Scientific Papers. Series D. Animal Science. Vol. LXV, No. 1, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PERFORMANCE AND EGG QUALITY OF LAYING HENS FED WITH DIETARY RAW MATERIALS RICH IN PUFA Ω:3

Tatiana Dumitra PANAITE[1], Gabriela Maria CORNESCU[1], Raluca Paula TURCU[1], Petru Alexandru VLAICU[1], Gabriel Claudiu RADULESCU[2]

[1]Laboratory of Nutrition and Physiology, National Research-Development Institute for Animal Biology and Nutrition (IBNA), 1 Calea Bucuresti, Balotesti, 077015, Ilfov, Romania
[2]AGROPREST 2005 SRL, 16 Iasomiei Street, Dragos Voda, Calarasi, Romania

Corresponding author email: tatiana.panaite@ibna.ro

Abstract

The effect of dietary flaxseed meal, rapeseed meal and fodder peas on layers' performances and egg quality was investigated in a 6-wk feeding trial on 168 Tetra SL layers (65 weeks) assigned to 4 groups (C, E1, E2, E3). The commercial (C) diet had 2750 kcal ME and 16.4% CP. Compare to diet C, the experimental groups were fed with flaxseed meal (3%, E1), flaxseed meal-fodder peas (3%; 10%; E2), flaxseed meal-rapeseed meal (3%; 10%; E3) which increased the dietary level of the total polyunsaturated fatty acids PUFA n-3 (% of total fat) to 5.72(E1), 6.87 (E2) and 5.65 (E3) compared to group C (1.19), in diet. At the end of the trial, 18 eggs/group were collected to determine the eggs nutritional and quality parameters. The results showed that egg intensity was lower (P≤0.05) in all experimental groups. Egg weight (g) in E1 (65.39) was higher (P≤0.05) compared to all other groups. Also, yolk colour intensity increased significantly (P≤0.05) in all experimental groups compared to C group. Similarly, the results showed that PUFA n-3 acid content was higher in all experimental groups (3.14%; 3.38% and 3.53%) compared to C group (1.13%). In conclusion, using dietary raw materials rich in PUFA n-3 had a positive influence on laying hens' egg quality and improved the nutritional quality indices of the lipids in egg yolks.

Key words*: eggs quality, flaxseed meal, fodder peas, rapeseed meal, layers' performance, PUFAn-3 acid, yolk indices.*

INTRODUCTION

Eggs are consumed by millions of people around the world, being considered a complete food for the human diet due to the large amounts of essential nutrients they hold, such as protein, lipoproteins (ovalbumin, ovotransferine, HDL and LDL), a wide variety of minerals (potassium, phosphorus, calcium, iron, magnesium), vitamins (A, D, E, K, B6, B9, B12, riboflavin), lipids (MUFA, PUFA, carotenoids, choline and phospholipids) and other bioactive compounds. Genetic factors and diet can alter the chemical composition of chicken eggs(Layman & Rodriguez, 2009; Ruxton et al., 2010; Conrad et al., 2017; Franco et al., 2020). Omega-3 polyunsaturated fatty acidsenriched eggs provide the consumer with a value-added product that shows a clear and functional benefit for an increasingly health-conscious population. Humans require a ratio of omega-6 fatty acids: omega-3 of 4:1 (Sittiprapaporn, 2020). In the recent years people have become more conscious of the relation between food and their health and started to show more interest in the quality of eggs that they consume (Siro et al., 2008). The main concern of consumers is that the food they consume to be safe and healthy, to have reduced content of substances which can pose risk to human health and to have additional benefit by enriching with substances beneficial for their health (Carocho et al., 2014; Sireesha and Prasanna, 2019, Untea et al. 2021).

Functional foods are designed to encourage the consumer to change their diets, instead of taking pills or capsules that could harm the body (Karelakis et al., 2020). Poultry eggs have huge potential in this aspect (Fernandez & Lemos, 2019). The most common practices to obtain such food products is to include flaxseed, rapeseed, canola or their by-products into poultry feeding with the purpose to increase the concentration of polyunsaturated fatty acids (Gheorghe et al., 2019; Świątkiewicz et al., 2020). From the polyunsaturated fatty acids (PUFA) linoleic and α-linolenic acids are lipids with important physiological roles

considered essential to adults. Clinical studies have shown that replacing saturated fatty acids by polyunsaturated fatty acids produces beneficial effects on the cardiovascular system (Turcu et al., 2019).

In this regard many attempts have been made to focus on different methods of improving the nutritional quality of poultry eggs by enhancing levels of omega-3 fatty acids content, to obtain functional eggs through poultry feeding manipulation (Tocher et al., 2019; Goldberg et al., 2013; Panaite et al. 2021). Moreover, it was reported that current Western diets are generally deficient in PUFA, especially omega-3 fatty acids. For this reason, consuming foods enriched in omega-3 fatty acids, such as eggs, it is essential for human health nutrient deficiency (Simopoulos, 2002; Panaite et al., 2019). Enrichment of eggs in omega-3 PUFA presents increased susceptibility of yolk lipid peroxidation, which could affect the quality of eggs and may have deleterious effects on humans (Alagawany et al., 2019; Panaite et al., 2021). The fatty acids composition of eggs is dependent on the fatty acid composition of the feed given to the hens which plays an important role on in the prevention and regulation of different disorders and can modulate lipid metabolism in a beneficial way (Vlaicu & Panaite, 2021).

The richest sources to obtain polyunsaturated fatty acids enriched eggs, are those with a high content of alpha linolenic acid as rapeseed (respectively rapeseed meal), soya (soyabean meal), walnuts and flax (Harris et al., 2009), or flaxseed meal (Khan et al., 2019). To obtain polyunsaturated fatty acids enriched eggs, these raw materials enriched in omega-3 polyunsaturated fatty acids must be added into animals' diet (Franczyk-Żarów et al., 2019).

