PROCESSING AND PRODUCTS

Effect of yellow lupine (*L. luteus*) on the egg yolk fatty acid profile, the physicochemical and sensory properties of eggs, and laying hen performance

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ABSTRACT The aim of this study was to evaluate the effects of different dietary inclusion of raw yellow lupine seed meal (YLM) on laying hen performance, the fatty acid (FA) profile, physicochemical, and sensory properties of eggs. A total of 224 Lohmann Brown laying hens at 32 wk age were fed isonitrogenous and isocaloric diets for 16 wk. The control diet contained soybean meal (SBM), and in study diets SBM was replaced with YLM at 100, 200, or 300 g/kg. In comparison with soybean, lupine seeds had a higher content of nonstarch polysaccharides (NSP) and raffinose family oligosaccharides (RFO) (29.5 vs. 14.0 and 8.56 vs. 5.91% DM). The dietary 300 g/kg lupine seeds increased the content of NSP and RFO in the ration, from 9.34 to 13.39 and 1.36 to 2.54%, respectively. The YLM inclusion level had no adverse effect on laying performance, including feed intake, FCR, egg production, and egg weight. The final BW of hens fed lupine-based diets were significantly higher compared with the control ($P = 0.039$). Throughout the study, dietary treatments had no effect on eggshell and albumen quality. An increase in the inclusion rate of YLM was followed by a linear increase ($P < 0.001$) in yolk color intensity. Dietary treatments had no influence on the aroma, taste, and texture of eggs evaluated in laying hens at 46 wk age. The inclusion of lupine seeds in experimental diets caused a linear increase in n-6 polyunsaturated FA (PUFA) content and the n-6/n-3 ratio (all $P < 0.001$), but it had no influence on the atherogenic and the thrombogenic indices of egg yolk lipids. The results of this study indicate that YLM can be included at 300 g/kg in layer diets as a partial substitute for soybean meal without compromising laying performance, the physicochemical, and sensory properties of eggs.

Key words: yellow lupine, laying performance, egg-yolk fatty acid profile, sensory properties of eggs

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INTRODUCTION

Soybean meal (**SBM**) is probably the best quality vegetable protein source used as livestock feed in the world. Increased worldwide demand for SBM used as a protein source in pig and poultry diets drives up its prices. In Europe, recent years have witnessed an increased interest in alternative vegetable protein sources that could partially or totally replace soybean protein in poultry diets (Jankowski et al., 2011; Mikulski et al., 2012).

Seeds of modern sweet lupine varieties (*Lupinus* sp.) are characterized by a relatively high protein content, a low alkaloid content (Jezierny et al., 2011), a desirable fatty acid (**FA**) profile (Chiofalo et al., 2012), and high nonstarch polysaccharide (**NSP**) concentrations (Petterson, 2000; Mierlită, 2013). According to van Barneveld (1999), optimum egg production can be maintained if dietary lupine inclusion levels do not exceed 100 to 200 g/kg. Hammershøj and Steenfeldt (2005) reported that introducing blue lupine meal in a proportion of 150 g/kg in laying hen diets did not affect egg production, egg quality, or feed utilization. According to the cited authors, feeding a diet containing 250 g/kg lupine seeds, without methionine supplementation, significantly reduced layer performance, including feed intake and feed conversion, egg weight, and egg production. Studies by other authors (Perez-Maldonado et al., 1999; Prinsloo et al., 1992) demonstrated that blue or white lupine seeds can be included at up to 250 to 300 g/kg in layer diets without a negative effect on laying performance or egg quality. In this context, the effectiveness of yellow lupine seed meal (**YLM**) remains insufficiently researched. Therefore, the objectives of this study were to determine the dose response effect of layer diet supplementation with 100, 200, or 300 g yellow lupine seeds/kg on: 1) hen performance and egg traits during a 16-wk period from 33 to 48 wk age, 2) the FA profile of egg yolk lipids and the related

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health lipid indices of egg yolk at 48 wk age, and 3) the sensory properties of eggs.

MATERIALS AND METHODS

All procedures used in this study were approved by the Animal Ethics Committee at the University of Warmia and Mazury (Olsztyn, Poland).

Birds, Management, and Diets

A total of 224 Lohmann Brown laying hens aged 32 wk, with almost identical initial BW, were randomly assigned to 4 treatment groups. Each treatment consisted of 56 laying hens that were individually caged in Big Dutchman double-sided, 3-tier battery cages (40 × 35 × 60 cm, with a floor slope of 12°; 740 cm^2 per hen). The treatment groups were equally distributed between upper, middle, and lower tiers to minimize cage-level effect. In pre-experimental period, 18-week-old birds were purchased from a local commercial flock, where they had been vaccinated against infectious bronchitis, Newcastle disease virus, and egg drop syndrome disease. All hens were housed in a windowless environmentally controlled room, at ambient temperature of 20 to 22°C. In the light cycle, the number of hours of light was increased from 12L:12D at 18 wk age to 16L:8D at the rate of 1 h/wk (incandescent lighting, 12 lx).

