

## ORIGINAL ARTICLE

# Influence of isomalto-oligosaccharides on intestinal microbiota in rats

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inulin, isomalto-oligosaccharides (IMO), lactobacilli, rat

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**Abstract****Aims:** Isomalto-oligosaccharides (IMO) with  $\alpha(1 \rightarrow 6)$  and  $\alpha(1 \rightarrow 4)$  glucosidic linkages are produced by enzymatic conversion of starch. IMO are only partially digestible but data on their influence on intestinal microbiota are limited. It was the aim of this study to investigate the effect of IMO diet on intestinal microbiota and short-chain fatty acids production (SCFA) in rats.**Methods and results:** Three groups of F344 rats, each consisting of six animals, were fed IMO, inulin or a control diets for six weeks. A qualitative assessment of the intestinal microbiota was achieved by PCR-denaturing gradient gel electrophoresis (DGGE). Major bacterial taxa were quantified by quantitative PCR (qPCR), and SCFA were measured using gas chromatography. Quantitative PCR demonstrated that lactobacilli were one of the dominant bacterial taxa in faecal samples from rats. IMO increased the number of lactobacilli and the total number of intestinal bacteria in rats fed IMO compared with animals receiving control and inulin diets. Furthermore, PCR-DGGE with lactobacilli-specific primers showed an altered biodiversity of lactobacilli in rats fed IMO compared with control diet.**Conclusions:** IMO selectively stimulates lactobacilli and increases their diversity in rats.**Significance and impact of study:** Isomalto-oligosaccharides specifically stimulate growth of intestinal lactobacilli in a rat model system.**Introduction**

Dietary nondigestible oligosaccharides (NDO) modulate the composition and activity of intestinal microbiota and they may also exert health benefits in the host. They improve bowel function, may prevent overgrowth of pathogenic bacteria through selective stimulation of non-pathogenic members of intestinal microbiota and increase production of short-chain fatty acids (SCFA). SCFA reduce the luminal pH and provide energy for colonocytes (Topping and Clifton 2001; Meyer and Stasse-Wolthuis 2009). Intestinal fermentation and health benefits of fructo-oligosaccharides and galacto-oligosaccharides have been well documented in animal and human studies (Meyer and Stasse-Wolthuis 2009; Gibson *et al.* 2010). However, information regarding intestinal fermentation

of other oligosaccharides that are present in food or are used as food additives such as isomalto-oligosaccharides (IMO), soybean oligosaccharides and lactulose, is limited (Kolida and Gibson 2008). The diverse structure, monomer composition and degree of polymerization of dietary oligosaccharides influence not only intestinal fermentation and SCFA production, (Kleessen *et al.* 2001; Nilsson and Nyman 2005) but also affect their technological properties in food.

IMO with  $\alpha(1 \rightarrow 6)$  and  $\alpha(1 \rightarrow 4)$  glucosidic linkages are used as alternative low-calorie sweeteners in food products (Kohmoto *et al.* 1992). IMO are only partially digested and the undigested portion is fermented in the colon. The caloric content of a commercial IMO preparation was about 75% when compared with maltose (Kohmoto *et al.* 1992). IMO improve constipation similar

to other fibres (Wang *et al.* 2001). NDO and polysaccharides usually have a laxative effect when taken in high dosage. However, IMO are tolerated at higher dosages compared with other NDO (Kaneko *et al.* 1994). Commercial IMO preparations consist of isomaltose, isomalto-triose, panose and isomalto-tetraose as major compounds. Different products differ substantially in their composition, particularly the proportion of digestible carbohydrates (maltose and glucose), the proportion of  $\alpha$ -(1  $\rightarrow$  4) linkages and the degree of polymerization (Kohmoto *et al.* 1991, 1992; Yen *et al.* 2010), and these differences in composition likely influence digestibility, caloric content and their effect on intestinal microbiota.

Several human studies indicate bifidogenic properties of IMO (Kohmoto *et al.* 1988; Kohmoto *et al.* 1991, 1992; Kaneko *et al.* 1994). However, the majority of these studies relied on culture-dependent methods that targeted only a few bacterial groups in the colon. Information regarding the *in vivo* effect of IMO on intestinal microbiota using culture-independent methods is limited (Yen *et al.* 2010). The aim of this study was to investigate the effect of IMO on microbiota composition and SCFA production in the intestine of rats using culture-independent molecular techniques.

