

The effect of addition of yellow lupin seeds (*Lupinus luteus* L.) to laying hen diets on performance and egg quality parameters

A. Rutkowski¹, M. Hejdysz^{1,5}, S. Kaczmarek¹, M. Adamski³, S. Nowaczewski² and D. Jamroz⁴

Poznań University of Life Sciences ¹ Department of Animal Nutrition and Feed Management, Wołyńska 33, 60-637 Poznań, Poland ² Department of Animal Breeding and Product Quality Assessment, Złotniki, Słoneczna 1, 62-002 Suchy Las, Poland ³ University of Technology and Life Sciences in Bydgoszcz, Department of Poultry Breeding Mazowiecka 28, 85-084 Bydgoszcz, Poland ⁴ Wrocław University of Environmental and Life Sciences, Department of Animal Nutrition and Feed Quality Chełmońskiego 38C, 51-630 Wrocław, Poland

KEY WORDS: *Lupinus luteus*, seeds, hens, performance, egg quality Received: 6 February 2017

Revised: 22 June 2017 Accepted: 31 August 2017

⁵ Corresponding author: e-mail: marhej@up.poznan.pl ABSTRACT. The aim of the present study was to determine the influence of a new variety of yellow lupin (Lupinus luteus L.) var. Mister seeds in laying hens diet on their performance and egg quality parameters. The experiment was performed on 17-week old 360 Hy-Line Brown hens housed in cages. Hens were randomly assigned to five treatments, each with 24 replications. Starting from 21 week of age birds were fed five different diets: control (without lupin seeds) and with 10, 15, 20 and 25% inclusion of yellow lupin seeds. Dietary inclusion of 25% lupin seeds significantly decreased the mean laying rate and egg weight. Also, the lowest dietary consumption and the worst feed conversion ratio were found in birds fed diet with 25% lupin seeds addition; the best results were found in the control animals. As the yellow lupin seeds content in the diets increased, the pH value of yolk and albumen increased, also Haugh unit scores and yolk pigmentation were significantly higher but shell thickness and strength was decreased. So, the inclusion of up to 20% yellow lupin seeds into diet did not decrease laying rate and egg weight. In line with the increasing lupin content in diets the egg quality was deteriorated (except for yolk pigmentation).

Introduction

Soyabean meal (SBM) is the main vegetable protein source in poultry diet. However, in countries that are not climatically adapted to soyabean production the inclusion of home-grown legume seeds into diets for non-ruminant animals as an alternative to soyabean products has been receiving attention (Laudadio and Tufarelli, 2011b; Kaczmarek et al., 2015; Rutkowski et al., 2015; Hejdysz et al., 2016, 2017; Kasprowicz-Potocka et al., 2016). In the past, the use of yellow lupin as a source of protein for poultry was limited due to its high alkaloid, nonstarch polysaccharides (NSP) and other anti-nutrients contents that negatively affected the bird performance (Gdala and Buraczewska, 1996; Knudsen, 1997; Gdala, 1998; Wasilewko and Buraczewska, 1999; Jamroz and Kubizna, 2008; Laudadio and Tufarelli, 2011a). However, in the last decades, plant breeders have succeeded in developing lupin cultivars characterized by a very low alkaloid content. The NSP level in lupin is almost twice as high as in other protein-rich plants (Gdala and Buraczewska, 1996; Knudsen, 1997, 2014) and is still a nutritional problem. These authors claimed that the soluble NSP content in some old Polish cultivars of yellow lupin seeds ranged from 2.0 to over 12.0%. Yet, according to later research, lupin protein is digested to the same degree as from SBM (Hughes and Kocher 1998; Hammershoj and Steenfeldt, 2005) or even better (Kaczmarek et al., 2014, 2015).

The effect of raw materials on yolk colour is a well-known phenomenon: maize contains lutein and zeaxanthin at concentrations of 20 to 25 mg of xanthophyll \cdot kg⁻¹, carrot meal contains 54 to 65 mg of xanthophyll \cdot kg⁻¹ (Sikder et al., 1998; Dvořák et al., 2007). Maize silage (grains and cobs) exert a positive effect on yolk colour when included in diets for laying hens.

Therefore, the aim of the present study was to determine the influence of a new variety of yellow lupin seeds (var. Mister) added into laying hen diets on their performance and egg quality parameters.

Material and methods

Animals and diets

The study was conducted accordingly to the guidelines of the Local Ethics Commission with respect to animal experimentation and care of animals under study.

