PRODUCTION PERFORMANCES AND NUTRITIONAL EGG QUALITY WHEN FEEDING FLAXSEED MEAL AND DIFFERENT SOURCES OF ANTIOXIDANTS IN LAYING HENS' DIET

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Abstract

Nowadays, the evaluation of the fat content of dietary components in the poultry sector carries considerable significance in assessing their utilization and deposition within the animal body and the resulting products. The effect of dietary flaxseed meal and different antioxidant sources on layers' performances and egg quality was investigated in a 6-wks feeding trial on 120 Tetra SL layers (38 weeks) assigned to 4 groups (C, E1, E2, E3). The control (C) diet was characterized by 2800 kcal metabolisable energy (ME) and 17.8% crude protein (CP), 1.13 g α-linolenic acid/100g FAME. Compare to C diet, all three experimental groups were supplemented with flaxseed meal 5%, and different antioxidant sources: 27 mg vitamin E/kg diet (E1), 100 mg vitamin E/kg diet (E2), and 2% red grape pomace meal (E3). The flaxseed meal supplementation increased up to 10 times the α-linolenic acid dietary concentration. At the end of the experiment, 18 eggs/group were collected to determine the eggs' nutritional and quality parameters. The ingesta of polyunsaturated fatty acids omega 3 (PUFA) concentration increased highly significantly (p=0.0001) by 7.5 times at all experimental groups (0.60; 0.60; 0.61 g FA/ingesta) compared to C group (0.08 g FA/ingesta). The fat concentration of yolk significantly decreased ($p \leq 0.05$) in E2 and E3 groups compared to C. The same trend was noticed for the cholesterol level which significantly decreased (p = 0.039) on all experimental groups (0.25; 0.25; 0.23g/100g egg) compared to C group (0.29g/100g egg). A highly significantly level (p = 0.0001) of Ω3 fatty acids was recorded on E1 (62.59 mg/ whole egg), E2 (64.45 mg/ whole egg), and E3 (59.97 mg/ whole egg) groups compared to C group (17.61 mg/ whole egg) which determined a low Ω6/Ω3 ratio. The index of thrombogenicity (TI), important indicators thrombogenic potential of fatty acids, registered lower values ($p \leq 0.05$) on E1, E2 and E3 (0.54, 0.57, 0.55) compared to C group (0.70).

In conclusion, the dietary flaxseed meal inclusion and the antioxidants supplements registered a high transfer rate of omega-3 fatty acids to egg yolk therefore enhancing the egg nutritional quality with real benefits for human health.

Key words: *eggs quality, flaxseed meal, fatty acids, layers' performances*

INTRODUCTION

In recent years, there has been growing interest in enhancing the eggs' nutritional quality while promoting the well-being of laying hens. As the awareness of health among consumers continues to grow, there is an increasing desire for sustainably produced eggs that also contain essential nutrients and antioxidants [1]. Obtaining an enhanced egg quality led to the investigation of various feed ingredients approaches, including flaxseed meal and

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different antioxidant sources in laying hens' diets [2]. In recent years, the importance of flaxseed (*Linum usitatissimum*) has also increased due to the newer trend of veganism among consumers around the world and the perception of consumers in terms of societal concerns associated with the consumption of products of animal origin [3]. In the same time, flaxseed is recognized as a functional food source, with significant health benefits for consumers due to the well-established biological effects of α-linolenic acid (ALA) and its high content (aprox. 55%) although this triggers a poor oxidative stability of linseed oil [4]. After the oil extraction the by-product resulted, flaxseed meal has impressive nutritional value, but research on its potential is limited. It offers significant benefits as a non-traditional protein source for livestock and poultry. However, its recent introduction to the feed industry faces a major challenge due to the presence of an anti-nutritional factor as cyanogenic glycosides [5, 6]. The studies carried out on livestock have revealed that incorporating n-3 PUFA rich dietary sources into their diet not only improves their growth and fatty acid metabolism but also enriches the nutritional value of animal products [7, 8]. This aspect presents promising possibilities for developing functional livestock products by utilizing flaxseed oil or meal. Another study [9] stated that supplementing hens' diets with flaxseed (7.5 mg/kg) in combination with a plant polyphenol extract can be regarded as an effective method for boosting the n-3 PUFAs concentration in egg yolks. Other researchers [10] highlighted the positive impact of flaxseed meal dietary inclusion, either alone or in combination with rapeseed meal or rice bran, on eggs' nutritional quality due to a significant reduction in saturated fatty acid (SFA) content, as well as a decrease in the ΣSFA/ΣUFA ratio and the PUFA ω6: ω3 ratio. The most favorable results were observed using 2.5% flaxseed meal and 10% rice bran, which exhibited the lowest values for lipid indices in egg yolks, indicating a potential health benefit for consumers. Vitamin E serves as an important cellular antioxidant, protecting cells from oxidative damage. Enhancing yolk vitamin E levels and adding tocopherols to layers' diets can prevent lipid oxidation. Furthermore, vitamin E supplementation notably enhances egg production in laying hens under heat stress [11]. Red grape pomace meal, derived from grape industry waste, presents a significant antioxidant capacity due to its rich content of phenols, flavonoids, anthocyanins, catechins, resveratrol, luteolin, quercetin, and kaempferol. It has been shown to have favorable impacts on the productive performance of livestock and the nutritional quality of animal products, as a natural source antioxidants, dietary fiber and pigments [12].

This study investigates, the dietary flaxseed meal inclusion and the antioxidants supplements in laying hens' diet and their effects on laying performances, eggs' fatty acid profile and health lipid index.

MATERIALS AND METHODS

Experimental Ethics

The study was conducted at Laboratory of Animal Nutrition Physiology of the National Research-Development Institute for Animal Biology and Nutrition (IBNA) Balotesti, according to the principles of the animal welfare stated by the EC Directive 63/2010/EEC regarding the protection of the animals for the experimental trials. All experimental procedures were in accordance with an internal protocol no. 1250/28 February 2019 approved by the Authors' Institution Ethics Committee.

Animals management

A 6-week feeding trial was performed on 120 Tetra SL (38 weeks) laying hens, assigned in a completely randomized design with four dietary treatments with 15 cages (replicates, n=2 birds, 50×50×40 cm, with a

floor slope of 12°) per treatment. The hens were housed in an experimental hall, in a Big Dutchman double-sided, 3-tier battery cages. Each cage was equipped with individual nipple drinker (two nipples/cage), positioned on the back of each cage. At the front of the cages were mounted feed gutters made of galvanized metal. At each battery level, the feed line was divided for each cage so as to ensure that the hens were not able to consume feed assigned to the adjoining replicate. Moreover, the cages' structure allowed the daily recording of the feed administered and of the leftovers in the feed trough, 24 hours after the administration of the feed. Prior to the birds purchase and accommodation, the experimental space, cages, watering system, feeding gutters and egg collecting tape were cleaned and disinfected properly using Virkon® S disinfectant (1% solution with 50% Pentapotassium active substance).

