INFLUENCE OF CHICORY PREPARATION CONTAINING FRUCTANS AND POLYPHENOLS ON NITROGEN EXCRETION PATTERNS AND ILEAL MINERAL ABSORPTION IN RATS

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Abstract. In two experiments on Wistar rats, the influence of chicory fructans and their dietary combination with polyphenols on nitrogen excretion routes and mineral absorption in the ileum was examined. In the first experiment, during 15 days, twenty four rats were randomly divided into three equal groups of eight animals. The rats were assigned to the following diets: control (C), with 7.5% of chicory fructooligosaccharides (F), and that containing 7.5% of fructooligosaccharides combined with 0.05% of polyphenols (FP) were applied to 24 animals. In the second experiment on 30 rats, an *in situ* technique in an open system based on controlled flow of perfusion fluid through the small intestine of anaesthetized rats was used for assessing gut absorption. The content of fructooligosaccharides in the F fluid was 9.7 g/100 ml, while in the FP group the fluid contained similar amount of FOS and 62.5 mg of chicory polyphenols per 100 ml. Feeding fructooligosaccharides caused, typical for this type of dietary fibre, higher nitrogen excretion in faeces and lower N losses in urea as compared to the control group. Simultaneous dietary addition of polyphenols slightly increased these effects, however, the N digestibility and utilization indices did not differ significantly between F and FP groups (Experiment I). The chicory preparation containing both fructooligosaccharides and polyphenols decreased the ileal absorption of glucose and magnesium from the perfusion fluid in comparison to the F group. It was reflected in the lowest serum concentration of glucose in the FP group measured just after the perfusion period. Both preparations F and FP similarly increased the rate of calcium absorption when compared to the control treatment.
Key words: chicory, fructans, polyphenols, nitrogen balance, ileal absorption, rat.

CIKORIJOS PREPARATŲ, TURINČIŲ FRUKTANŲ IR POLIFENOLIŲ, ĮTAKA ŽIURKIŲ ORGANIZMUI IŠSISKIRIANT AZOTUI IR MINERALINIŲ MEDŽIAGŲ ABSORBCIJAI KLUBINĖJE ŽARNOJE

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Santrauka. Atlikti du bandymai su *Wistar* linijos žiurkėmis. Buvo tiriama cikorijos fruktanų ir jų racionų derinių su polifenoliais įtaka žiurkių organizme išskiriamo azoto kiekiui ir mineralinių medžiagų absorbcijai klubinėje žarnoje. Pirmuoju bandymu, kuris tęsėsi 15 dienų, 24 žiurkės buvo šeriamos trimis racionais: kontroliniu (C), papildytu 7,5 proc. cikorijos fruktooligosacharidų (F), ir papildytu 7,5 proc. fruktooligosacharidų bei 0,05 proc. polifenolių (FP) mišiniu. Antruoju bandymu su 30 žiurkių žarnyno absorbcijai įvertinti taikytas *in situ* atviros sistemos metodas, pagrįstas kontroliuojamu perfuzijos skysčio srautu per anestezuotų žiurkių plonąsias žarnas. F grupės skystyje buvo 9,7 g fruktooligosacharidų 100 ml, FP grupės skystyje buvo tiek pat fruktooligosacharidų ir 62,5 mg cikorijos polifenolių 100 ml skysčio. Šėrimas fruktooligosacharidais parodė (tai būdinga šio davinio ląstelienos tipui), kad su išmatomis azoto išskiriama daugiau, o su šlapalu išskiriamo azoto nuostoliai mažesni palyginti su kontrolinės grupės žiurkėmis. Raciono papildymas polifenoliais šiek tiek sustiprino šį poveikį, o azoto virškinamumo ir panaudojimo rodikliai tarp grupių F ir FP skyrėsi nežymiai (I bandymas). Cikorijos preparatai, kurių sudėtyje buvo ir fruktooligosacharidų, ir polifenolių, sumažino gliukozės ir magnio absorbciją iš perfuzinio skysčio klubinėje žarnoje palyginti su F grupe. FP grupėje tas pasireiškė mažesne gliukozės serumo koncentracija, kuri nustatyta iškart po perfuzijos laikotarpio. Abu preparatai – F ir FP – beveik vienodai padidino kalcio absorbcijos laipsnį.
Raktažodžiai: cikorija, fruktanai, polifenoliai, azoto balansas, absorbcija klubinėje žarnoje, žiurkės.

