Study of Fatty Acids Profile and Oxidative Stability of Egg Yolk from Hens Fed a Diet Containing White Lupine Seeds Meal

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The aim of this study was to evaluate the effects of different dietary inclusion of raw white lupine seed meal (WL) in diets of laying hen on fatty acids (FA) profile, health lipid indices and oxidative stability of egg yolk. A total of 160 TETRA-SL LL laying hens (30-week old) were used in a ten week trial. The laying hens were randomly assigned to four dietary treatments: control diet which contained soybean meal (C), and study diets in which soybean meal was replaced with WL at 150 g/kg (E_{15}), 200 g/kg (E_{20}) and 250 g/kg (E_{25}). Each treatment was replicated 5 times with 8 birds each. The inclusion of WL in experimental diets caused a linear increase in n-6 and n-3 polyunsaturated FA (PUFA) content and a decrease in monounsaturated FA (MUFA), but it had no influence on the saturated FA (SFA) of egg yolk lipids. The data suggested that the partial replacement of soybean meal with WL of in laying hen diet it significant increase h/H (hypocholesterolemic/Hypercholesterolemic) and PUFA/SFA ratio, and decrease thrombogenic index (TI), which improves human health because of the beneficial effect on the cardiovascular system. The authors determined the concentration of α-tocopherol by high performance liquid chromatography (HPLC) and assessed the degree of oxidative degradation of lipids using the classical test based on the concentration of malondialdehyde (MDA). Inclusion of WL in diets led to increases in α-tocopherol and decrease in MDA in eggs ($P < 0.05$). Egg storage for 20 d or 40 d led to linear reduction in egg á-tocopherol and increase of MDA content ($P < 0.05$). The results of the present study suggest that the seeds of modern white lupine varieties can be included at 250 g/kg in layer diets as an effective substitute for soybean meal, improving fatty acids profile, health lipid indices and oxidative stability of egg yolk.

Keywords: α-linolenic acid, cholesterol, thrombogenic index, α-tocopherol, malondialdehyde

Layers diets typically contain soybean meal due to its high crude protein content but used can result in large increases in cost of feed, especially, in countries that are not climatically adapted to soyabean production. Lupin seeds have high protein content (comparable with soya) but previous experience with white lupins has shown toxic effects in poultry due to high levels alkaloids and poor performance due to anti-nutritional non-starch polysaccharides (NSP) [1-3]. However, in the last decades, plant breeders have succeeded in developing lupin cultivars characterized by very low alkaloid content. The NSP level in lupin is almost twice as high as in other protein-rich plants [4] but yet, according to later research, lupin protein is digested to the same degree as from soybean meal [5] or even better [6].

Apart from low levels of anti-nutritional factors, modern lupine varieties are also characterized by higher concentrations of high-quality protein [7], and a higher yield [8]. The seeds of white lupine can be a valuable ingredient of laying hen diets due and to high concentrations of unsaturated fatty acids (UFAs) [2, 7] which can enrich the egg yolk with essential FAs.

Eggs are a principle food for human consumption and contain most of the nutrients needed by human. However, egg consumption should be limited due to high content of SFA (mainly C16:0 which has hypercholesterolaemic effect) and cholesterol that increases the risk of developing many diseases [9]. In contrary, recent evidences suggested their omega-3 and omega-6 fatty acids, especially α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, C22:6n-3) and linoleic acid (LA, C18:2n-6) have positive effects on human health. They reduce the risk of cardiovascular disease, are anti-carcinogenic, and have anti-sclerosis properties [10, 11]. Therefore, maximizing ALA, EPA, DHA (PUFA n-3) and LA in egg would benefit human health.

Major changes in cholesterol and fatty acid concentrations of egg yolk can be induced by manipulating nutrition, such as use of different oil sources that are commonly used as energy sources in the diets of laying hens [12, 13]. Some of the oil sources and oil seeds are rich in long chain PUFA that can change the fatty acids profile of egg yolk [14]. It is well known that through dietary manipulation, fatty acids profile and several nutrients with important health implication can be affected [9, 15, 16].

Following research showing the beneficial effects of specific FAs, different health indexes have been suggested as indicators for the health benefit of egg. The indexes indicate the relationship between FAs in food and their contribution to the prevention of coronary diseases [17]. These include the atherogenicity (AI), thrombogenicity (TI) and the hypocholesterolemic/Hypercholesterolemicratio (h/H) indexes.

