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Impact of feeding mixture containing lupin meal on improvement of polyunsaturated fatty acids in egg yolk

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Abstract: The aim of the experiment was to determine how the content of lupin meal in the diet for commercial laying hens would affect the quality of fat in the egg yolk. A total of 210 Isa Brown laying hens was divided into three groups: the control group C (fed a mixture containing only soybean meal as a source of protein) and two experimental groups: EN 50% (fed a mixture containing 50% of soybean meal and 50% of white lupin seed meal, Zulika variety) and EN 100% (fed a mixture containing only white lupin seed meal as a source of protein). The results of the experiment using lupin seed meal in the feed mixture as a 50% and 100% replacement of extracted soybean meal confirmed the positive effect of lupin-based diets on egg yolk fat composition. Although the diets did not affect the fat content of the egg yolk, some other changes in the quality of the egg yolk were demonstrated during laying. These changes in egg yolk fat were characterized by a decrease ($P \leq 0.05$) of saturated fatty acids (SFA), an increase ($P \leq 0.05$) of monounsaturated fatty acids (MUFA), but only in some of them (C17:1 – heptadecenoic acid; C20:1n9 – eicosenoic acid and C22:1n9 – erucic acid) and, what is important, by a significant ($P \leq 0.05$) increase of polyunsaturated fatty acids (PUFA) from the n-6 group (C18:2n6 – linoleic acid and C20:2n-6 – eicosadienoic acid) and n-3 group (C18:3n3 – α-linolenic acid; C20:5n3 – eicosapentaenoid acid and C22:5n3 – docosapentaenoic acid). From these results it is evident that using lupin meal in the feed mixtures for commercial laying hens increases the nutritional value and health benefit of the egg through the improvement of the levels of saturated and unsaturated fatty acids.

Keywords: fat content; health benefit; layers; *Lupinus albus*; omega-3; omega-6

Feed represents one of the principal costs in animal breeding, and understanding the effect of nutrition strategy on the quality and marketability of livestock farming and products are essential to farming profitability. Nutrition can affect the health of the animals and the qualitative and quantitative aspects, even from 70% (Zhaleh et al. 2019). The current status puts emphasis on high-quality and healthy outputs together with maintaining the favourable state of animal health. Nowadays there are trends of production of not even safe foodstuffs but also high-quality foodstuffs contributing to the health of human population (Lund 2013). One of the factors influencing the feedstuff

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quality is content of saturated fatty acids (SFA) and unsaturated fatty acids (UFA), their ratio and spectrum. Monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids belong in the UFA class. PUFA contain essential fatty acids which are required in human as well as animal nutrition, especially n-3 long-chain HUFA [highly unsaturated fatty acids where exactly eicosapentaenoic acid EPA (n-3/omega-3) and docosahexaenoic acid DHA (n-6/omega-6) belong] have an indisputable effect on human health. Both (n-3 and n-6) fatty acids are important components of cell membranes and they are necessary for the correct function of cells. They are essential for health and normal physiological functioning of the human body, especially for prevention of human coronary diseases and weight reduction (Adamkova et al. 2011), arrhythmia prevention (Leaf et al. 2003) and plasma triacylglycerol reduction (Harris et al. 2008), with regard to these facts they are irreplaceable. Recommendations about the mutual ratio of n-3 and n-6 in diet were presented by von Schacky (2003). For this reason there exist efforts aimed at the production of foodstuffs of animal origin (meat, eggs) with higher levels of PUFA.

Foods that provide n-6 fatty acids include soybean, palm, sunflower and rapeseed oils, whereas foods that provide n-3 fatty acids include certain nuts, and plant and fish oils (Gomez et al. 2011). Fats make the energetic part of feed and they can be added to the mixture in the form of plant oil, animal fat or feed ingredients. For the poultry nutrition, the most commonly used fats are oils of plant origin as a source of PUFA (Kalakuntla et al. 2017). To fulfil the task of balanced and high-yield diet for animals it is necessary to know the demands on nutrition and use components of fine nutritive quality. As mentioned by Sun et al. (2019) and Milinsk et al. (2003), suitable plant oil can improve the composition of fatty acids in final animal products (meat, eggs). Up to the present, promising results were achieved by Konieczka et al. (2017) with rapeseed oil in combination with flaxseed oil and by Baeza et al. (2013) with soybean oil and their effect on chicken meat quality. Ahmad et al. (2012) decreased the cholesterol content of eggs when feeding hens the diet supplemented with n-3 fatty acids.

