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PHYSIOLOGICAL EFFECT OF LOW DIGESTIBLE OLIGOSACCHARIDES IN DIETS FOR ANIMALS AND HUMANS

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The article reviews literature data and results of own investigations concerning biological properties of low digestible carbohydrates (LOOs): lactulose, inulin and different type of oligosaccharides. The following problems: (1) sources of oligosaccharides in animal and human diets, (2) behaviour of oligosaccharides in the gastrointestinal tract, (3) physiological effect of LOOs in diets for laboratory rodents, (4) physiological effect of LOOs in diets for non-ruminat livestock and (5) nutritional advances of LOOs in diets for humans were presented. The above-mentioned reports indicate that oligosaccharide preparations can be considered as feed additives in pigs and poultry feeding, *e.g.* being as effective as antibiotics in the control of pathogens and enhancement of growth performance. However much more research are needed to determine the appropriate role of these oligosaccharides in animal feeding. LDOs may influence animals and humans in various ways: improve intestinal health, modify lipid metabolism, modulate the immune response, and decrease the risk of intestinal and systemic diseases. The present state of knowledge on the mechanism of dietary action of prebiotic LDOs and physiological effects of different types and doses of low-digestible carbohydrates is not sufficient.

INTRODUCTION

Among scientific concepts of functional food in Europe, one of the six major areas in human nutrition is gastrointestinal physiology and function [Diplock *et al.*, 1999]. The gut is an obvious target for the development of functional foods, acting as it does as the interface between the diet and the metabolic events which sustain life [Salminen *et al.*, 1998]. Three perspectives are useful to examine the importance of the physiology and functions of the gastrointestinal tract in mediating the effect of functional foods: (1) meal- - induced responses of the gastrointestinal tract caused by food-derived factors, which may result in longer-term adaptive changes; (2) the ability of foods or mixtures of foods to alter the digestive and absorptive function of the gastrointestinal tract in a manner that influences metabolism; and (3) the impact that the gastrointestinal tract has, through its adaptation to a diet, on risk factors of diseases [Schneeman, 2002]. One of the important factors affecting the gastrointestinal tract physiology are dietary components which cannot be digested by the enzymes in the small intestine. This group of components includes chains of carbohydrates which contain other than α(1-4) links between monosaccharides. It refers tonon-starch polysaccharides containing β(1-4) links between sugars and different types of oligosaccharides containing α(1-6), β(1-2), β(1-4), and β(1-6) bonds [Delzene & Roberfroid, 1994]. While non-digestible, they do affect the digestive process, *e.g.* they provide bulk in the intestinal contents, hold water, delay gastric emptying, and stimulate profitable effect of colonic microflora on the nutritional status and health of the host. Bifidogenic (prebiotic) effect is the main criterion in the evaluation of the functional properties of oligosaccharides. The prebiotic concept is based on the assumption that particular large intestine (colon) microflora, such as bifidobacteria and lactobacilli considered beneficial to the host health (human and animals), may be selectively stimulated by non-digestible but easily fermentable carbohydrates [Delzenne & Roberfroid, 1994; Gibson & Roberfroid, 1995; Gibson, 1998; Roberfroid, 1998; Cummings & Macfarlane, 2002]. In respect of potential functional effects of prebiotics and probiotic, Roberfroid [1998] has shown that the strategy for research and development of a "functional food" required: (1) the demonstration of an interaction with (a) function(s) in the body; (2) some understanding of the mechanism thereof; (3) the establishment of the effect in relevant biological systems; (4) the formulation of sound hypotheses; (5) the testing of these hypotheses in human nutrition studies.

To bring closer the realization of the above-mentioned postulates has been the aim of extensive studies in many research centers, mainly in Japan, USA and Europe. Results of ample studies published within the last 2 decades are summarised in this work.

SOURCES OF OLIGOSACCHARIDES IN ANIMAL AND HUMAN DIETS

By chemical definition oligosaccharides (OS) are carbohydrates of 3–10 linked monomer sugars in size. They are highly divergent in their molecular size, monosaccharide units structure, and linkage between units. In terms of phys-

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iological properties, OS can by classified as non-digestible carbohydrates. Due to those properties, two other carbohydrates are included into this group: disaccharide lactulose and long-chain polymer of fructose – inulin.

Lactulose is present in small amounts in unheated milk, but its content has been found to increase when milk was subjected to heat treatment to prepare milk products [Andrews, 1984]. Inulin is naturally present in a large variety of plants, high contents of inulin have been reported in chicory (*Cichorium intybus*) and other plants belonging to the *Compositae* family. Inulin is a polyfructan with a degree of polymerization ranging from 2 to 60. Many plants contain fructans with a lower degree of polymerization (to 25), namely fructooligosaccharide (FOS). The FOS are widely distributed throughout the plant kingdom. Extensive analysis of Campbell *et al.* [1997], concerning one hundred of fruits, vegetables and feeds, has shown that more than 50% of the plants analysed contained from 0.0 to 0.2 of total FOS, determined as sum 1-Kestose, Nystose, and 1^F-β-Fructofuranosylnystose (Table 1). More FOS can be found in roots, tuber, and fruits of plants of the *Compositae* (for example Globe artichoke, Jerusalem artichoke, and chicory), the *Amaryllidadeae* (onion, garlic, leek, shallot, and Chinese chive), and the *Gramineae* families (rye, wheat, barley, and oat). The highest content of total FOS, reaching 58.4 mg and 45.0 mg/g, was reported in the Jerusalem artichoke and onion powder, respectively [Campbell *et al.*, 1997].

Fructooligosaccharides are a part of conventional or everyday foods, to be consumed with the normal/usual diet of humans. The daily consumption of FOS has been estimated to range from 1 to 8 g per capita per day in the North American population [Egan & Petersen, 1992; MoshFegt *et al.*, 1999], and between 3 and 11 g in Europe [Loo *et al.*, 1995], with the most common sources of FOS in the diets being wheat, onion, banana, garlic and leek.

Other group of oligosaccharides which is present in human and animal diets are raffinose family oligosaccharide (RFO, α-galactoside) consisting of raffinose, stachyose and verbascose. This type of oligosaccharides occurs in grain legumes. An average content of RFO in seeds of grain legumes consumed in Europe is 39.6 g/kg (Table 2). Due to a low consumption of grain legumes in Europe (average 2.43 kg per capita and year), daily consumption of RFO is lower than 0.3 g. In animal diets, the content of RFO is higher because these substances occur in the main protein sources. Diets for growing pigs with the standard content of soybean meal (16%) contain *ca.* 8 g/kg of RFO (Table 3). The content of RFO in diets increases when instead of soybean, pea or lupine seeds are used. The total substitution of soybean protein with protein from pea seeds increases RFO content in diets from *ca.* 8 to over 16–20 g/kg. Compared with soybean, pea seeds contain similar amount of RFO but also less crude protein. Whereas compared with pea seeds, lupine seeds contain more crude protein but also more RFO. It is known that other dietary components also contain RFO. According to Carré *et al.* [1984], the content of RFO in diets for poultry usually ranges from 0.5 to 3%, with the main sources being, in a decreasing order, soybean meal (6%), pea (5%), faba beans (4%), rapeseed meal (3%), and sunflower meal (2% of dry matter).

Apart from natural food components, an additional source of oligosaccharides in human diets can be food additives. Since inulin and oligofructose are naturally occurring substances, they can by extracted and applied in different food products. Fructan preparations are usually obtained from chicory root which contains approximately 150 to 200 g/kg of inulin and 80 to 120 g/kg of oligofructose [Flickinger *et al.*, 2003]. In Europe, the main producers of inulin are Belgium and the Netherlands, providing 98.8% of all the inulin production from chicory of the EU-15 Member States [Askew, 2002]. The extraction of fructan fraction

TABLE 2. Annual consumption of raffinose family oligosaccharides (RFO) in legumes in Europe [Kozłowska *et al*., 2001].

