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Evaluation of commercial microbial hydrocolloids concerning their effects on plasma lipids and caecal formation of SCFA in mice

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ABSTRACT

Exopolysaccharides (EPS) are excreted by microorganisms into the surrounding environment and have been shown to have various physiological effects and are commonly used as food additives due to their rheological properties. Four commercially available microbial EPS with different polymeric structures and composition were tested in LDL receptor knock-out mice to investigate their effect on blood cholesterol, lipoproteins and caecal formation of SCFA. After four weeks on a Western diet supplemented with 4% EPS there were significant increases in caecal content and caecal tissue weight for the EPS groups compared to the control. The total pool of caecal short chain fatty acids was increased when mice were fed scleroglucan, xanthan and dextran. There were no differences in plasma cholesterol levels on the experimental diets compared to the control. Plasma triglycerides did not differ between groups. The results indicate that EPS supplementation to a Western diet may help in maintaining a healthy intestinal environment.

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1. Introduction

Various microorganisms produce polysaccharide structures as capsular or extracellular slime (Sutherland, 1998). Exopolysaccharides (EPS) have different structural and chemical composition and may be homo- or heteropolysaccharides (Sutherland, 1982). These hydrocolloids are invaluable in food formulations due to their rheological properties and are used as gelling and viscosifying agents (Sutherland, 1994). They may be produced *in situ* thus providing functional properties without the use of additives (De Vuyst & Degeest, 1999; Ruas-Madiedo, Hugenholtz, & Zoon, 2002). EPS have similar structures to dietary fibres originating from plant material. Dietary fibres have on many occasions been shown to regulate body weight, glucose homeostasis, insulin sensitivity and other risk factors of cardiovascular disease like the plasma lipid profile (Galisteo, Duarte, & Zarzuelo, 2008). Elevated levels of serum cholesterol are associated with atherosclerosis and coronary heart disease (CHD) (Karnik, 2001). Four hydrocolloids that have a well documented hypocholesterolemic effect in humans (Theuwissen & Mensink, 2008) and animals (Anderson, Jones, & Riddell-Mason, 1994) are psyllium, pectin, guar gum and beta-glucans, all from different origin and of dissimilar composition structurally and chemically (Theuwissen & Mensink, 2008). A cholesterol lowering effect is also found with fungal beta-glucans (Nicolosi et al., 1999), seaweed (Jiménez-Escrig & Sánchez-Muniz, 2000) and various EPS (Levrat-Verny & Behr, 2000; Maeda, Zhu, Suzuki, Suzuki, & Kitamura, 2004; Zou, Guo, & Sun, 2009).

There are several different mechanisms proposed to explain the beneficial effects of soluble fibres on physiological functions. Firstly, the presence of soluble fibres in the intestine leads to absorption of fluid and an increase in viscosity of intestinal contents (Mäkeläinen et al., 2007). This increase may make the unstirred layer adjacent to the mucosa thicker, leading to impaired absorption of 1) bile acids (Kerckhoffs, Brouns, & Hornstra, 2002) and possibly dietary cholesterol and 2) glucose, causing the blood sugar to rise slowly giving a low insulin response (Mäkeläinen et al., 2007). This leads to decreased insulin-stimulated hepatic HMG-CoA reductase activity and consequently cholesterol synthesis (Theuwissen & Mensink, 2008). The binding of bile acids by soluble fibres leads to increased faecal bile acid excretion which is followed by a stimulation of hepatic bile acid

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synthesis from circulating cholesterol thereby lowering blood cholesterol levels (Beylot, 2005). Further the increased production of SCFA in the large intestine due to fermentation of non-digestible carbohydrates may alter hepatic lipogenesis. Acetate stimulates lipogenesis and propionate is an inhibitory molecule that might compete with acetate for the transporter of acetate into hepatocytes. Propionate has also been suggested to directly inhibit hepatic cholesterol synthesis (Beylot, 2005). Other beneficial effects of SCFA are that butyrate affects absorptive and metabolic functions of enterocytes thus slowing down intestinal fat transport (Marcil, Delvin, Garofalo, & Levy, 2003). In addition SCFA dose dependently stimulate mucin production by intestinal epithelial cells. Since the mucus layer protects the mucosa from chemical, mechanical and microbial challenges a high concentration of SCFA plays a mucoprotective role (Willemsen, Koetsier, & Van Deventer, 2003). The total SCFA content and regional differences in the intestinal tract consequently affect the health state of the colon and are important factors in cancer development and gastrointestinal disorders, which often occurs distally where SCFA concentrations are low. Increased SCFA production and delivery of especially butyrate may help preventing these colonic diseases (Wong, de Souza, Kendall, Emam, & Jenkins, 2006). SCFA also control intraluminal homeostasis by affecting water and electrolyte absorption and maintaining colonic osmolarity. Hence is useful in the treatment of different types of diarrhoea (Vernia, 2007).