## Materials and methods

### Determination of IMO components with high-performance anion exchange chromatography – pulsed amperometric detection (HPAEC-PAD)

IMO were obtained from BioNeutra Inc. (Edmonton, Canada). The composition of the IMO preparation was specified by the supplier as IMO with predominantly  $\alpha$ -(1  $\rightarrow$  6) linkages and a degree of polymerization (DP) of 2 (18–25%), DP 3 (15–23%), DP 4 (14–22%), DP 5 (8–10%), DP 6 (6–8%), DP 7 (2–4%) and DP 8 (2–3%). Isomalto-oligosaccharides were analysed by HPAEC-PAD with a CarboPac PA20 column coupled to an ED40 chemical detector (Dionex, Oakville, Canada) using water (A), 200 mmol l<sup>-1</sup> NaOH (B) and 1 mol l<sup>-1</sup> Na-acetate (C) as solvents at a flow rate of 0.25 ml min<sup>-1</sup> and a temperature of 25°C. The gradient was as follows: 0 min 30.4% B, 1.3% C, 22 min 30.4% B and 11.34% C followed by washing and regeneration. Isomaltose, isomalto-triose and panose were identified and quantified by use of external standards (all obtained from Sigma, ON, Canada). Other peaks were tentatively identified by synthesizing oligosaccharides of the panose series with dextranase of *Weissella minor* ATCC35912 (Galle *et al.* 2010). In brief, *W. minor* was grown for 24 hour in modified de Man, Rogosa and Sharpe medium (MRS) containing 230 mmol l<sup>-1</sup> sucrose as glucosyl-donor and

55 mmol l<sup>-1</sup> maltose as glucosyl-acceptor for synthesis of oligosaccharides. Bacterial cells were removed by centrifugation, and oligosaccharides in the supernatant were analysed by HPAEC-PAD as described earlier.

### Experimental design

Five-week-old F344 rats were housed in specific pathogen-free conditions (SPF). Rats in three treatment groups were fed either IMO (BioNeutra), commercial inulin (Raftiline HP, Raffinerie Tirlemontoise, Tienen, Belgium) or a control diet. Each treatment group consisted of six rats and one or two animals were housed per cage. The base diet was a commercial laboratory rodent diet (5001; Lab Diet Inc., Leduc, Canada) with the following composition: 23% crude protein; 4.5% crude fat; 6% fibre; 8% ash. Inulin or IMO were added to this base diet at 8 g (kg body weight)<sup>-1</sup> for 6 weeks. Rats were given free access to water. Sampling of stool from individual animals was performed at 5, 8 and 11 weeks of age. Faecal samples were immediately snap-frozen at -80°C for analysis of intestinal microbiota and SCFA. Experiments were approved by the University of Alberta Animal Policy and Welfare Committee (UAPWC) in accordance with the Canadian Council on Animal Care (CCAC) guidelines.

### PCR-denaturing gradient gel electrophoresis (DGGE)

PCR-DGGE analysis with universal primers was performed as previously described (Tannock *et al.* 2000). In brief, DNA from faecal samples was extracted using the Qiagen DNA extraction kit and the DNA concentration was adjusted to 50–70 mg l<sup>-1</sup>. Universal primers HAD1-GC (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG T-3') and HAD2 (5'-GTA TTA CCTG CGG CTG CTG GCA C-3') were used to amplify bacterial rDNA. DGGE was performed by using a DCode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA) in 6% acrylamide gels with a denaturing gradient of 30–55%. Electrophoresis was performed at 150 V and 60°C for about 3 hours. Gels were stained with ethidium bromide and viewed by UV transillumination. Patterns were normalized by including PCR products from one sample on all gels. Cluster analysis was performed by unweighted pair group method with arithmetic mean (UPGMA) algorithm based on the Dice correlation coefficient using an optimization coefficient of 1% (Bionumerics software, version 3; Applied Maths, Sint-Martens-Latem, Belgium).

