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Research Article
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Sunflower cake mixed with crude glycerine in the diet of Semi-heavy laying hens[1]

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ABSTRACT - The use of alternative feeds in the diet of laying hens can reduce costs and add nutritional value to the eggs; however, information on the combined effects of sunflower cake and crude glycerine remains limited. This study evaluated the individual and combined effects of including both ingredients on productive performance, egg quality and the oxidative stability of egg yolks from semi-heavylaying hens, based on the hypothesis that moderate levels do not compromise performance and may improve the antioxidant quality of the eggs. A total of 320 Hy-Line Brown laying hens were used in a completely randomised design, employing a 4 x 2 factorial scheme with five replications, each of eight hens. Four levels of sunflower cake and two levels of crude glycerine were evaluated. The data were submitted to analysis of variance, with the mean values compared using the SNK test (5%) and the levels of sunflower cake evaluated by regression analysis. The addition of 27% sunflower cake reduced egg mass and production, had an adverse effect on feed conversion, the specific gravity of the eggs and yolk colour, while increasing the levels of phenolic compounds and antioxidant capacity, and reducing lipid oxidation in the yolk. Glycerine had no effect on productive performance, but reduced specific gravity, increased the occurrence of shell blemishes and increased lipid oxidation in the yolk. Regardless of the use of glycerine, the addition of up to 18% sunflower cake is viable, increasing antioxidant capacity and reducing lipid oxidation in fresh eggs and eggs stored for up to 28 days.

Keywords: Alternative feeds. Antioxidant activity. Anti-nutritional factors. Lipid oxidation.

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INTRODUCTION

The search for alternative ingredients that can replace those commonly used in poultry feed to minimise costs and increase the availability of by-products generated by the biodiesel industry has encouraged research into the use of these ingredients in the composition of poultry feed.

Some of the by-products generated in the biodiesel industry, such as crude glycerine and sunflower cake, are rich in nutrients and can be included in poultry feed to reduce production costs and enrich the products (meat and eggs) with polyunsaturated fatty acids or antioxidant activity (Jesus; Oliveira JR; Silva, 2020). In addition, the use of these by-products reinforces sustainability indices by reducing the amount of waste disposed of in the environment and allowing the diet of the hens to be varied.

Sunflower cake is the result of the mechanical extraction of the oil, and excels as a protein-rich feed (208.5 to 277.9 g/kg of crude protein) that contains considerable levels of ether extract (167.2 to 265.5 g/kg) and apparent metabolisable energy for poultry, ranging from 1.711 to 3.217 kcal/kg (Kargopoulos *et al*., 2017; Pinheiro *et al*., 2013; Souza, D., *et al*., 2020).

The residual oil found in this ingredient is rich in polyunsaturated fatty acids, especially linoleic and oleic acids (Rakita *et al*., 2023; Saleh *et al*., 2021). Sunflower products can also be a potential source of natural antioxidants due to the levels of α-tocopherols and chlorogenic acid (Žilić *et al*., 2010). However, the high, predominantly insoluble, fibre content is a limiting factor for including this ingredient in diets, as it can reduce nutrient digestibility (Sakomura; Rostagno *et al*., 2016). It also contains both phytate, which can form insoluble salts with nutritionally important cations, reducing the availability of minerals (Franceschina *et al*., 2016), and chlorogenic acid, which can inhibit enzymes such as trypsin and lipase, reducing the availability of proteins (González-Pérez *et al*., 2002).

Crude glycerine can be obtained through transesterification. Despite differences in composition resulting from different fat sources, processing methods and the reagents used in biodiesel production, several authors have shown that for poultry, the energy value of glycerol is high (Souza, C., *et al*., 2020; Tavernári *et al*., 2022). Furthermore, glycerol can have a positive effect on amino acid retention, as it inhibits the activity of the enzymes phosphoenolpyruvate carboxykinase and glutamate dehydrogenase, and may result in a saving of gluconeogenic amino acids, favouring the deposition of body proteins (Ghayas *et al*., 2023). It can also influence the lipid profile of the final product due to fatty acids (Duarte *et al*., 2014). However, when using glycerine in animal diets, the levels of sodium and such contaminants as methanol need to be monitored to avoid affecting the health of the animals (Gianfelici *et al*., 2011).

Various studies have evaluated the effects of including glycerine (Avellaneda; Ariza-nieto; Afanador-Téllez, 2020; Duarte *et al*., 2014) and the by-products of sunflower processing, cake (Kargopoulos *et al*., 2017; Pinheiro *et al*., 2013) or meal (Baghban-Kanani *et al*., 2018; Koçer *et al*., 2021; Saleh *et al*., 2021), in poultry feed, with varying recommendations for their inclusion, demonstrating the feasibility of using these products in the diets of laying hens. However, there is a maximum limit on the use of these ingredients to replace more traditional foodstuffs such as maize and soya beans due to the anti-nutritional factors mentioned above. More than one alternative ingredient, each with different characteristics, can be used as a way of diversifying the diet and affording the producer good economic return.

In this context, the aim of the present study was to verify whether the individual or combined addition of sunflower cake and crude glycerine to the diet of semi-heavylaying hens compromises zootechnical performance and egg quality, or whether these ingredients can be used safely and effectively. The hypothesis was that moderate levels of sunflower cake, individually or combined with crude glycerine, do not harm the productive performance of the hens and may result in improvements in the antioxidant quality of the eggs.

MATERIAL AND METHODS

The experimental procedure was approved by the Ethics Committee on the Use of Animals (CEUA-UFC) of the Federal University of Ceará, under protocol no. 46/2017. The experiment was conducted in the Poultry Sector of the Department of Animal Science (DZ) of the Centre for Agricultural Sciences (CCA) at the Federal University of Ceará (UFC) in Fortaleza.

A total of 320 Hy-Line Brown laying hens were used, each aged 40 weeks. The hens were selected based on weight (1.68 ± 0.017 kg) and egg production (74.48% laying rate), and distributed in cages as per the recommendations of Sakomura and Rostagno (2016).

The experimental design was completely randomised in a 4 x 2 factorial scheme, including eight treatments with five replications of eight hens per experimental unit. The treatments comprised four levels of sunflower cake (0%, 9%, 18% and 27%) and two levels (0% and 7%) of crude glycerine, giving a total of eight treatments.

The sunflower cake was obtained by mechanically pressing sunflower seeds with their husks to remove the oil using a Scott Tech ERT 40-V1

mechanical press, where each kilogram of processed seed yielded 29.20% oil and 70.80% cake. Crude glycerine from cotton was supplied by the Biodiesel Plant of the Centro de Tecnologias Estratégicas do Nordeste, located in the district of Caetés, Pernambuco.