The experimental test was performed in the spring, between March and April, but the hens were kept on under controlled environmental conditions and monitored by ViperTouch computer (temperature: 18.32±1.31 °C; humidity: 54.81±4.84%; ventilation: 3.07±0.77%). The light regimen was in agreement with Tetra SL-LL Hybrid Commercial Layer Management Guide (2018) (16h light/8h darkness). Hens' had free access to water and diets. The feed was weighed daily and administered to the birds in the morning at 08:30 h. All productive parameters were monitored throughout the experimental period and calculated using the method described previously by Panaite et al., [13]. The body weight of the birds was realized twice, at the beginning and at the end of the experimental period. Feed intake and eggs from hens were daily collected and weighed. The egg production were calculated as rate of production per hen per day and FCR were calculated as feed intake/egg mass. No vaccination treatments were applied during the entire experimental period.

The intake of n-3 PUFA fatty acids was calculated as the product between feed intake, the dietary fat concentration, and the concentration of the fatty acid per 100 grams of feed, according to the folowing equation:

$$\text{AG ingested} = \frac{\text{FI x \% EE x FA (g)}}{100}$$

where: FI, feed intake (g/day/hen); EE, ether extract concentration (%); FA, fatty acid concentration (% of ether extract)

Experimental diets

A dedicated software HYBRIMIN® Futter 2008, (Hybrimin GmbH & Co., Hessisch Oldendorf, Germany) was used to design the diets in agreement with the feeding requirements of laying hens as given by [14] and optimized according to the Commercial Layer Management Guide [15]. The formulation of the basic diet was similar for all four groups. The C diet had a conventional structure, based on corn and soybean meal (2800 kcal/kg ME and 178 g CP/kg). Compared to C diet, the three experimental diets included flaxseed (5%) as a source enriched in omega-3 PUFA. To protect the lipid feed structure, experimental diets with flaxseed meal included 27 mg vitamin E/kg feed (E1); 100 mg vitamin E/kg feed (E2) and 2% red grape pomace meal as a natural antioxidant. The feed was manufactured in a single batch, in IBNA Balotesti pilot station, the bags being labeled for each group and stored in special conditions of humidity and temperature. After the manufacture of compound feed, samples were collected from each batch (approx. 500 g/sample), in order to perform basic chemical analyzes and fatty acids profile, according to standards. The ingredients and the calculated analysis of the experimental diets are presented in Table 1.

Table 1 Diet formulation and estimated chemical composition of experimental diet

Ingredients, g/kg diet	Dietary treatments			
	C	E1	E2	E3
Corn	537.40	560.20	560.20	540.20
Soybean meal	238.50	256.20	256.20	256.20
Sunflower meal	80.00	-	-	-
Flaxseed meal	-	50.00	50.00	50.00
Red grape pomace meal	-	-	-	*20.00*
Vitamin E	-	-	*0.10*	-
Sunflower oil	27.00	14.80	14.80	14.80
Monocalcium phosphate	13.50	14.40	14.40	14.40
Calcium carbonate	87.90	88.00	88.00	88.00
Salt	4.00	4.10	4.10	4.10
Methionine	1.20	1.80	1.80	1.80
Choline	0.50	0.50	0.50	0.50
Premix*	10.00	10.00	10.00	10.00
Calculated analysis				
Metabolizable energy, kcal/kg	2800.00	2800.00	2800.00	2800.00
Crude protein, %	178.0	178.0	178.0	178.0
Ether extract, %	44.6	37.4	37.4	37.43
Fatty acids composition, %				
Miristic(C 14:0)	0.14	0.11	0.12	0.13
Palmitic (C 16:0)	8.63	8.49	8.68	8.56
Stearic (C 18:0)	2.64	2.60	2.67	2.61
Lignoceric (C 24:0)	0.11	0.08	0.08	-
Total SFA	11.52	11.28	11.55	11.3
Palmitoleic (C 16:1)	0.37	0.19	0.19	0.13
Oleic (C 18:1)	32.64	29.63	29.80	29.49
Total MUFA	33.01	29.82	29.99	29.62
Linoleic (C 18:2n6)	52.92	44.53	44.14	46.21
γ-Linolenic (C 18:3n6)	-	0.05	0.05	0.05
Eicosadienoic (C 20:2n6)	0.15	0.20	0.17	0.16
Arachidonic (C 20:4n6)	0.50	0.35	0.36	0.35
Total n-6	53.57	45.13	44.72	46.77
α-Linolenic (C 18:3n3)	1.13	13.29	13.19	11.93
Octadecatetraenoic (C18:4n3)	0.31	0.24	0.24	0.24
Eicosapentaenoic (C 20:5n3)	0.09	0.08	0.08	0.13
Total n-3	1.53	13.61	13.51	12.3
Total PUFA	55.1	58.74	58.23	59.07
n-6/n-3	34.88	3.32	3.31	3.80

where: C,control diet; E1, flaxseed meal + 27 mg vitamin E; E2, flaxseed meal supplemented + 100 mg vitamin E; E3, flaxseed meal supplemented with 2% red grapeseed pomace meal; ΣSFA: sum of saturated fatty acids; ΣPUFA: sum of polyunsaturated fatty acids; ΣPUFA-ω3=C18:3ω-3+C20:3ω-3+C20:5ω-3+C22:6ω-3; ΣPUFA-ω6 = C18:2ω- 6 +C20:2ω-6+C20:4ω-6;

*Per kg premix contained: 1350000 IU/kg vitamin A; 300000 IU/kg vitamin D3; 2700 IU/kg vitamin E; 200 mg/kg vitamin K; 200 mg/kg vitamin B1; 480 mg/kg vitamin B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium.

Feed and eggs sampling collection and analysis

After manufacturing, the feed samples have been collected and chemically analyzed using the methods from Regulation (CE) 152/2009 (Methods of sampling and analysis for the official control of feed) for CP using the Kjeldahl method; *ether extract* (EE) determined by extraction in organic solvents and *fatty acids* concentration (FA) determined by gas chromatography according to standards SR CEN ISO/TS 17764-1/ 2008 (Feeds. Determination of the fatty acids content 1. Preparation of the fatty acids methyl esters) and SR CEN ISO/TS 17764-2/ 2008 (Feeds.

Determination of the fatty acids content 2. Method of gas chromatography).

Eighteen eggs samples from each treatment were colected randomly to determine external and internal egg quality parameters. The egg measurements were carried out the next day, after the eggs were previously kept in the refrigerator for 24 hours at 4 °C. Before the measurements, the eggs were weighed individually using an analytical scale (Kerm scales, precision 0.001) and before the eggs were broken, the shell thickness and the breacking strength were measured using Egg Shell Thickness Gauge (ORKA Technology LLC) and, Egg Force Reader (ORKA Technology LLC) respectively. The egg components weight (g/egg) represented by albumen, yolk and shell was registered using the same balance as for the whole egg. The pH-ul (albumen and yolk) was measured using a pH-meter portable (kit Five Go F2-Food with LE 427IP67, Sensor Metler Tolledo). Albumen height (mm) was measured by using stage micrometer manually. And based on albumen height, Haugh unit was calculated using the equation proposed by [16]:

$$\text{Haugh unit} = 100 \log (H - 1.7W^{0.37} + 7.6)$$

where: H is albumen height (mm) and W is the egg weight (g)

The yolk colour (colour scale from 15, dark orange, to 1, light pale) was determined by comparing yolk colour with the DSM yolk color fan (DSM Nutritional Products Ltd., Basel, Switzerland), according to the CIE standard colorimetric system.

