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CONCENTRATES MADE FROM LEGUME SEEDS (*LUPINUS ANGUSTIFOLIUS*, *LUPINUS LUTEUS* AND *PISUM SATIVUM*) AND RAPESEED MEAL AS PROTEIN SOURCES IN LAYING HEN DIETS*

Andrzej Rutkowski[1♦], Sebastian A. Kaczmarek[1], Marcin Hejdysz[1], Sebastian Nowaczewski[2], Dorota Jamroz[3]

[1]Department of Animal Nutrition and Feed Management,
[2]Department of Poultry Science,
Poznan University of Life Sciences, Wołyńska 33, 60-637 Poznań, Poland
[3]Department of Animal Nutrition and Feed Quality,
Wrocław University of Environmental anf Life Sciences, J. Chełmońskiego 38c, 51-630 Wrocław, Poland
♦Corresponding author: rutkowski@jay.au.poznan.pl

Abstract

The aim of the study was to determine the usefulness of two protein concentrates composed of rapeseed meal and new cultivars of narrow-leaved and yellow lupine, peas in laying hen diets and their influence on birds' performance and egg characteristics. The experiment was conducted with 180 layers kept in individual cages. The birds were randomly assigned to three treatments, with 60 hens in each and during the period of 17 weeks, they were fed diets: I – containing soybean meal as a protein source, II – containing protein concentrates composed of mixture of lupines, peas (19.48% of diet) and rapeseed meal, III – containing protein concentrates composed of a mixture of lupines and peas (27.68% of diet). The body weight, laying rate, egg weight, feed intake and feed conversion ratio (FCR) and egg characteristics were registered. After 9 weeks of experiment, a decrease of laying rate was recorded in treatment III. The mean value of laying rate for 17 weeks amounted to 82.7 (I), 82.5 (II) and 75.9% (III) ($P<0.01$). The egg weight was diversified already after 4 weeks of egg production and averaged 57.9 (I), 55.9 (II) and 54.9 g (III) ($P<0.05$). Feed intake amounted to 108 (I), 111 (II) and 104 g per hen/day (III), and FCR was 2.05, 3.17 and 2.23 kg/kg egg weight, respectively. As to egg characteristics, increases of white index ($P<0.05$), Haugh unit score and yolk colour in treatment III were observed but egg shell thickness was found significantly reduced. In conclusion, the use of about 27.68% of legume seed in laying hen diet affected negatively performance results but about 19.48% of these seeds and 8% rapeseed meal in diets could be accepted as a soybean meal substitute.

Key words: lupine seeds, peas, performance, layers

Soybean meal constitutes the main dietary protein source in poultry feeding. Laying hen diets may contain about 20–25% and growing bird feeds more than 30% soybean. Soya, derived mainly from Brazil or the USA, is mostly genetically modified giving rise to strong protests of poultry product consumers in many countries.

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Although according to other authors, soya (GMO) has no negative influence on animal organisms (Świątkiewicz and Arczewska-Włosek, 2011).

Complete substitution of soybean meal in poultry feeds is difficult due to low protein concentration or/and high anti-nutrient content in lupines and pea seeds (Castanon and Perez-Lanzac, 1990; Fru-Nji et al., 2007; Nalle et al., 2011). Nevertheless, in recent years, the interest in the use of native legume seeds as protein components in diets for monogastric animals increased again (Perez-Maldonado et al., 1999; Jul et al., 2003; Diaz et al., 2005; Sujak et al., 2006; Laudadio and Tufarelli, 2011).

The area of cultivation of legume plants: field beans, narrow-leaved lupine (*Lupinus angustifolius* L.), yellow lupine (*Lupinus luteus* L.), white lupine (*Lupinus albus* L.) or peas (*Pisum sativum* L.) is currently low in Poland. However, the relative high crude protein level in these feeds is about 30–40% in lupine and 20–30% in other legume seeds (pea, faba bean) (Perez-Maldonado et al., 1999; Diaz et al., 2005; Smulikowska and Rutkowski, 2005; Duranti, 2006; Martinez-Villaluenga et al., 2006; Jamroz and Kubizna, 2008; Nalle et al., 2011).