Atherogenic index (AI): indicating the relationship between the sum of the main SFA and that of the main classes of unsaturated FA, the former being considered pro-atherogenic (favoring the adhesion of lipids to cells of the immune and circulatory systems), and the latter being considered anti-atherogenic (inhibiting plaque aggregation and reducing the levels of esterified FAs, cholesterol and

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phospholipids, thereby preventing the occurrence of coronary vascular diseases) [18]. Thrombogenic index (TI) is an indicator of the tendency to form clots in blood vessels and is defined as the relation ship between the pro-thrombogenetic (C14:0, C16:0, C18:0) and the anti-thrombogenetic FAs (MUFA, n-6 and n-3 PUFA) [19]. The h/H ratio index is associated with cholesterol metabolism in which the ratio of hypocholesterolemic FAs (MUFA and PUFA) to hypercholesterolemic FAs (C14:0 and C16:0) are of relevance.

An increased proportion of unsaturated fat in egg yolks may augment its oxidative susceptibility. To maintain a high quality, the concentration of antioxidants should therefore be elevated. In egg yolks, the concentration of á-tocopherol and carotenoids as antioxidants is believed to be important for oxidative stability [20].

The effects of used of white lupine seed as laying hens feed options in terms of their effects on the FA profile of the egg yolks, with particular reference to health lipid indices and oxidative stability of egg yolk, which affect human health, are not well documented. Therefore, the aim of the present study was to determine the influence of a new variety of white lupine seeds (cv. Amiga) added into laying hen diets on fatty acids profile, health lipid indices and oxidative stability of egg yolk.

Experimental part

Experimental design, animals and diets

A total of 160, 30 weeks old Tetra-SL LL laying hens were assigned to four dietary treatments with five replicates having eight hens (40 laying hens/treatment), in a completely randomized design. Before starting the trial, egg production of hens was measured individually and hens with equal egg production were placed in each replicate. Treatments consisted of four levels (0, 15, 20 and 25%) of white lupine seed meal (WL) inclusion.

Birds were fed a balanced commercial diet as their daily requirement for two weeks prior to commencement of the study to allow them to adapt and reach a standard level of egg production. The experiment lasted 10 wk, from 30 to 40 wk age. Feed and water were offered ad libitum during the experiment. Light was provided for 16 h per day.

The seeds of white lupine (cv. Amiga) were obtained under the pedo-climatic conditions of Transylvania (Romania) and were also analyzed for CP (33.80%), crude fat content (9.84%, as-is basis) and DM (92.18%) in the laboratory. This cultivar is characterized by a low alkaloid content of seeds [7]. The white lupine seed meal was used as a substitute for soybean meal in the diets of laying hens.

All diets were ground and formulated to be balanced for energy, crude protein, crude fiber, essential amino acids and minerals as required for layers hens [21]. The metabolizable energy (ME) content of white lupine seed meal (WL) was estimated based on the following equation [22]:

$$MEn = 26.7\ (DM) + 77\ (EE) - 51.22\ (CF),$$

where: DM, EE and CF are dry matter, ether extract and crude fiber percentage of WL, respectively.

Samples collection

Eggs were collected for a period of 8 consecutive days from all of the pens under each of the 4 treatments (40 eggs/treatment) during wk 9 and 10 for egg yolk fatty acid analysis. The egg yolks were separated and stored at -20 ^{0}C until analyzed. The white lupine seed meal (WL) and diet samples were also stored at -20 ^{0}C until analyzed for fatty acids.

Ten eggs per group (two eggs per replicate) were collected at random and analyzed at 0, 20, and 40 days of storage (2-4 ^{0}C) for α-tocopherol content and oxidative stability (content of malondialdehyde - MDA) of egg yolks.

Chemical analyses

The analyses of fatty acid compositions of the WL, diets, and egg yolks were determined using standard gas chromatographic techniques of the fatty acid methyl esters (FAME) [23].

Total fat of egg yolk for fatty acid analysis was extracted with a chloroform/methanol mixture according to Folch et al. [24]. At the first stage fat was saponified using a 0.5N KOH methanol solution at 70^{o}C. Esterification with methanol was conducted in the presence of sulphuric acid as a catalyst.

Chromatographic analysis was performed using a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Tokyo, Japan) equipped with a DB-23 column (60x0.25 mm i.d. and 0.25μm film thickness). Column and detector temperatures were 190 and 240^{o}C, respectively. Carrier gas was helium at 1.0 mL/min ratio. The temperature of injection port was 230^{o}C with the split ratio of 1:80, temperature program was 80^{o}C/5 min, 200^{o}C/30 min, and 230^{o}C/15 min [7]. The FAME were identified using external standards (Supelco 37 Component FAME mix; Supelco Bellefonte, PA, USA), and the FA contents were calculated from the peak area of the corresponding fatty acid in relation to the total area of all peaks.