After completing the measurements on the eggs, six samples of yolk (3 eggs/sample) for each group were subjected to drying in the etuva for 48 h at a constant temperature of 65 °C for processing to determine the content of the total fat egg yolk, the cholesterol content (g col./100g whole egg) and the fatty acids profiles (g FAME% fat). Fatty acids profiles and cholesterol concentration were determined as described by [13]. Fatty acids were determined by gas chromatography were the working principle of gas chromatography is to convert fatty acids from a sample into methyl esters, which are subsequently separated on a chromatography column, identified by comparison with a standard chromatogram, and determined the percentage of fatty acid esters. To measure the cholesterol content it was used the AOAC International Standard 2002 method (Cholesterol in Multicomponent Foods - Gas Chromatography Method, Assoc. Off. Anal. Arlington Chemistry, Virginia). This method's underlying principle involved saponifying the sample, followed by extracting it with petroleum ether, concentrating it, and adding chloroform.

Health lipid indices of yolk eggs

The fatty acid profile is important for the nutritional quality of lipids in yolk eggs. The health indices of yolk eggs were calculated using the following formula: AI and TI indices [17, 18]; hypocholesterolemic fatty acids, hypercholesterolemic fatty acid and ratio between hypocholesterolemic/hypercholesterolemic (h/H) [19]; health-promoting index (HPI) [20]; cholesterol index (CI) [21]; cholesterol-saturated fat index (CSI) [22] and catalysis enzymes of long-chain polyunsaturated fatty acids: elongase; thioesterase; Δ9-desaturase (16+18) and Δ5 + Δ6 - desaturase (Ω:6).

$$AI = [C12:0 + (4 \times C14:0) + C16:0] / (\Sigma MUFA + \Sigma PUFA\ n\text{-}6 + \Sigma PUFA\ n\text{-}3)$$

$$TI = (C14:0 + C16:0 + C18:0) / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n\text{-}6\ PUFA) + (3 \times \Sigma n\text{-}3\ PUFA) + (n\text{-}3/n\text{-}6)]$$

$$h/H = (cis\text{-}C18:1 + \Sigma PUFA)/(C12:0 + C14:0 + C16:0)$$

$$HPI = \Sigma UFA/[C12:0 + (4 \times C14:0) + C16:0]$$

$$CI = 1.01\ (\text{g of SFA}/100\ \text{g of fresh matter} - 0.5 \times \text{g of PUFA}/100\text{g of fresh matter}) = (0.06 \times \text{mg of cholesterol}/100\ \text{g of fresh matter})$$

CSI = (1.01×g of SFA/100 g of fresh matter) + (0.05×mg of cholesterol/100 g fresh matter)

Elongase = C18:0/C16:0

Thioesterase = C16:0/C14:0

Δ9-desaturase (16+18) = [C16:1 + C18:1 /(C16:1 + C16:0 + C18:1 + C18:0)] x 100

Δ5 + Δ6 - desaturase (Ω:6) = [(C 20:2n 6 + C 20:4n6)/(C 18:2n6 + C 20:2n6 + C 20:4n6)] x 100

Δ5 + Δ6 - desaturase (Ω:3) = [(C 20:5n 3 + C 22:5n3 + C 22:6n3)/(C 18:3n3 + C 20:5n3 + C 22:5n3 + C 22:6n3)] x 100

The first two indices, IA and IT, are most often used to evaluate fatty acids composition. The AI index reflects the connection between saturated fatty acids that promote lipid adhesion to immune and circulatory system cells, promoting atherosclerosis, and unsaturated fatty acids that have anti-atherogenic properties, reducing the risk of coronary heart disease through plaque inhibition and lowering blood cholesterol and phospholipid levels. The TI index shows the tendency to form clots in the blood vessels, and is defined as the relationship between pro-thrombogenetic fatty acids (saturated) and anti-thrombogenetic (unsaturated).

Statistical analysis

All experimental data were analysed using one-way analysis of variance (ANOVA) procedure of the SPSS version 20 (Inc., Chicago IL, USA). The following linear model was used: Yij =μ + Aj + eij, where: Yij = value of trait (dependent variable); μ = overall mean; Aj = the treatment effect; and eij = random observation error. Tukey test was used to compare differences among treatment means. The probabilities lower than 0.05 were considered as statistically significant ($p < 0.05$). The results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSIONS

The proximal chemical composition and fatty acid profile from flaxseed meal and red grapeseed pomace meal are presented within Table 2. Flaxseed meal has a good nutritional value and can be used as a high-quality non-conventional protein feed for livestock and poultry [6]. The flax seeds contain up to 23% CP of the total weight of the seeds, a significantly higher quantity being noticed in the flaxseed meal obtained after oil extraction (up to 35 - 40%) as reported by [23]. In the present study, the flaxseed meal utilizsed is characterized by a high level of CP (34.57 ± 0.063%) and EE (9.79 ± 0.133), the results being similar to the data presented by [2].

Meanwhile the high quality of the protein is given by the balanced content of amino acids, much preferable in flaxseed compared to soy [24], however, the nutrient profile may vary depending on factors such as the flaxseed variety, oil extraction method and the manufacturing process [25, 26]. Compared to soybean, the lysine/arginine ratio of flax seeds is significantly lower (0.37 *vs.* 0.88), correlated with lower thrombogenic and atherogenic potential, having a beneficial effect on the cardiovascular system health [24]. Flaxseed meal is an important source of additional n-3 PUFA [27]. It is characterized by a high content of unsaturated fatty acids (UFA; 90.76±0.078%) compared to saturated fatty acids (SFA; 9.20±0.122%). Of the total UFA, PUFA represent 68.40±0.062%, of which α-linolenic acid (ALA) is found in the largest amount of 51.67±0.062% (Table 2). Therefore flaxseesd meal is considered a higher source of n-3 PUFA compared to fish, corn, soybean or seaweed oil [28, 29, 30], however, environmental and farming conditions may influence the fatty acid profile [31, 32].