The nutritive value of legume seeds, especially of lupines, is restricted by the activity of naturally occurring antinutritive substances which are recognized as harmful factors, i.e. oligosaccharides, trypsin inhibitors, alkaloids, tannins, phytoestrogens, inositol phosphates, lectins and saponins (Zduńczyk et al., 1998; Wasilewko and Buraczewska, 1999; Buckeridge et al., 2000; Miecznikowska et al., 2004; Martinez-Villaluenga et al., 2006; Erdemouglu and Ozkan, 2007; Guillamon et al., 2008; Jamroz and Kubizna, 2008). However, some of these constituents of leguminous plants, tocopherols and tannins, show a beneficial activity as antioxidants (Lampart-Szczapa et al., 2003; Duranti, 2006; Martinez-Villaluenga et al., 2006; Erdemouglu and Ozkan, 2007; Viveros et al., 2007; Jamroz and Kubizna, 2008). Legume proteins are rich in lysine, although poor in methionine and isoleucine. Oligosaccharides from the raffinose family, small amounts of tannins or lectins participate in regulation of microbial populations in the digestive tract and in the immune response of organisms (Jul et al., 2003; Erdemouglu and Ozkan, 2007; Jamroz and Kubizna, 2008). Seeds, particularly those of lupines, are rich in microelements (Sandberg, 2002) and antioxidants, namely tocopherol (Lampart-Szczapa et al., 2003; Viveros et al., 2007).

Investigations concerning the use of legume seeds in laying hen and broiler diets have a long tradition and the number of published results is considerable, although the inclusion of different cultivars of those feeds in diet has in many cases decreased the performance results in poultry (Castanon and Perez-Lanzac, 1990; Igbasan and Guenter, 1997; Hughes and Kocher, 1998; Perez-Maldonado et al., 1999; McNeill et al., 2004; Diaz et al., 2005; Hammershøj and Steenfeldt, 2005; Orda et al., 2006; Fru-Nji et al., 2007; Laudadio and Tufarelli, 2011).

Rapeseed and its byproducts have a high pectin content and complex fibre matrix structure, which results in its high water-holding capacity and poor nutrient availability for monogastric animals, including poultry. But due to high soybean meal prices, there is growing interest within the feed industry in using rapeseed byproducts in poultry feeding (Mikulski et al., 2012) and full-fat oilseeds in poultry diets (Rutkowski et al., 2012). According to the above authors, rapeseed and its byproducts could be a valuable source of energy and protein for poultry.

The aim of the study was to evaluate the effect of replacement of soybean meal with a mixture of narrow-leaved or yellow lupine and pea seeds in hen diets on performance and egg quality.

Material and methods

Animals and diets

One hundred eighty Hy-line Brown hens at the age of 17 weeks were weighed, placed in individual cages and fed pre-laying diets based on wheat, maize and soybean meal and containing 16% crude protein and 11 MJ ME/kg. Before the beginning of the laying period, the birds were randomly assigned to three treatments, each with 60 hens. The birds had free access to drinking water and feed. The lighting program was 14 h of light and 10 h of darkness.

Table 1. Composition of concentrates

Components (%)	Concentrate	
	I	II
Yellow lupine	5.07	23.70
Narrow-leaved lupine	22.22	22.22
Peas	15.56	11.11
Rapeseed meal	17.90	-
Maize	-	4.44
Rapeseed oil	13.30	12.22
Limestone	18.87	18.89
Dicalcium phosphate	2.89	3.11
Vitamin-mineral premix[1]	1.11	1.11
$NaHCO_3$	0.89	0.78
NaCl	0.38	0.36
DL-Methionine	0.40	0.47
L-Lysine	0.56	0.56
Threonine	0.42	0.43
Tryptophan	0.10	0.09
L-Valine	0.33	0.51
Metabolizable energy (MJ/kg)	10.64	10.64
Crude protein (%)	19.28	19.30
Ca (%)	8.20	8.19
P-available (%)	0.73	0.74
Na (%)	0.40	0.37
Cl (%)	0.36	0.36
Lys (%)	1.46	1.45
Met+Cys (%)	0.99	0.98
Try (%)	0.28	0.27
Thr (%)	1.15	1.13
Val (%)	1.23	1.24
Arg (%)	1.61	1.66
Linol. acid (%)	3.36	3.34