Each cage was equipped with an individual nipple drinker. A continuous, metal feed trough was divided by replicate to ensure that the hens were not able to consume feed assigned to the adjoining replicate. A wire egg collector was installed in the front of each cage to prevent mixing of eggs from separate replicates.

The trial lasted for 16 wk, from 33 to 48 wk age. During the study period, the birds were fed four isonitrogenous and isocaloric diets supplemented with 0 (control), 100, 200, and 300 g/kg YLM as a substitute for soybean meal (Groups L_0, L_{100}, L_{200}, and L_{300}, respectively; Table 1). Seeds of sweet (low-alkaloid) yellow lupine [*Lupinus luteus* (*L. luteus*) cv. Mister] from the 2011 harvest were purchased from the Plant Breeding Station in Tulce, Wiatrowo (Poland). The AMEn content of yellow lupine seeds was assumed at 1,990 kcal/kg based on the Polish feedstuff analysis tables (Smulikowska and Rutkowski, 2005). The analyzed chemical composition of soybean meal and YLM, and laboratory procedures used in this study were described elsewhere (Zduńczyk et al., 2014). Before inclusion in the diets, raw lupine seeds with hulls were ground to pass through a 3-mm sieve in a hammer mill (Jesma Company, Sprout Matador, Denmark). Diets in mash form were produced in the "Agrocentrum" feed mill in Kaleczyn (Poland), and were formulated to meet the nutrient requirements of laying hens, i.e., to contain 2,700 kcal AMEn/kg and 16.5% CP (Smulikowska and Rutkowski, 2005). The content of lysine, methionine, threonine, tryptophan, minerals, and vitamins was similar in all dietary treatments. The average content of crude fiber, NSP, and raffinose family oligosaccharides (RFO) in L_0, L_{100}, L_{200}, and L_{300} diets was as follows: 2.56 to 6.64, 9.34 to 13.4, and 1.36 to 2.54%, respectively. Study diets and water were provided ad libitum throughout the 16-wk study.

Table 1. Ingredient composition and calculated nutrient content (percent, as-fed basis) of complete diets containing different levels of yellow lupine seed meal (YLM) and fed to laying hens from 33 to 48 wk age.

	Treatment[1]			
	C	L_{100}	L_{200}	L_{300}
Diet composition				
Wheat (12.0% of CP)	24.10	19.80	15.50	11.20
Triticale (12.0% of CP)	25.00	25.00	25.00	25.00
Corn (8.5% of CP)	20.00	20.00	20.00	20.00
Soybean meal (46.0% of CP)	19.45	13.02	6.58	0.15
YLM (34.7% of CP)	–	10.00	20.00	30.00
Soybean oil	0.56	0.93	1.31	1.69
Lard	0.56	0.93	1.31	1.69
Sodium chloride	0.37	0.37	0.37	0.36
Limestone	9.00	8.98	8.96	8.94
Monocalcium phosphate	0.55	0.56	0.57	0.58
DL-Methionine (99.0%)	0.15	0.14	0.13	0.12
L-Lysine HCl (78.0%)	0.01	0.02	0.02	0.02
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25
Calculated analysis[3]				
AMEn, kcal/kg	2,700	2,700	2,700	2,700
CP	16.50	16.50	16.50	16.50
Crude fiber	2.56	3.92	5.28	6.64
Nonstarch polysaccharides	9.34	10.69	12.04	13.39
Raffinose family oligosaccharides	1.36	1.75	2.15	2.54
Arginine	0.99	1.16	1.33	1.50
Lysine	0.78	0.78	0.78	0.78
Methionine and cysteine	0.70	0.70	0.70	0.70
Calcium	3.60	3.60	3.60	3.60
Available phosphorus	0.25	0.25	0.25	0.25
Sodium	0.16	0.16	0.16	0.16
Analyzed fatty acid content				
Oleic acid (C18:1 cis 9)	24.10	29.54	29.33	29.23
Linoleic acid (C18:2 n-6)	43.00	40.33	41.93	44.52
α-Linolenic acid (C18:3 n-3)	3.32	2.18	2.05	2.19
n-6 PUFA	43.06	40.41	42.03	44.63
n-3 PUFA	3.32	2.18	2.05	2.19
n-6/n-3 PUFA ratio	12.97	18.54	20.50	20.38

[1]YLM was applied at the following dietary levels: 0, 100, 200, and 300 g/kg (C, L_{100}, L_{200}, and L_{300}, respectively).