Moreover, increasing dietary levels of fish oil and milled flaxseed improved the concentration of linoleic acid (LA), EPA, and DHA in the yolk, and the fatty acid deposition from fish oil was found to be twice higher than that from milled flaxseed when fed at the same dietary levels (Sihvo et al. 2014).

White lupin (*Lupinus albus*) belongs to plants with the ability to influence the parameters of health and production. One of the main topics of the Czech Republic and its agricultural policy is searching for internal sources of protein feeds and reduce the import of protein feeds (mainly soybean). There were three reasons why to focus on lupin: it is a feed rich in proteins and what is the benefit, rich in omega-3 PUFA, climatic conditions for its cultivation are good in the Czech Republic and the tested Zulika variety originates from the Czech Republic. Zapletal et al. (2015) stated that lupin seed oil is an important source of PUFA, mainly the n-3 and n-6 group. Lupin seeds find a wide range of utilization in feed mixtures for poultry nutrition (Jeroch et al. 2016), primarily in the fattening of broiler chickens (Suchy et al. 2010; Olkowski 2018), or rearing of laying hens (Rutkowski et al. 2017). Laudadio and Tufarelli (2011) investigated the impact of substitution of lupin seed protein for soybean protein in feed mixtures for early phase laying hens on egg production.

The aim of this study was to find out if the addition of lupin seed meal to feed mixture for layers would improve:

1. the fat content of egg yolk,
2. composition and ratio of SFA and
3. composition and ratio of PUFA in egg yolk.

MATERIAL AND METHODS

A total of 210 Isa Brown laying hens was divided into three groups: the control group C (fed a mixture containing only soybean meal as a source of protein) and two experimental groups: EN 50% (fed a mixture containing 50% of soybean meal and 50% of white lupin seed meal, Zulika variety) and EN 100% (fed a mixture containing only white lupin seed meal as a source of protein). Each group included 70 hens. Hens were in colony layer cages (10 hens per cage, living space of 750 cm^2 per hen, 12 cm of trough feeder, two accessible nipple drinkers, hen-roost and space for grubbing and dust bathing) in a stable certified for layer farming when they were 17 weeks old; the initial body weight was 1 456 g (C), 1 441 g (EN 50%) and

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1 443 g (EN 100%) on average. The laying cycle lasted 12 months, and started at the 20th week of age of the hens. The experiment was done in accordance with technological guidelines for Isa Brown laying hens, with a controlled light (16 h of light and 8 h of dark), ventilation and temperature regime (19–23 °C). Fifty eggs were tested from each group laying period (10 eggs for one monitored period). All necessary documents for the experiment were approved by Ministry of Education, Youth and Sport of the Czech Republic.

Hens were fed complete feed mixtures *ad libitum* during the laying period: N1 starter (in the 1st month of laying), N1 (from the 2nd to the 5th month of laying), N2 (from the 6th to the 12th month of laying). Each feed mixture was in three variants (according to the source of protein). Commercially produced complete feed mixtures were administered in a loose state from the trough feeders. Cages were equipped with nipple drinkers (two drinkers per one cage). The nutritional composition of the feed mixtures is shown in Table 1. The ingredient composition is the producer's know-how and the firm would not like to publish it.

The health condition of the laying hens was monitored daily by checking their vital functions, symptoms of any disease were apparent. In every group, two layers died during the experiment (mortality 2.9%).

During the laying cycle, every 8th week 10 eggs were taken (five times) from each group (50 eggs per group) in which the fatty acids were analysed. The yolk was separated from the egg, the fat content was determined, and its percentage was calculated. Samples for analysis were prepared according to Hara and Radin (1978). Fat from the samples was extracted with a mixture of the solvents *n*-hexane and isopropanol (at the ratio of 6 : 4). The solvents were evaporated in a vacuum evaporator, then transesterified with BF_3 (transformation of fatty acids to volatile esters). Fatty acid esters were detected by gas chromatography using a Gas Chromatograph GC-2010 analyser (Shimadzu, Kyoto, Japan) with a flame ionisation detector and evaluated in the GC PostRun program. Important fatty acids from groups of SFA, MUFA and PUFA detected in the yolks are listed individually in Results and in Tables. Some fatty acids in the egg yolk reached very low values below the detection limit, therefore their values are expressed as zero. Individual values are cited to three decimal places because even in such low values there were statistically significant differences.

