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Effect dietary inulin on microbial ecosystem and concentrations of volatile fatty acids in rat's caecum*

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ABSTRACT

The effect of inulin on diet intake, excreta output, microbial ecosystem and concentrations of volatile fatty acids in rat's caecum was investigated on twenty one, 8-week old male Wistar rats. The animals were divided randomly into three experimental groups of 7 rats each, which were kept in individual cages. Three experimental isoprotein and isoenergetic diets were prepared providing different source and amount of fermentable carbohydrate. The control diet contained 5% of potato starch, while the IN-5 and the IN-10 diet contained 5 and 10% of inulin, respectively. Rats were fed these diets and provided distilled water *ad libitum* for 10 weeks. Dietary intake was monitored daily, weight gain was recorded twice a week. Between 30 and 41 day of experiment 10-day balance study was performed to determine diet intake and excreta output. Transit time was determined with the help of chlorophiline (0.5% diet weight) as a colour marker. At the end of experiment, after 12-h starvation, rats were sacrificed by thiopental injection and dissected to obtain samples of caecal digesta. Inclusion of 5 and 10% of inulin in the rat's diet did not affect significantly feed intake, excreta output, its water content and transit time. However, in rats fed high-inulin diet a tendency to a higher water content of excreta and decrease of transit time was observed. Concentration of the volatile fatty acids in caecum, specially the butyrate, was significantly higher in rats fed inulin supplemented diets, however without changing their mutual proportions. Inulin inclusion in the diet, led to changes in caecal microflora populations, total counts of the *coli* form and anaerobic bacteria dropped. Comparing to control group the 10% inclusion of inulin led lowered the count of total *coli* form and anaerobic bacteria.

KEYWORDS: rat, inulin, intake, excreta, caecum, microflora, volatile fatty acids

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INTRODUCTION

Prebiotics are generally classified as indigestible feed agents that are potentially beneficial to the health of the host, due to their fermentable properties which may stimulate the growth and/or activity of one or limited number of bacteria in colon or caecum (Gibson and Roberfroid, 1995). According to this definition prebiotics might be a very variable and wide range of chemical substances however most of the authors are in agreement that these are carbohydrates which are not hydrolysed by the host endogenous enzymes thus available for microbial fermentation in lower parts of non-ruminant gastrointestinal tract. In rat, caecum is the main fermentative chamber (Stevens and Hume, 1998) where these carbohydrates are fermented by different groups of microbes mainly to volatile fatty acids (VFA) but also carbon dioxide and methane (Stevens and Hume, 1998; Le Blay et al., 1999).

Fructooligosaccharides and inulin found in plants have been recognized as prebiotics in human (Cummings and Macfarlane, 1997) and animal nutrition (Mikkelsen, 2001). While they improve colonic health, reduce cholesterol, increase glucose metabolism, improve insulin response, reduce blood lipids (Cummings and Macfarlane, 1997; Cook and Sellin, 1998; Prosky, 2000), it is suggested that most of mentioned benefits are correlated with fermentation end-products (Cummings and Macfarlane, 1997; Mikkelsen, 2001), which also may interact with resident microflora (Józefiak et al., 2004). On the other hand fructooligosaccharides and inulin may also improve laxation by several ways. Increasing excreta weight (as the fibre appears in stool), increased bacterial mass in stool, and faecal water content. The effect on stool output depends on the dose and the amount of fibres in the diet (Brighenti et al., 1999). Certain dietary fibres can also stimulate motility and reduce colonic transit time (Cherbut, 2002). However, inulin that is almost completely fermented in large bowel does not appear to exert such effect. Nevertheless, some authors (Gibson et al., 1995; Kleessen et al., 1997) reported that inulin and oligofructose intake has increased faecal water content in human subjects. Listed effects of dietary fibre fractions including inulin are recognized to be beneficial in human nutrition however in farm animals could be totally adverse. Since increased water content in excreta is affecting negatively quality of animal products (i.e. eggs) and cause poorer litter quality thus worst hygiene conditions in the house.