In total, 360 17-week old Hy-Line Brown hens were weighed and randomly allocated into cages (3 birds in each cage) with ad libitum access to drinking water and feed. Animals were fed pre-laying feed mixture containing 14.5% of crude protein and 11 MJ \cdot kg⁻¹ metabolizable energy from the 17 to 21 week of age. Before the beginning of egg production, birds were randomly assigned to five treatments, 24 replicates in each. The experiment started at the 21 week of age. Birds from the control group were fed a diet based on wheat/native grain in which soyabean meal and rapeseed meal were the main protein sources. Experimental diets consisted of a constant content of peas var. Trachalska (10%); soyabean meal and rapeseed meal were replaced with yellow lupin seeds var. Mister (Table 1) in the amounts of 10, 15, 20 and 25% (Table 2). Animals had free access to water, and isonitrogenous and isoenergetic diets in a mash form. The 14 h light/10 h dark cycle was provided. The chemical composition of diets (Table 2) was calculated using linear optimalization on the basis of our own chemical analysis of the used components.

Yellow	lupin	seeds	in	laving	hen	nutrition

Table	1.	CI	nemica	composition	of	yello	w lupin	seeds	var.	M	ist	ter
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Indices	Content
Dry matter, g · kg⁻¹ seeds	890.1
g · kg⁻¹ DM	
crude ash	41.5
crude protein	389.8
crude fibre	192.3
ADF	242.4
NDF	282.4
crude fat	52.6
carotenes	2.3
xanthophylls	13.6
Gross energy, MJ · kg ⁻¹	20.49
Viscosity, cP	1.09
Amino acid, g amino acid/16 g N	
Asp	8.81
Glu	24.46
Ala	2.83
Cys	2.56
Gly	3.47
lle	3.20
Leu	6.50
Met	0.81
Phe	4.24
Pro	6.08
Ser	4.24
Thr	3.17
Tyr	3.24
Val	3.17
Arg	10.12
Lys	4.76
His	3.32
Antinutrients	
total alkaloids, g · kg⁻¹ DM	0.027
lupinine, in total alkaloids, %	63.29
sparteine, in total alkaloids, %	33.6
ammodendrine, in total alkaloids, %	3.12
oligosaccharides, g · kg ⁻¹ DM	8.57
rafinose, $g \cdot kg^{-1} DM$	1.10
stachyose, g · kg ⁻¹ DM	4.94
verbascose, g · kg ⁻¹ DM	2.53
P-phytate, g · kg ⁻¹ DM	7.0
P-phytate/P total, %	75.0
1 -phylate/1 total, /0	15.0

Collected data

In the study the following parameters were examined:

- initial and final body weight of hens (for each replicate),
- week feed intake and feed conversion ratio (FCR) (for each replicate),
- egg weight with 0.1 g accuracy (g), determined weekly for 20 eggs from each replicate,
- egg shape index (ESI, %) calculated according to the formula:

 $ESI = egg width (mm) \times 100 / egg height (mm),$

Table 2. Composition of experimental diets

	Treatme	ents					
Indices	control	yellow lupin, g · kg⁻¹					
	control	100	150	200	250		
Components, g · kg ⁻¹							
wheat	611.5	536.7	502.8	483.7	479.8		
soyabean meal	144.3	110.0	86.0	50.0	-		
yellow lupin var. Miste	r -	100.0	150.0	200.0	250.0		
peas var. Trachalska	-	100.0	100.0	100.0	100.0		
rapeseed meal	70.0	-	-	-	-		
rapeseed oil	51.5	47.0	55.0	60.0	63.0		
limestone	95.1	81.0	81.0	80.5	80.6		
calcium phosphate	12.8	12.8	12.9	13.0	13.1		
NaCl	1.8	1.9	2.0	2.0	1.9		
NaHCO ₃	3.5	3.0	2.9	2.9	2.9		
DL-Methionine	1.5	1.5	1.5	2.0	1.5		
L-Lysine	1.5	0.2	-	0.1	0.8		
L-Threonine	0.5	0.2	0.2	0.1	0.4		
L-Valine	1.0	0.7	0.7	0.7	1.0		
premix 0.5% ¹	5.0	5.0	5.0	5.0	5.0		
Metabolizable energy, $MJ \cdot kg^{-1}$	11.61	11.62	11.68	11.64	11.62		
Analysed nutritional valu	ie, g · kg	-1					
crude protein	163.6	165.2	164.3	168.9	164.1		
Са	35.0	35.3	35.2	35.0	35.0		
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- weight of yolk, thick and thin albumen and eggshell (g),
- content of yolk, thick and thin albumen and shell in the egg mass (%),
- yolk colour (points) measured every two weeks visually using a La Roche Yolk Colour Fan (from 1 – the lightest to 15 – the darkest) (Roche, Bern, Switzerland) and with a Minolta CR-410 colorimeter (Konica Minolta, Tokyo, Japan),
- Haugh units (HU) score was calculated according to the formula:

 $HU = 100 \log (h - 1.7 W^{0.37} + 7.6)$ where: h – average thick albumen height (mm), W – egg weight (g),

- pH of yolk and albumen using a CP-401 pH meter (Elmetron, Bydgoszcz, Poland),
- eggshell thickness (mm) together with shell membranes at the equatorial part of the egg, using a micrometre screw with 0.01 mm accuracy,
- eggshell strength (kg) measured with an egg crusher (ORKA, Tel Aviv, Israel).

