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PHYSIOLOGICAL EFFECTS OF DIETARY INULIN, XYLITOL AND β-GALACTOSYL-DERIVATIVES OF SUGAR ALCOHOLS IN RAT

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Rats were fed for 4 weeks with diets containing 5% sucrose or the following preparations: inulin, xylitol or β-galactosyl-derivatives of polyol (β-galactosyl-xylitol, -sorbitol, -erythritol). Except for β-galactosyl-erythritol, all preparations caused an enlargement of caecum weight (tissue: 0.41–0.51, digesta: 1.28–1.80 g/100 g BW), compared to the control group (0.28 and 1.00, respectively). The control caecal pH was close to 7.0, while in the experimental groups it ranged from 6.45 (xylitol) to 6.84 (β-galactosyl-erythritol). The caecal ammonia concentration was the lowest in the inulin group (0.45 mg g^{-1}) and the highest in β-galactosyl-sorbitol group (0.62). All preparations decreased the β-glucuronidase activity in the caecal digesta (0.59–0.81 U g^{-1}) compared to rats fed sucrose-diet (1.00). The highest concentration of SCFAs in the caecum was in inulin and β-galactosyl-erythritol groups (68.57 and 68.36 μmol g^{-1}), and the lowest one – with xylitol (52.41). The total production of SCFAs in the caecum (μmol/100 g BW) was the lowest in the control group (64.8).

Keywords: inulin, polyols, caecum, enzyme activity, short-chain fatty acids, rats

Dietary low-digestible carbohydrates (LDCs) represent a large complex group of food components, which play an important nutritional role. All the LDCs resist digestion (to a different extent) in the small intestine of human and monogastric animals, and they are potential substrates for the bacteria colonizing the large intestine. This way, LDCs indirectly provide the host with nutrients, e.g. short-chain fatty acids (SCFA), vitamins and other bacterial metabolites (SCHEPPACH et al., 2001). Inulin is the most recognizable indigestible carbohydrates with prebiotic properties. It was observed that inulin had beneficial effects on gut physiology and that this was associated with the production of SCFA and a lower pH in the intestinal lumen (NYMAN, 2002). In turn, xylitol is a sugar alcohol and, as other polyols, it is slowly and incompletely absorbed from the small intestine into blood (ELLWOOD et al., 1999). Undigested xylitol reaches the caecum and is fermented rapidly by the intestinal flora, but prebiotic properties of dietary xylitol are rather low (WÜRSCH et al., 1990; ZDUŃCZYK et al., 2002).

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The β-galactosyl-derivatives of sugar alcohols (β-galactosyl-xylitol, β-galactosyl-sorbitol and β-galactosyl-erythritol) are a new group of low-digestible carbohydrates (KLEWICKI, 2000), whose functional properties are insufficiently recognized. The chemical composition of β-galactosyl-derivatives of polyols suggests that they can be fermented in the large intestine and are likely to have an effect on the caecal/colonic metabolism and/or activity of the intestinal flora, but this has not been tested enough in biological trials so far.

The presented study was conducted to determine the effect of different types of dietary low-digestible carbohydrates (inulin, xylitol and β-galactosyl-derivatives of polyols) on various caecal parameters including SCFA production and glycolytic enzymes activity.

1. Material and methods

Diets were formulated to meet or exceed the nutrient requirements for rats (NRC, 1995). The control diet contained ca. 13.5% total protein (casein with DL-methionine), 10% soybean oil, 5% sucrose, 0.8% cholesterol, 3% mineral mixture (AIN-93G Mineral Mix), 2% vitamin mixture (AIN-93G Vitamin Mix) and maize starch as the rest. Inulin (Frutafit-Tex, SENSUS), xylitol (XYRAFIN OY, Marbis Co.) and preparations containing β-galactosyl-derivatives of polyols were added to replace sucrose. Preparations of β-galactosyl-derivatives of polyols (β-galactosyl-xylitol, -sorbitol and -erythritol) were prepared at the Institute of Chemical Technology of Food, Technical University of Łódź (Poland) according to KLEWICKI (2000). The composition of tested preparations is given in Table 1.