Egg yolks were also analyzed for total cholesterol content by the Washburn and Nix method [25]. Cholesterol was separated from fat after saponification with KOH and extraction with ethyl ether. The sample was subjected to chromatographic analysis under the following conditions: the length of a glass column, 1 m, internal diameter, 4 mm; film thickness, 0.25 μm; temperature of detector, 300^{o}C; temperature of injector, 290^{o}C; temperature of column, 260^{o}C; carrier gas, argon, flow rate, 50 cm^3/min [18]. Egg yolk cholesterol content was calculated and expressed as milligrams per gram of yolk.

Extraction of tocopherols was done with a mixture of diethyl ether and petroleum ether (1:1), in aliquots of 20 mL. The ether phase, after separation, was saponified with methanolic KOH solution (10%), after which tocopherols were extracted in hexane, washed with water in a separator funnel, and evaporated to dryness. After evaporation of the hexane phase, separation of the tocopherols was by high performance liquid chromatography (HPLC) using a system (Parkin-Elmer LC-295), equipped with Alltech C18 column (length 15 cm, 4.6 mm internal diameter and particle size 3 μm). The mobile phase for the various tocopherols was acetonitrile:methanol (85:15)/isopropanol 90/10. The concentrations of α-, γ-, and δ-tocopherol in the samples of WL, diets and egg yolks were calculated with external standards (Sigma-Aldrich, USA) through a linear regression from known standards.

Malondialdehyde (MDA) is the main product of oxidation of PUFAs. It is widely used as a marker of lipid peroxidation in food and biofluids. Malondialdehyde was determined based the 2-thiobarbituric acid (TBA) as described by Salih et al. [26], with some modifications [27]. Egg yolk samples (2 g) were weighed into test tubes, perchloric acid added. The samples were homogenized for 15 s at high speed. The butylated hydroxytoluene (BHT) was added to each sample during homogenization to control lipid oxidation. The homogenate was filtered through filter paper. Filtrate (2 mL) was mixed with 2 mL of 20 mM TBA in distilled water and incubated in a boiling water bath for 30 min. Absorbance was determined at 531 nm [27]. The oxidative stability was expressed as milligrams of malondialdehyde (MDA) per 100 g of yolk.

Health lipid indices calculation

Health lipid quality was assessed by calculating the atherogenic and thrombogenic indices as well as the ratio between the hypocholesterolemic and hyper cholesterolemic using the following equations:

a)AI(atherogenicity index) = [(4xC14:0) + C16:0 + C18:0]/(MUFA + + n-6PUFA + n-3PUFA), [19];

b) TI(thrombogenicity index)=(12:0+16:0+18:0)/[(0.5×MUFA)+ +(0.5×n-6PUFA)+ (3×n-3PUFA)+(n-3PUFA/n-6 PUFA)], [19];

c) h/H(hypocholesterolemic/Hypercholesterolemic)= =(C18:1+PUFA)/(C12:0+C14:0+C16:0), [28].

Statistical analysis

The results of the analysis were compared by analysis of variance (ANOVA) with the diet as principal effect [29]. The ANOVA for fatty acid profile, health lipid indices, total cholesterol content, α-tocopherol and MDA of yolk included the main effect of the diet and week and the interaction between these two main effects. When the effect of diet was significant, the means were separated using Duncan's test. In addition, Pearson correlations were used to evaluate relationships between egg yolk oxidative stability (MDA values) and α-tocopherol, PUFA and storage time. The level of significance to detect statistical differences was set at $P < 0.05$ for all analyses.

Results and discussions

Chemical composition of white lupine seed and diets

The diets compared in this study had an identical total protein and metabolizable energy (ME) content. The diets supplemented with 150-250 g·raw WL/kg contained 20.5 - 37.2% more ether extract, and 34.8 -58.0% more crude fiber, than the control diet (table 1).

Compared with the control diet, lupine seed meal based diets had higher concentrations of oleic (C18:1), linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acid and lower levels of total saturated fatty acids (25.41 vs. 18.04% of FAME) (Table 2). The decrease LA:ALA ratio in diets containing WL resulted from the fact that white lupine seed meal were a rich source of ALA (C18:3 n-3) and a poor source of LA (C18:2 n-6). Our results are consistent with the findings of Strakova et al. [30] and Mierlita et al. [7] who demonstrated that white lupine oil is characterized by the high concentrations of n-3 PUFA levels. White lupine seeds meal showed a substantial content of tocopherols (table 2) which can ensure high oxidative stability to fat from egg yolk.