Introduction. Numerous studies have reported effects of non-digestible carbohydrates and various phytochemicals, for instance polyphenols, added to a diet as single supplements on the upper and lower gastrointestinal ecosystem (Juśkiewicz et al., 2002, 2005; Wróblewska et al., 2008; Zduńczyk et al., 1998, 2002). Action, absorption and metabolism of phenolic compounds occur along the digestive tract. They might be

declycosylated and absorbed in the ileum, and they are often claimed to be modulators os small intestinal absorption on nutrients (Scalbert and Williamson, 2000; Zduńczyk et al., 2000). Glycosylated polyphenols, which are not hydrolyzed and absorbed in the small intestine, enter the caecal-colonic segments affecting the activity of microflora and modifying, for example, nitrogen excretion routes (Pastuszewska et al., 2000; Puupponen-Pimiä et al., 2002). In recent years, prebiotic oligofructose and inulin have been one of the most extensively investigated groups of non-digestible carbohydrates used in monogastric animal feeding (Ašmenskaitė et al., 2007; Juśkiewicz et al., 2008, Stańczuk et al., 2005). Considering the physiological properties of fructans, the main attention has mainly been paid to fermentation processes occurring in the large bowels. But some authors have additionally pointed out the physiologically-important action of several non-digestible oligosaccharides in the upper part of the gastrointestinal tract, e.g. their promotive effects on rat glucose and mineral absorption (Asvarujanon et al., 2005; Kim, 2005). Taking into account that non-digestible carbohydrates and phenolic compounds may influence both the upper and lower gastrointestinal tract metabolism, information on their biological response when applied in a dietary combination remains scarce. Our recent experiments have shown that a dietary combination of biologically active compounds may exert a different host's response when compared to a single dietary supplementation (Jurgoński et al., 2008; Juśkiewicz et al., 2007; Semaškaitė et al., 2006; Zduńczyk et al., 2006).

In the present study, the following hypothesis was advanced: the presence of polyphenol constituents in a chicory root preparation may affect the influence of dietary fructans on nitrogen excretion patterns and ileal mineral absorption in rats.

Material and Methods. The animal protocol used in this study was approved by the Local Council for Animal Experiments in Olsztyn, Poland. In both experiments as a source of fructooligosaccharides (F group) was commercial oligofructose preparation (Raftilose® P95) kindly provided by Orafti (Oreye, Belgium). The fructan-polyphenol preparation used in the FP treatment, from the Institute of Chemical Technology, Technical University of Łódź, Poland, was obtained from chicory roots through a multistage process comprising of ethanol extraction, lyophilisation and additional drying. The content of oligo-, di- and monosaccharide in both preparations was measured with HPLC method using Knauer (Berlin, Germany) chromatograph with RI detector and Animex HPX 87C (300 x 7.8 mm) column (Bio-Rad, Hercules, CA, USA) at 85°C (Król and Grzelak 2006). The composition of individual mono- to heptamers in oligofructose preparation was analyzed as follows: glucose and fructose: 5.1%, sucrose: 2.7%, DP 3 (kestose): 25.6%, DP 4 (nystose): 31.2%, DP 5 (fructosyl nystose): 18.7%, DP 6: 12.3% and DP 7: 4.4%. The FOS-polyphenol mixture contained 0.1% of glucose, 1.5% of sucrose, 90% of short-chain fructooligosaccharides (degree of polymerization 3-10) and 0.58% of polyphenolic constituents. For determination of total polyphenols Folin-Ciocalteau method was used (De Pascual-Teresa and Santos-Buelga, 2000). To the 0.5 mL of an extract 0.25 mL of Folin-Ciocialteau reagent was added, then 2.5 mL of 20% Na_2CO_3 and finally the volume was filled-up with water up to 25 mL and mixed. The incubation was carried at room temperature for 1h. Absorbance was measured with SP 880 Meratech spectrophotometer at λ=720 nm. Chlorogenic acid was used as a standard (SIGMA, Poznań, Poland).

