POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, SI 1, pp. 111–114

METHODOLOGICAL ASPECTS OF DETERMINATION OF THE PHYSIOLOGICAL EFFECTS OF PREBIOTIC PREPARATIONS

Zenon Zduńczyk

Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Key words: prebiotics, physiological effect, evaluation, methodology

Candidate prebiotics available for human consumption and animal nutrition represented a numerous and diverse group of substances with different amount of bifidogenic oligosaccharides (20-90% of dry matter). The application of prebiotic preparation caused a decrease in the energy value of diets, however the body weight gain of an animal remained unchanged with increased diet intake. One of the effects of oligosaccharide addition to a diet is an increase in the amount of digesta in the gastrointestinal tract, including caecum, which in turn lowers concentration of major products of oligosaccharide fermentation - short-chain fatty acids in fresh caecal digesta. Therefore, total SCFA pool in the whole caecum (converted into 100 g of body weight of rats) is a better indicator of the intensity of oligosaccharide fermentation in the large bowel. The content of probiotic preparations in a diet differentiates the activity of microbial enzymes (mainly α-and β-galactosidase as well α- and β-glucosidase) in the caecal digesta.

INTRODUCTION

Prebiotics are defined as "non-digestible food ingredients which beneficially affect the host by selective stimulation of the growth and/or activity of the one or a limited number of bacteria in the colon" [Gibson & Roberfroid, 1995]. In the last decade, many low-digestible carbohydrates were proposed as prebiotic preparations: new crystal form of lactulose, β-fructans isolated and purified from plant sources (mainly chicory inulin and oligofructose), α-galactosides extracted from grain legumes, oligosaccharides obtained by the enzymatic hydrolysis of polysaccharides (*e.g.* xylooligosacharides and isomalto-oligosaccharides), oligosaccharides produced by enzymatic transglycosylation (*e.g.* galactooligosaccharides or fructo-oligosacchraides) and resistant starch prepa-rations. Currently, over 20 different types of low--digestible carbohydrates are on the world market [Sako *et al.*, 1999]. Better understanding of physiological effects of this heterogeneous group of substances is necessary on the one hand and difficult on the other.

POTENTIAL PHYSIOLOGICAL EFFECTS OF PREBIOTIC PREPARATIONS

Behaviour of prebiotic preparation in the gastrointestinal tract should be as follows: resistance to digestion and hydrolysis, fermentation by colonic microflora and no excretion in stools (Figure 1). Such properties are shown by *e.g.* fructans (inulin and oligofructose) obtained from plant sources (mainly chicory root) or by biotechnological processing (enzymatic transglycosylation of sucrose). D-Fructofuranosyl β(1-2) links constituting the

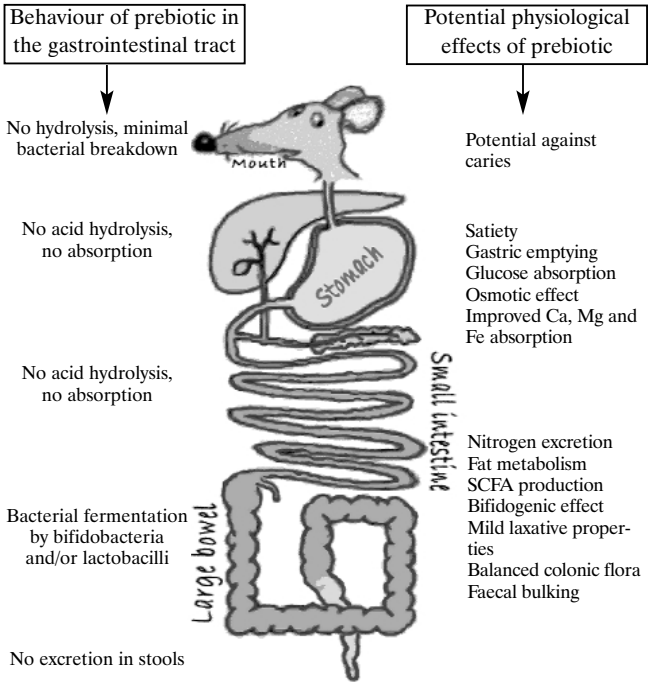

FIGURE 1. Behaviour and potential physiological effect of probiotic preparations [Scheppach *et al.*, 2001; Cummings & Macfarlane, 2002; Kolida *et al.*, 2002].

majority of glycosidic bonds in the fructans are resistant to hydrolysis by animal and human digestive enzymes (α-glucosidase, malto-isomaltase, sucrase) specific to α-glucosidic linkages [Roberfroid, 2000]. For this reason, fructans decreased energetic value of diet in the upper part of the gastrointestinal tract, *e.g.* caused satiety and gastric

*Author's address for correspondence: Zenon Zduńczyk, Institute of Animal Reproduction and Food Research, ul. Tuwima 10, 10-747 Olsztyn, Poland; tel.: (48 89) 523 46 71; fax: (48 89) 524 01 24; e-mail: zez@pan.olsztyn.pl

emptying. In mice fed diets with 10% of inulin or oligofructose, the small intestine was longer and heavier compared with mice fed diets with 10% of cellulose [Buddington *et al.*, 2000]. The content of inulin and oligofructose in the diets decreased the rates of glucose transport and absorption of leucine, proline and lycylsacrosine compared with a diet containing cellulose.

