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EVALUATION OF THE NUTRITIONAL VALUE OF YELLOW (*LUPINUS LUTEUS*) AND BLUE LUPINE (*LUPINUS ANGUSTIFOLIUS*) CULTIVARS AS PROTEIN SOURCES IN RATS

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Abstract

The aim of this study was to determine the nutritional value of protein from the seeds of yellow lupine (*Lupinus luteus*) and blue lupine (*Lupinus angustifolius*) cultivars, contained in diets fed to rats. The experimental diets were based on the seeds of three yellow lupine (Mister, Markiz, Taper) or three blue lupine (Sonet, Boruta, Elf) cultivars as the only or main protein source. The nutritional value of the diets was determined based on their chemical composition and alkaloid and oligosaccharide concentrations. Lupine seeds were fed to male Wistar rats with initial body weight of approx. 112 g. Alkaloid concentrations in yellow lupine and blue lupine seeds were 0.07–0.09 g kg^{-1} DM and 0.26–0.39 g kg^{-1} DM, respectively. Oligosaccharide concentrations in yellow and blue lupine seeds were 108.0–108.9 and 65.4–67.6 g kg^{-1} DM, respectively. The inclusion of lupine seeds in rat diets increased fecal and urinary nitrogen losses, and decreased nitrogen retention by approx. 10%. The diets based on yellow or blue lupine seeds were characterized by lower nitrogen digestibility and lower biological value of protein than the control diet containing casein.

Key words: lupine, alkaloids, carbohydrates, rats, protein value

Legumes have a high protein content and their nutritional value has been improved due to progress in breeding, which makes them a valuable source of vegetable protein for animal feed production and the food processing industry. This is an important consideration in view of the growing demand for vegetable protein and the efforts to become independent of imported genetically modified soybeans.

Lupines, in particular modern lupine varieties, have a high nutritional value and play an important role in organic farming (Sujak et al., 2006; Gulewicz et al., 2008). The protein content of lupine seeds is high, ranging from 28% to 48% DM depending on species (Flis et al., 1998; Jezierny et al., 2010). Lupine seed proteins are high in lysine and arginine. Lupines are also a valuable source of lipids, fiber, minerals and vitamins (Martinez-Villaluenga et al., 2006). The concentration of anti-nutritional

factors is low in lupine seeds, as compared with other sources of vegetable protein. The results of studies into the potential threats posed by anti-nutritional factors are inconclusive. Wäsche et al. (2001) and Champ (2002) demonstrated that alkaloids present in low concentrations in lupine seeds do not exert adverse effects on human and animal health. However, according to De Cortes-Sánchez et al. (2005) and Maknickiene et al. (2013), their presence remains a limiting factor to the wide use of lupine seeds. Oligosaccharides contained in lupine seeds, which are poorly absorbed in the small intestine and undergo bacterial fermentation in the cecum, often cause digestive system disorders in humans and animals (Gdala and Buraczewska, 1996). However, they may also stimulate digestion processes in the large intestine (Fooks and Gibson, 2002). Experiments performed with different animal species revealed a significant role of oligosaccharides in promoting the development of beneficial microflora (*Bifidobacteria*, *Lactobacillus*) and inhibiting the growth of harmful bacteria such as *Clostridium perfringens*, *Salmonella* spp. and *Escherichia* spp. (Topping and Clifton, 2001). A beneficial influence of oligosaccharides on metabolic processes in the intestines of rats was reported, among others, by Guillon and Champ (2002), Juśkiewicz et al. (2006) and Sobotka et al. (2013). As a consequence, already in 2003, lupine was included among eight potential vegetable protein sources for use in feed and food production in Europe (Dijkstra et al., 2003). Apart from soybean, also lupine seeds – which are considered the richest source of valuable vegetable protein – are increasingly used in the food processing industry in Western Europe and Australia (Chew et al., 2003; Papoti et al., 2005).