Although most Western people ingest EPS on a daily basis as food additives, these substances are scarcely studied concerning their physiological effects. If these functional ingredients were shown to be beneficial to health, food products with added value could be developed. The growing demand of healthy foods motivates the investigation of new and old substances to test their potential as health promoting ingredients. Clinical trials are costly and time-consuming to perform. Animal models are a cost-efficient alternative for the screening of candidate food components, and also enable mechanistic studies. Health beneficial effects of dietary fibres have been studied in both wild type and genetically modified mice, such as the LDL-receptor deficient mice ($LDLr^{-/-}$) (Andersson, Immerstrand et al., 2010; Andersson, Svedberg, Lindholm, Oste, & Hellstrand, 2010; Choi, Kim, Jung, Hong, & Song, 2010; Dupasquier et al., 2007; Immerstrand et al., 2010). In this study four commercially available EPS i.e. scleroglucan, xanthan, gellan and dextran were tested in mice to evaluate their effect on plasma cholesterol, lipoproteins, SCFA production in caecum and faecal bile acid excretion.

2. Experimental methods

2.1. Exopolysaccharides

Four commercially available microbial hydrocolloids were used for the experiment: scleroglucan (Actigum CS 11 GR, Cargill), xanthan (Keltrol T, C P Kelco), gellan (Kelcogel LT100, C P Kelco) and native dextran (GE Healthcare BioSciences AB). The EPS were analysed by the accredited laboratory Eurofins concerning their protein, fat and mineral content. Due to their low content of contaminants they were regarded as pure EPS when added to the diets.

2.2. Diets

The EPS were dissolved in water together with maltodextrin (C*Dry MD 01910, Cerestar) to aid solubilisation. Dissolved EPSs were frozen at −20 °C and sequentially freeze dried for 72 h. The freeze drier was programmed to start at −20 °C and increase the temperature by 10 °C per hour up to 20 °C. The dry EPS/maltodextrin mix was mortared and blended with a diet premix (Research Diets Inc., New Brunswich, NJ, USA) to an EPS concentration of 4% (w/w). Anhydrous butter (Arla, Sweden) was melted and heated to 50 °C before addition and final mixing of the diet. The diet was stored at 4 °C until use and was administered as powder. As negative control 4% (w/w) microcrystalline cellulose (Avicel PH 101, FMC Biopolymer) was used. The EPS and cellulose diets were all based on the same premix and all diets contained equal amounts of maltodextrin (Table 1 and Table 2).

2.3. Animals

The reduction of plasma cholesterol by dietary fibres has been relatively larger in $LDLr^{-/-}$ than in wild type mice (Andersson, Immerstrand et al., 2010; Andersson, Svedberg et al., 2010), possibly due to the higher magnitude of hypercholesterolaemia in the genetically modified mice. To be able to observe small changes in the plasma cholesterol level the $LDLr^{-/-}$ model was chosen for this experiment. At arrival female, homozygous, LDL-receptor deficient mice (B6.129s/-$Ldlr^{tm1Her}$/J, Charles River Laboratories, Sulzfeld, Germany) were acclimated to their new environment for two weeks while fed a commercial normal chow (R34 rodent chow, Lactamin, Vadstena, Sweden). At the age of 10 weeks the mice ($n = 49$, body weight 18.6 ± 1.5 g) were randomly divided into five groups, and fed a Western type diet, containing 4% dietary fibres, for four weeks. The mice were housed in plastic cages with housing material and free access to food and water (22 °C, relative air humidity 60% with a 12 h light/dark cycle). At baseline and after two and four weeks on the experimental diet blood was collected from the saphenous vein into EDTA-coated microvette tubes after 4 h fasting. The tubes were centrifuged at 5000× *g* for 10 min at 4 °C to yield plasma. In samples for analysis of lipoprotein patterns sucrose was added to a final concentration of 10%. The plasma was stored at −80 °C.