The main role prebiotics play through fermentation in the large bowel - production of short-chain fatty acids and lactate, increasing biomass, increasing fecal energy and nitrogen, mild laxative properties. They also affect microflora causing selective increase in bifidobacteria and lactobacilli in planctonic and biofilm communities, reduction in clostridia, increase in colonisation resistance to pathogens, and potential benefit in preventing pathogen invasion [Cummings & Macfralane, 2002].

Potential physiological effects of prebiotics, which are presented in Figure 1, are wide but in different degree are substantiate. Holzapfel and Schillinger [2002] confirmed effects/aspects with regard to prebiotics to announce: non--digestible and low energy (<9 kJ/g), increase in stool volume and modulation of the colonic flora by stimulation of benficial bacteria (*Bifidobacterium*, *Lactobacillus* and *Eubacterium* spp.), and inhibition of "undesirable" bacteria (*Clostridium* and *Bacteroides*). Other effects of prebiotics, including: prevention of intestinal infections, modulation of the immune response, prevention of colorectal cancer, reduction of the serum cholesterol level and improved bio-availability, Holzapfel and Schillinger [2002] recognize as "postulated effects that have not been finally confirmed". Also Roberfroid [2001] classifies the evidence that inulin-type fructans influence colonic flora and bowel function as "strong", whereas the evidence concerning improving calcium bioavailability and hypolipidemic effect of prebiotis as "promising". The quoted Robefroid's opinion concerned inulin-type fructans, substances which have been tested most frequently. The present state of knowledge concerning physiological effects of other types of low-digestible carbohydrates is not sufficient. For the sake of a wide range of potential physiological effect of a prebiotic, examination of the biological effect of new preparations brings many methodological problems. Some of these are discussed in this paper.

DIVERSITY IN COMPOSITION OF PREBIOTIC PREPARATIONS

At present, over 20 different types of low-digestible carbohydrates Are available for human consumption and animal nutrition. Several such preparations are presently under consideration by the industry for application (Figure 2). It is important that oligosaccharides with bifidogenic functions constitute only a part of these preparations: from 21.2% of dry matter of Pyrodextrin (commercialization envisaged by Matsutani, Japan) to 92-32% of dry matter of Raftiline (Orafti, Belgium) and fructooligosaccharides (Suntory, Japan). Preparations of inulin, available on the European market, may contain over 20% of mono-saccharides. For this reason, commercial inulin or oligofructose (and especially other low-digestible carbo-hydrates) should be called "prebiotic preparations" rather than "prebiotics".

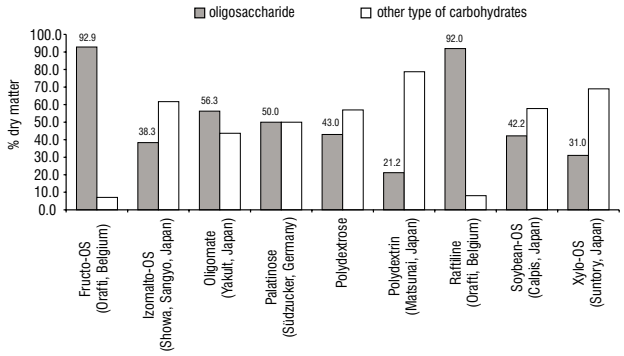

FIGURE 2. The content of oligosaccharides (OS) in commertial candidate probiotics available for human consumption [Cummings *et al.*, 2000; Holzapfel & Schillinger, 2002, modified].