The aim of this paper is to evaluate the performance and egg quality of laying hens fed with dietary raw materials rich in polyunsaturated fatty acids.

MATERIALS AND METHODS

Birds, housing and experimental diets

The efficiency diets' evaluation with vegetable raw materials rich in polyunsaturated fatty acids was carried out by an experimental study conducted during 6 weeks on 168 Tetra SL laying hens (65 weeks of age), individually weighed and randomized into 4 experimental groups (C, E1, E2 and E3).

The experiment was conducted in accordance with the Romanian legislation (Law 206/2004, Ordinance 28/31.08.2011, Law 43/11.04.2014, Directive 2010/63/EU according to an experimental protocol approved by the Ethics Committee of IBNA.

The hens were accomodated in cages (2 hens/cage; 21 cages/group) Big Dutchman two-sided with 3-tier cages (length x width x height; 50×50×40 cm), benefited from the same conditions of controlled microclimate (temperature: 23.08 ± 0.98^{0}C; humidity 66.35±5.68%; ventilation: 1.70±0.14% and a lighting program of 16 h/24 h).

The laying hens had free access to feed and water. For dietary feed formulation, the following were considered: the experiment objective, species, hybrid, age and nutritional requirements of the hybrid Tetra SL (Tetra-SL commercial Layer Management Guide, 2017).

The diets basic structure (Table 1) was the same for the 4 experimental groups, characterized by: 2750 kcal/kg metabolizable energy; 17.5% crude protein; the difference between the control group (M) and the experimental groups was given by the flaxseed meal inclusion (3%) as a source of polyunsaturated fatty acids.

The E1 diet included only flaxseed meal (3%) while E2 and E3 groups included, in addition to flaxseed meal, 10% peas (E2) and 10% rapeseed meal (E3), respectively. A nutritional optimization programme was used for diets' formulation (Table 1) (HYBRIMIN® Futter 5) in agreement with the feeding requirements of laying hens as given by NRC (1994).

The diets (table 1) were isocaloric and isonitrogenous.

Throughout the experiment were monitored the daily feed intake (DFI; g/day/layer), the feed conversion ratio (FCR; g feed/g egg), the laying intensity rate (LIR; %) and egg weight (EW; g).

Table 1. The experimental diets'structure

Specifications	C	E1	E2	E3
Flaxseed meal, %	-	*3.00*	*3.00*	*3.00*
Peas, %	-	-	*10.00*	-
Rapeseed meal, %	-	-	-	*10.00*
Corn, %	39.31	38.69	35.20	37.71
Wheat, %	20.00	20.00	20.00	20.00
Soyabean meal, %	16.26	16.36	17.39	14.28
Sunflower meal, %	10.00	7.00	-	-
Vegetal oil, %	2.48	2.86	2.32	3.17
Lysine, %	0.09	0.13	0.09	0.05
Methionine, %	0.14	0.18	0.16	0.12
$CaCO_3$, %	9.01	9.02	9.05	8.89
Monocalcium phosphate., %	1.29	1.33	1.36	1.34
Salt, %	0.38	0.38	0.39	0.39
Choline, %	0.05	0.05	0.05	0.05
Premix A5, %	1.00	1.00	1.00	1.00
Total	100	100	100	100
Calculated analysis,				
Metabolizable energy., kcal/kg	2750	2750	2750	2750
Lysine, %	0.80	0.80	0.80	0.80
Met.+cist, %	0.71	0.71	0.71	0.71
Chemical analysis, %				
Dry matter	90.79	90.88	90.33	90.87
Crude protein	17.25	17.27	17.37	17.21
Ether extract	4.01	4.60	4.05	5.02
Crude fiber	5.29	5.20	4.47	4.88
Ash	13.52	14.27	13.80	14.84
Non-nitrogen extractive substances	50.72	48.54	50.64	47.92
• *Fatty acid (% of the sum fatty acids)*				
Linoleic acid (C 18:2n6),	51.44	48.96	47.50	48.03
Linolenic α acid (C 18:3n3)	0.96	5.55	6.73	5.58
PUFA, of which:	52.75	54.94	54.56	53.80
Ω3	1.19	5.72	6.87	5.65
Ω6	51.56	49.22	47.69	48.15
Ω6/Ω3	45.29	8.63	6.99	8.52

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal;
*1kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg Vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg Vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.

Sampling collection and measurements

After experimental feed was manufactured, sample of 500 g were extracted from each batch for chemical analysis then the bags were packed, labelled and stored in optimal conditions for the conduct of the experiment. At the end of the experiment (week 6), 18 eggs/group were collected randomly from each group and used determine the internal and external quality parameters of the egg: the whole egg weight and its components: egg white, yolk, shell (Kern balance, accuracy 0,001); colour intensity expressed in value on a scale of 1 to 15 measured with an egg Analyser TM analyser and albumen pH and yolk measurements (using mobile pH-meter). After all internal and external quality measurements were determined 6 samples of yolk/group were formed (3 eggs/sample) dried for 48 h (ECOCELL oven), at 65^0C, and chemical composition were assayed (using the methods from Regulation (CE) 152/2009), fatty acids (FA) concentration (determined by gas chromatography described by Panaite et al, 2016), and cholesterol concentration.

