The effect of the administration of cellulose and fructans with different degree of polymerization to rats on caecal fermentation and biochemical indicators in the serum

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ABSTRACT: Research was conducted to determine the physiological effect of substituting 5% of maize starch with cellulose, inulin and oligofructose in the diet of rats. The feeding of cellulose and fructans did not influence the food intake and weight gains of animals. The addition of oligofructose and inulin induced an enlargement of the caecum (wall and digesta). Dry matter and pH of caecal digesta were significantly decreased in rats receiving fructan preparations compared to the cellulose and starch groups. Inulin and oligofructose caused a significant increase in the SCFA content produced in the whole caecum (calculated per 100 g BW). Cellulose as well as fructans decreased the ammonia concentration in the caecal digesta compared to control rats. The diet composition did not have a significant influence on the concentration of glucose, triglycerides and total cholesterol in the serum of rats. Compared to the control and cellulose, the investigated fructans were very efficient by inducing beneficial changes in the functioning of the caecal ecosystem.

Keywords: cellulose; inulin; oligofructose; caecum; digesta; serum; rats

Recently, great attention has been paid to non-digestible oligo- and polysaccharides and their effect on the health status of the host (Van Loo et al., 1999). The commercialization of inulin and oligofructose as food ingredients has made researchers focus their investigations on the influence of these substrates on the intestinal ecosystem (Flamm et al., 2001). Inulin is extracted on a commercial basis from chicory roots and the degree of polymerization (DP) of inulin typically ranges from 3 to 60. An inulin extract contains also mono- and disaccharides, mainly sucrose and fructose (Niness, 1999). Native inulin is processed by the food industry to produce short-chain fructans, namely oligofructose (DP 2 to 10; average DP 4), as a result of partial enzymatic (endoinulinase, EC 3.2.1.7) hydrolysis (Van Loo et al., 1995). Due to their β(2-1) linkages, inulin and oligofructose, similarly like all dietary fibres, are resistant to enzymatic hydrolysis in the upper gastrointestinal tract of humans and monogastric animals. Extensive research on *in vitro* and *in vivo* models has shown that both inulin and oligofructose are fermented by Bifidobacteria (Kolida et al., 2002). Bifidobacteria are widely recognized as microorganisms that have potential beneficial effects on their host. These health benefits include stimulation of the immune system, prevention of the growth of pathogenic species, increased mineral absorption, cancer inhibition and improvements in blood lipid metabolism (Delzenne et al., 2002).

Since the physiological action of non-digestible carbohydrates in the large bowel is still poorly known, the purpose of our study was to compare the caecal environmental changes in rats after supplementing their diets either with cellulose, which escapes digestion completely and is only poorly fermented in the caeco-colon, or with two fructans

with different DP (inulin and oligofructose), which should be completely fermented in the caeco-colon. Selected biochemical indicators in the serum of rats were also analysed as the indices of metabolic changes caused by dietary cellulose and fructans.

MATERIAL AND METHODS

The experiment was performed on 40 male Wistar rats kept under a 12-h light: dark cycle, controlled temperature of 21–22°C, relative air humidity of 50–70%, and intensive ventilation of the rooms (15×/h). The experiment was commenced on rats aged ca. 4 weeks and weighing 91.5 ± 3.1 g. The rats were assigned randomly to one of the four groups and had free access to experimental diets and tap water. The composition of experimental diets is presented in Table 1. The diets contained ca. 13.5% total protein, a standard amount of mineral mixture (according to AIN-93G Mineral Mix) and vitamin mixture (according to AIN-93G Vitamin Mix). The diets to be compared were administered for 4 weeks to 10 rats from each group housed individually in wire cages. In the experimental diets, 5% of maize starch was substituted with cellulose (Sigma, C8002) or two types of fructan preparations (inulin and oligofructose). The inulin and oligofructose preparations were obtained from ORAFTI (Frutafit EXL and Frutafit IQ, respectively). The composition of the preparations was determined by the HPLC method on an Animex HPX-87K packed column at 75°C. As a mobile phase 50 mmol/L KH_2PO was used and the eluted saccharides were subject to detection at an RI detector. Chromatographic peaks were identified on the basis of comparison with retention times of a mixture of standards.