Fatty acids profile and cholesterol concentrations of egg yolk

The results of fatty acid analyses were shown in Table 3. The lipid content ranged from 30.86 to 31.47% in egg yolks; fat content isn't influenced by supplementation with lupine seed meal of laiyng hens' diets. Similarly, Alatas and Citil [31] found that the lipid content of egg yolk was at a level of 30 - 32 %, and fat content isn't influenced by different oil sources of the diets.

The SFA contents were much higher than PUFA, and MUFA contents of egg yolks were higher than SFA in all groups. In general, MUFA is higher than SFA and PUFA [32].

The study has shown that palmitic acid, steric acid, oleic acid and linoleic acid were the fatty acids with highest content in the egg yolks and saturated fatty acids content was similar for all egg, regardless of diets. A decrease in monounsaturated fatty acids (MUFA) deposition in egg yolks was observed with increasing inclusion of the lupine seed meal in diets (table 3). Eggs laid by hens fed the diet containing white lupine seed meal had a large amount of

Table 1
CALCULATED NUTRIENT COMPONENT (PERCENT, AS-FED BASIS) AND CALORIC VALUE OF THE EXPERIMENTAL DIETS CONTAINING DIFFERENT LEVELS OF WHITE LUPINE SEED MEAL

Item	Dietary treatment*			
	C	E_{15}	E_{20}	E_{25}
Metabolizable energy (kcal/kg)	2800	2801	2800	2805
Crude protein (%)	17.00	17.00	17.02	17.06
Ether extract (%)	6.48	7.81	8.32	8.89
Crude fiber (%)	3.48	4.69	5.10	5.50
Lysine (%)	0.85	0.85	0.85	0.85
Methionine (%)	0.42	0.42	0.42	0.42
Calcium (%)	3.76	3.75	3.75	3.76
Available phosphorus (%)	0.40	0.40	0.40	0.40

* *Dietary treatment*/C: control diet with no lupine seed, E_{15}: diet containing lupine seed (150 g/kg), E_{20}: diet containing lupine seed (200 g/kg), E_{25}: diet containing lupine seed (250 g/kg).

Table 2
MAJOR FATTY ACID COMPOSITION OF THE LIPID AND TOCOPHEROLS CONTENT OF THE WHITE LUPINE SEED MEAL (WLS) AND DIETS USED IN THE EXPERIMENTS

	WL	Dietary treatment*			
		C	E_{15}	E_{20}	E_{25}
Fatty acid content (% of fatty acid metil esters):					
Palmitic, C16:0	6.10	15.21	14.88	14.05	14.37
Stearic, C18:0	3.12	3.48	3.62	3.58	4.34
Oleic, C18:1	48.80	23.34	28.59	28.87	29.31
Linoleic, C18:2 n-6 (LA)	20.12	44.83	45.07	45.90	46.39
Linolenic, C18:3 n-3 (ALA)	9.98	4.81	5.27	5.18	5.42
Saturated fatty acids (SFA)	13.47	25.41	18.19	18.27	18.04
Monounsaturated fatty acids (MUFA)	56.42	24.38	29.47	29.65	29.84
Polyunsaturated fatty acids (PUFA)	30.10	50.21	52.34	52.08	52.12
LA : ALA (n-6/n-3 PUFA ratio)	2.02	9.32	8.55	8.86	8.56
Tocopherols (mg/100 g DM):					
α-tocopherol	3.25	1.72	2.26	2.27	2.41
γ- tocopherol	46.53	18.36	24.96	25.15	25.07
δ- tocopherol	1.94	1.88	2.54	2.56	2.62

WL - white lupine seed meal; * Dietary treatment/C: control diet with no WL, E_{15}: diet containing WL (150 g/kg), E_{20}: diet containing WL (200 g/kg), E_{25}: diet containing WL (250 g/kg).

