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EFFECT OF FABA BEAN SEEDS WITH DIFFERENT CONTENT OF PROANTHOCYANIDINS ON GROWTH OF RATS, CAECAL ENZYME ACTIVITY AND METABOLISM INDICES*

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Rats were fed for 6 weeks with diets containing seeds or their fractions (cotyledons and hulls) of two types of faba bean: colour-flowered varieties (dark seeds) and white-flowered strain (light seeds). Compared to dark seeds, the light seeds contained more crude protein (29.68% vs. 26.96) and less fibre (CF: 7.79 vs. 8.54% and DF: 24.74 vs. 28.93%). The light seeds contained also more α-galactosides (3.88%) than the dark seeds (3.37%). Amino acid composition, e.g. the content of lysine, methionine and cystine in the seeds and cotyledons of different types of faba bean was similar. The content of total polyphenols in light seeds of white flowered faba bean (2.79 g kg^{-1}) was about five times lower than in dark seeds of colour-flowered varieties (10.95 g kg^{-1}). Proanthocyanidins (condensed tannins) consist about 65% of the content of total polyphenols in dark seeds and about 1% of polyphenols in light seeds. The content of proanthocyanidins in the diet (about 2.7 g kg^{-1}) significantly lowered the body weight gain of rats and protein efficiency ratio (PER). Proanthocyanidins decreased also the activity of β-glucuronidase in the caecal digesta, without affecting the activity of α- and β-glucosidase and α- and β-galactosidase. Different proanthocyanidin content in the diet had no effect on the content of glucose, triglycerides and total cholesterol in the serum and activity of selected enzymes: aspartate aminotransferase, alanine transferase, superoxide dismutase and glutathione peroxidase.

Keywords: *Vicia faba* L., proanthocyanidins, nutritional value, enzyme activity, caecal contents, rat

Condensed tannins (proanthocyanidins) are treated as the most important antinutritional components of faba bean seeds. A high content of tannins in the colour-flowered faba bean cultivars is responsible for generation of poorly soluble complexes with dietary proteins, digestive enzymes and proteins of the intestinal epithelium. This results in a lowered digestibility and absorption of protein and other nutrients in the upper gastrointestinal tract (JANSMAN, 1993). In many countries, the amount of tannins is decreased by selection of white-flowered varieties of faba bean. Along with decreased content of tannins, a decrease in seed hull content, the content and composition of fibre

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and even content of protein can be observed in the seeds (GROSJEAN et al., 1995; FREJNAGEL et al., 1997). Therefore, estimation of nutritional advantages, caused by lowering of the tannin content, is difficult and requires determination of many indices of biological response of the organism. In the assay of biological activity of tannins, attention is usually paid to availability of protein and amino acids (ORTIZ et al., 1993; YU et al., 1996), activity of the digestive enzymes (YUSTE et al., 1992) and absorption of food components from the alimentary tract (JANSMAN, 1993; ZDUNCZYK et al., 1996). There is relatively little knowledge on the effect of tannins in the seeds of colour- and white-flowered faba bean on functioning of the large intestinal ecosystem and metabolism. These aspects have been considered in the presented studies.

The aim of the study was to determine chemical differences between seeds of colour- and white-flowered varieties of faba bean and to compare the nutritional and biological properties of diets with a different content of faba bean proanthocyanidins.

1. Material and methods

In this study seeds of two types of faba bean were used: one of the new Polish colour-flowered variety Start (dark seeds) and one of the first white-flowered strain STH-19BK (light seeds). Seeds were obtained from the Polish Plant Cultivation Station, Strzelce from 1999 harvest. Whole seeds and their manually-obtained fractions (cotyledons and hulls) were milled and used for chemical analysis and feeding experiment. The basic ingredients of seeds, i.e. dry matter, crude protein, crude fibre (CF) and dietary fibre (DF) were determined by the A.O.A.C. (1990) methods. The content of α-galactosides (as a sum of raffinose, stachyose and verbascose) was determined by HPLC according to MUZQUIZ and co-workers (1992). Phenolic compounds were determined using a colour reaction with the Folin-Ciocalteau reagent (NACZK & SHAHIDI, 1989), and expressed as tannic acid equivalent. Proanthocyanidins (condensed tannins) were determined by a dye reaction of cyanidine in butanol-HCL (OSZMIAŃSKI et al., 1988). In order to determine the amino acid composition, samples were hydrolyzed under nitrogen with 10 ml of 6 M HCl at 110 °C for 24 h. The amino acids were determined using a Microtechna AAA 881 (Czech Republic) automatic amino acid analyser. Sulphur containing amino acids were determined after performing acid oxidation.