DGGE analysis of the diversity in *Lactobacillus–Pediococcus–Leuconostoc–Weissella* species was performed using the primers LAC1 (5'-AGC AGT AGG GAA TCT

TCC A-3') and LAC2- GC (5'- CGC CCG GGG CGC GCC CCG GGC GGC CCG GGG GCA CCG GGG GAT TYC ACC GCT ACA CAT G-3') with subsequent separation of amplicons by DGGE (Walter *et al.* 2001). Selected bands from DGGE gels were excised from the gel, used as template for PCR amplification with primers Lac1 and Lac2, and sequenced in the Molecular Biology Facility of Department of Biological Sciences at the University of Alberta. Sequences were deposited with accession numbers: HM765476 (*Lactobacillus animalis*), HM765477, HM765478 and HQ658983 (all *Lactobacillus reuteri*). Sequences were matched to type strain sequences available on RNA database project ([http://rdp.cme.msu.edu/seqmatch/seqmatch\\_intro.jsp](http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp)).

#### Quantification of microflora by quantitative PCR (qPCR)

Group-specific primers were used to quantify 16S rRNA gene copy numbers (rDNA) of *Lactobacillus–Pediococcus–Leuconostoc–Weissella* species (*Lactobacillus* group), *Bifidobacterium* spp., *Bacteroides–Prevotella–Porphyromonas* spp. (*Bacteroides* group), clostridial clusters I (*Clostridium perfringens* group), IV (*Clostridium leptum* group), XI (*Clostridium difficile* group) and XIV (*Clostridium coccooides* group), *Enterobacteriaceae* and total bacteria. Genes coding for butyrate CoA-CoA transferase and butyrate

kinase were quantified using degenerate primers (Table 1). PCR and calibration of qPCR were carried out on a Fast Real-Time PCR unit (Applied Biosystems, Streetsville, Canada) as described previously (Metzler-Zebeli *et al.* 2010). Samples from individual animals were analysed in at least duplicate. Results from samples obtained from the same treatment group and time point were averaged and results are reported as log(gene copy number per g).

#### Analysis of short-chain fatty acids in stool samples with gas chromatography (GC)

SCFA were extracted from 100 mg stool samples by adding 200  $\mu$ l of 5% phosphoric acid. Solids were removed by centrifugation at 17 000 g and supernatants were injected on a Stabilwax-DA column (30 m, 0.53 mm ID, 0.5  $\mu$ m df). The head pressure was 7.5 psi, and split vent flow was set to 20 ml min<sup>-1</sup> or adjusted as required. Injector temperature was 170°C. The column temperature was held at 90°C for 0.1 min, increased to 170°C at 10°C min<sup>-1</sup> and held for 2 min. The detector temperature was 190°C. Concentration of acetate, propionate, butyrate, isobutyrate, isovalerate, valerate and caproate was determined used external standards (obtained from Sigma, ON, Canada). Isocaproate was used as internal standard.

**Table 1** Oligonucleotide primers used in qPCR of faecal samples

Bacterial group (amplicon size)	Oligonucleotide sequence (5' → 3')	A <sub>T</sub> *	Reference
<i>Lactobacillus</i> group (341 bp)	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	62	Walter <i>et al.</i> 2001 Heilig <i>et al.</i> 2002
<i>Bifidobacterium</i> spp.(243 bp)	F: TCGCGTCYGGTGTGAAAG R: CCACATCCAGCRTCCAC	60	Rinttilä <i>et al.</i> 2004
Cluster XIV (438–441 bp)	F: AAATGACGGTACCTGACTAA R: CTTTGAGTTTCATTCTTGCGAA	60	Matsuki <i>et al.</i> 2002
Cluster IV (230 bp)	F: GCACAAGCAGTGGAGT R: CTTCTCCGTTTTGTCAA	60	Matsuki <i>et al.</i> 2004
Cluster I (120 bp)	F: ATGCAAGTCGAGCGAKG R: TATGCGGTATTAATCTYCCTTT	60	Rinttilä <i>et al.</i> 2004
<i>Bacteroides</i> group (140 bp)	F: GGTGTCGGCTTAAGTGCCAT R: CGGAYGTAAGGGCCGTGC	60	Rinttilä <i>et al.</i> 2004
<i>Enterobacteriaceae</i> family (195 bp)	F: CATTGACGTTACCCGCAGAAGAAGC R: CTCTACGAGACTCAAGCTTGC	53	Bartosch <i>et al.</i> 2004
Butyryl CoA-CoA transferase (530 bp)	F: CIGAIATTTACITGGAAAYWSITGGCAYATG R: CCTGCCTTTGCAATRTCIACRAANGC	53	Louis and Flint 2007
Cluster XI (bp) (~180 bp)	F: ACG CTA CTT GAG GAG GA R: GAG CCG TAG CCT TTC ACT	58	Song <i>et al.</i> 2004
Butyrate kinase (301 bp)	F: GTA TAG ATT ACT IRY IAT HAA YCC NGG R: CAA GCT CRT CIA CIA CIA CNG GRT CAN C	53	Louis and Flint 2007
Total bacteria (200 bp)	F: CGG YCC AGA CTC CTA CGG G R: TTA CCG CGG CTG CTG GCA C	60	Rinttilä <i>et al.</i> 2004