[2]Supplied the following per kilogram feed: 10,000 IU vitamin A, 2,500 IU vitamin D_3, 25 mg vitamin E, 2.0 mg vitamin K_3, 1.6 mg vitamin B_1, 6 mg vitamin B_2, 3 mg vitamin B_6, 0.02 mg vitamin B_{12}, 0.125 mg biotin, 30 mg nicotinic acid, 12 mg pantothenic acid, 1,240 mg choline, 82.5 mg Mn from magnesium oxide, 40 mg zinc from zinc oxide, 40 mg Fe from ferrous sulfate, 1.0 mg I from ethylene diamine dihydroiodide, 0.2 mg Se from sodium selenite, 0.03 mg Mo, 5 mg Cu.

[3]Calculated based on analyzed chemical composition of soybean meal and yellow lupine seed meal, and Polish feedstuff analysis tables (Smulikowska and Rutkowski, 2005).

Traits Recorded and Methods Applied: Laying Performance

The birds were weighed at the beginning (32 wk age) and at the end (48 wk age) of the trial. Egg production, egg weight, and feed consumption were monitored on a per hen basis. Daily feed consumption (DFI) per bird

was calculated on a total feed consumption basis for the entire study period, i.e., 16 wk and for the number of days in the period. FCR (kilogram feed / kilogram eggs) was calculated from egg production, egg weight, and feed consumption.

Eggs were collected daily and classified as either normal or damaged for calculating the rate of damaged eggs; the latter included misshapen eggs, broken eggs, cracked eggs, and shell-less eggs. A misshapen egg is defined as one that has major changes in the shape, i.e., it is round instead of oval, flat-sided, and body-checked with grooves and ridges. Egg production was expressed on a hen-day basis (percent hen-day). Individual egg weights were recorded by weighing individually 2 fresh eggs per hen at 2-wk intervals (a total of approximately 896 eggs per group during the study), and the data were used to calculate mean egg weight for the study period. Total egg mass was calculated by multiplying egg weight by egg production.

Egg Quality

The quality of eggs laid by hens was evaluated at 36 wk age, and then at 4-wk intervals (at 40, 44, and 48 wk age). For this purpose, eggs laid by 20 hens from each group were collected each time, between 9:00 a.m. and 12:00 p.m. (a total of 80 eggs per group during the study). Specific gravity, eggshell thickness and breaking strength, yolk weight and color, and albumen Haugh unit score were determined for each egg. Eggs were weighed individually, and the specific gravity of eggs, as an indicator of eggshell thickness, was measured using a densitometer (Axis Hydro AD, Gdansk, Poland). Eggshell thickness was the mean value of measurements at 3 locations on the eggs (air cell, equator, and sharp end), determined with an egg shell thickness gauge (**ESTG**; i.e., ESTG-1, ORKA Food Technology Ltd., Ramat Hasharon, Israel). Eggshell breaking strength was measured with an egg force reader (ORKA Food Technology Ltd., Ramat Hasharon, Israel). Next, the eggs were broken to assess albumen quality using an EggAnalyzer (ORKA Food Technology Ltd., Ramat Hasharon, Israel), based on albumen height and egg weight. Albumen quality was expressed as a Haugh unit score for each egg, according to Haugh's formula (1937). Yolk color intensity was evaluated and scored according to the DSM® yolk color fan (1, light yellow; 15, orange), with the use of the same device. The yolk was then separated from the albumen using a Teflon spoon. Before yolk weight was determined, the chalaza was removed with a spatula and each yolk was rolled on a blotting paper towel to remove adhering albumen. Yolk weight was expressed as a percentage of total egg weight. Egg internal and external quality analyses were completed within 24 h after egg collection. Over that period of time, eggs were stored at a temperature of 16°C.

Cholesterol Concentrations and the FA Profile of Egg Yolk

At 48 wk age, i.e. after 16 wk laying season, 20 eggs picked from each group for the assessment of quality attributes were used to determine the cholesterol content and the FA profile of egg yolks. Total cholesterol concentrations and the FA profile were determined on individual eggs, in the fat separated via extraction from the egg yolk with a chloroform and methanol mixture (2:1 vol/vol; Folch et al., 1957). Cholesterol was separated from fat after saponification with KOH and extraction with ethyl ether, by the modified method of the International Dairy Federation (1992). The sample was subjected to chromatographic analysis in a PU-4600 (Pye Unican, Cambridge, UK) chromatograph with a flame ionization detector, 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°C; temperature of injector, 290°C; temperature of column, 260°C; carrier gas, argon, flow rate, 50 cm^3/min; internal standard, dotriacontane (Sigma, St. Louis, MO). Egg yolk cholesterol content was calculated and expressed as milligrams per gram of yolk lipids.

The extracted fat was esterified with a chloroform, methanol, and sulfuric acid mixture, as described by Peisker (1964). The resulting fatty acid methyl esters were analyzed using a 7890A gas chromatograph from Agilent Technologies with a flame ionization detector and a Supelcowax 10 capillary column (column length, 30 m; internal diameter, 0.32 mm; film thickness, 0.25 μm; carrier gas, helium; temperature of detector, 250°C; temperature of injector, 230°C; temperature of column, 195°C). The peaks of FA were identified by comparing their relative retention times with those of individual FA methyl ester reference standards (Supelco) diluted in hexane (1:1, 1:2, 1:3, 1:4 vol/vol).

