EFFECT OF FABA BEAN SEEDS AND THEIR FRACTIONS ON RAT CAECUM PHYSIOLOGY

Jerzy Juśkiewicz, Monika Wróblewska, Sławomir Fertykowski, Zenon Zduńczyk, Łucja Brzuzan, Irena Godycka-Kłos
Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, 10 Tuwima Str., 10-747 Olsztyn, Poland

Summary. The influence of dietary supplementation (5%) of a casein diet with faba bean seeds, haba bean hulls and faba bean oligosaccharides extracted from cotyledons on the metabolism of the caecum was investigated in an experiment on Wistar rats. In rats receiving the diet with seeds and their fractions (hulls and oligosaccharides), a greater weight of caecal tissue (especially in oligosaccharide treatment) was recorded, compared with the control group. The significantly greater accumulation of digesta was observed only in the case of the oligosaccharide group. The highest hydration of digesta as well as activity of bacterial β-glucuronidase activity was in the control group. The highest activity of β-glucosidase was in group fed diet containing whole faba bean seeds. The oligosaccharide addition to a diet was associated with the highest activities of α-glucosidase and α-galactosidase, the highest production of short-chain fatty acids in the caecum as well as the most beneficial composition of particular acids.

Keywords: Bean, rats, caecal digesta, digestible.

‘FABA’ VEISLĖS PUPŲ SĖKLŲ IR JŲ FRAKCIJŲ ĮTAKA ŽIURKIŲ AKLOSIOS ŽARNOS FIZIOLOGIJAI

Jerzy Juśkiewicz, Monika Wróblewska, Sławomir Fertykowski, Zenon Zduńczyk, Łucja Brzuzan, Irena Godycka-Kłos
Gyvulių reprodukcijos ir Maisto tyrimų institutas, Lenkijos mokslų akademija, Tuwima gt., 10-747 Olštynas, Lenkija

Santrauka. Šiuo bandymu buvo tiriama kazeino, raciono papildyto (5 proc.) ‘Faba’pupų sėklomis, ‘Haba’pupų sėklaskiltėmis ir ‘Faba’pupų oligosacharidais, ekstrahuotais iš sėklaskilčių, įtaka Vistar veislės žiurkių medžiagų apykaitai aklojoje žarnoje. Žiurkių, gavusių racioną su sėklomis ir jų frakcijomis (sėklaskiltės ir oligosacharidai), organizme buvo nustatytas didesnis aklosios žarnos audinio svoris palyginti su kontroline grupe. Patikimai didesnis maisto medžiagų susikaupimas pastebėtas tik žiurkių grupėje, gavusioje oligosacharidų. Didžiausia pasisavintų maisto medžiagų hidratacija, taip pat bakterinės beta-gliukoronidazės aktyvumas nustatytas kontrolinėje grupėje. Didžiausias beta-gliukosidazės aktyvumas buvo grupėje, šertoje ‘Faba’ pupų sėklomis. Olisacharidų priedas racione buvo susijęs su didesniu alfa-gliukosidazės ir alfa-galaktosidazės aktyvumu, didesne žemo molekulinio svorio riebiųjų rūgščių gamyba ir palankesne tam tikrų rūgščių sudėtimi.

Raktažodžiai: pupos žiurkės, akloji žarna, mityba, pasisavinimas.