The experiment was conducted on 60 Wistar rats aged 28 days and weighing initially about 65 g. Each experimental group consisted of 10 male rats. Rats were fed diets with seeds and their fractions (cotyledons and hulls). DS and LS diets contained whole dark or white seeds, diets DC and LC contained cotyledons obtained from dark or white seeds. Diets DC+LH and LC+DH contained seed fractions from different types of faba bean: cotyledons from dark seeds (DC) and hulls from light seeds (LH), or cotyledons from light seeds (LC) and hulls from dark seeds (DH). Composition of the diet is presented in Table 1. The diets contained about 100 g kg^{-1} crude protein derived

Table 1. Composition of diets with dark[a] or light[a] faba bean seeds (DS or LS, respectively) and their fractions – cotyledons (DC or LC) and hulls (DH or LH) (%)

	Seeds		Cotyledons		Cotyledons and hulls	
	DS	LS	DC	LC	DC+LH	LC+DH
Whole dark seeds	37.20					
Whole light seeds		33.70				
Cotyledons from DS			32.40		31.50	
Cotyledons from LS				29.90		29.30
Hulls from DS						5.10
Hulls from LS					5.10	
Cellulose		3.40	5.00	6.50	0.60	2.30
DL-methionine	0.25	0.25	0.25	0.25	0.25	0.25
Soya oil	10.00	10.00	10.00	10.00	10.00	10.00
Mineral mixture[b]	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin mixture[c]	2.00	2.00	2.00	2.00	2.00	2.00
Maize starch	47.55	47.65	47.35	48.35	47.55	48.05
Crude protein	10.42	10.42	10.68	10.68	10.51	10.94
Dietary fibre	10.70	10.70	10.76	10.66	10.74	10.78
Proanthocyanidins[d]	2.65	0.01	0.025	0.01	0. 03	2.78

[a] Dark or light seeds obtained from colour- or white-flowered varieties of faba bean, respectively.
[b] Mineral mixture (NRC, 1976) containing in 100 g: 73.5 g $CaHPO_4$; 8.10 g K_2HPO_4; 6.80 g K_2SO_4; 3.06 g NaCl; 2.10 g $CaCO_3$; 2.14 g $NaHPO_4$; 2.50 g MgO; 558 mg ferric citrate; 81 mg $ZnCO_3$; 421 mg $MnCO_3$; 33.3 mg $CuCO_3$; 0.7 mg KJ and 705 mg citrate acid.
[c] Vitamin mixture (A.O.A.C., 1975) containing in 1 g: 2 000 IU vitamin A; 200 IU vitamin D_3; 10 IU vitamin E; 0.5 mg vitamin K; 200 mg choline; 10 mg *p*-aminobenzoic acid; 10 mg inositol; 4 mg niacin; 4 mg calcium pantothenate; 0.8 mg riboflavin; 0.5 mg thiamin; 0.5 mg pyridoxine; 0.2 mg folic acid; 0.04 mg biotin; 0.003 mg cobalamin; sucrose (supplement to 1 g).
[d] The content of proanthocyanidins expressed in g kg^{-1}

from the seeds of faba bean, about 110 g kg^{-1} dietary fibre (balanced by the addition of cellulose) and a standard amount of mineral mix (30 g kg^{-1}) and vitamin mixture (10 g kg^{-1}).