Health lipid indices of yolk eggs

The fatty acid profile of yolk fat is important for the nutritional quality of lipids in yolk eggs.Thehealth lipid indices such as UFA/SFA, PUFA/SFA, PUFA n-6/n-3, the hypocholesterolemic/hypercholesterolemic ratio (h/H), index of atherogenicity (IA), the index of thrombogenicity(IT), peroxidizability

index (PI) ratios, hypocholesterolemic acids (DFA), hypercholesterolemic acids (OFA), and nutritive value (NVI) indices, are widely used to evaluate the nutritional value of yolk fat.
They were calculated according to the following equations:

1) IA = (C 12:0 + 4 x C 14:0 + C 16:0)/∑UFA (Ulbricht and Southgate, 1991; Senso et al., 2007).
2) IT = (C 14:0 + C16:0 + C18:0)/[(0.5 x MUFA) + (0.5 x ∑ n-6) + (3 x ∑ n-3) + (∑ n-3/∑n-6)] (Ulbricht and Southgate, 1991;Senso et al., 2007).
3) h/H = [(C 18:1 n-9 + C 18:1 n-7 + C 18:2 n-6 + C 18:3 n-6 + C 18:3 n-3 + C 20:3 n-6 + C 20:4n-6 + C 20:5 n-3 + C 22:4 n-6 + C 22:5 n-3 + C22:6 n-3)/(C 14:0 + C 16:0)] (Fernandes et al., 2014).
4) PI = (monoenoic acid x 0.025) + (dienoic acid x 1) + (trienoic acid x 2) + (tetraenoic acid x 4) + (pentaenoic acid x 6) + (hexaenoic acid x 8) (Erickson, 1992).
5) NVI = (C 18:0 + C18:1)/C 16:0 (Chen et al., 2016).
6) DFA = (UFA+C18:0) (Medeiros et al., 2014)
7) OFA = (SFA-C18:0) (Skiepko et al., 2016)

Statistical analysis:
The measurements of all groups wereanalysed by the one-way analysis of variance (ANOVA) procedure of the SPSS version 20 (Inc., Chicago IL, USA), according to the following linear model:

$$Yij = \mu + Aj + eij$$

Where: Yij = value of trait (the dependent variable); μ = overall mean; Aj = the treatment effect; and eij = random observation error.We using a Tukey test to compare differences among treatment means and probabilities lower than 0.05 were considered as statistically significant ($P < 0.05$).

RESULTS AND DISCUSSIONS

The chemical composition presented in Table 1 shows that all diets were isoproteic and isoenergetic. The raw materials rich in PUFA-3 inclusion into the dietary feeding structure resulted in an increase in ALA content in all experimental groups compared to the control group, mainly in the E2 group which included flaxseed meal mixed with peas (Table 1). Of the experimental groups, the E2 diet (3% flaxseed meal+ 10% peas) administered to the laying hens had the highest PUFA-3 content, corroborated with a much-improved ratio of PUFA-6/3 fatty acids compared to C group.

Tabelul 2. Effect of dietary raw materials rich in PUFA ω:3 on laying hens performance and egg size classification (average values/group)

Specification	Experimental groups				SEM	P-value
	C	E1	E2	E3		
	n=42	n=42	n=42	n=42		
Daily feed intake (g/day/layer)	129.06^{a}	121.69^{b}	125.75^{ab}	124.45^{ab}	0.073	0.0505
Feed conversion ratio (g feed/g egg)	2.17	2.11	2.23	2.23	0.021	0.1521
Egg weight (g).	64.08^{b}	65.39^{a}	63.57^{c}	63.75^{bc}	0.102	<.0001
Laying intensity rate (%)	93.67^{a}	89.56^{b}	90.67^{b}	90.03^{b}	0.419	0.0018
*Eggs classification**						
"XL" (>73 g), %	3.11	7.20	4.73	1.35		
"L" (63 - 73 g), %	53.03	59.61	48.08	56.32		
"M" (53 - 63 g), %	43.27	33.06	44.84	42.07		
"S" (< 53 g), %	1.18	0.38	2.36	0.97		

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal; n=hens per group;
^{a,b,c} Means within a row with different superscripts differ significantly, $P \leq 0.05$.
* C.E Regulation no. 852/2004 on the general rules of food hygiene, with subsequent amendments and completions and Directive 2000/13 / C.E.

Concerning productive performances (Table 2), E1 group supplemented by 3% flaxseed meal recorded a significantly lower feed consumption ($P<0.05$) compared to C group corroborated with a low specific consumption (kg NC/kg egg). Following egg weight (g/egg) registration, the entire E1 group recorded the highest value of the egg weight, the differences

being significant (P<0.05) compared to both experimental groups and C group, but laying intensity percentage decreased by 4.38% compared to C. Significant differences (P <0.05) were also recorded between E1 compared to E2 and E3 groups. In literature, the results obtained for the productive parameters and the eggs chemical composition are extremely contradictory and varied following the use of flax as a source of polyunsaturated fatty acids in the laying hen's nutrition. For example, Hayat (2009) showed that the flaxseed utilization in laying hens reduced the feed ingesta, while Caston et al. (1994) noticed the opposite. With regard to egg weight some studies show a decrease of this parameter (Scheideler and Froning, 1996), while in other studies there are no changes in the same parameter (Aymond & Van Elswyk, 1995). Some researches has also shown an increase in egg weight (Rizzi & Simioli, 2009). With regard to the percentage of laying as in the case above, the results obtained are variable and contradictory. Thus, Aymond & Van Elswyk (1995) demonstrate the decrease of this parameter; Scheideler & Froning (1996) demonstrated egg laying percentage increasing while other researchers (Bean & Leeson, 2003) believed that flaxseed utilization laying hens diet does not alter the laying intensity. Regarding the dietary peas in laying hens diets, existing studies in the literature have shown that a large amount of peas inclusion into diets has a detrimental effect on their performance (Igbasan & Guenter, 1997; Świątkiewicz & Koreleski, 2006).