Introduction. Cultivated faba bean seeds are commonly used as a component of animal feed in many countries, but also as human food in developing regions. Moreover, the use of domestic legumes like faba beans and lupins, instead of imported soya bean meal, offers a possibility to increase the protein self-sufficiency in animal feeding in many countries (Frejnagel et al., 1997). It is well-known that a high content of tannins in the colour-flowered faba bean cultivars is responsible for lower digestibility and absorption of protein and other nutrients in the upper gastrointestinal tract (Zduńczyk et al., 2000). In this context, a great many studies have focused on the biological activity of faba beans proathocyanidins (Juśkiewicz et al., 2001; Reed, 1995; Zduńczyk et al., 1996). On the other hand, legume seeds are a rich-source of dietary fibre, including its insoluble and soluble fractions (Queiroz-Monici et al., 2005). It has been repeatedly observed that, the type of dietary fibre has a great impact on large bowel physiology (Juśkiewicz et al., 2005). Depending on a diet composition the metabolic processes in that site of GI could be considered as beneficial or detrimental (Remesy et al., 1992). The aim of this experiment was to evaluate the in vivo effects of diets containing 5% faba bean seeds or their fractions (hulls and oligosaccharides extracted from cotyledons) on caecal metabolism in growing rats.

Material and Methods. The experiment was conducted on Wistar rats aged 40-45 days and weighing 90 ± 3.10 g at the beginning of the test. The environment was well controlled with a 12-h light-dark cycle, a temperature of 21 ± 1°C, a relative humidity of 50 ± 5% and 20 air changes/h. Animals had free access to water and semi-purified powdered diets prepared from well-defined components. All diets provided sufficient amounts of vitamins, minerals, essential amino acids and lipids. Each experimental diet contained 10% crude protein, 8% soybean oil and 1% of cholesterol. Faba bean seeds (Nadwiślański cv.), hulls and oligosaccharides from faba bean seeds were used as experimental factors in three diets (50 g/kg). The compositions of the different diets are given in Table 1.

The hulls were obtained from hand-dehulled seeds and oligosaccharides were extracted from faba bean cotyledons using 80% aqueous ethanol according to the method described by Muzquiz et al. (1992). The extract contained 30% of oligosaccharides in dry matter.

The experimental protocol was approved by the Local Council for Animal Experiments in Olsztyn (Poland). The experiment lasted 14 days. At the termination of the experiment rats were anaesthetized with sodium pentobarbitone according to the recommendations for euthanasia of experimental animals (Close et al., 1997). After laparotomy, the caecum with contents was removed and weighed. Samples of fresh digesta were used for immediate analysis (dry matter, SCFA); the rest was transferred to tubes and stored at -70°C. The caecum wall was flushed clean with ice-cold saline, blotted on filter paper and weighed as the caecal tissue mass. The caecal pH was measured using a microelectrode and pH/ION meter (model 301, Hanna Instruments). Dry matter of the caecal digesta was determined at 105°C. Bacterial enzyme activity in the caecal digesta was measured by the rate of p- or o- nitrophenol release from their nitrophenylglucosides according to the modified method of Djouzi and Andrieux described by Juskiewicz et al. (2002). The following substrates were used: for β-glucuronidase: p-nitrophenyl-β-D-glucuronide, for α-galactosidase: p-nitrophenyl -α-D-galactopyranoside, for β-galactosidase: o-nitrophenyl-β-D-galactopyranoside, for α-glucosidase: p-nitrophenyl-α-D-glucopyranoside, and for β-glucosidase: p-nitrophenyl-β-D-glucopyranoside. The reaction mixture contained 0.3 mL of substrate solution (5 mM) and 0.2 mL of a 1:10 (v/v) dilution of the caecal sample in 100 mM phosphate buffer (pH 7.0) after centrifugation at 10000 × g for 15 minutes. Incubation was carried out at 37°C and p- or o-nitrophenol was quantified at 400 nm and at 420 nm, respectively, after addition of 2.5 mL 0.25 M cold sodium carbonate. The enzymatic activity (α- and β-glucosidase, α- and β-galactosidase, and β-glucuronidase) was expressed as μmol of a product formed per minute [U] per g of digesta in the fresh caecal sample.