		Dietary treatment[1]				SEM[2]	p-values
		C	E_{15}	E_{20}	E_{25}		D[3]
Egg yolk fat content (%)		31.32	30.94	30.86	31.47	0.214	ns
Fatty acid (% of FAME):							
Lauric	C12:0	0.06	0.06	0.05	0.05	0.030	ns
Myristic	C14:0	0.40^{a}	0.38^{a}	0.32^{b}	0.30^{b}	0.010	**
Palmitic	C16:0	25.23	24.28	24.84	24.50	0.255	ns
Stearic	C18:0	8.76	8.81	8.15	8.79	0.083	ns
Arachidic	C20:0	0.51	0.48	0.50	0.47	0.014	ns
Total saturated FA		34.96	34.01	33.86	34.11	0.231	ns
Myristoleic	C14:1	0.24^{a}	0.20^{ab}	0.18^{b}	0.15^{c}	0.005	***
Palmitoleic	C16:1	3.61^{a}	2.70^{b}	2.29^{ab}	1.97^{c}	0.136	***
Oleic	C18:1 *cis*-9	41.11^{a}	38.61^{b}	38.31^{bc}	37.87^{c}	0.358	**
Oleic	C18:1 *cis*-11	2.53^{a}	1.95^{b}	2.01^{b}	1.80^{bc}	0.021	***
Eicosenoic	C20:1	0.41^{a}	0.32^{b}	0.30^{b}	0.30^{b}	0.014	**
Erucic	C22:1	0.11	0.12	0.11	0.10	0.003	ns
Total monounsaturated FA		48.01^{a}	43.90^{b}	43.20^{b}	42.19^{b}	0.324	**
Linoleic	C18:2 n-6	13.97^{b}	18.29^{ab}	18.87^{ab}	19.33^{a}	0.518	***
Linolenic	C18:3 n-3	0.61^{b}	0.70^{a}	0.72^{a}	0.72^{a}	0.022	*
Arachidonic	C20:4 n-6	1.29^{d}	1.73^{c}	1.95^{b}	2.22^{a}	0.047	**
EPA	C20:5 n-3	0.38^{b}	0.51^{a}	0.52^{a}	0.55^{a}	0.018	***
DHA	C22:6 n-3	0.78^{b}	0.86^{a}	0.88^{a}	0.88^{a}	0.026	*
Total polyunsaturated FA		17.03^{c}	22.09^{b}	22.94^{ab}	23.70^{a}	0.330	***
PUFA n-6		15.26^{c}	20.02^{b}	20.82^{b}	21.55^{a}	0.227	***
PUFA n-3		1.77^{b}	2.07^{a}	2.12^{a}	2.15^{a}	0.024	*
Total unsaturated FA		65.04	65.99	66.14	65.89	0.748	ns
Hypercholesterolemic FA[4]		25.69	24.72	25.21	24.85	0.321	ns
Hypocholesterolemic FA[5]		60.67^{b}	62.65^{a}	63.26^{a}	63.37^{a}	0.582	*
Total cholesterol:							
- mg/g of yolks		16.35	15.84	15.40	15,71	0.417	ns
- mg/egg		240.02	232.06	224.07	225.28	12.385	ns

Means within a row with different superscripts are different (P<0.05);
[1]/C: control diet with no lupine seed, E_{15}: diet containing lupine seed (150 g/kg), E_{20}: diet containing lupine seed (200 g/kg), E_{25}: diet containing lupine seed (250 g/kg); [2]SEM: standard error of the mean
[3]D: effect of experimental diet (ns: P >0.05; *P <0.05; **P <0.01; ***P <0.001); FA: fatty acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; PUFA: polyunsaturated FA;
[4]Hypercholesterolaemic FA: (C12:0 + C14:0 + C16:0); [5]Hypocholesterolemic FA: (C18:1 + PUFA).

Table 3
THE EFFECT OF LUPINE SEED MEAL ON THE FATTY ACID PROFILE AND CHOLESTEROL CONCENTRATIONS OF EGG YOLKS IN LAYING HENS

PUFA. Eggs from hens fed WL had increased linoleic (C18:2 n-6), α-linolenic (18:3 n-3), eicosapentaenoic (20:5 n-3), docosahexaenoic acids (DHA, 22:6 n-3) and arachidonic acid (20:4 n-6) when compared with control eggs (P <0.001).

The inclusion of WL reduced the linoleic acid:linolenic acid ratio (LA:ALA) of the test diets from 9.32:1 in the control diet to 8.56:1 for the E_{25} diet (highest ALA diet; table 2) and produced significant shifts in the n-6 and n-3 FA content of the egg yolk lipids. For the experimental diets, the total n-6 content of the eggs increased with increasing inclusion of the lupine seed meal, and these increases were observed across the three n-3 FA measured: ALA, EPA, and DHA (table 3). The increase of intake of ALA to be the primary determinant of the total n-3 fatty acid content of table eggs. Delta-6 desaturase is responsible for converting ALA to EPA and DHA [33]. Similar results were obtained by Oliveira et al. [13] by the use of linseed oil in the hen diet which causes a nutritional enhancement because it is rich in C18:3 n-3, and also by the incorporation of EPA and DHA into the egg yolk. An increase in total n-3 FAs in white lupine seed meal based fed groups may be due to the antioxidant profile of lupine seed [34], which can regulate the elongase and desaturase pathway in a favourable way during egg yolk lipid metabolism [35].