The experiment lasted 4 weeks, and individual feed consumption and weight gains of rats were determined. At the termination of the experiment, the rats were anaesthetised using sodium pentobarbitone. After laparotomy, the caecum with digesta was removed and weighed. The caecal pH was measured using a microelectrode and pH/ION meter (model 301, Hanna Instruments). Samples of fresh digesta were used for immediate analysis (dry matter content, ammonia and SCFA concentrations), the rest was transferred to microfuge tubes and stored at –40°C. The caecum wall was flushed clean with ice-cold saline, blotted on filter paper and weighed as the caecum wall weight. Dry matter of the caecal digesta was determined at 105°C. In fresh caecal digesta, ammonia extracted and trapped in a solution of boric acid in Conway's

Table 1. Composition of experimental diets (%)

Ingredient	Group			
	polysaccharides		fructans	
	starch (control)	cellulose	inulin[2]	oligofructose[3]
Casein	14.8	14.8	14.8	14.8
DL-methionine	0.2	0.2	0.2	0.2
Soybean oil	10	10	10	10
Cellulose	–	5	–	–
Inulin (Frutafit EXL)	–	–	5	–
Oligofructose (Frutafit IQ)	–	–	–	5
Mineral mixture[1] (AIN-93G)	3	3	3	3
Vitamin mixture[1] (AIN-93G)	2	2	2	2
Maize starch	70	65	65	65

[1]Reeves (1997)

[2]Frutafit EXL contained 24% monosaccharides, 8% disaccharides, 36% oligosaccharides with DP 3–4, 12% oligosaccharides with DP 5–7 as well as 17% of polymers with DP > 7

[3]Frutafit IQ contained 3.8% monosaccharides, 4% disaccharides, 56.4% oligosaccharides with DP 3–4 as well as 35.8% oligosaccharides with DP 5–7

dishes was determined by direct titration with sulphuric acid (Hofirek and Haas, 2001).

The caecal digesta were analysed for SCFA concentration by gas chromatography (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, column temperature 110°C, detector FID temperature 180°C, injector temperature 195°C). The caecal digesta were weighed (sample of about 0.2 g), mixed with 0.2 ml formic acid, diluted with deionised water and centrifuged at 10 000 × g for 5 min. The supernatant was decanted for injection into the gas chromatograph. The caecal SCFA pool size was calculated as the product of SCFA concentration in digesta and caecal digesta weight.

Glycolytic activity in the caecal digesta was measured by the rate of *p*- or *o*-nitrophenol release from their nitrophenylglucosides according to the modified method of Djouzi and Andrieux described by Juśkiewicz et al. (2002). The following substrates were used: for β-glucuronidase: *p*-nitrophenyl-β-D-glucuronide, for α-galactosidase: *p*-nitrophenyl-α-D-galactopyranoside, for β-galactosidase: *o*-nitrophenyl-β-D-galactopyranoside, for α-glucosidase: *p*-nitrophenyl-α-D-glucopyranoside, and for β-glucosidase: *p*-nitrophenyl-β-D-glucopyranoside. The reaction mixture contained 0.3 ml substrate solution (5 mM) and 0.2 ml of a 1:10 (v/v) dilution of the caecal sample in 100 mM phosphate buffer (pH 7.0) after centrifugation at 10 000 × g for 15 minutes. Incubation was carried out at 37°C and *p*- or *o*-nitrophenol was quantified at 400 nm and at 420 nm, respectively, after the addition of 2.5 ml of 0.25 M cold sodium carbonate. Enzymatic activity (α- and β-glucosidase, α- and β-galactosidase, and β-glucuronidase) was expressed as μmol product formed per min (IU) per g of digesta in the caecal sample.

