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The chemical composition and nutritive value of low-alkaloid varieties of white lupin 2. Oligosaccharides, phytates, fatty acids and biological value of protein*

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ABSTRACT

The oligosaccharide content, fatty acid and amino acid compositions, protein digestibility and growth efficiency were determined in three low-alkaloid varieties of white lupin from the 1993 and 1994 harvest (Wat and Hetman) and the 1992 and 1993 harvest (Bardo). The α-galactoside content was relatively low (7.1-8.6% DM). Phytates constituted 0.7 to 0.9% of Wat and Bardo seeds, and from 1.2 to 1.6% of Hetman seeds. Saturated fatty acids comprised 13-18% of total fatty acids, monounsaturated fatty acids 51-57%, and polyunsaturated fatty acids, 27-35%. The lysine content was relatively low (4.70-5.25 g/16 g N), while the limiting amino acid was methionine. The chemical score (CS) calculated for methionine and cystine ranged from 35 to 45% as related to egg protein and 35 to 41% in relation to the ideal protein for pigs. The true digestibility coefficient (TD, 83-86%) and the protein efficiency ratio (PER) for seeds supplemented with methionine (1.99-2.19) did not differ significantly. The obtained results do not, however, point to intervarietal differences in oligosaccharide content and protein and fat quality. However, significant variability in the characteristics was found, depending on the year of harvest.

KEY WORDS: white lupin, oligosaccharides, phytates, fatty acids, amino acids, digestibility, PER

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INTRODUCTION

The practice of Polish plant breeding stations has shown that it is possible to obtain more than 1000 kg protein per hectare from white lupin, i.e. significantly more than from other lupin species (Pągowska, 1990).

The white lupin seeds contain 34-38% crude protein in DM (Buraczewska et al., 1993; Eggum et al., 1993; Alloui et al., 1994; Smulikowska et al., 1995; Zduńczyk et al., 1996) that is characterized by a low methionine, tryptophane and lysine contents (Prieto and Aguilera, 1986; Batterham, 1989; Donovan et al., 1991). After supplementation with these amino acids, lupin seeds can be a good source of protein in the diet of monogastric animals, under the condition that the alkaloid level in the seeds is sufficiently low and does not limit feed intake or utilization. During the early 1980s, white lupin varieties and lines contained from 0.4 to 1.7 mg/g total alkaloids (Wiatr, 1989). Hetman, a new variety registered later and Bardo, the newest variety, contain less alkaloids (under 1 mg/g) (Buraczewska et al., 1993; Alloui et al., 1994; Zduńczyk et al., 1996), and due to that the seeds of these varieties can be more widely used in the nutrition of monogastric animals. The small number of nutritional studies performed do not provide a full assessment of the chemical composition and nutritional value of the new varieties. This applies in particular to the oligosaccharide and phytate contents, as well as to protein and fat. According to many authors the oligosaccharide fraction should be classified as a lupin antinutrient (Eskin et al., 1980; Saini, 1989).

In this study we determined the α-galactoside and phytate contents, total fatty acid and amino acid compositions, protein digestibility and protein efficiency ratio (PER) of Polish low-alkaloid varieties of white lupin in order to better assess the nutritional value of this crop.

MATERIAL AND METHODS

White lupin seeds were obtained from Polish seed stations (Kosieczyn, Przebędowo and Wiatrowo) situated the western and south-western regions of Poland. Seeds of the Wat and Hetman varieties were from successive harvests in 1992 and 1993, while Bardo variety seeds from 1992 and 1994. The differences in size, weight, proportion of seed coat and chemical composition of the seeds, cotyledons and seed coat were described in the first part of this study (Zduńczyk et al., 1996).

Total protein and crude fat contents after acid hydrolysis were determined by standard methods (AOAC, 1990), sucrose and α-galactosides (raffinose, stachyose and verbascose) contents were determined by HPLC in samples prepared

according to Múzquiz (1992). Phytates separated into individual inositol phosphates (from tri- to hexaphosphates) were determined by HPLC after sample preparation according to Sandberg and Ahderinne (1986). The total fatty acid composition was determined using a Shimadzu GC-14A gas chromatograph.

The amino acid composition was determined using an automatic Beckman Model 6300 analyzer after hydrolyzing the samples in 6 N HCl. Sulphur-containing amino acids were determined after oxidizing the samples with formic acid, while tryptophane after hydrolysis with barium hydroxide. Protein quality was determined using the essential amino acid index according to Oser (EAAI), with egg protein composition as the standard. An additional index was calculated ($EAAI_{pig}$), in which the standard was the ideal protein for pigs developed by Wang and Fuller (1990).