The yolks of eggs from hens fed white lupine seed meal based diets had a higher arachidonic acid (AA; C20:4 n-6) content, than in the control group, probably because of the high level of its precursor, C18:2n-6 in diets (tabel 2) [13, 18].

Among the hens fed diets containing white lupine seed meal, those fed diet content 25% WL deposited more n-6 fatty acids in the egg yolks than those fed diet content 15% WL, which, in turn, deposited more n-6 FAs in the yolks than those fed control diet (P <0.001). Regarding the n-3FAs, the highest levels were observed in the egg yolks of laying hens fed diets containing WL (2.07 - 2.15% of FAME), which were statistically similar (P >0.05), and the lowest levels were observed with the control diet (P <0.05).

There were no significant differences in egg yolk cholesterol content between hens fed lupine-based diets and hens fed the control diet (table 4). Similarly, Krawczyk et al. [18] found that the cholesterol content of egg yolk isn't influenced by different level of yellow lupine seed meal of the diets of laying hens.

Lipid quality indices of egg yolk

The PUFA/SFA and n-6/n-3 PUFA ratios, atherogenity index (AI) and thrombogenity index (TI) are commonly used to assess the nutritional value and consumer health of animal fat [36]. In general, a ratio of PUFA to SFA above 0.45 and a ratio of n-6/n-3 below 4.0 are required in the diet to combat 'lifestyle diseases' such as coronary heart disease and cancers [10]. In the present study, the n-6/n-3 FA ratios (8.2 - 10.0) were considerably higher than the recommended values, whereas the PUFA/SFA ratios (0.48

- 0.69) were within the recommended levels (table 4). The PUFA/SFA ratios in the egg yolks from laying hens feeding diet containing lupin seed were significantly ($P < 0.001$) higher compared with the egg yolks of laying hens fed control diet. A low PUFA/SFA ratio in egg yolks of laying hens fed control diet resulted from a lower level of some PUFAs in the yolks, especially that of C18:2 n-6, C18:3 n-3 and C20:4 n-6 acids, whose consequence was a very lower total content of PUFA. The introduction of lupine seeds in laying hens feed groups provided the most beneficial ratios of PUFA/SFA in egg yolks, with the following hierarchy being set: $E_{25} \geq E_{20} \geq E_{15} \geq C$ (table 4).

In the present study, the egg yolks from laying hens feeding diet containing lupine seed had a significantly higher percentage of LA ($P < 0.001$) and ALA ($P < 0.05$) in the total content of FAs, whereas the egg yolks from laying hens fed diet control had a significantly ($P < 0.01$) higher percentage of MUFA. The LA/ALA ratio in the egg yolks from laying hens feeding diet containing lupin seed was significantly ($P < 0.01$) higher compared with the yolks of C group, which indicates a more less favourable proportion of these acids in the yolks from laying hens feeding diet containing lupine seed.

Antioxidant properties of lupine seed can stimulate the metabolic pathway of essential fatty acids like n-3 and n-6 [37] and ultimately lower the n-6 / n-3 ratio [35]. However, this conclusion is not supported by the results obtained in this study.

The atherogenic index (AI) and thrombogenic index (TI) take into account the effects that single FAs might have on human health and, in practice, on the probability of increasing the incidence of pathogenic phenomena such as atheroma and/or thrombus formation [36, 38]. In the present study, the TI in the egg yolks from laying hens feeding diet containing lupin seed was significantly ($P < 0.05$) lower compared with the egg yolks from laying hens fed diet control, which improves human health because of the beneficial effect on the cardiovascular system. The values of AI were not affected by the dietary supplementation of lupine seeds (table 4).

The data suggested that the partial replacement of soybean meal with white lupine seed of in laying hen diet did not show a positive effect on n-6/n-3 FA and LA/ALA ratios but it significant increase h/H ratio (hypo-cholesterolemic/Hypercholesterolemic). A higher h/H index value indicates better nutritional quality with a potential for lowering plasma cholesterol [17].

