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Original Article

Fish Waste Oil in Laying Hens* Diets

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ABSTRACT

The present study aimed to evaluate increasing levels of fish waste oil in laying hens diets on performance, egg quality, and sensory features of the eggs. 192 Hisex White laying hens with 29 weeks of age were used, with water and food *ad libitum*. The experimental design was completely randomized consisting of eight treatments corresponding to the inclusion levels of fish waste oil (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5%) in the diets, with four replicates of six birds each. Data collected were subjected to polynomial regression at 5% of significance. Differences (p<0.05) were observed in feed intake and egg mass. Feed intake increased until 2.50% of fish waste oil in the diets. Differences were not observed (p>0.05) in all variables analyzed. Differences were observed (p<0.05) in flavor. Eggs from birds fed diets up to 2.00% present better acceptance by the tasters. Above this level, there was a considerable drop in acceptance. From these results, the present study indicates that the use of fish waste oilin laying hens diets did not affect the egg quality. However, its high inclusion negatively affected the feed intake, egg mass, and egg flavor.

INTRODUCTION

In the last years, there was a significant growth in the Brazilian aquaculture, especially in the Northern region. This large volume of production indicates a considerable amount of waste that represents a significant potential to be used in animal feed (Anjos *et al.*, 2015; Arruda, 2017). However, the use of fish waste as alternative feed in poultry diets is not an innovation in the poultry industry, especially due to their large use in regions that present logistics barriers to grain and high-cost feedstuffs (Jafari *et al.*, 2006; Cruz *et al.* 2016).

In general, fish oils are rich sources of omega-3 fatty acids and poor sources of omega-6, and the contents of linoleic acid are also low (Mariod *et al.*, 2015). Currently, omega-3 fatty acid-rich fish oil has been widely used in food, pharmaceutical and in the health products industry, especially in the European and American market. At the same time, scientists in the animal nutrition field hoped to diversify the source of omega-3 fatty acid alternative to fish products (Dong *et al.*, 2018). Furthermore, previous studies pointed that the use of fish oils and fats in poultry feed may affect the energy metabolism, improve the diet's palatability, and regulate the digestibility of nutrients (Carvalho, 2006; Cherian *et al.*, 2007; Pita, 2007; Santos *et al.*, 2009; Feltesv *et al.*, 2010). Their investigations also indicated that the inclusion of various fish oils in hen diets result in significant increase in yolk omega-3 fatty acids.

However, studies about the effect of fish oil on performance and egg quality are relatively limited (Dong *et al.*, 2018), although some studies using fat sources (soybean oils, linseed, cottonseed, and sunflower, fish fat, animal fat, among others) in hen's diets did not present negative changes on performance and egg quality (Baucells *et al.*, 2000; Santos *et al.*, 2009; Mariod *et al.*, 2015). The same works also indicating that the major sources of n-6 polyunsaturated fatty acids are vegetable oils, whereas n-3 polyunsaturated fatty acids sources include fish by-products (Schmitz & Ecker, 2008; Mariod *et al.*, 2015). The inclusion of various fish oil in hen diets may result in a significant increase on fatty acids content in yolk, producing the named "enriched eggs" (Gonzalez-Esquerra & Leeson, 2000; Cherian *et al.*, 2002; Cherian *et al.*, 2007), a popular tend in the worldwide market (Dong *et al.*, 2018).

It is important to mention that lipid degradation may cause a deterioration of the biological content in the eggs and change several properties such as the sensory quality (flavor, aroma, texture, and color), nutritional value, storage time (Araújo, 1999; Saleh, 2013), among others. And the use of oils containing high polyunsaturated fatty acids content may help to avoid these problems and positively affect the egg quality (Pacheco, 2005). The dietary inclusion of fatty acids in poultry diets may be a great advantage to consumers and the poultry industry if commercially acceptable levels of their use could be established (Shin *et al.*, 2011).Thus, the objective of this study was to evaluate increasing levels of fish waste oil in diets for laying hens on performance, egg quality, and sensory features of the eggs.