The results were analysed using one-way ANOVA and significant differences between groups were determined by Duncan's multiple range test. Differences were considered significant at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

The addition of cellulose and fructan preparations did not have a significant influence on the food intake and weight gain of rats (Table 2). Average food intake during 4 weeks ranged from 418.9 g (control diet containing only maize starch) to 424.3 g (diet

Table 2. Body weight and caecal parameters in rats fed experimental diets

	Polysaccharides		Fructans		SEM
	starch[1]	cellulose	inulin	oligofructose	
Initial body weight (g)	91.5	91.6	91.5	91.5	0.63
Final body weight (g)	226.7	226.1	231.34	232.0	1.45
Diet intake (g)	418.9	424.3	420.6	422.6	2.60
Weight gain (g)	135.2	134.5	139.9	141.4	0.83
Whole caecum (g/100 g BW)	0.87^{B}	1.04^{B}	1.61^{A}	1.64^{A}	0.06
Caecal tissue (g/100 g BW)	0.16^{Cb}	0.19^{BCb}	0.38^{Aa}	0.31^{ABa}	0.02
Caecal digesta (g/100 g BW)	0.71^{B}	0.87^{B}	1.23^{A}	1.33^{A}	0.05
Caecal parameters:					
– dry matter (%)	26.77^{ABab}	29.39^{Aa}	26.21^{ABb}	25.01^{Bb}	0.54
– dry matter (g/100 g BW)	0.19^{Bc}	0.26^{ABb}	0.33^{Aa}	0.32^{Aa}	0.03
– pH	6.99^{Aa}	6.66^{ABb}	6.30^{BCc}	6.02^{Cd}	0.08
– ammonia (mg/g)	0.60^{A}	0.37^{B}	0.43^{B}	0.39^{B}	0.02
– ammonia pool (mg/100 g BW)	0.43^{ab}	0.32^{b}	0.53^{a}	0.53^{a}	0.02

a, b, c – Values within each row with the same superscript are not different at $P \leq 0.05$

A, B, C – Values within each row with the same superscript are not different at $P \leq 0.01$

[1]control group

with 5% cellulose), and weight gain from 134.5 g (diet with cellulose) to 141.4 g (diet with oligofructose). Significant differences in the weights of the caecum wall and caecal digesta were observed between groups fed both fructans and groups fed polysaccharides (control – exclusively with maize starch, and the group fed a diet containing 5% cellulose). The rats fed cellulose were characterised by the highest dry matter content in the caecal digesta (29.39%) while the groups receiving a diet with 5% addition of oligofructose and inulin preparations showed the highest hydration of the caecal digesta (dry matter content 25.01 and 26.21%, respectively). The caecal pH was 6.99 in the control diet and decreased to 6.66 ($P \leq 0.05$) in rats fed 5% cellulose. The administration of inulin and oligofructose preparations was accompanied by a significant decrease in the pH value of the caecal digesta (6.30 – $P \leq 0.05$ and 6.02 – $P \leq 0.01$, respectively), compared to the cellulose group. The difference in the caecal pH values between inulin and oligofructose groups was also significant ($P \leq 0.05$). The concentration of ammonia in the caecal digesta was the highest in the control group (0.60 mg/g), and significant differences ($P \leq 0.01$) were reported between the control and all experimental groups (0.37–0.43). The total ammonia pool (expressed as mg/100 g BW) was the lowest in the rats fed cellulose (0.32) and the highest in groups receiving fructans (0.53).

Table 3 shows the activities of particular glycolytic enzymes in the caecal digesta. Similar activities of α-galactosidase and α-glucosidase were observed in all groups. The control group, receiving exclusively maize starch as a dietary polysaccharide, was characterised by the highest activity of β-galactosidase and the lowest activity of β-glucosidase, compared to the other groups. The lowest activity of β-glucuronidase in the digesta was reported in the rats fed a diet containing cellulose and the highest in the inulin group. The rats fed the inulin preparation also had the highest β-glucosidase activity in the caecum. The oligofructose preparation caused the lowest activity of β-galactosidase, compared to the other groups.