The methods of egg quality determination were described by Adamski (2008).

Chemical analyses

According to AOAC International (2005), nitrogen concentration (method 976.05) with the use of a Kjel Foss Automatic 16210 (A/S N. Foss Electric, Hillerod, Denmark) and crude fat (method 920.39) using a Soxtex System HT 1043 Extraction Unit (Foss Tecator, Hillerod, Denmark) were determined in the diet. To determine crude protein concentration, the assayed nitrogen was multiplied by 6.25. Crude fibre concentrations in the diet were calculated in line with INRA standards (Sauvant et al., 2004). The concentration of carotenes and xanthophylls was analysed according to spectrophotometric method – PB 60 KLP. The amino acid (AA) content was determined by using an AAA-400 Automatic Amino Acid Analyser (Ingos, Prague, Czech Republic) with ninhydrin for post-column derivatization. Before analyses, the samples were hydrolysed with 6N HCl for 24 h at 110 °C (procedure 994.12; AOAC International, 2005).

Lupin alkaloids were extracted from the meal with trichloroacetic acid and methylene chloride. The determination was provided by the gas chromatography method (Shimadzu GC17A, Phenomenex Inc., Torrance, CA, USA) with a capillary column (Phenomenex Inc., Torrance, CA, USA). Raffinose family oligosaccharides (RFO) in lupin seeds were extracted and analysed using high-resolution gas chromatography, as described by Zalewski et al. (2001). Phytic phosphorus was determined by extracting the sample in hydrochloric acid. Next, iron-ammonium sulphate was added to the centrifuged extract which was heated and then centrifuged. Bipyridine solution was added to the supernatant and absorbance was determined using a Media spectrophotometer (Marcel Lamidey S.A., Chatillon, France) at 519 nm wavelength (Hang and Lantz, 1983). The water extract viscosity (WEV) of lupin seeds was measured in vitro. Prior to the determination of WEV, lupin samples were ground in a mill to pass through a sieve with 0.5 mm mesh, and then 1 g of each of the examined cultivar was mixed with 5 ml distilled water for 1 h at 40 °C. The samples were centrifuged at 10 000 g for 10 min at 4 °C, the supernatant was withdrawn and viscosity was determined in a Brookfield Digital DV-II+ cone/plate viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) maintained at 40 °C at a shear rate of $12 \cdot s^{-1}$ (mPas \cdot s = cP = 1 \times 100 dyne \cdot s \cdot cm⁻²; WEV units are mPas \cdot s). The metabolizable energy of diets was calculated based on Smulikowska and Rutkowski (2005).

Statistical analysis

All data were examined earlier to discard any possible outliers. The analyses were performed using the appropriate procedures of SAS Software (distribution analyses; outliers were defined as observations whose distance to the location estimate exceeded 3 times the standard deviation; SAS Institute Inc., Cary, NC, USA). The obtained results were subjected to one-factorial analysis of variance (ANOVA). Experimental data were compared using the Duncan test and differences were assumed to be significant at P < 0.05.

Regression analysis of the effects of laying period on the changes of yolk colour was performed using the following model:

$$y_i = \beta_0 + \beta_{1x_{i1}} + \varepsilon i$$

where: yi – yolk colour, β_0 , β_1 – regression coefficients, Xi – laying period, ϵi – random variable assumed to be normally distributed with mean 0 and variance δ^2 .

In the experiment, standard error of the mean (SEM) was used as a measure of error.

Results

Performance

The health status of hens was good and there was noted no mortality. Body weight of hens (Table 3) amounted on average 1.90–1.96 kg at the beginning and 1.63–1.66 kg after 22 weeks of experiment, without significant differences between treatments.

The dynamics of laying rate (Table 3) in the first 9 weeks of the experiment did not differ between groups. Between 10 and 18 week of egg production, there were observed differences between groups in laying rate (P < 0.05); only group containing 10% of vellow lupin seeds did not differ from the control group. The laying rate in group with 15% yellow lupin seed addition was lower in comparison to control group in weeks 12, 15, 16 and 18. The group with 20% yellow lupin seed addition also differed from control group but only in selected weeks: 11, 12 and 15. The laying rate in group with 25% yellow lupin seeds addition was lower than in the control group and group with 10% inclusion from 10 to 17 week and from all other treatment groups in weeks 10, 11, 14, 15, 16 and 17. For the whole experiment the laying rate was the highest in the control group, and in groups with 10 and 20% yellow lupin seeds addition; however groups with 10 and 20% addition also did not differ from groups fed diet with 15% addition. The lowest laying rate was obtained in birds fed diet with 25% inclusion of