The optimum use of protein sources in animal diets depends on the availability of detailed information on their chemical composition, biological properties and nutritional value, which may vary between species and varieties. Thus, further research is needed to investigate the chemical and biological properties of different lupine species and varieties, which change in response to breeding efforts aimed not only at increasing the yield potential, disease resistance and protein content of plants but also at improving protein quality (Schumacher et al., 2011). An increased interest in lupine seeds as an alternative source of vegetable protein in diets for monogastric animals, observed in recent years, has been reflected in studies of pigs (Hanczakowska and Świątkiewicz, 2014) and poultry (Smulikowska et al., 2014; Zduńczyk et al., 2014; Krawczyk et al., 2015).

Accordingly, the objective of this study was to determine the nutritional value of protein from the seeds of yellow lupine (*Lupinus luteus*) and blue lupine (*Lupinus angustifolius*) cultivars in a nitrogen balance trial with rats.

Material and methods

The animal protocol used in this study was approved by the Local Institutional Animal Care and Use Committee in accordance with resolution No. 07/2011, and the study was carried out in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Animals aged four weeks, with

initial body weight of approx. 112 g ± 5 g were selected for experimental groups so as not to exceed a 10 g difference between individuals within groups and a 5 g difference in total body weight per group. Rats from one litter were allocated to different groups. The animals were kept in individual metabolic cages, under constant microclimatic conditions: 12 h light, temperature 21–23°C, relative air humidity 50–60%, air exchange 10–15 times per hour. The housing conditions were described in detail by Zduńczyk et al. (1998).

The experimental diets (Table 1) contained the seeds of three yellow lupine cultivars (Mister, Markiz, Taper) and three blue lupine cultivars (Sonet, Boruta, Elf). The cultivars were selected for the study based on the chemical composition of seeds and the date they were registered by the Research Centre for Cultivar Testing and added to the Polish National List of Agricultural Plant Varieties. The main sources of protein were casein in the control diet and the seeds of yellow lupine or blue lupine in experimental diets. To balance dietary protein levels, blue lupine-based diets were supplemented with a small amount of casein.

Table 1. Composition of experimental diets (%)

Item	Control	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*		
		Mister	Markiz	Taper	Sonet	Boruta	Elf
Casein	11.25	-	-	-	2	2	2
Lupine	-	24.30	25.00	25.40	25.50	25.10	26.50
Maize starch	70.11	61.90	61.20	60.80	58.70	59.10	57.70
Soybean oil	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Mineral-vitamin premix[1]	5.50	5.50	5.50	5.50	5.50	5.50	5.50
DL-methionine	0.14	0.30	0.30	0.30	0.30	0.30	0.30
Cellulose[2]	5.00	-	-	-	-	-	-
Calculated composition (g kg^{-1} D.M.)[3]							
Crude protein	112.76	112.59	115.74	115.74	104.20	112.72	113.86
Met	4.19	3.96	4.01	4.00	4.12	4.06	4.16
Lys	5.94	5.55	5.49	5.49	5.25	5.53	5.67
Ca[4]	16.05	16.18	16.20	16.20	16.34	16.24	16.27
Total P[4]	4.46	4.90	4.95	4.95	4.59	4.39	4.43
Dietary fiber	32.2	37.7	41.1	41.8	38.0	3.97	3.94
Energy (kJ/g/DM)[4]	17.29	17.28	17.28	17.28	17.30	17.29	17.29
Alkaloids	-	0.019	0.022	0.020	0.097	0.098	0.069
Oligosaccharides	-	26.22	27.00	27.65	17.23	16.69	17.32

[1]Vitamin and mineral premix: g/kg mix – Ca, 250; P, 60; Mg, 5; Na, 51; Fe, 5; Zn, 5; Mn, 3; Cu, 0.5; mg/kg mix – Co, 20; I, 40; Se, 15; g/kg, L-Lysine, 9.4; methionine + cystine, 3.7; threonine, 2.3; tryptophan, 1.1; IU/kg: vitamin A, 500000; vitamin D_3, 100000; mg/kg, vitamin E, 2000; vitamin K, 150; vitamin B_1, 100; vitamin B_2, 300; vitamin B_6, 150; vitamin B_{12}, 1.5; nicotinic acid,1200; pantothenic acid, 600; folic acid, 50; biotin, 7.5; choline 10.