The results were processed by statistical methods using the Unistat v5.6 program. The mean values

Table 1. Nutrient composition of feed mixtures

Feed mixture	N1 starter			N1			N2		
(g/kg)	C	EN 50%	EN 100%	C	EN 50%	EN 100%	C	EN 50%	EN 100%
Dry matter	891.7	888.4	890.4	894.9	898.4	896.6	883.9	882.8	886.2
Crude protein	161.0	149.0	165.0	162.0	165.5	171.0	135.5	136.5	165.0
Crude fat	30.5	44.9	75.4	34.6	48.0	78.0	23.4	33.0	62.6
Ash	126.0	110.0	114.0	118.0	124.0	109.5	117.0	103.7	102.1
Fibre	25.8	34.7	51.3	29.8	36.8	50.9	30.3	35.0	48.0
Sugar	29.6	31.1	32.8	38.8	38.0	38.4	30.0	33.7	36.1
Starch	438.0	432.0	356.0	422.5	388.5	336.5	474.0	248.5	376.0
Ca	40.2	34.0	37.1	37.1	38.1	34.3	36.4	32.3	31.8
P	3.9	3.9	4.0	5.0	5.0	4.2	3.9	3.7	3.9
Mg	1.5	1.4	1.3	2.1	2.0	1.8	2.1	1.9	1.9
Methionine	3.7	3.6	4.7	3.9	3.9	3.4	2.8	2.4	2.8
Lysine	8.3	6.5	7.6	7.3	6.9	6.8	7.0	6.2	7.3
Arginine	10.5	9.2	12.4	9.5	9.2	11.4	7.8	8.1	11.4
Tryptophan	2.1	1.6	1.4	1.9	1.6	1.4	1.7	1.4	1.4

C = feed mixture containing only soybean meal as a source of protein; EN 50% = feed mixture containing 50% of soybean meal and 50% of white lupin seed meal; EN 100% = feed mixture containing only white lupin seed meal as a source of protein

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and their differences were evaluated by multiple comparisons, using the Tukey-HSD test, significance level $P \leq 0.05$.

RESULTS

Weight gain was not a subject of the research. The mean body weight at the end of the experiment was 2 163 g in group C (increase by 707 g; 49%), 2 057 g in group EN 50% (increase by 616 g; 43%) and 2 018 g in group EN 100% (increase by 575 g; 40%).

In general, regardless of the dietary treatment, the percentage of fat in the yolk was in a relatively narrow range of 25.49% to 25.68%. The results show that diets based on lupin meal did not affect the percentage of fat in the yolk quantitatively, but they affected the composition of fatty acids as follows.

Hen-day egg production was 86.1% (C), 88.4% (EN 50%) and 86.6% (EN 100%). Thus it can be assumed that the change of the protein source in the feed mixture did not influence (statistically) the production and number of eggs.

SFA

From the results in Figure 1 it is evident that laying hens fed lupin-based meal diets showed the same proportion of SFA in the egg yolk fat in group EN 50% (28.9 g/100 g of fat) compared to the control group (28.9 g/100 g of fat). The significantly lowest SFA content in the yolk fat (26.4 g/100 g of fat) was in group EN 100% (fed a mixture with 100% lupin meal as a source of protein).

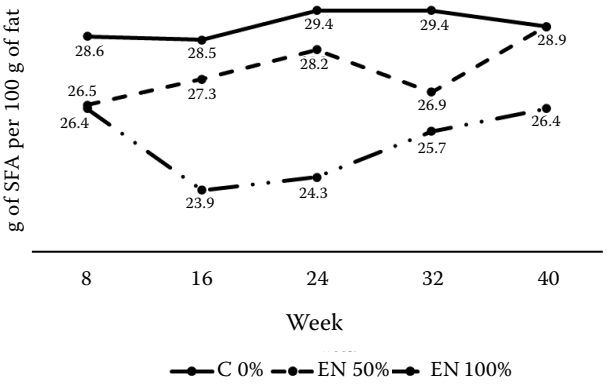

Figure 1. The levels of SFA during the experiment (mean values)

C = control group; EN 50% = group fed the feed mixture consisting of 50% of soybean and 50% of white lupin meal as a protein source; EN 100% = group fed the feed mixture with white lupin seed meal as a protein source

The yolk fat did not contain any butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0) from the SFA group. In the control group behenic acid (C22:0) was also absent while the value in group EN 50% was on the detectable limit 0.001 ± 0.002 g/100 g of fat (Table 2)