[1]Provided per kg diet: vit. A 10 000 IU, vit. D_3 2000 IU, vit. E 20 mg, vit. K_3 1.5 mg, vit. B_1 1 mg, vit. B_2 4 mg, vit. B_3 20 mg, vit. B_5 8 mg, vit. B_6 1.5 mg, vit. B_9 0.8 mg, choline 200 mg, Fe 45 mg, Mn 90 mg, Cu 8 mg, Zn 60 mg, I 1 mg, Co 0.5 mg, Se 0.25 mg, antioxidant 15 mg, vit. B_{12} 3300 mcg, biotin 50 mg.

Experimental diets were isoproteic and energetic and containing about 16.0% crude protein, 11.3 MJ ME/kg feed but differed in plant protein components. Control diet contained soybean meal as a protein source. The diets for treatment II and III consisted of protein concentrates, which accounted for 45% of the diet (Table 1). Protein concentrates were designed to provide high quality protein (amino acid composition) and minimal level of antinutrients. In the protein concentrate I, the following protein components were used: rapeseed meal (8%), narrow-leaved lupine (*Lupinus angustifolius*) cv. Boruta (10%), 2% yellow lupine cv. Mister and about 8% pea cv. Muza. In the protein concentrate II, yellow lupine (12%), narrow-leaved lupine (10%) and about 5% peas were used. The content of individual legumes depended on the amino acid content in these components and calculation and balancing of the amino acid level in the feed mixtures. The characteristics of chemical composition of the applied legume seeds and concentrates are presented in Table 3, the diet composition is shown in Table 2.

Table 2. Composition of experimental diets

Components (%)	Treatments – diets		
	I	II	III
	control	19.48% legumes	27.68% legumes
Maize	12.71	-	-
Wheat	55.0	55.0	55.0
Concentrate I	-	45	-
Concentrate II	-	-	45
Soybean meal	18.02	-	-
Rapeseed oil	3.24	-	-
Limestone	8.59	-	-
Dicalcium phosphate	1.14	-	-
Vitamin-mineral premix	0.5	-	-
$NaHCO_3$	0.305	-	-
NaCl	0.152	-	-
DL-Methionine	0.155	-	-
L-Lysine	0.135	-	-
Threonine	0.046	-	-
Tryptophan	0.002	-	-
Metabolizable energy (MJ/kg)	11.3	11.3	11.3
Crude protein (%)	16.2	15.8	15.9
Ca (%)	3.5	3.5	3.5
P-available (%)	0.39	0.39	0.39
Na (%)	0.16	0.16	0.16
Cl (%)	0.16	0.16	0.16
Lys (%)	0.75	0.75	0.77
Met+Cys (%)	0.63	0.64	0.68
Try (%)	0.16	0.16	0.16
Thr (%)	0.53	0.53	0.53
Val (%)	0.68	0.61	0.54
Arg (%)	0.96	1.05	1.19
Linol. acid (%)	1.56	2.01	2.03

Provided per kg diet: vit. A 10 000 IU, vit. D_3 2000 IU, vit. E 20 mg, vit. K_3 1.5 mg, vit. B_1 1 mg, vit. B_2 4 mg, vit. B_3 20 mg, vit. B_5 8 mg, vit. B_6 1.5 mg, vit. B_9 0.8 mg, choline 200 mg, Fe 45 mg, Mn 90 mg, Cu 8 mg, Zn 60 mg, I 1 mg, Co 0.5 mg, Se 0.25 mg, antioxidant 15 mg, vit. B_{12} 3300 mcg, biotin 50 mg.