Table 3. Body weight of hens and laying rate during experiment

Week of Treatments							
	control	yellow l	upin, g ·	kg⁻¹		SEM	P-value
laying	CONTROL	100	150	200	250		
Body we	ight, kg						
initial	1.91	1.96	1.92	1.93	1.90	0.01	0.549
final	1.63	1.63	1.66	1.65	1.65	0.01	0.441
Laying ra	ate, %						
1	74.0	64.8	64.0	68.4	70.0	1.66	0.310
2	91.5	85.5	87.6	93.5	86.9	1.01	0.067
3	96.5	96.2	94.3	96.8	94.6	0.51	0.411
4	99.0	95.8	95.8	97.4	96.7	0.43	0.125
5	97.3	95.6	94.8	97.0	95.4	0.52	0.328
6	98.6	94.4	96.5	94.2	94.4	0.55	0.056
7	95.4	95.2	91.9	93.5	93.2	0.63	0.341
8	98.8	95.6	95.5	96.2	94.4	0.54	0.133
9	97.7	94.6	94.4	95.8	92.5	0.63	0.123
10	97.3ª	94.4ª	94.1ª	94.4ª	87.0 ^b	0.82	0.001
11	94.8ª	90.9 ^{ab}	91.2 ^{ab}	88.0 ^b	78.9℃	1.00	<.0001
12	96.1ª	91.7 ^{ab}	89.3 ^b	87.9 ^{bc}	82.7⁰	1.02	0.001
13	95.8ª	94.1ª	90.6 ^{ab}	90.3 ^{ab}	85.5⁵	0.90	0.003
14	95.7ª	94.1ª	91.7ª	91.1ª	83.9 ^b	0.87	0.0001
15	95.1ª	94.4 ^{ab}	89.8 ^b	89.5 [⊳]	83.5°	0.84	<.0001
16	97.1ª	94.2 ^{ab}	92.6 ^b	93.7 ^{ab}	85.3°	0.71	<.0001
17	95.7ª	96.2ª	94.5ª	95.2ª	89.3 ^b	0.66	0.04
18	97.7ª	96.6 ^{ab}	93.6 ^b	98.0ª	95.4 ^{ab}	0.50	0.026
19	96.2	95.4	94.3	97.0	95.0	0.62	0.691
20	96.8	93.5	93.2	95.4	91.3	0.64	0.059
21	95.7	97.0	92.3	94.6	95.0	0.58	0.115
22	97.1	97.0	92.1	96.8	96.5	0.68	0.094
Mean	95.4ª	92.9 ^{ab}	90.8 ^{bc}	92.6 ^{ab}	88.8°	0.47	0.0001

SEM – standard error of the mean; abc – means with different superscripts within the same row are significantly different at $P \le 0.05$

yellow lupin but this result did not differ from group with 15% yellow lupin seeds supplementation.

The egg weight varied significantly between treatments after the first 5 weeks up to 20 week of the experiment (Table 4). The mean values for the whole study show that only hens fed diet containing 25% yellow lupin seeds produced significantly smaller eggs as compared to the control and other treatments.

Feed intake during the 22-week study varied significantly between treatments starting from week 5 of the experiment. For the whole experimental period the lowest dietary consumption was noted in the control group and in the group with 25% lupin seeds addition (Table 5); however there was observed no difference between groups with 25% and 10 or 15% addition of yellow lupin seeds.

The best FCR (Table 6) was obtained in the control hens. Inclusion of 25% yellow lupin seeds into diet negatively influenced the FCR increasing its value (P < 0.05). Also, the addition of 10, 15 and 20% lupin seeds significantly affected FCR in comparison to control hens.