Legume	Beans	Pea	Lentil	Chickpea	Total
Average content of RFO (g/kg)[1]	36.8	49.4	28.4	47.0	39.6
Annual consumption of legumes (kg per capita)	1.14	0.67	0.43	0.19	2.43
Annual consumption of RFO (g)	42.0	33.1	12.3	8.9	96.3

[1]RFO – Raffinose Family Oligosaccharides

TABLE 3. The content of RFO in diets for growing pigs with soybean or grain legumes as alternative sources of crude protein (CP) [Kozłowska *et al.*, 2001].

Species	Content in feed (g/kg)		Share in diet (%)	RFO content in diet (g)
	CP	RFO		
Soybean meal	440	49.0	16	7.8
Pea	214	49.4	33	16.4
Faba bean	259	24.2	27	6.5
White Lupin	333	78.9	21	16.6
Yellow Lupin	375	104.8	19	19.9
Narrow Lupin	328	75.5	21	15.8

TABLE 1. The content of fructooligosaccharides (FOS) in selected food and feedstuffs [Campbell *et al*., 1997].

Food, feedstuffs	FOS content (mg/g)						
	0.1–0.2	0.3–0.5	0.6–0.7	1–2	3–5	7–10	40–60
Fruits	apple, pear, blackberry, plum, grapes, gooseberry, raspberry, muskmelon	banana (red), orange, peach, plantain	banana (green)	banana	artichoke, chicory root	–	–
Vegetables	peas, bean, carrot, yam, radish	acorn squash carrot, lettuce	peas (snow)	onion, garlic powder	garlic, onion (white)	shallot	onion powder, Jerusalem artichoke
Feedstuffs	beet pulp, corn gluten, oat goats, rice bran	oat grain, clover hay	oat straw	barley, wheat grain, timothy hay	rye grain, wheat bran, wheat germ	–	–

from chicory root is generally similar to that of sucrose from sugar beets. Fructans are extracted using hot water. After ion exchange of semi-purified extracts and spray-drying, inulin preparation is obtained. To obtain oligofructose, a liquid extract of fructans is partially hydrolysed enzymatically and then spray-dried. Typical differences in the chemical composition of commercial inulin and oligofructose are presented in Table 4. By chemical definition, inulin is a polydisperse fructan with $\beta(1\rightarrow2)$ bonds between fructose units and $\alpha(1\rightarrow2)$ bonds between fructose and terminal glucose [Phels, 1965]. In oligofructose obtained from chicory inulin, both bonds: $\beta(1\rightarrow2)$ or $\alpha(1\rightarrow2)$, occur between fructose and terminal glucose. Synthesized oligofructose contains only $\beta(1\rightarrow2)$ bonds of fructose chain with terminal glucose [Loo *et al.*, 1995; Playne & Crittenden, 1996].

TABLE 4. The composition of inulin and oligofructose extracted from chicory root [BIOMATRIX, 2002].

Ingredient	Inulin	Oligofructose
Dry matter (%)	96	95
Carbohydrate content (%)	99.5	99.9
Ash (%)	0.5	0.1
Carbohydrates:		
Free sugars	0.86	8.2
- glucose	0.08	0.7
- fructose	0.32	2.9
- sucrose	0.46	4.6
DP 1–10	4.3	41.1
DP 11–20	27.0	30.0
DP 21–30	36.4	17.5
DP 31–40	20.8	7.7
DP 41–50	7.6	2.6
DP 51–60	3.2	1
DP >61	0.3	-
DP 3–20	30.4	61.8
Average DP_n	19.5	6.6

Table 5 compiles oligosaccharides whose industrial production processes have been established as extracting from natural sources (inulin, oligofructose, RFO), by hydrolysing polysaccharides (Pyrodextrin, XOS), and by enzymatic and chemical synthesis from disaccharide (*e.g.* oligofructose produced by transfructosylation of sucrose, and lactulose obtained by izomeration of lactose).

Soy-oligosaccharides, representing raffinose family oligosaccharide (RFO), are produced in relatively small amounts. Depending on plant sources and degree of purification, RFO preparations contain different amounts of stachyose, raffinose, verbascose and sucrose (Table 6). Of all oligosaccharides, lactulose is produced in the largest quantities. A chemical, alkali isomerization process is used to convert glucose moiety in lactose to a fructose residue [Playne and Crittenden, 1996]. The obtained synthetic keto analog of lactose (β-D-fructopyranosyl-D-fructofuranose) is used predominantly as a pharmaceutical, *e.g.* for the control of constipation. Lactulose has been also applied as a low-calorie sweetener and bifidogenic factor [Mizota *et al.*, 2002]. Large scale production (over 10 000 tones annually) refers to oligofructose, galacto-, malto- and isomalto-oligosaccharides [Playne & Crittenden, 1996]. The above- mentioned oligosaccharides are widely used as low-calorie sweetener in beverages, confectionery and candies, especially on a Japanese market [Playne & Crittenden, 1996]. Currently, over 20 different types of low-digestible carbohydrates are on the world market [Sako *et al.*, 1999].

TABLE 5. Oligosaccharides according to their origin and chemical structure [Delzenne & Roberfroid, 1994; Loo *et al*., 1999, Macfarlane & Cummings, 1999].

Oligosaccharide	Origin	Chemical structure	Chemical composition
Inulin	Chicory root extraction	$(Fru)_n$ – Glu	>99% oligosaccharides β (1→2) fructan, DP 10–50 (average 10–12)
Oligofructose	Hydrolysis of inulin or transfructosylation from sucrose	$(Fru)_n$ – Glu	95% oligosaccharides β (1→2), DP 2–10 (average 4–5)
RFO[1]	Raffinose and stachyose mixture extraction from soybean	$(Gal)_n$ – Glu – Fru 3→4	(α (1→6) linked fructose, galactose, glucose) raffinose and stachyose, DP 3 and 4, respectively
TOS[2]	Transgalactosylation of lactose	$(Gal)_n$ – Glu	β (1→4) linked oligolactose (85%), small amount of glucose, galactose and lactose, DP 2–8
ISOS[3]	Transgalactosylation of maltose	Glu-$(Isomal)_n$	Mixture of α (1→4) linked oligomers (isomaltose, panose, isomaltotriose), DP 2–4
Lactulose	Izomerization of lactose		β (1→4) linked disaccharide containing galactose and fructose
MOS[4]	Synthesized or derived from yeast cell walls	$(Man)_n$ 2→8	Mannose-glucose polymer
Palatinose	Enzymatically rearranged sucrose	$(Glu - Fru)_n$ 2→7	α (1→6) linked disaccharide isomaltulose
Pyrodextrin	Pyrolysis of maize or potato starch	Complex mixture	Complex mixture of β (1→6) linked glucose-containing oligosaccharides
XOS[5]	Enzymatic hydrolysis of xylan	$(Xyl)_n$ 2→4	β (1→4) linked xylose; 70% pure, DP of oligosaccharide fraction 2–4

[1]Raffinose Family Oligosaccharides, [2]Transgalactooligosaccharides, [3]Isomalooligosaccharide, [4]Mannaooligosaccharide, [5]Xylooligosaccharide
Glu = glucosyl, Fru = fructosyl, Gal = galactosyl, Isomal = Isomaltose, Man – mannopyranosyl

TABLE 6. Average content (%) of individual α–galactosides and sucrose in RFO preparations obtained from soybean, lupin and pea seeds.

	Soybean RFO preparations		Lupin RFO preparations		Pea RFO preparations	
	Semi-pure[1]	Pure[1]	Semi-pure[2]	Pure[3]	Semi-pure[2]	Pure[3]
Sucrose	44.0	2.0	35.0	9.1	24.5	20.3
Raffinose	7.0	20.0	3.1	30.2	12.3	4.0
Stachyose	23.0	71.0	32.7	47.0	40.9	51.5
Verbascose	-	-	-	10.7	-	19.5
Other	23.0	2.0	23.2	-	17.9	-
Moisture	3.0	5.0	6.0	3.0	4.4	4.7

[1][Masai *et al*., 1987]; [2][Juśkiewicz *et al*., 2003]; [3][Gulewicz *et al*., 2002]

In Europe, the most popular are fructans obtained from chicory root. In all countries, where inulin and oligofructose are used, they are well accepted for food without limitations [Coussement, 1999].

