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Effects of inulin supplemented to cellulose-free or cellulose-rich diets on caecal environment and biochemical blood parameters in rats

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ABSTRACT

The effects of feeding soluble dietary fibre (inulin, I) separately or in combination with insoluble fibre (cellulose, CEL) on caecal fermentation and biochemical blood parameters were studied in rats. Four diets: CEL-rich (10%) and CEL-free, each supplemented with 5% of sucrose or I (CR, CF, CRI and CFI diets, respectively) were fed over 4 weeks, each to 10 male growing Wistar rats. Supplementation with I caused the increase of the mass of caecal tissue and digesta (only on CF diet, interaction at $P<0.01$ and $P<0.05$, respectively), modification of short-chain fatty acids profile, decrease of caecal pH and ammonia concentration, increase of *Bifidobacteria* and depression of *E. coli* number, and decrease of β-glucuronidase activity. Feeding CEL reduced ammonia and total SCFA concentration and activity of all enzymes, particularly β-galactosidase, did not affect microbial population but slightly decreased number of anaerobic saccharolytic and proteolytic bacteria spores. Interactive effects of I and CEL were found for the concentration of branched-chain fatty acids which was more depressed by I on CF than on CR diet. Dietary treatments did not affect blood parameters.

KEY WORDS: cellulose, inulin, fermentation, microflora, caecum, rat

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INTRODUCTION

Although numerous reports have addressed the physiological response of the organism to ingestion of soluble and insoluble dietary fibre, this subject still constitutes a challenge for investigators (Topping, 2007). An important task of research is to clarify their mechanism of action, separately and in complex combination. The soluble type fibres, e.g., fructan-type, are generally present at high levels in vegetables and fruit, while whole grains are a major source for insoluble fibres, as exemplified by cellulose (Mussatto and Mancilha, 2007). The main area of dietary fibre action in the gastrointestinal tract is the large intestine (Tungland and Meyer, 2002). It has been reported that both types of fibre play an important role in decreasing the incidence of colon cancer, however, influence different parameters (Moore et al., 1998). It has been demonstrated that caecal metabolism in rats is affected by the dose, time and mode of soluble inulin administration (Juśkiewicz et al., 2005, 2007). Moreover, other biologically active substances like polyphenols or different dietary components may have an influence on the physiological effect of feeding inulin (Wróblewska et al., 2006; Zduńczyk et al., 2006). Some previous reports claimed that cellulose is relatively inert in the rat large intestine and does not significantly influence microbial proliferation (Reshef et al., 1990; Johansen and Bach Knudsen, 1994). On the other hand, recent studies have shown that insoluble dietary fibres such as cellulose are not completely inert and their breakdown in the lower gastrointestinal tract should be taken into account in the interpretation of nutritional experimental data (Takahashi et al., 2005; Jurgoński et al., 2008).

In the present investigation, the following hypothesis was advanced: the presence of insoluble cellulose in a diet may modify the effects of fermentable inulin. Caecum is the main site of bacterial fermentation in rats, therefore the indices of microflora metabolism including enzymatic activity, populations of bacteria and SCFA concentration, were determined in caecal digesta. Moreover, blood parameters related to lipid, carbohydrates and protein metabolism, were analysed.

MATERIAL AND METHODS

Diets

Four experimental diets were used: a cellulose-rich diet containing 10% of α-cellulose and cellulose-free diet were supplemented with 0 or 5% of inulin at the expense of sucrose (CR, CF, CRI and CFI groups, respectively) (Table 1).

Table 1. Composition of the diets fed to rats, %.