The protein efficiency ratio (PER) was determined using Wistar rats aged 27-30 days and weighing 65.9 ± 3.4 g at the start of the test. The experimental groups were composed of 8 rats of both sexes at a 1:1 ratio. The diets contained 10% crude protein derived from the seeds of the lupin varieties under study. The diets were supplemented with DL-methionine (0.25%), soyabean oil (7.5%) and the standard amount of a mineral mixture (according to NRC, 1976) and vitamin mixture (according to AOAC, 1975). The digestibility of crude protein was determined on the same rats in the last week of the growth test. The true protein digestibility coefficient was calculated by subtracting the amount of metabolic nitrogen, estimated according to Rakowska (1978).

The preferences of rats for the experimental diets were additionally determined using seeds from the 1994 harvest. The experiment was carried out according to Dayton and Morril (1974) on 15 males weighing 90.0 ± 3.5 g. Each rat was allowed free access to 3 containers with different diets. The consumption of each diet was measured daily, the order in which the containers were placed was changed.

The results were subjected to statistical analysis, using the Duncan test.

RESULTS AND DISCUSSION

The seeds of the analysed varieties contained from 1.7 to 3.3% sucrose and from 7.1 to 8.6% α-galactosides, i.e. raffinose, stachyose and verbascose in DM (Table 1). Eskin et al. (1980) and Cuadra et al. (1994) found similar amounts of the oligosaccharide fraction (7.4% DM). Comparing our results with the data from many studies compiled by Saini (1989), it can be seen that the seeds of Polish white lupin varieties have a low α-galactoside content. According to the data reported by Saini (1989), white lupin seeds contain from 7 to 14% α-galactosides,

TABLE 1

Content of saccharose and α-galactosides in seeds, % DM

Ingredient	Cultivar, year of harvest					
	Wat		Hetman		Bardo	
	1993	1994	1993	1994	1992	1994
Saccharose	2.94	3.04	1.67	2.83	3.07	3.30
Raffinose	0.68	0.62	0.75	0.92	0.59	0.83
Stachyose	6.25	6.53	7.34	6.09	6.87	6.65
Verbascose	0.38	0.68	0.48	0.79	0.34	0.56
Total α-galactosides	7.31	7.83	8.57	7.08	7.69	8.04

while in the seeds of other lupin species, then can reach even 20%. Our results do not, however, let us draw conclusions about differences between varieties in respect to the studied compounds. They do, however, show that different conditions of vegetation and harvest in different years can affect the oligosaccharide content in the seeds of the same variety.

The inositol hexaphosphate level, which is the main phytate fraction in the seeds of the studied varieties, was rather high and variable (Table 2). Total inositol phosphates ranged from 7 to 16 g/kg and was higher than values found by Burbano et al. (1995) and Caudra et al. (1994) in high alkaloid white lupin seeds. In the case of Wat and Bardo, total inositol phosphates contents (7-9 g/kg) were similar to values reported by Elkowicz and Sosulski (1982) and Caudra et al. (1992). The seeds of the Hetman variety were found to have a surprisingly high inositol phosphate content (16.2 and 12.2 g/kg). The elevated phytate content, especially IP-5 and IP-6 that form stable complexes with many elements can limit the availability of both phosphorus, as well as Ca, Mg, Zn and Cu (Fox and Tao, 1989).

TABLE 2

Content of particular and total of inositol phosphates (IP) in seeds

Ingredient	Cultivar, year of harvest					
	Wat		Hetman		Bardo	
	1993	1994	1993	1994	1992	1994
IP-3, μmol/g	–	–	–	–	–	–
IP-4, μmol/g	0.03	0.02	0.15	0.04	0.03	0.01
IP-5, μmol/g	0.40	0.23	0.71	0.23	0.29	0.13
IP-6, μmol/g	13.52	13.70	23.64	18.28	10.33	10.48
Total IP, μmol/g	13.95	13.95	24.50	18.55	10.65	10.62
Total IP, g/g	9.21	9.21	16.17	12.24	7.03	7.01