Table 2 Flaxseed and red grapeseed pomace meal nutritional characterization

Parameters	Flaxseed meal	Red grapeseed pomace meal
Calculated analysis		
Dry matter, %	90.24±0.083	93.55±0.339
Organic matter, %	84.95±0.085	88.71±0.123
Crude protein, %	34.57±0.063	7.88± 0.729
Crude fat, %	9.79±0.133	4.85 ± 0.899
Crude fiber, %	8.56±0.316	18.86± 0.827
Ash, %	5.29±0.002	4.83±0.784
Nitrogen-free extractive substances, %	32.02±0.597	57.11± 0.578
Antioxidant profile		
Total polyphenols (mg echiv ac galic/g)	nd	22.45±5.67
Antioxidant capacity (mM echiv Trolox)	nd	272.86±32.22
Lutein+zeaxanthin (mg/kg)	1.37±0.19	1.79±0.17
Vitamin E (mg/kg)	nd	20.62 ±3.24
Fatty acids profile		
Caproic (C 6:0)	nd	0.02±0.008
Miristic (C 14:0)	0.08±0.005	0.25±0.035
Pentadecanoic (C 15:0)	nd	0.06±0.021
Palmitic (C 16:0)	6.41±0.143	11.75±0.028
Heptadecanoic (C 17:0)	nd	0.09±0.007
Stearic (C 18:0)	2.52±0.004	3.72±0.396
Arachidic (C 20:0)	nd	0.08±0.106
Behenic (C 22:0)	0.19±0.267	nd
Lignoceric (C 24:0)	nd	0.09±0.021
∑SFA, %	9.20±0.122	16.04±0.510
Pentadecanoic (C 15:1)	nd	0.06±0.021
Palmitoleic (C 16:1)	0.07±0.009	0.34±0.007
Pentadecenoic (C 17:1)	nd	0.08±0.049
Oleic (C 18:1)	22.29±0.007	15.39±0.566
∑MUFA, %	22.36±0.016	15.87±0.474
Linoleic (C 18:2n6)	16.03±0.278	64.17±0.212
γ-Linolenic (C 18:3n6)	0.30±0.131	nd
Eicosadienoic (C 20:2n6)	0.16±0.052	0.13±0.134
Arachidonic (C 20:4n6)	0.13±0.191	0.60±0.219
∑PUFA-n6	16.73±0.001	64.89±0.141
α-Linolenic (C 18:3n3)	51.67±0.062	2.03±0.495
Octadecatetraenoic (C18:4n3)	0.10±0.007	0.63±0.134
Eicosapentaenoic (C 20:5n3)	nd	0.05±0.028
∑ PUFA-n3	51.77±0.054	2.71±0.601
∑ PUFA	68.40±0.062	67.60±0.742
∑UFA	90.76±0.078	83.46±0.269
SFA/UFA	0.10±0.001	0.19±0.007
PUFA/MUFA	90.76±0.08	83.46±0.269
n-6/n-3	0.32±0.001	24.59±5.412

n.d. – not detected,

The results on the chemical composition of red grapeseed pomace meal are presented within Table 2, and shows a polyphenols high content (22.45±5.67 mg equiv ac gallic/g), vitamin E (20.62±3.24 mg/kg), and a high antioxidant capacity (272.86±32.22 mM equiv Trolox), being among the most valuable sources of polyphenolic compounds [33]. Among the phenol group, in large quantities are found proanthocyanidins [34] its antioxidant activity being 20 times higher compared to

vitamin E and 50 times higher compared to vitamin C [35]. However, due to the large amounts of lignin, tannins and fibers, moderate use in poultry feed is recommended [36].

The effects of flaxseed meal and red grapeseed pomace meal evaluation on productive performances and egg classification are presented within Table 3. No statistical differences ($p > 0.05$) were noticed concerning the initial and final body weight. The total feed consumption (kg/hen/period) exhibited significant lower differences ($p < 0.05$) on E2 and E3 groups compared to C and E1. A significantly higher ($p < 0.05$) feed conversion ratio (g feed/g egg) was registered on E3 group compared to C and E1 groups. Egg weight parameter registered significantly higher values ($p < 0.05$) on C and E1 groups compared to E2 and E3. Egg production parameter was significantly higher ($p < 0.05$) on E3 compared to C and E1 groups. Contrary with our results Hayat et al., [37] registered no significant differences ($p > 0.05$) in egg production due to dietary flaxseed inclusion supplemeted with 2 types of antioxidants: α-tocopherols and butylated hydroxy toluene at 3 levels (50, 100, and 150 IU or mg/kg). Other authors, [38] found no significant differences ($p > 0.05$) concerning egg production, egg weight, feed conversion ratio when tested flaxseed meal supplemented with dried tomato and grape pomace on laying hens' diet. Also, Kara et al. [39] using 4 and 6% grape pomace dietary inclusion found no negative effects ($p > 0.05$) on laying hens performances.

Table 3 Effects of flaxseed and red grapeseed pomace meal on productive performances and egg classification

Parameters	Experimental diets				SEM	*p*-value
	C	E1	E2	E3		
Initial body weight (g/hen)	1744.33	1710.03	1794.19	1757.17	28.9	0.227
Final body weight (g/hen)	1841.83	1853.83	1844.33	1848.33	14.068	0.990
Total feed consumption, (kg/hen/period)	3.59^{b}	3.76^{a}	3.79^{a}	3.77^{a}	46.382	0.008
Average daily feed intake, (g/day/hen)	125.62^{a}	125.46^{a}	122.31^{b}	121.79^{b}	1.094	0.018
Feed conversion ratio, (g feed/g egg)	1.93^{b}	1.95^{b}	1.98^{ab}	2.02^{a}	0.020	0.011
Intake of PUFA n-3 fatty acid	0.08^{b}	0.60^{a}	0.60^{a}	0.61^{a}	0.005	0.0001
Egg weight, (g/egg)	66.21^{a}	66.57^{a}	65.49^{b}	64.30^{c}	0.110	0.0001
Egg production,(%)	96.27^{b}	97.94^{a}	95.93^{b}	97.54^{ab}	0.454	0.004
Egg size classification (European Council Directive, 2006):						
XL, (> 73 g)	5.66^{b}	9.97^{a}	7.15^{ab}	1.64^{c}	0.753	0.0001
L, (63–73 g)	72.90	65.25	67.19	64.58	2.21	0.058
M, (53–63 g)	21.02^{b}	24.53^{ab}	25.50^{ab}	31.48^{a}	2.19	0.023
S, (< 53 g)	0.42	0.25	0.16	2.30	0.631	0.051

where: C,control diet; E1, flaxseed meal + 27 mg vitamin E; E2, flaxseed meal supplemented + 100 mg vitamin E; E3, flaxseed meal supplemented with 2% red grapeseed pomace meal; SEM, standard error of the mean. $^{a-c}$Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$; XL-extra large egg, L-large egg, M-medium egg, S-small egg.

Within table 4 are presented the results concerning the effects of flaxseed meal and red grapeseed pomace meal on external and internal egg parameters. The egg components weight values (albumen, yolk, shell) registered no significant differences ($p > 0.05$) among groups during the whole experimental period. The pH albumen values decreased significantly ($p < 0.05$) on E2, E3 groups compared to C group. No significant differences ($p > 0.05$) were noticed between groups concerning yolk pH value and breakingshell strength. The yolk color parameter registered significant highly ($p < 0.05$) values for E2 and E3 groups compared to C and E1 groups.