After four weeks the mice were killed by cervical dislocation under isofluran anaesthesia. Caecum contents were collected and immediately frozen in liquid nitrogen before storage at −80 °C. Caecum tissue was rinsed in PBS, blotted between filter papers and weighed. All experiments followed national guidelines for the care and use of animals and were approved by the Malmö/Lund regional ethical committee for laboratory animals. The animals tolerated the study well.

Table 1
Formulation of the diets (g/kg diet).

Ingredient	Control	EPS diet
Casein, 80 mesh[a]	200	200
DL-methionine	3	3
Corn starch	281	281
Maltodextrin 10	100	100
Sucrose	100	100
EPS	0	44
Cellulose	44	0
Butter, anhydrous[b]	200	200
Corn oil	10	10
Mineral mix S10026	10	10
Dicalcium phosphate	13	13
Calcium carbonate	5.5	5.5
Potassium citrate $1H_2O$	16.5	16.5
Vitamin mix V10001	10	10
Choline bitartrate	2	2

EPS, exopolysaccharide.
[a] Casein is 88% protein.
[b] Anhydrous butter contains 230 mg cholesterol per 100 g The amount of cholesterol in all diets was 0.46 g/kg diet.

Table 2
Macronutrient and energy contents of the experimental diets.

	Control	EPS diet
Protein (% energy)	16	16
Carbohydrate (% energy)	43	43
Fat (% energy)	41	41
Energy (kJ/g diet)	19	19

EPS, exopolysaccharide.

2.4. Plasma cholesterol and triglycerides

Plasma concentrations of cholesterol and triglycerides were determined enzymatically in duplicates (Infinity Cholesterol/Triglyceride Liquid Stable Reagent, Thermo Scientific, Melbourne, Australia) according to the manufacturer's manual.

2.5. Lipoprotein

The lipid distribution between HDL and LDL + VLDL was calculated by separating plasma lipoproteins on agarose gels with barbital buffer as described by Andersson, Immerstrand et al. (2010). Duplicate samples were run.

2.6. Analysis of short chain fatty acids

The SCFA analysis was performed by gas chromatography according to Zhao, Nyman, and Ake (2006) with some modifications. Acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid were used as standards; 2-ethylbutyric acid was used as internal standard. Duplicate samples were run.

The chromatographic analysis was performed using an Agilent 6890N system equipped with a flame ionization detector and an N10149 automatic liquid sampler (Agilent Technologies Inc., USA). A fused-silica capillary column with a free fatty acid phase (DB-FFAP 125–3237, J&W Scientific, Agilent Technologies Inc., USA) of 30 m * 0.53 mm (inner diameter) coated with 0.50 μm film thickness was used. The SCFAs were identified on chromatograms by their specific retention times. Calibration curves for each SCFA were made using the standard SCFA mixture.

2.7. Bile acids in faeces

At baseline and at week 4 faeces were collected cage-wise after the animals had been on grills for 24 h. The faeces collected from each cage were freeze-dried, mortared and weighed. Three samples from each cage were run in duplicates for total bile acid measurements. Bile acids were extracted in 75% ethanol at 50 °C for 2 h (Yu et al., 2000). The solids were discarded after centrifugation and the supernatants were analysed according to the manufacturer's description using the Colorimetric Total Bile Acids Assay Kit (Diazyme Laboratories, CA, USA) modified to suit a 96-well plate assay.

2.8. Statistics

Data analysis was performed using SigmaPlot 11.0 (Systat Software Inc.) using one-way ANOVA for multiple comparisons followed by Tukey's test to determine significance of differences between groups. Results are expressed as mean values and their standard error. Values with a $P < 0.05$ were considered statistically significant. Data failing the normality test were analysed by the Kruskal–Wallis one-way analysis followed by Dunn's method for pairwise multiple comparisons between groups. Data are expressed as median values and the 25th and 75th percentiles.

3. Results

3.1. Body weight, feed intake and faecal excretion

The weight of the mice was recorded throughout the study. There was no significant difference in feed intake or the mean body weight gain. Mice fed dextran seemed to have less faecal output, but this could not be verified statistically since faeces were collected as a pooled sample from each cage (Table 3). Remarkably, gellan faeces contained 25% water while the other groups contained approximately 10%.

3.2. Plasma cholesterol and triglycerides

There were significant differences between the EPS-groups concerning plasma cholesterol levels after 4 weeks on the diets (Table 4). Both scleroglucan and gellan gave significantly lower plasma cholesterol concentrations than the xanthan. However none of the EPS were significantly different from the negative control. Feeding the mice EPS did not alter plasma triglyceride levels.

