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BIOLOGICAL ACTIVITY OF FABA BEANS PROANTHOCYANIDINS

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The objective of the experiment was to determine whether small amounts of proanthocyanidins (0.1 and 0.3%) may increase the antioxidative properties of the rat diet without exerting an antinutritional effect. Proanthocyanidins of faba bean seed coats were extracted with a mixture of acetone and water (70:30) and lyophilized. The amount of proanthocyanidins was two- or fourfold higher in the experimental diets as compared to the control diet. The addition of proanthocyanidin extract had no significant effect on the coefficients of digestibility of crude protein, daily nitrogen retention and the coefficient of biological value of diet protein. In the blood serum of rats fed diets supplemented with proanthocyanidin extract, there was a slightly higher content of vitamin E and alanine aminotransferase activity, while the content of vitamin A and aspartate aminotransferase activity were similar to those of the control group. In the contents of the rat gut (caecum), a lower activity of β-glucuronidase was found as compared to the control group, whereas β-galactosidase was unaffected. The addition of proanthocyanidin extract to diet caused a decrease in the malondialdehyde content in the heart, kidneys, erythrocytes and blood plasma of rats. The results obtained indicate that the amount of proanthocyanidins used did not exert any antinutritional effects, but extended the pool of diet antioxidants and beneficially affected the activity of the large bowel microflora.

Keywords: proanthocyanidins, nitrogen digestibility and retention, antioxidative activity, enzyme activity, rat

Polyphenols of seed coat, especially condensed tannins, are treated as the most important antinutritional components of faba bean seeds (JANSMAN, 1993). In polyphenol-rich diets, the availability of protein and aminoacids is decreased (ORTIZ et al., 1993; YU et al., 1996), and when such diets are consumed the activity of the digestive enzymes is lowered (YUSTE et al., 1992), and the absorption of food components from the alimentary tract is limited (JANSMAN, 1993; ZDUŃCZYK et al. 1996). On the other hand, those compounds are found to reveal antioxidative,

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bacteriostatic, anticarcinogenic and antimutagenic activity (BAGCHI et al., 1998; HAGERMAN et al., 1998; KAUL & KHANDUJA, 1998). There is, however, scarce information on what quantities of different groups of polyphenolic compounds are necessary for obtaining their beneficial or adverse biological properties.

The objective of the experiment was to determine whether a small amount of proanthocyanidins (0.1 and 0.3%) could increase the antioxidative properties of the rat diet without exerting an antinutritional effect.

1. Materials and methods

The experiment was conducted on 30 Wistar rats aged 40 days and weighing 110.1±2.2 g at the beginning of the test. The experimental groups were composed of 10 male rats. The rats were kept individually in metabolic cages at the temperature of 24 °C, 70% relative humidity and equal periods of dark and light. The composition of diets is presented in Table 1. The diets contained 150 g kg^{-1} crude protein (casein supplemented with DL-methionine) and standard amount of mineral mix (according to NRC, 1976) and vitamin mixtures (according to A.O.A.C., 1975). The experimental groups (II and III) were fed diets containing proanthocyanidin extract, 1 or 3 g kg^{-1}, respectively. The proanthocyanidin content in diet II and III was equal to their content in the diets containing 5 or 10% of seeds of colour-flowered faba bean. Proanthocyanidin extract was incorporated at the expense of potato starch.

Proanthocyanidins were isolated from the hulls of faba beans using method described by HUSSEIN and co-workers (1990). The hulls of faba beans were extracted with 70% aqueous acetone containing 2.8 mmol l^{-1} ascorbic acid as antioxidant. After semi-purification the extract contained 41% of proanthocyanidins in dry matter. The experiment lasted 10 days. Rats were anesthetized using urethane (140 mg×100 g^{-1} body wt.).

