0.450	0.450
Calcium, %	0.344	0.339
Available phosphorus, %	0.600	0.600
Sodium, %	0.156	0.155

[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.

Samples of the diets and excreta were analyzed following the methods described by Silva and Queiroz (2012). After analysis, the coefficients of nutrient digestibility, metabolizable energy, and coefficients of apparent metabolization of gross energy of the diets were calculated using the equations described by Sakomura and Rostagno (2016). Energy coefficients were determined according to Sakomura and Rostagno (2016) as well as Rostagno et al. (2017).

Before conducting statistical data analysis, all data were tested for normality and transformed if necessary. All data were subjected to one-way ANOVA using the R software (version 4.1.3). All commands were executed following Logan (2010). Tukey's Honestly Significant Difference test was used to determine significant differences among the mean values. The results are presented as means, and the significance level for differences was set at $p < 0.05$.

Results and discussion

The inclusion of fish waste oil did not have an effect ($p > 0.05$) on the digestibility of dry matter, crude protein, mineral matter, crude fiber, and non-nitrogenous extract in the diets for laying hens. Regardless of whether fish waste oil was included in the diet or not, the hens exhibited similar utilization of these nutrients (Table 3). These results are consistent with those of Batalha et al. (2017), who, while studying the use of acid silage of pirarucu in diets for laying hens, did not observe any effect ($p > 0.05$) on the digestibility of dry matter and crude fiber. The same authors also reported that the use of this fish by-product did not have a negative impact on nutrient digestibility for the hens.

Table 3. Coefficients of apparent digestibility of the control diet and the experimental diet containing 3.5% of fish waste oil to laying hens.

Coefficients of digestibility	Fish waste oil levels (%)		p-value	CV, %
	0	3.5		
Dry matter, %	67.52	67.71	0.91^{ns}	4.52
Crude protein, %	51.33	54.50	0.45^{ns}	13.17
Ashes, %	27.97	29.60	0.58^{ns}	17.20
Crude fiber, %	4.77	5.26	0.19^{ns}	11.90
Ethereal extract, %	59.49^{b}	83.59^{a}	0.01*	4.16
Non-nitrogenous extract, %	81.02	81.99	0.55^{ns}	3.40

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

Maia Junior and Sales (2013) mentioned that the use of fish by-products offers good cost-efficiency because it doesn't negatively impact the productive indexes of birds. However, according to Rahmi et al. (2008), the utilization of fish by-products in poultry diets may interfere with protein metabolism, particularly in the energy-to-protein ratio balance. On the other hand, fish waste oil had an effect ($p < 0.05$) on the digestibility of ether extract (fats), wherein hens fed diets containing 3.5% fish waste oil showed improved fat utilization. This outcome could be directly linked to the higher lipid content made available through the inclusion of fish waste oil in the diets.

Studies have reported that achieving maximum fat digestibility requires a minimal amount of supplemental fat in poultry diets to attain the desired energy level. For this reason, finding methods to enhance the digestibility of supplementary fat in poultry diets, such as through the incorporation of fish oil (Shin et al., 2011; Ravindran et al., 2016; Beckford et al., 2017; Tompkins et al., 2022), would be beneficial. This approach might stimulate specific emulsifying agents and elevate fat digestibility (Zhang, Haitao, Zhao, Guo, Barri, 2011; Tancharoenrat, Ravindran, Zaefarian, & Ravindran, 2014; Ravindran et al., 2016).

Brelaz et al. (2021) observed a significant increase in triglyceride and cholesterol levels in the blood due to the elevated inclusion of fish waste oil in diets for laying hens, suggesting a notable impact of this food on fat metabolism, which aligns with the findings of this study. It's noteworthy that the primary source of serum lipids is dietary lipids absorbed by the intestinal mucosa, and factors like the amount and type of food, as well as the fat profile present in the diet, directly influence lipid concentrations in the blood and subsequently impact metabolic pathways (Zhang et al., 2011; Silva, Moura, & Nogueira, 2012; Ravindran et al., 2016).

The literature indicates that the elevation of serum fats in certain situations may not be connected to pathological changes (Alonso-Alvarez, 2005; Rodriguez-Sanchez, Tres, Sala, Guardiola, & Barroeta, 2019; Saminathan et al., 2022), but rather to heightened lipid activity in the body. This insight also aids in comprehending the increased activity of these molecules in the digestibility of laying hens. The supplementation of fats in poultry diets can enhance the digestibility of other feedstuffs, thereby accelerating the passage rate and nutrient digestibility in the gastrointestinal tract (Rahman & Koh, 2016; Cruz & Rufino, 2017; Rodriguez-Sanchez et al., 2019).