At the end of the experiment, eggs collected throughout the whole experimental period were classified in accordance with Regulation No. 852/2004 of the General Rules on Food Hygiene, as subsequently amended and supplemented and Directive 2000/13/EC (table 2). Thus, most of the eggs were of classes "L" and "M". The highest percentage of 'L' eggs was recorded on groups E1 and E3, while for 'M' eggs the highest percentage was recorded in lot E2 followed by C, E3 and E1 groups. With regard to eggs of category "S" the highest percentage was recorded in lot E2 and the lowest value recorded in lot E1 with a percentage of 0.38%. The highest value for the category "XL" was recorded in lot E1, and the lowest value was seen in lot E3. This eggs classification is extremely important for the farmer, as there is a great emphasis on the egg price, which varies depending on its size. Economically, the best-selling eggs are those in categories "L" and "M". The EU has also created marketing standards for eggs. They have been designed to ensure consistent high product quality, protect consumers and ensure consistency of standards in the EU market. The EU Regulations (EC No 589/2008 of 23/06/2008) stipulates detailed rules that eggs must comply with in order to be marketed. Printing of such standards shall also specify the quality classes, that is, eggs of category AA or very fresh, eggs of category A or fresh eggs, eggs of category B or eggs of second quality.

Table3. Effect of dietary raw materials rich in PUFA ω:3 on egg quality parameters

Egg quality parameters	Experimental groups				SEM	P-value
	C	E1	E2	E3		
	n=18	n=18	n=18	n=18		
Egg weight, g	65.03	64.87	65.07	64.88	0.079	0.7469
Egg albumen weight, g	37.42^{c}	38.72^{b}	39.51^{ab}	37.89^{c}	0.206	0.0010
Egg yolk weight, g	18.22^{a}	17.01^{b}	16.87^{b}	17.62^{b}	0.167	0.0140
Eggshell weight, g	9.29	9.24	8.86	9.21	0.099	0.4042
pH albumen(value)	9.13	9.08	9.06	9.05	1.125	0.4039
pH yolk (value)	6.27^{ab}	6.25^{b}	6.33^{a}	6.23^{b}	0.013	0.0377
Yolk colour intensity	3.94^{b}	4.50^{a}	4.44^{a}	4.47^{a}	0.075	0.0205

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal;
^{a,b,c} Means within a row with different superscripts differ significantly, $P \leq 0.05$.

The data presented within Table 3 showed that eggs collected at the end of the experiment and analysed for internal and external quality parameters did not show significant differences (P>0.05) of their average weight. However, in the experimental groupsthe albumen weight

showed the highest values. For E1 and E2 groups, the differences were significant (P<0.05) compared to C group. Group E3 differed significantly (P<0.05) only from E2 group. For yolk weight values significant differences (P<0.05) were recorded only in the case of E1 and E2 groups compared to C group. The data obtained by us are inconsistent with those obtained by Cherian et al., (2016) when using camelina as a source of polyunsaturated fatty acids. The results of the study showed that the egg albumen size and mass were smaller when camelina was used, with no differences observed for the Haugh unit, the yolk: albumen ratio and the yolk weight (Cherian et al., 2016). For the shell weight no significant differences (P>0.05) were recorded in any of the groups. In case on E2 group pH yolk recorded significant values (p<0.05) compared to E1 and E3 groups. Yolk colour registered a significant increase (p<0.05) in all experimental groups of this compared with the values recorded for C group. Similar results were obtained by Świątkiewicz & Koreleski (2006) in an experiment on Lohman Brown laying hens, who received a peas by-product (5, 10, 15 and 20%) achieving an egg yolk increase intensity. Other studies (Roberson et al., 2005) reported improved yolk color intensity at a rate of 5% and 10% dietary inclusion of the peas by-product, while a dietary level inclusion of 15% had no influence on its color intensity.

Table 4. Fatty acid composition in total lipids of eggyolks (avarage values/group)

Fatty acid (g FAME/100 g Total FAME)	Experimental groups				SEM	P-value
	C	E1	E2	E3		
	n=6	n=6	n=6	n=6		
C14:0	0.253 ^{ab}	0.240 ^{ab}	0.259 ^{a}	0.222 ^{b}	0.009	0.027
C15:0	0.064 ^{b}	0.056 ^{c}	0.067 ^{ab}	0.074 ^{a}	0.002	<0.0001
C16:0	22.995 ^{a}	22.700 ^{a}	22.539 ^{a}	21.578 ^{b}	0.160	<0.0001
C17:0	0.153	0.149	0.169	0.175	0.0124	0.421
C18:0	11.066	11.016	10.849	11.318	0.321	0.778
∑ SFA	34.532	34.161	33.882	33.366	0.387	0.217
C14:1	0.044 ^{a}	0.043 ^{a}	0.044 ^{a}	0.023 ^{b}	0.003	<0.0001
C15:1	0.154 ^{a}	0.144 ^{ab}	0.107 ^{ab}	0.115 ^{b}	0.011	0.024
C16:1	2.665 ^{ab}	2.655 ^{ab}	2.891 ^{a}	2.374 ^{b}	0.086	0.004
C17:1	0.068	0.081	0.068	0.098	0.012	0.297
C18:1n9c	35.885	35.665	36.038	35.054	0.431	0.411
C22:1n9	0.087	0.068	0.084	0.071	0.009	0.377
C24:1n9	0.338 ^{a}	0.242 ^{b}	0.255 ^{b}	0.277 ^{b}	0.011	<0.0001
∑ MUFA	39.242	38.898	39.487	38.013	0.500	0.206
C18:2n6	18.686 ^{b}	18.938 ^{b}	18.044 ^{c}	20.160 ^{a}	0.118	<0.0001
C18:3n6	0.106	0.087	0.113	0.092	0.008	0.092
C18:3n3	0.187 ^{c}	0.690 ^{b}	0.793^{a}	0.781^{ab}	0.025	<0.0001
C20:2n6	0.176 ^{a}	0.141 ^{b}	0.167 ^{b}	0.177 ^{b}	0.006	0.002
C20:3n6	0.279	0.249	0.309	0.290	0.019	0.194
C20:3n3	0.217	0.296	0.269	0.261	0.029	0.309
C20:4n6	4.249	3.818	3.966	3.863	0.187	0.384
C22:4n6	1.572 ^{a}	0.549 ^{b}	0.483 ^{b}	0.483 ^{b}	0.029	<0.0001
C22:5n3	0.065 ^{b}	0.171 ^{a}	0.135 ^{a}	0.164 ^{a}	0.009	<0.0001
C22:6n3	0.658 ^{b}	1.986 ^{a}	2.188 ^{a}	2.322 ^{a}	0.096	<0.0001
∑ PUFA	26.193 ^{b}	26.925 ^{b}	26.468 ^{b}	28.593 ^{a}	0.187	<0.0001
∑ PUFA n-3	1.126 ^{b}	3.143 ^{a}	3.385 ^{a}	3.528 ^{a}	0.098	<0.0001
∑ PUFA n-6	25.067	23.782	23.083	25.065	0.119	<0.0001
∑ UFA	65.435 ^{a}	65.823 ^{b}	65.955 ^{c}	66.606 ^{a}	0.387	0.224
Other fatty acids	0.030^{a}	0.029^{a}	0.160^{b}	0.030^{a}	0.016	<0.0001