Table 2. Content of individual SFA in egg yolk, n = 50

SFA	C		EN 50%		EN 100%	
g FA/100 g of fat		SD		SD		SD
C12:0 (lauric acid)	0.002	0.004	0.002	0.004	0.001	0.003
C14:0 (myristic acid)	0.266^{a}	0.004	0.235	0.004	0.195^{b}	0.003
C16:0 (palmitic acid)	21.747^{a}	0.765	20.653^{bc}	1.180	18.686^{bd}	1.556
C17:0 (margaric acid)	0.163^{bd}	0.024	0.194^{bc}	0.029	0.244^{a}	0.028
C18:0 (stearic acid)	6.608^{a}	0.421	6.308^{b}	0.412	6.062^{b}	0.646
C20:0 (arachidic acid)	0.042^{a}	0.009	0.037	0.011	0.034^{b}	0.009
C21:0 (heneicosanoic acid)	0.099^{b}	0.013	0.103	0.010	0.109^{a}	0.016
C22:0 (behenic acid)	0.000^{b}	0.000	0.001	0.002	0.004^{a}	0.005
C23:0 (tricosanoic acid)	0.004^{a}	0.006	0.001^{b}	0.004	0.000^{b}	0.000
C24:0 (lignoceric acid)	0.005	0.034	0.002	0.017	0.000	0.000
∑SFA	28.938^{a}	0.980	27.537^{bc}	1.364	25.336^{bd}	2.120

C = feed mixture containing only soybean meal as a source of protein; EN 50% = feed mixture containing 50% of soybean meal and 50% of white lupin seed meal; EN 100% = feed mixture containing only white lupin seed meal as a source of protein

$^{a–d}$Means within a row with different superscript letters differ significantly ($P \leq 0.05$)

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and in group EN 100% it was 0.004 ± 0.005 g/100 g of fat (significantly $P \leq 0.05$ higher than in the control group). Palmitic acid (C16:0) and stearic acid (C18:0) were the most frequent from the SFA group. Both of them were significantly the highest 21.747 ± 0.765 g/100 g of fat (C16:0) and 6.608 ± 0.421 g/100 g of fat (C18:0) in the control group.

All the other saturated fatty acids were present in the yolk fat below 1%, but even in these minimal amounts there were some significant differences (see Table 2).

Generally, it is possible to note that feeding the lupin meal significantly ($P \leq 0.05$) decreased the sum of SFA from 28.938 ± 0.980% (C) to 27.537 ± 1.364% (EN 50%) and 25.336 ± 2.120 g/100 g of fat (EN 100%) in the fat of the yolk.

MUFA

The results of the MUFA analysis show different outcomes (Figure 2). Compared to the control group the mean values of MUFA in the fat of the yolk were comparable or slightly lower in the experimental groups during the laying period.

At the end of the experiment the lowest content of MUFA was in group EN 100% (42.9 g/100 g of fat), higher content of MUFA was in the control group (44.1 g/100 g of fat) and the highest levels were established in group EN 50% (46.1 g/100 g of fat).

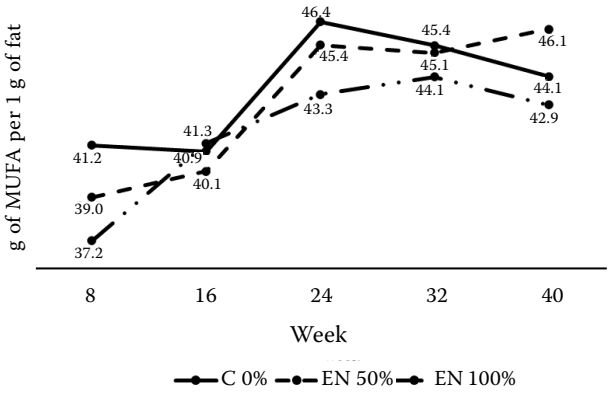

Figure 2. The levels of MUFA during the experiment (mean values)

C = control group; EN 50% = group fed the feed mixture consisting of 50% of soybean and 50% of white lupin meal as a protein source; EN 100% = group fed the feed mixture with white lupin seed meal as a protein source

The results in Table 3 show that the total values of MUFA ranged from 41.741 ± 4.853 g/100 g of fat to 43.597 ± 2.583 g/100 g of fat and did not differ significantly. Higher values in experimental groups than in the control group were only at C17:1 (heptadecenoic acid), C20:1n9 (gadoleic acid) and C22:1n9 (erucic acid). A dominant acid among MUFA was C18:1n9 (oleic acid) whose values reached 40.317 ± 2.469 g/100 g of fat (C), 40.351 ± 3.321 g/100 g of fat (EN 50%) and 39.721 ± 4.929 g/100 g of fat (EN 100%) and differences between the values were not statistically provable.

