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Regular paper

Dietary resistant dextrins positively modulate fecal and cecal microbiota composition in young rats*

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The objective of the present study was to demonstrate the effect of dietary resistant dextrins, as potential prebiotics, on the intestinal microflora of young rats. Enzyme-resistant dextrin, prepared by heating of potato starch in the presence of hydrochloric (0.1% dsb) and tartaric (40% dsb) acid at 130°C for 2 h (CA-dextrin). The experiment was performed on 24 Wistar male rats at 3-wk of age, divided by analogues in three experimental groups (control, starch and dextrin). Analyses determined the overall bacterial counts and the counts of *Lactobacillus, Bifidobacterium, Bacteroides* and *Clostridium* strains within the feces and cecal contents of rats using fluorescence *in situ* hybridization method. CA-dextrin had no effect on primary growth indicators (body weight, body weight gain, dietary consumption) or the mass of the small intestine and the cecum, but dextrins caused a reduction in pH and the concentration of ammonia within the cecal contents. That supplementation of diet with resistant dextrins had a positive effect on composition of intestinal microflora in rats. It increased the counts of *Bifidobacterium* and *Lactobacillus* strains both in the feces and in the cecum. Moreover, it reduced the counts of *Clostridium* and *Bacteroides* strains. These results may suggest that resistant dextrins exerted a prebiotic-like effect in the large intestine.

Key words: resistant dextrin, microflora, rats

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INTRODUCTION

In food science, increasing attention has been paid to the possibility of modulating the composition of intestinal microbiota in humans and animals by means of appropriate food supply. Gastrointestinal tract of healthy individuals is colonized predominantly by microorganisms that are neutral or beneficial for their health (autochthonous microorganisms) (Koboziev *et al.*, 2014). However, excessive proliferation of bacteria classified as neutral may disturb the systemic function. *Enterococcus*, *Streptococcus*, *Bacteroides*, and *Escherichia coli* strains, which are naturally present within the large intestine, may cause diseases when becoming predominant (Blaut *et al.*, 2007). Quantitative and qualitative composition of intestinal microorganisms may also be subject to changes or complete devastation due to exo- and endogenous factors. Disruption of normal equilibrium may be a result of chemotherapy or bacterial or viral infections, or surgeries (Kleessen *et al.*, 2000). The quantity and quality of intestinal microbiota is also determined by environmental conditions, overall health, mental stress and individual factors (age, gender, genotype, intestinal passage time and peristalsis) (Azad *et al.*, 2015; Preidis & Versalovic, 2009). Diet is one of the major factors affecting the quality of intestinal microorganisms (Guarner *et al.*, 2003). With this regard, wide possibilities are offered by the dietary use of amylolytic enzyme digestion-resistant large-molecular dextrins obtained by means of modification of potato starch.

Resistant dextrins are defined as short chain glucose polymers devoid of sweet flavor and characterized by significant resistance to the hydrolytic effects of digestive enzymes in humans (Ohkuma *et al.*, 1999). The first step in the process of preparation of resistant dextrins from starch includes pyroconversion consisting of four stages: thermolysis, transglucosylation, rearrangements and repolymerization. Thermolysis of starch leads to the cleavage of α-D-(1→4) and α-D-(1→6) glycosidic bonds, resulting in products characterized by lower molecular mass, higher viscosity and increased reducing sugar content. Transglucosylation is followed by recombination of hydrolyzed starch fragments with free hydroxyl groups leading to formation of highly-branched structures. Repolymerization of glucose and oligosaccharides leading to formation of large molecular compounds occurs at high temperatures and at the presence of acidic catalyst (hydrochloric acid). The resulting pyrodextrins are a mixture of poly- and oligosaccharides of varied degrees of polymerization and thus of varied molecular mass. Next, pyrodextrins are subjected to enzymatic hydrolysis of chromatographic separation, i.e stages aimed at separation of fractions other than those typical for starch, i.e. containing bonds other than α-(1→4) and α-(1→6)-glycosidic bonds (Berenal *et al.*, 2002; Wang *et al.*, 2001). Much is expected of the use of starch modification products, particularly resistant starches and resistant dextrins, as prebiotic substances. It has been reported that the consumption of prebiotic substances stimulates the growth of not only *Bifidobacteria* (bifidogenic effects), but also of strains be-