To formulate the different diets, the nutritional composition of the sunflower cake and the crude glycerine was determined by bromatological analysis (AOAC, 2005); the metabolisable energy had earlier been determined by metabolic testing using pullets (Table 1). The nutritional and energy values reported by Rostagno *et al*. (2017) were considered for the other ingredients.

To quantify the chlorogenic acid in the sunflower cake, Soxhlet hot extraction was carried out using hexane to degrease the material which was then extracted with methanol and concentrated in a rotary evaporator under reduced pressure. The extract was then subjected to high-performance liquid chromatography (HPLC) as per the IUPAC (1979).

The experimental diets used in feeding the laying hens (Table 2) were formulated to be isonutrient (except for fibre) and isoenergetic, as per the nutritional requirements recommended by the breed handbook.

The experiment lasted 126 days, divided into 6 periods of 21 days. Throughout the experimental period, environmental data were recorded using a datalogger, which registered minimum and maximum temperatures of 27.17 °C and 35.76 °C, and a minimum and maximum relative humidity of 30% and 89%. The hens received unlimited feed and water, with feeders refilled in the early morning and late afternoon, and 16 hours of lighting per day. The eggs were collected daily in the late afternoon.

To evaluate egg quality, three eggs with an average weight per batch were selected and submitted to the other analyses. The specific density of the eggs (g/cm^3) was determined by weighing each egg in air and in water, as per procedures described by Freitas *et al*. (2004). Albumen quality was evaluated by determining the Haugh unit (Haugh, 1937), for which the eggs were broken onto a flat glass surface and the height (mm) of the dense albumen was measured using a depth micrometer. The weight of the egg and height of the albumen were used in the following equation:

$$HU = 100 \cdot \log\left(H - 1.7 \cdot P^{0.37} + 7.6\right) \quad (1)$$

where: HU = Haugh unit; H = albumen height in mm; P = egg weight in g.

After measuring the height of the albumen, this was separated from the yolk, which was then removed and weighed. To obtain the percentage of yolk in relation to the egg, the weight of the yolk was divided by the weight of the egg, and the result multiplied by 100. The Digital YolkFan™ (Digital YolkFan™, DSM Company Heerlen, Limburg, Netherlands), based on the same colour tones as the colorimetric fan, was used to evaluate the colour of the yolk.

Table 1 - Nutritional composition and energy value of the sunflower cake and crude glycerine added to the feed of semi-heavylaying hens

Parameter	Sunflower cake	Crude glycerine
AME kcal/kg	2.774	3.582
Dry matter (%)	90.04	91.60
Crude protein (%)	26.26	0.18
Ether extract (%)	15.22	
Calcium (%)*	0.30	
Available phosphorus (%)*	0.10	
Sodium (%)*	0.03	0.06**
Chlorine (%)*	0.09	
Potassium (%)*	1.28	
Digestible lysine (%)*	0.70	
Digestible methionine (%)*	0.49	
Digestible methionine + cystine (%)*	0.80	
Digestible threonine (%)*	0.73	
Digestible tryptophan (%)*	0.27	
Chlorogenic acid (%)	2.08	
Methanol (ppm)		601**

AME - Apparent Metabolisable Energy, *Estimated by the authors based on tables from the Spanish Foundation for the Development of Animal Nutrition (FEDNA, 2010), expressed as natural matter; **Data provided by the supplier

Table 2 - Percentage composition of ingredients, calculated nutrient levels, and metabolisable energy values of the experimental diets formulated with different levels of sunflower cake (SC) and crude glycerine (CG), for semi-heavylaying hens

Ingredient (kg)	Diet							
	Without Glycerine				With Glycerine			
	0% SC	9% SC	18% SC	27% SC	0% SC	9% SC	18% SC	27% SC
Maize	64.70	60.03	55.37	50.71	57.64	52.70	47.77	42.83
Soya bean meal	22.84	18.30	13.74	9.19	21.82	17.73	13.65	9.57
Sunflower cake	0.00	9.00	18.00	27.00	0.00	9.00	18.00	27.00
Maize gluten 60%	0.00	0.00	0.00	0.00	1.60	1.31	1.01	0.71
Soya bean oil	0.74	0.92	1.10	1.28	0.18	0.47	0.77	1.06
Crude glycerine	0.00	0.00	0.00	0.00	7.00	7.00	7.00	7.00
Calcitic limestone	9.35	9.30	9.26	9.21	9.34	9.30	9.25	9.20
Dicalcium phosphate	1.57	1.59	1.61	1.64	1.60	1.62	1.64	1.65
DL-methionine	0.21	0.19	0.17	0.15	0.20	0.19	0.17	0.16
L-Lysine	0.05	0.11	0.19	0.26	0.08	0.14	0.20	0.26
L-Threonine	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03
Vitamin supplement[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral supplement[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Common salt	0.38	0.38	0.38	0.38	0.38	0.38	0.37	0.37
TOTAL	100	100	100	100	100	100	100	100
Calculated nutrients								
Metabolisable energy (kcal/kg)	2.780	2.780	2.780	2.780	2.780	2.780	2.780	2.780
Crude protein (%)	15.60	15.60	15.60	15.60	15.60	15.60	15.60	15.60
Dry matter (%)	89.33	89.53	89.72	89.92	89.60	89.80	90.01	90.22
ADF (%)	4.03	6.18	8.32	10.46	3.85	5.99	8.14	10.29
NDF (%)	10.87	13.74	16.60	19.47	9.99	12.67	15.75	18.63
Ether extract	3.48	4.79	6.09	7.39	4.12	5.52	6.94	8.35
Calcium (%)	4.20	4.20	4.20	4.20	4.20	4.20	4.20	4.20
Avail. phosphorus (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Sodium (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Chlorine (%)	0.28	0.28	0.28	0.29	0.27	0.27	0.28	0.28
Digestible lysine (%)	0.83	0.74	0.66	0.58	0.82	0.74	0.66	0.58
Dig. methionine + cystine (%)	0.71	0.62	0.53	0.44	0.71	0.62	0.53	0.44
Dig. methionine (%)	0.44	0.39	0.34	0.29	0.44	0.39	0.34	0.29
Dig. threonine (%)	0.83	0.52	0.44	0.35	0.61	0.52	0.43	0.35
Dig. tryptophan (%)	0.18	0.15	0.12	0.09	0.18	0.15	0.12	0.09
Chlorogenic acid (%)*	0	0.19	0.37	0.56	0	0.19	0.37	0.56
Cost of the feed (BRL/kg)	1.37	1.31	1.25	1.19	1.34	1.28	1.21	1.15