Figure 1 presents some of the oligosaccharide preparations currently available for human consumption that are candidate prebiotics [Cummings *et al.*, 2001]. An analysis of these preparations indicates that some are very pure, containing 86–87% of oligosaccharides (92–93% of dry matter, respectively), *e.g.* inulin and oligofructose. In other preparations the oligosaccharides, determined as a fraction which is soluble in 80% ethanol (at pH 2, at a temperature of 0°C for 30 min), are minor, even merely 20–30%. The rest being free monosaccharides, starch, and non-starch polysaccharides. For example, dry matter of xylooligosaccharide (Suntory, Japan) contains 31% of oligosaccharide, 43% of starch, and 16% of monosaccharide [Cummings *et al.*, 2001]. It is important that oligosaccharides with bifidogenic functions constitute only a part of these preparations. Inulin preparations available on the market can also contain over 20% of monosaccharides. For this reason, commercial inulin or oligofructose, and especially other preparations of low-digestible carbohydrates, should be called "prebiotic preparations" rather than "prebiotics". Prebiotic properties of these preparations will be determined by the content of pure oligosaccharides, chain length, type of bonds between monomeric sugars, structure of the chain (linear, branched, substitutes), linkage to non-carbohydrates (conjugates), and chemical properties of the substances (carbohydrates) occurring together with oligosaccharides.

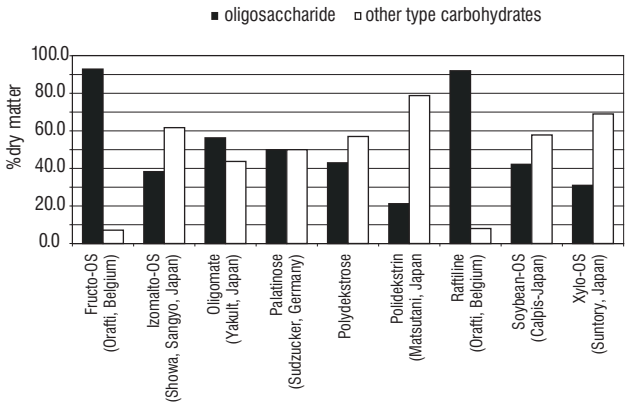

FIGURE 1. Content of oligosaccharides (OS) in commertial candidate probiotics available for human consumption [Cummings *et al*., 2001, modified].

BEHAVIOUR OF OLIGOSACCHARIDES IN THE GASTROINTESTINAL TRACT

In the small intestine of monogastric animals and humans only monosaccharides are absorbed directly. Disaccharide and longer chain of monosaccharide units (oligo- and polysaccharide) have to undergo earlier digestion achieved by enzyme-catalysed hydrolysis. Due to the presence of appropriate enzymes in the upper part of the gastrointestinal tract (Table 7), starch is the only important food polysaccharide that can be digested in the small intestine. Enzymes present therein (α-amylase, maltase-glucoamylase and sucrase-isomaltase) are not able to disrupt the α(1-6), β(1-2), β(1-4), and β(1-6) glycosidic linkages which are characteristic for different types of oligosaccharides. Studies in rats have shown that fructooligosaccharide (FOS) are not hydrolysed by salivary and pancreatic amylases [Oku *et al.*, 1984; Hidaka *et al.*, 1986], and that few or none of them are hydrolysed by intestinal brush border enzymes [Oku *et al.*, 1984; Nilsson *et al.*, 1988]. For this reason, recovery of FOS from rat small intestine was approximately the same as that of the non-absorbable marker. Results of studies carried out on ileostomized patients [Bach Knudsen & Hessov, 1995] and on normal subjects with aspiration of ileum contents [Molis *et al.*, 1996] indicated that about 86% of the ingested dose of inulin (7.07 g) and about 89% of the ingested oligofructose (20.1 g) were recovered in the terminal ileum. Only a small amount of ingested inulin (0.12%) was recovered in urine [Molis *et al.*, 1996]. Nevertheless, after oral ingestion of FOS, no changes in blood glucose levels were noted in humans [Hidaka *et al.*, 1986; Nilson *et al.*, 1988]. Incubation *in vitro* of fructooligosaccharides extracted from Jerusalem artichokes or various cereals with rat pancreatic homogenate or human gastric juice has shown the FOS to be "hardly digested" [Hidaka *et al.*, 1986; Nilsson *et al.*, 1988]. Taking this into account, numerous researchers have been describing this group of carbohydrates (*e.g.* frutans) as non-digestible

TABLE 7. Mammalian enzymes capable of digesting carbohydrates [Mountzouris *et al.*, 2002].

Place of action	Enzyme
Gastrointestinal lumen	Salivary α-amylase
	Pancreatic α-amylase
Enterocyte brush border	Lactase-phlorizin
	Maltase-glucoamylase
	Sucrase-isomaltase
	Trehalase

oligosaccharides [Delzenne & Roberfroid, 1994; Loo *et al.*, 1999; Buddington, 2001; Nyman, 2002]. Other authors [Howlett, 2001; Livesey, 2001; Marteau & Flourie, 2001; Storey & Lee, 2001] describe these oligosaccharides as "low-digestible" compounds. The term "low-digestible oligosaccharide" (LDO) refers especially to commercial preparations of oligosaccharides which contain from 20% to over 90% of pure oligosaccharides (Figure 1).

It is well known that the main property of the LDOs is their fermentability by the bacteria of the large intestine of animal and humans. The fermentation process provides stimulation of bacterial growth (biomass) and production of short-chain fatty acids (SCFA) and gases (H_2, CO_2, and CH_4). All SCFA are rapidly absorbed from the hindgut and stimulate salt and water absorption. They are metabolised mainly by the gut epithelium, liver and muscle, with virtually none appearing in urine and only small amount in faeces [Salminen *et al.*, 1998]. The SCFAs play a very important role in the functioning of the large bowel as a energy source for the colonic epithelium. Especially butyrate, which regulates epithelial cell growth and differentiation, is important for health of the large bowel.

The nutritional and healthy status of the host depend, to a high extent, on the amount and proportion of individual SCFA (acetate, propionate, and butyrate), bacterial enzyme activity (*e.g.* pro- or anti-carcinogenic activity), the content of different bacterial metabolites in faeces (*e.g.* phenols, cresols and bacterial breakdown of protein and urea), and the amount as well as bulking of stool [Salminen *et al.*, 1998; Loo *et al.*, 1999]. For the host the most profitable are these oligosacccharides which can selectively stimulate the growth and/or activity of one or a limited number of bacteria in the colon, thus improving the host's health. These LDOs which support the growth of the endogenous lactic acid bacteria and *Bifidobacteria* are defined as prebiotics [Delzene & Roberfroid, 1994; Gibson & Roberfroid, 1995; Walker & Duffy, 1998].

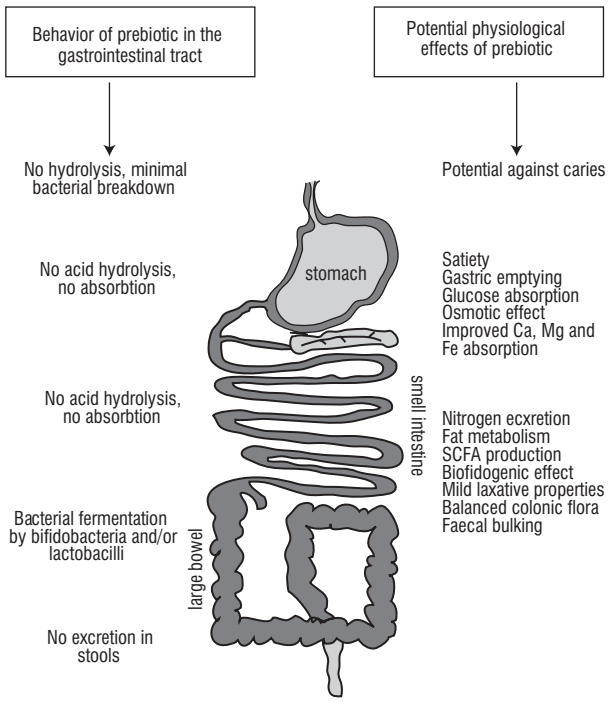

FIGURE 2. Behavior and potential physiological effect of probiotic preparations [Scheppach *et al*., 2001; Cummings & Macfarlane, 2002; Kolida *et al*., 2002].