The experiment lasted 4 weeks and was conducted on 60 four-week old Wistar rats weighing 69 ± 2 g. The groups consisted of ten male rats housed individually in metabolic cages in a room with a 12-h light-dark cycle, controlled temperature of 21–22 °C, relative humidity of 50 ± 5% and intensive ventilation (15 air changes h^{-1}).

Table 1. Chemical composition of preparations of β-galactosyl-polyol derivatives (% of dry matter)

	β-Galactosyl-		
	xylitol	sorbitol	erythritol
Xylitol	41.3	–	–
Galactosyl-xylitol	17.8	–	–
Digalactosyl-xylitol	1.6	–	–
Sorbitol	–	42.3	–
Galactosyl-sorbitol	–	19.8	–
Digalactosyl-sorbitol	–	2.0	–
Erythritol	–	–	35.0
Galactosyl-erythritol	–	–	24.2
Digalactosyl-erythritol	–	–	1.5
Lactose	25.5	22.4	13.5
Others (mono-, di- and trisaccharides)	13.8	13.5	25.8

During the experiment, individual feed consumption and body weight gains of rats were recorded. After laparotomy, the caecum with contents was removed and weighed. Samples of fresh digesta were used for immediate analysis (pH, dry matter content, ammonia and SCFAs concentrations), the rest was transferred to microfuge tubes and stored at –40 °C. Dry matter of the digesta was determined at 105 °C. In fresh caecal digesta, ammonia extracted and trapped in a solution of boric acid in Conway dishes was determined by direct titration with sulphuric acid. Protein content in the digesta was determined with the method of LOWRY and co-workers (1951).

The caecal digesta were analysed for SCFA concentration by GC (Shimadzu GC-14A with a glass column 2.5 m × 2.6 mm, containing 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb W AW, and column temperature was 110 °C, detector FID temperature 180 °C and injector temperature 195 °C). Glycolytic activity in the caecal digesta was measured by the rate of release of *p*- or *o*-nitrophenol from their nitrophenylglucosides according to the modified method of DJOUZI and ANDRIEUX (1997) described by JUŚKIEWICZ and co-workers (2002).

Results were analysed using one-way ANOVA and significant differences between groups were determined by the Duncan's multiple range test. Differences were considered significant at $P \leq 0.05$.

2. Results and discussion

The chemical analysis demonstrated that mixtures containing β-galactosyl-derivatives of polyols were not pure preparations and consisted of several ingredients (Table 1). β-Galactosyl derivatives of polyols contained 17.8% preparation in the case of galactosyl-xylitol and 19.8 and 24.2% in the case of galactosyl-sorbitol and galactosyl-erythritol, respectively. The tested preparations included also small amounts of digalactosyl-derivatives (1.5–2.0%), then primary sugar alcohols, i.e. xylitol (41.3%), sorbitol (42.3), erythritol (35.0) as well as lactose and mono-, di- and trisaccharides.

Diet intake and final body weights of rats did not differ significantly among the experimental groups (Table 2). In the experiment of OHTA and co-workers (1998), diets with 5 or 10% fructans with different sugar-chain lengths had no effect on dietary intake and growth in rats either. In earlier studies, it was observed that rats fed 1% or 2% sorbitol or xylitol did not show significant differences in food intake or food efficiency, but their body weight gain was significantly lower compared to rats fed an equivalent amount of glucose (KARIMAZADEGAN et al., 1979). On the other hand, ELLWOOD and co-workers (1999) observed a decrease in food intake in rats fed 10% or 20% xylitol for 8 weeks, compared to a diet with glucose. Our results suggested that, when applied in a 5% dose, the investigated LDC preparations did not lower the nutritive value of the diet.

Supplementation of diets with LDCs induced enlargement of the caecum (tissue and digesta) compared to the control group. Only in the case of β-galactosyl-erythritol, the caecal content (1.10 g/100 g BW) was similar to the control group (1.00), moreover the caecal tissue weight (0.33 g/100 g BW) was lower than in groups consuming other