Table 1. **Composition of experimental diets, %**

	Control	Faba bean		
		seeds	hulls	oligosaccharides
Casein	11.15	9.60	10.80	11.15
DL-methionine	0.15	0.20	0.15	0.15
Faba bean seeds	-	5	-	-
Faba bean hulls	-	-	5	-
Oligosaccharides	-	-	-	5
Soybean oil	8	8	8	8
Cholesterol	1	1	1	1
Mineral mixture[1]	3	3	3	3
Vitamin mixture[2]	2	2	2	2
Maize starch	74.7	71.2	70.05	69.7

[1]NRC, 1976
[2]AOAC, 1975

Tabela 2. **Body weight and caecal parameters of rats fed experimental diets**

	Control	Faba bean			SEM
		seeds	hulls	oligosacchar.	
Body weight, g	139.9	138.1	138.6	139.7	0.45
Caecal parameters:					
tissue, g/100g BW	0.237^{c}	0.283^{b}	0.279^{b}	0.316^{a}	0.01
digesta, g/100g BW	0.935^{b}	1.087^{b}	0.941^{b}	1.552^{a}	0.15
dry matter, %	22.7^{c}	24.5^{b}	27.7^{a}	25.7^{b}	0.25
pH of digesta	6.96^{a}	6.94^{a}	6.83^{a}	6.46^{b}	0.03
Enzyme activity[1]					
α-glucosidase	1.76^{b}	1.75^{b}	1.60^{b}	2.21^{a}	0.08
β-glucosidase	0.19^{b}	0.42^{a}	0.21^{b}	0.28^{b}	0.01
α-galactosidase	0.86^{bc}	1.43^{ab}	0.59^{c}	1.76^{a}	0.07
β-galactosidase	5.96^{a}	5.31^{a}	2.10^{b}	2.02^{b}	0.22
β-glucuronidase	2.05^{a}	1.65^{b}	0.96^{c}	0.77^{c}	0.09

[1]U/g fresh digesta
a, b, c - $P \leq 0.05$

Short-chain fatty acids (SCFA) in fresh caecal contents were determined by gas chromatography (Shimadzu GC-14A with a glass column 2.5m × 2.6 mm, containing 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW; column temperature 110°C; detector FID temperature 180°C; injector temperature 195°C). The known amount of caecal digesta was mixed with 0.2 mL of formic acid, diluted with deionised water, and centrifuged at 10000×g for 5 min. Supernatant was decanted for injection into a gas chromatograph.

The results were worked out statistically using one-way analysis of variance and significance of differences between groups was determined by the Duncan's multiple range test at the significance level of $P \leq 0.05$. The calculations were made using the STATISTICA (v. 6.0) software package.

Results and Discussion. The supplementation of diets with faba bean seeds, hulls nor oligosaccharides had no significant influence on final body weight (Table 2). The weights of caecal tissue and digesta of rats fed diet with oligosaccharides were significantly higher in other treatments. Moreover, the dietary treatments with seeds and hulls caused also a significant enlargement of caecal wall mass compared to the control group. The highest dry matter concentration in caecal digesta ($P \leq 0.05$ vs remaining groups) was observed in rats fed diet with hulls and the lowest ($P \leq 0.05$ vs remaining groups) was in the control group. The supplementation of diet with a raffinose family oligosaccharide extract from faba bean cotyledons significantly decreased caecal pH value when compared to other treatments. In the study of Zduńczyk et al. (2003), the removal of hulls from a diet did not have any effect on caecal tissue and digesta weights in comparison to rats fed a diet containing whole faba bean seeds. The main polysaccharide presents in faba bean hull is cellulose, which according to Brunsgaard et al. (1995), as a microbially inert compound, has no effect on the caecum in rats. Our results were not in agreement with those findings as the rats fed diet containing 5% hulls were characterised by significantly higher caecal tissue mass than in the control treatment. In our recent study (Zduńczyk et al., 2004) the replacement of sucrose with cellulose caused 20% enlargement of the caecum, both digesta and tissue. The results obtained in the group fed with oligosaccharides (the highest mass of caecal digesta and tissue) were in accordance with findings obtained by Brunsgaard et al. (1995), which suggested that the highly fermentable carbohydrates could induce rapid hypertrophy of the proximal part of large intestine, i.e. in the caecum. A decline in pH of caecal digesta, which was observed in the present study in the case of oligosaccharide treatment, is considered beneficial to the health condition, since such conditions of the caecal-colonic ecosystem enhance proliferation of beneficial bacteria (mainly *Bifidobacterium* and *Lactobacillus*), and limit the growth of detrimental micro-organisms (Roberfroid, 2000).