Ingredient	Cellulose-free diet		Cellulose-rich diet	
	sucrose	inulin	sucrose	inulin
Casein	11.5	11.5	11.5	11.5
Soyabean isolate	11.5	11.5	11.5	11.5
DL-methionine	0.2	0.2	0.2	0.2
Sucrose	5	-	5	-
Inulin[1]	-	5	-	5
Cellulose	-	-	10	10
Soyabean oil	7	7	7	7
Lard	7	7	7	7
Cholesterol	1	1	1	1
Mineral mix[2]	3.5	3.5	3.5	3.5
Vitamin mix[3]	2	2	2	2
Maize starch	51.3	51.3	41.3	41.3

[1] Frutafit-Tex (SENSUS, The Netherlands)

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

[3] AIN-93G (Reeves, 1997), per kg mix, g: nicotinic acid 3.0, Ca pantothenate 1.6, pyridoxine-HCl 0.7, thiamin-HCl 0.6, powdered sucrose 974.655, riboflavin 0.6, folic acid 0.2, biotin 0.02, vit. B_{12} (cyanocobalamin, 0.1% in mannitol) 2.5; IU, g: vit. E (all-rac-α-tocopheryl acetate, 500) 15.0, vit. A (all-trans-retinyl palmitate, 500000) 0.8, vit. D_3 (cholecalciferol, 400000) 0.25, vit. K-1 (phylloquinone) 0.075

As a source of inulin (I), a commercial preparation Frutafit-Tex purchased from Sensus (Roosendaal, The Netherlands) was used. Frutafit-Tex is a product containing 98% of long-chain inulin with higher degree of polymerization (DG>10). A preparation of pure α-cellulose (CEL) (Sigma Aldrich, Poznan, Poland) was used as a source of a dietary insoluble fibre. The chemical composition of inulin preparation was confirmed with the aid of HPLC method using Knauer (Berlin, Germany) chromatograph with RI detector and Animex HPX 87C (300 × 7.8 mm) column (Bio-Rad, Hercules, CA, USA) at 85°C (Król and Grzelak, 2006).

Experimental procedures

The experiment was conducted on forty male Wistar rats, with average initial body weight of 81.4±0.5 g, divided into four groups of ten rats each, and fed over

four weeks with experimental diets.

Animals were housed in individual cages under the standard conditions: temperature 21-22ºC, relative humidity 50-70%, intensive room ventilation (15x/h), 12 h light/dark cycle, permanent access to feed and water.

The experiment was conducted according to the recommendations of the Local Committee for Animal Experiments in Olsztyn (Poland).

At the end of the experiment animals were weighed and anaesthetized with sodium pentobarbitone (14 mg/100 g of body mass). Blood samples were taken from the superior *vena cava* and the caecum with contents was taken out and weighed. Blood was allowed to clot during 1.5 h at 37ºC, supernatant was collected and centrifuged at 2500 rpm, 10 min, 4ºC.

Measurements

The pH of the caecal content was measured using electrode and a pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal). Dry matter of digesta was determined at 105ºC. In fresh caecal digesta, ammonia was determined in Conway dishes, as described by Hofirek and Haas (2001). The intestine was rinsed with cold physiological saline, dried and weighed.

The concentration of short-chain fatty acids (SCFA) was analysed by gas chromatography: Shimadzu GC-14A (Shimadzu Co., Kyoto, Japan) with a glass column 2.6 mm × 2.5 m, containing 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb W AW (Supelco Co., Bellefonte, PA, USA), column temperature 110°C, detector FID temperature 180°C, injector temperature 195°C. Sample of caecal digesta was mixed with 0.2 ml of formic acid, diluted with deionized water and centrifuged at 10000 rpm for 5 min. Supernatant was injected into the gas chromatograph. Caecal SCFA pool was calculated as the product of SCFA concentration and digesta weight.

Activity of bacterial enzymes in the caecal digesta was measured as the rate of release of *p*- or *o*-nitrophenol from their nitrophenylglucosides according to the method described by Juśkiewicz et al. (2006). The following substrates (Sigma Chemical Co., St. Louis, MO, USA) 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. Enzymatic activity was expressed as μmol of product formed per g of digesta per h.