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal; n=egg yolk samples

^{a,b,c} Means within a row with different superscripts differ significantly, P ≤ 0.05.

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid;

The egg yolk FA composition for the four experimental treatments is summarized in Table 4. The results showed a significant increase (P<0.05) in their content in all experimental groups compared to C group. Significant concentrations (P<0.0001) of α-

linolenic acid (ALA) and docosahexaenoic acid (DHA) were recorded in E2 and E3 groups compared to C group. Long chain omega-3 polyunsaturated fatty acids (PUFA n-3), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential components for both human and animal nutrition, with well recognized beneficial effects for human health (Roy et al., 2020).

The fact that the new feed solutions tested within this study led to a total content PUFA n-3 enrichment, and also an ALA and DHA increasing concentrations in egg yolk, is of particular importance for improving the nutritional egg quality, and implicitly for human nutrition. For fatty acids according to the degree of non-saturation (Table 4), the concentration of PUFA n-3 increased significantly (P<0.0001) in all three experimental groups compared to C group, leading to a significant improvement (P<0.0001) in the ratio of PUFA n-6/n-3 fatty acids (Table 5).

Table 5. Nutritional quality indices of the lipids inegg yolks.

Item	Experimental groups				SEM	*P*-value
	C	E1	E2	E3		
	n=6	n=6	n=6	n=6		
Total yolk fat, (%)	26.05	26.05	26.76	26.35	0.163	0.378
Cholesterol, (g/100g whole egg)	0.234	0.199	0.214	0.229	0.014	0.300
UFA/SFA	1.896	1.930	1.947	1.999	0.034	0.214
PUFA/SFA	0.759^{b}	0.789^{b}	0.78^{b}	0.858^{a}	0.008	<0.0001
PUFA n-6/n-3	22.39^{a}	7.620^{b}	6.826^{b}	7.153^{b}	0.432	<0.0001
DFA	76.500^{b}	76.839^{b}	76.803^{b}	77.924^{a}	0.159	<0.0001
OFA	23.466^{a}	23.145^{a}	23.034^{a}	22.048^{b}	0.163	<0.0001
DFA/OFA	3.268^{a}	3.321^{a}	3.335^{a}	3.536^{b}	0.031	<0.0001
NVI	2.042^{b}	2.057^{b}	2.080^{ab}	2.150^{a}	0.020	0.005
IA	0.367^{a}	0.360^{a}	0.357^{a}	0.337^{b}	0.004	<0.0001
IT	0.964^{a}	0.830^{b}	0.809^{b}	0.785^{b}	0.013	<0.0001
h	61.624^{b}	62.20^{ab}	62.03^{ab}	63.180^{a}	0.336	0.025
H	23.249^{a}	22.940^{a}	22.798^{a}	21.800^{b}	0.162	<0.0001
h/H	2.654^{b}	2.711^{b}	2.723^{b}	2.902^{a}	0.032	<0.0001
PI (%)	50.355^{b}	57.078^{a}	58.281^{a}	61.076^{a}	1.320	<0.0001

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal; n = egg yolk samples

a,b,c Means within a row with different superscripts differ significantly, P ≤ 0.05.

Abbreviations: NVI, nutritive value index; AI, atherogenic index; TI, thrombogenic index; h/H, hypocholesterolemic/hypercholesterolemic index; PI, peroxidizability index; DFA,hypocholesterolemic acids; OFA, hypercholesterolemic acids;

Similar results were obtained by Franco et al. (2020), who tested three diets: conventional feed (CF), corn/pea/triticale mixture (CPT) and corn/wheat mixture (CW) on two hybrids of laying hens: Mos (native breed) and ISA Brown (commercial hybrid). The fatty acid profile was influenced by the type of diet, mainly the content of oleic and linoleic acids. Aguillón-Páez et al., 2020 conducted a laying hen study to evaluate sunflower or flaxseed seeds effects on performance, egg quality and fatty acids profile in yolk. The dietary inclusion of flaxseed (13.5%) resulted in a significant increase (P<0.05) of PUFA n-3 content and reduced the n-6:n-3 ratio without affecting performance parameters.Another study evaluated (Moghadam et al., 2020), the effects of dietary flax supplementation and flax in addition with enzymes laying hens feed. The new feeding solutions tested included: 15 g raw whole flaxseed/100 g feed, or 15 g heated whole flaxseed/100 g feed; flaxseed in addition with 0,1% enzyme. No dietary effects (P>0.05) on the total fatty acid content, respectively palmitic, stearic, α-linolenic, eicosapentaenoic acid, docosahexaenoic or arachidonic acid was observed. However, oleic acid and total monounsaturated fatty acids concentrations (mg/egg) were higher (P< 0.05. Concerning the eggs coming from hens which received only dietary flaxseed, compared to other experimental groups, the researchers concluded that heating flaxseed before consumption increases egg production while reducing the content of oleic acid and linoleic acid in the egg, but had

no effect on egg weight or the level of α-linolenic acid in the eggs.