Table 4 presents concentrations and total pool of short-chain fatty acids (SCFA) in the caecal digesta of rats. Compared to the control group, the addition of cellulose and fructan preparations did not increase the concentration of total SCFAs in the caecum digesta. On the other hand, in the rats fed the control diet the concentrations of isobutyrate, isovalerate and valerate were significantly higher ($P \leq 0.01$) than those reported in the other groups. In all substrates, however, it was found that acetic, propionic and butyric acids together accounted for more than 90% of SCFAs in the digesta, with isobutyric, isovaleric and valeric acids being present in relatively small amounts. The substitution of a part of maize starch with cellulose increased ($P \leq 0.05$) the content of acetate in 1 g of the caecal digesta, compared to the inulin group, while the control and oligofructose groups were intermediate between them. The propionate concentration was the highest in the rats fed oligofructose and differed significantly ($P \leq 0.05$) compared to the cellulose group. The addition of inulin and oligofructose increased ($P \leq 0.01$) the butyrate content in 1 g of the caecal digesta compared to the control group. Both fructan preparations in the diet caused a significant ($P \leq 0.05$ in the case of the cellulose group, and $P \leq 0.01$ in the case of the control group) increase in

Table 3. Glycolytic activity (U/g fresh caecal content) in the caecal digesta of rats fed experimental diets

Enzyme	Polysaccharides		Fructans		SEM
	starch[1]	cellulose	inulin	oligofructose	
α-galactosidase	1.48	1.16	1.32	1.14	0.07
β-galactosidase	6.51^{Aa}	3.55^{BCc}	5.45^{ABb}	3.40^{Cc}	0.34
α-glucosidase	2.20	2.09	2.10	2.32	0.11
β-glucosidase	0.30^{Bb}	0.55^{ABab}	0.72^{Aa}	0.66^{ABa}	0.05
β-glucuronidase	1.61^{ABa}	1.05^{Bb}	1.81^{Aa}	1.48^{ABab}	0.10

a, b, c – Values within each row with the same superscript are not different at $P \leq 0.05$

A, B, C – Values within each row with the same superscript are not different at $P \leq 0.01$

[1]control group

Table 4. Concentration (μmol/g fresh digesta) and total pool[2] of SCFA (μmol/ 100 g BW) in the caecum

Concentration	Polysaccharides		Fructans		SEM
	starch[1]	cellulose	inulin	oligofructose	
Total SCFA (μmol/g)	109.0	118.3	106.6	117.4	4.04
– Acetate	72.8^{ab}	80.5^{a}	61.9^{b}	71.0^{ab}	2.64
– Propionate	20.0^{ab}	19.8^{b}	23.2^{ab}	26.3^{a}	0.88
– Isobutyrate	2.4^{Aa}	1.7^{Bb}	1.4^{BCc}	1.1^{Cc}	0.07
– Butyrate	7.0^{B}	11.6^{AB}	16.5^{A}	15.8^{A}	0.81
– Isovalerate	3.6^{Aa}	2.2^{Bb}	1.7^{Bbc}	1.4^{Bc}	0.13
– Valerate	3.2^{Aa}	2.5^{Bb}	1.9^{BCc}	1.7^{Cc}	0.10
Profile C_2:C_3:C_4	55:25:20	50:21:29	41:29:30	42:31:27	–
Total pool[2]	77.39^{Cc}	102.92^{BCb}	156.14^{Aa}	131.12^{ABa}	4.22

[1]control group

[2]caecal SCFA pool was calculated as SCFA concentration × caecal digesta/100 g BW

a, b, c – Values within each row with the same superscript are not different at $P \leq 0.05$

A, B, C – Values within each row with the same superscript are not different at $P \leq 0.01$

the SCFA content produced in the whole caecum of rats (calculated per 100 g BW). The total SCFA content in the caecal digesta increased from 77.4 in the control group and 102.9 μmol/100 g BW in the cellulose group up to ca. 131 μmol/100 g BW in the group fed the oligofructose preparation, and up to ca. 156 μmol/100 g BW when a part of maize starch was substituted with inulin.

The diet composition had no significant influence on the concentration of glucose, triglycerides and total cholesterol or on the activity of alanine aminotransferase and aspartate aminotransferase in the serum of rats (Table 5). The inulin preparation (containing also monosaccharides) insignificantly increased glucose concentration and insignificantly decreased triglyceride concentration in the serum, compared to the concentrations observed in the other groups.