\*A<sub>T</sub>, annealing temperature in °C, F, forward; R, reverse.

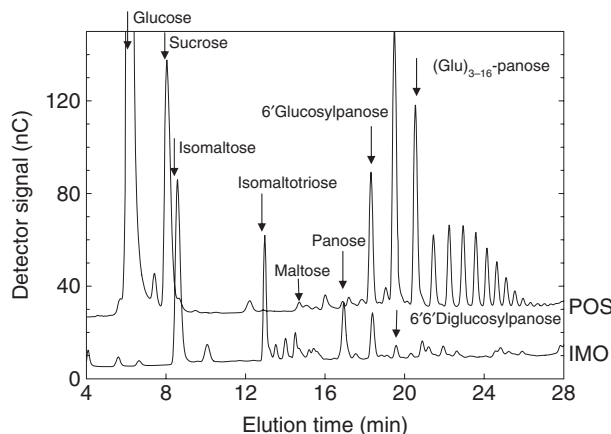
## Statistical analysis

A mixed procedure with repeated measures was used to analyse the effect of diet, time and the interaction of time and diet using SAS software. Data from five-week-old rats were used as covariates. Results were reported as mean values and their standard errors. The least significant difference test was used to identify differences between treatments. Differences were considered to be significant if  $P < 0.05$ .

## Results

### Characterization of the IMO preparation

Carbohydrates in the IMO preparation were separated and quantified by HPAEC-PAD (Fig. 1). Isomaltose, isomaltotriose and panose accounted for  $11.3 \pm 2.9$ ,  $5.8 \pm 1.2$  and  $5.6 \pm 2.2\%$  (w/w) of the IMO preparation, respectively; glucose and maltose were essentially absent. 6'Glucosylpanose and 6'6'diglucoylpanose were also identified using enzymatically synthesized standards (Fig. 1). Dextranucrase from *W. minor* produces oligodextran from sucrose and maltose, a homologous series of linear oligosaccharides composed of  $\alpha$ -(1  $\rightarrow$  6) linked glucose moieties and a maltose residue at the reducing end. These oligosaccharides elute with increasing degrees of polymerization (Galle et al. 2010, Dols et al., 1997). Other disaccharides and higher oligosaccharides were



**Figure 1** high-performance anion exchange chromatography – pulsed amperometric detection separation of isomalto-oligosaccharides (lower trace) and of oligosaccharides of the panose series (POS) synthesized with dextranucrase of *Weissella minor* ATCC 35912 with maltose as acceptor carbohydrate. Glucose, sucrose, isomaltose, isomaltotriose, maltose and panose were identified and quantified by use of external standards; 6'glucosylpanose and 6'6'diglucoylpanose were tentatively identified by enzymatic synthesis of oligosaccharides of the panose series.

additional components of the IMO preparation, but these could not be identified with external or enzymatically synthesized standards.

### Qualitative analysis of faecal microbiota with DGGE

PCR-DGGE was employed to initially assess qualitative effects of IMO or inulin on the faecal microflora. Cluster analysis of PCR-DGGE at 8 weeks of age showed that almost all faecal samples from inulin-fed rats were separated from those fed control or IMO diets. There was no clear separation between treatment groups at 11 weeks of age (Fig. 2a,b).