Week of	Treatme	ents					
laying	control	yellow	lupin, g ·	SEM	P-value		
aying	CONTION	100	150	200	250		
1	50.9	50.5	51.3	51.3	50.8	0.18	0.583
2	52.3	53.5	53.7	53.7	52.8	0.23	0.180
3	55.1	56.2	56.2	56.4	54.9	0.24	0.151
4	56.8	57.5	57.7	57.8	56.3	0.21	0.097
5	57.5	57.6	57.5	57.5	56.2	0.21	0.158
6	57.7ª	58.2ª	57.3ª	57.4ª	55.8 ^b	0.25	0.021
7	58.5ª	58.3ª	58.8ª	57.8 ^{ab}	56.6 ^b	0.23	0.023
8	59.1ª	59.7ª	60.1ª	59.5ª	57.1⁵	0.24	0.001
9	59.7ª	60.1ª	60.5ª	59.7ª	57.5⁵	0.24	0.001
10	59.6ª	60.2ª	60.4ª	59.1ª	56.5⁵	0.28	<.0001
11	59.7 ^{ab}	59.9ª	60.6ª	58.3 ^b	56.7°	0.27	<.0001
12	60.1ª	60.5ª	59.6ª	60.5ª	57.2 [⊳]	0.35	0.012
13	59.8 ^{bc}	61.4ª	61.2 ^{ab}	59.6 ^{cd}	58.3 ^d	0.25	0.0001
14	60.0 ^{bc}	64.8ª	61.0 ^b	60.0 ^{bc}	57.9°	0.41	<.0001
15	60.6 ^b	65.5ª	61.5 [⊳]	60.4 ^{bc}	58.3°	0.38	<.0001
16	61.4 ^{bc}	65.7ª	62.5 ^b	61.6 ^{bc}	59.8°	0.33	<.0001
17	61.7 ^{bc}	63.5ª	64.0ª	62.8 ^{ab}	60.8°	0.27	0.0004
18	62.6 ^{bc}	63.9 ^{ab}	64.7ª	63.5 ^{abc}	62.0°	0.26	0.008
19	62.8 ^{bc}	64.5 ^{ab}	65.1ª	63.2 ^{bc}	62.6°	0.26	0.006
20	63.0 ^{ab}	63.6 ^{ab}	64.6ª	63.2 ^{ab}	61.7 ^b	0.29	0.029
21	63.1	64.2	64.4	64.1	62.8	0.26	0.169
22	63.0	64.3	64.8	64.2	63.3	0.29	0.228
Mean	59.3ª	60.5ª	60.4ª	59.6ª	58.0 ^b	0.19	0.0001

 Table 4. Egg weight during experiment, g

SEM – standard error of the mean; abc – means with different superscripts within the same row are significantly different at $P \le 0.05$

Table 6. Feed conversion ratio during experiment, $kg \cdot kg^{-1}$ eggs

Week of	Treatm	ents					
	control	yellow	lupin, g		SEM	P-value	
laying	CONTROL	100 150		200 250			
2	2.18	2.35	2.27	2.17	2.34	0.03	0.202
3	2.00	2.06	2.08	1.95	2.06	0.02	0.064
4	1.89	1.96	1.92	1.89	1.98	0.01	0.231
5	1.86	1.92	1.89	1.84	1.81	0.02	0.160
6	1.87	2.00	2.04	1.96	1.95	0.02	0.091
7	1.97	2.00	2.07	2.09	2.03	0.02	0.229
8	1.73°	1.91 ^b	1.87 ^b	1.88 ^b	2.06ª	0.02	<.0001
9	1.88	1.95	1.95	1.90	1.97	0.02	0.259
10	1.97 ^b	2.14ª	2.05 ^{ab}	1.98 ^b	2.07 ^{ab}	0.02	0.027
11	1.84	1.93	1.92	1.97	2.08	0.03	0.062
12	1.96°	2.11 ^{bc}	2.21 ^{ab}	2.13⁵	2.35ª	0.03	<.0001
13	1.85⁵	1.95 ^{ab}	1.98ª	1.97ª	2.05ª	0.02	0.015
14	1.92⁵	1.98⁵	2.03 ^b	2.03 ^b	2.18ª	0.02	0.003
15	1.95°	2.06 ^{bc}	2.19 ^{ab}	2.17 ^{ab}	2.31ª	0.03	<.0001
16	1.96°	2.05 ^{bc}	2.05 ^{bc}	2.09 ^b	2.27ª	0.02	<.0001
17	1.99 ^b	2.01 ^b	2.06b	2.10 ^b	2.35ª	0.02	<.0001
18	1.90°	1.96 ^{bc}	2.05 ^b	2.00 ^b	2.17ª	0.02	<.0001
19	1.88	1.86	1.95	1.88	2.02	0.02	0.072
20	2.01°	2.17⁵	2.17⁵	2.15⁵	2.30ª	0.02	<.0001
21	1.84 ^b	1.87 ^{ab}	1.97ª	1.91 ^{ab}	1.99ª	0.02	0.037
22	2.27 ^b	2.37 ^{ab}	2.42ª	2.34 ^{ab}	2.46ª	0.02	0.022
Mean	1.94°	2.03	2.06 ^b	2.02 ^b	2.14ª	0.01	<.0001

SEM – standard error of the mean; abc – means with different superscripts within the same row are significantly different at $P \le 0.05$

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Table 5. Feed intake during experiment, $g \cdot d^{-1} \cdot hen^{-1}$