Behaviour and physiological effect of prebiotic preparation in the gastrointestinal tract should be as follows: (1) it must be neither hydrolysed nor absorbed in the upper part of the gastrointestinal tract, (2) selectively ferment potentially beneficial bacteria in the colon, (3) alternate the composition of the colonic microbiota towards a healthier composition, and (4) preferably, induce effects which are beneficial to the host's health (Figure 2). Such properties are displayed by *e.g.* fructans (inulin and oligofructose) obtained from plant sources (mainly chicory root) or by biotechnological processing (enzymatic transglycosylation of sucrose). Potential physiological effects of prebiotics, which are presented in Figure 2, are wide but of different significance. Results obtained *in vivo* depend on the experimental model (*e.g.* species of animal), indices analysed and first of all type and dose of oligosaccharide preparation.

PHYSIOLOGICAL EFFECT OF LDOs IN DIETS FOR LABORATORY RODENTS

Physiological effect of different oligosaccharide preparations has been confirmed in ample *in vivo* experiments with the use of laboratory rats. The main criteria of physiological response of the gastrointestinal tract were caecum weight, caecal pH, content and composition of short-chain fatty acids (SCFAs), and microbiota concentration in faecal or caecal digesta (Table 8).

The supplementation of rat diets with lactulose resulted in a higher caecum weight, lower caecal pH, higher caecal SCFAs production with higher proportion of propionic and butyric acids in the sum of SCFAs [Rémésy & Demigné, 1989]. Data presented in the Table 8 show that similar tendencies were observed in experiments of other authors. In numerous experiments, administration of LDOs preparation had no significant effect on the SCFAs concentration in the caecal digesta [Campbell *et al.*, 1997; Rémésy & Demigné, 1989; Levrat *et al.*, 1991; Zduńczyk *et al.*, 2004]. It was most likely due to the fact that a higher production of SCFA in the caecum caused a higher quantity of caecal digesta. Usually the content of oligosaccharides in a diet increases water holding capacity and the amount of biomass in the large intestine. In the experiment of Zduńczyk *et al.* [2004], 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. Total substitution of cellulose with 8% of inulin or lactulose caused an over fourfold increase in the output of caecal digesta. Such a high increase in the caecal digesta amount brings about a decrease in the concentration of major products of oligosaccharide fermentation – short-chain fatty acids (SCFA). For this reason, the concentration 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. Conversion of the results into body weight of rats enables increasing differences between results of individual experiments which are conducted on animals with different weight and age.

Results of ample experiments on rats have indicated that chicory fructans were able to affect large bowel mass,

TABLE 8. Selected results illustrating the influence of different oligosaccharides on the functioning of the gastrointestinal tract of rats.

Carbohydrate	Level (%)	Caecum weight (g)	Caecal pH	Total SCFA) (μmol/caecum	SCFA–profile (%)	*Bifidobacteria* in caeca ($\log_{10}$ CFU/g)	References
Fibre-free	-	2.79	7.29	127	-		[Remesy &Demigne, 1989]
Lactulose	10	6.41	6.10	429	-		
Control	-	2.8	7.37	93.3	59:27:14	-	[Younes *et al.*, 1995]
FOS	7.5	5.98	6.28	306.9	49:29:22	-	
XOS	7.5	5.71	6.28	312.4	65:27:8	-	
Cellulose	5	2.91	6.70	82.5	72:12:17	8.8	[Campbell *et al.*, 1997]
Chicory FOS	6	5.79	6.17	238.6	70:9:21	9.2	
Synthetic FOS	6	5.14	6.19	261.7	75:9:16	9.2	
XOS	6	7.16	5.91	268.4	76:11:13	10.2	
Sucrose	10	-	-	218.1	66:21:14	-	[Sakaguchi *et al.*, 1998]
FOS	10	-	-	788.3	36:21:43	-	
TOS	10	-	-	803.9	74:8:18		
Sucrose	4	5.1	6.4	160.6	61:23:16	7.4	[Djouzi & Andrieux, 1997]
GOS	4	7.4	5.9	444.7	65:24:11	7.0	
FOS	4	6.3	5.6	356.0	66:13:21	9.2	
TOS	4	6.6	5.8	396.0	69:13:18	9.6	
Inulin	0	3.06	6.98	156	65:23:12	-	[Levrat *et al.*, 1991]
Inulin	5	4.13	6.37	357	45:34:21	-	
Inulin	10	5.38	5.96	524	43:37:20	-	
Inulin	20	9.96	5.65	657	40:34:26	-	
Control	-	5.76	6.40	84.4	59:25:16	8.6	[Klessen *et al.*, 2001]
FOS	5	7.78	5.71	134.3	56:23:21	9.2	
Inulin	5	7.24	5.65	130.5	44:31:25	7.4	
Cellulose	5	2.35	6.66	232.7	50:21:29	-	[Juśkiewicz *et al.*, in press]
Inulin	5	3.72	6.30	361.2	41:31:27	-	
Oligofructose	5	3.80	6.02	304.2	42:31:27	-	
Cellulose	8	2.91	6.83	168.6	73:16:11	-	[Zduńczyk *et al.*, 2004]
Lactulose	4/8	4.19/13.75	5.79/5.81	264.3/611.3	66:23:11/66:33:6	-	
Inulin	4/8	3.86/12.03	5.81/5.47	257.0/582.7	55:24:21/59:32:9	-	

caecal and faecal SCFAs, pH and microflora [Campbell *et al.*, 1997; Kleessen *et al.*, 2001]. Bulking index for inulin and oligofructose (increase in faecal wet weight in gram per gram of added indigestible carbohydrate) ranged between 1.1 and 1.2 and was similar as that of gums and pectins [Nyman, 2002]. Inulin and oligofructose have been found to modulate lipids in rats [Delzenne *et al.*, 2002]. Animal experiments with FOS have suggested their possible benefit in lowering blood sugar levels in people with diabetes and reducing elevated blood cholesterol and triglyceride levels [Delzenne, 1999].

Result of the same experiments have suggested that oligosaccharides, such as lactulose, although very effective in the acidification of the contents of the large intestine, may enhance caecal ammonia concentration and absorption [Rémésy & Demigné, 1989]. In another experiment, administration of FOS and XOS with a diet induced a 20 to 30% decrease in blood urea and renal nitrogen excretion, compared to the control [Younes *et al.*, 1995] It indicates the possibility of LDOs-diet therapy in chronic renal diseases.

Numerous investigations performed in animal models in the last decade have shown repeatedly that NDOs, such as inulin, FOS or TOS, stimulate mineral absorption, mainly that of calcium and magnesium [Otha *et al.*, 1998; van den Heuvel *et al.*, 1999; Scholz-Ahrens & Schrezenmeir, 2002]. Less numerous investigations have indicated that profitable changes in the intestinal microflora that occur with the consumption of prebiotic LDOs may potentially mediate immune changes via: the direct contact of lactic acid bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the intestine; the production of SCFAs or by changes in mucin production [Buddington *et al.*, 2002b; Schley & Field, 2002]. Results of single experiments have also shown that LDOs can induce reduced absorption of environmental contaminants (*i.e.* mirex and methylmercury), increase their faecal elimination and transformation to forms that are excreted in the urine [Kimura *et al.*, 2002].

Few studies in animal models have demonstrated that incorporation of LDOs to a diet can reduce the risk of colon cancer. This is most likely attributed to the butyric acid which is the preferred energy substrate for the colonic mucosa and has been suggested to protect against colonic disease, *e.g.* uncreative colistis and cancer [Gamet *et al.*, 1992; Scheppach *et al.*, 1992]. Reddy *et al.* [1997] examined the effect of diets containing 10% of oligofructose or inulin on the development of aberrant crypts in azoxymethane rats. Both fructans reduced the number of aberant crypts per colon, with inulin being somewhat more effective than oligofructose. Oligosaccharide with prebiotic properties has been reported to: induce changes in the population and metabolic characteristics of gastrointestinal bacteria, modulate enteric and systemic immune functions, and provide laboratory rodents with resistance to carcinogens that pro-

mote colorectal cancer [Buddington *et al.*, 2002a]. The best results were obtained when carcinogen-treated rats were fed a diet with a mixture of bifidobacteria and oligofructose [Gallaher & Khil, 1999]. In this experiment, however, the effect of soybean oligosaccharide on colon cancer risk was not confirmed.