[2]The source of cellulose was powdered cellulose (Vitacel®, Rettenmaier, Poland).

[3]Calculated based on own study.

[4]Calculated based on data on the nutritional value of feed, Pig Nutrient Requirements (2014).

The protein content of diets was equalized to approx. 11.5%, and it satisfied the protein requirements of rats. Nitrogen content was converted into protein content in rat diets using the conversion factor of N × 6.25. The diets were supplemented with synthetic methionine to meet the amino acid requirements of rats (NRC, 1997). The diets were fed to 56 male Wistar rats (eight animals per group). The animals had free access to feed and water. In the second week of the 28-day experiment, a five-day digestibility and balance trial was carried out to determine feed intake, and the total amount of feces and urine excreted. Feces samples were frozen and urine samples were preserved with sulfuric acid. Lupine seeds were assayed for proximate chemical composition (AOAC, 2003), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), according to the methods proposed by Van Soest and Wine (1967) and Van Soest (1973), using the Fibertec M system. ADL was determined by ADF hydrolysis with 72% sulfuric acid.

Lupine seeds were analyzed to determine their proximate chemical composition, the amino acid profile of protein, and the levels of alkaloids and oligosaccharides. The amino acid profile of protein was determined using the Biochrom 20 Plus analyzer (PN-EN ISO 13903, 2006). Samples were hydrolyzed in 6 M HCl at 110ºC for 24 h. Cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, following sample oxidation with performic acid and hydrolysis in 6 M HCl at 110ºC for 24 h. Tryptophan was determined after hydrolysis in 1.07 mol/dm^3 barium hydroxide, at 110°C for 16 h (PN-EN ISO 13904, 2005). All analyses were performed in duplicate. Reagents were supplied by Biochrom Ltd. (Cambridge, England, UK) and Sigma-Aldrich.

Oligosaccharide concentrations were determined by ion exchange chromatography (Shimadzu HPLC–RID), as described by Gulewicz et al. (2000). Alkaloid levels were measured by capillary gas chromatography (Varian CP-3800) and gas chromatography coupled with mass spectrometry (Varian 450-GC, 220-MS) with a flame-ionization detector (FID), as described by Wink et al. (1995). Urinary and fecal N excretion was estimated by the Kjeldahl method using the Kjeltec 8400 Analyzer and the Kjeltec 8420 Sampler systems (FOSS). All analyses were performed in duplicate.

The results of the rat N balance experiment were processed statistically by one-way ANOVA. The statistical significance of differences between the mean values of the analyzed parameters in experimental groups was estimated by Duncan's multiple range test, using the STATISTICA PL 10.0 software package.

Results

The seeds of yellow and blue lupines differed considerably in chemical composition (Table 2).

Yellow lupine cultivars were characterized by similar protein content values (387.5, 391.1 and 398.5 g kg^{-1} DM in cv. Markiz, Taper and Mister, respectively). The protein content of blue lupine seeds was numerically lower and more variable (279.9, 300.7 and 317.9 g kg^{-1} DM in cv. Sonet, Elf and Boruta, respectively). Blue

lupine seeds contained numerically higher concentrations of N-free extracts than yellow lupine seeds (from 308.0 in cv. Sonet to 390.4 g kg^{-1} DM in cv. Elf, and from 276.1 in cv. Markiz to 291.6 g kg^{-1} DM in cv. Taper, respectively), and the difference reached 113.4 g. Differences were also found between the two lupine species in the amounts of crude fiber and ADF, which were numerically higher in yellow lupine. Both yellow and blue lupine cultivars differed also in their content of NDF, ADL and hemicellulose. The seeds of yellow lupine cv. Taper had a relatively low content of hemicellulose and ADL. The seeds of yellow and blue lupines differed in chemical composition, and differences were also noted within lupine species, between the analyzed cultivars.