TABLE 3

Fatty acid composition of the fatty extract from lupin seeds, % of methyl esters

Specification	Cultivar, year of harvest					
	Wat		Hetman		Bardo	
	1993	1994	1993	1994	1992	1994
Ether extract, % DM	9.67	9.53	11.01	8.87	10.61	10.17
Saturated fatty acids:						
– palmitic acid, $C_{16:0}$	8.07	10.83	7.23	11.30	8.50	9.41
– stearic acid, $C_{18:0}$	1.34	1.56	1.47	1.91	1.68	1.70
– eicosenoic acid, $C_{20:0}$	0.66	0.82	0.75	0.84	0.78	0.85
– behenic acid, $C_{22:0}$	3.76	4.47	3.09	3.85	3.51	4.92
– total	13.83	17.68	12.54	17.90	14.47	16.88
Mononusatutrated fatty acids:						
– palmitoleic acid, $C_{16:1}$	0.51	0.91	0.32	0.88	0.51	0.91
– oleic acid, $C_{18:1}$	48.77	51.79	53.11	52.40	54.79	51.07
– erucic acid, $C_{22:1}$	1.76	1.95	1.38	2.13	2.13	2.74
– total	51.04	54.65	54.81	55.41	57.43	54.72
Polyunsaturated fatty acid:						
– linoleic acid, $C_{18:2}$	21.83	15.54	19.63	14.78	15.46	16.93
– eicosenoic acid, $C_{20:2}$	0.35	0.23	0.25	0.23	0.25	0.32
– linolenic acid, $C_{18:3}$	12.95	11.90	12.77	11.68	12.18	11.46
– total	35.13	27.67	32.65	26.69	27.89	28.71
Ratio-saturated fatty acid (1.0):						
– monounsaturated fatty acid	3.7	3.1	4.4	3.1	4.0	3.2
– polyunsaturated fatty acid	2.5	1.6	2.6	1.5	1.9	1.7

The crude fat content in seed dry matter of the lupin varieties equaled about 10% (Table 3). In all, saturated fatty acids constituted about 12.5 to 17.9%, the proportion of monounsaturated fatty acids exceeded 50%, while polyunsaturated fatty acids accounted for 26.7 to 35.1% of the total. In comparison with the data of other authors (Mason et al., 1990; Singh et al., 1995), the proportion of saturated fatty acids in our study was higher (by 3-7 percentage units), while the proportion of monounsaturated fatty acids was lower, especially in seeds from 1994. In the white lupin seed oil analyzed by Mason et al. (1990), the proportions of the assayed fractions were, respectively, 10, 60 and 30%.

The amino acid composition of seed protein is given in Table 4. The lysine content varied from 4.70 to 5.25 g/16 g N and was in the range reported by others (Brenes et al., 1993; Eggum et al., 1993; Buraczewska et al., 1994). However, in many studies (Batterham et al., 1986; Yanez, 1990; Eggum et al., 1993), the lysine content was lower (3.7-4.6 g/16 g N). The differences in the lysine content in the same variety, but in different years (Hetman, 4.70 and 5.00 g/16 g N and Bardo,

TABLE 4

Crude protein content in seeds and amino acid composition of protein

Specification	Cultivar, year of harvest					
	Wat		Hetman		Bardo	
	1993	1994	1993	1994	1992	1994
Crude protein, % DM	35.77	34.30	36.22	38.16	37.90	38.07
Amino acid (g/16 g N)						
His	2.86	3.12	2.89	3.18	2.80	3.17
Lys	4.93	4.94	4.70	5.00	5.25	4.80
Phe + Tyr	8.90	8.93	7.83	8.53	8.43	8.63
Met	0.86	0.80	0.81	0.74	0.82	0.78
Cys	1.55	1.79	1.55	1.78	1.42	1.74
Thr	3.81	3.35	3.49	3.33	3.77	3.24
Trp	0.84	0.83	0.83	0.85	0.88	0.86
Ile	4.65	4.33	4.38	4.10	4.58	4.18
Leu	7.84	7.62	7.76	7.54	7.90	7.36
Val	4.60	4.02	4.20	3.90	4.69	4.11
CS[1]	37.7	43.8	36.9	44.8	35.0	44.1
EAAI	69.0	67.4	65.6	66.7	68.9	66.6
CS^2_{pig}	41.0	38.1	38.6	35.2	39.0	37.1
$EAAI_{pig}$	83.0	81.0	80.6	80.7	82.9	80.0

[1] methionine with cystine

[2] methionine with cystine (methionine 55% sum S-AA)

5.25 and 4.80 g/16 g N) are difficult to explain. It is not possible, however, to conclude that there are differences in the amino acid composition between the studied varieties. All of the studied seeds had the low content of sulphur amino acids characteristic for white lupin, and higher content of cystine than methionine. Because of the low methionine content (0.74-0.86 g/16 g N), the value of the lupin protein is limited by sulphur-containing amino acids, regardless if the sum of both amino acids was compared with egg protein, or with the „ideal" protein for pigs (Table 4). In comparison with the „ideal" protein for pigs (Wang and Fuller, 1990), the essential amino acid index ($EAAI_{pig}$) was high and exceeded 80 in all samples.

No significant differences were found in digestibility and PER of the seed protein among the varieties (Table 5). The true digestibility coefficients of protein were within the range of 83-86% which is in agreement with the results of Eggum et al. (1993). The PER ranged from 1.99 to 2.19. Only by differences in the value of the limiting amino acid index can the higher PER values of 1994 seeds be explained, including the differences in the PER of Bardo seed protein (1.99 and 2.16). This may be an indication that the methionine supplement was insufficient and sulphur-containing amino acids limited the biological value of protein in the experimental diets.