Table 3. Content of individual MUFA in egg yolk, n = 50

MUFA	C		EN 50%		EN 100%	
g FA/100 g of fat		SD		SD		SD
C14:1 (myristoleic acid)	0.061^{a}	0.011	0.047^{bc}	0.013	0.027^{bd}	0.007
C15:1 (pentadecenoic acid)	0.040	0.010	0.039	0.011	0.037	0.009
C16:1 (palmitoleic acid)	2.935^{a}	0.384	2.381^{bc}	0.497	1.547^{bd}	0.222
C17:1 (heptadecenoic acid)	0.090^{bd}	0.025	0.108^{c}	0.022	0.127^{a}	0.019
C18:1n9t (oleic acid)	40.317	2.469	40.351	3.321	39.721	4.929
C20:1n9 (gadoleic acid)	0.140^{bd}	0.024	0.193^{bc}	0.032	0.258^{a}	0.039
C22:1n9 (erucic acid)	0.012^{b}	0.015	0.016^{b}	0.006	0.022^{a}	0.005
C24:1n9 (nervonic acid)	0.002	0.014	0.002	0.014	0.002	0.014
∑MUFA	43.597	2.583	43.134	3.510	41.741	4.853

C = feed mixture containing only soybean meal as a source of protein; EN 50% = feed mixture containing 50% of soybean meal and 50% of white lupin seed meal; EN 100% = feed mixture containing only white lupin seed meal as a source of protein

$^{a–d}$Means within a row with different superscript letters differ significantly ($P \leq 0.05$)

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PUFA

From the results shown in Figure 3 it is apparent that lupin-based mixtures fed to experimental groups of laying hens had a positive effect on the increase of n-6 PUFA in the yolk compared to the control group. At the end of the experiment, the highest content of n-6 PUFA was found in the egg yolk of experimental group EN 100% (14.3 g/100 g of fat) and the lowest in the control group (10.1 g/100 g of fat) while the value of EN 50% was between them (11.1 g/100 g of fat). Changes of n-6 FA in the egg yolk were very dynamic. A gradual decrease of n-6 PUFA in the yolk fat was observed in all groups until the 24th week of laying. From the 24th week, the n-6 PUFA levels stabilized and had an increasing tendency until the end of the experiment.

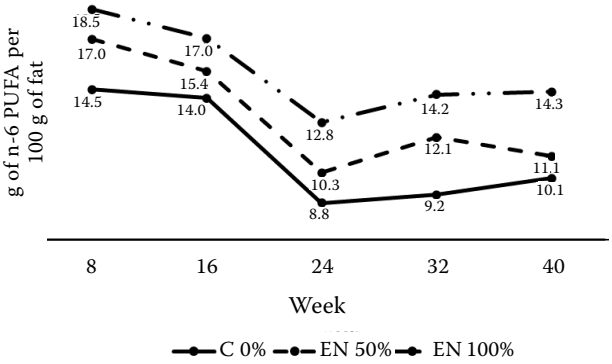

Figure 3. The levels of n-6 PUFA during the experiment (mean values)

C = control group; EN 50% = group fed the feed mixture consisting of 50% of soybean and 50% of white lupin meal as a protein source; EN 100% = group fed the feed mixture with white lupin seed meal as a protein source

Similarly, the total values of n-6 PUFA were significantly ($P \leq 0.05$) higher in experimental groups EN 50% (13.172 ± 2.755 g/100 g of fat) and EN 100% (15.322 ± 3.091 g/100 g of fat) than in the control group (11.323 ± 2.612 g/100 g of fat) (Table 4). Among n-6 PUFA linoleic acid (C18:2n6) dominated with low values in the control group (9.611 ± 2.551 g/100 g of fat) and the growing tendency in accordance with the growing content of lupin meal 11.492 ± 2.647 g/100 g of fat (EN 50%) and/or 13.991 ± 2.290 g/100 g of fat (EN 100%). The inverse trend was apparent in arachidonic acid (C20:4n6) with no significant decrease of values in experimental groups EN 100% (1.359 ± 0.191 g/100 g of fat) and EN 50% (1.396 ± 0.213 g/100 g of fat) compared with control group (1.420 ± 0.256 g/100 g of fat).