Table 2. Chemical composition of lupine seeds (g kg^{-1} DM)

Item	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*		
	Mister	Markiz	Taper	Sonet	Boruta	Elf
Crude protein[1]	398.5	387.5	391.9	279.9	317.9	300.7
Crude fat	34.2	36.0	34.9	41.9	34.3	32.6
N-free extracts	278.2	276.1	291.6	308.0	364.2	390.4
Crude fiber	161.3	171.0	171.3	155.1	164.9	155.6
NDF[2]	237.9	249.4	223.1	235.6	229.4	214.6
ADF[3]	207.2	211.1	209.8	184.2	193.0	176.8
ADL[4]	12.9	14.5	7.7	11.2	14.7	10.6
Hemicellulose[5]	30.7	38.3	13.3	51.4	36.5	37.8
Cellulose[6]	194.3	196.6	202.1	173.0	178.3	166.2
Alkaloids	0.08	0.09	0.07	0.38	0.39	0.26
Oligosaccharides	108.1	108.0	108.9	67.6	66.5	65.4

[1]Nitrogen content (N) was multiplied by 6.25 – N × 6.25.
[2]Neutral Detergent Fiber.
[3]Acid Detergent Fiber.
[4]Acid Detergent Lignin.
[5]Hemicellulose = Neutral Detergent Fiber – Acid Detergent Lignin.
[6]Cellulose = Acid Detergent Fiber – Acid Detergent Lignin.

The seeds of yellow and blue lupines differed in alkaloid content, which was numerically lower in yellow lupine (from 0.07 in cv. Taper to 0.09 g kg^{-1} DM in cv. Markiz) than in blue lupine (0.26, 0.38 and 0.39 g kg^{-1} DM in cv. Elf, Sonet and Boruta, respectively). Distinct differences in the concentrations of oligosaccharides were noted between yellow and blue lupines, whereas differences between cultivars were smaller. Yellow lupine seeds contained numerically higher concentrations of oligosaccharides than blue lupine seeds (108.0–108.9 g kg^{-1} DM vs. 65.4–67.6 g kg^{-1} DM). The inclusion of yellow and blue lupine seeds in rat diets (Table 1) led to differences in the levels of alkaloids and oligosaccharides. Diets with yellow lupine contained 0.019–0.022 g alkaloids and 26.22–27.65 g oligosaccharides per kg DM, and diets with blue lupine had numerically higher concentrations of alkaloids (0.069–0.097 g kg^{-1} DM) and lower levels of oligosaccharides (16.69–17.32 g kg^{-1} DM).

The amino acid profile of yellow and blue lupines (Table 3) was characterized by high lysine levels that were slightly higher in yellow lupine (5.15–5.35 g/16 g N vs.

4.60–4.80 g/16 g N). The geometric mean of the ratio of essential amino acids in the tested protein relative to their respective amounts in whole egg protein (EAA Index according to Oser, 1959) points to high protein quality, which was comparable in five lupine cultivars (70-71), whereas the lowest EAAI (68) was noted in the protein of yellow lupine cv. Mister.

Table 3. Amino acid composition of protein and nutritional value of lupines (g/16 g N)

Item	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*		
	Mister	Markiz	Taper	Sonet	Boruta	Elf
Amino acid:						
His	2.61	2.68	2.73	2.70	2.50	2.60
Lys	5.35	5.29	5.15	4.75	4.60	4.80
Met	0.81	0.88	0.85	0.89	0.77	0.79
Cys	2.01	2.10	2.09	1.37	1.19	1.33
Thr	3.25	3.25	3.40	3.52	3.57	3.68
Trp	0.79	0.80	0.79	0.88	0.82	0.81
Ile	3.75	3.96	3.74	3.81	4.01	3.98
Leu	7.56	7.69	7.61	6.84	6.81	7.20
Phe	4.00	4.05	3.99	3.78	3.86	3.80
Tyr	3.67	3.88	3.88	3.96	4.46	4.25
Val	3.62	3.84	3.66	3.85	4.08	3.84
Arg	11.43	10.93	10.27	10.16	10.61	10.48
EAAI[1]	68	71	70	71	70	71
$CS_{(met)}$[2]	29	31	31	32	29	29

[1]Essential Amino Acid Index according to Oser – geometric mean of the ratio of EAA in the test protein relative to their respective amounts in whole egg protein (Oser, 1959).