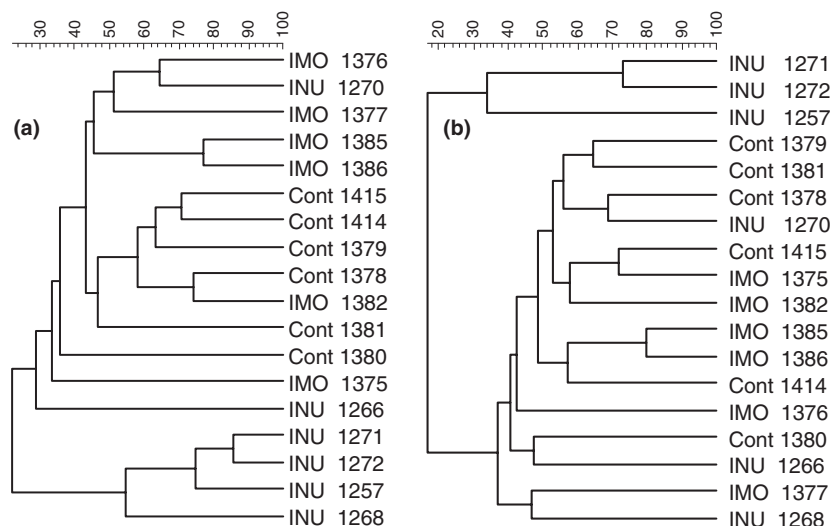
### Quantitative analysis of faecal microbiota with qPCR

Quantitative differences between bacterial taxa in faecal samples from IMO-fed and control animals were assessed using qPCR and group-specific primers (Table 2). The *Lactobacillus* group was one of the dominant bacterial taxa in the samples; feeding IMO significantly increased rDNA copy numbers of faecal organisms in the *Lactobacillus* group compared with rats on control diet (Table 2). In contrast, the number of bifidobacteria in faecal samples from rats fed IMO was low and significantly different from animals fed the control diet at eleven weeks of age. Faecal clostridial cluster XI and *Enterobacteriaceae* decreased over time in both treatment groups. The *Bacteroides* group as well as clostridial clusters I, IV and XIV was not affected by diet or time. Total number of faecal bacteria was increased in rats fed IMO compared with control diet.

Quantitative analysis of genes encoding key enzymes of bacterial butyrate metabolism demonstrated that genes encoding butyrate kinase were below the detection limit of  $10^4$  gene copies per g in all samples (data not shown). Copy numbers of genes encoding butyrate CoA-CoA transferase were unaffected by diet or time.

### Qualitative assessment of organisms in the *Lactobacillus* group by PCR-DGGE with group-specific primers

To determine whether the increased abundance of organisms in the *Lactobacillus* group in rats fed IMO was associated with an increased biodiversity, PCR-DGGE analysis with primers specific for the *Lactobacillus* group was performed (Fig. 3). The number of bands in faecal samples of rats fed IMO diet after 8 or 11 weeks of age was not significantly increased compared with those from the same time points in rats fed control diets, and cluster analysis did not clearly separate the banding patterns according to the diet. However, one specific band was observed almost exclusively in rats fed IMO. Four bands



**Figure 2** PCR-denaturing gradient gel electrophoresis of faecal samples of rats at 8 weeks of age (Panel a) and at 11 weeks of age (Panel b) fed IMO, inulin or control diet with lactobacilli-specific primers (Dice correlation coefficient, Tol, position tolerance 1%; Opt, optimization 0.5%). IMO, isomalto-oligosaccharides; INU, inulin; Cont, control; #, rat number.

differing in their abundance in IMO-fed and control animals were subjected to sequence analysis. Band a, identified as *L. reuteri*, was present in most animals. Band b, also identified as *L. reuteri*, was present in most samples from rats fed IMO but was essentially absent in rats fed the control diet. Band c, attributed to *L. animalis*, was present in few animals fed either IMO or control diets. Band d, also identified as *L. reuteri*, was present in most of the control rats but was absent in rats fed IMO diet (Fig. 3).

#### Short-chain fatty acids

SCFA were analysed in the faecal samples of rats fed IMO or inulin diet to determine the effect of nondigestible carbohydrates in colonic carbohydrate fermentation. Acetate, butyrate and propionate were the dominant end products of bacterial fermentation in faecal samples, whereas isobutyrate, isovalerate, valerate and caproate were minor components of SCFA. Inulin did not change faecal SCFA concentrations compared with rats on a control diet. However, IMO significantly decreased faecal acetate compared with rats fed control diet at 11 weeks of age. Total SCFA was also decreased in rats fed IMO diet, compared with control and inulin treatments. Propionate, butyrate, isobutyrate, isovalerate, valerate and caproate were not affected by dietary intervention treatments (Fig. 4a,b).