	Treatme						
Week of	control	yellow	lupin, g ·	SEM	P-value		
laying	control	100 150		200	200 250		
1	99	103	101	102	100	0.73	0.342
2	102	106	104	108	106	0.83	0.207
3	106	111	108	106	107	0.64	0.055
4	105	107	106	106	106	0.55	0.830
5	104ª	105ª	101 ^{ab}	102ª	97 ^b	0.77	0.009
6	106 ^{ab}	109ª	110ª	107 ^{ab}	102 ^b	0.72	0.006
7	108 ^{bc}	111 ^{ab}	113ª	112 ^{ab}	105°	0.69	0.003
8	100 ^b	108ª	109ª	107ª	111ª	0.71	<.0001
9	108 ^{ab}	110ª	112ª	108 ^{ab}	104 ^b	0.74	0.010
10	114 ^{ab}	117ª	115 ^{ab}	110 ^b	103°	0.90	<.0001
11	103ª	104ª	106ª	100ª	93 [⊳]	0.97	0.0002
12	111 ^{bc}	117ª	115 ^{ab}	112 ^{abc}	109°	0.84	0.021
13	104 ^{bc}	112ª	110 ^{ab}	106 ^{bc}	101°	0.90	0.001
14	109 ^{ab}	114ª	112ª	110ª	104 ^b	0.82	0.003
15	110 ^b	120ª	117 ^{ab}	117 ^{ab}	111 ^b	1.11	0.018
16	113⁵	120ª	117 ^{ab}	120ª	115 ^{ab}	0.95	0.046
17	115⁵	123ª	121ª	125ª	127ª	0.92	0.0004
18	113°	121 ^₅	122 ^b	124 ^{ab}	128ª	0.95	<.0001
19	110°	114 ^{bc}	115 ^{ab}	115 ^{ab}	119ª	0.79	0.006
20	120 ^b	128ª	126ª	128ª	129ª	0.84	0.001
21	107 ^b	116ª	114ª	115ª	118ª	0.87	0.001
22	116⁵	126ª	123ª	124ª	128ª	0.87	<.0001
Mean	108°	114ª	112 ^{ab}	112 ^{ab}	110 ^{bc}	0.49	0.004

SEM – standard error of the mean; abc – means with different superscripts within the same row are significantly different at $P \le 0.05$

Egg quality

The inclusion of yellow lupin seeds into laying hen diet had no significant effect on egg quality parameters such as: shape index, percentage share of yolk and thick albumen content (Table 7). Significant differences between treatments for percentage share of thin albumen content were noted between the control group and group with 15% yellow lupin seeds addition. In comparison to the control group, the egg shell content decreased in all lupin treatments (P < 0.05).

Parallel to the rise in lupin seeds content in the diets, the pH value of egg content and HU units were increasing. The egg yolk colour was improved from 2.01 points in the control group to 4.61 points of the La Roche fan scale in the group fed diet containing 25% yellow lupin seeds (P < 0.01), also when the colour was measured with a Minolta device the same tendency was stated (Table 8; Figures 1 and 2). In contrast, the egg shell thickness and strength decreased significantly with the increasing lupin seeds content in the diets (Table 8). Significantly better shell quality was found in eggs from control hens, and from those receiving 10% yellow lupin seeds.

	Treatm	ents						
Indices	control	yellow	lupin, g		SEM	P-value		
	CONTROL	100	00 150 200		250			
Egg shape	72.9	72.5	72.8	72.8	73.1	0.07	0.079	
Weight, g								
yolk	14.2 ^{ab}	14.2 ^{ab}	14.3ª	14.0 ^{bc}	13.9⁰	0.06	0.004	
thick albumen	21.9ª	22.3ª	22.3ª	22.2ª	21.4 ^b	0.09	0.002	
thin albumen	18.2 ^{bc}	18.0 ^{bc}	19.1ª	18.4 ^b	17.8℃	0.09	<.0001	
eggshell	6.0ª	5.8⁵	5.9 ^b	5.8 ^b	5.6°	0.02	<.0001	
Content, % of eg	gg weigl	ht						
yolk	23.5	23.6	23.2	23.2	23.6	0.07	0.051	
thick albumen	36.3	36.8	36.2	36.7	36.5	0.11	0.249	
thin albumen	30.2 [♭]	29.9 ^b	31.0ª	30.5^{ab}	30.3 ^{ab}	0.12	0.028	
eggshell	9.9ª	9.6 ^b	9.5 ^b	9.6 ^b	9.5 ^b	0.03	<.0001	
		6 41-		abc				

Table 7. Morphology and physical traits of eggs

Table 8. Egg quality characteristics

significantly different at $P \le 0.05$

	Treatments							
Indices	control	yellow	lupin, g	SEM	P-value			
	CONTITION	100	150	200	250			
pН								
yolk	5.59 ^d	5.65°	5.69 ^b	5.74ª	5.73ª	0.01	<.0001	
albumen	8.01°	8.09 ^{at}	° 8.07⁵	8.12ª	8.11ª	0.01	<.0001	
HU	94.17 ^b	93.22 ^b	95.36ª	95.52ª	96.43ª	0.20	<.0001	
Yolk colour ¹	2.01°	2.97 ^d	3.69°	4.15⁵	4.61ª	0.01	<.0001	
L*	78.89ª	77.46 ^b	77.53 [♭]	76.56°	75.84 ^d	0.16	<.0001	
a*	-3.21°	-1.77 ^d	-0.84°	-0.23 ^b	0.38ª	0.01	<.0001	
b*	51.42 ^d	54.68°	56.99 ^b	58.18ª	58.23ª	0.12	<.0001	
Eggshell								
thickness, mm	0.38ª	0.37 ^b	0.37 ^b	0.37 ^b	0.36°	0.001	<.0001	
strength, kg	4.27ª	4.03ª	3.92 ^b	3.95 ^{bd}	° 3.83°	0.03	<.0001	