The effect of lupin meal in the feed mixture on the increase of n-3 FA in the egg yolk fat was similar like in n-6 FA. A gradual decrease of n-3 FA in the yolk fat was observed in all groups until the 24th week of laying and then barely change (Figure 4).

In summary, it is apparent that the content of n-3 FA was increasing significantly together with the higher content of dietary lupin meal not only in the most frequent α-linolenic acid (C18:3n3) – 0.371 ± 0.138 g/100 g of fat (C), 0.650 ± 0.178 g/100 g of fat (EN 50%) and 1.017 ± 0.257 g/100 g of fat (EN 100%) but also in the total n-3 content (Table 5).

Table 4. Content of individual n-6 PUFA in egg yolk, $n = 50$

n-6 PUFA	C		EN 50%		EN 100%	
g FA/100 g of fat		SD		SD		SD
C18:2n6 (linoleic acid)	9.611^{bd}	2.551	11.492^{bc}	2.647	13.991^{a}	2.290
C18:3n6 (γ-linolenic acid)	0.061	0.014	0.059	0.013	0.059	0.012
C20:2n6 (eicosadienoic acid)	0.069^{bd}	0.019	0.086^{bc}	0.021	0.108^{a}	0.020
C20:3n6 (eicosatrienoic acid)	0.041^{a}	0.012	0.027^{b}	0.011	0.016^{b}	0.009
C20:4n6 (arachidonic acid)	1.420	0.256	1.396	0.213	1.359	0.191
C22:2n6 (docosadienoic acid)	0.001	0.004	0.001	0.004	0.002	0.004
C22:4n6 (docosatetraenoic acid)	0.119^{a}	0.017	0.110	0.019	0.087^{b}	0.015
∑n-6 PUFA	11.323^{bd}	2.612	13.172^{bc}	2.755	15.322^{a}	3.091

C = feed mixture containing only soybean meal as a source of protein; EN 50% = feed mixture containing 50% of soybean meal and 50% of white lupin seed meal; EN 100% = feed mixture containing only white lupin seed meal as a source of protein

$^{a–d}$Means within a row with different superscript letters differ significantly ($P \leq 0.05$)

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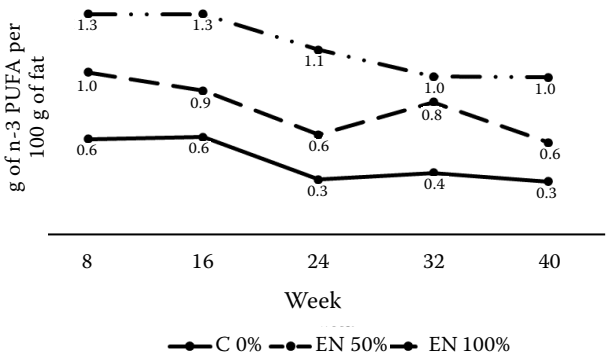

Figure 4. The levels of n-3 PUFA during the experiment (mean values)

C = control group; EN 50% = group fed the feed mixture consisting of 50% of soybean and 50% of white lupin meal as a protein source; EN 100% = group fed the feed mixture with white lupin seed meal as a protein source

Table 6 documents the changes in values of the most frequent fatty acids present in groups of SFA, MUFA, n-3 PUFA, n-6 PUFA with respect to the protein source in the feed mixture.

Data on the fatty acid composition of lupin and other substances which are often used in feed mixtures for the improvement of egg quality are in Table 7.

DISCUSSION

The nutritional value and health benefits of eggs can be improved by adjusting feeding strategies in poultry (Kassis et al. 2010). Hen eggs are not naturally rich in n-3 PUFA; therefore, n-3 PUFA supplementation of poultry feed mixtures

Table 5. Content of individual n-3 PUFA in egg yolk, n = 50

n-3 PUFA	C		EN 50%		EN 100%	
g FA/100 g of fat		SD		SD		SD
C18:3n3 (α-linolenic acid)	0.371^{bd}	0.138	0.650^{bc}	0.178	1.017^{a}	0.257
C20:3n3 (dihomo γ-linolenic acid)	0.000	0.000	0.000	0.000	0.000	0.000
C20:5n3 (eicosapentaenoic acid)	0.001^{bd}	0.003	0.003^{bc}	0.005	0.006^{a}	0.005
C22:6n3 (docosahexaenoic acid)	0.003	0.016	0.000	0.000	0.001	0.010
C22:5n3 (docosapentaenoic acid)	0.067^{bd}	0.060	0.112^{c}	0.030	0.117^{a}	0.033
∑n-3 PUFA	0.442^{bd}	0.138	0.765^{bc}	0.186	1.141^{a}	0.268