PHYSIOLOGICAL EFFECT OF LDOS IN DIETS FOR NON-RUMINAT LIVESTOCK

Effects of the supplementation of diets for pigs with oligosaccharide preparations are very diversified, depending on the type of preparation (*e.g.* the content of pure oligosaccharides) and the experimental model (Table 9). In the last experiment of Liying *et al.* [2003], diet supplementation with 1% stachyose (responses to the sum of stachyose and raffinose in soybean meal diet) resulted in a higher content of lactobacilli in the ileum and bifidobacteria in the caecum and colon, however weight gain of piglets was lower, compared with the control group. A higher content of stachyose in diet (2%) had a depressing effect on piglets weight gain and a number of lactobacilli and bifidobacteria in the caecum. It indicates that at least a part of the growth depression, observed when soybean is included in the diet of weaning pigs, can be attributed to the presence of α-galactosides (mainly stachyose) in soybean meal. In a similar experiment of Juśkiewicz *et al.* [2003], in rats fed diets with RFO extracts from pea or lupin seeds, dry matter of caecal digesta was significantly lower and activities of bacterial α- and β-galactosidase, β-glucosidase and α-glucuronidase were significantly higher. Compared with cellulose, the total production of SCFAs in the caecum was significantly higher when the diet contained 4.87% of RFO and insignificantly higher with a lower content of RFO (3.89%).

The supplementation of diets for weaning pigs (9.1–13.8 kg) with 0.5% TOS preparation or 0.87% GOS preparation (0.2% pure oligosaccharides in both experimental diets) did not affect nutrients digestibility and bacterial populations in the small intestine of pigs (Table 9) [Gabert *et al.*, 1995]. Similarly, a small amount of FOS (3 g/L) in a liquid diet for neonatal pigs or 0.4% FOS in a semi-synthetic diet for newly weaned piglets had no effect on the physiological indices analysed [Howard *et al.*, 1995; Mikkelsen *et al.*,

TABLE 9. Selected results illustrating the influence of different oligosaccharides on the functioning of the gastrointestinal tract and feeding results of pigs.

Carbohydrate	Level, %	Model of experiment	The main results	References
Corn starch TOS GOS	1.0 0.5 0.87	35 d-old barrows, 13.8 kg BW, fitted with the simple T-cannula.	Supplementation of diets with oligosaccharides did not affect the monosaccharide concentration in illeal digesta. The daily illeal output of monosaccharides, pH, ammonia and VFA concentrations, bacterial populations and incidence of diarrhoea were not affected.	[Gabert *et al.*, 1995]
FOS-free FOS	- 1.4 g/d	Neonatal pigs fed liquid formula without or with 3 g FOS/L for15 days	Supplementation with FOS did not alter neither the cell count of viable bifidobacteria in the organisms nor the total anaerobic microbiota, caecal pH, or concentration of SCFA. Caecal mucosal cell density and labelled increased with FOS consumption.	[Howard *et al.*, 1995]
Cellulose FOS TOS Cellulose	0.4 0.4 0.4 0.4	Newly weaned 4-week-old piglets fed a semi-synthetic diet for 4 weeks	There was no effect of diet on faecal pH and total organic acid concentration, but the pH decreased and the total organic concentration increased during the period after weaning. Faecal bacteria populations (total culturable anaerobic bacteria, lactic acid bacteria, lactobacilli, bifidobacteria, and coliforms) were not affected by the diet.	[Mikkelsen *et al.*, 2003]
Control FOS FOS TOS TOS	- 0.75 1.5 1.0 2.5	9-week-old castrated pigs with an initial BW of 15.6±0.3 kg, fed different diets for 6 weeks	FOS- and galacto-oligosaccharides did not affect the mean growth performance in week one to six. FOS and GOS could not be detected in the faeces. Supplementation of diets with FOS and GOS resulted in a temporary depressed feed intake with litter or no effect on faecal dry matter content and pH.	[Houdijk *et al.*, 1998]
Control FOS FOS TOS	- 0.75 1.5 1.0	57-day-old castrated pigs, with an initial BW of 15.9±0.6 kg, fed different diets for 44 days	Supplementation of diets with FOS and GOS tended to lower pH of the stomach content from 4.5 to 4.2. Compared to GOS-fed pigs, FOS-fed pigs had a higher proximal colon pH (6.5 *vs*. 6.2), lower proximal colon VFA concentration (131 *vs*. 166 mmol/L) and lower portal VFA concentration (0.9 *vs*. 1.6 mmol/L), with the control pigs being intermediate. The amount of colonic VFAs was similar.	[Houdijk *et al.*, 2002]
Control FOS fluorine FOS	- 1.5 1.5	28-d-old piglets fed for 4 weeks	No significant difference occurred between dietary treatments in colonic concentration of total anaerobes (7.95, 8.06 and 8.13 $\log_{10}$ CFU/g), total aerobes (5.20, 7.04 and 4.97 $\log_{10}$ CFU/g), total bifidobacteria (5.91, 6.86 and 6.79 $\log_{10}$ CFU/g), or coliform (7.95, 8.06 and 8.13 $\log_{10}$ CFU/g) for control FOS fluorine and FOS, respectively.	[Franworth *et al.*, 1992]
TOS Soybean meal Stachyose Stachyose	2.5 2.0 1.0 2.0	144 pigs weaned at 28 days (n=7), a three-week trail	Pigs fed 1% stachyose had more lactobacilli in the ileum as well as more bifidobacteria in the caecum and colon than the control pigs. Pigs fed 2% stachyose had fewer lactobacilli and bifidobacteria in the jejunum, ileum an caecum than compared to the control pigs. SCFA contents in the ileum, caecum and colon were the highest for pigs fed 1% stachyose and lowest for pigs fed 2% stachyose.	[Liying *et al.*, 2003]

2002]. Higher contents of FOS or TOS (0.75 and 1.5% of air dry matter) have bee reported to result in a temporarily depressed feed intake with a litter or in no effect on faecal dry matter content and pH [Houdijk *et al.*, 1998], however both preparations exerted a considerable effect on the fermentation in the large intestine of pigs [Houdijk *et al.*, 2002].

The possibility of increasing the number of bifidobacteria in the intestinal tract of pigs is a very important question because in pig intestinal microflora the number of bifidobacteria can be smaller than that of lactobacilli [Farnworth *et al.*, 1996]. Results of a number of experiments have shown that bifidobacteria population of piglets can be increased as a result of inulin and oligofructose incorporation to diets [Flickinger *et al.*, 2003]. From the practical point of view, however, costs make their use in pig diets undesirable.

It has been reported that low-digestible oligosaccharide addition to a diet for poultry can effectively improve health and performance of birds [Patterson & Burkholder, 2003]. On the other hand, there are still disagreements between research results even when the same oligosaccharide had been fed to the same class of poultry. Durst [1996] compared the nutritional response to the incorporation of fructo-and galacto-oligosacharides into broiler diets, and indicated that besides a negligible effect on production indices those oligosaccharides considerably affected metabolism and health condition of the birds. Juśkiewicz *et al.* [2002] reported that the addition of 0.4% oligosaccharides (inulin, mannan or FOS) to a diet had no significant effect an the body weight of young (4-week old) turkeys and in a small degree changed SCFA concentration in the caecal digesta. Higher contents of FOS administered to broilers over a 6-week period in the form of spray-dried Jerusalem artichoke flour (2.8% flour corresponded to 2% of carbohydrates) or refined fructo-oligosaccharide had no effect on the pH in the upper parts of the gastrointestinal tract, but reduced the caecal pH by 0.4 units [Farnworth *et al.*, 1996]. In the case of TOS, XOS and maltosyl cyclodextrin preparations given to broiler for 6 weeks (2.5% of diet), the following results were observed: (1) all probiotic factors had no influence on the caecal weight, but significantly increased the caecal volume, (2) only TOS compounds significantly reduced *Salmonella* scores at 42 day of age [Farnworth *et al.*, 1996]. In other experiment [Patterson *et al.*, 1997], a diet containing thermally-produced kestoses (2% kestoses and 8% other sugars) had no effect on weight gain, feed conversion, concentration of total aerobic bacteria, coliforms, aerobically-enumerated lactobacilli, total anaerobes, or clostridia, but increased caecal bifidobacterial concentrations.