tested LDCs preparations (0.41–0.51). The preparation with β-galactosyl-erythritol contained also a high amount of erythritol, which is extensively absorbed in the small intestine (OKU & NODA, 1990). It was probably the reason of the observed minor effect of that preparation on caecal digesta. The highest enlargement of caecal tissue and digesta was observed in rats fed a diet with 5% xylitol (0.51 and 1.80, respectively). The xylitol preparation decreased the dry matter concentration in the caecum (18%), while inulin preparation showed the lowest hydration of the caecal digesta (dry matter 21.3%), compared to the other groups (19.5–20.7). The dry matter content in the caecum (expressed as g/100 g BW) of rats, in consequence of caecal digesta amounts, was the highest in inulin and xylitol groups (0.32), and the lowest in the case of control and β-galactosyl-erythritol groups (0.20 and 0.21, respectively). The caecal pH in the control group was about 7 and was decreased by LDCs treatments. Administration of xylitol and inulin preparations was accompanied by the strongest decrease in caecal pH value (6.45 and 6.50, respectively), while the mixtures containing β-galactosyl derivatives of polyols caused a lower decrease of pH (to the level of 6.68–6.84). Compared to the control rats (0.55 mg g^{-1}), the concentration of ammonia in the caecum was found to decrease in the case of inulin (0.45), and to increase in the case of β-galactosyl-sorbitol preparation (0.62), but it was not verified statistically. When caecal ammonia concentration is considered, significant difference was reported between β-galactosyl-sorbitol and inulin groups. The lowest protein concentration was found in rats fed a diet containing sucrose, and it differed significantly from inulin, β-galactosyl-xylitol and β-galactosyl-sorbitol treatments.

When non-digestible oligosaccharide (NDO) consumption is considered, a typical digesta bulking effect is evaluated of 1.5–2 g increase per gram NDO ingested (VAN LOO et al., 1999). An increase in the digesta weight was most probably caused by an increased bacterial population in the caecum, which was confirmed by greater protein content in the digesta when LDC preparations were added to a diet. In our study, the greatest trophic effect on caecal tissue was observed in xylitol group, which suggested that a high amount of xylitol reached the large bowel where it was fermented. A smaller effect in β-galactosyl-xylitol group was noted, which was probably because the mixture contained, besides β-galactosyl-xylitol, digalactosyl-xylitol and xylitol, and also well-digestible lactose as well as mono-, di- and trisaccharides. Great hyperplasia of the caecal mucosa was presented in the study of ELLWOOD and co-workers (1999), wherein rats received 10 or 20% dietary xylitol or sorbitol. The main mechanism of intestinal action of LDC is based on the modification of bacterial ecosystem, higher production of lactic acid and SCFA, thus lower pH of digesta in the hind gut is probably responsible for the proliferation of beneficial bacteria (GIBSON et al., 1995). In our experiment, all investigated preparations lowered caecal pH compared to sucrose-fed rats, and an acidification process in the caecal digesta was observed in xylitol and inulin groups.

Table 2. Body weight, daily diet intake, caecal parameters and glycolytic activity in the caecal digesta in rats fed with experimental diets

	Control	Inulin	Xylitol	β-Galactosyl-			SEM
				xylitol	sorbitol	erythritol	
Final body weight, g	234.8	226.0	231.4	231.8	225.6	234.4	1.84
Diet intake, g/rat/day	13.96	13.81	13.53	14.01	13.78	14.26	0.35
Caecal tissue, g/100 g BW	0.28^{d}	0.42^{b}	0.51^{a}	0.41^{b}	0.41^{b}	0.33^{c}	0.01
Caecal digesta, g/100 g BW	1.00^{e}	1.52^{b}	1.80^{a}	1.40^{bc}	1.28^{cd}	1.10^{de}	0.05
Caecal dry matter, g/100 g BW	0.20^{b}	0.32^{a}	0.32^{a}	0.28^{ab}	0.26^{ab}	0.21^{b}	0.06
DM of caecal digesta, %	19.5^{b}	21.3^{a}	18.0^{c}	19.8^{b}	20.7^{ab}	19.5^{b}	0.60
pH of caecal digesta	7.02^{a}	6.50^{cd}	6.45^{d}	6.71^{b}	6.68^{bc}	6.84^{b}	0.04
Ammonia, mg g^{-1}	0.55^{ab}	0.45^{b}	0.59^{ab}	0.57^{ab}	0.62^{a}	0.54^{ab}	0.02
Lowry's protein, mg g^{-1}	0.17^{c}	0.22^{ab}	0.19^{bc}	0.22^{ab}	0.25^{a}	0.18^{bc}	0.01
Glycolytic activity, U g^{-1} fresh caecal content							
β-Glucuronidase	1.00^{a}	0.81^{ab}	0.59^{b}	0.65^{b}	0.85^{ab}	0.71^{ab}	0.15
α-Glucosidase	1.20^{b}	0.86^{b}	3.35^{a}	4.39^{a}	4.33^{a}	3.20^{a}	0.28
β-Glucosidase	0.14	0.21	0.14	0.22	0.33	0.22	0.02
α-Galactosidase	0.45^{c}	0.73^{bc}	0.78^{bc}	1.64^{ab}	2.02^{a}	1.17^{ab}	0.18
β-Galactosidase	2.64^{c}	2.20^{c}	10.09^{b}	19.93^{a}	23.42^{a}	8.59^{b}	2.05