Table 3. Chemical composition of legume seeds, in DM

Item	Narrow-leaved lupine cv. Boruta	Yellow lupine cv. Mister	Pea cv. Muza
Dry matter (%)	88.62	89.01	86.65
Crude ash (%)	3.78	4.15	3.14
Crude protein (%)	36.88	38.98	27.57
Crude fiber (%)	15.09	19.23	6.34
ADF (%)	21.43	24.24	7.97
NDF (%)	25.92	28.24	13.88
Crude fat (%)	5.81	5.26	1.32
Starch (%)	-	-	44.23
Gross energy (MJ/kg)	20.73	20.49	19.45
Viscosity (cP)	1.21	1.09	1.29
Amino acid (% protein)			
Asp	8.91	8.81	10.49
Thr	3.15	3.17	3.54
Ser	4.11	4.24	4.38
Glut	23.77	24.46	19.46
Pro	6.52	6.08	5.77
Gly	4.01	3.47	3.83
Ala	3.33	2.83	3.81
Val	3.72	3.17	4.35
Iso	3.68	3.20	3.66
Leu	6.64	6.50	6.63
Tyr	3.07	3.24	3.26
Phe	3.46	4.24	5.00
His	2.91	3.32	3.37
Lys	4.49	4.76	6.52
Arg	11.65	10.12	8.82
Total	39.39	39.29	42.53
Minerals (g/kg DM)			
Ca	3.33	2.95	1.27
K	13.45	12.66	12.72
P	6.84	7.47	5.10
Na	0,08	0.08	0.062
Mg	2,10	3.14	1.47
Mn	0.13	0.08	0.02
Cu	0.04	0.02	0.02
Fe	0.07	0.13	0.07
Zn	0.07	0.07	0.06
Antinutrients			
Total alkaloid (TA) (mg/kg)	440	270	-
Angustifoline (% of TA)	12.54	-	-
Isolupanine (% of TA)	4.56	-	-
Lupanine (% of TA)	56.17	-	-
130H lupanine (% of TA)	26.73	-	-
Sparteine (% of TA)	-	33.6	-
Lupinine (% of TA)	-	63.29	-
Oligosaccharide (g/kg)	8.77	8.56	8.34
Rafinose (g/kg)	1.20	1.10	0.90
Stachiose (g/kg)	5.61	4.94	3.86
Verbascose (g/kg)	1.96	2.53	3.59
P phyt. (%)	0.42	0.70	0.44
P phyt./P total	61	75	62

Analytical methods

For chemical analysis, the representative samples of seeds were ground to pass through a 0.5 mm sieve. Seeds were analyzed in duplicate for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), crude ash (CA), acid detergent fiber (ADF) and neutral detergent fiber (NDF) using methods 934.01, 976.05, 920.39, 978.10, 942.05, 973.18 respectively, according to AOAC (2007).

The amino acid content was determined using a type AAA-400 Automatic Amino Acid Analyzer employing ninhydrin for post-column derivatization. Before the analysis, samples were hydrolyzed with 6 N HCl for 24 h at 110°C (procedure 994.12; AOAC (2007)). Sugars were analyzed according to PN-R-64784 method. Gross energy was determined using an adiabatic bomb calorimeter (KL 12Mn, Precyzja-Bit PPHU, Poland) standardized with benzoic acid. Mineral composition (Ca, P, Na, K, Zn, Mg, Cu, Mn, Fe) was analyzed by ICP-OES (P.10I35-ICP method) after microwave mineralization.

Lupine alkaloids were extracted from flour by trichloroacetic acid and methylene chloride. The determination was provided by gas chromatography method (Shimadzu GC17A) with a capillary column (Phenomenex). Raffinose family oligosaccharides (RFO) were extracted and analyzed by high-resolution gas chromatography as described previously by Zalewski et al. (2001). Phytate content was analyzed according to AOAC (2007) (methods 986.11) after extraction in HCl and by the use of bipiridine. The absorbance was measured with Spectrophotometer Marcel at wavelength of 519 nm.