¹ La Roche points; SEM – standard error of the mean; HU – Haugh

unit; abc - means with different superscripts within the same row are

SEM - standard error of the mean; $^{\rm abc}$ - means with different superscripts within the same row are significantly different at $P \le 0.05$

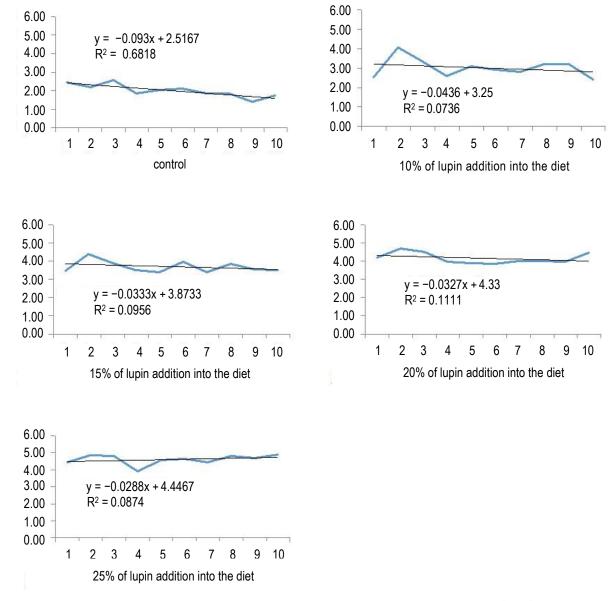


Figure 1. Changes of yolk colour during laying period (La Roche points) measured every two weeks of laying (from 2nd to 20th week of experiment)

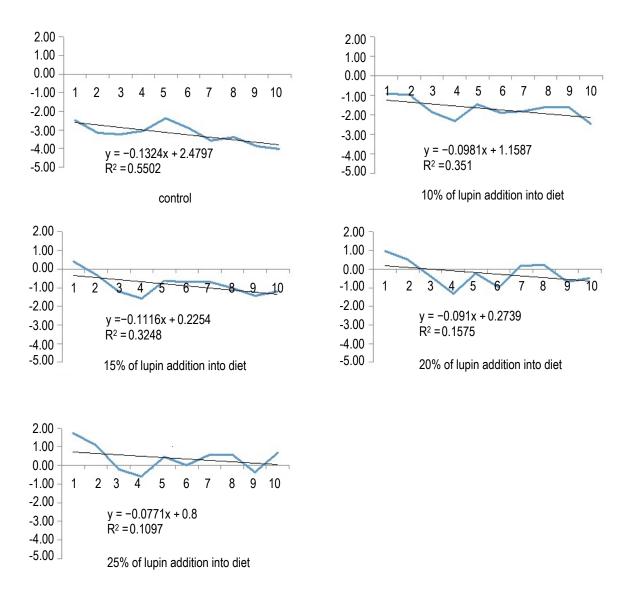


Figure 2. Changes of yolk colour during laying period (LAB - a*) measured every two weeks of laying (from 2nd to 20th week of experiment)

The dynamics of changes of yolk pigmentation were measured ten times every two weeks (the first time after two weeks of feeding with experimental diets), and significant differences were visible between treatments (Figures 1 and 2). In the control group, a significant aggravation of yolk pigmentation was observed. In eggs from hens fed diets with 15, 20, 25% of yellow lupin seeds the yolk colouring was relatively stable at the level of \pm 4.0 points on the La Roche scale.

Discussion

The yellow lupin seeds (var. Mister) used in this study were of good quality, with a low content of alkaloids and saccharides from the raffinose family in comparison to data presented by Gdala and Buraczewska (1996) and Wasilewko and Buraczewska (1999). Moreover, the amino acid and phosphorus contents show that Mister seeds are a profitable component of laying hen diets. Chemical composition results obtained in this study were better than those presented by Sandberg (2002).