The addition of faba bean seeds, hulls and oligosaccharides had a beneficial influence on the activity of the caecal microflora, resulting in a decrease in the β-glucuronidase activity compared to the control treatment (Table 2). The oligosaccharide group was associated with the lowest activity of that enzyme, which is involved in the generation of toxic and carcinogenic metabolites in the hindgut (Reddy et al., 1992) as well as the highest levels of microbial α-glucosidase and α-galactosidase. The activity of the latter enzyme, which potentially allows the metabolism of raffinose family oligosaccharides, has been localised in high range in bifidobacteria (Desjardins et al., 1990). The activity of bacterial β-galactosidase was significantly higher in the control and the group with faba bean seeds than in rats fed with faba bean hulls and oligosaccharides. The β-glucosidase activity was enhanced by specific substrate induction by faba bean seeds. β-Galactosidase and α-glucosidase activities can improve fermentation of resistant starch and lactose leading to SCFA and lactic acids which are a source of energy for the tissues (Cummings and Macfarlane, 1991). The increase in β-glucosidase activity is more ambiguous because this hydrolytic activity is responsible both for the generation of toxins (Mallet and Rowland, 1988), and for the production of bacterial glucoside derivatives which are assumed to be responsible for protection against chemically induced cancer (Roland et al., 1993).

All faba bean ingredients added to the diets did not evoke an increase in the concentration of total SCFA in the caecal digesta, compared to the control group (Table 3). However, the oligosaccharide preparation was significantly effective ($P \leq 0.05$ vs remaining groups) in the increasing of butyrate concentration. The highest concentration of valeric acid was observed in the control group ($P \leq 0.05$ vs remaining groups). An analysis of SCFA C_2:C_3:C_4 profile points to beneficial changes in the proportions of individual major acids under the influence of oligosaccharide-supplemented diet (especially in comparison to the hull-diet), i.e., a lower ration of acetic acid and higher ratios of propionic and butyric acids. The ratios of individual SCFA are likely very important as each SCFA impacts host metabolism differently. Propionate has been demonstrated to lower cholesterol synthesis, both in vitro in isolated rat hepatocytes (Demigne et al., 1995) and in vivo in rats (Chen et al., 1984), likely by inhibiting gluconeogenesis, stimulating glycolysis and inhibiting biosynthesis of fatty acids. Conversely, acetate stimulates gluconeogenesis, inhibits glycolysis, and is a well known precursor of cholesterol (Remesy et al., 1992). Butyrate and to a lesser extent, propionate may have a role in preventing certain types of colitis (Scheppach, 1994). The SCFA may also inhibit proliferation of colon cancer cells (Scheppach, 1995). It is also known that the concentrations of branched-chain fatty acids (iso-butyrate, iso-valerate and valerate) are increased by bacterial proteolytic degradation (Swanson et al., 2002). In our study, diet supplementation with faba bean seeds, faba bean hulls and faba bean oligosaccharides decreased the concentration of branched-chain fatty acids to 4.48, 4.30 and 4.01 μmol/g, respectively, compared with the control group (5.41 μmol/g). Many authors postulate that at diet supplementations with non-digestible carbohydrates, the

short-chain fatty acid pool size rather than fatty acid concentration is a better indicator of the intensity of the large intensive fermentation (Berggren et al. 1993, Campbell et al. 1997). In our study, the supplementation of a diet with oligosaccharides extracted from faba bean cotyledons was associated with the highest total SCFAs as well as particular acids pools.