The nutritional value of dietary fat of poultry eggs can be enhanced by nutritional manipulation, changing the fatty acids ratio, especially those of PUFA n-3 (Franczyk-Żarów et al., 2019).

From the results presented in Table 5, although there was a decrease in cholesterol concentration in the experimental groups compared to C group, this was not statistically assured (P = 0.300). The most effective feeding diet concerning lowering the cholesterol levels in relation to the whole egg was found to be in E1 group in which flaxseed meal was included, the obtained results being in agreement with Basmacıoğlu et al. (2003).

Of the experimental groups, but also compared to C group, E3 presented the best PUFA/SFA ratio (0.858; P<0.0001), the results being in agreement with those obtained by Tomaszewska et al., (2021). At the same time, it showed a significantly higher content (77.924; P<0.0001) of cholesterol-lowering FA (DFA) and the lowest (22.048; P<0.0001) amount of hypercholesterolemic FA (OFA), leading to a significant increase (3.536; P < 0.0001) of DFA/OFA ratio. Similar results were obtained by Skiko et al., (2016); Walczak et al., (2017).

Examining the values reported in Table 5, it can be shown that both atherogenic indices (AI), considered pro-atherogenic with a role in coronary heart disease, and thrombogenic indices (TI) ones considered pro-thrombogenic with a tendency to form clots in blood vessels, recorded decreases compared to C group registered values. However, a significant decrease was recorded only for E3 in AI case (0.337; P<0.0001), while for TI all 3 experimental groups differed (P< 0.0001) significantly from C group. Similar results were obtained by other researchers (Omri et al., 2019; Dedouusi et al., 2022).

With regard to the h/H ratio, the data presented in Table 5 shows an increasing trend of the recorded values for E1 and E2 groups, while for E3 group the differences are significant compared to C (2.902 vs. 2.654; P<0.0001). The results are consistent with those obtained by Dedouusi et al, (2022), but contradictory to those obtained by Omri et al., (2019), (Panaite et al., 2020)

The peroxidability indices (PI) was significantly (P<0.0001) higher in all experimental groups (E1, E2, E3) compare with C group.Our results were similar with those recorded by Vlaicu et al. (2021), but contrary with those registrated by Zita et al. (2022)

CONCLUSIONS

In conclusion, the utilization of raw materials rich in polyunsaturated fatty acids into laying hens diet increased the polyunsaturated fatty acids concentration within the egg and improved the nutritional quality indices of the lipids in egg yolks by decreasing the content of undesirable hypercholesterolemic acids and increasing the proportion of essential cholesterol acids, as well as DFA/OFA and UFA/SFA ratios, increasing the peroxides index and decreasing the hypercholesterolemic index.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Research, Innovation and Digitalisation, Project PN 19 09 0102, and the National Research Development Project to Finance Excellence (PFE)-8/2021.

REFERENCES

Aguillón-Páez, Y.J., Romero, L.A., & Diaz, G.J. (2020). Effect of full-fat sunflower or flaxseed seeds dietary inclusion on performance, egg yolk fatty acid profile and egg quality in laying hens. *Animal Nutrition*, *6*(2), 179-184.

Alagawany, M., Elnesr, S. S., Farag, M. R., El-Hack, A., Mohamed, E., Khafaga, A. F., ...& Dhama, K. (2019). Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals, 9(8), 573.

Aymond, W. M., & Van Elswyk, M. E. (1995). Yolk thiobarbituric acid reactive substances and n-3 fatty acids in response to whole and ground flaxseed. *Poultry Science*, 74(8), 1388-1394.

Bean, L. D., &Leeson, S. (2003). Long-term effects of feeding flaxseed on performance and egg fatty acid composition of brown and white hens. *Poultry Science*, *82*(3), 388-394.

Carocho, M., Barreiro, M. F., Morales, P., & Ferreira, I. C. (2014). Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Comprehensive reviews in food science and food safety*, 13(4), 377-399.

Chen, Y., Y. Qiao, Y. Xiao, H. Chen, L. Zhao, M. Huang, and G. Zhou. (2016). Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial broilers and spent hens. *Asian-australas*. *J. Anim. Sci*., 29, 855–864.

Cherian, G., & Quezada, N. (2016). Egg quality, fatty acid composition and immunoglobulin Y content in eggs from laying hens fed full fat camelina or flax seed. *Journal of Animal Science and Biotechnology*, 7(1), 1-8.

Conrad, Z., Johnson, L. K., Roemmich, J. N., Juan, W., & Jahns, L. (2017). Time trends and patterns of reported egg consumption in the US by sociodemographic characteristics. *Nutrients*, 9(4), 333.

Dedousi, A., Kritsa, M. Z., ĐukićStojčić, M., Sfetsas, T., Sentas, A., & Sossidou, E. (2022). Production Performance, Egg Quality Characteristics, Fatty Acid Profile and Health Lipid Indices of Produced Eggs, Blood Biochemical Parameters and Welfare Indicators of Laying Hens Fed Dried Olive Pulp. *Sustainability*, *14*(6), 3157.