C = feed mixture containing only soybean meal as a source of protein; EN 50% = feed mixture containing 50% of soybean meal and 50% of white lupin seed meal; EN 100% = feed mixture containing only white lupin seed meal as a source of protein

$^{a–d}$Means within a row with different superscript letters differ significantly ($P \leq 0.05$)

Table 6. Changes in the values of the most frequent fatty acids present in groups of SFA, MUFA, n-3 PUFA, n-6 PUFA with respect to the source of protein in feed mixture, n = 50

Feed mixture	N1 starter			N1			N2		
g FA/100 g of fat	C 0%	EN 50%	EN 100%	C 0%	EN 50%	EN 100%	C 0%	EN 50%	EN 100%
∑SFA	3.60	4.72	8.53	4.69	7.06	10.12	2.38	3.85	6.02
C16:0	2.63	3.44	5.59	3.41	4.91	6.48	1.94	2.81	3.89
∑MUFA	5,.71	10.05	27.46	6.13	12.48	29.01	2.79	9.96	19.33
C18:1n9	5.10	9.47	25.62	6.01	11.69	27.21	2.72	9.32	17.96
∑n-6 PUFA	10.40	13.21	21.32	14.41	19.08	25.42	8.02	10.71	14.19
C18:2n6	10.37	13.14	21.14	14.37	19.00	25.26	8.01	10.64	14.05
∑n-3 PUFA	1.07	2.16	5.47	1.39	2.76	5.91	0.64	1.97	3.94
C18:3n3	0.94	1.79	4.31	1.28	2.38	4.69	0.61	1.57	3.04

C = feed mixture containing only soybean meal as a source of protein; EN 50% = feed mixture containing 50% of soybean meal and 50% of white lupin seed meal; EN 100% = feed mixture containing only white lupin seed meal as a source of protein

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Table 7. Content of fatty acids in white lupin seeds, soybean oil*, fish oil* and microalgae* [*modified from Kralik et al. (2020), available from https://doi.org/10.2141/jpsa.0190076, the average value of the results of two sample analyses]. Fatty acids in lupin are the average value of the results of three sample analyses

Fatty acids (% of total fatty acids)	White lupin Zulika	Soybean oil*	Fish oil*	Microalgae *Schizochytrium limacinum**
C12:0 (lauric acid)	0.01	0.00	0.00	0.19
C14:0 (myristic acid)	1.65	0.00	2.15	5.62
C16:0 (palmitic acid)	3.02	10.31	9.40	57.18
C17:0 (margaric acid)	0.50	0.00	0.00	0.58
C18:0 (stearic acid)	0.83	6.12	2.87	2.24
C20:0 (arachidic acid)	0.15	0.00	0.00	0.37
C23:0 (tricosanoic acid)	0.09	0.00	0.00	0.16
C24:0 (lignoceric acid)	0.52	0.00	0.00	0.14
∑SFA	6.77	16.43	14.42	66.48
C16:1 (palmitoleic acid)	0.29	0.00	2.78	0.31
C17:1 (heptadecenoic acid)	0.04	0.00	0.00	0.05
C18:1n9 (oleic acid)	26.05	26.89	40.25	3.45
C20:1n9 (gadoleic acid)	3.64	0.00	4.82	0.00
C22:1n9 (erucic acid)	1.40	0.00	3.19	0.00
∑MUFA	31.42	26.89	51.04	3.81
C18:2n6 (linoleic acid)	10.18	49.42	14.41	2.97
C20:2n6 (eicosadienoic acid)	0.13	0.00	0.00	0.13
C20:4n6 (arachidonic acid)	0.00	0.00	0.00	0.79
∑n-6 PUFA	10.31	49.42	14.41	3.89
C18:3n3 (α-linolenic acid)	5.56	5.69	6.33	1.47
C20:3n3 (dihomo γ-linolenic acid)	0.19	0.00	0.51	0.00
C20:5n3 (eicosapentaenoic acid)	1.46	0.00	3.81	0.43
C22:6n3 (docosahexaenoic acid)	0.05	0.00	5.23	21.33
∑n-3 PUFA	7.26	5.69	15.88	23.23

