

The influence of extrusion of faba bean seeds and supplementation of sulphur amino acids on performance, pancreatic trypsin activity, and morphological parameters of the jejunum in rats

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ABSTRACT

The effect of extrusion of faba bean seeds and supplementation of sulphur amino acids (AA) on trypsin inhibitor (TI) and lectin activity, and the histology and microbiology of the jejunum were studied. Male Wistar rats were fed semi-purified diets containing 10% faba bean seeds, either raw (R) or extruded at 160°C (E); the diets were either unsupplemented or supplemented with 0.3% sulphur AA (met + cys, 1:1). Extrusion reduced TI activity of seeds from 1.8 (FR) to 0.5 with TIU/mg (FE) and completely eliminated agglutination activity against human red blood cells (RBC). Extrusion of faba bean seeds did not affect rat performance or pancreatic trypsin activity (5255 vs 4065 U/100 g protein), and decreased ($P<0.05$) dry matter digestibility (89.7 vs 92.9%). Supplementing sulphur amino acids improved performance of rats fed both raw and extruded seeds. Extrusion of faba bean and supplementing sulphur amino acids significantly improved intestinal crypt depth, villi length and width.

KEY WORDS: lectins, trypsin inhibitors, extrusion, methionine, cystine, faba bean, rat

INTRODUCTION

Although faba bean seeds have fewer protease inhibitors and lower lectin activity than soyabean seeds (Valdebouze et al., 1980; Leontowicz et al., 2001b),

they contain tannins, which reduce digestibility by forming complexes with proteins and/or digestive enzymes (Griffiths and Moseley, 1980) and also depress animal performance. Different technologies (boiling, autoclaving, extrusion) are used for inactivation of thermolabile ANFs present in legumes (Licner, 1994). The low level of sulphur amino acids limits growth of animals fed on diets with legumes but supplementation with synthetic amino acids usually improves performance.

The aim of our study on rats was to estimate the effect of extruding faba bean seeds and supplementing sulphur amino acids on performance, protein digestibility, pancreatic trypsin activity, morphometry of the jejunal mucosa, and total counts of bacteria colonizing mucosa.

MATERIAL AND METHODS

Animals and diets

Male Wistar rats of about 86 ± 7 g were divided into four groups of ten and fed, *ad libitum*, for 28 days semi-purified diets composed of (%) wheat starch, 64.3; casein, 10; cellulose, 1; soyabean oil, 10; mineral-vitamin mixture, 4.7 (NAS, 1972) and 10% faba bean seeds (*Vicia faba* L.) var. Nadwiślański (F), raw (R) or extruded (E) (extruder INSTA-PR0 600, temp. 160°C , exposition time 30-60 s). Half of each diet was supplemented with 0.3% sulphur amino acids (AA) (met + cys, 1:1). Digestibility of dry matter and protein were estimated.

Measurements

Trypsin inhibitor activity (TIA) in the seeds was analyzed according to Valdebouze et al. (1980). Pancreatic trypsin activity (Erlanger et al., 1961) and protein content (Lowry et al., 1951) were determined.

Lectins were extracted from raw and extruded seeds according to Paredes-Lopez et al. (1989) and the agglutination test with human RBC (groups O, A, B, AB) was performed according to Liener (1989).

In the jejunum, the depth of crypts, length and width of villi, and thickness of the tunica mucosa were measured and total bacteria and *E. coli* counts were determined (except group FE) as described in a previous paper (Leontowicz et al., 2001a).

Statistical analysis

The results were evaluated by ANOVA and differences between arithmetic means ($x \pm \text{SD}$) were evaluated by the Tukey multiple range test.

RESULTS

Trypsin inhibitor activity in faba bean seeds was reduced by extrusion from 1.8 to 0.5 TIU/mg (Table 1). The lectin extract obtained from raw seeds agglutinated human red blood cells without a clear-cut affinity for a specific blood group; the smallest activity was found against group B (Table 2). Extrusion eliminated haemagglutination activity.