Blood was collected from the abdominal artery in order to obtain plasma samples and red blood cells (RBC). Blood was collected in heparinized tubes, plasma was prepared by centrifugation at 1500×g for 15 min at 4 °C and stored at –40 °C until analysis. The muscles of samples and internal organs (heart, lung, kidney and liver) were frozen in liquid nitrogen and stored at –40 °C until analysed. Samples were homogenized with 1.15% KCl until the assay of MDA. The malondialdehyde (MDA) concentration was determined using the colorimetric method described by UCHIYAMA and MIHARA (1978). The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined using the kinetic methods with an Alpha Diagnostics kit and an Epoll-20 photometer. The content of vitamins was determined by the HPLC method according to CUESTA SANZ and CASTRO SANTA-GRUZ (1986).

Table 1

Composition of diets without or with different content of tannin extract (g kg^{-1})

Ingredients	Experimental group		
	I	II	III
Casein	115.0	115.0	115.0
DL-methionine	2.0	2.0	2.0
Soya oil	100.0	100.0	100.0
Potato starch	100.0	99.0	97.0
Mineral mixture[a]	30.0	30.0	30.0
Vitamin mixture[b]	10.0	10.0	10.0
Proanthocyanidins extract	–	1.0	3.0
Maize starch	643.0	643.0	643.0

[a] 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 citric acid

[b] 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)

The glycolytic activity was measured by the rate of release of *p*-(*o*-)nitrophenol from its *p*-(*o*-)nitrophenylglucosides. The reaction mixture contained 0.3 ml substrate solution (5 mmol) and 0.2 ml of a dilution 1:10 (v/v) caecal sample in a phosphate buffer (pH 6.4, 0.1 mol l^{-1}). Incubation proceeded at 37 °C and the *p*-nitrophenol concentration was measured as the optical absorbance at 400 nm (β-glucuronidase) and the *o*-nitrophenol concentration at 420 nm (β-galactosidase) after the addition of 2.5 ml 0.25 mol sodium carbonate. The enzyme activity (β-galactosidase and β-glucuronidase) was expressed as μmol of product formed per min (IU) per g of caecal sample (DJOUZI & ANDRIEUX, 1997).

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

2. Results and discussion

The nitrogen balance for rats fed casein diets supplemented with proanthocyanidins is presented in Table 2. The addition of the proanthocyanidin extract reaching 0.1 or 0.3% caused a slight increase (by about 7%) in the amount of nitrogen

expelled with faeces, as compared to the control group. The coefficient of crude protein digestibility reached 94.7% in the control group and was insignificantly higher than that of the experimental groups (94.0%). The nitrogen losses in urine of rats given a diet supplemented with proanthocyanidins were higher by about 10% as compared to the control group. Subsequently, a small decrease in the nitrogen content retention in the organisms of rats from those groups was observed (68.3 and 68.7% of nitrogen absorbed) in comparison to the control group (71.6%). The biological value (BV) of diet protein was similar in all groups (from 84.8 to 87.5%).

The addition of proanthocyanidins did not have any antinutritional effects observed in the case of their increased content in a diet (ORTIZ et al., 1993; YU et al., 1996). It corresponded to the studies of LONGSTAFF and MCNAB (1991), who stated that a low proanthocyanidins content can even increase the digestibility coefficients of some components, e.g. fat.

In the blood plasma of rats fed diets supplemented with proanthocyanidins, a higher (by 20–20%) activity of alanine aminotransferase (ALT) was observed, in comparison with the control group (Table 3). The activity of aspartate aminotransferase (AST) was similar in all groups tested. The vitamin A content in blood plasma was similar in all groups, while that of vitamin E was higher in the experimental groups. The vitamin E content in blood plasma reached 838 mg l^{-1} for the control group, 902 mg l^{-1} for rats fed a diet supplemented with 0.1% proanthocyanidin extract, and 954 mg l^{-1} for rats given a diet with a higher amount of the proanthocyanidin extract.