3.3. Lipoprotein

At the start of the study the LDL + VLDL fraction was high as expected for the LDLr$^{-/-}$ mice, the HDL fraction being about 34% in all groups. After 4 weeks on the experimental high fat diets the HDL fraction was further decreased in all groups to constitute less than 27% of the lipoproteins. Xanthan gave the lowest level (18%) which was significantly lower than the control (Table 4).

3.4. Caecum content, caecal tissue weight and SCFA formation

The EPS-containing diets induced a significant increase in caecum content and caecal tissue weight compared to the negative control (Table 5). The caecum contents were visibly different concerning the colour and viscosity between groups. Gellan ingestion resulted in a caecum content with a cuttable gel like consistency. Scleroglucan, xanthan and dextran induced an increase in the total

Table 3
Initial weight, body weight gain, feed intake and dry faeces for mice fed experimental diets for 4 weeks.

	Initial weight (g)			Body weight gain (g)			Feed intake (g/mouse and day)			Dry faeces (g/mouse and 24 h)	
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	*n*
Control	17.6[a]	0.39	10	2.7[a]	0.85	10	2.0[a]	0.10	3	2.0	1*
Scleroglucan	19.2[a]	0.43	10	1.8[a]	1.2	10	2.1[a]	0.03	3	1.7	1*
Xanthan	19.2[a]	0.44	10	2.1[a]	0.74	10	2.4[a]	0.17	3	2.5	1*
Gellan	18.4[a]	0.57	10	1.6[a]	0.73	10	2.2[a]	0.16	3	2.5	1*
Dextran	19.2[a]	0.45	9	2.0[a]	0.51	9	2.0[a]	0.03	3	0.93	1*

n, number of observations. Statistics were calculated using one-way ANOVA for multiple comparisons. Tukey's test was used for pairwise comparisons of means. Mean values with unlike superscript letters were significantly different ($P < 0.05$).
*Figure refers to number of cages (9–10 mice per cage).

Table 4
Plasma lipid profile and bile acid excretion in faeces.

	Plasma cholesterol levels (mmol/L)			Plasma triglycerides (mmol/L)			Plasma HDL (%)			Bile acids in faeces[a] (μmol/mouse and 24 h)			
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Median	25%	75%	*n*
Negative control	17.6[ab]	0.65	10	5.2[a]	0.45	10	26.5[a]	1.3	9	1.7[ab]	1.6	2.0	3
Scleroglucan	16.5[b]	0.85	10	6.1[a]	0.53	10	22.1[ab]	1.7	9	2.5[ab]	2.2	2.6	3
Xanthan	19.6[a]	0.57	10	6.5[a]	0.37	10	17.6[b]	0.97	10	2.5[ab]	2.5	3.2	3
Gellan	15.3[b]	0.58	10	5.3[a]	0.27	10	23.6[a]	1.3	10	2.9[a]	2.8	3.5	3
Dextran	17.2[ab]	0.63	9	5.0[a]	0.28	9	22.1[ab]	1.6	9	1.4[b]	1.3	1.8	3

n, number of observations. Statistics were calculated using one-way ANOVA for multiple comparisons. Tukey's test was used for pairwise comparisons of means.
[a] These data failed the normality test so a Kruskal–Wallis one-way analysis of variance on the ranks was run followed by Dunn's method for pairwise multiple comparisons. Data are expressed as median and 25th and 75th percentiles. Groups with unlike superscript letters were significantly different ($P < 0.05$).

amount of caecal SCFA where acetic acid constituted the major part (Table 5). The butyric acid level was increased by scleroglucan and propionic acid levels were increased upon consumption of xanthan and dextran. Increased SCFA production may lower the intestinal pH leading to increased mineral solubility and reduced formation of secondary bile acids (Wong et al., 2006). Unfortunately the pH was not measured in the present study due to small samples of caecal content.

3.5. Bile acids

Although the scleroglucan, xanthan and gellan groups showed increased bile acid excretion compared to the control, none of them were statistically significant (Table 4). Dextran induced lower bile acid excretion than the other EPS-groups, but this was significant only relative to gellan.