Tufarelli et al. [38] stated that dietary flaxseed meal supplemented with tomato pamace and grapeseed pomace as natural antioxidant sources showed no effects (p>0.05) on eggshell thickness, strength, Haugh unit. On the other hand, statisticaly significant ($p < 0.05$) results, similar with our study, were noticed on yolk color intensity. Taking into consideration that egg color intensity represents a major criteria for consumers, as expected, the results showed that the highest intensity was recorded on the E3 group due to the yolk-coloring agent of grape pomace properties.

Table 4 Effects of flaxseed and red grapeseed pomace meal on external and internal egg parameters

Parameters	Experimental diets*				SEM	p-value
	C	E1	E2	E3		
Egg Weight and components (g)						
Whole Egg	65.84	67.23	66.32	66.61	1.024	0.590
Albumen	40.62	41.75	41.32	41.04	0.910	0.654
Yolk	16.47	16.51	16.57	16.86	0.383	0.742
Shell	8.76	8.97	8.42	8.71	0.263	0.228
Breakingshell strength	4.02	4.05	4.04	4.19	0.238	0.881
Fresh Egg (Value)						
pH-albumen	7.58^{a}	7.54^{ab}	7.42^{bc}	7.35^{c}	0.055	0.0001
pH-yolk	6.29	6.15	6.16	6.12	0.115	0.473
Albumen height	8.80	8.48	8.32	8.23	0.416	0.537
Yolk color	5.06^{b}	5.50^{b}	6.22^{a}	6.39^{a}	0.271	0.0001
Haugh Units	91.90	89.65	89.05	88.53	1.56	0.442

where: C, control diet; E1, flaxseed meal + 27 mg vitamin E; E2, flaxseed meal supplemented + 100 mg vitamin E; E3, flaxseed meal supplemented with 2% red grapeseed pomace meal; SEM, standard error of the mean. $^{a-c}$Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$.

The effects of flaxseed and red grapeseed pomace meal on cholesterol and fatty acids profile are presented in table 5. Fatty acid, total lipid, and cholesterol profiles in the egg yolk showed significant differences ($p < 0.05$) among of experimental groups compared to the C group. The results obtained from the yolk samples from the experimental trials showed higher levels ($p = 0.006$) of palmitoleic acids (C16:1) as part of saturated fatty acids (SFA) and omega-3 polyunsaturated fatty acid (PUFA n-3) ($p < 0.0001$) which included the α-linolenic (C18:3n3), docosapentaenoic (C22:5n3) and docosahexaenoic acids (C22:6n3), but lower ($p < 0.0001$) levels of heptadecanoic (C17:0), nervonic (C24:1n9) and omega-6 polyunsaturated fatty acid (PUFA n-6) like linoleic (C18:2), γ-linolenic (C18:3n6), eicosatrienoic (C20:3n6), arachidonic (C20:4n6) or docosatetraenoic (C22:4n6) in the yolk of eggs. For human health, a significant importance is given to PUFA n-3 fatty acids concentration. The significant increase ($p < 0.0001$) in omega-3 fatty acids in the egg yolks from the groups fed with flaxseed meal (5%) demonstrates an efficiency in the of omega-3 acids yolk transfer up to 3.65 times compared to the control group. Among the total PUFA n-3 acids, α-linolenic acid (C18:3n3) increased significantly ($p < 0.0001$), by 7.04 times higher in E1, 5.64 times higher in E2, and 6.16 times higher in E3 compared to C group. Furthermore, docosapentaenoic acid (C22:5n3) was significantly higher ($p < 0.0001$), while docosahexaenoic acid (C22:6n3) increased significantly ($p < 0.0001$), up to 4 times in the experimental groups compared to the C group. No significant differences ($p > 0.05$) were recorded between the concentrations of ALA and DHA determined in the egg yolks from the experimental groups (Table 5). Increased Σn-3 fatty acids and PUFA in egg yolk and liver were obtained by [40] when added 50

and 100 g/kg dietary flaxseed meal in laying hens aged 24 to 36 weeks of age. In a study conducted by [41], Hy-Line Brown hens were fed a diet supplemented with 10% flaxseed. Starting from the second week of the experiment, it was noticed a significant ($p < 0.05$) increase in DHA and total n-3 PUFA concentration in eggs. Additionally, there was a decrease in total n-6 PUFA and the n-6/n-3 ratio. Other researchers [42] supplemented 10% flaxseed in laying hens diet and obtained improved eggs (above 600 mg of n-3 PUFA, from which ALA accounts above 80% of the total n-3 PUFA). Some authors [43] used dried tomato waste (7.5%) as another antioxidant source in addition to flaxseed supplementation (5%), and noticed that the n-3 fatty acids content significantly increased after 4 weeks of experimentation. Additionally, the dried tomato waste decreased the enhanced eggs' lipid oxidation.

Table 5 Effects of flaxseed and red grapeseed pomace meal on cholesterol and fatty acids profile

Parameters	Experimental diets*				SEM	*p*-value
	C	E1	E2	E3		
Crude fat, %	30.71^{a}	29.37^{ab}	29.06^{b}	28.15^{b}	0.556	0.002
Cholesterol, mg/egg	290.40^{a}	245.60^{b}	247.56^{b}	236.03^{b}	0.017	0.039
Fatty acids, mg/egg						
Miristic C14:0	3.25	3.24	2.91	3.26	0.143	0.279
Pentadecanoic C15:0	0.93^{a}	0.83^{ab}	0.79^{ab}	0.74^{b}	0.037	0.011
Palmitic C16:0	312.74	297.53	306.58	302.88	7.170	0.511
Heptadecanoic C17:0	2.23^{a}	1.92^{b}	1.63^{c}	1.68^{bc}	0.069	0.0001
Stearic C18:0	156.80	132.47	148.45	132.49	7.890	0.102
∑SFA	475.95	436.00	460.36	441.04	13.900	0.192
Miristioleic C14:1	0.41	0.55	0.47	0.54	0.039	0.056
Pentadecenoic C15:1	2.06^{a}	1.58^{ab}	1.59^{ab}	1.33^{b}	0.170	0.045
Palmitoleic C16:1	28.60^{b}	36.32^{a}	32.79^{ab}	36.94^{a}	1.630	0.006
Heptadecenoic C17:1	1.99^{a}	1.59^{ab}	1.56^{ab}	1.32^{b}	0.158	0.050
Oleic C18:1	426.40	451.60	440.40	447.80	10.300	0.348
Erucic C22 (1n9)	1.08	0.63	0.84	1.63	0.412	0.374
Nervonic C24 (1n9)	4.68^{a}	2.16^{b}	2.20^{b}	2.03^{b}	0.177	0.0001
∑MUFA	465.18	494.46	479.82	491.60	11.400	0.280
Linoleic C18:2	269.44^{a}	229.82^{b}	224.61^{b}	236.68^{b}	4.940	0.0001
Linolenic γ C18:3n6	1.75^{a}	1.34^{b}	1.28^{b}	1.43^{ab}	0.009	0.009
Eicosadienoic C20 (2n6)	2.61^{a}	2.09^{ab}	1.72^{b}	2.06^{ab}	0.171	0.013
Eicosatrienoic C20 (3n6)	4.32^{a}	2.62^{b}	2.79^{b}	2.76^{b}	0.242	0.000
Arachidonic C20 (4n6)	56.84^{a}	38.02^{b}	44.98^{ab}	41.78^{b}	3.110	0.002
Docosatetraenoic C22 (4n6)	18.50^{a}	1.97^{b}	2.57^{b}	2.27^{b}	0.519	0.0001
Linolenic α C18:3n3	3.23^{b}	22.73^{a}	18.23^{a}	19.90^{a}	1.150	0.0001
Eicosatrienoic C20 (3n3)	3.54	2.94	3.33	2.70	0.373	0.395
Docosapentaenoic C22 (5n3)	1.01^{c}	2.38^{ab}	2.61^{a}	1.93^{b}	0.164	0.0001
Docosahexaenoic C22 (6n3)	9.82^{b}	34.54^{a}	40.28^{a}	35.43^{a}	2.760	0.0001
∑ PUFA	371.05	338.45	342.40	346.94	8.570	0.059
∑Ω6	353.45^{a}	275.86^{b}	277.95^{b}	286.97^{b}	6.370	0.0001
∑Ω3	17.61^{b}	62.59^{a}	64.45^{a}	59.97^{a}	2.500	0.0001
Ω6/Ω3	264.15^{a}	56.87^{b}	55.41^{b}	61.46^{b}	2.330	0.0001
Ω3/Ω6	0.65^{b}	2.87^{a}	2.98^{a}	2.68^{a}	0.101	0.0001