The percentage content of FA and the total pool of polyunsaturated FA (**PUFA**), including n-3 PUFA (C18:3, C22:5, and C22:6) and n-6 PUFAs (C18:2, C20:2, C20:3, and C20:4), were determined by relative FA quantification, and percent the total FA peak area was calculated. The remaining FA categories were calculated in terms of saturated FA (**SFA**) and monounsaturated FA (**MUFA**) as:

$$\begin{aligned}\text{SFA} = sum\,of\, C13{:}0 + C14{:}0 + C15{:}0 + C16{:}0 \\ + C17{:}0 + C18{:}0 + C20{:}0 + C22{:}0 \\ + C23{:}0 + C24{:}0\end{aligned}$$

$$\begin{aligned}\text{MUFA} = sum\,of\, C16{:}1 + C17{:}1 + C18{:}1n-9 \\ + C20{:}1n-9 + C20{:}1n-7 + C22{:}1n-9.\end{aligned}$$

From the data on FA composition, the following were calculated:

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 phospholipids, thereby preventing the occurrence of coronary microvascular and macrovascular diseases). The following equation was applied:

AI = C12:0 + 4*C14:0 + C16:0/MUFA + PUFA, where C12:0, C14:0, C16:0, MUFA, and PUFA are the content (percent total FA) of C12:0, C14:0, C16:0, MUFA, and PUFA, respectively.

Thrombogenic index (**TI**): reflecting the tendency to form clots in the blood vessels, defined as the relationship between the prothrombogenetic (saturated) and the anti-thrombogenetic FA (MUFA, n-6 PUFA, and n-3 PUFA). The following equation was applied:

TI = C14:0 + C16:0 + C18:0/(0.5*MUFA) + (0.5*n-6 PUFA) + (3*n-3 PUFA) + (n-3 PUFA/n-6 PUFA), where C14:0, C16:0, C18:0, MUFA, n-6 PUFA, and n-3 PUFA are the content (percent total fatty acids) of C14:0, C16:0, C18:0, MUFA, n-6 PUFA, and n-3 PUFA, respectively (Ulbricht and Southgate, 1991).

Sensory Evaluation

In wk 16 of the laying season, i.e., at 48 wk age, 20 eggs were randomly selected from each group for a sensory analysis. A 6-member trained panel (ISO 8586-2 1996), experienced in a descriptive analysis of different food products and familiarized with the sensory quality of eggs, performed the assessments. The assessments were carried out in a sensory laboratory room that fulfilled the requirements of the ISO standard (ISO 8589 1998), in individual booths equipped with a computerized system for data collection and processing (FIZZ, Biosystemes, Counternon, France), as described elsewhere (Horszwald et al., 2009).

For a sensory evaluation, eggs were cooked in boiling water for 12 min, cooled to an external temperature of approx. 20°C, shelled and cut in half, and the yolks were placed in transparent plastic containers covered with lids to preserve odor compounds. The samples were presented in random order to the assessors from 3-digital code. Together with the samples, the panelists received a cup of room temperature spring water for cleaning their palates. Panelists were asked to focus first on the aroma and color, and next on the taste and texture of the separated yolk.

A quantitative descriptive analysis was performed to determine the sensory characteristics of the samples. The panelists, together with the panel leader, established the descriptions of the main sensory attributes of eggs using a standard procedure (ISO/DIS 13299 1998). Eleven attributes related to the color, aroma, taste, and mouth feel (adhesiveness) of eggs were selected and thoroughly defined for profiling. The panelists evaluated intensity perceived for each of the attributes on a continuous unstructured graphical scale. The scale was 10-cm long and verbally anchored at each end. The left side of the scale corresponded to the lowest intensity and the right side to the highest intensity of the attribute. The results were automatically converted to numerical values (from 0 to 10 U) by a computer. All samples were evaluated in 3 replications (3 sessions) preceded by an introductory session.

Statistical Analysis

One-way ANOVA was performed to determine the effect of the inclusion level of lupine in study diets. For performance data, a cage (each laying hen) was considered as a replicate study unit for the statistical analysis. The model assumptions of normality and homogeneity of variance were examined by the Shapiro–Wilk and Levene tests, respectively. The Newman–Keuls multiple comparison test was used to separate different means among treatments. In addition, linear polynomial contrast was used to evaluate the effect of lupine inclusion. For statistical comparisons, BW gain data were subjected to arcsin transformation followed by one-way analysis of covariance with feed consumption as the dependent variable (covariate). All calculations were made using the STATISTICA software system ver. 10PL (StatSoft Inc., 2011). The effects were considered to be significant at $P \leq 0.05$, and were expressed as mean values with pooled SE.