#### Discussion

Isomalto-oligosaccharides are produced commercially by transglycosylation of maltodextrins obtained by starch hydrolysis (Pan and Lee 2005). Starch hydrolysis and transglycosylation activity determine the degree of

polymerization as well as the ratio of  $\alpha$ -(1  $\rightarrow$  4) to  $\alpha$ -(1  $\rightarrow$  6) linkages of IMO. The composition of commercial IMO preparations differs substantially; products contain up to 40% disaccharides and a substantial proportion of maltose and glucose (Kohmoto *et al.* 1992; Kaneko *et al.* 1994; Yen *et al.* 2010). The IMO preparation employed in this study did not contain maltose and glucose, whereas 6'glucosylpanose and 6'6'diglucosylpanose were identified by enzymatic synthesis of oligosaccharides of the panose series (Dols *et al.* 1997; Galle *et al.* 2010). Isomaltose is hydrolysed by brush border enzymes in the intestinal epithelium, the digestibility of isomaltotriose and panose is unclear and longer-chain oligosaccharides are considered nondigestible (Kohmoto *et al.* 1992; Kaneko *et al.* 1995). The composition of commercial IMO preparations thus affects digestibility and their effect on the composition of intestinal microbiota.

This study assessed the influence of an IMO preparation on intestinal microbiota of rats by PCR-DGGE and qPCR targeting dominant bacterial groups of the rodent intestine (Benson *et al.* 2010). The effect of inulin on intestinal microbiota of rodents is well established (Kleessen *et al.* 2001; Meyer and Stasse-Wolthuis 2009; Gibson *et al.* 2010), and samples from rats fed inulin were therefore analysed using DGGE, and qPCR quantification of bifidobacteria and lactobacilli only. In keeping with previous studies, inulin significantly increased numbers of bifidobacteria from 5.8 to 6.5 log copy numbers, whereas the abundance of the *Lactobacillus* group remained unchanged (data not shown, Kleessen *et al.* 2001). Dietary IMO exhibited a remarkable specificity towards the stimulation of the *Lactobacillus* group. *Lactobacillus* species colonize the rodent forestomach (Walter 2008) and are a dominant bacterial groups in faecal microbiota of rodents

Bacterial group diet	16S rRNA genes [log (copy number) per g] before and after dietary intervention				Statistical significance of effect: <i>P</i>		
	5 weeks	8 weeks	11 weeks	Pooled SEM	Diet	Time	Diet × Time
<i>Lactobacillus</i> group							
Control	7.82	7.74	7.46	0.17	*	ns	*
IMO	8	8.14	8.57	0.17			
Bifidobacteria							
Control	6.07	6.36	6.08	0.09	*	*	*
IMO	6.13	5.84	5.51	0.09			
Cluster XIV							
Control	8.18	8.45	8.13	0.14	ns	ns	ns
IMO	8.48	8.26	8.29	0.14			
Cluster IV							
Control	8.10	8.30	8.11	0.14	ns	ns	ns
IMO	8.08	8.33	8.30	0.14			
<i>Bacteroides</i>							
Control	9.84	9.99	9.61	0.17	ns	ns	ns
IMO	9.82	9.61	9.58	0.17			
Cluster I							
Control	8.69	8.81	8.58	0.16	ns	ns	ns
IMO	8.34	8.59	8.35	0.16			
<i>Enterobacteriaceae</i>							
Control	8.02	8.03	7.34	0.16	ns	ns	*
IMO	7.94	7.98	7.54	0.16			
Cluster XI							
Control	7.43	7.18	6.93	0.15	ns	ns	*
IMO	7.60	6.99	7.04	0.15			
But CoA- CoA transferase							
Control	6.64	6.89	6.59	0.19	ns	ns	ns
IMO	6.77	6.53	6.54	0.19			
Total bacteria							
Control	10.10	10.15	9.85	0.14	*	ns	ns
IMO	10.44	10.28	10.28	0.14			

IMO, isomalto-oligosaccharides; ns, not significant; SEM, standard error of mean.