Performance and egg quality

At the beginning of the experiment, laying hens were at the age of 21 weeks. The laying performance was recorded weekly in the period of 17 weeks of experiment for each of randomly selected 60 hens per treatment. The length of this period was associated with an attempt to establish the moment at which positive or negative effects of protein sources on the above mentioned variable could be seen. The feed consumption was registered in the same way. The average egg weight was determined also weekly on the basis of randomly collected 30 eggs from each treatment. Mortality was recorded daily.

In the fifth and thirteenth weeks of the experiment 30 eggs from each treatment and time were randomly selected. The egg quality was determined by taking into consideration the following parameters:

– Egg weight (g) with 0.01 g accuracy with the assistance of the WPS 360C type balance,

– Yolk colour (points) was measured visually using Roche Yolk Colour Fan from 1 (the lightest) to 15 (the darkest) points of scale,

– White index (%) was calculated according to the formula:

White index = thick egg white height (mm) × 100/thick white width (mm),

– Haugh units were calculated according to the formula:

$$HU = 100 \log (h - 1.7\, W^{0.37} + 7.6)$$

where:

HU – Haugh unit score,

h – average thick white height (mm),

W – egg weight (g).

– Eggshell thickness (μm) together with shell membranes at the sharp, blunt and equatorial part of the egg using a screw micrometer for this purpose.

Statistical analysis

Statistical calculations were performed using SAS® v.9.1 package. Mean values as well as the standard error of the mean (SEM) were calculated for all traits. Differences among treatments with regard to egg quality traits were determined employing the two-way linear model of ANOVA:

$$Y_{ijk} = \alpha_j + \beta_k + (\alpha\beta)_{jk} + e_{ijk}$$

where:

Y_{ijk} is value of the analyzed trait,

α_j is constant effect of ith group of hens (i=I, II, III),

β_k is constant effect of jth week (j=5,13),

$(\alpha\beta)_{jk}$ is interaction between α and β,

e_{ijk} is effect of experimental error.

For all traits, the significance of differences between groups of hens was verified using Fisher's test. Differences between weeks, within groups, were determined by the Student t-test.

Results

The health status of hens during the entire experimental time was good and without visible disease symptoms and mortality cases. The initial body weight of hens amounted in treatments to about 1.75 kg and at the end of experiment it was 1.88, 1.89 and 1.69 kg, respectively, in individual treatments ($P \leq 0.004$).

From the tenth week on, the egg production was significantly ($P<0.01$) lower in treatment III (75.9%) in comparison with the laying rate of hens fed the control diet (82.7%) or the diet containing about 19.48% legume seeds and 8% rapeseed meal (82.5%) (Table 4).

The significant variability of egg weight among treatments was noted. At the beginning of the seventh week, the egg weight of hens from the control treatment (I) was significantly higher ($P<0.01$) than of eggs derived from the experimental treatments (II and III) (Table 5).

For feed consumption (Table 6), irregular variability was observed; however, in the majority of weeks, hens fed diets with greater legume seed content (diet III) consumed lower amounts of feed mixture. Mean values of this parameter calculated for

17 weeks varied non-significantly in treatments I and II, while in treatment III they were lower than in control treatment (P<0.05).

Table 4. Laying rate of hens during first 17 weeks of egg production, %

Weeks	Treatments			SEM	P-Value
	I	II	III		
1	0.71	0.48	1.19	0.42	0.799
2	17.86	12.38	16.91	1.03	0.059
3	67.14	64.52	63.33	1.83	0.711
4	92.86	89.05	86.19	1.32	0.115
5	95.00	97.38	93.57	1.01	0.313
6	96.19	97.62	95.71	0.72	0.563
7	97.14	95.95	94.05	1.25	0.623
8	95.48	96.43	91.19	1.56	0.572
9	95.48	96.43	91.19	1.38	0.272
10	95.24 a	96.43 a	89.05 b	1.20	0.015
11	91.19 ab	97.14 a	83.81 b	2.18	0.032
12	91.67 a	95.95 a	85.00 b	1.69	0.017
13	93.81 A	95.71 B	85.95 B	1.46	0.006
14	94.29 a	89.76 ab	81.67 b	2.12	0.037
15	92.62 A	90.48 A	69.76 B	2.87	<.0001
16	94.52 A	90.24 A	78.10 B	2.01	<.0001
17	95.48 A	97.86 A	83.81 B	1.93	0.001
Mean	82.70 A	82.54 A	75.94 B	1.05	0.004