RESULTS

Chemical Composition of Yellow Lupine and Diets

Raw yellow lupine seeds contained a moderate level of CP (39.98% DM vs. 46.75% in SBM), and relatively low levels of lysine (4.71% CP vs. 6.03% in SBM) and methionine (0.46% CP vs. 1.32% in SBM). The total alkaloid content of lupine seeds was low (270 mg/kg), including 170.9 mg lupanine as the main alkaloid. The concentrations of structural carbohydrates, determined as crude fiber and NSP, were 19.23 and 29.51%, respectively (vs. 3.82 and 14.05%, respectively, in SBM). Stachyose was the main RFO, and total RFO content was 8.56% (vs. 5.91% in SBM). The diets compared in this study had an identical total protein and ME content. The diets supplemented with 300 g raw YLM/kg contained 40.8 g/kg more crude fiber, 40.5 g/kg more NSP, and 11.8 g/kg more RFO, respectively, than the control diet (Table 1). Compared with the SBM diet, YLM-based diets had higher concentrations of oleic acid (C18:1 *cis* 9; 29.4 vs. 24.1%) and lower levels of total n-3 PUFA (2.2 vs. 3.3%).

Table 2. The effect of different levels of yellow lupine seed meal (YLM) on the production parameters of hens during the laying period from 33 to 48 wk age[1].

	Treatment[2]				Polled	*P* - value		
	C	L_{100}	L_{200}	L_{300}	SEM	Groups	Linear	Quadratic
Hen-day egg production (%)	97.34	97.95	97.67	97.78	0.119	0.314	0.331	0.281
Average egg weight (g)	64.26	64.42	63.71	64.50	0.694	0.694	0.998	0.541
Total egg mass (kg of eggs/hen)	7.00	7.07	6.98	7.07	0.030	0.580	0.705	0.816
Daily feed intake (g/hen)	127.6	125.0	123.5	127.8	0.714	0.095	0.866	0.016
Rate of damaged eggs (%)	1.36	0.60	0.80	1.10	0.136	0.212	0.629	0.058
Feed conversion ratio (kg of feed /kg of eggs laid)	2.044	1.983	1.986	2.026	0.010	0.087	0.575	0.014
Final body weight (kg/bird) [3]	1.92[b]	2.02[a]	2.00[a]	1.99[a]	0.012	0.039	0.090	0.051
Body weight change (%)	2.20[b]	5.96[a]	7.59[a]	5.80[a]	0.467	< 0.001	< 0.001	0.001

[1]Data represent mean values of 56 hens per treatment. SEM = standard error of the mean (SD divided by the square root of replication number, n = 224).

[2]YLM was applied at the following dietary levels: 0, 100, 200, and 300 g/kg (C, L_{100}, L_{200}, and L_{300}, respectively).

[3]After 16 wk feeding (48 wk age).

[a,b]Means within the same row with different superscripts differ significantly ($P < 0.05$).

Laying Performance

Laying performance parameters are summarized in Table 2. There were no significant differences in egg production, average egg weight, and the rate of damaged eggs between hens fed lupine-based diets and hens fed the control diet. The lupine-based diets did not significantly affect feed intake or feed efficiency in hens. However, it was found that DFI and FCR decreased at the 0 to 200 g YLM content of the diets, and increased when the inclusion level of YLM reached 300 g/kg (quadratic contrast $P = 0.016$ and 0.014, respectively). During the study period, bird deaths were not recorded in any of the groups. The initial BW of hens was comparable in all groups (approx. 1.89 kg at 32 wk age), whereas the final BW of hens in Study Groups L_{100} to L_{300} (1.99 to 2.02 kg) were significantly higher than in the control group (1.92 kg, $P = 0.039$).

Egg Components and Egg Quality

No differences were found between the groups with respect to eggshell characteristics, egg albumen quality expressed as Haugh units, and relative yolk weight (Table 3). In all analyzed periods and throughout the study, yolk color intensity increased significantly ($P < 0.001$) in response to increasing YLM inclusion level.

Cholesterol Concentrations, the FA Profile, and Sensory Properties of Egg Yolk

There were no significant differences in egg yolk cholesterol content between hens fed lupine-based diets and hens fed the control diet (Table 4). An analysis of yolk FA revealed that the major FA were oleic acid (C18:1 *cis*9), palmitic acid (C16:0), and linoleic acid (**LA**; C18:2 n-6). Lupine-based diets contributed to a significant decrease ($P \leq 0.001$) in the concentrations of myristoleic acid (C14:1), palmitoleic acid (C16:1), and oleic acid (C18:1 *cis*9), and to a significant increase ($P \leq 0.05$) in the levels of α-linolenic acid (C18:3 n-3) and all n-6 PUFA in egg yolks. The inclusion of YLM at up to 300 g/kg in layer diets did not affect the proportion of n-3 PUFA in yolk fat, but it significantly increased the share of n-6 PUFA and, consequently, the n-6/n-3 PUFA ratio (both $P < 0.001$), compared with the control group. Despite the above, the values of the AI and TI were similar in control and YLM groups.