The experiment I was conducted on 24 male Wistar rats aged 6 weeks and weighing 102.8 ± 3.1 g at the beginning of the test. Diet composition (Table 1) was based on recommendations of AIN-1993 (Reeves, 1997). The control group was fed with the casein diet containing 5% of sucrose, and experimental groups were fed with the diets in which sucrose and part of maize starch were replaced with chicory preparations. The diets contained 135 g/kg crude protein (casein supplemented with DL-methionine) and standard amount of a mineral mix (according to AIN-93G Mineral Mix) and vitamin mixtures (according to AIN-93G Vitamin Mix). Experimental and control groups consisted of eight male rats housed individually in metabolic cages. The selection of the animals and their maintenance over the 15-d experiment followed common regulations. The environment was controlled with a 12-h light-dark cycle, a temperature of 21±1°C, relative humidity of 50±5% and 20 air changes/h. Animals had free access to water and diets. The initial 10-day preliminary period was aimed at adapting the gut microflora to the preparations examined, followed by a 5-day experimental period when faeces and urine were collected once daily. Nitrogen in faeces and urine was determined for each rat according to Kjedhal's method. Apparent nitrogen digestibility and nitrogen retention were used as criteria of nutritional quality of diets.

In the second experiment, 30 adult male rats weighing about 310 ± 12 g were used (3 groups, 10 rats in each of them). Rats were anaesthetized by means of an intraperitoneal injection of sodium pentobarbital and body temperature was maintained at 37°C with an electric heating pad. The technique of the small intestine perfusion, precisely described in the work by Zduńczyk et al. (1998), was used to determine the absorption of glucose and minerals from the intestine of rats receiving perfusion fluid without or with the addition of chicory preparations (the oligofructose preparation and the FOS-polyphenol extract in the F and FP groups, respectively). Krebs-Henseleit fluid (HSE Biomesstechnik I/83), supplemented with proper amount of glucose to level off its concentration in each group, was used as the control perfusion fluid. In the experimental F and FP groups, the amount of dissolved fructooligosaccharides was estimated at 9.7 g/100ml, and the FP fluid, besides similar amount of FOS, additionally contained 62.5 mg of chicory polyphenols in 100 ml. Following tracheotomy and laparotomy, two catheters were inserted in the small intestine, with the inlet immediately below duodenum and the outlet above the caecum. Perfusion fluids at a

temperature of 37°C were administered for 60 minutes into this limited section of the small intestine, following washing with physiological saline solution (0.9% NaCl) and 20-minute introductory period. Regular administration (1 ml/min), close to a physiological rate, was ensured by a peristaltic pump PA-SK 8 (IKA-Labortechnik, Janke & Kunkel, Germany). Anaesthesia was maintained by administration of intraperitoneal pentobarbital throughout the experimental period. The amount of compounds (glucose, calcium, magnesium and phosphorus) absorbed from the intestine was determined on the basis of differences in the composition of fluid given through the inlet drain and flowing out through the outlet drain. At the termination of the perfusion period, blood samples were taken from the *inferior vena cava* into test tubes, and then serum was obtained after solidification for 90 min at 37°C and centrifugation at 2000 g for 15 min at 4°C. The concentrations of glucose, calcium, magnesium and phosphorus in the serum as well as perfusion fluids were determined with commercial diagnostic kits (Alpha Diagnostics, Warsaw, Poland).

Table 1. **Composition of experimental diets fed to rats, %** (Experiment I)

	C	F	FP
Casein[1]	14.8	14.8	14.8
DL-methionine	0.2	0.2	0.2
Sucrose	7.5	-	-
Cellulose	0.6	0.6	0.6
Soybean oil	5	5	5
Lard	5	5	5
Cholesterol	0.5	0.5	0.5
Mineral mix[2]	3.5	3.5	3.5
Vitamin mix[3]	1	1	1
FOS preparation[4]	-	7.9	-
FOS-PP preparation[5]	-	-	8.3
Maize starch[6]	61.9	61.5	61.1
Calculated ingredients:			
Crude protein, %	13.5	13.5	13.5
Fructooligosaccharides	-	7.5	7.5
Polyphenols	-	-	0.048

[1]Casein preparation: crude protein 89.7%, crude fat 0.3%, ash 2.0%, and water 8.0%.