^{a,b,c,d,e} Values in one row with different superscripts are significantly different at $P \leq 0.05$.

Table 3. The concentration (μmol/g fresh digesta) and total pool[A] of SCFA (μmol/100 g BW) in the caecum

	Control	Inulin	Xylitol	β-Galactosyl-			SEM
				xylitol	sorbitol	erythritol	
SCFA concentration:							
Total SCFA	64.41^{ab}	68.57^{a}	52.41^{b}	60.70^{ab}	64.22^{ab}	68.36^{a}	1.80
Acetate	45.27^{ab}	34.72^{cd}	27.85^{d}	36.93^{bcd}	39.99^{bc}	49.25^{a}	1.51
Propionate	6.07^{b}	12.82^{a}	12.80^{a}	11.80^{a}	13.06^{a}	7.61^{b}	0.57
Isobutyrate	1.35^{a}	0.81^{c}	0.71^{c}	0.94^{bc}	0.82^{c}	1.05^{b}	0.04
Butyrate	7.41^{b}	17.29^{a}	8.11^{b}	7.46^{b}	6.95^{b}	6.52^{b}	0.60
Isovalerate	1.81^{a}	1.28^{b}	1.35^{b}	1.61^{ab}	1.58^{ab}	1.88^{a}	0.06
Valerate	2.50^{a}	1.65^{bc}	1.59^{c}	1.96^{bc}	1.82^{bc}	2.05^{b}	0.07
C_2:C_3:C_4 profile[B]	70^{a}:9^{b}:11^{c}	51^{c}:19^{a}:25^{a}	53^{c}:24^{a}:15^{b}	61^{b}:19^{a}:12^{bc}	62^{b}:20^{a}:11^{c}	72^{a}:11^{b}:10^{c}	–
SCFA pool[1]:							
Total SCFA	64.8^{c}	104.2^{a}	94.3^{ab}	85.0^{abc}	82.2^{abc}	75.2^{bc}	3.17
Acetate	45.3	52.8	50.1	51.7	51.2	54.2	2.01
Propionate	6.07^{b}	19.5^{a}	23.0^{a}	16.5^{a}	16.7^{a}	8.4^{b}	0.99
Isobutyrate	1.35	1.23	1.28	1.32	1.05	1.15	0.05
Butyrate	7.41^{c}	26.3^{a}	14.6^{b}	10.4^{c}	8.90^{c}	7.17^{c}	0.97
Isovalerate	1.81	1.95	2.43	2.25	2.02	2.07	0.08
Valerate	2.50	2.51	2.86	2.74	2.33	2.25	0.09

[A] Caecal SCFA pool was calculated as SCFA concentration × caecal digesta/100 g BW; [B] μmol/100 μmol total SCFA.
[a,b,c] Values in one row with different superscripts are significantly different at $P \leq 0.05$.

Inulin preparation was also the most effective in decreasing of caecal ammonia concentration. Many of the putrefactive compounds have adverse effects on large intestinal health, e.g. high ammonia concentration may promote tumorigenesis (LIN & VISEK, 1991). The fermentation affects nitrogen metabolism in the large intestine. Carbohydrate substrates stimulate bacterial proliferation, which leads to incorporation of nitrogen (from ammonia and other sources) into bacterial cell walls and consequent excretion in faeces. SCFA, as end products of carbohydrate fermentation, lower caecal pH, which in turn reduces diffusion of (ionized) ammonia into portal blood (SCHEPPACH et al., 2001).