[2]Chemical Score according to Mitchell and Block (1946) – the amount of the most limiting amino acid present in the test protein relative to the amount of that amino acid in reference egg protein.

As shown in Table 4, both lupine species and cultivar had a significant effect on nitrogen balance. The lowest nitrogen intake (0.183 g/100 g BW) was found in rats fed a diet with the seeds of blue lupine cv. Boruta. Nitrogen loss in urine was higher in rats fed yellow lupine. Less satisfactory results with respect to nitrogen digestibility, nitrogen retention and the biological value of protein were noted for the diet containing the seeds of blue lupine cv. Boruta, which was less willingly consumed by rats.

Urinary nitrogen excretion was also affected by lupine cultivar. The inclusion of blue lupine seeds in rat diets reduced nitrogen loss in urine, and the highest urinary nitrogen excretion was observed in rats fed seeds of blue lupine cv. Elf (0.08 g/100 g), which had the highest nitrogen intake (0.281 g/100 g BW). Nitrogen retention was approx. 10% lower in rats fed lupine-based diets than in those fed the control diet, and the noted difference was statistically significant. More pronounced differences were observed in rats fed blue lupine seeds, and the lowest nitrogen retention was determined in rats fed a diet with seeds of blue lupine cv. Boruta with the highest alkaloid content.

Table 4. Nitrogen balance in rats

Item	Diet[1]			Significance level	Yellow lupine (LL)			Significance level	Blue lupine (LA)			Significance level
	C	LL	LA	P	Mister	Markiz	Taper	P	Sonet	Boruta	Elf	P
N intake (g/100 g BW)	0.245	0.259	0.232	**	0.264	0.257	0.257	**	0.234	0.183	0.281	**
N excretion in feces (g/100 g BW/day)	0.020	0.039	0.043	**	0.036	0.043	0.039	*	0.044	0.044	0.044	ns
N excretion in urine (g/100 g BW/day)	0.068	0.080	0.057	**	0.082	0.075	0.083	**	0.054	0.037	0.080	**
Apparent N digestibility[2] (%)	91.83	84.94	81.47	**	86.36	83.27	84.22	**	81.20	76.00	84.34	**
N retention[3] (g per day)	0.157	0.140	0.132	**	0.146	0.139	0.135	**	0.136	0.102	0.15	**
BV4 (%)	88.77	78.18	74.73	**	80.28	76.70	77.5 A	**	76.54	69.41	78.25	**

[1]Diets: C – control, LL – yellow lupine, LA – blue lupine.
LL – the values of the analyzed traits are arithmetic means for yellow lupine cultivars (Mister, Markiz, Taper).
LA – the values of the analyzed traits are arithmetic means for blue lupine cultivars (Sonet, Boruta, Elf).
[2][(N intake – fecal N)/N intake] × 100.
[3][(N intake – fecal N – urinary N)/N intake] × 100.
[4][N intake – (fecal N – metabolic N[5])-(urinary N – endogenous N[5])] × 100/[N intake – (fecal N – metabolic N[5])].
[5]Metabolic N, endogenous N according to Lehmann and Bergner (1968) .
*Significant at P≤0.05. ** Significant at P≤0.01.

Discussion

The proximate chemical composition of lupine seeds determined in the present experiment is comparable with those reported by Sujak et al. (2006) and Jezierny et al. (2010), except for the concentrations of NDF, ADF and ADL, which were lower in our study. Distinct differences in the concentrations of oligosaccharides were noted between yellow and blue lupines, whereas differences between cultivars were smaller. Yellow lupine seeds contained higher concentrations of oligosaccharides than blue lupine seeds. The inclusion of yellow and blue lupine seeds led to differences in the levels of alkaloids and oligosaccharides in rat diets.