Table 2

Digestibility and retention of nitrogen of diets

	Experimental group			SEM
	I	II	III	
Diet intake, g/week	66.9	67.3	67.0	0.15
Nitrogen intake, mg	1124.3	1130.6	1126.3	2.51
Nitrogen excretion:				
– faecal, mg	125.0	134.5	134.9	5.56
– urinary, mg	260.5	290.2	285.3	15.03
N digestibility, %	94.7	94.0	94.0	0.49
Nitrogen retention:				
– mg/day	114.9	110.3	110.4	1.96
– % of nitrogen intake	71.6	68.3	68.7	1.26
BV, %	87.5	84.8	85.7	1.39

Table 3

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity and vitamin A and E content in plasma

	Experimental group			SEM
	I	II	III	
AST (U l^{-1})	99.4	97.8	99.8	2.55
ALT (U l^{-1})	15.8 b	19.0 ab	20.4 a	0.83
Vitamin A, mg l^{-1}	368.6	350.6	358.8	18.8
Vitamin E, mg l^{-1}	838.3	901.8	953.6	29.0

a, b: significant at $P<0.05$

These results may indicate the antioxidative activity of the proanthocyanidin extract. Plant proanthocyanidins are natural biological antioxidants (HAGERMAN et al., 1998; PLUMB et al., 1998). When added to diet, they can limit the consumption of other antioxidants, including vitamin E.

The malondialdehyde (MDA) content in rat tissues is presented in Table 4. The addition of the proanthocyanidin extract to diet caused a decrease in the MDA concentration in heart, kidneys, erythrocytes and most of all in blood plasma. Only in the case of lung tissue, a significant increase in the malondialdehyde content was observed in groups fed a diet with faba bean proanthocyanidins. The decreased MDA content in tissues can be explained by the antioxidative activity of the proanthocyanidin extract. Polyphenols belong to the biologically active diet components which affect the oxidative status of tissues (DECKER, 1995).

The addition of the proanthocyanidin extract had a beneficial influence on the activity of the blind gut microflora, resulting in a decrease in the β-glucuronidase activity (Table 5). The hydrolysis of glucuronide bonds, caused by the presence of that enzyme, increases the content of substances revealing potential toxic and carcinogenic activity in the large bowel (REDDY et al., 1992). The addition of 0.1% and 0.3% tannin extract into a diet decreased the activity of β-glucuronidase by about 30% and 70%, respectively, as compared to the control group. No effect of the extract examined on the β-galactosidase activity was noted. The results obtained are in agreement with the studies of other authors (DE BRUYNE et al., 1999; CHUNG et al., 1998; TEBIB et al., 1996) indicating the beneficial effect of tannins on the microbiological activity of the blind gut and colon by having a limited growth of harmful bacteria.

Table 4

Malondialdehyde (MDA) concentration in internal organs and plasma

	Experimental group			SEM
	I	II	III	
Muscles, mg/100g	1.714	1.806	1.904	0.08
Lung, mg/100g	0.678 b	0.860 a	0.820 a	0.03
Kidney mg/100g	3.417 A	2.304 B	2.406 B	0.17
Liver, mg/100g	1.959	1.669	1.999	0.15
Heart, mg/100g	1.642 a	1.278 b	1.448 ab	0.06
Erythrocytes, mg/100g	4.377 A	3.001 B	2.998 B	0.22
Plasma, μg/100ml	133.2 A	44.3 B	47.28 B	11.35

A, B: significant at $P<0.01$
a, b: significant at $P<0.05$

Table 5

The activity of β-galactosidase and β-glucuronidase in the caecum content

Enzyme activity	Experimental group			SEM
	I	II	III	
β-Galactosidase, Ug^{-1}	2.48	2.58	2.19	0.18
β-Glucuronidase, Ug^{-1}	1.99 Aa	1.44 ABa	0.62 Bb	0.20

A, B: significant at $P<0.01$
a, b: significant at $P<0.05$

3. Conclusions

The results of the study indicate that a small amount of proanthocyanidins (0.1 and 0.3%) increases the antioxidative properties of diets without exerting antinutritional effects. The addition of proanthocyanidin extract had no effect on the digestibility and biological value of the proteins, it decreased the MDA content in most tissues and the β-glucuronidase activity in the blind gut contents, and increased the level of vitamin E in plasma.

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