[1]Composition per kg of product: Vit. A – 9,000,000.00 IU; Vit. D3 – 2,500,000.00 IU; Vit. E – 20,000.00 mg; Vit. K3 – 2,500.00 mg; Vit. B1 – 2,000.00 mg; Vit. B2 – 6,000.00 mg; Vit. B12 – 15.00 mg; Niacin – 35,000.00 mg; Pantothenic acid – 12,000.00 mg; Vit. B6 – 8,000.00 mg; Folic acid – 1,500.00 mg; Selenium – 250.00 mg; Biotin – 100.00 mg. [2]Composition per kg of product: Iron – 100,000.00 mg; Copper – 20.00 g; Manganese – 130,000.00 mg; Zinc – 130,000.10 mg; Iodine – 2,000.00 mg. *Calculated based on the amount of chlorogenic acid found in the sunflower cake

The shells were separated, washed, and left to dry in the open air for 72 hours. Once dried, they were weighed on a semi-analytical balance with a sensitivity of 0.01 g. To obtain the percentage of shell, the weight of the shell was divided by

the weight of the egg and the result was multiplied by 100. The percentage of albumen was obtained by difference, where:

$$\%\ albumen = 100 - (\%\ yolk + \%\ shell) \quad (2)$$

To determine the thickness of the shell (mm), shell fragments were taken from each end (blunt and narrow) and from the equatorial region of the eggs. These fragments were measured using a calliper (Mitutoyo Company, Kawasaki, Japan) with a resolution of 0.01 mm. The shell thickness was determined as the average thickness of the three regions.

The percentage of eggs with dark blemishes on the shell was determined by observation during weighing. The number of blemished eggs in each batch was divided by the total number of eggs in the batch throughout the experimental period. Eggs with several pigmented areas were considered blemished (Roberts, 1998).

Three eggs were selected from each batch for the sixth experiment, to determine the level of phenolic compounds and evaluate the antioxidant capacity of the eggs. These were mixed together and dried in a forced-air oven at 55 °C for 72 hours. The dried sample was then crushed, packed in jars and stored in a freezer.

To obtain the extracts from the diets and the dehydrated eggs for use in the tests, 2 gm of material was first reconstituted with 6 ml of water and then extracted in methanol (1:10 v/v) for 1 hour under agitation (Smet; Raes; Smet, 2006) - adapted. The extract was centrifuged for 10 minutes at 1000 rpm and the supernatant filtered using filter paper.

The phenolic compounds in the extracts were quantified as per Genovese *et al*. (2008), where 0.25 ml Folin-Ciocalteu reagent, 2 ml distilled water, and after three minutes, 0.25 ml saturated sodium carbonate solution were added to a 0.25 ml aliquot of the extract. The mixture was homogenised and incubated in a water bath at 37 ºC for 30 minutes and then centrifuged for 10 minutes at 1000 rpm. The absorbance was read using a spectrophotometer (750 nm). The phenolic compounds were expressed in mg of gallic acid/mL of sample.

Antioxidant capacity, measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method in methanolic egg extract, was carried out as per the methodology described by Arabshahi-Delouee and Urooj (2007), with adaptations. During the assay, 1 ml methanolic egg extract was mixed with 3 ml DPPH solution in methanol (6.10-5 Mol/L) and left for 30 minutes at room temperature in the absence of light. The reading was taken using a spectrophotometer (517 nm), with the antioxidant capacity expressed as a percentage of eliminated DPPH relative to the control, using the following equation:

$$DPPH(\%) = \frac{absorbance\ of\ the\ control - absorbance\ of\ the\ sample}{absorbance\ of\ the\ control} \times 100 \quad (3)$$

Antioxidant capacity, measured using the ABTS°+ (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging method in methanolic egg extract, was carried out following the methodology described by Chen *et al*. (2011), with adaptations. First, the radical was obtained by the reaction of 5 mL ABTS (7 mmol/L) with 88 µL potassium persulfate (2.45 mmol/L). This system was then left to rest at room temperature (25 °C) for 16 hours in the absence of light. Once the ABTS°+ radical was formed, it was diluted with ethanol and the absorbance verified until reaching a value of 0.70 ± 0.05 at 734 nm. From the resulting extract, a 0.3 ml aliquot of each sample was prepared in test tubes in a dark environment with 3.0 ml of the ABTSº+ radical and homogenised in a vortex mixer. The reading was taken six minutes after preparing the mixture using a spectrophotometer at 734 nm. Ethyl alcohol was used as the standard to calibrate the spectrophotometer. The antioxidant capacity was expressed as a percentage of eliminated ABTSº+ in relation to the control, using the above equation for DPPH.

Three eggs were selected from each batch to determine the lipid stability of the yolks of the fresh eggs and of the eggs stored for 28 days. The yolks were evaluated for lipid oxidation by determining the concentration of thiobarbituric acid reactive substances (TBARS) using aqueous acid extraction (Cherian *et al*., 2002). Approximately 2 g of fresh egg yolk (without the membrane) was placed in a 15 ml tube and weighed. Then, 6.75 mL perchloric acid (3.86%) and 18.75 µL BHT (4.5%) were added and the contents homogenised in a vortex mixer for 30 seconds. The tubes were then centrifuged at 8500 rpm for 10 minutes. The supernatant was filtered using filter paper (Whatman no 1).

One millilitre of the filtrate was then placed in Eppendorf tubes and 1 mL of aqueous TBA solution (20 mM) was added. The tubes were heated (Eppendorf ThermoMixer) for 30 minutes at 95 °C with no agitation. To reduce the temperature, the tubes were placed in a refrigerated centrifuge at 4 °C. The optical density was then read using a spectrophotometer at 531 nm. The TBARS concentration was calculated using a malondialdehyde (MDA) standard curve, with the results expressed in µg of MDA per g of sample.

For the statistical analysis, the data underwent tests of normality and homoscedasticity, and any outliers were removed. The (SAS Institute Inc., Cary, NC, USA) was used, with the data submitted to analysis of variance in a 4 x 2 factorial design, including four levels of sunflower cake and two levels of crude glycerine. The mean values were compared using the Student-Newman-Keuls (SNK) test at 5% significance.

Whenever there was any interaction between the factors, the mean values in the breakdown were compared using Tukey's test at 5% significance. The following statistical model was adopted: $Yijk = \mu + \alpha i + \beta j + (\alpha\beta ij) + Eijk$, where Yijk is the k-th response that received the i-th level of factor α and the j-th level of factor β; μ is the overall mean of the experiment; αi is the effect of the i-th level of sunflower cake; βj is the effect of the j-th level of crude glycerine; αβij is the interaction effect between the factors; Eijk is the experimental error. To determine the optimal inclusion level for the sunflower cake, the data were submitted to regression analysis, giving the following statistical model: $Y = \beta 0 + \beta 1X + \beta 2X2$, where: Y is the dependent variable; X is the level of sunflower cake; β0 is the intercept; β1 is the coefficient; β2 is the quadratic coefficient; E is the residual or regression error.