TABLE 5

Protein digestibility (TD) and protein efficiency ratio (PER)

Cultivar, year of harvest	TD	PER
Wat		
1993	83.5±0.56	2.15±0.12
1994	85.1±1.99	2.19±0.25
Hetman		
1993	82.8±1.10	2.10±0.11
1994	86.1±3.16	2.19±0.21
Bardo		
1992	83.6±0.92	1.99±0.16
1994	85.2±2.46	2.16±0.24

The newer varieties, Hetman and Bardo, were not inferior in terms of PER to the old variety, Wat. The experiments of Wasilewko et al. (1995) also showed that the BV of protein of Bardo did not differ from the BV of Wat seed protein (76.0). The differences in alkaloid and fibre content of the particular varieties discussed in the first part of this study (Zduńczyk et al., 1996), i.e. the higher content of both fractions in Wat seeds, did not affect the results of growth test on rats. In the test on feed preferences, lupin variety was found to have an effect on feed intake if

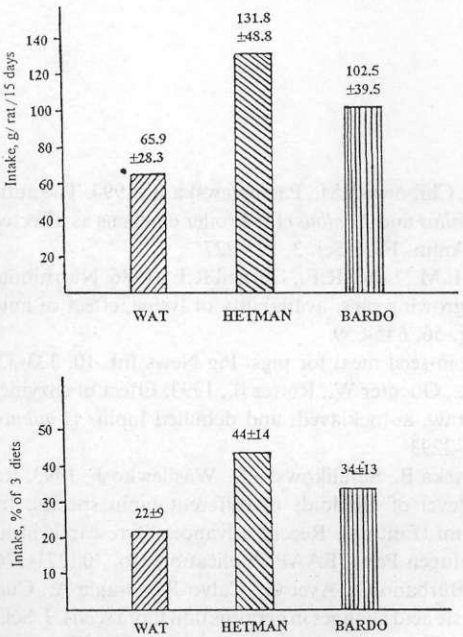

Figure 1. Intake of diets containing seeds of different sweet lupin varieties in a free choice test

the rats were allowed to freely choose among rations: intake of feeds containing Wat seeds was, on average, half that of Hetman and Bardo varieties (Figure 1). This may have been caused by the lower alkaloid content in these seeds than in Wat seeds (Zduńczyk et al., 1996). This effect was not noted, however, in the standard growth test. The results of comparative experiments show that rats are less sensitive than young pigs to the level of alkaloids in the diet (Buraczewska et al., 1993). The results of our analyses (including that of alkaloid, oligosaccharide and phytate contents) did not explain why rats preferred the diet containing Hetman seeds over that of Bardo.

CONCLUSIONS

In conclusion the results of the study show that the seeds of the white lupin varieties Wat, Hetman and Bardo have a similar content and composition of oligosaccharide fractions and fat and protein. Hetman seeds contained more phytates. In growth tests on rats, no differences were found in protein digestibility or PER. When rats had free choice of diets, they ate less of the ration containing seeds of the older variety, Wat.

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STRESZCZENIE

Skład chemiczny i wartość pokarmowa nasion niskoalkaloidowych odmian łubinu białego 2. Oligosacharydy, fityniany, kwasy tłuszczowe i wartość biologiczna białka

Oznaczono zawartość oligosacharydów, skład kwasów tłuszczowych i aminokwasowy oraz strawność i wydajność wzrostową białka trzech niskoalkaloidowych odmian łubinu białego ze zbiorów 1993 i 1994 r (Wat i Hetman) oraz 1992 i 1993 r (Bardo). Zawartość α-galaktozydów była stosunkowo niska (7,1-8,6% s.m.). Zawartość fitynianów wynosiła 0,7-0,9% w nasionach odmiany Wat i Bardo, do 1,2-1,6% w nasionach odmiany Hetman. Kwasy nasycone stanowiły 13-18%, jednonienasycone 51-57% oraz wielonienasycone 27-35% sumy kwasów tłuszczowych. Udział lizyny w białku był stosunkowo niski (4,70-5,25 g/16 g N), a aminokwasem ograniczającym wartość białka była metionina. Wskaźnik aminokwasu ograniczającego (CS), obliczony dla metioniny z cystyną, wynosił od 35 do 45% w stosunku do białka jaja kurzego i 35 do 41% w stosunku do idealnego białka dla świń. Współczynniki strawności rzeczywistej TD (83-86%) oraz wskaźniki wydajności wzrostowej białka PER nasion uzupełnionych metioniną (1,99-2,19) nie różniły się istotnie. Uzyskane wyniki nie pozwalają jednak na wskazanie odmianowych różnic w zawartości oligosacharydów oraz jakości białka i tłuszczu. Stwierdzono natomiast dużą zmienność analizowanych cech w zależności od roku zbioru nasion.