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Abbreviations: BW, final body weight; CA-dextrin, enzyme-resistant citric acid-modified dextrin; Cy-3, cyanine-3; DAPI, 4′,6-diamidino-2-phenylindole; dsb, dry starch basis; FISH, fluorescence in situ hybridization; Fluo, fluorescein; PI, prebiotic index; RS, resistant starch.

Table 1. Study groups and relevant diet compositions (%).

Component	Control group	Starch group	Dextrin group
Casein (>85%)	20.0	20.0	20.0
DL-methionine	0.3	0.3	0.3
Cellulose	5.0	5.0	5.0
Sucrose	10.0	–	–
Potato starch	–	10.0	–
Dextrin	–	–	10.0
Soya bean oil	7.0	7.0	7.0
Mineral-mix (AIN-93G-MX)	3.5	3.5	3.5
Vitamin-mix (AIN-93G-VM)	1.0	1.0	1.0
Corn starch	53.2	53.2	53.2
TOTAL	100	100	100

longing to the phyla *Bacteroides* and *Actinobacteria*, while inhibiting *Firmicutes* strains (Martinez *et al.*, 2010).

The objective of this study was therefore to demonstrate the effect of dietary resistant dextrins, as potential prebiotics, on the intestinal microflora of young rats. The scope of the study included determination of overall bacterial counts as well as of the counts of *Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Clostridium* strains in the feces and cecal contents of rats fed with resistant dextrins-supplemented diet.

MATERIALS AND METHODS

Materials. The study was conducted on 24 male Wistar rats divided into 3 experimental groups, 8 animals per group, according to the study diet (Table 1). The experiments were conducted at the Institute of Animal Reproduction and Food Research, Polish Academy of Science in Olsztyn. All experimental procedures involving animals were conducted according to the Polish legal regulations concerning experiments on animals (following a decision issued by the Local Ethical Committee for Experiments on Animals No. 61/2009/N of 21 June 2009). Rats were kept in standard conditions, at ambient temperature of 21–22°C, relative humidity of 50–70%, with extensive ventilation and 12-hour artificial daylight. In the fourth week of diet administration, animals were anesthetized, weighted and subjected to laparotomy. Next, specimens of selected gastrointestinal tract segments (small intestine, cecum, and colon) were collected. After dissection, the cecum was weighed and its contents were collected for analysis. After removal of contents, the cecum was rinsed under running water, dried, and weighed again. Dry mass content, pH and ammonia content were determined in cecal content immediately after collection. Dry mass of cecal contents was determined by means of drying the samples at not more than 60°C for 24 hours and then at 105°C to a constant mass. Ammonia was determined by Conway method (Hofirek & Haas, 2001) consisting in expulsion of ammonia by means of saturated potassium carbonate solution, subsequent binding of ammonia by means of boric acid and then in titration of ammonium borate with sulfuric acid against bromocresol green and methyl red. The pH values in the intestinal contents were measured using a Hanna Instruments pH-meter (model 301, Portugal).

Preparation of dextrin. Enzyme-resistant citric acid-modified dextrin (CA–dextrin) was prepared following the method of Kapusniak *et al.* (2008). Thus, potato starch was sprayed with hydrochloric acid solution (0.5% w/v) to obtain a final HCl concentration of 0.1% on a dry starch basis (dsb). The citric acid solution (0.5% w/v) was then added to obtain a final organic acid concentration of 0.1% dsb. Thoroughly mixed sample was dried at 110°C to obtain a final moisture content below 5%. Dried sample (10 g) was placed in an anti-pressure bottle (SIMAX), capped and heated at 130°C for 3 h in an ELF 11/6 EUROTHERM CARBOLITE oven (Hope, England). Product was cooled in a desiccator and milled to powder. Dextrin was then washed with 80% EtOH to remove excess of citric acid, and low molecular weight material formed during dextrinization, dried overnight at 50°C, and then at 110°C for 1 h, and finally milled in a cyclone lab sample mill (UDY Corp., Fort Collins, CO, USA).