4. Discussion

Hydrocolloids are used in food design due to their rheological properties. There is a difference between viscous and gelling fibres in that a gel does not flow but elastically stretches when deformed. The properties of viscous fibres are well known whereas the role of gelation in relation to physiological effects is unknown (Wood, 2007). Gellan is an anionic, multifunctional gelling agent produced by the genus *Sphingomonas*. It has a linear structure based on a tetrasaccharide repeating unit composed of two D-glucose, one L-rhamnose and one D-glucoronic acid. It is natively esterified with acyl substituents. Gellan is approved for use in the food and medical sector in the US and the EU (Fialho et al., 2008). In this study gellan was shown to hold more water (25%) in the faeces compared to the other groups (10%) and the caecal contents appeared as a hard gel. A high viscosity of the intestinal contents may impair the absorption of nutrients and other compounds (Kerckhoffs et al., 2002). However, in this study the mean food intake and body weight gain were the same for all groups indicating that there was no difference in nutrient availability. No difference was seen in the SCFA profile of gellan compared to the control indicating the same level of fermentation. The higher content of water contained by gellan does not influence the results since the concentration of SCFA (μmol/g caecal content) was multiplied with the caecal content (g). Cellulose is not or only slightly fermented (Barry et al., 1995) and the same applies to gellan, consequently allowing it to be used as a substitute to agar when growing bacteria. Furthermore gellan induced a significant increase in both caecal tissue weight and caecal contents, comparable to the non-gel forming EPS (Table 5). This increase in caecum contents demonstrates a bulking effect commonly seen with fibre diets (Levrat-Verny & Behr, 2000; Yamamoto et al., 2000). Oat containing diets have been shown to induce growth of caecum tissue in mice and rats leading to enlargement of the organ (Forsythe, Chenoweth, & Bennink, 1978; Immerstrand et al., 2010). The present results indicate that this phenomenon occurs also for EPS in mice.

Xanthan is produced by *Xanthomonas campestris* and is approved as a food additive without any quantity limitation. It exhibits pseudoplastic behaviour and is extensively used as stabilizer in sauces and dressings (Palaniraj & Jayaraman, 2011). Xanthan showed a tendency to increase plasma triglycerides in agreement with an earlier study (Castro, Tirapegui, & Benedicto, 2003). Castro et al. could not detect any significant hypolipidemic effect in rats by xanthan supplemented at a concentration of 1.5%. Higher levels were tested by Yamamoto et al. using xanthan at a concentration of 3% in rats, which however did not have any effect on cholesterol and triglyceride levels (Yamamoto et al., 2000). In the present study using a concentration of 4% xanthan, total plasma cholesterol was not different compared with the negative control. The cholesterol level on xanthan was however significantly higher than on scleroglucan and gellan, indicating that xanthan does not have a positive effect on plasma cholesterol levels in contrast to the results from Levrat-Verny et al. showing a cholesterol lowering effect of xanthan at a level of 1% (Levrat-Verny & Behr, 2000). The HDL lipoprotein fraction decreased in all groups during the study period, which was expected due to the high fat diet. Xanthan gave however significantly lower HDL-levels than the control, indicating a disadvantageous effect of xanthan on lipoprotein distribution. The structure of xanthan is composed of a linear backbone with β-(1,4)

Table 5
Caecal content, caecal tissue weight and caecal SCFA in mice fed experimental diets.

	Acetic acid[a] (μmol)			Propionic acid[a] (μmol)			Butyric acid[a] (μmol)			Total SCFA[a] (μmol)			Caecum content (mg)			Caecal tissue weight (mg)		
	Median	25–75%	*n*	Median	25–75%	*n*	Median	25–75%	*n*	Median	25–75%	*n*	Mean	SEM	*n*	Mean	SEM	*n*
Control	5.1[a]	4.3–5.6	10	0.83[a]	0.60–0.93	10	0.45[a]	0.29–0.66	10	6.9[a]	5.4–7.4	10	150[a]	10	10	33[a]	2.2	10
Scleroglucan	10.3[bc]	8.5–13.5	10	1.2[ab]	1.1–1.4	10	2.1[b]	0.88–2.5	10	14.5[b]	10.7–17.5	10	290[b]	16	10	63[b]	2.4	10
Xanthan	12.1[b]	9.6–13.8	10	1.3[bc]	1.2–1.6	10	0.92[ab]	0.74–1.0	10	14.6[b]	12.1–16.9	10	330[b]	27	10	66[b]	4.6	10
Gellan	6.1[ac]	4.9–6.7	9	0.88[ac]	0.71–1.1	9	0.48[a]	0.29–0.71	9	7.7[ac]	6.2–8.7	9	270[b]	22	10	68[b]	4.5	9
Dextran	9.7[bc]	6.9–14.6	9	1.9[b]	1.4–2.8	9	0.54[ab]	0.39–1.1	9	12.2[bc]	9.4–19.1	9	270[b]	14	9	55[b]	3.2	9

n, number of observations.
[a] The concentration of SCFA (μmol/g caecal content) multiplied with the caecal content (g). Since these data failed the normality test a Kruskal–Wallis one-way analysis of variance on the ranks was run followed by Dunn's method for pairwise multiple comparisons. Data are expressed as median and 25th and 75th percentiles. Groups with unlike superscript letters were significantly different ($P < 0.05$).