In comparison to many publications presenting the results of the use of yellow lupin, pea, faba beans in animal nutrition (e.g., Olkowski et al., 2005; Olkowski, 2011; Kocher et al., 2000; Kaczmarek et al., 2014, 2015; Hejdysz et al., 2015, 2016; Rutkowski et al., 2016), studies concerning the inclusion of lupin seeds into laying hen diets are not so common (Watkins and Mirosh, 1987; Hughes and Kocher, 1998; Mierlita, 2013; Krawczyk et al., 2015; Rutkowski et al., 2016). The inclusion of 25% yellow lupin seeds into diet caused a decrease in the laying rate of hens as compared to the effects of control diet and treatments with 10 and 20% lupin addition. The negative influence of lupin addition to hen diet was recorded by Rutkowski et al. (2015) – the 11% yellow lupin seed inclusion evoked decreased egg production. This effect was observed after 8 weeks of feeding. In our study similar effect was observed at week 10 of the experiment. In contrast, Krawczyk et al. (2015) did not confirm the negative effect of 30% vellow lupin seeds inclusion on laving performance, feed intake, FCR and egg weight. In this study, the feed intake amounted on average $126 \text{ g} \cdot \text{d}^{-1} \cdot \text{hen}^{-1}$ and FCR -2.03 kg \cdot kg⁻¹ of egg weight. In our experiment, these indices were lower and amounted on average to 111 g \cdot d⁻¹ \cdot hen⁻¹ and FCR – 2.06 kg \cdot kg⁻¹ egg, respectively; hens consumed more feed with lower content of seeds or without seeds. Using 15% Lupinus angustifolium L. in laying hen diets, Hammershoj and Steenfeldt (2005) did not observe any negative effects; however, with 25% lupin addition reduced egg production was recorded. Hughes and Kocher (1998) fed hens diets with 7.5, 15 or 22.5% narrow-leafed lupin seeds and at the end of the feeding period no negative effects were found. However, the increased lupin amounts in hen feed negatively influenced egg production indices (Mierlita, 2013); following 80% isoprotein substitution of diet components with lupin an enormous decrease in these parameters was noted. In our study only 25% inclusion of lupin into the diet led to a significant decrease in egg weight in comparison to the remaining treatments. In earlier investigations (Rutkowski et al., 2015), similar effect was presented. Krawczyk et al. (2015) recorded no differences in egg weight, shell thickness or strength after inclusion of 10-30% yellow lupin into laying hen diets.

In the study of Rutkowski et al. (2015), the use of increased amounts of yellow lupin seeds in hen diets decreased the egg quality parameters. Not only higher values of egg content pH and of HU units were determined, but also there was a decrease in the thickness, strength and share of egg shell in the egg weight. Dvořák et al. (2007) observed improved egg quality parameters after adding lupin to hen feed. Watkins and Mirosh (1987) confirmed the lack of significant differences in the HU units of eggs among treatments of hens fed diets containing 10, 15, 20, 25 and 30% white lupin seeds, but 30% share of this feed component in the diet led to a decrease in egg production. Increased feed intake was found in animals fed diet with 15% lupin seeds inclusion.

In comparison to the study of Calik (2013) on the quality of eggs from Yellowleg partridge laying hens, eggs from Hy-Line Brown hens used in our study were characterized by higher HU units but lower values of albumen and yolk pH. The increase in HU units resulting from increasing content of lupin in the diets is connected with better elasticity of thick albumen, stronger bond of ovomucins with lysozyme and better technological properties of egg albumen (Wells, 1968). Wells (1968) and Williams (1992), however, presented the view that hen nutrition does not appear to have a significant effect on albumen characteristics. Nevertheless, in our study, the presence of yellow lupin seeds in laying hen diets improved some egg quality parameters. Sikder et al. (1998) and Dvořák et al. (2007), and also many other authors, have underlined the fact that dietary composition significantly influences yolk colour - an important egg quality index for consumers. In our experiment, significant improvement of yolk colour (La Roche scale) was obtained as an effect of the rise in yellow lupin seeds content in the diets and increased levels from 1.59 (in group with 10% yellow lupin seeds addition) to 3.97 (in group with 25% yellow lupin seeds addition) in 1 kg of feed mixture. These differences between 'lupin' treatments, calculated as percentages of the La Roche points rise amounted from 49 to 249%. Yellowness of yolk increased together with increasing yellow lupin seeds level in diets. More yellow yolks were noted in eggs from hens fed diets with 15, 20 and 25% yellow lupine seeds inclusion than in the remaining groups. It is possible, that inclusion of yellow lupin seeds into very poor in carotenoids wheat-pea diets, enriched the feed mixtures in those ingredients from 48.84, 206 to 229% in comparison to the control group (except for yolk pigmentation).

Conclusions

The use of up to 20% of yellow lupin seeds in diet instead of soyabean meal did not decrease laying rate and egg weight. However, the egg quality was decreased in line with the increasing yellow lupin seeds content in the diets (except for yolk pigmentation).

Acknowledgements

This project was supported by the founds from the programme 'Improvement of native plant protein feeds, their production, trade turnover, and utilisation in animal feed' initiated by the Ministry of Agriculture and Rural Development of Poland, No. 505 037 07.

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