where: C,control diet; E1, flaxseed meal + 27 mg vitamin E; E2, flaxseed meal supplemented + 100 mg vitamin E; E3, flaxseed meal supplemented with 2% red grapeseed pomace meal; ∑SFA, sum of saturated fatty acids; ∑MUFA, sum of monounsaturated fatty acids; ∑PUFA, sum of polyunsaturated fatty acids; ∑n-6 omega, sum of 6 fatty acids, ∑n-3, sum of omega 3 fatty acids; SEM, standard error of the mean; n=6 samples/group; $^{a-c}$Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$.

The lipid indices of eggs' yolk are presented within Table 6. In terms of human nutrition, saturated and polyunsaturated fatty acids have different metabolic effects. They can promote or prevent atherosclerosis and coronary thrombosis, based on their effects on serum cholesterol and low-density lipoprotein-cholesterol levels. Therefore, very low AI and IT values are recommended for a healthy diet.

In our research, there were no statistically significant differences observed in the lipid indices of egg yolks, except the TI index which showed significantly lower values ($p < 0.0001$) and the Δ9-desaturase (16+18) index which exhibited significantly higher values ($p < 0.007$) in all experimental groups compared to C group.

The fatty acids C14: 0 and C16: 0 are known to be among the most atherogenic, while C18: 0 is considered neutral in terms of atherogenicity, but is considered instead, to be thrombogenic [44].

Preserving favorable AI and TI indices, and maintaining an appropriate n-6/n-3 PUFA ratio, promotes consumers' health. Therefore, the health indices, AI and TI, introduced by [17], showing low values were recommended for a healthy diet [45]. The Δ9-desaturase is involved in MUFA biosynthese used to synthesize the PUFA, phospholipids, triacylglycerols and cholesterol esters [46]. On the other hand, high values of peroxidability index (PI), ratio of hypocholesterolemic and hypercholesterolemic (HH), are recommended as well as beneficial healthy promoters for a healthy diet [47].

Some authors [48] reported that moderate consumption of hen eggs, up to two per day, does not appear to increase the risk of heart disease in healthy people. A 2007 human study showed no correlation between moderate consumption (6 per week) eggs and cardiovascular disease, except for the subpopulation of diabetic patients, who had an increased risk of coronary heart disease [49]. It is important to note, however, that the increase in both atherogenic LDL cholesterol and anti-atherogen HDL cholesterol occurs, resulting in virtually no change in the LDL/HDL ratio, a major determinant of the risk of cardiovascular disease.

Table 6 Effects of flaxseed and red grapeseed pomace meal on lipid indices of eggs' yolks

Lipid indices of eggs' yolk	Dietary treatments				SEM	*p*-Value
	C	E1	E2	E3		
Cholesterol index (CI)	0.31	0.28	0.31	0.28	0.010	0.275
Cholesterol-saturated fat index	0.49	0.45	0.48	0.46	0.012	0.202
Indices of athergenicity (AI)	0.39	0.37	0.39	0.38	0.0048	0.129
Indices of thrombogenicity (TI)	0.70 ^{a}	0.54 ^{b}	0.57 ^{b}	0.55 ^{b}	0.0115	0.0001
Hypocholesterolemic Fatty Acids	75.50	75.97	75.59	75.66	0.167	0.364
Hypercholesterolemic Fatty Acid	24.04	23.66	24.08	23.83	0.171	0.419
Hypocholesterolemic/ Hypercholesterolemic Ratio	2.21	2.35	2.21	2.31	0.0445	0.174
Health-promoting index	2.27	2.37	2.28	2.35	0.0299	0.120
Elongase	0.50	0.44	0.48	0.44	0.0177	0.096
Thioesterase	96.37	93.82	107.23	93.22	4.300	0.195
Δ9-desaturase (16+18)	263.74^{b}	336.91^{a}	302.64^{a}	336.68^{a}	12.90	0.007
Δ5 + Δ6 - desaturase (Ω:6)	18.08	14.67	17.27	15.59	0.879	0.104

where: C, control diet; E1, flaxseed meal + 27 mg vitamin E; E2, flaxseed meal supplemented + 100 mg vitamin E; E3, flaxseed meal supplemented with 2% red grapeseed pomace meal;

CONCLUSIONS

Experiments conducted recently on poultry nutrition concluded that flaxseed dietary utilization can significantly improve health consumers' due to positive biological effects of α-linolenic acid content. The study observed that the flaxseed meal dietary inclusion combined to different antioxidant sources, increased the PUFA ingesta up to 7.5 times at all experimental groups compared to C group. Also, a highly significant level of Ω3 fatty acids was recorded on all experimental groups, determining a low Ω6/Ω3 ratio. The findings indicated a significant transfer of omega-3 fatty acids to the egg yolk, resulting in an improved nutritional quality of the eggs. This egg enrichment presents advantages for human health, especially when considering that all experimental groups displayed lower thrombogenic index values.