RESULTS AND DISCUSSION

There was no significant interaction between the factors under analysis (sunflower cake and crude glycerine) for feed intake, laying percentage, egg weight, egg mass or feed conversion. There was, however, a significant effect from the level of sunflower cake on feed conversion, laying percentage and egg mass (Table 3).

The addition of sunflower cake to the diets promoted a linear reduction in laying percentage ($Y = 89.571-0.1588x$; $R^2 = 0.9823$) and egg mass ($Y = 54.905 - 0.1361x$; $R^2 = 0.9955$), with a consequent negative effect on feed conversion ($Y = 1.992 + 0.0074x$; $R^2 = 0.948$). Based on the mean comparison test, egg production and egg mass were significantly reduced when sunflower cake was included in the diet at a level of 27% compared to the diet with no cake, while a loss in feed conversion occurred at a level of 18%.

It can be inferred that the lower egg mass of the hens fed 27% sunflower cake is a result of the lower egg production and lower egg weight of these hens. On the other hand, since feed intake was similar between treatments, the reduction in egg mass resulted in poorer feed conversion in the hens fed the diet containing more than 18% sunflower cake.

The negative result for production, egg mass and feed conversion can be attributed to the increased levels of insoluble fibre and such anti-nutritional factors as phytates and chlorogenic acid in the diets with added sunflower cake. The greater amount of insoluble fibre can increase the rate at which the feed passes through the gastrointestinal tract, minimising enzyme access to the feed and affecting nutrient use efficiency (Sakomura; Rostagno 2016). Furthermore, chlorogenic acid, found in sunflower seeds, when oxidised

Table 3 - Mean values for feed intake, laying percentage, egg weight, egg mass and feed conversion in semi-heavylaying hens fed diets containing different levels of sunflower cake and crude glycerine

Factor	Intake (g/hen/day)	Laying percentage (%)	Egg weight (g)	Egg mass (g/hen/day)	Feed conversion (g/g)
			Sunflower cake (%)		
0	109.95	89.77 A	61.12	54.98 A	2.01 B
9	109.08	87.97 AB	60.81	53.53 AB	2.04 AB
18	110.25	86.46 AB	60.76	52.53 AB	2.11 A
27	111.94	85.51 B	59.91	51.23 B	2.21 A
			Crude glycerine (%)		
0	111.14	87.81	61.26	53.83	2.08
7	109.47	87.04	60.08	52.30	2.11
SEM[1]	0.594	0.524	0.410	0.482	0.024
ANOVA[2]			p-value		
Sunflower cake	0.4074	0.0197	0.7143	0.0317	0.0080
Crude glycerine	0.1719	0.4230	0.1510	0.0878	0.3691
Cake x Glycerine	0.6937	0.4083	0.1819	0.4136	0.5409
Regression			p-value		
Linear	0.1827	0.0014	0.2928	0.0032	0.0005
Quadratic	0.2845	0.6573	0.7829	0.9344	0.4343

[1]SEM – Standard error of the mean; [2]Analysis of variance; Mean values followed by different letters in a column differ statistically by the SNK test ($P < 0.05$)

by polyphenol oxidase, results in substances that react with protein, potentially altering its availability and reducing amino-acid digestibility (González-Pérez *et al*., 2002).

Studies related to the addition of sunflower by-products in the diet of laying hens have shown variable results due to variability in their chemical composition and the different experimental conditions, particularly the breeds and ages of the hens used in the experiments. The results for hen performance obtained with the present research are therefore in line with various reports in the literature on the use of these by-products. Koçer *et al*. (2021) reported that it is possible to add up to 9.7% sunflower cake to the diet of white laying hens with a significant benefit to egg weight and production. Saleh *et al*. (2021) found an increase in production with up to 10% sunflower cake in the diet. However, Pinheiro *et al*. (2013), evaluating the addition of sunflower cake (0%, 7%, 14% and 21%) for semi-heavylaying hens, concluded that it is possible to include up to 21% in the diet. This differs from the findings of the present study, since the hens showed poorer feed conversion when sunflower cake was included in the diet at levels of 18% and above.

The addition of 7% crude glycerine had no effect on feed intake, laying percentage, egg mass or feed conversion. Glycerine has been used in the diet of laying hens at levels of 5% to 10% (Cufadar; Göçmen; Kanbur, 2016; Duarte *et al*., 2014) with no effect on performance parameters. However, Avellaneda, Ariza-Nieto and Afanador-Téllez (2020) evaluated the addition of increasing levels of glycerine in the diet (0%, 3%, 6% and 9%) and saw a quadratic effect on egg production, with maximum production up to a level of 4.5% and reducing at higher levels.

When evaluating egg quality, there was a significant interaction between the levels of sunflower cake and crude glycerine in the diet for yolk colour only. In terms of the effect of sunflower cake, neither Haugh units, percentage of components, shell thickness or shell blemishes were influenced by including this ingredient in the diet (Table 4). However, the addition of sunflower cake had the effect of reducing the specific density of the eggs, which was lowest at a level of 18%. This differs from the results obtained from diets containing 0% or 9% sunflower cake.

These results are in line with the findings of Saleh *et al*. (2021), who used sunflower meal in the diet up to a level of 10%, and Pinheiro *et al*. (2013), who tested up to 21% added sunflower cake. Neither study reported any significant effect on eggshell quality from the addition of these ingredients.

Table 4 - Mean values for specific density (SD), haugh unit (HU), percentage of albumen, yolk and shell, yolk colour, shell thickness (ST) and percentage of eggs with shell blemishes, in fresh eggs from semi-heavylaying hens fed diets containing sunflower cake (SC) and crude glycerine (CG)

Factor	SD (g/cm^3)	HU	Albumen (%)	Yolk (%)	Shell (%)	Yolk colour	ST (mm)	Shell blemishes (%)
SC (%)								
0	1.083 A	93.28	66.11	23.90	9.99	8.47	0.392	1.60
9	1.084 A	92.70	66.20	24.05	9.75	8.38	0.395	1.94
18	1.079 B	93.62	66.36	23.83	9.81	8.20	0.387	1.77
27	1.082 AB	93.01	66.39	23.59	10.02	7.44	0.397	2.05
CG (%)								
0	1.089 A	92.88	66.31	23.80	9.90	7.96	0.391	1.58 B
7	1.075 B	93.42	66.23	23.89	9.88	8.28	0.396	2.10 A
SEM[1]	0.001	0.265	0.093	0.081	0.033	0.055	0.001	0.093
ANOVA[2]	p-value							
SC	0.0436	0.6588	0.7183	0.2716	0.1023	< 0.0001	0.3548	0.3005
CG	< 0.0001	0.3154	0.6982	0.5839	0.8915	< 0.0001	0.4879	0.0062
SC x CG	0.0906	0.3096	0.8956	0.8148	0.9446	< 0.0001	0.4295	0.9948
Regression	p-value							
Linear	0.4435	0.9686	0.2329	0.1132	0.0817	-	0.7249	0.1656
Quadratic	0.6885	0.9847	0.8768	0.2145	0.0519	-	0.4353	0.8687