Impact of experimental factors on the caecal microecosystem was assessed by measuring the number of bacteria in several populations of probiotic and potentially pathogenic microorganisms. The populations of bacteria were

enumerated after their cultivation on selective culture media and/or in specific conditions of incubation. *Bifidobacterium* counts were determined on Garche's agar medium after incubation at 37°C for 48 h under anaerobic conditions (anaerostat + AnaeroGen, Oxoid) (Teraguchi et al., 1982). Lactic acid bacteria was enumerated on MRS medium (BTL, Poland) after cultivation at 37°C for 72 h under partially anaerobic conditions. Quantity of *E. coli* was evaluated after 24 h incubation at 37°C on Mc Conkey-agar medium (Merck) in an oxidative atmosphere. Saccharolytic spores were counted after growth onto SPC medium (Harrigan and Mc Cance, 1976) in anaerobic conditions for 7 days at 37°C, while proteolytic spores were enumerated in broth-gelatin medium, after 5 days of incubation at 22-25°C under anaerobic atmosphere (Bielecka et al., 2002).

Blood levels of glucose, total cholesterol, HDL cholesterol, triglycerides, urea, magnesium, calcium, phosphorous and activities of alanine and asparagine aminotransferases were assessed using Alpha Diagnostics sets (Warsaw, Poland).

Statistical analysis

The STATISTICA software package version 6.0 (StatSoft Corp., Krakow, Poland) was used. Two-way ANOVA was performed to assess the effects of inulin, cellulose and interaction. Means were evaluated using Duncan's multiple range test. Differences were considered to be significant at $P \leq 0.05$. Results are presented as mean values with their standard errors.

RESULTS

Body weight, caecal parameters. Diet intake (not shown) and final body weight of rats did not differ among the groups (Table 2). Feeding I increased relative mass of caecal tissue and caecal digesta while CEL did not affect these parameters (Table 2). However, for both parameters significant interactions of CEL and I were found: the effects of I were greater and statistically significant only in rats fed on diets not supplemented with CEL; on the other hand, supplementation with CEL did not affect tissue and digesta mass in rats fed on diets without inulin while it significantly depressed mass of caecal tissue (and tended to depress digesta mass) in rats fed on diets supplemented with I. Dry matter content of caecal digesta was not affected by I while it was significantly increased by CEL irrespectively of the presence of I.

Table 2. Body weight and caecal parameters of rats

Item	Cellulose-free diet		Cellulose-rich diet		ANOVA (P value)		
	sucrose	inulin	sucrose	inulin	cellulose (CEL)	inulin (I)	CEL × I
Body weight, g	255.6 ± 2.45	256.6 ± 2.65	257.3 ± 2.21	258.5 ± 2.25	0.709	0.821	0.983
Caecum							
tissue mass[1]	0.246 ± 0.03^{c}	0.442 ± 0.02^{a}	0.297 ± 0.02^{bc}	0.327 ± 0.03^{b}	0.251	<0.001	<0.01
digesta mass[1]	0.711 ± 0.05^{b}	1.239 ± 0.06^{a}	0.963 ± 0.06^{ab}	1.056 ± 0.06^{a}	0.747	<0.01	<0.05
dry matter, %	20.5 ± 0.80	22.0 ± 0.79	28.0 ± 0.77	27.4 ± 0.85	<0.001	0.627	0.310
ammonia, mg/100 g	64.1 ± 1.85	44.2 ± 2.22	45.7 ± 2.42	40.0 ± 2.11	<0.01	<0.01	0.065
pH of digesta	6.97 ± 0.05	6.58 ± 0.02	7.01 ± 0.04	6.65 ± 0.04	0.266	<0.001	0.752
µmol/h/g digesta							
α-glucosidase	18.12 ± 1.08	19.20 ± 0.84	13.68 ± 0.96	13.2 ± 0.84	<0.01	0.872	0.657
β-glucosidase	6.60 ± 0.48	4.80 ± 0.36	4.20 ± 0.24	4.20 ± 0.24	<0.05	0.126	0.162
α-galactosidase	13.92 ± 0.60	10.92 ± 0.84	10.8 ± 0.72	7.08 ± 0.84	<0.05	<0.05	0.808
β-galactosidase	66.84 ± 5.28	60.72 ± 4.80	39.48 ± 4.68	21.96 ± 2.64	<0.001	0.109	0.438
β-glucuronidase	25.92 ± 2.52	9.72 ± 1.32	13.56 ± 1.80	8.28 ± 1.08	<0.05	<0.001	0.063