Erickson, M. C. (1992). Variation of lipid and tocopherol composition in three strains of channel catfish (*Ictalurus punctatus*). *Journal of the Science of Food and Agriculture*, *59*(4), 529-536.

Fernandes, C. E., da Silva Vasconcelos, M. A., de Almeida Ribeiro, M., Sarubbo, L. A., Andrade, S. A. C., & de MeloFilho, A. B. (2014). Nutritional and lipid profiles in marine fish species from Brazil. *Food chemistry*, *160*, 67-71.

Fernandez, M. L., &Lemos, B. (2019). Eggs are natural functional food. *Eggs as Functional Foods and Nutraceuticals for Human Health*, *14*, 22.

Franco, D., Rois, D., Arias, A., Justo, J. R., Marti-Quijal, F. J., Khubber, S., ... & Manuel Lorenzo, J. (2020). Effect of breed and diet type on the freshness and quality of the eggs: A comparison between MOS (Indigenous Galician breed) and Isa brown hens. *Foods*, *9*(3), 342.

Franczyk-Żarów M., Szymczyk B., Kostogrys R.B. (2019). Effects of dietary conjugated linoleic acid and selected vegetable oils or vitamin E on fatty acid composition of hen egg yolks. *Ann. Anim. Sci.*, 19, 173–178.

Gheorghe, A., Vlaicu, P. A., Olteanu, M., Vișinescu, P., & Criste, R. D. (2019). Obtaining eggs enriched in polyunsaturated fatty acids (PUFA). 1. Use of vegetable sources rich in PUFA as functional ingredients in laying hens diets: A review. *Archiva Zootechnica,* 22(2), 54-85.

Goldberg, E. M., Ryland, D., Gibson, R. A., Aliani, M., & House, J. D. (2013). Designer laying hen diets to improve egg fatty acid profile and maintain sensory quality. *Food Science & Nutrition*, 1(4), 324-335.

Harris, W. S., Mozaffarian, D., Lefevre, M., Toner, C. D., Colombo, J., Cunnane, S. C., ...& Whelan, J. (2009). Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. *The Journal of nutrition*, 139(4), 804S-819S.

Hayat, Z., Cherian, G., Pasha, T. N., Khattak, F. M., & Jabbar, M. A. (2009). Effect of feeding flax and two types of antioxidants on egg production, egg quality, and lipid composition of eggs. *Journal of Applied Poultry Research*, *18*(3), 541-551.

Iannotti, L. L., Lutter, C. K., Bunn, D. A., & Stewart, C. P. (2014). Eggs: the uncracked potential for improving maternal and young child nutrition among the world's poor. *Nutrition reviews*, 72(6), 355-368.

Igbasan, F. A., and W. Guenter. 1997a. The influence of feeding yellow–, green– and brown–seeded peas on production performance of laying hens. *J. Sci. Food Agric.*, 73, 120–128.

Jiang, Z., Ahn, D. U., & Sim, J. S. (1991). Effects of feeding flax and two types of sunflower seeds on fatty acid compositions of yolk lipid classes. *Poultry Science*, 70(12), 2467-2475.

Karelakis, C., Zevgitis, P., Galanopoulos, K., & Mattas, K. (2020). Consumer trends and attitudes to functional foods. *Journal of International Food & Agribusiness Marketing*, 32(3), 266-294.

Khan, I. A., Parker, N. B., Löhr, C. V., & Cherian, G. (2021). Docosahexaenoic acid (22: 6 n-3)-rich microalgae along with methionine supplementation in broiler chickens: Effects on production performance, breast muscle quality attributes, lipid profile, and incidence of white striping and myopathy. *Poultry Science*, *100*(2), 865-874.

Layman, D. K., & Rodriguez, N. R. (2009). Egg protein as a source of power, strength, and energy. *Nutrition Today*, 44(1), 43-48.

Medeiros, E., Queiroga, R., Oliveira, M., Medeiros, A., Sabedot, M., Bomfim, M., & Madruga, M. (2014). Fatty acid profile of cheese from dairy goats fed a diet enriched with426 castor, sesame and faveleira vegetable oils. *Molecules*, 19(1), 992-1003.https://doi.org/10.3390/molecules19010992.

Moghadam-Behesti, M.H., Shehab, A., & Cherian, G. (2020). Production performance, quality and lipid composition of eggs from laying hens fed heated flaxseed with carbohydrase enzymes. *J Appl Poult Res*., 1–9. In press. doi: 10.3382/japr/pfz034

Omri, B., Chalghoumi, R., Izzo, L., Ritieni, A., Lucarini, M., Durazzo, A., ...& Santini, A. (2019). Effect of dietary incorporation of linseed alone or together with tomato-red pepper mix on laying hens' egg yolk fatty acids profile and health lipid indexes. *Nutrients*, *11*(4), 813.

Panaite, T. D., Nour, V., Saracila, M., Turcu, R. P., Untea, A. E., & Vlaicu, P. A. (2021). Effects of linseed meal and carotenoids from different sources on egg characteristics, yolk fatty acid and carotenoid profile and lipid peroxidation. *Foods*, 10(6), 1246.

Panaite, T. D., Olteanu, M., Ropota, M., Soica, C., Vlaicu, P. A., Visinescu, P., & Criste, R. D. (2019). Enriching the diet in polyunsaturated fatty acids for laying hens using flaxseed meal and rice bran. *Scientific Papers, Series D. Animal Science*, 62(2), 88-96.

Panaite, T. D., Turcu, R. P., Soica, C., & Visinescu, P. (2020). Nutritional parameters of eggs from laying hens fed with flaxseed meal or mixture with rapeseed meal or rice bran. *Journal of Applied Animal Research*, *48*(1), 566-574.