Oxidative stability of egg yolk

Results of present study indicate that the fatty acid profile of eggs could be altered by used WL in diets for laying hens. However, an improvement of omega-6 and omega-3 type fatty acids of eggs will result in a higher susceptibility to lipid oxidation and possibly a shorter shelf-life of stored eggs [39, 40, 41]. This could affect egg quality negatively, mainly due to a decrease in organoleptic and nutritional properties of eggs [42]. Dunn-Hurrocks et al. [43] reported that using dietary flaxseed oil in layer diets to manipulate the fatty acid profile of eggs resulted in higher MDA content with a consequent negative impact on the egg quality. The oxidative process in egg yolk depends on the PUFA content and on the balance between anti- and pro-oxidant compounds [44, 45]. The present study investigates the effect of feeding lupine seed on tocopherol (antioxidant lipofilic) content and oxidative stability of eggs yolks during storage (0, 20 and 40 day of storage).

Inclusion of WL in diets led to 23- to 30% increases in α-tocopherol in eggs, maximum á-tocopherol concentration was observed in the eggs from hens fed content 250 g WL/kg. The α-tocopherol content in egg yolk decreased significantly ($P < 0.001$) in all groups by day 20 and 40 compared to the fresh eggs. By day 0, 20, and 40, á-tocopherol content in egg yolk were significantly higher ($P < 0.05$) in experimental groups compared to the control (table 5).

Egg storage for 40 day led to over 35-40% reduction in egg α-tocopherol. These findings corroborate previous results of Hayat et al. [37], who observed about a over 50% decline in α-tocopherol concentration of eggs after 40 day of storage at 4°C. The difference in the extent of α-tocopherol decrease and duration of storage may be due to the fatty acid composition of the eggs. The decrease in á-tocopherol concentration of eggs upon storage may suggest the PUFA-protecting roles of α-tocopherol against oxidative damage, confirming previous results obtained by other authors [37].

Inclusion of WL in diets led to increases in á-tocopherol in eggs and decrease in MDA in eggs ($P < 0.05$). The concentration of MDA was higher in egg yolk samples from the control diet compared with white lupine seed meal based diets groups (table 5). In the present study, the lowest MDA production in yolk was found in the E_{25} group in fresh egg. These egg yolks also had the highest mean α-tocopherol, which could contribute to highest egg antioxidant activity. The higher levels of tocopherol in yolks fat for white lupine seed meal groups might have positive

Health lipid indices	Dietary treatment[1]				SEM[2]	p-values
	C	E_{15}	E_{20}	E_{25}		D[3]
PUFA/SFA	0.487[b]	0.649[a]	0.677[a]	0.695[a]	0.012	***
MUFA/SFA	1.373[a]	1.290[b]	1.276[b]	1.237[b]	0.022	*
n-6/n-3 FA	8.26[c]	9.67[b]	9.82[ab]	10.02[a]	0.173	*
HFA/UFA	0.395	0.375	0.381	0.377	0.009	ns
h/H	2.361[b]	2.534[a]	2.509[a]	2.550[a]	0.037	*
AI	0.547	0.524	0.518	0.523	0.005	ns
TI	0.928[a]	0.874[b]	0.865[b]	0.874[b]	0.009	*
LA/ALA	22.90[b]	26.13[a]	26.21[a]	26.85[a]	0.412	**

Means within a row with different superscripts are different ($P<0.05$);
[1]C: control diet with no lupine seed, E_{15}: diet containing lupine seed (150 g/kg), E_{20}: diet containing lupine seed (200 g/kg), E_{25}: diet containing lupine seed (250 g/kg);
[2]SEM: standard error of the mean; [3]D: effect of experimental diet (ns: $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$); FA: fatty acid; SFA: saturated FA; UFA: unsaturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; LA: linoleic acid; ALA: linolenic acid; HFA: hypercholesterolemic FA (C12:0 + C14:0 + C16:0); h/H: hypocholesterolemic/Hypercholesterolemic ratio; AI: atherogenicity index; TI: thrombogenicity index.