TABLE 1

Trypsin inhibitors and haemagglutination titre of protein extract from faba bean seeds

Faba bean seeds	Trypsin inhibitors, TIU/mg	Haemagglutination titre of human red blood cells			
		group			
		O	A	B	AB
Raw	1.8	1:200	1:100	1:50	1:200
Extruded	0.5	0	0	0	0

O = no agglutination at 1 mg/ml concentration of initial protein extract,
1: 50, 1: 100, 1: 200 – dilution of initial (1mg/ml) protein extract

TABLE 2

The influence of faba bean extrusion and sulphur amino acids supplementation on performance, dry matter and protein digestibility in rats (n=10)

Item	Diet FR ¹	Diet FE ²	Diet FRAA ³	Diet FEAA ⁴
Food intake, g/d	11.3 ± 1.6 ^a	11.9 ± 1.8 ^a	14.5 ± 1.3 ^b	15.9 ± 1.5 ^c
Body gain, g/d	2.7 ± 0.4 ^a	3.0 ± 0.5 ^a	5.2 ± 0.8 ^b	5.6 ± 0.8 ^b
Feed/gain, g	4.0 ± 1.2 ^b	4.0 ± 0.3 ^b	2.8 ± 0.2 ^a	2.9 ± 0.3 ^a
Digestibility, %				
dry matter	92.9 ± 1.5 ^b	89.7 ± 2.0 ^a	92.7 ± 0.9 ^b	92.8 ± 0.9 ^b
crude protein	85.0 ± 2.9	86.0 ± 1.1	85.2 ± 2.1	85.5 ± 2.2

^{a,b,c} means in the rows tagged with different letters differ significantly at P<0.05

¹ faba bean raw

² faba bean extruded

³ faba bean raw +amino acids

⁴ faba bean extruded + amino acids

The protein content in the diets amounted to: 12.12; 12.15; 12.77; 12.59 % on a DM basis in FR, FE, FRAA and FEAA, respectively. Extrusion of faba bean seeds did not affect feed intake, body gain, or FER (Table 2). Sulphur AA supplementation of diets with raw and extruded seeds significantly improved

performance. Dry matter (DM) digestibility was lower ($P < 0.05$) in rats fed FE than FR (89.7 vs 92.9), AA supplementation significantly increased DM digestibility in FEAA. Crude protein digestibility was not affected by either extrusion or AA supplementation.

The proteolytic activity in the pancreas tended to be higher in rats fed extruded beans (Table 3). The protein content in the pancreas increased significantly in group FEAA in comparison with group FE.

TABLE 3
The influence of raw or extruded seeds and amino acids on pancreatic parameters in rats (n=10)

Item	Diet FR ¹	Diet FE ²	Diet FRAA ³	Diet FEAA ⁴
Pancreas, % BW	0.31 ± 0.03	0.34 ± 0.05	0.35 ± 0.07	0.36 ± 0.07
Trypsin activity, U/100 g protein	4065 ± 581	5255 ± 1010	4146 ± 1217	4999 ± 1184
Protein, mg/organ	4.85 ± 0.81 ^{ab}	4.03 ± 0.79 ^a	4.85 ± 1.01 ^{ab}	5.59 ± 0.76 ^b

^{a,b} means in the rows tagged with different letters differ significantly at $P < 0.05$
^{1,2,3,4} as in Table 2

Extrusion of seeds and addition of AA to the diet with raw faba bean significantly increased crypt depth, suggesting an increase of proliferative processes in the mucosa epithelium. Villi length, width, as well as thickness of the tunica mucosa significantly increased due to extrusion (Table 4).

TABLE 4
Morphometry analysis of the small intestine (jejunum) of rats (n=10)

Item	Diet FR ¹	Diet FE ²	Diet FRAA ³	Diet FEAA ⁴
Crypt depth, µm	148 ± 6 ^a	161 ± 6 ^b	165 ± 6 ^b	160 ± 6 ^b
Villi length, µm	368 ± 15 ^a	394 ± 15 ^{ab}	376 ± 12 ^a	401 ± 15 ^b
Villi width, µm	101 ± 3 ^a	117 ± 6 ^{bc}	114 ± 6 ^{ab}	122 ± 6 ^c
Thickness of tunica mucosa, µm	537 ± 18 ^a	563 ± 18 ^b	532 ± 12 ^a	576 ± 15 ^b

^{a,b,c} means in the rows tagged with different letter differ significantly at $P < 0.05$ and SD
^{1,2,3,4} as in Table 2

Total culturable bacterial counts decreased ($P < 0.05$) in group FEAA in comparison with group FRAA, but there was no difference ($P > 0.05$) in *E. coli* counts between groups (Table 5).