MATERIAL AND METHODS

The experimental procedures were developed in the facilities of the Poultry Sector, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas State, Brazil. All procedures were approved by the Ethics Committee in Use of Animals (protocol number 012/2017) of Federal University of Amazonas.

The experiment period lasted 105 days divided into five periods of 21 days. The birds were subjected to a previous adaptation period of seven days to the diets and facilities. The aviary (17.0 x 3.5 m) used had galvanized wire cages, trough feeders, and nipple drinkers.

192 Hisex White laying hens with 29 weeks-of-age were used. Birds were weighed in the beginning of the experimental period to standardize the plots, presenting an average weight of 1.45± 0.0025kg. Egg collection was performed two times a day (9 a.m. and 3 p.m.). The temperature and relative humidity were recorded two times a day (9:00 a.m. and 3:00 p.m.) using a digital term-hygrometer positioned above the birds' cage, with average results of 30.38 °C and 67.13%, respectively. The experimental design was completely randomized constituted by eight treatments corresponding to the inclusion levels of fish waste oil (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5%) in the diets, 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 fish waste oil was from the fish processing plant of RIOMAR©, Itacoatiara town, Amazonas State, Brazil. This product was obtained from pressing of freshwater fish waste (head, bone structure, fins, tissue, and visceral residue) in the industrial level. The composition and fatty acids profile of fish waste oil was determined in the CBO Laboratories© (Campinas, São Paulo, Brazil), and its results are present in Table 1.

Table 1 – Composition of fish waste oil.

Components	Composition
Palmitic acid (C16:0), %	29.01
Stearic acid (C18:0), %	9.62
Oleicacid (C18:1n9c), %	18.48
Linoleicacid (C18:2n6c)	4.39
Alpha Linolenicacid LNA (C18:3n3)	4.51
Arachidonicacid AA (C20:4n6)	3.09
Eicosapentaenoicacid5,8,11,14,17- EPA (C20:5n3)	3.26
Docosahexaenoicacid 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	4.36
Iodine index, meq/kg	85.37

The experimental diets were formulated according to the nutritional requirements of laying hens according to Rostagno *et al.* (2017), using the composition obtained for fish waste oil (Table 2).

From data collected during the 105 days of the experimental period, we calculated the feed intake (g/bird/day), egg production (%), feed efficiency (kg of feed used / kg of egg), feed efficiency (kg of feed used / dozen eggs), and egg mass (g). In the end of each 21 days period, four eggs of each plot were randomly selected to evaluate egg weight (g), albumen (%), yolk

Table 2 – Diets composition containing fish waste oil.

Ingredients	Fish waste oil (%)							
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Corn (7.88%)	65.0529	63.7356	62.5469	60.5696	58.2469	55.2459	54.3412	52.1422
Soybean meal (46%)	23.2198	23.2741	23.3068	23.1381	22.6938	22.5048	22.6845	22.0501
Wheat meal	0.0000	0.5000	1.0000	2.5000	5.0000	7.5000	7.5000	10.0000
Fish waste oil	0.0000	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000	3.5000
Limestone	8.8070	9.0744	9.2352	9.3998	9.2005	9.4005	9.6488	9.5122
Dicalcium phosphate	1.9812	1.9760	1.9706	1.9520	1.9195	1.9095	1.8877	1.8568
Vit. min. supplement [1]	0.5000[1]	0.5000[1]	0.5000[1]	0.5000[1]	0.5000[1]	0.5000[1]	0.5000[1]	0.5000[1]
Salt	0.3500	0.3500	0.3500	0.3500	0.3500	0.3500	0.3500	0.3500
DL-methionine (99%)	0.0891	0.0899	0.0905	0.0905	0.0893	0.0893	0.0878	0.0887
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient	Nutritional levels							
M.E., kcal.kg^{-1}	2,727.3	2,736.7	2,750.5	2,750.5	2,750.5	2,745.7	2,750.5	2,750.5
Crude protein, %	16.000	16.000	16.000	16.000	16.000	16.000	16.000	16.000
Calcium, %	3.900	3.900	3.900	3.900	3.900	3.900	3.900	3.900
Available phosphorus, %	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450
Methionine, %	0.344	0.344	0.344	0.344	0.342	0.340	0.340	0.339
Met. +cystine, %	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
Sodium, %	0.156	0.156	0.156	0.156	0.156	0.156	0.155	0.155

[1] Guaranteed levels 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.