\*Significant ( $P < 0.05$ ).

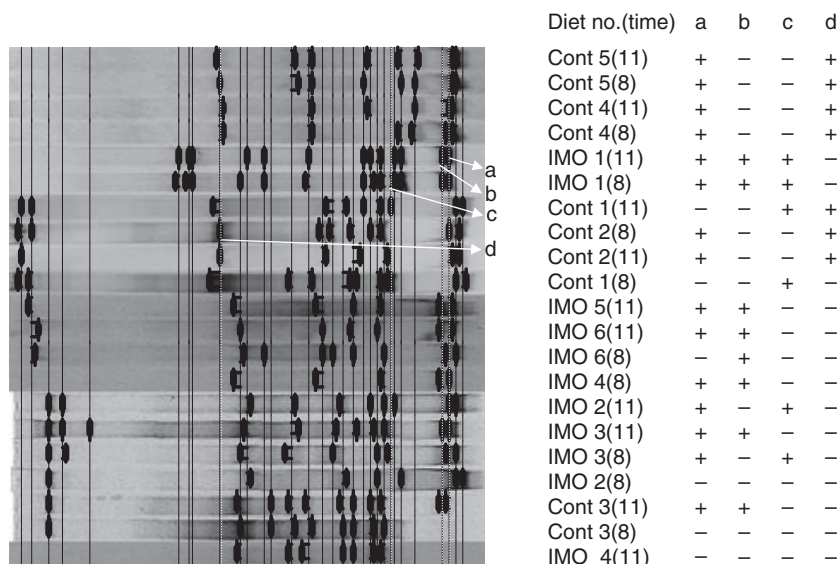
(Benson *et al.* 2010). Previous studies in rodent models also reported increased numbers of lactobacilli as a result of dietary intervention with IMO (Kaneko *et al.* 1990). *Lactobacillus animalis*, *Lactobacillus johnsonii* and *L. reuteri* are dominant *Lactobacillus* species in the rodent intestine. Other *Lactobacillus* spp., pediococci, *Leuconostoc* spp. and *Weissella* spp., which are also detected by the *Lactobacillus* group primers, are substantially less abundant (Walter 2008; Benson *et al.* 2010). Analysis of PCR-DGGE patterns generated with primers specific for the *Lactobacillus* group indicates that a strain of *L. reuteri* was specifically stimulated by IMO.

The number of bifidobacteria decreased in rats fed an IMO diet. However, previous reports indicated that IMO increased faecal bifidobacteria in BALB/c mice (Kaneko *et al.* 1990). This discrepancy can be attributed to the low numbers of bifidobacteria in rodent intestines, in contrast to lactobacilli, a stable and more abundant genus in rodent intestinal microbiota (Walter 2008). *Bifidobacteri-*

*um* spp. have extracellular enzymes hydrolysing polymeric  $\alpha$ -(1 → 4) and  $\alpha$ -(1 → 6)-linked glucans (Ryan *et al.* 2006). In contrast, enzymes for IMO metabolism in lactobacilli are unknown; however, lactobacilli have only few, if any, extracellular glycosyl hydrolases and preferentially metabolize disaccharides using intracellular hydrolases or phosphorylases (Gänzle *et al.* 2007). Lactobacilli are thus expected to preferentially metabolize low molecular weight IMO, whereas bifidobacteria are capable of hydrolysis of larger polymeric glucans. Similarly, lactobacilli and bifidobacteria exhibited preference towards metabolism of low and high molecular weight galacto-oligosaccharides, respectively (Gopal *et al.* 2001).

The total number of faecal bacteria increased in rats fed IMO diet compared with control diet. Other groups of bacteria were not affected by the IMO diet. Faecal *Enterobacteriaceae* decreased at 11 weeks of age in all animals irrespective of the diet. Inulin or fructo-oligosaccharides altered the numbers of organisms in the

**Table 2** Effect of diet, time and interaction of diet and time on faecal microbiota



**Figure 3** Denaturing gradient gel electrophoresis of faecal samples of rat at 8 and 11 weeks of age fed IMO or control diets run with lactobacilli-specific primers. Band assignment was carried out with BioNUMERICS with a 1% tolerance for the position of the band in the gels; presence (+) or absence (-) of bands that were identified by sequencing is shown to the right of the gel. IMO, isomalto-oligosaccharides; Cont, control; #, rat number, (age of rats in weeks). Selected bands were excised from the gel and identified by sequencing, a, *Lactobacillus reuteri*; b, *Lactobacillus reuteri*; c, *Lactobacillus animalis*; d, *Lactobacillus reuteri*.