[1]SEM – Standard error of the mean; [2]Analysis of variance; Mean values followed by different letters in a column differ statistically by the SNK test ($P < 0.05$)

The addition of crude glycerine to the diet had no effect on the Haugh Unit or the percentage of egg components, and did not affect shell quality, measured by percentage and thickness; it did however reduce the specific gravity of the eggs and increase the occurrence of eggs with blemished shells.

In line with the results of the present study, there are no reports in the literature of any significant effect from including crude glycerine at levels between 5% and 10% on the specific gravity of eggs (Cufadar; Göçmen; Kanbur, 2016; Duarte *et al*., 2014; Fontinele *et al*., 2017). However, Pinchai, Songserm and Ruangpanit (2021) reported a reduction in the proportion and thickness of the shell with the inclusion of 7.5% glycerine in the diets of laying hens. This effect may be related to the fact that use of the glycerol molecule by the hens is limited, since the enzyme glycerol kinase has a saturation point that restricts the transformation of glycerol into glycerol-3-phosphate (Min *et al*., 2010). As a result, unmetabolised glycerol is eliminated by the kidneys in the urine and, because it is hydrophilic, it carries water with it when excreted, leading to increased urine production (Gianfelici *et al*., 2011). This can cause electrolyte imbalance, which affects mineral absorption. In addition, the presence of excess fatty acids (8% to 12%) in the diet of laying hens can lead to the formation of insoluble calcium salts in the small intestine, which hinders the mobilisation of this mineral by the hens (Çelebi; Karaoğlu, 2024).

Consumer preference suggests that brown coloration is an important parameter of shell quality. Pigment deposition in the shell is influenced by the type of housing, age, breed, diet, stress factors, and diseases such as infectious bronchitis (Lu *et al*., 2021). The addition of crude glycerine increased the appearance of blemishes, affecting the uniformity of the shell colour. This may have been due to the metal content of the crude glycerine, e.g. lithium (239ppm), aluminium (172 ppm), sulphur (30 ppm), and particularly vanadium (< 10 ppm), which is known to have a detrimental effect on eggshell pigmentation when added to the diet of semi-heavylaying hens (Odabasi *et al*., 2006).

Breaking down the interaction for yolk colour (Table 5) revealed a quadratic effect from the addition of sunflower cake ($Y= 8.3175 + 0.0492X - 0.0036X^2$; $R^2 = 0.9839$) in the eggs of laying hens that received no glycerine, showing an increase in the value of this variable for an increase in the level of sunflower cake in the diet that reached a maximum estimated value at a level of 6.83%, reducing at higher levels. On the other hand, in the eggs of laying hens that received crude glycerine, there was a linear reduction ($Y = 8.617 - 0.0248X$; $R^2 = 0.9539$) in the intensity of the yolk colour as the amount of sunflower cake increased.

The results of the mean value comparison test demonstrated that the effect of sunflower cake in the diets without the addition of crude glycerine resulted in a significant reduction in yolk colour at a level of 27% only. However, in diets with 7% crude glycerine, the addition of sunflower cake led to a significant reduction in yolk colour from 18% onwards.

This reduction in yolk colour intensity in treatments with added sunflower cake may be associated with the reduction in the amount of maize in the diet when the sunflower cake is added. This is because maize is rich in carotenoids, which contribute to yolk pigmentation, unlike sunflower cake. This effect has also been reported by other researchers (Pinheiro *et al*., 2013).

Table 5 - Breakdown of the interaction between sunflower cake and crude glycerine on the mean values for yolk colour in eggs from semi-heavylaying hens

		Crude Glycerine		
		0	7	Mean
Sunflower Cake (%)	0	8.35 Ab	8.59 Aa	8.47
	9	8.37 Aa	8.39 ABa	8.38
	18	8.13 Aa	8.26 Ba	8.20
	27	6.98 Ba	7.89 Ca	7.44
Mean		7.96	8.28	
Regression		p-value		
Linear		< 0.0001	< 0.0001	
Quadratic		< 0.0001	0.1737	

Mean values followed by lowercase letters in a row differ statistically by Tukey's test ($P < 0.05$); Mean values followed by uppercase letters in a column differ statistically by Tukey's test ($P < 0.05$)

Only those treatments that contained no added sunflower cake showed any significant effect from the addition of crude glycerine, resulting in a more-intense yolk colour. This is not associated with the addition of the crude glycerine, but rather with the fact that to obtain isonutrient diets it was necessary to include maize gluten, which, in addition to its high protein content, is an excellent source of carotenoid pigment, as it concentrates the pigments found in the maize (Damasceno *et al*., 2020).

In determining antioxidant capacity using the DPPH and ABTS methods, and the level of phenolic compounds in the diets containing sunflower cake and crude glycerine (Table 6), it was found that the phenolic compounds increased with the addition of sunflower cake, resulting in increased antioxidant activity.

When evaluating the level of phenolic compounds, antioxidant capacity by the DPPH and ABTS methods, and lipid oxidation (TBARS) in the eggs (Table 7), there was no significant interaction between the factors under study for the variables under evaluation. However, the addition of sunflower cake had a significant effect on the level of phenolic compounds, the antioxidant capacity of the eggs by the DPPH and the ABTS methods, and lipid oxidation in the egg yolks (TBARS), both fresh and stored. The addition of crude glycerine had a significant effect only on lipid oxidation in the fresh and stored eggs.

There was a linear increase in the level of phenolic compounds ($Y = 58.615 + 3.684X$; $R^2 = 0.99$) due to the increase in sunflower cake added to the diets. A 9% increase in sunflower cake was enough to significantly increase the level of phenolic compounds compared to the control group.

The addition of sunflower cake to the diet promoted an increase in the antioxidant activity of the eggs, detected by the DPPH method at a level of 18% or 27%, and by the ABTS method at a level of 27%. However, adding crude glycerine to the diets had no significant effect on the antioxidant capacity or activity of the eggs.