INTAKE AND NUTRITIONAL VALUE OF DIETS WITH PREBIOTIC PREPARATIONS

Calculations from predictive theoretical equation have proposed the energy value of oligofructose to range between 4.6 and 9.5 kJ/g [Roberfroid *et al.*, 1993], while 8 kJ/g is assumed as an average value [FAO/WHO, 1998]. Energy value of inulin and oligofructose is lower than that of digestible carbohydrates (17 kJ/g). For this reason, substitution of starch with 5% or 10% fructan (inulin or oligofructose) decreased the energy value of daily diets from 210 to 203 and 198 kJ, *i.e.* on average 3.2% and 6.4%, respectively (Figure 3). In calculation presented in Figure 2, the mean data for young rats in 4-8 weeks of live (energy value of diet - 14 kJ/g, daily diet intake 14 g) were assumed. In many experiments into the application of oligosaccharides in diets, both energy value of diets and daily diet intake were different. In an experiment of Daubioul *et al.* [2002], wherein obese Zucker rats obtained control diet or diet with 10% of fructan for 8 weeks, the energy value of diet amounted to 13.84 or 12.98 kJ, respectively and daily food intake to 24.1 or 22.5 g. Daily energy intake decreased consequently from 334 to 292 kJ and body weight of rats from 394 to 346 g. In the experiment of Sakaguchi *et al.* [1998], the application of 10% of fructo- or galacto-oligosaccharides in a diet did not differentiate the feed intake and weight of rats compared with control group. Young, fast growing rats may increase the diet intake with lower energy value [Lopez-Guisa *et al.*, 1988]. For this reason, a lower energy value of diets containing low--digestible carbohydrates did not inhibit the animal growth.

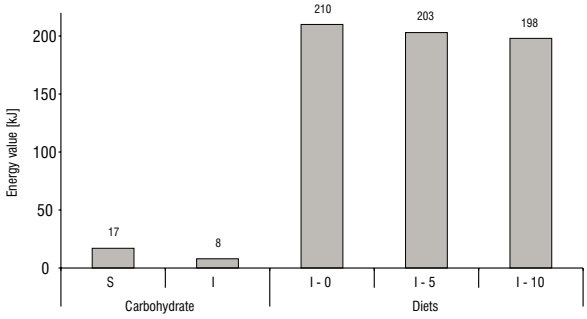

FIGURE 3. Energy value of starch (S), inulin (I) and daily diets without (I-0) or with 5% (I-5) and 10% of inulin (I-10%) (own calculation, discussion in the text).

INFLUENCE OF PREBIOTIC PREPARATIONS ON FUNCTIONING OF THE LARGE BOWEL

One of more important aspects of application of oligosaccharides in a diet is their laxative effect. The increase in faecal output, reported in numerous studies, is likely to be due to increased biomass in the lower part of the gastrointestinal tract. It can also be observed in an increased amount of caecal digesta in rats fed a diet supplemented with prebiotic preparations. In the experiment of Wróblewska [2003], the substitution of 4% cellulose with the same amount of inulin or lactulose increased the output of caecal digesta in rats from 0.90 g to 1.16-1.33 g/100 g BW (Figure 4). Total substitution of cellulose with inulin or lactulose caused an over fourfold increase in the output of caeacal digesta. Such a high increase in the caeacal digesta amount brings about a decrease in the concentration of major products of oligosaccharide fermentation - short-chain fatty acids (SCFA). For this reason, the content of SCFA in fresh caecal digesta is not a good indicator of the intensity of oligosaccharide fermentation in the large bowel. A better indicator of that trait is total SCFA pool in the whole caecum converted into 100 g body weight of rats (Figure 5).

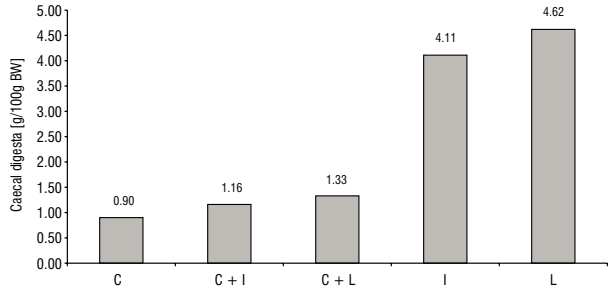

FIGURE 4. The influence of partial or total substitution of cellulose (C) with inulin (I) or lactulose (L) on the mass of caecal digesta [Wróblewska, 2003].

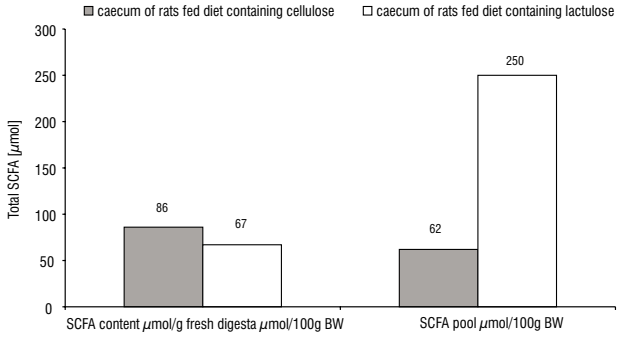

FIGURE 5. The concentration (μmol/fresh digesta) and total pool of SCFA (μmol/100 g BW) in the cellulose or inulin [Juśkiewicz & Zduńczyk, 2002].