The first attempts to apply FOS as a substitute for subtherapeutic level of antibiotics were tested with broilers over ten years ago [Ammerman *et al.*, 1988; 1989]. Bailey *et al.* [1991] investigated the influence of two levels of FOS on the ability of *Salmonella typhimurium* to grow and colonize the gut of chickens. When FOS was added to a diet at a 0.375% level, little influence on *Salmonella* colonization was observed. Whereas at the 0.75% level, 12% fewer FOS-fed birds were colonized with *Salmonella* compared to the control group. A higher level of FOS (0.75%) was also very effective in the reduction of *Salmonella* incidence when chickens were stressed by temporary water and feed deprivation. The authors concluded that feeding chickens with FOS-supplemented diet may lead to a shift in the intestinal gut microflora, and under some circumstances may result in reduced susceptibility to *Salmonella* colonization. In turn, Fukata *et al.* [1999] showed that low doses of FOS (0.1%) incorporated into a diet for chicks may evoke a decrease in *Salmonella* colonization, but with few changes in the number of total bacteria, *Bifidobacterium*, *Bacteroides*, *Lactobacilli* and *E. coli*.

Although mannan-oligosaccharides (MOS) have been used in the same manner as the dominant prebiotics, as fructooligosaccharide products, they do not selectively enrich beneficial bacterial populations and affect them to a different extent. Results of ample experiments compiled in a review of Hooge [2003] have indicated that mannan-oligosaccharide (MOS) can be an alternative for antibiotic growth promoters. The MOS, derived from mannans on yeast cell surfaces can act as high-affinity ligands, offering a competitive binding site for the bacteria [Fairchild *et al.*, 2001]. In the presence of dietary MOS, the pathogen cannot attach itself to the receptors on the cell membrane of the intestinal epithelium and is flushed from the body with the excreta [Van Immerseel *et al.*, 2002]. It has been reported that MOS can also affect the immune system of the host [Savage & Zakrzewska, 1997] It is not proved that a supplementation of poultry diet with 0.1% MOS (with the costs comparable to the use of an antibiotic) is enough to protect against pathogens under different environmental conditions. That amount of MOS in the diet had no significant influence on neither SCFA production not pH level in broiler [Spring *et al.*, 2000] and turkey chickens [Juśkiewicz *et al.*, 2003]. In the study of Fritts and Waldroup [2003], 0.1% dietary treatments of MOS significantly improved feed conversion in turkeys compared to negative control or MOS 0.05%, but body weight, mortality and breast meat yield were unaffected by dietary treatments.

The above-mentioned reports indicate that oligosaccharide preparations can be considered as feed additives in pigs and poultry feeding, *e.g.* being as effective as antibiotics in the control of pathogens and enhancement of growth performance. However, as emphasised by Flickinger *et al.* [2003], much more research are needed to determine the appropriate role of these oligosaccharides in animal feeding.

NUTRITIONAL ADVANCES OF LDOs IN DIETS FOR HUMANS

Most of human studies presented in Table 10 involve healthy volunteers given 3-40 g doses of oligosaccharides per day for 3-6 weeks. The results obtained were differentiated, depending on the type and dose of oligosaccharides, and the criterion of the physiological response of the subjects to LDOs ingested.

In vitro and *in vivo* studies of Masai *et al.* [1997] have indicated that soybean oligosaccharides ingested in the amount of 3 or 9 g/day were utilised very well by bifidobacteria and enhanced the growth of bifidobacteria in the intestine. Furthermore, the intake of soybean oligosaccharides was demonstrated to reduce the activities of harmful bacterial enzymes and the amount of putrefactive products. It should be emphasised that information on dietary use of

TABLE 10. Selected results illustrating the physiological effect of different oligosaccharides in human diets.

Carbohydrate, dose/d	The main results	References
RFO 3 or 9 g	After 3 weeks ingestion of RFOO, number of bifidobacteria increased significantly, but that of *Clostridium*- other and *C. perfringens* reduced significantly by the ingestion 3 or 9 g of RFO. RFO significant decreased azoreductase activity. The water holding capacity of faces tended to increase by the ingestion RFO, but the increase was not significant.	[Masai *et al.*, 1987]
TOS 10 g	In the eight volunteers, administration of TOS (during 21 days) led a significant decrease in faecal concentration of bifidobacteria from 8.6 to 9.5 $\log_{10}$CFU/g faecal concentration of enterobacteria, as well as stool weight, faecal water and pH did not change.	[Bouhnik *et al.*, 1997]
TOS 7.5 or 15 g	The number of bifidobacteria increased after both placebo and TOS ingestion, but the differences between these increases were not significantly different. TOS did not significantly affect bowel habits, stool composition, the concentration of ammonia, indoles, or skatoles in faeces, faecal pH or the composition of the intestinal microflora.	[Alles *et al.*, 1999]
Inulin 9 g	No changes in dietary habits, faecal and bile acid output, faecal SCFA and pH, were observed, whereas plasma total cholesterol and triacylglycerol significantly decrease after four weeks ingestion of inulin. Total facultative anaerobes significantly decreased after test, and bifidobacteria increased alter correlation for total anaerobes ($P<0.05$).	[Brighenti *et al.*, 1999]
FOS 8 g	Bacterial count for bifidobacteria increased by a mean of 2.8 $\log_{10}$CFU/g faeces after 3 weeks of supplementation, and decrese by a mean of 1.1 $\log_{10}$CFU/g faeces after the period without FOS. Unexpected changes in non-specific immunity were observed: decrease phagocytic activity of granulocytes and monocytes, as well as a decreased expression of interleukin-6-mRNA in peripheral blood monocytes.	[Guigoz *et al.*, 2002]
Inulin, FOS, GOS 15 g	As compared to the control treatment: higher concentration of faecal acetate (inulin and GOS $P<0.05$) and valerate (inulin $P<0.05$), significantly lower the faecal deoxycholic acid (inulin and FOS $P<0.05$) and β-glucuronidase activity (inulin and GOS $P<0.05$). Other changes of faecal parameters and those of blood lipids and glucose absorption were not significant.	[Dokkum *et al.*, 1999]
Inulin 15 g	In healthy volunteers with low stool frequency ingestion of inulin increase stool frequency from 4.0 to 6.5 per week. The mean increase in stool weight was observed, *i.e.* 22 g/day, represent a value of approximately 1.5 g weight increase per 1 g inuline consumed.	[Den Hond *et al.*, 2000]
Inulin 18 g	Men and women with baseline LDL increased significantly (7.4 and 12.4%, respectively) during the control phase. There were small, insignificant declines in total (1.3%) and LDL-C (2.1%) during the inuline phase. Thus, differences in response between periods (inulin-control) were significant ($P<0.05$) for LDL-C (-14.4%) and total cholesterol (-8.7%).	[Davidson & Maki, 1999]
Inulin 22–34 g	Long-term consumption of inulin significantly increased bifidobacteria from 9.8 to 11.0 $\log_{10}$/g dry faeces and caused a moderate increase in gastrointestinal symptom such as flatulence and bloatednees, whereas blood lipids and short-chain fatty acids remained essentially unaffected.	[Kruse *et al.*, 1999]
Inulin 20–40 g	Inulin increased bifidobacteria significantly from 7.9 to 9.2 $\log_{10}$/g dry faeces, but decreased enterococci in number and enterobacteria in frequency. No changes in the concentration of faecal SCFAs and lactate were observed. SCFAs showed a slight trend toward higher molar rations of acetate to butyrate, the faecal pH and the β-glucosidase and β-glucuronidase activities were not influenced by inulin intake.	[Kleessen *et al.*, 1997]

soybean or grain legumes oligosaccharides (RFO) are sparse, however the results of experiments on animals administered with higher doses of RFO are ambiguous.