The beneficial result for the level of phenolic compounds and antioxidant activity found in the present study may be related to the levels of phenolic compounds and α-tocopherols in sunflower cake, especially in the residual oil present in this by-product. Chlorogenic acid is the principal phenolic compound in sunflower cake, together with small amounts of caffeic, cinnamic, coumaric, ferulic, sinapic and hydroxycinnamic acids (Žilić *et al*., 2010). Both chlorogenic and caffeic acid are known as effective antioxidants, capable of eliminating free radicals and inhibiting the oxidation of various lipid substrates (Shahidi; Chandrasekara; Zhong, 2010). Also, α-tocopherol has been extensively studied as a natural antioxidant which, added to poultry diets, not only prevents lipid oxidation but also increases the natural antioxidant content of the eggs (Kralik *et al*., 2023).

The addition of sunflower cake led to a linear reduction in TBARS values in the yolks of both the fresh ($Y = 1.034 – 0.005X$, $R^2 = 0.6621$) and stored eggs ($Y = 0.749 - 0.0223X$, $R^2 = 0.6621$). According to the mean value comparison test, there was a reduction in TBARS values in the fresh eggs for all levels of added sunflower cake compared to the diets that did not contain this ingredient. However, for the stored eggs, the only significant difference, compared to the diet with no added sunflower cake, was seen when 27% sunflower cake was added to the diet, affording a reduction in lipid oxidation in the yolks of the eggs from hens fed this diet.

The concentration of thiobarbituric acid reactive substances (TBARS) in the yolk of fresh eggs may be due to both the ingestion of these substances from the diet and their subsequent transfer to the yolk, and the endogenous production of the hens (Romero *et al*., 2022). However, during storage, eggs undergo lipid oxidation, increasing the concentration of primary and secondary peroxidation products, with the TBARS level in the eggs being directly related to the lipid composition of the yolk and transfer of the antioxidant to the eggs (Sărăcilă *et al*., 2017). The addition of antioxidants to the feed of laying hens is

Table 6 - Mean values for total phenolic compounds and antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging) and ABTS (2,2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid radical scavenging) methods in diets with different levels of sunflower cake and crude glycerine used in poultry feed

Sunflower cake (%)	Crude glycerine (%)					
	0	7	0	7	0	7
	Total phenolics (µg /mL)		% DPPH		% ABTS	
0	94.24	84.24	11.44	10.76	6.92	8.91
9	124.00	119.12	31.19	32.35	11.47	15.93
18	227.61	183.82	60.73	42.32	27.60	24.13
27	251.41	194.78	65.38	55.54	29.63	30.06

Table 7 - Mean values for phenolic compounds, antioxidant activity and lipid oxidation in fresh and stored eggs from semi-heavylaying hens fed diets containing different levels of sunflower cake and crude glycerine

Factor	Phenolic	DPPH[1]	ABTS[2]	TBARS[3] in eggs	
	Compounds (µg/mL)	(%)	(%)	(mg MDA/ Kg Yolk)	
				Fresh	Stored
Sunflower cake (%)					
0	62.09 C	19.92 B	6.85 B	1.051 A	1.656 A
9	66.04 B	22.23 AB	7.82 AB	0.960 B	1.558 AB
18	70.18 A	23.31 A	9.33 AB	0.938 BC	1.532 AB
27	72.99 A	25.18 A	10.02 A	0.898 C	1.482 B
Crude glycerine (%)					
0	68.19	22.86	8.57	0.880 B	1.456 B
7	67.46	22.46	8.45	1.043 A	1.658 A
SEM[4]	0.954	0.507	0.388	0.017	0.028
ANOVA[5]			p-value		
Sunflower cake	< 0.0001	0.0011	0.0199	< 0.0001	0.0412
Crude glycerine	0.4596	0.5920	0.8642	< 0.0001	< 0.0001
Cake x Glycerine	0.9587	0.9379	0.9481	0.1527	0.3182
Regression			p-value		
Linear	0.0028	0.0676	0.1838	0.0007	0.0243
Quadratic	0.5129	0.7429	0.8195	0.3866	0.6521

[1]Elimination of the 2,2-diphenyl-1-picrylhydrazyl radical; [2]Elimination of the 2,2-diphenyl-1-picrylhydrazyl radical; [3]Concentration of thiobarbituric acid reactive substances; [4]Standard error of the mean; [5]Analysis of variance; Mean values followed by different letters in a column differ statistically by Tukey's test ($P < 0.05$)

therefore an important resource for reducing the effects of lipid oxidation in stored eggs, especially when enriched with long-chain and unsaturated fatty acids from the diet (Faitarone *et al*., 2016; Sărăcilă *et al*., 2017).

The effects on the antioxidant activity of the eggs and on the lipid stability of the yolks from the addition of sunflower cake in the diet of laying hens may be associated with the increased levels of phenolic compounds in the feed and, consequently, in the eggs, as shown above (Table 6). These results are consistent with those in the literature, since several researchers have reported the benefits of the antioxidant action of different phenolic compounds found in various plant species when added to the diet of laying hens, improving antioxidant activity (Sărăcilă *et al*., 2017) and reducing the oxidation of lipids in the yolk (Romero *et al*., 2022; Sărăcilă *et al*., 2017).

As for the effect of glycerine, it can be assumed that the increase in lipid oxidation indicated by the increase in TBARS values in the egg yolks of hens fed this by-product may be related to the greater endogenous production of free radicals during glycerol metabolism (Min *et al*., 2010). It may also be due to the increase in the number of unsaturated fatty acids in the yolk resulting from the addition of glycerine to the diet (Duarte *et al*., 2014).

Studies show that including lipid sources in the diet of laying hens alters the fatty acid profile of the yolk, resulting in an increase in unsaturated fatty acids, especially polyunsaturated fatty acids, which can increase the susceptibility of the end product to lipid oxidation due to the instability of these molecules in the face of oxidative reactions (Dotas *et al*., 2023). However, this effect was not seen in the present study, indicating that the presence of antioxidant compounds in sunflower cake, particularly α-tocopherols and phenolic compounds such as chlorogenic acid, was sufficient to prevent lipid oxidation in the yolks (Žilić *et al*., 2010).

CONCLUSIONS

1. Regardless of the addition of crude vegetable glycerine, including up to 18% sunflower cake in diets for semi-heavylaying hens does not compromise either productive performance or the principal quality parameters of the eggs, thereby confirming the proposed hypothesis. In addition, sunflower cake promotes an increase in the level of phenolic compounds and in the antioxidant capacity of the eggs, resulting in less lipid oxidation in the yolks, in both fresh eggs and those stored for up to 28 days;

2. The addition of 7% crude vegetable glycerine does not affect the performance of the hens; it does, however, reduce the specific density of the eggs and promotes the appearance of dark blemishes on the shell. It also intensifies lipid oxidation in the yolk in both fresh and stored eggs. This shows that the antioxidant benefits predicted in the hypothesis are not confirmed at this level, which limits the use of 7% crude vegetable glycerine when considering the quality and oxidative stability of the eggs.