TABLE 5

Microbiological parameters of the jejunum of rats (n=7)

Diets	Total culturable bacterial count log CFU*/g	<i>E. coli</i> log CFU/g	<i>E. coli</i> /total culturable bacterial count
FR ¹	5.54 ± 0.77 ^b	2.16 ± 1.84	0.390
FRAA ³	5.09 ± 0.17 ^b	1.74 ± 1.27	0.342
FEAA ⁴	4.16 ± 1.11 ^a	1.23 ± 0.60	0.296

* CFU – colony forming unit

^{a,b} means in the rows tagged with different letter differ significantly at P<0.05^{1,3,4} as in Table 2

DISCUSSION

Extrusion of faba bean seeds at 160°C eliminated about 72% of trypsin inhibitor activity and total hemagglutination activity towards human RBC. Similar effects of extrusion (Kostyra et al., 1999) or boiling (Leontowicz et al., 1998) of faba bean and pea seeds have been observed. Soyabean seeds are less sensitive to extrusion at the same temperature than faba bean (Leontowicz et al., 2001a). Sulphur AA (0.3%) significantly improved performance of rats fed both raw or extruded faba bean seeds, which is in agreement with other studies. The lack of a positive effect of extrusion of faba bean on digestibility parameters and body gain results from its lower ANF activity or possible negative effects of temperature on lysine and protein availability. Also AA supplementation did not increase pancreatic trypsin activity (P>0.05), which was reported by Friedman (1994).

Lower lectin activity in raw faba bean and lower *E. coli* number in the rat jejunum caused less significant morphological changes in the small intestine than in rats fed on raw soyabean (Leontowicz et al., 2001a) or pea (Leontowicz et al., 2000). Elimination of lectin activity during extrusion of faba bean seeds and AA supplementation significantly improved intestinal crypt depth, villi length and width.

CONCLUSIONS

Extrusion of faba bean seeds (160°C) completely eliminates lectin activity, but TI activity only partially. Rat performance is not affected by extrusion but is significantly improved by amino acid supplementation of both raw and extruded seeds. A synergistic effect of extrusion and amino acid supplementation is observed in respect to feed intake. Both extrusion and amino acid supplementation improve morphological parameters of the small intestine (jejunum).

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STRESZCZENIE

Wpływ ekstruzji nasion bobiku i dodatku aminokwasów siarkowych na wzrost, aktywność trypsyny w trzustce i morfologię jelita czczego szczurów

Badano wpływ ekstruzji nasion bobiku oraz dodatku do diety aminokwasów siarkowych (AA) na aktywność inhibitorów trypsyny (TI) i lektyn, histologię i mikrobiologię jelita czczego szczurów. Szczury Wistar karmiono półsyntetycznymi dietami zawierającymi 10% nasion bobiku: surowych lub ekstrudowanych (w temp. 160°C) bez lub z dodatkiem 0,3% aminokwasów siarkowych (met + cys, 1:1). Ekstruzja obniżała aktywność TI z 1,8 (FR) do 0,5 TUI/mg (FE) i całkowicie eliminowała aktywność aglutynacji określaną względem ludzkich erytrocytów. Ekstruzja nie wpływała na wzrost i wykorzystanie paszy (FER), zwiększała aktywność trypsyny w trzustce (5255 vs 4065 u/100 g białka) i obniżała ($P<0,05$) strawność suchej masy (89,7 vs 92,9%). Dodatek AA siarkowych poprawiał przyrosty m.c. i FER zarówno w grupach otrzymujących surowe jak i ekstrudowane nasiona bobiku. Zarówno ekstruzja jak i dodatek AA siarkowych powodowały istotne pogłębienie krypt oraz wydłużenie i pogrubienie kosmków jelita czczego.