[2]AIN-93G-MX according (Reeves, 1997), per kg mix: 357g calcium carbonate anhydrous (40.04% Ca), 196 g potassium phosphate monobasic (22.76% P, 28.73% K), 70.78 g potassium citrate, tripotassium monohydrate (36.16% K), 74g sodium chloride (39.34% Na, 60.66% Cl), 46.6 g potassium sulfate (44.87% K, 18.39% S), 24g magnesium oxide (60.32% Mg), 6.06 g ferric citrate (16.5% Fe), 1.65 g zinc carbonate (52.14% Zn), 1.45 g sodium meta-silicate · $9H_2O$ (9.88% Si), 0.63 g manganous carbonate (47.79% Mn), 0.3 g cupric carbonate (57.47% Cu), 0.275 g chromium potassium sulfate · $12H_2O$ (10.42% Cr), 81.5mg boric acid (17.5% B), 63.5 mg sodium fluoride (45.24% F), 31.8 mg nickel carbonate (45% Ni), 17.4 mg lithium chloride (16.38% Li), 10.25 mg sodium selenate anhydrous (41.79% Se), 10 mg potassium iodate (59.3% I), 7.95 mg ammonium paramolybdate · $4H_2O$ (54.34% Mo), 6.6 mg ammonium vanadate (43.55% V), 221.026 g powdered sucrose.

[3]AIN-93G-VM (Reeves, 1997), g/kg mix: 3.0 nicotinic acid, 1.6 Ca pantothenate, 0.7 pyridoxine-HCl, 0.6 thiamin-HCl, 0.6 riboflavin, 0.2 folic acid, 0.02 biotin, 2.5 vitamin B-12 (cyanocobalamin, 0.1% in mannitol), 15.0 vitamin E (all-rac-α-tocopheryl acetate, 500 IU/g), 0.8 vitamin A (all-trans-retinyl palmitate, 500000 IU/g), 0.25 vitamin D-3 (cholecalciferol, 400000 IU/g), 0.075 vitamin K-1 (phylloquinone), 974.655 powdered sucrose.

[4]Raftilose P95 (Orafti, Oreye, Belgium): water 3.6%, ash 0.83%, glucose 0.6%, fructooligosaccharides (DP 3-7) 95%.

[5]FOS-polyphenols preparation (Institute of Chemical Technology, Technical University of Łódź, Poland): water 5.9%, ash 1.9%, glucose 0.1%, sucrose 1.6%, fructooligosaccharides (DP 3-10) 90%, polyphenols (expressed as chlorogenic acid) 0.58%.

[6]Maize starch preparation: crude protein 0.6%, crude fat 0.9%, ash 0.2%, total dietary fibre 0%, and water 8.8%.

The results are presented as mean values and (pooled) standard errors of the mean (SEM). The results were analysed using the Shapiro-Wilk normality test and one-way ANOVA. Significant differences between the groups were determined with Duncan's multiple range test and considered significant at $p<0.05$. The calculations were made using the STATISTICA software package version 6.0 (StatSoft Corp., Kraków, Poland).

Results and Discussion. The nitrogen balance of rats fed casein diets supplemented with sucrose (C), or

fructooligosaccharides (F), or fructooligosaccharides and polyphenols (FP) obtained from chicory roots is presented in Table 2. The rats given a diet supplemented with fructooligosaccharides (group F) excreted more nitrogen in faeces, expressed as mg per 5 days, than those fed with control sucrose ($P<0.05$). This effect was more pronounced when the FP preparation was added to a diet ($P<0.05$ vs. F and C groups). The contrast between F and FP treatments was a simple result of a significantly higher intake of dietary nitrogen by rats fed on the FP diet ($P<0.05$ vs. other groups). When faecal N output was expressed as a percentage of N intake, there were no differences between F and FP treatments, yet they differed when compared to the control treatment. As a result, the coefficient of apparent nitrogen digestibility was significantly diminished in both experimental treatments. When the F and FP groups are considered, the dietary addition of chicory polyphenol constituents in the amount of 0.05 % of a diet did not change the nitrogen digestibility. We suggest that in the case of differentiated N intake amongst groups, the normalization of nitrogen excretion (faecal and urinary) by its expression as a percentage of N intake seems to be more accurate reflection of physiological response. The effect of experimental preparations on urinary nitrogen losses, expressed in this way, was also significant. The F and FP treatments similarly reduced the amount of N in urine when compared to C group ($P<0.05$). The coefficient of nitrogen utilization in the body of rats, which takes into account both faecal and urinary N output, was not influenced, regardless of the type of dietary additive. The group fed on a diet containing FOS and polyphenols was characterized by the highest, although not significant ($P = 0.068$), nitrogen utilization index. The study conducted by Pastuszewska et al. (2000) showed that nitrogen excretion patterns were strongly influenced by a diet's composition, especially by different types of dietary carbohydrates but also by dietary polyphenols. Fructooligosaccharides are known to act as a fermentable substrate to the caecum and colon which provides energy for microflora and in turn the bacteria utilize N in various forms (ammonia, peptides, amino acids, etc.) for growth, thus trapping nitrogen in the form of bacterial protein (Howard et al., 2000; Matusevičius et al., 2005). Our results are in accordance with those reported by Younes et al. (1995) and Juśkiewicz et al. (2006) who demonstrated that more fermentable oligosaccharides reduced N excretion in urine, which was linked to the fact that, under such conditions, blood urea constitutes the most readily available source of N for bacterial growth. Pastuszewska et al. (2000) reported on findings where dietary polyphenols increased the amount of nitrogen excreted in faeces and decreased its urinary excretion; these effects were more pronounced on the gluten than on the casein diets.