glycosidic linkages like cellulose. The branches consist of mannose and glucoronic acid. Cellulose is not fermented (Barry et al., 1995) which would suggest that the microbial action is performed on the side chains of xanthan. In the present study fermentation of xanthan produced the greatest proportion and level of acetate. This may be the result of polysaccharide deacetylation since the EPS is partially acetylated (Bourquin, Titgemeyer, & Fahey, 1996). In agreement with Bourquin et al. butyric acid was produced at around 6% of the total SCFA concentration.

Dextran is an α-(1,6) linked glucan that is branched mainly at the α-(1,3) position. It is produced by several bacterial genera but for commercial production *Leuconostoc mesenteroides* is the most commonly used species. Dextran is used as a blood plasma substitute and is present in many food systems through *in situ* production (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). No reports have been found on cholesterol levels in relation to ingestion of dextran. In this report native dextran did not have any effect on plasma cholesterol or triglyceride levels. However dextran was significantly increasing the total pool of SCFA including butyric and propionic acid levels. High levels of SCFA and butyric acid in particular may have a mucoprotective role in the gut (Willemsen et al., 2003).

The basidiomycete *Sclerotium rolfsii* produces scleroglucan composed of a β-(1,3) linked glucopyranose backbone with single β-(1,6) linked glucopyranosyl branches on every third subunit. Scleroglucan is not approved for food use in the US and EU but it is extensively used in Japanese food products (Schmid, Meyer, & Sieber, 2011). No reports on physiological effects of scleroglucan have been found in the literature. In this study scleroglucan at a concentration of 4% had a positive effect on the mice gut since a clear bulking effect was seen together with an increased level of total SCFA in caecum including acetic and butyric acid. Butyric acid is responsible for the maintenance of colonic osmolarity and modulates mucus production and is capable of reducing inflammatory responses making it a substance that is promising in the treatment of inflammatory bowel diseases like ulcerative colitis (Vernia, 2007).

It has been suggested that the formation of SCFA is dependent on physico-chemical characteristics of the carbohydrate reaching the colon, such as monomeric composition, type of linkages, molecular weight and solubility (Lambo-Fodje, Öste, & Nyman, 2006). It has for example been shown that fructo-oligosaccharides differing in degree of polymerization and level of solubility give different SCFA patterns (Nilsson & Nyman, 2005), displaying the complexity of intestinal fermentation of polysaccharides. Perhaps due to this complexity, no conclusive results exist in the literature where SCFA patterns are correlated with substrate properties. All branched EPS included in the present study (xanthan, dextran, scleroglucan) increased both the total amount of SCFA and levels of acetic and propionic acid in the caecal content, as opposed to the linear gellan. Gellan showed a lower solubility than the other EPS studied (results not shown) and it is also known for its gel-forming ability; two characteristics that might influence the level of fermentation based on sterical hindrance. More studies are needed to find out whether gel-forming polysaccharides are fermented to a lesser extent than non-gelling polysaccharides. The result from the present study provide new information on SCFA patterns *in vivo* from four structurally different polysaccharides, but it is hard to draw conclusive correlations between their structural characteristics and the formation of SCFA. Further studies are needed before any general conclusions can be drawn between substrate properties and SCFA patterns.

Elevated bile acid excretion with a concomitant increase in bile acid synthesis from cholesterol in the liver is proposed as one of the mechanisms behind the cholesterol lowering effect of oat and barley β-glucans (Andersson, Ellegård, & Andersson, 2002; Andersson, Immerstrand et al., 2010; Zhang et al., 1992). Bile acid excretion in faeces was measured in the present study but there was no difference between the control and experimental groups concerning either plasma cholesterol or bile acid levels indicating no binding of bile acids by the EPS.

In conclusion the tested food hydrocolloids do not impart any negative effects on the investigated parameters in mice. All EPS showed an increased bulking effect leading to an increase in caecal tissue weight comparable to what is known for plant dietary fibre. That together with the increased SCFA production may help in the maintenance of a healthy gastrointestinal tract.

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