Dietary treatments did not influence the sensory properties of eggs evaluated in laying hens aged 46 wk, including aroma, taste, and texture (Table 5).

DISCUSSION

Previous research (Watkins and Mirosh, 1987) has shown that alkaloids present in diets with 30% lupine seeds usually reduce feed palatability due to their bitter taste, thus negatively affecting feed intake and depressed egg production. Our results are consistent with the findings of other authors who demonstrated that the inclusion of sweet lupine seeds to laying hen diets did not depress egg production or other performance parameters (Perez-Maldonado et al., 1999; Laudadio and Tufarelli, 2011; Mierlită, 2013) and had no negative influence on the analyzed egg quality parameters (Laudadio and Tufarelli, 2011). It should be stressed that in our study, feed efficiency did not deteriorate, despite the fact that the relatively high amount of 300 g YLM/kg provided a dietary load of NSP and RFO in comparison with the control treatment. The BW of laying hens is known to be negatively correlated with egg production (Akhtar et al., 2003), but in the present study, an increase in the final BW of hens fed YLM-based diets did not decrease hen-day egg production. In an earlier study by Hammershøj and Steenfeldt (2005), the inclusion of 25% blue lupine in layer diets reduced the final BW of hens, but it also decreased egg production, egg weight, and feed intake. However, as suggested by the cited authors, those adverse effects were due to dietary amino acid imbalance.

Egg yolk color is one of major parameters linked to consumer requirements concerning eggs. Increased yolk

Table 3. The effect of different levels of yellow lupine seed meal (YLM) on the physicochemical properties of eggs.[1]

	Treatment[2]				Pooled	*P* - value		
	C	L_{100}	L_{200}	L_{300}	SEM	Groups	Linear	Quadratic
Egg specific gravity (g/cm^{-3})								
36 wk	1.083	1.085	1.086	1.084	0.007	0.513	0.537	0.169
40 wk	1.085	1.086	1.087	1.085	0.001	0.819	0.945	0.373
44 wk	1.084	1.085	1.086	1.084	0.001	0.404	0.863	0.107
48 wk	1.090	1.087	1.087	1.089	0.001	0.207	0.421	0.049
From 36 to 48 wk	1.085	1.086	1.086	1.085	0.001	0.704	0.986	0.280
Eggshell thickness (mm)								
36 wk	0.350	0.351	0.360	0.350	0.002	0.381	0.651	0.251
40 wk	0.356	0.359	0.371	0.351	0.003	0.213	0.927	0.094
44 wk	0.354	0.362	0.373	0.364	0.002	0.068	0.068	0.096
48 wk	0.373	0.370	0.368	0.378	0.003	0.657	0.597	0.294
From 36 to 48 wk	0.358	0.361	0.366	0.361	0.002	0.286	0.324	0.195
Eggshell breaking strength (N)								
36 wk	39.71	40.31	41.30	39.66	0.876	0.906	0.917	0.530
40 wk	37.90	40.15	40.29	38.83	0.915	0.771	0.724	0.321
44 wk	39.44	41.86	41.64	38.87	0.990	0.631	0.829	0.198
48 wk	41.58	39.87	41.82	41.81	0.800	0.799	0.717	0.599
From 36 to 48 wk	39.66	40.55	41.26	39.79	0.449	0.565	0.781	0.191
Albumen - Haugh units								
36 wk	85.28	85.31	85.53	84.22	0.569	0.854	0.565	0.563
40 wk	84.94	86.41	84.62	86.30	0.748	0.776	0.737	0.944
44 wk	84.10	84.85	83.43	83.16	0.639	0.798	0.466	0.697
48 wk	84.26	83.74	83.80	84.76	0.612	0.932	0.778	0.554
From 36 to 48 wk	84.65	85.08	84.35	84.61	0.323	0.885	0.772	0.899
Yolk color scores								
36 wk	8.35^{c}	9.45^{b}	9.75^{a,b}	10.30^{a}	0.132	<0.001	<0.001	0.202
40 wk	8.15^{c}	9.25^{b}	9.50^{a,b}	9.95^{a}	0.130	<0.001	<0.001	0.140
44 wk	8.02^{b}	9.15^{a}	9.60^{a}	9.80^{a}	0.146	<0.001	<0.001	0.069
48 wk	9.25^{b}	9.50^{b}	9.60^{b}	10.15^{a}	0.095	0.005	<0.001	0.402
From 36 to 48 wk	8.44^{c}	9.34^{b}	9.61^{b}	10.05^{a}	0.064	<0.001	<0.001	0.040
Yolk percentage (%)[3]								
36 wk	25.46	25.60	25.75	25.87	0.148	0.792	0.312	0.985
40 wk	24.82	25.98	26.00	26.16	0.201	0.065	0.024	0.207
44 wk	26.03	25.26	26.12	25.61	0.175	0.280	0.799	0.711
48 wk	25.89	25.46	25.66	26.22	0.170	0.441	0.440	0.152
From 36 to 48 wk	25.55	25.57	25.87	25.97	0.087	0.220	0.045	0.843

[1]Data represent mean values of 20 hens per treatment (one egg per hen). SEM = standard error of the mean (SD divided by the square root of replication number, n = 80).