[1] g/100 g body weight; ^{a,b,c} - values within each row with the same superscript letter are not different at P≤0.05; SEM - standard error of the mean (SD for all animals divided by the square root of rat number; n=40)

Ammonia concentration was the highest on the unsupplemented control diet (64.1 mg/100 g) and was decreased to similar levels by I and CEL given as single supplements and slightly more when given in combination (44.2, 45.7 and 40.0 mg/100 g, respectively). pH of digesta was decreased only by I and this effect did not depend on the presence of CEL.

Enzymatic activity of caecal microflora. Supplementation of experimental diets with CEL resulted in lower activity of all microbial enzymes while I reduced only α-galactosidase and β-glucuronidase activities (Table 2). The effect of CEL was the greatest on the activity of β-galactosidase (decrease from 66.84 to 39.48 and from 60.72 to 21.96 micromol/h/g of digesta) whereas I affected more strongly β-glucuronidase than β-glucosidase. The interactive effects of both supplements were not confirmed statistically ($P>0.05$).

Short-chain fatty acids. Supplementation with CEL depressed concentration of total and single SCFA except acetate. I did not influence total SCFA and acetate, increased propionate and butyrate and decreased isobutyrate, isovalerate and valerate concentrations (Table 3). The depressive effect of I on the concentration of branched-chain fatty acids was greater on CEL-free than on CEL supplemented diet (significant CEL × I interaction).

The SCFA profile was affected by both supplements. CEL slightly increased while I considerably depressed proportion of acetate in total SCFA (from 71 to 62% and from 74 to 66% on CEL free and CEL supplemented diets, respectively). Proportion of propionate was not affected by CEL while it was increased by I. I had the greatest effect on the proportions of butyric acid which were increased from 11 to 21% on CEL free and from 11 to 18% on CEL supplemented diets. Significant interactive effects of CEL and I on SCFA profile were not found.

CEL did not influence the SCFA pool (μmol/100 g BW) while I increased pool of total and single SCFA except branched-chain fatty acids.

Caecal microflora. Inulin supplementation contributed to the increase of *Bifidobacterium* population but did not affect lactic acid bacteria, and had depressive effect on *E. coli* (Table 4). The number of anaerobes producing saccharolytic and proteolytic spores was higher on diet supplemented with CEL and was not affected by I.

Blood parameters. Only serum urea concentration was significantly greater on CEL supplemented diets (Table 5).