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REFERENCES

1. El-Sabrout, K; Aggag, S; Mishra, B. Advanced practical strategies to enhance table. *Hindawi Scientifica* **2022**, 17 pages, https://doi.org/10.1155/2022/1393392
2. Attia, YA; Al-Harthi, MA; Al-Sagan, AA; Alqurashi, AD; Korish, MA; Abdulsalam, NM; Olal, MJ; Bovera, F Dietary supplementation with different ω-6 to ω-3 fatty acid ratios affects the sustainability of performance, egg quality, fatty acid profile, immunity and egg health indices of laying hens. *Agriculture* **2022**, *12*(10), 1712. https://doi.org/10.3390/agriculture12101712
3. Mueed, A; Shibli, S; Korma, SA; Madjirebaye, P; Esatbeyoglu, T; Deng, Z. Flaxseed Bioactive Compounds: Chemical composition, functional properties, food applications and health benefits-related gut microbes. *Foods*, **2022**, *11*(20), 3307, https://doi.org/10.3390/foods11203307
4. Gruia, A; Raba, DN; Dumbrava, D; Moldovan, C; Bordean, D; Mateescu, C. Fatty acids composition and oil characteristics of linseed (Linum Usitatissimum L.) from Romania. *Journal of Agroalimentary Processes and Technologies*, **2012**, *18*(2), 136-140.
5. Bekhit, AEA; Shavandi, A; Jodjaja, T; Birch, J; Teh, S; Ahmed, IAM; Al-Juhaimi, FY; Saeedi, P; Bekhit, AA. Flaxseed: Composition, detoxification, utilization, and opportunities. *Biocatal. Agric. Biotech.* **2018**, *13*, 129–152. [Google Scholar] [CrossRef]
6. Xu, L; Wei, Z; Guo, B; Bai, R; Liu, J; Li, Y; ... Pi, Y. Flaxseed meal and its application in animal husbandry: A Review. *Agriculture*, **2022**, *12*(12), 2027. https://doi.org/10.3390/agriculture12122027
7. Ehr, IJ; Persia, ME; Bobeck, EA. Comparative omega-3 fatty acid enrichment of egg yolks from first-cycle laying hens fed flaxseed oil or ground flaxseed. *Poult. Sci.* **2017**, *96,* 1791–1799. https://doi.org/10.3382/ps/pew462
8. Rodríguez, M; G. Rebollar, P; Mattioli, S; Castellini, C. n-3 PUFA sources (precursor/products): A review of current knowledge on rabbit. *Animals*, **2019**, *9*(10), 806. https://doi.org/10.3390/ani9100806
9. Tadesse, D; Retta, N; Girma, M; Ndiwa, N; Dessie, T; Hanotte, O; ... Maak, S. Yolk fatty acid content, lipid health indices, and oxidative stability in eggs of slow-growing sasso chickens fed on flaxseed supplemented with plant polyphenol extracts. *Foods*, **2023**, *12*(9), 1819; https://doi.org/10.3390/foods12091819
10. Panaite, TD; Turcu, RP; Soica, C; Visinescu, P. Nutritional parameters of eggs from laying hens fed with flaxseed meal or mixture with rapeseed meal or rice bran. *Journal of Applied Animal Research*, **2020,** *48*(1), 566-574. https://doi.org/10.1080/09712119.2020.1848846
11. Pečjak, M; Leskovec, J; Levart, A; Salobir, J; Rezar, V. Effects of dietary vitamin E, vitamin C, selenium and their combination on carcass characteristics, oxidative stability and breast meat quality of broiler chickens exposed to cyclic heat stress. *Animals*, **2022**, *12*(14), 1789. https://doi.org/10.3390/ani12141789
12. Baroi, AM; Popitiu, M; Fierascu, I; Sărdărescu, ID; Fierascu, RC Grapevine wastes: A rich source of antioxidants and other

biologically active compounds. *Antioxidants* **2022**, *11*(2), 393; https://doi.org/10.3390/antiox11020393
13. Panaite, T; Criste, RD; Ropota, M; Cornescu, GM; Alexandrescu, DC; Criste, V; Vasile, G; Olteanu, M; Untea, A. Effect of layer diets enriched in omega-3 fatty acids supplemented with Cu on the nutritive value of the eggs. *Rom Biotech Lett*. **2016**, *21*, 11754–11762
14. National Research Council. Nutrient requirements of poultry: **1994**. National Academies Press.
15. Tetra-SL LL commercial layer management guide. **2018**.
16. Haugh, RR. The Haugh unit for measuring egg quality. *United States Egg Poultry Magazine*, **1937**, *43*, 552-555.
17. Ulbricht, TLV; Southgate, DAT. Coronary heart disease: seven dietary factors. *The Lancet*, 199, *338*(8773), 985-992. https://doi.org/10.1016/0140-6736(91)91846-M
18. Senso, L; Suárez, MD; Ruiz-Cara, T; García-Gallego, M. On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (Sparus aurata). *Food Chemistry*, **2007**. *101*(1), 298-307. https://doi.org/10.1016/j.foodchem.2006.01.036
19. Fernandes, CE; da Silva Vasconcelos, MA; de Almeida Ribeiro, M; Sarubbo, LA; Andrade, SAC; de MeloFilho, AB. Nutritional and lipid profiles in marine fish species from Brazil. *Food chemistry*, **2014**, *160*, 67-71. https://doi.org/10.1016/j.foodchem.2014.03.055
20. Erickson, MC. Variation of lipid and tocopherol composition in three strains of channel catfish (Ictalurus punctatus). *Journal of the Science of Food and Agriculture*, **1992**, 59(4), 529-536. https://doi.org/10.1002/jsfa.2740590416
21. Zilversmit, DB. Cholesterol index of foods. *Journal of the American Dietetic Association*, **1979**, *74*, 562–565.
22. Connor, SL; Gustafson, JR; Artaud-Wild, SM; Favell, DP; Classick-Kohn, CJ; Hatcher, LF; The cholesterol/saturated-fat index an indication of the hypercholesterolemic and atherogenic potential of food. *Lancet,* **1986,** *27*,1229–1232. https://doi.org/10.1016/S0140-6736(86)91384-x
23. Rajan, A; Devika, VM; Shabana, A; Krishnan, N; Dey, A; Sharma, AK; Dhama, K; Chandran, D. Positive impacts of integrating flaxseed meal as a potential feed supplement in livestock and poultry production: Present scientific understanding. *Journal of Experimental Biology and Agricultural Sciences*, **2023**, *11*(2), 264 – 279 http://dx.doi.org/10.18006/2023.11(2).264.279
24. Wu, S; Wang, X; Qi, W; Guo, Q. Bioactive Protein/Peptides of Flaxseed: A review. *Trends in Food Science & Technology*, **2019**, *92*, 184-193. https://doi.org/10.1016/j.tifs.2019.08.017
25. Prakash, P; Kumar, M; Pundir, A; Puri, S; Prakash, S; Kumari, N; Thakur, M; Rathour, S; Jamwal, R; Janjua, S; Ali, M. Documentation of commonly used ethnoveterinary medicines from wild plants of the high mountains in Shimla District, Himachal Pradesh, India. *Horticulturae*, **2021**a, *7*(10), 351. https://doi.org/10.3390/horticulturae7100351
26. Prakash, P; Kumar, M; Kumari, N; Prakash, S; Rathour, S; Thakur, M; Jamwal, R; Janjua, S; Ali, M; Pundir, A; Puri, S. Therapeutic uses of wild plants by rural inhabitants of Maraog region in district Shimla, Himachal Pradesh, India. *Horticulturae*, **2021**b, 7(10), 343. https://doi.org/10.3390/horticulturae7100343
27. Shim, YY; Gui, B; Wang, Y; Reaney, MJT. Flaxseed (Linum usitatissimum L.) oil processing and selected products. *TrendsFoodSci. Tech*. **2015**, *43*, 162–177. https://doi.org/10.1016/j.tifs.2015.03.001
28. Kajla, P; Sharma, A; Sood, DR. Flaxseed - a potential functional food source. *Journal of Food Science & Technology*, **2014**, *52*(4), 1857-1871. https://doi.org.10.1007/s13197-014-1293-y
29. Lan, Y; Ohm, JB; Chen, B; Rao, J. Physicochemical properties and aroma profiles of flaxseed proteins extracted from whole flaxseed and flaxseed meal. *Food Hydrocolloids*, **2020**, *104*, 105731. https://doi.org/10.1016/j.foodhyd.2020.105731
30. Chandran, D; Veterinary phytomedicine in India: A review. *International Journal of ScientificResearch in Science, Engineering and Technology*, **2021**, *8*(3), 598-605. https://doi.org/10.32628/IJSRST2183135
31. Rubilar, M; Gutiérrez, C; Verdugo, M; Shene, C; Sineiro, J. Flaxseed as a source of functional ingredients. *J. Soil. Sci Plant Nutr*. **2010**, *10*, 373–377.