[2]YLM was applied at the following dietary levels: 0, 100, 200, and 300 g/kg (C, L_{100}, L_{200}, and L_{300}, respectively).

[3]Measured as a proportion (weight/weight) of the whole egg (including the shell), expressed as a percentage.

[a–c]Means within the same row with different superscripts differ significantly ($P < 0.05$).

color intensity in hens fed YLM-based diets, observed in our study, may be related to the presence of natural pigments (lutein, zeaxanthin, and β-carotene) in lupine seeds (Wang et al., 2008). Other authors (Watkins and Mirosh, 1987; Hammershøj and Steenfeldt, 2005; Laudadio and Tufarelli, 2011; Drażbo et al., 2014) also reported that diets supplemented with 10 to 30% white or blue lupine seeds increased the deposition of yellow pigments in the egg yolk. On the other hand, it is known that preferences for a certain yolk color may vary widely depending on geographical location, culture, and local tradition. For instance, consumers in European countries, including Germany, Belgium, the Netherlands, and Spain, prefer orange egg yolks. Consumers in France, U.K., and Finland prefer yellow egg yolks, whereas in Ireland and Sweden a light color of egg yolk is accepted (Dvořák et al., 2012).

It is important to know that there are considerable differences in the lipid fraction composition between lupine species (Rusníková et al., 2013; Chiofalo et al., 2012). In comparison with white and blue lupines, yellow lupine oil has a higher content of n-6 PUFA, mostly LA (C18:2 n-6) and eicosadienoic acid (C20:2 n-6). White lupine oil is characterized by the highest n-3/n-6 PUFA ratio (Chiofalo et al., 2012), which is why diets containing white lupine seeds increased n-3 PUFA concentrations and decreased n-6 PUFA levels in chicken meat (Straková et al., 2010). It seems that the above differences resulted from the fact that YLM diets were a rich source of LA (C18:2 n-6) and a poor

Table 4. The effect of different levels of yellow lupine seed meal (YLM) on the fatty acid profile (percent total fatty acid content) and cholesterol content of egg yolks in laying hens aged 48 wk.[1]

	Treatment[2]				Pooled	*P* - value		
Fatty acid	C	L_{100}	L_{200}	L_{300}	SEM	Groups	Linear	Quadratic
Myristic (C14:0)	0.41[a]	0.40[a]	0.34[b]	0.29[c]	0.011	<0.001	<0.001	0.275
Myristoleic (C14:1)	0.10[a]	0.07[b]	0.05[c]	0.04[d]	0.005	<0.001	<0.001	0.117
Pentadecanoic (C15:0)	0.07[b]	0.07[a,b]	0.07[a,b]	0.08[a]	0.002	0.047	0.009	0.567
Palmitic (C16:0)	25.69	24.76	24.09	23.78	0.303	0.131	0.022	0.587
Palmitoleic (C16:1)	4.21[a]	2.87[b]	2.54[b,c]	2.14[c]	0.163	<0.001	<0.001	0.530
Margaric (C17:0)	0.15[c]	0.21[b]	0.23[b]	0.27[a]	0.009	<0.001	<0.001	0.657
Margaroleic (C17:1)	0.15	0.15	0.15	0.16	0.003	0.844	0.448	0.693
Stearic (C18:0)	7.49[b]	7.81[a,b]	8.25[a]	8.18[a]	0.092	0.007	0.001	0.222
Oleic (C18:1 *cis*9)	43.25[a]	40.97[b]	40.41[b]	38.60[b,c]	0.447	0.001	<0.001	0.727
Oleic (C18 :1 *cis*11)	2.69[a]	2.16[b]	1.82[c]	1.73[c]	0.077	<0.001	<0.001	0.830
Linoleic (C18:2 n-6)	12.08[d]	16.44[c]	17.97[b]	20.66[a]	0.630	<0.001	<0.001	0.076
α-Linolenic (C18:3 n-3)	0.56[b]	0.60[b]	0.63[a,b]	0.69[a]	0.015	0.015	0.002	0.372
Arachidic (C20:0)	0.01	0.01	0.01	0.01	0.002	0.829	0.477	0.746
Eicosenoic (C20:1)	0.21	0.20	0.20	0.19	0.004	0.402	0.114	0.564
Eicosadienoic (C20:2 n-6)	0.09[c]	0.13[b]	0.14[a,b]	0.16[a]	0.007	<0.001	<0.001	0.478
Eicosatrienoic (C20:3 n-6)	0.11[b]	0.11[b]	0.12[a,b]	0.13[a]	0.003	0.015	0.002	0.706
Arachidonic (C20:4 n-6)	1.75[b]	2.00[a]	2.02[a]	1.99[a]	0.034	0.017	0.012	0.027
DPA (C22:5 n-3)[3]	0.39[b]	0.48[a]	0.42[a,b]	0.33[b]	0.018	0.012	0.107	0.006
DHA (C22:6 n-3)[4]	0.59	0.57	0.56	0.58	0.011	0.862	0.663	0.459
SFA[5]	33.82	33.25	32.97	32.62	0.271	0.499	0.134	0.846
MUFA[6]	50.61[a]	46.42[b]	45.17[b]	42.84[c]	0.615	<0.001	<0.001	0.156
PUFA[7]	15.57[d]	20.33[c]	21.86[b]	24.54[a]	0.664	<0.001	<0.001	0.157
n-3 PUFA	1.54	1.65	1.61	1.60	0.027	0.572	0.567	0.252
n-6 PUFA	14.03[d]	18.68[c]	20.25[b]	22.94[a]	0.655	<0.001	<0.001	0.057
n-6/n-3 PUFA ratio	9.14[d]	11.35[c]	12.67[b]	14.35[a]	0.401	<0.001	<0.001	0.552
Atherogenic index	0.41	0.40	0.38	0.37	0.006	0.114	0.018	0.674
Thrombogenic index	0.91	0.88	0.87	0.86	0.011	0.451	0.121	0.700
Total cholesterol, mg/g fat	38.57	33.54	32.71	29.63	1.244	0.086	0.015	0.332