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids

is required to obtain enriched n-3 PUFAs eggs (Laudadio et al. 2015). Such enriched eggs are more beneficial to humans and have positive health effects in the human nutrition (Kassis et al. 2012) because they increase PUFA content in the egg yolk and help to decrease the bad cholesterol content (Fraeye et al. 2012). Enriched eggs offer well balanced ratios of n-6/n-3 PUFA (1 : 1) and provide more than 600 mg of n-3 PUFA (Ahmad et al. 2012). The content of n-3 PUFA in eggs can be increased by supplementing the diets of laying hens with certain dietary supplements, such as oils (most often fish oil), meals (linseed meal) or algae (Baiao and Lara 2005) or lupin meal as shown by the results of this study. Also Cachaldora et al. (2006) concluded that dietary fatty acid saturation influenced the fatty acid composition without any other impact on the egg production.

Alagawany et al. (2018) stated that not only the increase of PUFA in egg yolk but also the stability of n-3 PUFA are important. They tried to raise the stability by vitamin E and/or organic selenium, which reduces oxidation in raw eggs and protects their quality during storing and cooking. Ceylan et al. (2011) evaluated the effect of dietary supplementation of soybean oil, rapeseed oil, and linseed oil on two levels (15 g/kg and 30 g/kg diet) for 12 weeks in laying hens. They observed no changes

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in egg production and egg weight, but hens receiving sunflower oil produced eggs with yolks of fair colour, which customers considered as an unintended effect. Despite of this, the results of changes in fatty acid composition after the addition of sunflower oil showed that their composition was significantly ($P < 0.01$) affected by the treatment. Cachaldora et al. (2006) concluded that dietary fatty acid saturation influenced the fatty acid composition without any other impact on the egg production. In this longer-time study, feeding lupin meal helped to increase the values of UFA in yolks compared with feeding soybean meal without any other side-effects (production, health of the layers). Comparable results were described by Da Silva Filardi et al. (2005), who studied the effects of the 12-week dietary inclusion of different fat sources (cottonseed oil, soybean oil, lard, sunflower oil or canola oil) on egg quality and egg yolk lipid profile. The various fat sources affected the lipid composition of the egg yolk. Lipids changed based on dietary fat sources. Optimal changes were lower levels of SFA and LA, and higher levels of ALA and DHA – results in conformity with replacement of soybean protein by lupin protein. Ebeid et al. (2008) stated that hens fed a diet containing different concentrations of n-3 PUFA showed a linear decrease and increase ($P < 0.05$) in the egg yolk content of n-6 PUFA and n-3 PUFA, respectively, compared to the control hen group. In this study, levels of n-3 and n-6 PUFA also increased in accordance with the increasing lupin meal content in the feed mixture.

By contrast Buitendach et al. (2013) did not find any impact of supplementing fatty acids into the feed mixture for hens on their productivity. They added to the feed mixtures linseed oil, fish oil, sunflower oil and tallow. The results of the study failed to indicate the impact of dietary fatty acid saturation on the feed intake of birds. On the other hand, it could be concluded that the long-term (54-week) exposure to a range of fatty acid saturation levels had no negative effect on hen performance.

Fish is the richest dietary source of EPA and DHA, but a large part of the population all over the world consumes little or no fish, mostly from the countries without access to the seashore (Welch et al. 2010). Therefore, other dietary sources of EPA and DHA are being sought. Food enrichment with long-chain n-3 PUFA is probably the best long-term solution to boost their intake (Molendi-Coste et al. 2011). An interesting route is n-3 PUFA enrichment of eggs through dietary supplementation of laying hens. An important benefit of this advance is their wide acceptability as human food and food component. People consume eggs worldwide, without restriction for example by religion. The amount of SFA or MUFA in eggs is hardly influenced by the lipids in the feed (Herber and Van Elswyk 1996; Baucells et al. 2000). In contrast, producers are able to increase the PUFA content and profile in the egg through dietary supplementation.

As a result, lupin protein can be substituted for soybean protein (either partly – 50% or totally – 100%) in feed mixtures for laying hens. Lupin meal as a protein source in the diet reduced the SFA content and increased the UFA content in the yolk, which means also the fat quality compared with soybean protein.

The implication of these findings indicated that lupin dietary inclusion may be suitable for laying hens without influencing the productivity or layers' health.

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Conflict of interest

The authors declare no conflict of interest.

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