(%), eggshell (%), yolk height (mm), albumen height (mm), yolk index, eggshell thickness (μm), specific gravity (g/cm3), Haugh unit, and yolk color.

The eggs were stored for one hour in room temperature and weighed using an electronic balance (0.01 g). The eggs were placed in wire baskets and immersed in buckets containing different levels of sodium chloride (NaCl) with density variations from 1.075 to 1.100 g/cm^3 (interval of 0.005) to evaluate the specific gravity.

Then, the eggs were placed on a flat glass plate to determine albumen and yolk height, and yolk diameter using an electronic caliper. The yolk values (diameter and height) were used to determine the yolk index. To separate the albumen and the yolk a manual separator was used. Each one was placed in a plastic cup and weighted in an analytical balance.

Eggshells were washed, dried in anoven (50 °C) for 48 hours, and weighed. Dry eggshells were used to determine the eggshell thickness using a digital micrometer. Average eggshell thickness was analyzed considering three regions: basal, meridional, and apical.

The yolk color was evaluated using a ROCHE© colorimetric fan with a scale of 1 to 15. Haugh unit was calculated using the egg weight and albumen height values in the formula H_{unit} = 100 x log (H + 7.57 – 1.7 x W0.37), where H = albumen height (mm), and W = egg weight (g).

To evaluate the sensory features of the eggs, a 9-point hedonic test (acceptability) was used. The scale considered the extremes "I liked it very much" (9) and "disliked it very much" (1), characterizing a preference test (Dutcosky, 1996). 45 untrained and voluntary tasters evaluated a sample (half of a boiled egg in hot water for 10 minutes) of each treatment. The aroma, color, flavor, and appearance were evaluated.

All data collected in this study were analyzed using the GLM procedure of SAS (Statistical Analysis System, v. 9.2) and estimates of treatments were subjected to ANOVA and subsequent polynomial regression analysis. Results were considered significant at $p \leq 0.05$.

RESULTS

Feed intake ($y = -1.5262x^2 + 2.6893x + 102.74$ $R^2 = 0.77$) decreased 3.50% with fish waste oil in the diets. There was also a considerable reduction in egg production ($y = -1.3417x^2 + 1.7665x + 94.482$ $R^2 = 0.72$), and egg mass ($y = -1.6148x^2 + 2.1279x + 54.563$ $R^2 = 0.65$) of 3.50% with the inclusion of fish waste oil in the diets. The inclusion level of 2,00% of fish waste oil presented more balanced results than other inclusion levels (Table 3).

Differences were not observed ($p>0.05$) in all variables analysed. The inclusion of fish waste oil in the diets did not affect the egg quality (Table 4).

Table 3 – Feed intake (FI), egg production (EP), feed efficiency (FE, $kg.kg^{-1}$ and $kg.dz^{-1}$), and egg mass (EM) of laying hens fed diets containing fish waste oil.

Variables	Fish waste oil levels (%)								*p*-value	Effect	CV, %
	0.00	0.50	1.00	1.50	2.00	2.50	3.00	3.50			
FI, g/bird/day	101.52	103.83	102.02	102.98	103.25	102.19	101.75	94.78	0.01	Q	2.41
EP, %	94.76	94.60	94.12	94.52	93.45	93.45	93.57	84.96	0.01	Q	1.94
FE, $kg.kg^{-1}$	1.78	1.79	1.79	1.80	1.77	1.77	1.75	1.81	0.68	-	2.55
FE, $kg.dz^{-1}$	1.28	1.32	1.30	1.30	1.32	1.31	1.30	1.36	0.21	-	2.91
EM, g	54.05	54.96	53.51	54.01	54.37	53.83	54.16	44.09	0.01	Q	2.49

CV – Coefficient of variation. p-value – Coefficient of Probability. Q – Quadratic.