Table 3. Caecal short-chain fatty acids (SCFA) concentration, profile and pool

Item	Cellulose-free diet		Cellulose-rich diet		ANOVA (P value)		
	sucrose	inulin	sucrose	inulin	cellulose (CEL)	inulin (I)	CEL × I
Concentration, µmol/g							
total SCFA	50.99 ± 1.36	49.45 ± 1.01	43.03 ± 1.22	47.92 ± 1.45	<0.05	0.475	0.175
acetate	36.31 ± 1.02	30.67 ± 0.77	31.72 ± 0.93	31.87 ± 0.88	0.350	0.136	0.116
propionate	4.34 ± 0.09	5.67 ± 0.12	3.59 ± 0.16	4.81 ± 0.25	<0.001	<0.001	0.819
isobutyrate	1.25 ± 0.09[a]	0.61 ± 0.05[c]	0.84 ± 0.05[b]	0.70 ± 0.02[c]	<0.05	<0.001	<0.001
butyrate	5.78 ± 0.32	10.57 ± 0.98	4.59 ± 0.50	8.59 ± 0.77	<0.05	<0.001	0.496
isovalerate	1.54 ± 0.04[a]	0.80 ± 0.05[b]	1.02 ± 0.06[b]	0.83 ± 0.04[b]	<0.01	<0.001	<0.001
valerate	1.75 ± 0.08[a]	1.14 ± 0.04[b]	1.27 ± 0.05[b]	1.13 ± 0.06[b]	<0.05	<0.001	<0.05
Proportion C_2, % of total	71 ± 0.85	62 ± 0.79	74 ± 0.94	66 ± 0.58	<0.01	<0.001	0.315
C_3, % of total	9 ± 0.39	12 ± 0.52	8 ± 0.37	10 ± 0.41	0.153	<0.001	0.247
C_4, % of total	11 ± 1.01	21 ± 1.22	11 ± 0.87	18 ± 0.96	<0.05	<0.001	0.070
Pool, µmol/100 g BW							
total SCFA	36.01 ± 3.05	61.66 ± 4.22	41.19 ± 3.24	50.86 ± 3.45	0.627	<0.01	0.174
acetate	25.50 ± 1.25	38.22 ± 2.11	30.21 ± 1.99	33.95 ± 1.85	0.954	<0.001	0.247
propionate	3.11 ± 0.38	6.98 ± 0.51	3.43 ± 0.38	5.06 ± 0.45	0.151	<0.001	0.059
isobutyrate	0.81 ± 0.05	0.74 ± 0.06	0.91 ± 0.04	0.74 ± 0.05	0.617	0.229	0.638
butyrate	4.53 ± 0.91	9.05 ± 1.05	4.13 ± 0.66	13.36 ± 1.11	0.137	<0.001	0.076
isovalerate	0.98 ± 0.06	0.88 ± 0.03	1.11 ± 0.05	0.96 ± 0.04	0.370	0.298	0.879
valerate	1.22 ± 0.07	1.18 ± 0.07	1.25 ± 0.06	1.40 ± 0.05	0.402	0.726	0.528

[a,b,c] - values within each row with the same superscript letter are not different at P≤0.05; SEM – standard error of the mean (SD for all animals divided by the square root of rat number; n=40)

Table 4. Caecal microflora, log cfu/g digesta

Item	*Cellulose-free diet*		*Cellulose-rich diet*		*ANOVA (P value)*		
	sucrose	inulin	sucrose	inulin	cellulose (CEL)	inulin (I)	CEL × I
Bifidobacterium	9.33 ± 0.07	10.30 ± 0.09	9.14 ± 0.07	10.35 ± 0.08	0.215	<0.05	0.758
Lactic acid bacteria	9.86 ± 0.10	9.35 ± 0.09	9.43 ± 0.07	9.20 ± 0.07	0.121	0.658	0.485
E. coli	7.83 ± 0.14	6.94 ± 0.11	8.30 ± 0.19	6.14 ± 0.18	0.097	<0.001	0.524
Spores of anaerobic saccharolytic bacteria	1.75 ± 0.06	1.77 ± 0.05	1.31 ± 0.05	1.12 ± 0.04	<0.05	0.658	0.845
Spores of anaerobic proteolytic bacteria	1.29 ± 0.09	1.32 ± 0.14	1.19 ± 0.12	<1.0	< 0.05	0.078	0.254

SEM - standard error of the mean (SD for all animals divided by the square root of rat number; n=40)