Diversified results have been obtained when 7.5–15 g of transgalacto-oligosaccharides (TOS) were introduced into diets for healthy volunteers [Boughnik *et al.*, 1997; Alles *et al.*, 1999]. In an experiment by Boughnik *et al.* [1997], prolonged administration of TOS (for 21 days), at a dose of 10 g/day, did not induce digestive symptoms, but increased the number of bifidobacteria (from 8.6 to 9.5 $\log_{10}$CFU/g) and altered the fermentative activity of colonic flora in humans. In an experiment of Alles *et al.* [1999], the number of bifidobacteria increased after both placebo and TOS ingestion, but the differences between these increases were not significantly different. The TOS had no significant effect on bowel habits, stool composition, the concentration of ammonia, indoles, or skatoles in faeces, faecal pH or the composition of the intestinal microflora.

Ingestion of 5 g/day of FOS resulted in close to one log cycle increase in bifidobacteria numbers after 11 days, however no further increase was observed after the next 10 days [Rao, 2001]. A lower dose of 3 g/day of FOS resulted also in beneficial tendencies in bowel functioning and a decrease in faecal protein catabolites without changes in the faecal score, faecal pH and SCFA concentration [Swanson *et al.*, 2002]. Data presented in Table 10 demonstrate also that higher doses of FOS (8 or 9 g/day) evoked beneficial changes in the population of microflora (increase in bifidobacteria number), however did not influence faecal SCFA nor pH [Brighenti *et al.*, 1999; Guigoz *et al.*, 2002]. In a single experiment, 3-week ingestion of 8 g of FOS resulted in unexpected changes in the non-specific immunity: a decreased phagocytic activity of granulocytes and monocytes, as well as a decreased expression of interleukin-6-mRNA in peripheral blood monocytes [Guigoz *et al.*, 2002].

Ingestion of 15 g/day of inulin, FOS or DOS, *i.e.* medium level supplementation of diets with different LDOs, caused intensive fermentation in the large bowel, however the results obtained were differentiated (Table 10) [Dokkum *et al.*, 1999]. Physiological effect of inulin and GOS was generally similar and apparently stronger than that of FOS. Results obtained by Dokkum *et al.* [1999] indicate that the ingested LDOs were fermented in the human colon, but in healthy young men the effects were limited, *e.g.* LDOs did not seem to alter blood lipid concentration. Results of other experiments concerning the ingestion of 18 g/day of inulin were summarised as follows: it is not possible to draw any firm conclusions, inulin may have blunted the hypercholesterolemic effect observed during consumption of control foods [Davidson & Maki, 1999]. A higher dose of inulin (20–40 g/day) significantly increased bifidobacteria number, whereas blood lipids and SCFA remained essentially unaffected (Table 10) [Kleessen *et al.*, 1997; Kruse *et al.*, 1999].

In many experiments with volunteers obtaining diets containing inulin or oligofructose, their stool weigh increased by 1.5–2 g per gram of oligosaccharide ingested [Van Loo *et al.*, 1999; Nyman, 2002]. An increased stool weight and stool frequency have been reported with oligofructose administered at a level as low as 3 g/day to subjects having a low stool frequency [Tominaga *et al.*, 1999]. The laxative effect of inulin has also been reported when inulin was given in doses from 20 to 40 g [Kleessen *et al.*, 1997]. In contrast, other studies have reported minor effects with oligofructose and inulin given in doses from 5 to 15 g/day [Alles *et al.*, 1996].

The evaluation of the potential effect of prebiotic LDOs on blood lipids was the aim of numerous experiments. This possibility has been supported by the observation that in animals dietary fructooligosaccharides cause suppression of hepatic triglycerides and VLDL synthesis, resulting in substantial reduction in triglycerides, and to a lesser extent in cholesterol levels [Taylor & Williams, 1998]. The panel of putative mediators of the systemic effect of prebiotic oligosaccharides consists in either modifications in glucose/insulin homeostasis, the end-product of their colonic fermentation (*i.e.* propionate) reaching the liver by the portal vein, incretins and/or the availability of other nutrients [Delzenne *et al.*, 2002]. Significant reduction in the insulin concentration (suggesting an improvement in blood-glucose control) and significantly lowered triglyceride level were observed in middle-aged men after ingestion of 10 grams of inulin per day for eight weeks [Jackson *et al.*, 1999]. However, in another trial in people with type 2 diabetes administered for 20 days with 15 g of FOS per day f no effect on blood-glucose or lipid level was observed [Roberfroid, 1993]. Also the consumption of 15 g of galacto-oligosachcarides does not seem to alter blood lipid concentrations and glucose absorption in young healthy adults [Dokkum *et al.*, 1999]. It seems likely that in people with normal or low cholesterol or triglyceride level, FOS or inulin produced little effect [Luo *et al.*, 1996; Pedersen *et al.*, 1997; Dokkum *et al.*, 1999].

Pre-clinical studies have suggested that prebiotic saccharides may have promising properties in inflammatory disease [Szilagyi, 1998] and prevention of infections of intestinal origin [Dai & Walker, 1999]. Protective effects against colon cancer have also been suggested [Taper & Roberfroid, 2002; Pool-Zobel *et al.*, 2002]. the mechanism of anticancer effect of inulin and oligofructose is still unclear. Probably a very important role is played by butyrate which is capable of facilitating apoptosis of carcinoma cells [Hague *et al.*, 1995].

Changes in the intestinal microflora that occur with the consumption of prebiotic saccharides may potentially mediate immune changes *via*: the direct contact of lactic bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the intestine; the production of short-chain fatty acids; or by changes in mucin production [Schley & Field, 2002]. Ample investigations performed with animal models have demonstrated repeatedly that non-digestible oligosacccharides, such as inulin, oligofructose or transgalactooligosaccharides, stimulate mineral absorption, mainly that of calcium and magnesium [Scholz-Ahrens *et al.*, 2001]. In male adolescents ingestion of fifteen grams of oligofructose per day stimulates colonic calcium absorption [Heuvel *et al.*, 1999]. In healthy adult men, 40 g of inulin was observed to have a positive effect of on apparent calcium absorption [Coudray *et al.*, 1997]. It is assumed that the effects of NDO on mineral metabolism may be based on the enhancement of passive and active mineral transport across the intestinal epithelium, mediated by an increase in certain metabolites of the intestinal flora and reduction of pH [Scholz-Ahrens & Schrezenmeir, 2002]. However, more research is needed to explore this issue, *e.g.* the possible impact of bone mineral accumulation and bone quality.

Taking all these information together, low-digestible carbohydrates (prebiotics) bring a lot of benefits to animal and human nutrition. LDOs may influence animals and humans in various ways: improve intestinal health, modify lipid metabolism, modulate the immune response, and decrease the risk of intestinal and systemic diseases. The present state of knowledge on the mechanism of dietary action of prebiotic LDOs and physiological effects of different types and doses of low-digestible carbohydrates is not sufficient. It has also been emphasised in conclusions summarising numerous reviews concerning this topic. 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 beneficial bacteria (*Bifidobacterium*, *Lactobacillus* and *Eubacterium* spp.), and inhibition of "undesirable" bacteria (*Clostridium* and *Bacteroides*). Other beneficial 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] recognized as "postulated effects that have not been finally confirmed". Also Roberfoid [2001] classified the evidence that inulin-type fructans influence colonic flora and bowel function as "strong", but evidence concerning improving calcium bioavailability and hypolipidemic effect of prebiotis as "promising". Flickinger [2003] confirmed these data and stated that scientists have only begun to scratch the surface regarding the use of prebiotic LDOs as components of human diets.

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FINAL REPORT

Title of the research project:
THE METHODOLOGICAL BASES OF THE EVALUATION OF THE QUALITY AND SAFETY OF THE NEW GENERATION FOOD (PBZ-KBN-020/P06/1999).

Title of the individual project:
Characterization of functional properties of diets enriched with oligosaccharide preparations with the use of model experiments on rats.

Institution:
Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland.

Leader:
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Co-workers:
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Key words:
Oligosaccharide, prebiotics, physiological effect, evaluation, rat.