The knowledge regarding fructooligosaccharides and specifically inulin with emphasis on rat's hindgut microflora and its fermentation products in combination with diet passage is limited.The objective of this study was to determine the effect of varying amount of inulin addition to the diet on feed intake, excreta output, its water content, transit time and changes in VFA concentrations and microbial populations in the caecal content of rats.

MATERIAL AND METHODS

Animals and diets

The experiment was performed on twenty one, 8-week old male Wistar rats (150 g initial body weight, purchased from Licensed Animal Breeding Company, Brwinów, Poland), divided randomly into three experimental groups of 7 rats each. Rats were kept in individual cages, with standard conditions: air temperature 20-22°C, humidity 55-60% and 12-h light-cycle. Three experimental isoprotein and isoenergetic diets were prepared according to the recommendation of American Institute of Nutrition (AIN-93) (Reeves et al., 1993), providing different source and amount of non-digestible carbohydrate. The control diet contained 5% of potato starch, while the IN-5 and the IN-10 diet contained 5 and 10% of inulin (Inulin Frutafit TEX, min. 90% inulin, purchased from HORTIMEX Company), respectively, as a source of bulking substance. The composition of diets is presented in Table 1. Rats were fed the diets and provided distilled water *ad libitum* for 10 weeks. Dietary intake was monitored daily, weight gain was recorded twice a week. Between 30 and 41 day of experiment 10-day balance study was performed to determine diet intake and excreta output. Transit time was

TABLE 1

Composition and nutritional value of diets, g kg^{-1} dry matter

Components	Experimental diets		
	control	IN-5	IN-10
Casein	200	200	200
Soya oil	70	70	70
Sucrose	100	150	100
Wheat starch	532	482	482
Potato starch	50	-	-
Inulin[1]	-	50	100
Vitamin mix[2]	10	10	10
Mineral mix[2] (AIN-93)	35	35	35
L-cystine	3	3	3
Calculated			
ME in MJ/kg	7.65	7.65	7.65
dry matter	910.00	910.0	910.0
crude protein	175.00	175.0	175.0
crude fat	70.00	70.0	70.0
total carbohydrates	730.00	730.0	730.0
crude ash	3.45	3.45	3.45
Ca	7.20	7.20	7.20

[1] inulina Frutafit TEX (min. 90% inulin, HORTIMEX Co.)
[2] prepared according to AIN-93 formulation (Reeves et al., 1993)

determined with the usage of chlorophiline (0.5% diet weight) as a colour marker. At the end of experiment, after 12-h starvation, rats were sacrificed by thiopental injection and dissected to obtain digesta samples, 1 g of total digesta from caecum was stored at -20°C using sterile plastic tubes for further VFA analyses and 1g for enumeration of microflora was immediately transferred under a stream of CO_2 into flasks containing 90 ml of a pre-reduced salt medium (Holdeman et al., 1977). The experimental protocol was approved by the Local Bioethical Commission in Poznań (Approval No 93/2001).

Statistical analysis of results was performed using the SAS (1996) System ver. 6.12. It was also used for single factor analysis of variance. Differences were considered significant at $P \leq 0.05$.