Table 5. Biochemical serum parameters

Item	Cellulose-free diet		Cellulose-rich diet		ANOVA (P value)		
	sucrose	inulin	sucrose	inulin	cellulose (CEL)	inulin (I)	CEL × I
mmol/l, glucose	14.72 ± 0.37	14.15 ± 0.40	15.40 ± 0.48	13.66 ± 0.51	0.927	0.253	0.561
cholesterol	4.11 ± 0.08	3.89 ± 0.10	4.03 ± 0.08	3.87 ± 0.07	0.792	0.319	0.874
HDL	0.87 ± 0.01	0.92 ± 0.02	0.87 ± 0.02	0.87 ± 0.02	0.551	0.496	0.620
triglycerides	2.28 ± 0.13	2.05 ± 0.11	2.30 ± 0.12	2.23 ± 0.09	0.693	0.548	0.761
urea	14.78 ± 0.44	13.96 ± 0.67	18.38 ± 0.55	16.99 ± 0.51	<0.01	0.261	0.763
Mg	1.36 ± 0.02	1.38 ± 0.04	1.37 ± 0.03	1.34 ± 0.03	0.806	0.949	0.733
Ca	2.37 ± 0.03	2.43 ± 0.02	2.26 ± 0.02	2.37 ± 0.02	0.053	0.086	0.617
P	2.64 ± 0.07	2.70 ± 0.06	2.61 ± 0.08	2.61 ± 0.10	0.721	0.864	0.894
ALT, U/l	27.9 ± 1.1	34.4 ± 1.3	33.2 ± 1.1	32.3 ± 1.2	0.444	0.200	0.084
AST, U/l	120 ± 5.1	125 ± 3.9	128 ± 4.8	126 ± 4.2	0.677	0.847	0.714

SEM - standard error of the mean (SD for all animals divided by the square root of rat number; n=40)

DISCUSSION

The results of our experiment showing that neither cellulose (CEL) nor inulin (I) supplementation significantly affected final body weight of rats are in agreement with the observations reported in the earlier study, where diets were supplemented with 2.5, 5 and 10% of cellulose or fructooligosaccharides (FOS) (Jurgoński et al., 2008).

Our results demonstrate that the response of the caecum to inulin may be to some extent affected by the coingestion of cellulose. CEL itself did not but inulin significantly enhanced the relative mass of caecal tissue or digesta, and the trophic effect of inulin on caecal tissue was lower on the CEL-rich treatment (significant interaction between I and CEL). The observed differences could probably be explained by a lower caecal SCFA concentration on the CEL diet. Purified cellulose has been considered to be a diet's diluent, whereas the other complex carbohydrates are more likely to be extensively degraded by the caecal microflora into SCFA (Juśkiewicz et al., 2005). All SCFA are claimed to act as trophic agents on caecal/colonic epithelium, but butyric seems to be the most and propionic the least efficient (Berggren et al., 1993). Another possible way of increased epithelium proliferation was indicated in a study by Whiteley et al. (1996). These authors found that mucosal volume was correlated with digesta mass rather than with SCFA levels, concluding that physical stretching of the caecal/colonic epithelium might be a more important causal factor. In our study the caecal tissue mass was well correlated with digesta mass so it may be assumed that both ways, *via* SCFA and increased bulk of digesta, might be involved in differentiated caecal tissue mass.

Recent results showed that inulin and cellulose as single supplements impair the formation of caecal ammonia. An additional reduction in the level of this toxic compound was observed on combined ingestion of I and CEL preparations ($P=0.065$, for interaction CEL × I). Ammonia is considered to have deleterious influence on the intestinal mucosa, including stasis of microcirculation, disruption of the surface epithelial cells and necrosis of the mucosa (Hambly et al., 1997). The mechanism of reducing ammonia concentration in the caecal contents by dietary inulin and cellulose may be different. The fermentation of prebiotic inulin affects nitrogen metabolism in the large intestine by stimulating the bacterial requirement for nitrogen due to an enhanced growth. One of the sources of N is ammonia derived from blood urea (Kim et al., 1998). This process may lead to the lowering of blood urea, and sometimes cause a temporary rise in intracaecal ammonia concentration (Juśkiewicz et al., 2008). The latter effect, occasionally recorded while feeding with inulin (Juśkiewicz et al., 2007), was not observed in the present experiment. One of possible ways of a decrease of caecal ammonia

concentration by cellulose is the dilution of microbial population by this dietary supplementation (Kim et al., 1998).