The rats were kept individually in organic glass cages at 24 °C, 70% relative humidity and equal periods of dark and light. After 6 weeks of the experiment, the rats were anaesthetised, then blood was sampled and the caecum was separated. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in blood were assayed using kits from Randox Laboratories Ltd (United Kingdom). The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as the serum glucose, total cholesterol and triglycerides were determined using kits from Alpha Diagnostics (Poland). Glycolytic activity was measured by the rate of release of *p*-(*o*-) nitrophenols from their *p*-(*o*-) nitrophenylglucosides (DJOUZI & ANDRIEUX, 1997). The reaction mixture contained 0.3 ml substrate solution (5 mM) and 0.2 ml of a dilution

1:10 (v/v) caecal sample in a phosphate buffer (pH 7.0, 0.1 M l^{-1}). Incubation was carried out at 37 °C and *p*-nitrophenol concentration was measured as the optical absorbance at 400 nm and at 420 nm (*o*-nitrophenol concentration) after addition of 2.5 ml 0.25 M l^{-1}-sodium carbonate. Enzyme activity (glucosidase, galactosidase and glucuronidase) was expressed as μmol product formed per min (IU) per g of caecal sample.

The results of the experiment were analysed using the one-way ANOVA test, and significant differences between groups were determined by the Duncan's multiple range test. Differences were considered significant at $P \leq 0.05$ and $P \leq 0.01$.

2. Results and discussion

Chemical composition of seeds and their fractions (cotyledons and hulls) obtained from colour- and white-flowered varieties of faba bean (dark or light seeds, respectively) are presented in Table 2. Compared to dark seeds, the light seeds contained more crude protein (29.68% vs. 26.96) and less fibre (CF: 7.79 vs. 8.54% and DF: 24.76 vs. 28.93%). The light seeds contained also more α-galactosides (3.88%) than the dark seeds (3.37%). Cotyledons contained more crude protein (DC: 30.91%, LC: 33.45%) and less crude and dietary fibre (CF: 1.56 and 1.30%, DF: 17.77 and 13.92%, respectively). Hulls obtained from light seeds contained more crude protein and fibre. The protein of the seeds and cotyledons of different types of faba bean had a similar amino acid composition, e.g. the similar content of lysine, methionine and cystine.

The content of crude protein in the light seeds was similar to that determined in the seeds of the majority of white-flowered Dutch varieties of faba bean (GROSJEAN et al., 1995), however, it was higher compared to Caspar variety imported to Poland (FREJNAGEL et al., 1997). The dietary fibre contents in the whole seeds were higher, but in the cotyledons they were similar to the amounts determined in other studies (BJERGEGAARD & SØRENSEN, 1992; FREJNAGEL et al., 1997). The contents of lysine, methionine and cystine in the seeds and cotyledons were characteristic of faba bean (PASTUSZEWSKA et al., 1993).

The content of total polyphenols in light seeds of white-flowered faba bean (2.79 g kg^{-1}) was about five times lower than that in the dark seeds of colour-flowered varieties (10.95 g kg^{-1}). Polymers of polyphenols (proanthocyanidins) consist about 65% of total polyphenols of dark seeds and about 1% polyphenols of light seeds. Cotyledons obtained from dark seeds contained similar amount of polyphenols and more proanthocyanidins compared to cotyledons from light seeds. The highest differences between content of polyphenolic compounds were determined in dark and light hulls of faba bean.

Table 2. Chemical composition of the seeds (DS and LS), cotyledons (DC and LC) and hulls (DH and LH) of colour- and white-flowered faba bean, respectively

	Whole seeds		Cotyledons		Hulls	
	DS	LS	DC	LC	DH	LH
Dry matter (%)	90.66	90.65	90.61	90.53	91.05	91.54
Crude protein (%)	26.96	29.68	30.91	33.45	4.20	5.34
Crude fibre (%)	8.54	7.79	1.56	1.30	44.54	49.94
Dietary fibre (%)	28.93	24.76	17.77	13.92	87.29	89.00
α-Galactosides (%)	3.37	3.88	x	x	x	x
Amino acid (g/16 g N)						
Lys	6.06	6.08	6.00	6.06	x	x
Cys	1.26	1.27	1.22	1.21	x	x
Met	0.99	0.98	1.00	1.01	x	x
ΣEAA	37.41	37.74	37.31	37.47	x	x
Total polyphenols (g kg^{-1})	10.95	2.79	2.77	2.67	100.16	2.86
Proanthocyanidins (g kg^{-1})	7.12	0.03	0.78	0.03	66.17	0.03