SYNTHESIS OF RESULTS

Three research tasks were accomplished in a series of *in vivo* experiments: (1) functional properties of diets with different prebiotic preparations administered to rats, (2) influence of prebiotic properties on physiological properties of diets with different content of protein, fat and cholesterol, and (3) caecal adaptation in the rat in response to prebiotic and carboxymethylcellulose or flavonoid extract.

The low digestible carbohydrates assessed (inulin, lactulose, fructooligosaccharides, oligofructose, α-galactosides, starch, and carboxymethylcellulose) as well as control saccharides: saccharose or cellulose, were applied in standard diets for rats aged 4–8 weeks. Experimental groups were made of 8 individually-fed rats kept under controlled environmental conditions (temperature, humidity, and air-condition). In all experiments, the main attention was paid to caecal metabolism, as that part of the gastrointestinal tract is the main site of fermentation processes in rats.

The influence of replacing cellulose in a casein diet with oligosaccharides from lupin and pea seeds (3.89 or 4.87% of α-galactosides, respectively) on the metabolism of the caecum was determined [Juśkiewicz *et al.*, 2003]. Compared with cellulose, the total production of short chain fatty acids (SCFAs) in the caecum (expressed as mol/100 g BW) was significantly higher when the diet contained a higher amount of pea -galactosides and insignificantly higher when the diet contained less -galactosides extracted from lupin seeds. Diet supplementation with lupin and pea oligosaccharides caused significant changes in the functioning of the caecal ecosystem (an increased amount of water, enhanced bacterial glycolytic activity, production of SCFA and ammonia concentration), however without decreasing pH, ammonia content and activity of potential harmful bacterial enzymes (β-glucuronidase and β-glucosidase).

Parameters of rat caecum functioning were found similar when either a disaccharide (lactulose) or a polysaccharide (inulin) were administered with diets [Zduńczyk *et al.*, 2004]. The 8% addition of lactulose and inulin preparations increased significantly the content of caecal digesta (4.62 and 4.11 g/100g BW, respectively) and the weight of the caecal wall (1.10 and 0.86, respectively), compared to the groups with saccharose and cellulose (0.73, 0.90 and 0.24, 0.28, respectively). The group with the 8% addition of lactulose was characterised with the highest activities of microbiological α- and β-galactosidase and β-glucosidase in the caecal digesta compared with the inulin group. The administration of lactulose and inulin preparations was accompanied by a significant pH drop (5.47-5.81), compared to the cellulose and saccharose groups (6.83-6.91), and a decrease in ammonia concentration in the caecal digesta, compared to the cellulose control (0.27-0.40 and 0.62 mg/g, respectively). The total production of SCFAs in the caecum (μmol/100g BW) was fourfold higher with the 8% addition of lactulose and inulin preparations (254.7 and 236.4, respectively) than that in both controls groups (65.1 and 67.8, respectively).

The addition of a lower amount (5%) of oligofructose and inulin induced the enlargement of the caecum and significantly decreased dry matter and pH of caecal digesta in rats fed fructan preparations compared to the cellulose and starch (control) groups [Juśkiewicz *et al.*, 2004]. Inulin and oligofructose caused a significant increase in the SCFAs content produced in the whole caecum (calculated per 100 g BW). Cellulose as well as fructans decreased ammonia concentration in the caecal digesta compared to control rats. Diet composition had no significant influence on the concentration of glucose, triglycerides nor total cholesterol in the serum of rats. Compared to control and cellulose, the investigated fructans were very effective in causing beneficial changes to the functioning of the caecal ecosystem.

An increment of the mass of both caecal wall and caecal content was observed in rats fed diets with 5% addition of different low digestible carbohydrate preparations: lactulose, inulin, oligofructose, fructooligosaccharides, and maltitol [Wróblewska *et al.*, 2004]. The addition of LDC's to the diet of rats increased dry matter and ammonia pool in digesta but decreased pH in the caecal content, compared with rats fed the control diet. There was a marked enlargement of short chain fatty acids pool in the caecal content of rats fed a diet supplemented with inulin compared with other groups. When expressed as percent of total SCFAs and compared to control, acetate decreased in experimental groups with the exception of the group supplemented with lactulose, almost 10% increase in propionate was reported in the group with maltitol, and butyrate increase 2-fold in inulin, raftilose and FOS fed rats. The activities of bacterial α- and β- galactosidase and β-glucosidase were especially high when the diet was supplemented with lactulose. Within 5 groups of the caecal microflora determined, *Bifidobacterium* counts significantly increased in the lactulose group, *E. coli* populations significantly decreased in the maltitol group and showed a tendency to decrease in inulin, oligofructose and fructooligosaccharide, whereas enterococci tended to decrease in all groups.

The addition of 5% inulin preparation (Raftilose Synergy) significantly increased *Bifidobacterium* counts in the caecal digesta (from 9.46 to 10.36 log cfu/g), compared with rats fed the diet with cellulose [Majkowska *et al.*, in preparation]. Inulin preparation was found to insignificantly increase *Lactobacillus* counts and decrease *E. coli* counts. Diet supplementation with inulin and probiotic preparations (*Bifidobacterium animalis* Bi30 and *Lactobacillus acidophilus Bs*) negligibly increased the prebiotic influence of inulin. *Bifidobacterium* and *Lactobacillus* counts in the caecal content increased insignificantly. Diet supplementation with probiotic preparation, especially *Bifidobacterium animalis*, decreased IgG and IgA concentration in the small intestine, however had no influence on the concentration of these immunoglobulins in serum.

The next experiment indicated that the effects of prebiotic oligosaccharides (inulin preparation) are determined by the contents of other dietary components, including proteins and lipids [Zduńczyk *et al.*, in preparation]. Increasing the protein content in a diet from 13.5 to 20% diminished the trophic effect of the prebiotic on tissue and digesta of the caecum, still had no effect on such parameters of digesta as pH, concentration of dry matter and ammonia. A higher protein content resulted in enhanced microbiological activity of β-glucuronidase in the digesta, especially in the case of saccharose-supplemented diet. A twofold increase in fat content of diet (from 7.5 to 15%) lowered the concentration of production of SCFAs in the caecum to a great extent in the diet with saccharose and negligibly- in the diet with prebiotic. A higher fat content in the diet had no significant effect on the other parameters of the caecum: pH, dry matter content, glycolytic activity of intestinal microflora.

The functioning of the caecum ecosystem in rats was significantly affected by increasing viscosity of caecal digesta resulting from diet supplementation with carboxymethylcellulose [Juśkiewicz & Zduńczyk, Compar. Biochem. Physiol., in review]. Animals given carboxymethylcellulose (CMC) or in combination with inulin had watery caecal digesta and some of them suffered from diarrhoea. In the case of CMC, the caecal enlargement was due to tissue hypertrophy and digesta accumulation mostly in response to an increased bulk of contents. Unlike inulin applied with cellulose, the dietary combination of CMC and inulin enhanced fermentation in the caecum of rats, however the proportion of acetate, propionate and butyrate was less beneficial. Compared to CMC, inulin yielded a higher concentration of SCFA, especially of butyrate and propionate.

Considerable changes in the caecum metabolism were reported upon supplementation of diet with 0.3% extract of grapefruit phenolics [Zduńczyk *et al.*, in preparation]. Both in groups fed a diet without and with a prebiotic, the addition of phenols significantly increased the weight of the caecal wall and caecal digesta, and alkalinity of the digesta. On the other hand, it significantly reduced concentrations of Lowry's protein, dry matter and ammonia in the caecum. Phenolic compounds of the diet diminished the microbial activities of β-glucosidase and β-glucuronidase. A significant decrease was also observed in SCFAs concentration, especially in the concentration and content of butyric acid.

The main achievements of the presented studies include the following:

1. Determining that the best indicator of physiological activity of low digestible saccharides in the gastrointestinal tract of rats was the VFA pool size of the caecum, *i.e*. total content of VFA calculated taking into account their concentration in caecal digesta, the bulk of digesta and body weight of rat. Important indicators of physiological activity of LDS were also pH, glycolytic activity of microflora and ammonia content in the caecal digesta.

2. Determining that diet composition, including contents of protein and phenolic compounds, exerted a significant influence on the physiological activity of the prebiotic preparations administered with diets.