Ralatively little is known about the mechanism of microbiological digestion of oligosaccharides in the large bowel. Figure 6 indicates that the activity of microbiological α-and β-galactosidase, and to a lower extent also of α- and β-glucosidase, was diversified upon the application of cellulose or lactulose in a diet. In the study of Wróblewska [2003], significant differences were reported between exogenic digestion of inulin and lactulose. Depolymerisation of lactulose was to a higher extent affected by α- and β-galactosidase as well as by α- and β-glucosidase. Differences in the activities of microbial enzymes did not, however, affect the content of SCFA produced in the caecum of rats.

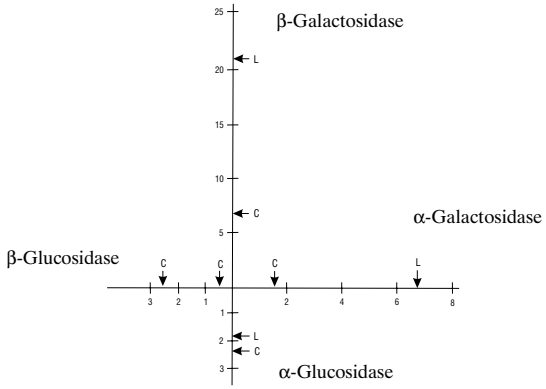

FIGURE 6. The glycolytic activity in caecal digesta (μ/10 mg protein) in rats fed diets containing 8% cellulose (C) or lactulose (L) [Juśkiewicz & Zduńczyk, 2002]

ACKNOWLEDGEMENTS

The research was supported by the State Committee for Scientific Research, grant No. PBZ-KBN/020/P06/1999.

REFERENCES

1. Buddington R.K., Donahoo J.B., Williams C.H., The colonic bacteria and rates of small intestinal nutrient transport of mice fed diets with inulin and oligofructose. Microbial Ecology in Health and Disease, 2000, 12, 233–240.
2. Cummings J.H., Macfarlane G.T., Gastrointestinal effects of prebiotics. Brit. J. Nutr., 2002, 87, Suppl. 2, S145–S151.
3. Daubioul C., Rousseau N., Demeure R., Gallez B., Taper H., Declerck B., Delzenne N., Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. J. Nutr., 2002, 132, 967–973.
4. FAO, Food and Agriculture Organization of the United Nations. Carbohydrates in human nutrition. Report of a joint FAO/WHO expert consultation. 1998. No. 99, Rome: FAO.
5. Gibson G.R., Roberfroid M.B., Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr., 1995,125, 1401–1412.
6. Holzapfel W.H., Schillinger U., Introduction to pre- and probiotics. Food Rese. Intern., 2002, 35, 109–116.
7. Juśkiewicz J., Zduńczyk Z., Lactulose-induced diarrhoea in rats: effect on caecal development and activities of microbial enzymes. Comp. Biochem. Physiol., Part A, 2002, 133, 411–417.
8. Kolida S., Tuohy K., Gibson G.R., Prebiotic effects of inulin and oligofructose. Brit. J. Nutr., 2002, 87 (Suppl. 2), S193–S197.
9. Lopez-Guisa J.M., Harned M.C., Bubielzig R., Rao S.C., Marlett J.A., Processed oat hulls as potential dietary fiber source in rats. J. Nutr., 1988, 118, 953–962.
10. Roberfroid M.B., Prebiotics: preferential substrate for

specific germs? Am. J. Clin. Nutr., 2001, 73 (Suppl.), 406S–409S.
11. Roberfroid M.B., Gibson G.R., Delzenne N., The biochemistry of oligofructose, a nondigestible fiber: an approach to caculate its caloric value. Nutr. Rev., 1993, 51, 137–146.
12. Sakaguchi E., Sakoda C., Toramaru Y., Caecal fermentation and energy accumulation in the rat fed on indigestible oligosaccharides. Brit. J. Nutr., 1998, 80, 469–476.
13. Sako T., Matsumoto, K., Tanaka R., Recent progress on research and applications of non-digestible galacto-oligosaccharides. Intern. Dairy J., 1999, 9, 69–80.
14. Scheppach W., Leuhrs H., Menzel T., Beneficial health effects of low-digestible carbohydrate consumption. Brit. J. Nutr., 2001, 85 (Suppl. 1), S23–S30.
15. Wróblewska M., Nutritional and wholesome properties of diets enriched in preparations of oligo- and polysaccharides. Doctoral thesis, Olsztyn, 2003.