DISCUSSION

In the present study, substituting a part of maize starch with cellulose or fructan preparations (inulin and oligofructose) did not differentiate the food intake and weigh gains of rats. These results are in agreement with the previous studies of Campbell et al. (1997). Ohta et al. (1998) also observed that

Table 5. Glucose, triglyceride and cholesterol content and the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of rats fed experimental diets

	Polysaccharides		Fructans		SEM
	starch[1]	cellulose	inulin	oligofructose	
Glucose (mg/l)	1 952	1 894	2 140	1 869	77.3
Triglycerides (mg/l)	2 024	2 136	1 777	1 918	109
Cholesterol (mg/l)	946	947	974	962	27.1
ALT (U/l)	19.3	19.4	17.6	18.5	0.55
AST (U/l)	143.8	150.6	146.4	150.9	3.05

a, b, c – Values within each row with the same superscript are not different at $P \leq 0.05$

A, B, C – Values within each row with the same superscript are not different at $P \leq 0.01$

[1]control group

the supplementation of a diet with 5 or 10% FOS of different sugar-chain lengths did not have any effects on diet intake and weight gains in rats. On the other hand, a compensatory increase in the intake of a diet when its nutritive value was decreased through the addition of dietary fibre was reported in the study of Lopez-Guisa et al. (1988). Our results suggested that neither cellulose nor fructan preparations lowered the nutritive value of a diet.

The content of both fructan preparations increased the weight of the caecal wall and digesta. In the case of inulin, it was consistent with the results reported by Klessen et al. (2001). In their study, the 5% addition of inulin into a diet caused a considerable increase in the weight of caecal digesta in rats. This increase in the caecal content weight was likely caused by the increased bacterial proliferation and by the capacity of those carbohydrates to hold water in the caecum. This is in agreement with the presented investigation as inulin and oligofructose decreased dry matter concentration in the digesta, compared to cellulose group. In other studies, it was observed that the distal part of the gastrointestinal tract tissue adapted to a diet containing fermentable polysaccharides by increasing the tissue weight (Johnson and Gee, 1986). The structural arrangement and the degree of polymerization of the carbohydrates may be important for the degree of fermentation (Nyman, 2002). In our experiment, in the case of the cellulose diet, the caecum weight changed very little compared to the control rats. The results obtained by Brunsgaard et al. (1995) suggested that the highly fermentable carbohydrates could induce rapid hypertrophy of the proximal part of the large intestine, i.e. in the caecum, while in the case of less fermentable polysaccharides (such as resistant starch), the hypertrophy would be located more distally, i.e. in the colon. Cellulose, as a microbially inert polysaccharide, has no effect on the caecum and low or no effect on the colon in rats (Brunsgaard et al., 1995).

Like in the studies of numerous authors (Djouzi and Andrieux, 1997; Kleessen et al., 2001), the addition of the investigated fructan preparation was accompanied by a considerable decrease in pH of the caecal digesta, especially in the case of oligofructose. In all experimental groups supplemented with cellulose and fructans the lower ammonia concentration was found in the caecal digesta, compared to the control group. Ammonia is potentially harmful for the host as this substance can alter the morphology and intermediate metabolism of epithelial cells (Visek, 1978). Ammonia concentration in the gut lumen can be reduced by active carbohydrate fermentation which stimulates the bacterial requirement for nitrogen due to an increased growth (Cummings and MacFarlane, 1991). In the present study, the increased ammonia amount in the whole caecal digesta of rats (expressed as ammonia pool, mg/100 g BW) receiving the investigated fructan preparations resulted from the accumulation of digesta in the caecum.