Determination of the number of bacteria. Analyses determined the overall bacterial counts and the counts of *Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Clostridium* strains within the feces and cecal contents of rats. Feces were collected before as well as in successive weeks of study diet administration. In the last week, rat cecal contents were collected for microbial analysis. Composition of intestinal microbiota was determined by means of fluorescent *in situ* hybridization (FISH).

Table 2. Characteristics of FISH probes used in the analysis.

Probe	Identified microorganisms	Sequence (5'->3')	Fluorescent label	Temp (°C)	Time (h)
Lab 158	Lactobacillus	GGT ATT AGC A(T?C)CTGT TTC CA	5' Fluo	56	24
Bif 164	*Bifidobacterium* spp.	CAT CCG GCA TTA CCA CCC	5'Cy3	58	18
Bac 303	Bacteroides	CCA ATG TGG GGG ACC TT	5'Cy3	55	3
Erec 484	*Clostridium coccoides*	GCT TCT TAG TCA GGT ACC G	5'Cy3	57	16
Eub 338	Total Bacteria Count	GCT GCC TCC CGT AGG AGT	5' Fluo	61	24

Fluo, fluorescein (excitation 490 nm, emission 520 nm, molecular weight 389 Da); Cy3, cyanine-3 (excitation 550 nm, emission 570 nm, molecular weight 766 Da)

Fluorescence *in situ* hybridization. In FISH studies used the following probe: Eub338, Lab 158, Erec 484, Enter 1432 (Table 2). In addition, the total number of microorganisms was determined by DAPI staining. To 0.5 g intestinal contents, 4.5 ml PBS and glass beads of a diameter of 4 mm, were added. The samples were vortexed followed by centrifugation at 2000 rpm for 5 min. A 4% paraformaldehyde was added to the supernatant at a ratio of 1:3. Incubation was conducted for 18 h at 4°C. Then, the precipitate was centrifuged (10000 rpm, 10 min, 4°C), and washed 3 times with PBS. The precipitate was stored in 1 ml 50% ethanol (in PBS) at 4°C until the proper analysis. To a small PCR tube, 50 µl was transferred followed by the addition of 20 µl of lysozyme in TRIS-EDTA. After vortexing, the samples were incubated at 37°C for 30 min. The supernatant was removed and the precipitate was washed with 100 µl PBS. Then, 50 µl of hybridization buffer and 10 µl of the appropriate probes were added. Hybridization was conducted in a humid chamber at a temperature and times specific to the molecular probes applied (Table 2). In order to determine the total number of microorganisms instead of the probe reagent was added 100 µl DAPI (4',6'-diamidino-2-phenylindole). After hybridization, the samples were centrifuged, the supernatant was removed. The amount of 150 µl of wash buffer was added followed by incubation for 30 min at a temperature suitable for the proper probe. The precipitate was washed in 100 µl PBS, centrifuged (14000 rpm, 5 min, 4°C) followed by the removal of supernatant. The precipitate was suspended in 50 µl PBS and stored at a temperature of 4°C until preparation of microscope slides.

Microscopic observations were performed using Eclipse E-400 fluorescence microscope (Nikon, Japonia) combined with COHU 4910 camera (Cohu Inc., USA) and coupled with a computer. Measurement of the amount of microbial cells was performed using NIS Elements BR version 3.2 competer program (Nikon, Japan).

Determination of prebiotic index (PI). Prebiotic fermentation of resistant dextrins were analyzed using quantitative equation (prebiotic index – PI). The PI equation is based on the changes in key bacterial groups during fermentation. The bacterial groups incorporated into this PI equation were bifidobacteria, lactobacilli, clostridia and bacteroides. The equation assumes that an increase in the populations of bifidobacteria and/or lactobacilli is a positive effect while an increase in bacteroides and clostridia is negative (Palframan *et al*., 2003).