DATA AVAILABILITY STATEMENT

Data-available-upon-request: The research data is only available upon request to the corresponding author.

REFERÊNCIAS

ARABSHAHI-DELOUEE, S.; UROOJ, J. A. Antioxidant properties of various solvent extracts of mulberry (Morus indica L.) leaves. **Food Chemistry**, v. 102, n. 4, p. 1233-1240, 2007. DOI: https://doi.org/10.1016/j.foodchem.2006.07.013.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (ed.). **Official methods of analysis.** 15th ed. Washington, DC, USA: AOAC Int., 2005. Disponivel em: https://www.researchgate.net/publication/292783651_AOAC_2005. Acesso em 28 maio 2016.

AVELLANEDA, Y.; ARIZA-NIETO, C.; AFANADOR-TÉLLEZ, G. Crude glycerin and energy density of diets for growing, pre-lay and pre-peak Backcob Brown egg-laying hens. **Brazilian Journal of Poultry Science**, v. 22, n. 2, p. 1-14, 2020. DOI: https://doi.org/10.1590/1806-9061-2019-1179.

BAGHBAN-KANANI, P. *et al*. Effect of different levels of sunflower meal and multi-enzyme complex on performance, biochemical parameters and antioxidant status of laying hens. **South African Journal of Animal Science**, v. 48, n. 2, p. 390-399, 2018. DOI: http://dx.doi.org/10.4314/sajas.v48i2.20.

ÇELEBI, Ş.; KARAOĞLU, M. Effects of various fat sources added into the diets of laying hens on calcium and phosphorus metabolism. **Journal of Agricultural Production**, v. 5, n. 3, p. 163-169, 2024. DOI: https://doi.org/10.56430/japro.1469620.

CHEN, Y. *et. al.* In vitro and in vivo antioxidant effects of the ethanolic extract of Swertia chirayita. **Journal of Ethnopharmacology**, v. 136, n. 2, p. 309-315, 2011. DOI: http://dx.doi.org/10.1016/j.jep.2011.04.058.

CHERIAN, G. R. K. *et al*. Muscle fatty acid composition and thiobarbituric acid-reactive substances of broilers fed different cultivars of sorghum. **Poultry Science**, v. 81, n. 9, p. 1415-1420, 2002. DOI: https://doi.org/10.1093/ps/81.9.1415.

CUFADAR, Y.; GÖÇMEN, R.; KANBUR, G. The effect of replacing soya bean oil with glycerol in diets on performance, egg quality and egg fatty acid composition in laying hens. **Animal**, v. 10, n. 1, p. 19-24, 2016. DOI: https://doi.org/10.1017/S1751731115001950.

DAMASCENO, J. L. *et al*. Corn distillers' dried gains with solubles to feed broiler chickens from 22 to 42d off age. **Journal off Applied Poultry Research**. v. 29, n. 3, p. 273-583, 2020. DOI: https://doi.org/10.1016/j.japr.2020.03.004.

DOTAS, V. *et al*. Perfil de ácidos graxos e estabilidade oxidativa da gema de ovos de galinhas poedeiras em função da suplementação alimentar com beldroega fresca e da adição de óleos essenciais de plantas aromáticas à água potável. **Sustainability**, v. 15, n. 15, e. 11539, 2023. DOI: https://doi.org/10.3390/su151511539.

DUARTE, C. R. A. *et al*. Mixed crude glycerin in laying hen diets: live performance and egg quality and fatty acid profile. **Brazilian Journal of Poultry Science**, v. 16, n. 4, p. 351-358, 2014. DOI: https://doi.org/10.1590/1516-635x1604351-358.

FAITARONE, A. B. G. *et al.* Yolk color and lipid oxidation of the eggs of commercial white layers fed diets supplemented with vegetable oils**. Brazilian Journal of Poultry Science,** v. 18, n. 1, p. 9-16, 2016. DOI: https://doi.org/10.1590/1516-635X1801009-016.

FOOD AND DRUG ADMINISTRATION**. Code of Federal Regulations**, Title, 21, 2024. Disponivel em: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm. Acesso em: 17 jan. 2016.

FONTINELE, G. S. P. *et al*. Glicerina oriunda do biodiesel na alimentação de poedeiras de ovos vermelhos. **Semina: Ciências Agrárias**, v. 38, n. 2, p. 1009-1016, 2017. DOI: https://doi.org/10.5433/1679-0359.2017v38n2p1009.

FRANCESCHINA, C. S. *et al.* Utilização de fitase na dieta de poedeiras. **Nutritime**, v. 13, n. 1, p. 4529-4534, 2016. Disponível em: nutritime.com.br/wp-content/uploads/2020/02/Artigo-358. pdf. Acesso em: 23 maio 2018.

FREITAS, E. R. *et al*. Comparação de métodos de determinação da gravidade específica de ovos de poedeiras comerciais. **Pesquisa Agropecuária Brasileira**, v. 39, n. 5, p. 509-512, 2004. DOI: https://doi.org/10.1590/S0100-204X2004000500014.

FUNDACIÓN ESPAÑOLA PARA EL DESARROLLO DE LA NUTRICIÓN ANIMAL. **Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos**. 3ª ed. Madrid: **FEDNA,** 2010. 502 p. Disponivel em: https://fundacionfedna.org/tablas-fedna-composicion-alimentos-valor-nutritivo. Acesso em: 17 fev. 2016.

GENOVESE, M. I. *et al*. Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil. **Food Science Technology International**, v. 14, n. 3, p. 207-214, 2008. DOI: https://doi.org/10.1177/1082013208092151.

GHAYAS, A. *et al*. Glycerin: an alternative energy source for poultry. **World's Poultry Science Journal**, v. 3, p. 467-484, 2023. DOI: https://doi.org/10.1080/00439339.2023.2225796.

GIANFELICI, M. F. *et al*. Determination of apparent metabolizable energy of crude glycerin in broiler chickens. **Brazilian Journal of Poultry Science**, v. 13, n. 4, p. 255-258, 2011. DOI: https://doi.org/10.1590/S1516-635X2011000400006.

GONZALEZ-PEREZ, S. *et al*. Isolation and characterization of undenatured chlorogenic acid free sunflower (Helianthus annuus) proteins. **Journal of Agricultural and Food Chemistry**, v. 50, n. 6, p. 1713-1719, 2002. DOI: https://doi.org/10.1021/jf011245d.