The amino acid profile of protein from yellow and blue lupine seeds was characterized by high lysine levels that were only slightly higher in yellow lupine, which corroborates the findings of Jezierny et al. (2010) and Stanek et al. (2012). It should be noted that lupine seeds contain large amounts of arginine that plays an important role in protein biosynthesis (Wu and Morris, 1998; Tong and Barbul, 2004) and detoxifying processes related to ammonia excretion and urea production (Campbell et al., 2004), and whose availability determines the rate of nitric oxide synthesis (Mori and Gotoh, 2000). The amino acid profile of lupine protein revealed that methionine was the first limiting amino acid for the quality of protein in all lupine cultivars, which is consistent with the findings of Dixon and Hosking (1992).

The effect of yellow and blue lupine seeds on N balance in rats, observed in our study, is partially consistent with that described in the literature. The lowest N intake was noted in rats fed a diet with seeds of blue lupine cv. Boruta, which could result from rats' sensitivity to high concentrations of alkaloids and crude fiber (particularly lignin) in lupine seeds. The rate of degradation of amino acids that had not been used for protein synthesis is reflected in the amounts of nitrogen excreted in the feces and urine. Nitrogen excretion in the feces was high in rats fed diets containing seeds of all analyzed cultivars of both lupine species. Large amounts of N were excreted in the feces, which could result from inadequate quality of lupine seed protein and the presence of oligosaccharides in lupine seeds. According to Younes et al. (1995), the increased rate of oligosaccharide fermentation, compared with cellulose, promotes bacterial growth, thus increasing fecal nitrogen excretion. Our results corroborate the findings of Wróblewska et al. (2004) and Juśkiewicz et al. (2006), who fed oligosaccharide extracts from lupine seeds to rats and observed enhanced fecal excretion of nitrogen. According to Eggum et al. (1993), fecal nitrogen includes nitrogen from undigested bacteria, dietary proteins, secreted enzymes and exfoliated mucosal cells.

In rats fed lupine-based diets, urinary and fecal losses of nitrogen from the breakdown of amino acids that had not been used for protein synthesis, at decreased protein synthesis and increased catabolism, can be associated with inadequate quality of lupine protein, which does not satisfy the protein requirements of animals (Rubio et al., 1995), and the production of urea that is excreted in the urine (Benevenga et al., 1993). The effect of dietary oligosaccharides on urinary and fecal nitrogen losses was also reported by Sudzinová et al. (2009) who observed higher fecal nitrogen excretion and lower urinary nitrogen excretion in rats fed diets containing yellow lupine seeds.

Nitrogen retention was significantly lower in rats fed lupine-based diets than in those fed the control diet. Our results do not agree with the findings of Wróblewska et al. (2004) in whose study fecal N loss was higher in rats fed diets with oligosaccharides which, however, had no effect on N retention. Lupine seeds contributed to a decrease in apparent N digestibility, which was higher in diets with blue lupine. The digestibility of lupine-based diets (yellow lupine – 84.94, blue lupine – 81.47%), although lower than in the control group, was similar to that reported by Molving et al. (1997) and considerably higher than that noted by Lubowicki et al. (2000).

The effect of dietary oligosaccharides on total N metabolism was described by Younes et al. (1995) and Juśkiewicz et al. (2006) who demonstrated that it was associated with changes in gut microbiota composition. The inclusion of yellow and blue lupine seeds in rat diets decreased the biological value of dietary protein, which was lower in lupine-based diets than in the control diet with casein. According to Rubio et al. (1995), the lower nutritional value of lupine seeds may result from the chemical structure of protein globulins and their negative influence on N metabolism rather than from the presence of anti-nutritional factors.

Conclusions

The results of our study indicate that the use of yellow or blue lupine seeds as protein sources in rat diets is determined by both lupine species and cultivar. Differences in the chemical composition of the analyzed lupines affect nitrogen balance and nitrogen retention in rats, and the biological value of dietary protein. In view of the progress in legume crops breeding, research into the nutritional value of lupine seeds should be continued to ensure their rational use as a potential source of vegetable protein in human and animal nutrition.

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