The PI equation is described below:

$$PI = (Bif/Total) - (Bac/Total) + (Lac/Total) - (Clos/Total)$$

where PI is prebiotic index; Bif, bifidobacterial numbers at sample time/numbers at inoculation; Bac, bacteroides numbers at sample time/numbers at inoculation; Lac, lactobacilli numbers at sample time/numbers at inoculation; Clos, clostridia numbers at sample time/numbers at inoculation; Total, total bacteria numbers at sample time/numbers at inoculation.

Statistical Analysis. The data were analyzed using the STATISTICA 8.0 software package (Statsoft Co., Krakow, Poland). A two-way analysis of variance (ANOVA) was applied to assess the effects of diets on the intestinal microflora and development indicators. If the analysis revealed a significant interaction or that dietary factors had a significant influence ($p \leq 0.05$), the differences among the individual groups were then analyzed with Duncan's multiple range post hoc test ($p \leq 0.05$).

RESULTS AD DISCUSSION

When used in rat diet, resistant dextrin had no effect on primary growth indicators (final body weight, body weight gain, dietary consumption) or the mass of the small intestine with digesta and the colonic tissue (Table 3). However, the addition of dextrin to a diet led to a significantly increase in the relative cecal tissue weight (Table 3), possibly indicating physiological response to increased digesta accumulation in the large intestine. Dextrins had the effect of lowering of the pH and the concentration of ammonia within the cecal contents. One may suppose that this was due to beneficial changes in microflora composition including reduction in the activity of proteolytic bacteria as shown in further studies.

Microbial analysis of rat feces revealed a statistically significant increase in the counts of *Lactobacillus* (Fig. 1) and *Bifidobacterium* (Fig. 2) strains after 3 and 4 weeks of administration of resistant dextrin-supplemented diet as compared to the control group. The increase in the counts of these strains was also observed within the cecal contents, with statistically significant differences being observed only in case of *Lactobacillus* strains. An reverse correlation was observed for the fecal and cecal content of *Clostridium* (Fig. 3) and *Bacteroides* (Fig. 4) strains. A statistically significant reduction in the *Clostridium* counts in the dextrin group as compared to the control group

Table 3. Indicators of the growth and basic functions of the gastrointestinal tracts of rats fed with the control diet, the starch diet and the resistant dextrin-supplemented diet.

	Group		
	Control (K)	Starch (S)	Dextrin (D)
Animal growth indicators			
Baseline body weight, g	79.09 ± 0.78	79.03 ± 0.87	79.08 ± 0.98
Final body weight, g (BW)	271.2 ± 4.10	264.1 ± 2.04	266.7 ± 4.05
Body weight gain, g	192.1 ± 3.93	185.1 ± 1.68	187.7 ± 4.65
Dietary intake, g	496.1 ± 5.04	492.0 ± 5.29	491.4 ± 9.30
Gastrointestinal tract			
Small intestine with contents[1]	2.535 ± 0.058	2.663 ± 0.094	2.480 ± 0.063
Cecal tissue[1]	0.180 ± 0.006[a]	0.211 ± 0.010[a]	0.302 ± 0.018[b]
Cecal contents[1]	0.876 ± 0.042[a]	0.955 ± 0.069[a]	1.846 ± 0.125[b]
Colon tissue[1]	0.363 ± 0.012	0.396 ± 0.018	0.357 ± 0.014
Cecal contents			
Dry matter, %	25.51 ± 0.326	25.03 ± 0.997	26.14 ± 1.317
Ammonia, mg/g	0.263 ± 0.012[a]	0.240 ± 0.012[ab]	0.206 ± 0.013[b]
pH of digesta	7.54 ± 0.044[a]	6.08 ± 0.197[b]	7.13 ± 0.072[c]

[1]g/100g BW; the results are expressed as means ± SEM (n = 8/group); Values not sharing the same superscript letters within a row are significantly different at $p \leq 0.05$.