HAUGH, R. R. The Haugh unit for measuring egg quality. **United States Egg Poultry Magazine**, v. 43, p. 552-555, 1937.

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY. **Standard methods for the analysis of oils, fats and derivatives**. 6 ed. Oxford: 1979. 188 p. Disponivel em: https://www.degruyter.com/document/doi/10.1351/pac197951122503/html. Acesso em: 27 jan 2017.

JESUS, M. R. R.; OLIVEIRA JR, G. M.; SILVA, C. M. Utilização de coprodutos do biodiesel na alimentação de aves. **Ciência Animal**, v. 30, n. 1, p. 77-91, 2020. Disponível em: https://revistas.uece.br/index.php/cienciaanimal/article/view/9654. Acesso em: 7 jan 2026.

LU, M.-Y. *et al*. Mechanisms associated with the depigmentation of brown eggshells: a review. **Poultry Science**, v. 100, n. 8,e101273, 2021. DOI: https://doi.org/10.1016/j.psj.2021.101273.

KARGOPOULOS, A. *et al*. Use of sunflower cake in the diet of isa brown pullets. **Journal of Agricultural Science and Technology**, v. 7, n. 2, p. 129-134, 2017. DOI: https://doi.org/10.17265/2161-6256/2017.02.008.

KRALIK, Z. *et al*. Natural multi-enriched eggs with n-3 polyunsaturated fatty acids, selenium, vitamin E, and lutein. **Animals**, v. 13, n. 2, p. 321, 2023. DOI: https://doi.org/10.3390/ani13020321.

KOÇER, B. *et al.* Effects of sunflower meal supplementation in the diet on productive performance, egg quality and gastrointestinal tract traits of laying hens. **British Poultry Science**, v. 62, n. 1, p. 101-109, 2021. DOI: https://doi.org/10.1080/00071668.2020.1814202.

MIN, Y. N. *et al*. Glycerin: a new energy source for poultry. **International Journal of Poultry Science**, v. 9, n. 1, p. 1-4, 2010. DOI: https://doi.org/10.3923/ijps.2010.1.4.

ODABASI, A. Z. *et al.* Vitamin C overcomes the detrimental effect of vanadium on brown egg shell pigmentation. **The Journal of Applied Poultry Research**, v. 15, n. 3, p. 425-432, 2006. DOI: https://doi.org/10.1093/japr/15.3.425.

PINCHAI, B.; SONGSERM, O.; RUANGPANIT, Y. The utilization of crude glycerol with low methanol content on production performance and blood chemistry of laying hens. **Journal of Mahanakorn Veterinary Medicine**, v. 16, n. 1, p. 119-128, 2021. Disponível em: https://li01.tci-thaijo.org/index.php/jmvm/article/view/251586/173082. Acesso em: 7 jan 2026.

PINHEIRO, J. W. *et al*. Torta de girassol na alimentação de poedeiras semipesadas. **Semina: Ciências Agrárias**, v. 34, n. 6, p. 3959-3970, 2013. DOI: https://doi.org/10.5433/1679-0359.2013v34n6Supl2p3959.

RAKITA, S. *et al.* Cold-pressed oilseed cakes as alternative and sustainable feed ingredients: a review**. Foods,** v. 12, n. 3, p. 432, 2023. DOI: https://doi.org/10.3390/foods12030432.

ROBERTS, J. R. **Egg shell quality problems: causes and solutions**. *[S. l.]*: University of New England, 1998. 44 p.

ROMERO, C. *et al*. Desempenho produtivo, qualidade dos ovos e oxidação lipídica da gema em galinhas poedeiras alimentadas com dietas contendo bagaço de uva ou extrato de uva. **Animals**, v. 12, n. 9, e. 1076, 2022. DOI: https://doi.org/10.3390/ani12091076.

ROSTAGNO, H. S. *et al*. **Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais**. 4. ed. Viçosa, MG: Universidade Federal de Viçosa, 2017. 488 p.

SAKOMURA, N. K.; ROSTAGNO, H. S. **Métodos de pesquisa em nutrição de monogástricos.** 2. ed. Jaboticabal: FUNEP, 2016. 262 p.

SALEH, A. A. *et al*. Efeitos da suplementação com farelo de girassol como fonte protéica complementar na dieta de poedeiras sobre o desempenho produtivo, qualidade dos ovos e digestibilidade dos nutrientes. **Sustentabilidade**, v. 13, n. 6, e3557, 2021. DOI: https://doi.org/10.3390/su13063557.

SĂRĂCILĂ, M. *et al*. Evaluation of oxidation stability and antioxidant activity in eggs enriched in ω-3 polyunsaturated fatty acids. **Archiva Zootechnica**, v. 20, n. 1, p. 51-64, 2017. Disponível em: http://www.ibna.ro/arhiva/AZ-20-1/04-MSaracila.pdf. Acesso em: 07 jan 2026.

SHAHIDI, F.; CHANDRASEKARA, A.; ZHONG, Y. Bioactive phytochemicals in vegetables. In: Sinha, N., *et al.* **Handbook of vegetables and vegetable processing**. [*S. l.*]: John Wiley & Sons, 2010. p. 125-158.

SMET, K.; RAES, K.; SMET, S. Novel approaches in measuring the antioxidative potential of animal feeds: the FRAP and DPPH methods. **Journal of the Science of Food and Agriculture**, v. 86, n. 14, p. 2412-2416, 2006. DOI: https://doi.org/10.1002/jsfa.2632.

SOUZA, C. *et al.* Energy values of crude glycerin for broilers. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 72, n. 6, p. 2348-2354, 2020. DOI: https://doi.org/10.1590/1678-4162-11751.

SOUZA, D. H. *et al*. Sunflower cake in brown-egg laying pullet diets: Effects on the growing phase and on the beginning of production cycle. **Animal Feed Science and Technology**, v. 269, n. 40, e114663, 2020. DOI: https://doi.org/10.1016/j.anifeedsci.2020.114663.

SOUZA, D. H. *et al*. Sunflower cake in the diets of lightweight laying pullets: effects on the growth phase and the beginning of production cycle. **Ciência e Agrotecnologia**, v. 46, n. 7, e004822, 2022. DOI: https://doi.org/10.1590/1413-7054202246004822.

TAVERNARI, F. C. *et al*. Metabolizable energy value of crude glycerin and effects on broiler performance and carcass yield. **Livestock Science,** v. 263, n. 4, e. 105017, 2022. DOI: https://doi.org/10.1016/j.livsci.2022.105017.

ŽILIĆ, S. *et al*. The content of antioxidants in sunflower seed and kernel. **Helia**, v. 33, n. 52, p. 75-84, 2010. DOI: https://doi.org/10.2298/HEL1052075Z.