Analytical methods

The content of dry matter, crude protein and crude fat, ash and minerals was determined using the AOAC procedure (1990). The concentration of the volatile fatty acids (VFA) in the fresh caecal content was determined using gas chromatography (Varian 3380) described by Jensen et al. (1995). Dilluted caecal digesta for enumeration of microflora suspension was further homogenized for 2 min in CO_2-flushed plastic bags using stomacher homogenizer (Interscience, France). Subsequently the samples were serially diluted in 10-fold steps using pre-reduced salt medium according to technique of Miller and Wolin (1974). The number of bacteria capable of anaerobic growth was determined using anaerobic conditions in plate system with ruminal fluid-glucose-cellobiose agar (Holdeman et al., 1977) incubated at 37°C for seven days. Presumptive total number of lactic acid bacteria were enumerated on de Man Rogosa and Sharp agar (Merck 1.10660, Darmstad, Germany) and incubated in anaerobic conditions at 37°C for two days. Total count of *coli* form bacteria on 1 MacConkey agar (Merck 1.05465), yeast were enumerated on malt chloramphenicol agar (10g/L glucose Mreck 1.08337.1000; 5g/L malt extract Difco 0186-17-7,Detroit, MI; 10 g/L peptone Difco 0118-17-0; 50 mg/L chloramphenicol Sigma-Aldrich Chemie GmbH C-0378, Steinheim, Germany; 15 g/L agar Merck 1.01614); both media were incubated in aerobic conditions at 37°C for one and two days, respectively.

RESULTS AND DISCUSSION

Inulin supplementation did not affect significantly the diet intake, stool output and water content in stool as well as transit time (Table 2). However the water

content of excreta slightly increased while transit time decreased in the group fed diet with 10% of inulin compared to the control group.

TABLE 2

Diet intake, excreta output and transit time determined during the balance study in rats

Group	Parameter, mean ± SEM			
	diet intake g/10 days/rat	wet weight of excreta g/10d/rat	water content in excreta %	transit time min
Control	252.3 ± 13.6	13.5 ± 1.0	29.3 ± 1.8	842 ± 70
IN-5	235.3 ± 18.7	11.9 ± 0.8	25.9 ± 2.6	764 ± 65
IN-10	233.9 ± 15.1	16.1 ± 0.9	33.6 ± 1.5	708 ± 103

Inclusion of the different levels of inulin in the diet, resulted in increased VFA concentration in caecal digesta (Table 3). In comparison with the control group total concentration of the VFA increased by 53 and 65% ($P<0.05$) in the IN-5 and the IN-10 groups, respectively. Moreover, in the IN-10 group increased amount of the butyrate by 74% (comparing to the control group and the IN-5 group), also acetate and propionate concentrations gradually raised after inulin supplementation (Table 3). However molar ratio between individual acids have not been affected. The increase of total VFA as well as butyrate after inulin implementation is in agreement with findings of Le Blay et al. (1999), however the reason for this butyrogenic effect is currently unknown, while other sugars also composed of fructose and glucose do not induce similar effects on butyrate concentration (Le Blay et al., 1999).

TABLE 3

Concentrations of the volatile fatty acids (mM/kg wet digesta) and their molar ratios (% of total VFA) in the caecal digesta

Group	Concentrations			
	acetate	propionate	butyrate	total
Control	11.67[a]	4.38[a]	0.76[a]	16.82[a]
IN-5	17.24[b]	7.31[b]	0.76[a]	25.72[b]
IN-10	18.57[b]	7.98[b]	1.32[b]	27.80[b]
Pooled SEM		5.94		

Molar ratio, %

group	acetate	propionate	butyrate
Control	69.40	26.03	4.56
IN-5	67.02	28.44	4.52
IN-10	66.60	28.64	4.74
Pooled SEM		7.90	

* SEM- standard error of mean

[a,b] - $P \leq 0.05$

Total VFA concentration increase, particullary butyrate, may be one of the most important effects of inulin in the diet. Butyrate is one of the most important source of energy for colonocytes, stimulates water and sodium absorption (Cherbut et al., 1997). Beneficial effects of VFA, particularly butyrate, are recognized in human nutrition (Cummings and Macfarlane, 1997; Cook and Sellin, 1998). However importance of gut health is also growing in farm animals, therefore more research is carried out to understand interaction between diet and gastrointestinal tract (GIT) microflora (Mikkelsen, 2001; Józefiak et al., 2004). In those aspects VFA seem to have an important role. Respectively Van der Wielen et al. (2002) demonstrated a bacteriostatic effect of VFA to some enteric bacteria and *S. typhimurium*, but no inhibition of beneficial GIT bacteria such as *Lactobacillus* spp.This is why it is suggested that gradual increase of total VFA concentrations is due to enhanced microbial density/activity while mode of inulin action is mainly recognized by their ability to increase number of *Lactobacillus* spp. (Gibson and Roberfroid, 1995). In this study inulin inclusion in the diet was reflected by statistically significant ($P<0.05$) lower count of total anaerobic as well as *coli* form bacteria (Table 3). Lactic acid bacteria were not affected in the IN-10 group but in the IN-5 group were slightly lower comparing to the control group but in both cases those differences were not statistically significant. In all treatments total number of yeast was not affected by inulin supplementation. Comparing to control group as well as IN-5, 10% inclusion of inulin in the diet led to stabilization of the microbial ecosystem (Table 4).