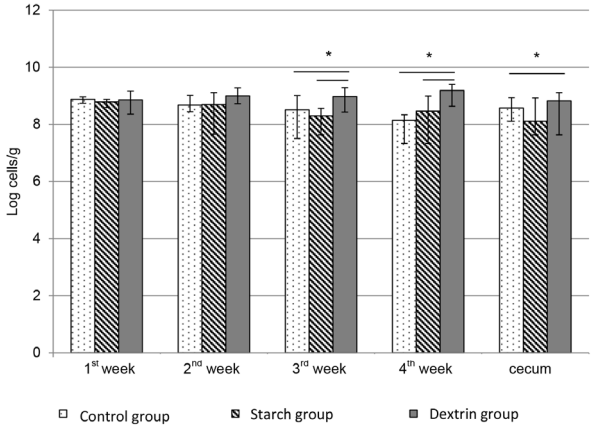

Figure 1. The effect of resistant dextrin-supplemented diet on *Lactobacillus* counts in the feces (1–4 weeks) and cecal digesta.
Asterisks indicate significant differences ($p \leq 0.05$). The results displayed are means (±S.D.) of three independent experiments.

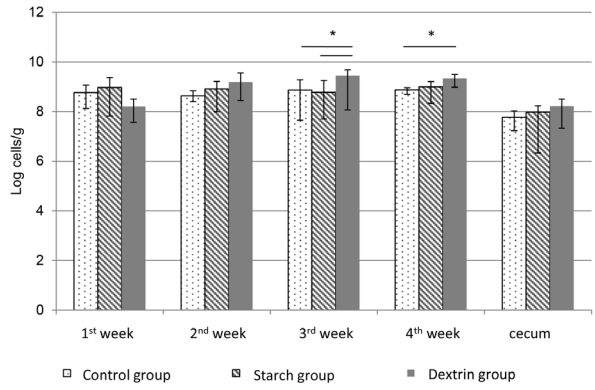

Figure 2. The effect of resistant dextrin-supplemented diet on *Bifidobacterium* counts in the feces (1–4 weeks) and cecal digesta.
Asterisks indicate significant differences ($p \leq 0.05$). The results displayed are means (±S.D.) of three independent experiments.

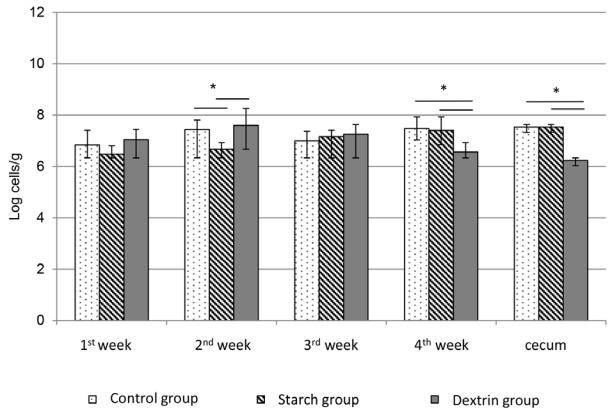

Figure 3. The effect of resistant dextrin-supplemented diet on *Clostridium* counts in the feces (1–4 weeks) and cecal digesta.
Asterisks indicate significant differences ($p \leq 0.05$). The results displayed are means (±S.D.) of three independent experiments.