This availability of nutrients from other feedstuffs combined with an additional metabolic effect results in improved energy efficiency (Sakomura et al., 2004; Costa et al., 2009b; Viana et al., 2009; Ravindran et al., 2016). Furthermore, Torres and Dreher (2015) noted a significant impact of fats from fish by-products on meat and eggs due to the fatty acid profile of fish fats. Other studies also observed that increasing the fat content in poultry diets could enrich the nutritional and sensory aspects of meat and/or eggs.

Earlier studies have highlighted that diets supplemented with high levels of fish oil significantly affected layer performance, particularly feed intake (Saleh, 2013; Brelaz et al., 2019). This outcome has been attributed to the high percentages of long-chain polyunsaturated fatty acids in fish oil that transfer their sensory characteristics to the diets, altering their palatability (Ayed, Attia, & Ennouri, 2015). Silva et al. (2017) similarly reported substantial effects on performance and egg quality resulting from the inclusion of fish by-products in laying hens' diets.

Normally, oils of animal origin, such as fish oil, tend to exert a more pronounced influence on feed intake compared to oils of plant origin (Faitarone et al., 2013; Bertipaglia et al., 2016; Faitarone et al., 2016; Silva et al., 2017). This effect can be attributed to the distinctive odor and flavor that these by-products convey to the diets through their polyunsaturated fatty acids, which naturally influence digestibility and nutrient utilization, ultimately impacting productive outcomes (Freitas et al., 2013, Nogueira et al., 2014). However, despite the impact of fish waste oil on the digestibility of fats for laying hens, no effect ($p > 0.05$) was observed on apparent metabolizable energy and apparent metabolizable energy corrected by nitrogen balance (Table 4).

Table 4. Apparent metabolizable energy (AME) and apparent metabolizable energy corrected by nitrogen balance (AMEn) of the control diet and the experimental diet containing 3.5% of fish waste oil to laying hens.

Energy metabolism	Fish waste oil levels (%)		p-value	CV
	0	3.5		(%)
AME, kcal.kg^{-1}	2,850.69	2,903.42	0.65^{ns}	6.87
AMEn, kcal.kg^{-1}	2,713.72	2,751.79	0.70^{ns}	6.20

CV – Coefficient of variation; ns – non significant.

These results are disagreed with those of Batalha et al. (2017), who observed a significant effect of a 3% inclusion of acid silage of pirarucu on the energy metabolism of laying hens. They attributed this result to the greater utilization of its fish by-product as an energy source in the diet. However, Oliveira et al. (2014b), while using fish silage in broiler diets, also did not detect any influence of this product on energy metabolism. The variations in results observed across studies that employed fish by-products in poultry diets, including the outcomes of this study, highlight that factors like the type of fish by-product, its processing, and chemical composition play crucial roles in nutrient digestibility and utilization.

Moreover, these factors are directly connected to the birds' energy metabolism (Calderano et al., 2010; Fernandes et al., 2015). According to Torres and Dreher (2015), lipid sources exhibit similar chemical structures, as they are composed of the same biomolecules. Nevertheless, the authors also noted that their physical, chemical, and nutritional characteristics vary based on the profile of unsaturated and saturated fatty acids. Vasconcelos, Mesquita, and Albuquerque (2011) and Oliveira, Jesus, Batista, and Lessi (2014a) also affirmed that the chemical composition of fish by-products differs according to fish species, rearing system, developmental stage of fish, sex, and other factors.

These variations, although subtle, can significantly impact the birds' nutrient utilization (Ravindran et al., 2016; Silva et al., 2017). To maintain high productivity, hens must consistently convert feed into energy. For this purpose, the movement of feed through the digestive tract is assessed through various means, such as the time required to empty the crop, the duration before the feed appears in the droppings, and the time needed to completely clear the digestive tract. All of these aspects are also indicative of nutrient utilization by the hens and their energy metabolism (Tancharoenrat et al., 2014; Bogusławska-Tryk, Piotrowska, Szymeczko, & Burlikowska, 2016; Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019).

Conclusion

Based on the obtained results, it can be concluded that the inclusion of 3.5% fish waste oil in laying hens' diets led to satisfactory nutrient digestibility, enhancing the digestibility of fats, without adversely affecting energy metabolism.

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