The activities of glycolytic enzymes in the caecal digesta are presented in Table 2. The groups receiving xylitol and β-galactosyl derivatives of polyols were characterized by highly increased activity of α-glucosidase and β-galactosidase, compared to groups receiving sucrose or inulin. The highest activities of α-galactosidase and β-glucosidase were reported in the β-galactosyl-sorbitol group, while the lowest in the control group. All the investigated preparations caused a decrease in the activity (per g fresh digesta) of β-glucuronidase in the caecal digesta, especially in the case of xylitol and β-galactosyl-xylitol (0.59 and 0.65, respectively), compared to the control rats (1.00). MONSAN and PAUL (1995) observed that oligosaccharides stimulated bacterial flora to enzyme production, especially glycolytic ones. On the other hand, DJOUZI and ANDRIEUX (1997) showed that neither the change of bacterial population nor the acidity in the caecal pH is sufficient to explain the glycolytic activity variations. In the present investigation, in all experimental groups the activity of potentially harmful β-glucuronidase was lower than in the control group. β-Glucuronidase is involved in alterations of glucuronide metabolites of the carcinogenic food contaminants, as they need to be cleaved by β-glucuronidases before being activated to the ultimate carcinogens (TURESKY et al., 1991).

Compared to the control group, the addition of low-digestible carbohydrates preparations did not increase the total concentration of SCFAs produced in the caecum of rats, moreover the xylitol group was characterized by the lowest total concentration of SCFAs (Table 3). The concentration of acetic acid was the highest in the group receiving β-galactosyl-erythritol preparation (49.25 μmol g^{-1} digesta), and the lowest in rats fed xylitol (27.85). Acetate concentration in the control group was close to that reported for β-galactosyl-erythritol preparation (45.27 and 49.25, respectively). A significant decrease in propionate concentration in the digesta was observed for the control and β-galactosyl-erythritol groups (6.07 and 7.61, respectively), compared to other preparations (11.80–13.06). Feeding inulin caused a substantial increase in the concentration of butyrate (17.29 μmol g^{-1} digesta), compared to the control and other LDC groups (6.52–8.11). The supplementation of diet with inulin caused a significant increase in the SCFAs content produced in the whole caecum of rats (calculated per 100 g BW), compared to the control group and rats fed β-galactosyl-erythritol preparation. The total SCFAs content in the caecal digesta increased from ca. 65 μmol/100 g BW in the control group up to ca. 104 μmol/100 g BW when inulin was

added to the diet. The sucrose and β-galactosyl-erythritol groups were characterized by the lowest total content of propionate (propionate pool), while rats fed inulin demonstrated the greatest butyrate pool.

The short-chain fatty acids (SCFA) are the major products of carbohydrate fermentation in the large bowel both of human and non-ruminant animals. The fermentation of different carbohydrates results in the formation of different proportions of acetate, propionate and butyrate (VAN LOO et al., 1999). For example, the inulin-type fructans typically increased the production of acetate and butyrate, which indicated that populations other than the bifidobacteria also benefit, as bifidobacteria do not produce butyrate (NYMAN, 2002). Some researchers have suggested that values for the caecal SCFA pool appear to be a more accurate reflection of caecal fermentation than SCFA concentrations when feeding with carbohydrates of various fermentabilities (BERGGREN et al., 1993). Possibly related to the increase in the pool of short-chain fatty acids is the effect of some LDC on the intestinal tissue, leading to hyperplasia of the mucosa and increased wall thickness in the caecum (REMESY et al., 1992).

3. Conclusions

In conclusion, compared with sucrose-fed rats, the investigated preparations strongly influenced caecal parameters. All preparations (in a different degree) increased the caecal (tissue and digesta) weight, lowered the pH of caecal digesta and enhanced production of SCFAs. The xylitol preparation was the most effective in lowering of caecal pH. Compared to the control group, only the supplementation of a diet with inulin led to a decrease in ammonia concentration in the caecum. The caecal glycolytic activity was enhanced the most by feeding β-galactosyl-sorbitol and β-galactosyl-xylitol preparations, however all preparations lowered the activity of potentially harmful bacterial β-glucuronidase. Except the mixture containing β-galactosyl-erythritol, all preparations were very effective in promoting propionate production in the caecum, while butyrate production was enhanced only in the case of inulin.

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