Table 4 – Egg weight (EW), %albumen (AB), %yolk (YO), %shell (SH), albumen height (AH), yolk height (YH), yolk index (YI), shell thickness (ST), specific gravity (SG), Haugh unit (HU), and yolk color (YC) of eggs from laying hens fed diets containing fish waste oil.

Variables	Fish waste oil levels (%)								*p*-value	Effect	CV, %
	0.00	0.50	1.00	1.50	2.00	2.50	3.00	3.50			
EW, g	57.56	58.68	57.14	57.71	58.79	57.98	58.46	58.14	0.39	-	1.82
AB, %	60.45	61.22	60.38	60.69	61.50	61.12	60.89	60.39	0.57	-	1.53
YO, %	26.55	26.34	26.94	27.26	26.72	27.37	26.75	27.11	0.28	-	2.31
SH, %	9.81	9.73	9.95	9.91	9.86	9.73	9.85	9.71	0.50	-	1.86
AH, mm	9.72	9.58	9.69	9.53	9.71	9.99	9.52	9.48	0.14	-	2.57
YI	0.38	0.40	0.40	0.40	0.40	0.41	0.40	0.40	0.17	-	3.24
ST, µm	0.45	0.45	0.45	0.46	0.46	0.45	0.45	0.46	0.06	-	1.51
SG, g/mL	1092.1	1091.1	1091.9	1092.1	1091.4	1090.8	1091.3	1090.7	0.06	-	0.80
HU	61.39	62.61	60.77	61.36	61.90	61.54	62.12	61.81	0.65	-	2.11
YC	6.01	6.11	5.91	5.86	6.10	6.16	6.00	5.81	0.09	-	2.97

CV – Coefficient of variation. p-value – Coefficient of Probability.

Differences were observed ($p<0.05$) in flavor. Eggs from birds fed diets up to 2.00% of fish oil presented better acceptance by the tasters. Above this level, there was a considerable drop in acceptance of the eggs. The inclusion level of 2,00% of fish waste oil provided eggs with better flavour (Table 5).

Table 5 – Sensory features of eggs from laying hens fed diets containing fish waste oil.

Variables	Fish waste oil levels (%)								*p*-value	Effect	CV, %
	0.00	0.50	1.00	1.50	2.00	2.50	3.00	3.50			
Aroma	6.75	6.86	6.43	6.68	7.18	6.75	6.68	6.25	0.38	-	26.53
Color	7.02	7.11	6.61	6.91	6.89	6.98	6.98	6.50	0.58	-	22.61
Flavor	7.36	7.43	7.36	7.39	7.73	7.07	6.95	6.45	0.01	Q	22.87
Appearance	7.29	6.95	7.16	7.18	6.93	7.20	7.02	6.59	0.51	-	22.31

CV – Coefficient of variation. p-value – Coefficient of Probability. Q – Quadratic.

DISCUSSION

The present study indicated that high levels of fish waste oil decreased hen's performance due to the negative–effect caused on feed intake. Different fat sources are available in poultry diets, being from vegetable or animal sources, where the industry observed that diets containing fish oil caused lower feed intake and body weight, and worst feed efficiency (Sahito *et al.*, 2012; Ayed *et al.*, 2015). Previous studies indicated that dietary supplemented with high levels of fish oil had only significant effects on layer performance, especially on feed intake (Saleh, 2013). Silva *et al.* (2017) reported a significant effect in feed intake, egg production, and egg weight from inclusion of fish by-product in laying hen's diets, corroborating with our results. Faitarone *et al.* (2013) also commented that the high inclusion of oil in hens' diets cause a significant reduction on the feed intake, attributing this result to high percentages of long-chains of polyunsaturated fatty acids in fish oil that transfer their sensory characteristics to the diets, changing their palatability (Ayed *et al.*, 2015). Normally, oils of animal origin, such as fish oil, tend to cause a larger effect in feed intake than oils of vegetal origin (Bertipaglia *et al.*, 2016; Silva *et al.*, 2017), attributing this effect to peculiar odor and flavor that these by-products transfer to the diets from their polyunsaturated fatty acids (Freitas *et al.*, 2013, Nogueira *et al.*, 2014).