The differences among means for treatments in rows designated with a, b – significant at P<0.05; A, B – significant at P<0.01.

Table 5. Changes of egg weight during first 17 weeks of egg production, g

Weeks	Treatments			SEM	P-Value
	I	II	III		
1	47.50	57.00	47.60	3.83	0.634
2	51.25	48.00	48.28	1.12	0.449
3	50.66 a	49.10 b	49.00 b	0.31	0.036
4	51.27	52.19	52.53	0.82	0.829
5	55.68	51.69	51.38	1.35	0.370
6	56.91	55.49	55.03	0.37	0.095
7	58.69 A	56.58 B	56.81 B	0.38	0.010
8	59.39 a	57.58 ab	56.71 b	0.44	0.026
9	59.45 a	57.66 ab	57.03 b	0.42	0.037
10	59.83	58.26	57.61	0.51	0.186
11	59.48 a	57.60 ab	55.44 b	0.74	0.039
12	60.33	56.21	56.20	0.91	0.096
13	60.82 A	57.03 B	57.63 B	0.57	0.005
14	61.09 a	58.25 ab	56.79 b	0.68	0.019
15	61.09 A	58.21 B	56.71 B	0.63	0.006
16	62.24 A	60.24 A	55.85 B	0.81	0.001
17	61.85 a	60.24 ab	58.05 b	0.62	0.037
Mean	57.92 a	55.94 b	54.99 b	0.44	0.012

The differences among means for treatments in rows designated with a, b – significant at P<0.05; A, B – significant at P<0.01.

For feed conversion ratio per kg of eggs (Table 7) during the last three weeks of the experiment, differences among treatments were significant (P<0.05).

Table 6. Average feed intake during first 17 weeks of egg production, g/hen/week,

Weeks	Treatments			SEM	P-Value
	I	II	III		
1	79	86	84	1.63	0.243
2	88 a	96 ab	105 b	2.86	0.047
3	95	100	94	1.24	0.129
4	104	106	103	1.50	0.674
5	99	104	99	1.31	0.133
6	124 A	124 A	116 B	1.23	0.003
7	111	114	107	1.39	0.074
8	117	120	112	1.57	0.110
9	109 ab	112 a	102 b	1.71	0.038
10	116	119	112	1.56	0.229
11	107	115	106	2.37	0.236
12	114	117	108	1.72	0.084
13	114	111	104	1.99	0.122
14	107 ab	108 a	98 b	1.92	0.056
15	123 A	116 A	108 B	2.18	0.003
16	110 ab	115 a	105 b	1.74	0.043
17	121	122	99	4.84	0.081
Mean	108 a	111 a	104 b	1.11	0.013

The differences among means for treatments in rows designated with a, b – significant at P<0.05; A, B – significant at P<0.01.

Table 7. Feed conversion ratio during first 17 weeks of egg production, kg/kg of egg weight

Weeks	Treatments			SEM	P-Value
	I	II	III		
1	-	-	-	-	-
2	-	-	-	-	-
3	2.84	3.17	3.05	0.08	0.199
4	2.22	2.29	2.28	0.06	0.863
5	1.88	2.11	2.09	0.06	0.293
6	2.26	2.29	2.29	0.03	0.406
7	1.95	2.11	2.01	0.03	0.083
8	2.08	2.18	2.17	0.03	0.469
9	1.93	2.01	1.97	0.03	0.639
10	2.04	2.12	2.19	0.03	0.098
11	1.97 A	2.07 B	2.33 B	0.05	0.004
12	2.07	2.19	2.28	0.05	0.277
13	2.00	2.02	2.11	0.02	0.144
14	1.86	2.10	2.13	0.06	0.117
15	2.19 A	2.21 A	2.73 B	0.07	<.0001
16	1.88 A	2.12 B	2.41 C	0.06	<.0001
17	2.05 a	2.07 a	2.35 b	0.06	0.043
Mean	2.05 a	2.17 ab	2.23 b	0.03	0.042