Table 2. **Nitrogen balance in rats** (Experiment I)

	C	F	FP	SEM
N intake, g/5 days	1.39^{b}	1.46^{b}	1.69^{a}	0.212
N excretion, mg/5 days				
in faeces	86.4^{c}	148.4^{b}	187.9^{a}	8.014
in urine	444^{a}	394^{b}	415^{ab}	14.02
N excretion, % of intake				
in faeces	6.21^{b}	10.18^{a}	11.15^{a}	0.528
in urine	31.9^{a}	27.0^{b}	24.6^{b}	0.949
N apparent digestibility[1], %	93.79^{a}	89.81^{b}	88.85^{b}	0.508
N utilization[2], %	61.90	62.77	64.22	0.945

[1]Apparent digestibility: [N intake – N faecal / N intake] × 100;
[2]Retention: [N intake – N faecal – N urinary / N intake] × 100;
a, b – Values within each row with the same superscript are not different at $p<0.05$

The results of perfusion of rat small intestine with the fluid not containing or containing chicory polyphenols and/or fructooligosaccharides are presented in Table 2. The presence of fructooligosaccharides in the perfusion fluid did not influence the glucose, magnesium nor phosphorus absorption compared with the control group. On the other hand, the ileal absorption of calcium was significantly enhanced by fructans, regardless of the simultaneous addition of polyphenols. The latter caused a significant reduction in glucose and magnesium absorption in comparison to the F and C treatments. These data were partly reflected in the parameters measured in the serum (Table 3). Glucose concentration in the serum was the lowest in the FP group, and it differed statistically from groups C and F. Calcium concentration was significantly higher in the F and FP treatments *versus* the control group ($P<0.05$). The serum level of Mg and P remained unaffected by the experimental components of the F and FP perfusion fluids. Our above-presented findings concerning the hypoglycaemic effect of chicory polyphenols are in accordance with some recent studies which have shown that diets rich in dietary phenols may result in an altered pattern of intestinal glucose uptake (Johnston et al., 2005; Jurgoński et al., 2008). Polyphenolic compounds decrease glucose transport, possibly by sodium-dependent glucose transporter 1 (SGLT1) inhibition (sodium-dependent active transport) and/or an inhibition of the facilitated diffusion glucose

transporters (e.g. GLUT1 and GLUT2) (Johnston et al. 2005). Our results suggest that chicory fructooligosaccharides, but not polyphenols, promote ileal calcium absorption and its serum concentration in rats. It has been reported that non-digestible oligosaccharides stimulate absorption of several minerals, mainly calcium, and improve mineralization of bone. These effects seem to be specific for the type of carbohydrate and are likely to be related to the rate of fermentation by the intestinal flora (Scholz-Ahrens et al., 2001). On the other hand, some authors have reported several non-digestible saccharides to have promotive effects on calcium absorption in the small intestine (Asvarujanon et al., 2005; Goda et al., 1993). The transcellular calcium transport system has been shown to consist of several steps, and the factors involved include not only calbindin-D9k (CaBP) – a cholecalciferol-induced calcium-binding protein, but also the brush border membrane, calmodulin and a calcium pump (Ohta et al., 1998; Wasserman and Fullmer, 1995). Ohta et al. (1998) observed that FOS feeding increased levels of CaBP in the large intestine, but decreased those in the small intestine of rats. Therefore we may speculate that an increased absorption of calcium observed in the present study was via the paracellular, CaBP-independent, route.