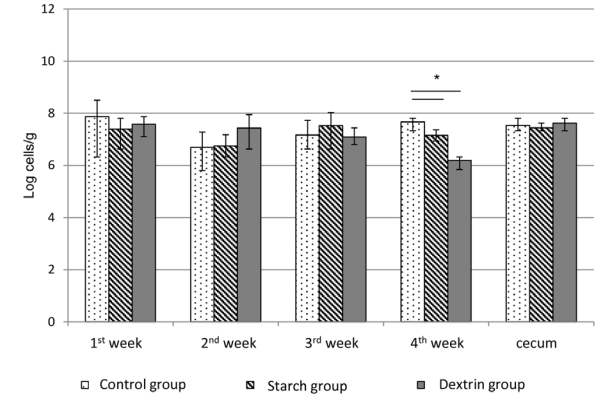

Figure 4. The effect of resistant dextrin-supplemented diet on *Bacteroides* counts in the feces (1-4 weeks) and cecal digesta.
Asterisks indicate significant differences ($p \leq 0.05$). The results displayed are means (±S.D.) of three independent experiments.

was observed in the feces of rats after 2 and 4 weeks of diet administration and in the cecal contents; reduction in *Bacteroides* counts was observed in the feces after 4 weeks of diet administration. The increase in beneficial bacteria of geni *Lactobacillus* and *Bifidobacterium* may be an evidence of the beneficial effect of resistant dextrins on modulation of intestinal microflora in young rats. Resistant dextrin-supplemented diet was also found to have no effect on the total bacterial counts which were comparable in all study groups.

The obtained results were consistent with those obtained by Klessen *et al.* (1997) who determined the effect of the diet supplemented with resistant starch RS1 (physically unavailable for digestive enzymes) and RS2 (native enzyme-resistant starch) on the intestinal microflora of rats. Increased counts of *Bifidobacterium* strains were observed in the feces of rats fed with both RS1, and RS2-supplemented diets while increased counts of *Lactobacillus* strains were observed only in rats fed with RS2-supplemented diet. The authors also determined a reduction in the counts of *Bacteroides* strains in the feces of rats, albeit only in the RS1-supplemented diet group. On the other hand, Hong *et al.* (2005) observed increased *Bifidobacterium* counts in the feces of mice fed with resistant starch RS3 (retrograded starch resistant to amylolytic enzymes).

Berard and coworkers (2009) determined the effect of the resistant dextrin (maltodextrin)-containing commercial product Nutriose® on the intestinal microflora in humans. The study included the analysis of the effect of ingestion Nutriose® at the doses of 8, 10, 15 or 20 g/day for 14 days as well as at the dose of 30 and 45 g/day for 35 days. Fourteen days of administration of the product at 8 and 10 g/day resulted in an increase in *Bacteroides* counts as compared to the control group. In case of the dose of 45 g/day, increase in *Lactobacillus* counts was observed. On the other hand, the dose of 15 g/day (administered for 14 days) reduced the counts of *Clostridium perfringens*. Similar correlations were observed by Pasman *et al.* (2006) in other studies involving the use of Nutriose®.

The studies led to a conclusion that supplementation of diet with resistant dextrins has a positive effect on the composition of intestinal microflora in rats. It increases the counts of *Bifidobacterium* and *Lactobacillus* strains both in the feces and in the small intestine. Moreover, it reduced the counts of *Clostridium* and *Bacteroides* strains. The prebiotic index (PI) was calculated to confirm the

Table 4. The influence of dextrin on Prebiotic Index.

Group	Faecal				Cecum
	1st week	2nd week	3rd week	4th week	
Control	0.307	0.264[a]	0.320	0.190[a]	0.137[a]
Starch	0.312	0.319[b]	0.341	0.288[b]	0.113[a]
Dextrin	0.397	0.326[b]	0.399	0.598[c]	0.320[b]

The results displayed are the mean three independent experiments. Values not sharing the same superscript letters within a column are significantly different at $p \leq 0.05$

beneficial effect of resistant dextrins on the intestinal microflora (Table 4). PI values were shown to be the highest in the feces of rats fed resistant dextrin-supplemented diet as compared to the control group and the starch diet group.

The calculated PI values for dextrin were higher than those reported by Olano-Martin *et al.* (2003) for pectin and pectic-oligosaccharides or by Kordyl (2010) for inulin and oligosaccharides which shows than CA-dextrin may act as a prebiotic substance.

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