http://dx.doi.org/10.4067/S0718-95162010000100010
32. Zhang, ZS; Wang, LJ; Li, D; Li, SJ; Özkan, N. Characteristics of flaxseed oil from two different flax plants. *Int. J. Food Prop*. **2011**, *14*, 1286–1296. https://doi.org/10.1080/10942911003650296
33. Azizi, M; Seidavi, AR; Ragni, M; Laudadio, V; Tufarelli, V Practical applications of agricultural wastes in poultry feeding in Mediterranean and Middle East regions. Part 1: citrus, grape, pomegranate and apple wastes. *World's Poult Sci J*. **2018**;*74*(3), 489–498.
34. Özacar, M.; Şengil, İA. The use of tannins from turkish acorns (valonia) in water treatment as a coagulant and coagulant aid. *Turkish Journal of Engineering and Environmental Sciences*, 2002; *26*(3), 255-264.
35. Shi, J; Yu, J; Pohorly, JE; Kakuda, Y. Polyphenolics in grape seeds-biochemistry and functionality. *J Med Food*. **2003**, *6*(4):291–299. https://doi.org/10.1089/109662003772519831
36. Mirghelenj, SA; Kianfar, R; Janmohammadi, H; Taghizadeh, A. Effect of different levels of grape pomace on egg production performance and egg internal quality during different keeping times and temperatures. *Animal Production Research*, **2017**, *6*(4), 81-91
37. Hayat, Z; Cherian, G; Pasha, TN; Khattak, FM; Jabbar, MA. Effect of feeding flax and two types of antioxidants on egg production, egg quality and lipid composition of eggs. *Journal of Applied Poultry Research*, **2009**, *18*(3), 541 - 551. https://doi.org/10.3382/japr.2009-00008
38. Tufarelli, V; Baghban-Kanani, P; Azimi-Youvalari, S; Hosseintabar-Ghasemabad, B; Slozhenkina, M; Gorlov, I; Viktoronova, FM; Seidavi, A; Laudadio, V. Effect of dietary flaxseed meal supplemented with dried tomato and grape pomace on performance traits and antioxidant status of laying hens. *Animal Biotechnology*, **2022**, *33*(7), 1525-1532. https://doi.org/10.1080/10495398.2021.1914070
39. Kara, K; Kocaoğlu Güçlü, B; Baytok, E; Şentürk, M. Effects of grape pomace supplementation to laying hen diet on performance, egg quality, egg lipid peroxidation and some biochemical parameters. *Journal of Applied Animal Research*, **2016**, *44*(1), 303-310. https://doi.org/10.1080/09712119.2015.1031785
40. Shafey, TM; Al-Batshan, HA; Farhan, AM. The effect of dietary flaxseed meal on liver and egg yolk fatty acid profiles, immune response and antioxidant status of laying hens. *Italian Journal of Animal Science*, **2015**, *14*(3), 3939. https://doi.org/10.4081/ijas.2015.3939
41. Shahid, MS; Raza, T; Wu, Y; Hussain Mangi, M; Nie, W; Yuan, J. Comparative effects of flaxseed sources on the egg ALA deposition and hepatic gene expression in Hy-Line brown hens. *Foods*, **2020**, *9*(11), 1663. https://doi.org/10.3390/foods9111663
42. Cherian, G. Chapter 34 - Supplemental flax and impact on n3 and n6 polyunsaturated fatty acids in eggs. In Egg innovations and strategies for improvements, *Academic Press*, **2017**, pp. 365-372. https://doi.org/10.1016/B978-0-12-800879-9.00034-2
43. Panaite, TD; Nour, V; Vlaicu, PA; Ropota, M; Corbu, AR; Saracila, M. Flaxseed and dried tomato waste used together in laying hens diet. *Archives of animal nutrition*, **2019**, *73*(3), 222-238. https://doi.org/10.1080/1745039X.2019.1586500
44. Salobir, K. The role of meat in balanced nutrition. *Animal products and human health*, **2000**, 16
45. Grela, ER; Ognik, K; Czech, A; Matras, J. Quality assessment of eggs from laying hens fed a mixture with lucerne protein concentrate. *Journal of Animal and Feed Sciences*, **2014**, *23*(3), 236-243.
46. Nagao, K., Murakami, A., & Umeda, M. (2019). Structure and function of Δ9-fatty acid desaturase. *Chemical and Pharmaceutical Bulletin*, *67*(4), 327-332. https://doi.org/10.1248/cpb.c18-01001
47. Santos-Silva, J; Bessa, RJB; Santos-Silva, FJLPS. Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. *Livestock Production Science*, **2002**, *77*(2-3), 187-194. https://doi.org/10.1016/S0301-6226(02)00059-3
48. Schärer, M; Schulthess, G; Eierkonsum und kardiovaskuläres Risiko [Egg intake and cardiovascular risk]. *Ther Umsch*, **2005**, *62*(9):611-3. http://dx.doi.org/10.1024/0040-5930.62.9.611
49. Qureshi, AI; Suri, FK; Ahmed, S; Nasar, A; Divani, AA; Kirmani, JF. Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. *Med Sci Monit*. **2007**, *13*(1):CR1-8.