Table 3. **Ileal absorption of glucose, calcium, magnesium and phosphorus from perfusion fluids and biochemical serum parameters of rats** (Experiment II)

	C	F	FP	SEM
Ileal absorption rate:				
Glucose, mg/rat/h	88.1[a]	70.9[a]	47.8[b]	4.25
Calcium, mg/rat/h	0.867[b]	0.956[a]	0.958[a]	0.06
Magnesium, mg/rat/h	0.296[a]	0.281[a]	0.239[b]	0.01
Phosphorus, mg/rat/h	0.508	0.436	0.488	0.07
Serum parameters:				
Glucose, mg/dl	236[a]	236[a]	217[b]	19.2
Calcium, mg/dl	8.26[b]	9.23[a]	9.61[a]	0.11
Magnesium, mg/dl	3.35	3.61	3.20	0.03
Phosphorus, mg/dl	5.68	5.72	5.55	0.04

a, b – Values within each row with the same superscript are not different at $p<0.05$

Our data suggest that when the nitrogen balance is considered the presence of chicory polyphenols in a diet (0.05%) did not change the action of dietary fructooligosaccharides. However, there was a tendency of increasing N utilization in the body of rats fed on diet FP ($P = 0.068$). Concerning the accepted hypothesis of the work, the inclusion of chicory polyphenols to diet decreased small intestinal absorption of glucose and magnesium but their presence in the perfusion fluid did not disturb the promotive action of fructooligosaccharides on calcium absorption. It could be concluded that chicory root extracts containing fructans as well as polyphenols may serve as safe feed additives.

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References

1. Ašmenskaitė L., Juśkiewicz J., Zduńczyk Z., Staniškienė B., Budreckienė R., Sinkevičienė I., Žilinskienė A., Matusevičius P. Influence of chicory flour (*Cichorium intybus* L.) on physiology of digestive tract and health in rabbits. Vet. Zootec. 2007.40, 3–8.

2. Asvarujanon P, Ishizuka S., Hara H. Promotive effects of non-digestible disaccharides on rat mineral absorption depend on the type of saccharide. Nutrition 2005. 21, 1025–1035.

3. De Pascual-Teresa S., Santos-Buelga C. Quantitative analysis of flavan-3-ols in Spanish food stuffs and beverages. J. Nutr., 2000. 129, 1662–1668.

4. Goda T., Takase S., Hosoya N. Maltitol-induced increase of transepithelial transport of calcium in rat small intestine. J. Nutr. Sci. Vitaminol. 1993. 39, 589–595.

5. Howard M.D., Kerley M.S., Sunvold G.D., Reinhart G.A. Source of dietary fiber fed to dogs affects nitrogen and energy metabolism and intestinal microflora populations. Nutr. Res. 2000. 20, 1473–1484.

6. Johnston K., Sharp P., Clifford M., Morgan L. Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. FEBS Lett. 2005. 1653–1657.

7. Jurgoński A., Juśkiewicz J., Zduńczyk Z.. Comparison of the effects of chokeberry fruit extract, chicory flour and their dietary combination on blood parameters and antioxidant status of healthy and diabetic rats. Pol. J. Food Nutr. Sci. 2008. 58, 273–278.

8. Juśkiewicz J., Ašmanskaitė L., Zduńczyk Z., Matusevičius P., Wróblewska M., Žilinskienė A. Metabolic response of the gastrointestinal tract and serum parameters of rabbits to diets containing

chicory flour rich in inulin J. Anim Physiol An. N. 2008. 92, 113–120.

9. Juśkiewicz J., Godycka-Kłos I., Matusevičius P., Zduńczyk Z., Juśkiewicz M. Influence of pea and lupin oligosaccharides on caecal short-chain fatty acids production and nitrogen excretion patterns in rats. Pol. J. Food Nutr. Sci. 2006. 15, 77–82.

10. Juśkiewicz, J., Semaskaite A., Zduńczyk Z., Wróblewska M., Gruzauskas R., Juśkiewicz M. Minor effect of the dietary combination of probiotic *Pediococcus acidilactici* with fructoolingosaccharides or polysaccharidases on beneficial changes in the cecum of rats. Nutr. Res. 2007. 27, 133–139.