On the other hand, there was no effect of fish waste oil on the quality of the egg, both lower and higher levels. Previous studies reported that the quality of the eggs is the most affected feature by the inclusion of fat sources in poultry diets, especially the yolk (Oliveira *et al.*, 2010). Grobas *et al.* (2001) reported an increase in the weight of eggs produced by laying hens fed diets with different lipid sources, indicating that this addition was sufficient to promote this increase. This small increase in egg weight may occur due to a little deposition of fat in the yolk, especially linoleic acid (Keshavarz & Nakajima, 1995; Grobas *et al.* 1999). According to Keshavarz & Nakajima (1995), this increase in egg weight may also be related to changes in the nutrients absorption by the hens caused by the increase of passage rate provided by the fat source used. Furthermore, it is known that the fatty acids deposited in the yolk are reflects of dietary fatty acid levels, where these variations in yolk fatty acids ~~are~~ directly caused to changes in the dietary fatty acids levels (Cachaldora *et al.*, 2006; Kralik *et al.*, 2008; Nogueira *et al.*, 2014).

Hester (1999) and Muramatsu *et al.* (2005) suggested that the inclusion of oils in laying hens diets should be carefully calculated due to the fact that high levels of inclusion may negatively affect the eggshell quality. The same authors reported that a high inclusion of oils in poultry diets interfere on mineral metabolism, especially in the retention and absorption of calcium due to the formation of insoluble soaps during the digestion process, hindering its good use by the birds. However, some studies pointed that the addition of oils in laying hen's diets may not represent a significant change in some internal or external structures of the egg (Mazalli *et al.*, 2004; Filardi *et al.*, 2005; Dong *et al.*, 2018), such as observed in this study.

And even the inclusion of fish waste oil did not affect the egg quality, we observed a significant effect on the sensory features, especially in the flavor. Previous research has shown that hens fed with fish by-products resulted in tainted eggs with a "fishy" taste (Holdas & May, 1966; Cherian *et al.*, 2002; Hargis *et al.*, 1993; Coorey *et al.*, 2014; Dong *et al.*, 2018). However, Cherian *et al.* (2007) reported that the inclusion of fish oil in the hens' diets resulted in worst egg flavor and indicated that this fish by-product is directly responsible for sensory changes in the eggs.

Naturally, fish oil contains high percentages of long-chain polyunsaturated fatty acids, which accounts for the oxidative instability and the transference of characteristic fish flavor to the meat or eggs of birds fed fish oil. In general, fish oils are rich sources of omega-3 fatty acids and poor sources of omega-6, and the contents of linoleic acid are also low (<2%) (Baião & Lara, 2005). For hens, the fatty acids are incorporated into the yolk, making it more susceptible to lipid degradation caused by oxidation, hydrolysis, polymerization, or pyrolysis. These processes change several features of the egg, especially the flavor, aroma, texture, and color (Bobbio & Bobbio, 1992; Araújo, 1999; Coorey *et al.*, 2014).

The enriched eggs with a great amount of fatty acids may present most susceptible to lipid oxidation, mainly due to polyunsaturated fatty acids and its double bonds (Gómez, 2003). Faitarone (2010) reported that eggs enriched with polyunsaturated fatty acids from soybean, canola, and linseed oils presented a high degree of lipid oxidation. Furthermore, the oxidative stability depends on unsaturation degree of fatty acids present or their concentration (Pacheco, 2005). The enrichment of eggs through fatty acids from animal or vegetable oils is a positive tool to the poultry industry. However, the oil level cannot be extrapolated, because it may cause a high lipid degradation, and reduce the sensorial acceptance of these eggs by the consumer (Seibel *et al.*, 2010).

CONCLUSIONS

The present study indicates that the use of fish waste oil in laying hens diets does not affect~~ed~~ the egg quality. The inclusion level of 2.00% of fish waste oil provided more balanced results of performance and better egg flavour. However, its high inclusion negatively affects the feed intake, egg mass, and egg flavor.

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