Inulin stimulated proliferation of beneficial *Bifidobacterium* and inhibited growth of potentially pathogenic bacteria (*E. coli*), both on high- and low-cellulose diets. This was in accordance with acidification of digesta of inulin fed rats. However, experimental diets did not influence lactic acid bacteria population in the caecum. Some investigators have even reported that while enhancing *Bifidobacterium* growth, at the same time dietary fructans may reduce *Lactobacilli* number (Rao, 1999). In present experiment an increased population of anaerobic spores in rats fed cellulose-free diet, regardless inulin addition, was observed. A variety of factors such as accessibility to nutrients, pH, media composition, ionic strength and aeration, are known to affect sporulation. We conclude that a lack of structural fibre in a diet may result in somewhat depletion of nutrients for microflora inhabiting the lower GIT, thus enhanced sporulation in bacteria. The ability to produce spores is of ecological advantage to the bacteria as it enables it to survive under adverse conditions (Bielecka et al., 2002).

In the present study, when rats were fed CEL-free diet, bacterial enzymes activities were surprisingly elevated, possibly due to the higher hydration of caecal contents. Dietary inulin significantly reduced activities of α-galactosidase and β-glucuronidase. Decreased level of the latter enzyme is of particular importance, since its activity has been associated with production of toxic substances in the intestine thus supporting neoplasia development (Goni et al., 2005).

In the present experiment, inulin, irrespectively of cellulose addition, considerably increased the total SCFAs pool, due to the increase of three main acids: acetic, propionic and butyric. Many studies on diets enriched in non-digestible saccharides have demonstrated that SCFA pool size, which takes into account the amount of digesta in the intestine, is a parameter reflecting more accurately the intensity of the bacterial fermentative processes, than the SCFA concentration (Berggren et al., 1993; Jurgoński et al., 2008). The increased concentration of butyrate after prebiotic treatment is of particular significance. Apart from being an important fuel for colon cells, this acid has been shown to posses the ability to reverse cancerous cells (Gibson et al., 1992). The reduction in caecal isobutyrate, isovalerate and valerate concentrations, especially when was fed with CF diet, should be considered as a beneficial effect of such a supplementation. However, a dietary combination of inulin and cellulose was not concomitant with an additional decrease in branched-chain fatty acids concentration. Higher production of these acids points at the intensification of anaerobic bacterial polypeptide and amino acid fermentation (Swanson et al., 2002). Addition of cellulose did not influence total SCFAs pool. The latter observation is in agreement with results demonstrating that CEL affects caecal fermentative processes only to small extent; they suggested

that CEL-diet can be applied as a suitable negative control while studying the properties of potentially prebiotic carbohydrates (Jurgoński et al., 2008).

Although many authors indicate that prebiotic saccharides favourably influence lipid metabolism (Parks, 2002) and mineral absorption (Lobo et al., 2006) our results did not demonstrate such effects. CEL addition led to a significant increase in serum urea. In addition to the probable reduced microbial density mentioned above, dilution of the diet with cellulose may have increased nitrogen flow into the caecum; hence more nitrogen was available for microbial protein synthesis, requiring less nitrogen from ureolysis (Kim et al., 1998). This was also in agreement with our findings referring to the caecal concentration of branched-chain fatty acids which was more depressed by inulin upon the cellulose-free treatment than upon the cellulose-rich diet. During the caecal fermentation of endogenous and undigested amino acids, several putrefactive compounds are produced, e.g., branched-chain fatty acids which are partly responsible for the malodour of digesta (Swanson et al., 2002).

CONCLUSIONS

In conclusion, inulin as a diet supplement, has modified rats' caecal metabolism beneficially, especially in terms of reducing the risk factors, i.e. luminal ammonia, β-glucuronidase, or pathogenic microorganisms. All these effects confirm the suggestion that inulin has the potential to be introduced to a common diet as an agent promoting better bowel integrity and function. Generally, the course of these changes was not modified by addition of cellulose, except the mass of caecum and the concentration of branched-chain fatty acids. However, in authors' opinion obtained results should not question the fact that judicious application of both fibre types is necessary to maintain gut health.

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