Table 4
THE EFFECT OF LUPINE SEED MEAL ON THE HEALTH LIPID INDICES OF EGG YOLKS

Storage time (days)	Contant of yolk	Dietary treatment[1]				SEM[2]	p-values
		C	E_{15}	E_{20}	E_{25}		D[3]
0	- α-tocopherol (µg/g of yolk)	27.6^{b}	34.1^{a}	34.6^{a}	36.2^{a}	0.314	**
	- MDA (mg/100 g of yolk)	3.09^{a}	2.86^{ab}	2.45^{b}	2.08^{b}	0.041	**
20	- α-tocopherol (µg/g of yolk)	20.8^{b}	28.5^{a}	29.8^{a}	28.2^{a}	0.286	**
	- MDA (mg/100 g of yolk)	3.28^{a}	3.05^{ab}	2.70^{b}	2.57^{b}	0.058	**
40	- α-tocopherol (µg/g of yolk)	17.1^{b}	22.4^{a}	23.7^{a}	23.3^{a}	0.332	*
	- MDA (mg/100 g of yolk)	4.89^{a}	3.77^{b}	3.32^{b}	3.81^{b}	0.094	*
Source of variation ---------- p-values ----------							
Storage time:							
	- α-tocopherol	0.001					
	- MDA	0.017					

Means within a row with different superscripts are different (P<0.05);
[1]C: control diet with no lupine seed, E_{15}: diet containing lupine seed (150 g/kg), E_{20}: diet containing lupine seed (200 g/kg), E_{25}: diet containing lupine seed (250 g/kg);
[2]SEM: standard error of mean; [3]D: effect of experimental diet (*p <0.05, **p < 0.01);
MDA: malondialdehyde

Table 5
EFFECT OF FEEDING LUPINE SEED ON α-TOCOPHEROL CONTENT AND OXIDATIVE STABILITY OF EGG YOLK DURING STORAGE

Correlation		Pearson's (r)	p*
MDA	- storage time	-0.143	0.37
	- PUFAs	0.524	< 0.001
	- FA n-6	0.094	0.53
	- FA n-3	0.362	< 0.01
	- α-tocopherol	-0.287	< 0.01

MDA: malondialdehyde; PUFAs: polyunsaturated fatty acids;
FA: fatty acids
*p value indicates the significance of the correlation.

Table 6
CORRELATION BETWEEN OXIDATIVE STABILITY (CONCENTRATION OF MDA), STORAGE TIME AND CONTENT OF PUFAS AND α-TOCOPHEROL CONTENT IN EGG YOLKS

implications in human nutrition as these substances, besides the protective PUFAs, reduce cholesterol oxidation, and therefore its cytotoxicity and atherogenicity [46].

Egg storage for 20 d or 40 d led to linear reduction in egg á-tocopherol and increase of MDA content (P <0.05). These studies demonstrate that the level and type of fatty acids polyunsaturated, level of á-tocopherol and duration of egg storage significantly affected the oxidative stability of eggs. There is an inverse relationship between MDA content, an indicator of egg yolk lipid peroxidation [47], and tocopherols level in poultry diet [48].

Subsequent analysis showed that yolks fat MDA concentrations were positively correlated with PUFAs concentrations (r = 0.524, P <0.001) and especially n-3 FAs (r = 0.362, P <0.01). In contrast, there was an inverse correlation between yolks fat MDA concentrations and yolks á-tocopherol levels (r = -0.287, P <0.01) (table 6). Similar results were reported by Vitas et al. [49] for fermented milk products.

Conclusions

The fatty acid compositions of the egg yolks of laying hens fed diets containing white lupine seed meal have been improved in accordance with their proportion in diets. The hens fed the control diet laid eggs showing higher percentages of monounsaturated fatty acids and lower amounts of polyunsaturated fatty acids than the egg yolks of hens fed diets containing white lupine seed meal (P <0.01). There was a linear decrease of approximately 12% in the amount of total MUFA in the egg yolks of laying hens fed lupine seed and a increase of total PUFA approximately 39% compared of control diet. The yolks of eggs from hens fed diets with white lupine seeds had a higher n-3 and n-6 PUFA content, regardless of their inclusion levels. The lowest n-6/n-3 PUFA ratio was noted in the yolks of eggs from hens fed control diet (P <0.05). The inclusion of white lupine seeds in layer diets contributed to an decrease in the thrombogenicity index (TI) and not affected the atherogenicity index (AI) and cholesterol content in egg yolk. The high concentrations of PUFAs and especially n-3 FA were associated with an increasing risk of lipid oxidation. The inclusion of WL in the diet of laying hens increased the α-tocopherol content and antioxidant activity in egg yolks. These parameters revealed that white lupine seed has good potential as a natural antioxidant and could contribute to preventing lipid oxidation in egg yolks. Therefore, the inclusion of white lupine seeds in layer diets at 15-25% could improve the fatty acid profile, health lipid indices and oxidative stability of egg yolk lipids.

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