ΣEAA: total essential amino acids without tryptophan;
x: not determined

The content of polyphenols in dark seeds was similar to the value determined by ORTIZ and co-workers (1993). In comparison with data from the literature (WANG & ÜBERSCHÄR, 1990; LONGSTAFF et al., 1993), the studied Polish colour-flowered varieties (dark seeds) had a lower proanthocyanidins content than many European varieties. The total polyphenols contents in dehulled seeds were lower than these found by ORTIZ and co-workers (1993). The lower and similar results of proanthocyanidins contents compared to those of CANSFIELD and co-workers' (1980) experimental data show that the white-flowered strain of faba bean differs only slightly in its polyphenolic content which is said to account for antinutritional effect. From the experiment of WANG and ÜBERSCHÄR (1990), it is evident that white-flowered faba bean variety, defined as a low-tannin variety, may contain more tannins (7–18 g kg^{-1}) than the amount determined in the studied light seeds. In the dark seeds hulls the proanthocyanidins content (6.62%) was lower than that determined by other authors (WANG & ÜBERSCHÄR, 1990; YUSTE et al., 1992; LONGSTAFF et al., 1993, ranging from 7.4 to 21%).

Different composition of diet affected feed intake only to some extent, while it had a significant effect on the body weight gain of rats (Table 3). The body weight gain of rats fed diets with dark faba bean was significantly lower compared to rats fed diet containing light seeds (groups DS and LS). The body weight gain of rats fed diet with cotyledons of both types of faba bean was similar (groups DC and LC). The addition of dark hulls (group LC+DH) decreased the body weight gain of rats compared to group LC fed diet containing cotyledons from light seeds. The addition of light hulls had no significant effect on body weight of rats. The lower PER was noted in the case of the diet DS and LC+DH containing whole seeds or hulls obtained from dark seeds. The obtained results support observations of other authors (BJERG et al., 1988;

ALZUETA et al., 1992; JANSMAN, 1993; PASTUSZEWSKA et al., 1993) that high content of condensed tannins decreased protein efficiency of diet containing seeds of colour-flowered faba bean.

Table 3. Diet intake and body weight gain of rats (g/rat/6 weeks) and protein efficiency ratio (PER) of diet containing different faba bean seeds and their fractions

	Seeds		Cotyledons		Cotyledons and hulls		SEM
	DS	LS	DC	LC	DC+LH	LC+DH	
Diet intake	725.6	735.4	741.5	729.9	733.5	737.5	3.2
Body weight gain	124.1^{Bc}	140.3^{Aa}	130.1^{Bbc}	132.0^{ABb}	131.6^{ABbc}	124.4^{Bc}	1.21
PER	2.08^{CDc}	2.33^{Aa}	2.18^{BCb}	2.25^{ABab}	2.28^{ABa}	1.98^{Dd}	0.02

PER was calculated according to protein consumption and body weight gain of rats recorded during four weeks of the experiment.
Values in one row having different superscripts are significantly different at $P \leq 0.05$ (a,b,c) or at $P \leq 0.01$ (A,B,C,D)

Diet composition, including tannin content, had no significant influence on the amount of caecal contents and caecal tissue weight (Table 4). Caeca of rats fed diet containing high-proanthocyanidins faba bean seeds (DS) were more plump, with the caecal contents containing more dry matter. Probably, for this reason pH of caecal content of rats from group DS was lower than that of the LS group. A higher pH of the caecal contents in rats from the other groups resulted partly from higher amount of water in the contents. Substitution of faba bean seed coat with cellulose (DC and LC groups) evoked lowering of the dry mass content and a slight pH increase, compared to the LS group in which rats were given diet containing low-tannin seeds. High content of proanthocyanidins groups DS and LC+DH caused lowering of β-glucuronidase activity in the caecal digesta. The activity of α-galactosidase was affected to a lower degree by the content of tannin in diets. A negative effect of faba bean proanthocyanidins was found in our other studies (JUSKIEWICZ et al., 2001*)*. The results collated in Table 4 indicate that antimicrobial properties of tannins are rather low in vivo compared to the effects found in vitro (CHUNG et al., 1998).