[1]Data represent mean values of 20 hens per treatment (one egg per hen). SEM = standard error of the mean (SD divided by the square root of replication number, n = 80).

[2]YLM was applied at the following dietary levels: 0, 100, 200, and 300 g/kg (C, L_{100}, L_{200}, and L_{300}, respectively).

[3]Docosapentaenoic acid.

[4]Docosahexaenoic acid.

[5] SFA = Saturated fatty acid.

[6]MUFA = Monounsaturated fatty acid.

[7] PUFA = Polyunsaturated fatty acid.

[a–d]Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 5. Average intensity values of egg sensory attributes.[1]

	Treatment[2]				*P* - value
Attribute	C	L_{100}	L_{200}	L_{300}	
Appearance					
Yolk color	5.9	6.8	6.7	7.4	0.081
Egg-like	6.7	6.7	6.8	6.8	0.767
Buttery	3.0	3.1	3.0	3.2	0.417
Sulfur-like	0.8	0.8	0.8	0.8	0.254
Slightly sweet	1.4	1.4	1.4	1.4	0.376
Taste					
Egg-like	7.0	7.1	6.9	6.9	0.797
Buttery	3.4	3.3	3.4	3.4	0.725
Slightly salty	1.4	1.4	1.3	1.4	0.307
Slightly sweet	1.4	1.3	1.3	1.3	0.553
Aftertaste	5.3	5.3	5.2	5.2	0.427
Texture					
Adhesiveness	5.6	5.7	5.8	5.8	0.668

[1]Data represent mean values of 20 hens per treatment (one egg per hen).

[2]YLM was applied at the following dietary levels: 0, 100, 200, and 300 g/kg (C, L_{100}, L_{200}, and L_{300}, respectively).

source of n-3 PUFA (Table 1), which increased their n-6/n-3 PUFA ratio. Increasing inclusion levels of YLM in layer diets were accompanied by a linear increase in n-6 PUFA concentrations and the n-6/n-3 PUFA ratio in yolk lipids. The LA (C18:2 n-6) is the precursor of arachidonic acid (**AA**; C20:4 n-6), which is reported to be advantageous to consumer's cardiovascular health only when it is present in low levels, due to its antagonistic effect on the health benefits of n-3 FA (Ozogul and Ozogul, 2007). The yolks of eggs from hens fed YLM-based diets had a low AA content, although their concentrations of both AA and LA were significantly higher than in the control group. It should be stressed, however, that YLM-based diets did not affect the AI and TI of the egg yolk, which is another positive aspect.

Previous studies (Vogt et al., 1983) demonstrated that the taste of eggs deteriorated when laying hens were fed diets with 16% bitter lupine seeds. The results of more recent experiments involving laying hens fed diets with sweet blue lupine (Hammershøj and

Steenfeldt, 2005; Drażbo et al., 2014) and our findings with YLM suggest that unlike traditional bitter lupine varieties, modern low-alkaloid varieties have no adverse effects on the aroma, taste, or texture of eggs.

In conclusion, the results of the present study suggest that the seeds of modern yellow lupine varieties can be included at 300 g/kg in practical layer diets as an effective substitute for SBM without compromising layer performance and egg quality, including their sensory properties and the health related lipid indices of egg yolk. Yellow lupine seeds added to layer diets intensified egg yolk color.

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