In conclusion, among all experimental treatments the oligosaccharide preparation changed the most beneficially caecal metabolism in rats (the lowest pH of digesta and activity of bacterial β-glucuronidase, the highest SCFA production in the caecum as well as the most beneficial proportion of major acids). The faba bean seeds and hulls addition to a diet did not increase the acidity of digesta as well as production of short-chain fatty acids in the caecum, but also beneficially decreased β-glucuronidase activity in comparison to the control treatment.

Tabela 3. **Short-chain fatty acids concentration and production (pool) in the caecum of rats**

	Control	Faba bean			SEM
		seeds	hulls	oligosacchar.	
Concentration, μmol/g					
Total SCFA	71.10	62.90	70.13	67.53	4.11
Acetate	50.30	45.05	53.32	45.84	3.15
Propionate	10.53	8.83	8.23	11.07	0.44
Iso-butyrate	1.39	1.20	1.19	1.05	0.06
Butyrate	4.86^{b}	4.45^{b}	4.28^{b}	6.59^{a}	0.32
Iso-valerate	1.86	1.54	1.33	1.35	0.06
Valerate	2.16^{a}	1.74^{b}	1.78^{b}	1.61^{b}	0.08
Profile[1] C_2:C_3:C_4					
C_2	70^{ab}	71^{ab}	76^{a}	67^{b}	0.90
C_3	15^{ab}	14^{ab}	11^{b}	16^{a}	0.22
C_4	7^{ab}	7^{ab}	6^{b}	9^{a}	0.11
Pool, μmol/100g BW					
Total SCFA	63.90^{b}	65.01^{b}	65.21^{b}	87.74^{a}	3.33
Acetate	44.92^{b}	46.55^{b}	49.57^{ab}	59.42^{a}	2.54
Propionate	9.63^{b}	9.11^{b}	7.65^{b}	14.86^{a}	0.75
Iso-butyrate	1.25^{ab}	1.24^{ab}	1.10^{b}	1.37^{a}	0.08
Butyrate	4.46^{b}	4.72^{b}	3.98^{b}	8.30^{a}	0.31
Iso-valerate	1.68^{a}	1.59^{a}	1.24^{b}	1.79^{a}	0.11
Valerate	1.96^{a}	1.80^{a}	1.66^{b}	2.00^{a}	0.09

a, b, c - $P \leq 0.05$
[1]μmol/100μmol total SCFA

References

1. Berggren A.M., Bjorck I.M.E., Nyman E.M.G.L. and Eggum B.O., Short-chain fatty acid content and pH in caecum of rats given various sources of carbohydrates. J. Sci. Food Agric., 1993. 63, 397-406.
2. Brunsgaard G., Eggum B.O., Sandstrom B., Gastrointestinal growth in rats as influenced by indigestible polysaccharides and adaptation period. Comp. Biochem. Physiol., 1995. 111A, 369-377.
3. Campbell J.M., Fahey Jr. G.C., Wolf B.W., Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J. Nutr., 1997. 127, 130-136.
4. Chen, W.J., Anderson, J.W., Jenning, D.J., Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. Proc. Soc. Exp. Biol. Med., 1984. 175, 215-218.
5. Close B., Banister K., Baumans V., Bernoth E-M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D., Warwick C., Recommendations for euthanasia of experimental animals: Part 2. Laboratory Animals, 1997. 31, 1-32.
6. Cummings J.H., Macfarlane G.T., The control and consequences of bacterial fermentation in the human colon. J. Appl. Bacteriol., 1991. 70, 443-459.
7. Demigne C., Morand C., Levrat, M.A., Besson C., Moundras, C., & Remesy, C., Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. Br. J. Nutr., 1995, 74, 209-219.
8. Desjardins M.L., Roy D., Goulet J., Growth of bifidobacteria and their enzyme profiles. J. Diary Sci., 1990. 73, 299-307.
9. Frejnagel S., Zduńczyk Z., Krefft B., The chemical composition and nutritive value of low- and high-tannin faba bean varietes. J. Anim. Feed Sci., 1997. 6, 401-412.
10. Juśkiewicz J., Wróblewska M., Zhaki K., Zduńczyk Z., Hussein L., Bilogical activity of faba beans proathocyanidins. Acta Aliment. Hung., 2001. 30, 63-69.
11. Juśkiewicz J., Zduńczyk Z., Wróblewska M., Oszmiański J., Hernandez T., The response of rats to feeding with diets containing grapefruit flavonoid extract. Food Res. Int., 2002. 35, 201-205.
12. Juśkiewicz J., Zduńczyk Z., Wróblewska M., The effect of the administration of cellulose and fructans with different degree of polymerization to rats on caecal fermentation and biochemical indicators in the serum. Czech J. Anim. Sci., 2005. 50, 273-280.
13. Mallet A.K., Rowland I.R., Factors affecting the gut microflora. In: Role of the gut flora in toxicity and cancer, London, Academic