Panaite, T., Criste, R. D., Ropota, M., Cornescu, G. M., Alexandrescu, D. C., Criste, V., ...& Untea, A. (2016). Effect of layer diets enriched in Omega-3 fatty acids supplemented with Cu on the nutritive value of the eggs. *Romanian Biotechnological Letters*, 21(4), 11754-11762.

Rizzi, L., Bochicchio, D., Bargellini, A., Parazza, P., & Simioli, M. (2009). Effects of dietary microalgae, other lipid sources, inorganic selenium and iodine on yolk n-3 fatty acid composition, selenium content and quality of eggs in laying hens. *Journal of the Science of Food and Agriculture*, *89*(10), 1775-1781.

Roberson K.D., Kalbfleisch J.L., Pan W., Charbenau R.A. (2005). Effect of corn distiller's dried grains with solubles at various levels on performance of laying hens and egg yolk color. *Int. J. Poultry Sci*., 4, 44-51.

Roy, J., Larroquet, L., Surget, A., Lanuque, A., Sandres, F., Terrier, F., ... & Skiba-Cassy, S. (2020). Impact on cerebral function in rainbow trout fed with plant based omega-3 long chain polyunsaturated fatty acids enriched with DHA and EPA. *Fish & Shellfish Immunology*, *103*, 409-420.

Ruxton, C. H. S., Derbyshire, E., & Gibson, S. (2010). The nutritional properties and health benefits of eggs. *Nutrition & Food Science*, 40, 263–279.

Senso, L., Suárez, M. D., Ruiz-Cara, T., & García-Gallego, M. (2007). On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (Sparusaurata). *Food Chemistry*, *101*(1), 298-307.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & pharmacotherapy*, *56*(8), 365-379.

Sireesha, P., & Prasanna, S. (2019). Designer eggs and poultry meat as functional foods–an overview. *Pharm Innov J*, 8, 829-31.

Siro, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. *Appetite*, 51(3), 456-467.

Sittiprapaporn, P. (2020). The effect of Plukenetia Volubilis residue on Omega-3 enriched eggs. *Asian Journal of Medical Sciences*, 11(1), 26-32.

Skiepko, N., Chwastowska-Siwiecka, I., Kondratowicz, J., & Mikulski, D. (2016). Fatty acid profile, total cholesterol, vitamin content, and TBARS value of turkey breast muscle cured with the addition of lycopene. *Poultry Science*, *95*(5), 1182-1190.

Świątkiewicz, S., & Koreleski, J. (2006). Effect of maize distillers dried grains with solubles and dietary enzyme supplementation on the performance of laying hens. *Journal of Animal and Feed Sciences*, 15: 253–260.

Świątkiewicz, S., Arczewska-Włosek, A., Szczurek, W., Calik, J., Bederska-Łojewska, D., Orczewska-Dudek, S., ...& Józefiak, D. (2020). Algal oil as source of polyunsaturated fatty acids in laying hens nutrition: Effect on egg performance, egg quality indices and fatty acid composition of egg yolk lipids. *Annals of Animal Science*, *20*(3), 961-973.

Tocher, D. R., Betancor, M. B., Sprague, M., Olsen, R. E., & Napier, J. A. (2019). Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: bridging the gap between supply and demand. *Nutrients*, 11(1), 89.

Tomaszewska, E., Muszyński, S., Arczewska-Włosek, A., Domaradzki, P., Pyz-Łukasik, R., Donaldson, J., & Świątkiewicz, S. (2021). Cholesterol content, fatty acid profile and health lipid indices in the egg yolk of eggs from hens at the end of the laying cycle, following alpha-ketoglutarate supplementation. *Foods*, *10*(3), 596.

Turcu, R. P., Saracila, M., Vlaicu, P. A., Visinescu, P., & Criste, R. D. (2019). Obtaining eggs enriched in polyunsaturated fatty acids (PUFA) 2. Clinical studies, consumer perception and market of the PUFA-enriched eggs: A review. *Archiva Zootechnica*, 22(2), 86-119.

Ulbricht, T.L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *The lancet*, *338*(8773), 985-992.

Untea, A. E., Panaite, T. D., Varzaru, I., Turcu, R. P., Gavris, T., & Lupu, A. (2021). Study on the influence of dietary sea buckthorn meal on nutritional properties of laying hen eggs. *Czech Journal of Animal Science*, *66*(6), 225-234.

Vlaicu, P. A., & Panaite, T. D. Effect of dietary pumpkin (*Cucurbita moschata*) seed meal on layer performance and egg quality characteristics. *Animal Bioscience*. Preprint. Revised: 6 May 2021 Accepted: 19 June 2021, https://doi.org/10.5713/ab.21.0044

Vlaicu, P. A., Panaite, T. D., & Turcu, R. P. (2021). Enriching laying hens eggs by feeding diets with different fatty acid composition and antioxidants. *Scientific Reports*, *11*(1), 1-12.

Walczak, J., Bocian, S., Kowalkowski, T., Trziszka, T., & Buszewski, B. (2017). Determination of omega fatty acid profiles in egg yolk by HILIC-LC-MS and GC-MS. *Food Anal. Methods*, 10, 1264–1272.

Zita, L., Okrouhlá, M., Krunt, O., Kraus, A., Stádník, L., Čítek, J., & Stupka, R. (2022). Changes in Fatty Acids Profile, Health Indices, and Physical Characteristics of Organic Eggs from Laying Hens at the Beginning of the First and Second Laying Cycles. *Animals*, *12*(1), 125.

*Romanian laws (Law 206/2004, Ordinance 28/31.08.2011, Law 43/11.04.2014 and Directive 2010/63/EU

** Regulation (CE) no. 152 /2009 (Sampling and analytical methods for the official inspection of feeds)