Different tannin content in diet had no effect on the content of glucose, triglycerides and total cholesterol in the serum and activity of selected enzymes: aspartate aminotransferase, alanine transferase, superoxide dismutase and glutathione peroxidase (Table 5). In our other studies (JUSKIEWICZ et al., 2001), a higher content of faba bean proanthocyanidins increased activity of alanine aminotransferase in plasma.

Table 4. The amount of caecal contents, caecal tissue weight and activity of microbial enzymes in the caecal digesta

	Seeds		Cotyledons		Cotyledons + hulls		SEM
	DS	LS	DC	LC	DC+LH	LC+DH	
Caecal content[c]	1.20	1.02	0.95	0.92	1.05	0.99	0.04
Caecal tissue weight[c]	0.27	0.26	0.23	0.23	0.26	0.24	0.01
Dry matter (%)	26.8^{a}	26.4^{ab}	24.4^{b}	24.5^{b}	26.4^{ab}	25.6^{ab}	0.31
pH	6.19^{b}	6.36^{ab}	6.38^{ab}	6.43^{a}	6.38^{ab}	6.32^{ab}	0.03
α-Glucosidase[d]	2.19^{b}	2.14^{b}	2.12^{b}	2.58^{ab}	2.06^{b}	2.94^{a}	0.09
β-Glucosidase[d]	0.84	1.00	0.91	1.01	0.77	0.97	0.05
α-Galactosidase[d]	3.15^{b}	4.40^{ab}	2.97^{b}	3.94^{ab}	5.19^{a}	3.60^{ab}	0.25
β-Galactosidase[d]	6.17	6.95	6.99	6.45	6.16	7.10	0.33
β-Glucuronidase[d]	1.43^{Bc}	2.66^{Aa}	2.04^{ABabc}	2.42^{ABab}	2.14^{ABab}	1.75^{ABbc}	0.12

[c] g/100 g BW.
[d] U g^{-1} caecal content.
Values in one row having different superscripts are significantly different at P≤0.05 (a,b,c) or at P≤0.01 (A,B)

Table 5. Glucose, triglycerides, cholesterol content and activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in serum[a] or blood[b]

	Seeds		Cotyledons		Cotyledons + hulls		SEM
	DS	LS	DC	LC	DC+LH	LC+DH	
Glucose[a] (mg dl^{-1})	190.1	181.0	190.0	183.6	191.4	177.8	3.9
Triglycerides[a](mg dl^{-1})	91.9	98.8	93.0	99.1	96.0	91.1	2.6
Cholesterol[a] (mg dl^{-1})	94.6	96.9	97.6	98.8	97.7	99.0	1.2
AST[a] (U l^{-1})	148.5	148.1	149.0	151.6	147.5	142.0	2.9
ALT[a] (U l^{-1})	17.8	18.4	19.0	19.3	17.2	17.0	0.6
GPx[b] (U l^{-1})	18.3	19.7	18.3	19.7	18.2	18.8	0.5
SOD[b] (U ml^{-1})	304	316	339	328	365	341	9.2

3. Conclusions

The results obtained in the present study suggest that seeds of white-flowered faba bean were characterised by more beneficial chemical composition, i.e. the higher content of crude protein and lower content of dietary fibre and first of all repeatedly lower content of proanthocyanidins. High content of proanthocyanidins in diet significantly decreased protein utilization, but only to some extent affected functioning of the caecal ecosystem (decreasing of dry matter content, increasing pH and microbial β-glucuronidase activity), and had no influence on biochemical and enzymatic blood indices of rats.

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