The differences among means for treatments in rows designated with a, b – significant at P<0.05; A, B, C – significant at P<0.01.

Table 8. Main effects of protein concentrate and laying period on egg characteristics

Item	Egg weight (g)	Yolk weight (g)	Yolk content (%)	White weight (g)	White content (%)	Shell weight (g)	Shell content (%)
Treatments							
diets							
I	59.4 a	12.8	21.5	39.8	67.2	5.8 A	9.8 A
II	58.0 ab	12.6	21.6	38.1	65.8	5.5 B	9.6 B
II	57.0 b	12.6	22.1	39.0	68.4	5.4 B	9.5 B
weeks							
5th	55.1 A	11.0 A	20.1 A	38.6	70.2 A	5.3 A	9.7 a
13th	61.4 B	14.3 B	23.4 B	39.3	64.0 B	5.9 B	9.5 b
SEM	0.40	0.21	0.29	0.47	0.67	0.04	0.05
P-Value							
for diet	0.015	0.828	0.619	0.324	0.281	<.0001	0.009
for weeks	<.0001	<.0001	<.0001	0.493	<.0001	<.0001	0.036
interaction							
diet × week	0.638	0.523	0.256	0.820	0.488	0.870	0.888

Within treatment effects means in columns designated with different letters differed at a, b – $P<0.05$; A, B, C – $P<0.01$.

The used plant protein sources affected egg parameters. The increased legume seed content in the diets led to a decrease of egg weight (57.0 g) ($P<0.05$) and shell weight (5.3 g) and their share in egg (9.5%) compared to the control treatment ($P<0.01$) (Table 8). After 13 weeks of application of experimental diets, significant ($P<0.01$) increases in the egg and yolk weight and proportion were noted (Table 9).

Table 9. Main effects of protein concentrate and laying period on egg quality traits

Item	Yolk index (%)	Yolk colour (points)	White index (%)	Haugh units (points)	Shell thickness (μm)
Treatments					
diets					
I	48.6	13.3	10.3 A	86.4 A	370 A
II	49.5	13.4	10.9 A	88.7 A	360 B
III	49.9	13.5	12.0 B	91.7 B	354 B
weeks					
5th	52.1 A	12.7 A	12.5 A	93.5 A	357 A
13th	46.4 B	14.0 B	9.6 B	84.3 B	366 B
SEM	0.30	0.07	0.20	0.65	1.77
P-Value diets	0.052	0.163	0.0004	0.0004	0.001
Weeks	<.0001	<.0001	<.0001	<.0001	0.008
Interaction diet × week	0.324	0.216	0.573	0.506	0.730

The differences among means in columns designated with a, b – significant at $P<0.05$; A, B, C – significant at $P<0.01$.

Use of diet III increased ($P<0.05$) white index and HU score, but decreased ($P<0.05$) shell thickness in comparison with treatments I and II. Thirteen weeks of feeding hens with experimental diets caused an increase in yolk color ($P<0.01$) and a decrease in egg shell thickness ($P<0.01$).

Discussion

In the available literature, considerable variations in legume seed chemical composition, as well as their nutritional value depending on cultivars or varieties, as well as variation in content of antinutritive substances were previously reported (Zduńczyk et al., 1996; Igbasan and Guenter, 1997; Hughes and Kocher, 1998; Perez-Maldonado et al., 1999; Jul et al., 2003; Sujak et al., 2006; Fru-Nji et al., 2007; Laudadio and Tufarelli, 2011; Laudadio and Tufarelli, 2012).