TABLE 4

Microbial populations

Group	Bacterial counts in caecal digesta, log cfu × g^{-1}digesta			
	anaerobic bacteria	*coli* form bacteria	lactic acid bacteria	yeast
Control	8.43[a]	6.99[a]	8.33	5.27
IN-5	7.88[ab]	5.94[ab]	8.04	5.38
IN-10	7.41[b]	5.22[b]	8.44	5.51
Pooled SEM	0.23	0.27	0.15	0.82

* SEM- standard error of mean

[a,b] - $P \leq 0.05$

Observed changes in populations of the lactic acid bacteria are probably in relation to higher concentrations of VFA and this is in agreement with findings of Le Blay et al. (1999). However, in contrast to Le Blay et al. (1999), in the experiment also lower count of total anaerobic bacteria was found. It is also suggested that one of other effects of the inulin action in the rat's caecum, is observed reduction of the *coli* form bacteria which may be represented by some pathogens. McHan and Shotts (1993) observed a toxic effect of VFA to some *Enterobacteriacae*, and

in vitro studies showed a 50 to 80% reduction in *Salmonella typhimurium* counts in the presence of VFA.

Even though the inulin was at very high level in the experimental diets we could not observe its significant effect on feed intake, excreta output as well as transit time. However it was recorded that end products of hindgut fermentation changed with special effect on butyrate concentration, thus it is possible that higher modification appeared in those microflora populations which were not evaluated in the experiment (i.e. *Bifidobacterium* spp.) or some others which are not possible to grow in *in vitro* conditions. On the other hand the high inclusion of the inulin affects diet composition and level of nutrients and metabolizable energy thus it should be considered only if marked changes in hindgut fermentation are enough beneficial to the host animal.

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STRESZCZENIE

Wpływ inuliny na spożycie diety, przepływ treści, mikroflorę i stężenie lotnych kwasów tłuszczowych w jelicie ślepym szczurów

Celem pracy było zbadanie wpływu dodatku inuliny do diety szczurów (Wistar) na spożycie paszy, przepływ treści pokarmowej, wybrane populacje mikroflory oraz stężenie lotnych kwasów tłuszczowych w jelicie ślepym. Dwadzieścia jeden 8-tygodniowych, indywidualnie utrzymywanych samców podzielono na trzy grupy: dieta kontrolna bez inuliny, i dwie doświadczalne: IN-5 i IN-10, zawierające 5 i 10% inuliny. Między 30 a 41 dniem doświadczenia przeprowadzono 10 dniowe doświadczenie bilansowe. Wraz ze zwiększaniem udziału inuliny wystąpiła tendencja do obniżenia suchej masy odchodów i zwiększenia tempa przepływu treści pokarmowej. Spożycie diet doświadczalnych nie różniło się. Stężenie lotnych kwasów tłuszczowych, szczególnie masłowego, znacznie wzrosło ($P \geq 0,05$), choć nie stwierdzono zmian w ich stosunkach molarnych. W porównaniu z grupą kontrolną, udział 10% inuliny w diecie spowodował zmiany w populacji mikroflory, obniżenie bakterii z grupy *coli* i wzrost ogólnej liczby bakterii fermentacji mlekowej.