In general, the supplementation of diet with cellulose or fructan preparations did not markedly change the glycolytic potential in the caecal digesta. In the rats fed the control diet, an odd increase in β-galactosidase activity was observed, compared to the other groups, especially to the oligofructose diet. It was contrary to the assumption of Monsan and Paul (1995), who suggested that a small addition of oligosaccharides to the diet was enough to stimulate the intestinal bacterial flora to produce enzymes, especially glycolytic ones. The results obtained by Djouzi and Andrieux (1997) showed that neither the change of bacterial population nor the decrease in the caecal pH were sufficient to explain the glycolytic activity variations. These authors observed that the glycolytic activities could be modified in a beneficial way without any change in the *Bifidobacterium* level. In the presented investigation, the activity of potentially harmful β-glucuronidase (Reddy et al., 1992), the enzyme involved in the generation of toxic and carcinogenic metabolites in the hindgut, was the lowest in the control group. The addition of inulin and oligofructose to the diet did not lower the activity of β-glucuronidase in the caecal digesta. Some results also showed that the caecal β-glucuronidase activity remained unaffected (Kleessen et al., 1996) or was slightly higher (Juśkiewicz et al., 2003) after consumption of a diet containing non-digestible oligosaccharides.

Inulin and oligofructose, appearing to be highly fermented both *in vitro* and *in vivo*, yield high levels of short-chain fatty acids (Nyman, 2002). In the present study, the concentration of total SCFAs in all groups was similar, but the total SCFA pool, which was calculated as SCFA concentration and caecal digesta per 100 g BW, was significantly increased by the fructan preparations added. Some researches have suggested that the values for the caecal SCFA pool appear to be a more accurate reflection of caecal fermentation than SCFA concentrations when feeding oligosaccharides of various

fermentabilities (Berggren et al., 1993; Campbell et al., 1997). In our study, the addition of inulin and oligofructose increased the butyrate content in 1 g of the caecal digesta, compared to the control group. Moreover, the propionate concentration was the highest in the rats fed oligofructose and differed significantly when compared to the cellulose group. Berggren et al. (1993) reported that oligofructose promoted a high production of butyric acid in rats while Campbell et al. (1997) observed that oligofructose at a level of 6% gave a higher caecal concentration of butyrate compared with the same amount of xylo-oligosaccharides. A higher caecal concentration of butyrate with oligofructose was also obtained by Poulsen et al. (2002) when compared with the rats fed inulin. On the other hand, Levrat et al. (1991) found that in rats receiving inulin the proportion of formed propionic acid was very high compared with studies on oligofructose, and it may be speculated that it was caused by the higher molecular weight of inulin. Butyric acid appears to be essential in the maintenance of the healthy caecum and colon, it is the preferred energy substrate for the mucosa cells and has been suggested to protect against hindgut diseases, e.g. ulcerative colitis and cancer (Nyman, 2002; Scheppach et al., 1992). Propionic acid is increasingly connected with beneficial effects on carbohydrate and lipid metabolism (Nyman, 2002). The proportion of propionic and acetic acid appears to affect glucose and lipid metabolism beneficially and the higher the amount of propionate the more pronounced the effects (Wolever et al., 1991).

In the present study, the absence of significant differences between groups was observed in the biochemical parameters in the serum of rats. Merely, the inulin addition insignificantly increased glucose concentration and insignificantly decreased triglyceride concentration in the serum, compared to the other groups. The inulin preparation used in our study contained a large amount of monosaccharides (24%) as well as disaccharides (8%), and this is probably the reason for an increasing level of glucose in the serum. Fiordaliso et al. (1995) observed that feeding a diet supplemented with oligofructose (10% in the diet) to male Wistar rats significantly lowered serum triacyloglycerol and phospholipid concentrations. Some studies (Delzenne et al., 2002) reported that oligofructose was able to positively modulate triglyceride metabolism disorders caused by dietary manipulation in animals: oligofructose reduced post-prandial triglyceridaemia by 50% and prevented an increase in the serum free cholesterol level occurring in rats fed a Western-type high fat diet.

In conclusion, compared with the control diet and with cellulose, fructan preparations (inulin and oligofructose) strongly influenced the caecal ecosystem in a beneficial way. Both preparations decreased the caecal pH and ammonia concentration (oligofructose > inulin) compared to the control group. The addition of cellulose was less efficient to decrease caecal pH, but it significantly decreased the ammonia concentration in the caecal digesta compared to control rats. Inulin and oligofructose increased the butyrate concentration in the digesta as well as the total SCFA content in the whole caecum of rats compared to the control and cellulose groups.

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