The nutritional value of the used seeds of narrow-leaved lupine cv. Boruta was good when compared to older cultivars cultivated in Poland (Wasilewko and Buraczewska, 1999). Protein content amounted to about 37% compared to 31–34% in the cited publications. ADF and NDF contents were similar to data concerning other Polish cultivars. It is worth drawing attention to the distinctly higher content of Cu, Zn, Mn in cv. Boruta than in Emir, Sur, Saturn and Polonez cultivars and the lower total amounts of oligosaccharides compared to data reported by Mosenthin

(2001). The alkaloid content was typical for sweet lupine (Petterson and Mackintosh, 1994).

In comparison with narrow-leaved lupine, yellow lupine cv. Mister contains more protein, fiber fractions, more methionine and lysine, phosphorus and Fe, but clearly lower amounts of Mn and total alkaloids. The oligosaccharide level in both lupine cultivars was relatively similar; however, the verbascose share in total oligosaccharides was about 30% higher in yellow than in narrow-leaved lupine.

The protein content in pea cv. Muza amounted to 27.6% and was higher than values given by Pettersen and Mackintosh (1994). The concentration of oligosaccharides was a little lower than in lupine seeds, although the verbascose share in total oligosaccharides was two times higher than in narrow-leaved and 25% higher than in yellow lupine.

In our own study, a mixture of narrow-leaved and yellow lupine and pea seeds was used in layer diets instead of soybean meal. By the inclusion of about 19.46% of these legumes and 8% rapeseed in diets, it was possible to obtain positive effects in the laying rate and feed intake; however, a decrease of egg weight and poorer FCR (kg/kg egg weight) indices were also recorded. Further increase of legume content to 27.68% (yellow lupine from 2.3% in diet II to 12% in diet III) caused a significant decrease in the performance in comparison with the control (by about 7–8%) after nine weeks of experiment. The lower egg weight was registered already after 4 weeks of the use of experimental diets for layers. Egg weight (1–17 weeks of experiment) was negatively affected in experimental treatments. This result was similar to the findings of Hammershøj and Steenfeldt (2005); 25% content of narrow-leaved lupine in hen feed lowered egg production, egg weight and also reduced feed intake were noted. The level of 15% of lupine in the hen diet did not have negative effects. In our study, increased content of legume seeds in diets for hens led to a decrease in egg weight, egg shell share in the egg and shell thickness. Moreover, greater changes in egg quality were observed as a result of time of feeding of experimental diets. After 13 weeks, yolk colour and shell thickness in egg from hens fed the diet with 27.68% of legumes were better than after 5 weeks.

Nalle et al. (2011) did not observe any decrease by feeding broilers with diets containing 20% of narrow-leaved lupine. McNeill et al. (2004) reported that the inclusion of 10% peas exerted only a small influence on feed intake and broiler growth; higher content of peas (20%) reduced the feed intake. Castano and Perez-Lanzac (1990) found a significant negative relationship between dietary concentrations of legumes and feed intake, egg production and feed to egg ratio.

The results obtained in our study suggest that the presence of antinutritive substances in investigated legume seeds negatively affects feed intake. This is clearly seen in the case of high content of yellow lupine (diet III).

It is still difficult to explain these effects on the basis of chemical characteristics of complete diets, all the more that the feed mixture compositions were optimized by taking into consideration many nutrients. Dietary fiber or alkaloid fractions may partly explain the results obtained. But the total α-galactosides content in yellow lupine cv. Mister was similar to data presented by Martinez-Villauenga et al. (2006). Moreover, the stachyose level in yellow lupine cv. Mister was significantly lower

(4.9 mg/g) than its levels presented by the cited authors (7–8 mg/g). Probably, the observed worse results can be attributed to the response of hens – mainly in treatment III – to the complex of harmful substances present in about 27.68% content of the used legume seeds. In conclusion, the use of about 27.68% of legume seed in laying hen diet affected negatively performance results but about 19.48% of these seeds in diets could be accepted as a soybean meal substitute.

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