11. Juśkiewicz J., Zduńczyk Z., Matusevicius P., Brzuzan Ł. Effect of low-digestible carbohydrates on caecal ammonia concentration in rats and turkeys. Vet. Zootec. 2005. 31, 63–66.

12. Juśkiewcz J., Zduńczyk Z., Wróblewska M., Oszmiański J., Hernandez T. The responce of rats to feeding with diets containing grapefruit flavonoid extract. Food Res. Int. 2002. 35, 201–205.

13. Kim M., 2005. High-methoxyl pectin has greater enhancing effect on glucose uptake in intestinal perfused rats. Nutrition 21, 372–377.

14. Król B, Grzelak K. Qualitative and quantitative composition of fructooligosaccharides in bread. Eur Food Res Technol 2006. 223:755–758.

15. Matusevičius P., Januškevičius A., Zduńczyk Z., Juśkiewicz J., Jankowski J., Žiliskiene A. Effectivity of assimilation of dietary protein in rabbits digestive tract. Vet. Zotec. 2005. 29, 93–97.

16. Ohta A., Motohashi Y., Ohtsuki M., Hirayama M., Adachi T., Sakuma K. Dietary fructooligosaccharides change the concentration of calbidin-D9k differently in the mucosa of the small and large intestine of rats. J. Nutr. 1998. 128: 934–939.

17. Pastuszewska B., Kowalczyk J., Ochtabińska A. Dietary carbohydrates affect caecal fermentation and modify nitrogen excretion patterns in rats. II. Studies with diets differing in protein quality. Arch. Anim. Nutr. 2000. 53, 335–352.

18. Puupponene-Pimia R., Aura A.M., Oksan-Caldentey K.M., Myllarinen P., Saarela M., Mattila-Sandholm T., Poutanen K. Development of functional ingredients for gut health. Trends Food Sci. Tech. 2002. 13, 3–11.

19. Reeves P.G. Components of the AIN-93 diets as improvements in the AIN-76A diet. J. Nutr. 1997. 127, S838–S841.

20. Scalbert A., Williamson G. Dietary intake and bioavailability of polyphenols. J. Nutr. 2000. 130, 2073S–2085S.

21. Scholz-Ahrens K.E., Schaafsma G., van den Heuvel E. and Schrezenmeir J. Effects of prebiotics on mineral metabolism. Am. J. Clin. Nutr. 2001. 73, S459–S464.

22. Semaškaitė A., Juśkiewicz J., Zduńczyk Z., Gružauskas R. Physiological influence of a probiotic, prebiotic and enzymatic preparation or a combination of these supplements in diets for growing rats. Pol. J. Natur. Sci. 2006. 3, S569–S574.

23. Stańczuk J., Zduńczyk Z., Juśkiewicz J., Jankowski J. Indices of response of young turkeys to diets containing mannanoligosaccharide or inulin. Vet. Zootec. 2005. 31, 98–101.

24. Wasserman R.H., Fullmer C.S. Vitamin D and intestinal calcium transport: facts, speculations and hypotheses. J. Nutr. 1995. S1971–S1979.

25. Wróblewska M., Juśkiewicz J., Frejnagel S., Oszmiański J., Zduńczyk Z. Physiological influence of chokeberry phenolics in model diet. Acta Aliment. Hung. 2008. 37, 221–232.

26. Younes H., Garleb K., Behr S., Remesy C., Demigne C. Fermentable fibers or oligosaccharides reduce urinary nitrogen excretion by increasing urea disposal in the rat cecum. J. Nutr. 1995. 125, 1010–1016.

27. 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. 9/50, 3S, 95–98.

28. Zduńczyk Z., Frejnagel S., Juśkiewicz J., Gomez-Villalve E., Klewicki R. Nutritional and functional properties of diets containing low-digestible carbohydrates. Pol. J. Food Nutr. Sci. 2002. 10/52, SI 2, 65–68.

29. Zduńczyk Z., Juśkiewicz J., Estrella I. Cecal parameters of rats fed diets containing grapefruit polyphenols and inulin as single supplements or in combination. Nutrition 2006. 22, 898–904.

30. Zduńczyk Z., Juśkiewicz J., Frejnagel S., Gulewicz K. Influence of alkaloids and oligosaccharides from white lupin seeds on utilisation of diets by rats and absorption of nutrient in the small intestine. Anim. Feed Sci. Technol. 1998. 72: 143–154.

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