Press., 1988. pp. 347-382.

14. Muzquiz M., Rey C., Cuadrado C., Fenwick G.R., Effect of germination on the oligosaccharide content of lupin species. J. Chromatogr., 1992. 607, 349-352.
15. Queiroz-Monici K.S., Costa G.E.A., da Silva N., Reis S.M.P.M., de Oliveira A.C., Bifidogenic effect of dietary fiber and resistant starch from leguminous on the intestinal microbiota of rats. Nutrition, 2005. 21, 602-608.
16. Reddy B.S., Engle A., Simi B., Goldman M., Effect of dietary fiber on colonic bacterial enzymes and bile acids in relation to colon cancer. Gastroenterology, 1992. 102, 1475-1482.
17. Reed J.D., Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci., 1995. 73, 1516-1528.
18. Remesy C., Behr S.R., Levrat M-A., Demigne C., Fiber fermentability in the rat cecum and its physiological consequences. Nutr. Res., 1992. 12, 1235-1244.
19. Roberfroid M.B., Chicory fructooligosaccharides and the gastrointestinal tract. Nutrition, 2000. 16, 677-679.
20. Roland N., Nugon-Baudon L., Rabot S., Interactions between the intestinal flora and xenobiotic metabolizing enzymes and their health consequences. World Rev. Nutr. Diet., 1993. 74, 123-148.
21. Scheppach W., Effects of short chain fatty acids on gut morphology and function. Gut., 1994. 1, 535-538.
22. Scheppach W., Bartram H.P., Richter F., Role of short-chain fatty acids in the prevention of colorectal cancer. Eur. J. Cancer., 1995. 31A, 1077-1080.
23. Swanson K.S., Grieshop C.M., Flickinger E.A., Bauer L.L., Healy H.P., Dawson K.A., Merchen N.R., Fahey G.C., Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. J. Nutr., 2002.132, 980-989.
24. Zduńczyk Z., Frejnagel S., Amarowicz R., Juśkiewicz J., Effect of faba bean tannins on nutrient absorption in the rat small intestine, Acta Aliment. Hung., 1996, 25, 37-46.
25. Zduńczyk Z., Frejnagel S., Juśkiewicz J., Influence of proanthocyjanidins from faba bean on utilisation of protein by rats and absorption of nutrients in the small intestine. Pol. J. Food Nutr. Sci., 2000. 9, 95-98.
26. Zduńczyk Z., Juśkiewicz J., Wróblewska M., Flis M., Effect of faba bean seeds with different content of proathocyanidins on growth of rats, caecal enzyme activity and metabolism indices. Acta Aliment. Hung., 2003. 32, 161-168.
27. Zduńczyk Z., Juśkiewicz J., Wróblewska M., Król B., Physiological effects of lactulose and inulin in the caecum of rats. Arch. Anim. Nutr., 2004. 58, 89-98.