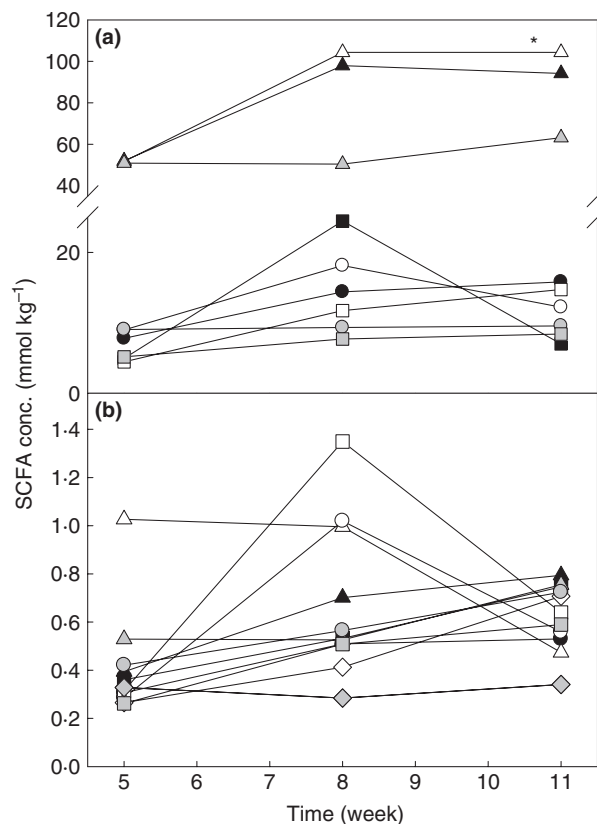
*C. coccoides* cluster as well as enterococci in the rat intestine. (Kleessen *et al.* 2001).

Differences between the microbiota of rats and humans relate to the physiology of the digestive tract. Rats have a forestomach with nonsecretory epithelium which is absent in humans; moreover, fibre fermentation occurs in the caecum in rats and in the colon in humans (Tiihonen *et al.* 2008). Bifidobacteria occupy narrow environmental niches compared with lactobacilli, belong to the dominant bacteria in humans, and colonize the intestine of infants shortly after birth (Biavati *et al.* 2000; Lamendella *et al.* 2008). In contrast, lactobacilli are abundant throughout the rodent digestive tract, but are much less abundant in human intestines (Walter 2008; Walter *et al.* 2008). Initial numbers of lactobacilli and bifidobacteria seem to be an important factor in stimulation of bacteria by NDO regardless of the host (Tiihonen *et al.* 2008). Despite these differences between rodent models and humans, the bifidogenic effect of fructans in rats (Kleessen *et al.* 2001; Rodriguez-Cabezas *et al.* 2010) matches results in human studies (Tuohy *et al.* 2001; Bouhnik *et al.* 2004; Whelan *et al.* 2005). Studies in humans to determine the effect of dietary IMO on intestinal microbiota relied predominantly on culture-dependent methods. Isomalto-oligosaccharides were bifidogenic at a dose of 10 g per day and stimulated lactobacilli in a dose-dependent fashion (Kohmoto *et al.* 1991; Kaneko *et al.* 1994). However, cultivation media for enumeration of bifidobacteria and

lactobacilli have a questionable selectivity (Mikkelsen *et al.* 2003; Simpson *et al.* 2004) and do not allow the quantification of other major bacterial groups in the intestine. The use of fluorescent-in-situ-hybridization with four group-specific probes indicated that dietary IMO stimulated bifidobacteria and particularly lactobacilli in elderly, constipated patients, whereas the abundance of *Bacteroides* spp. and *Clostridium* spp. decreased (Yen *et al.* 2010). In summary, an increased abundance of lactobacilli as observed in this study corresponds to human studies, whereas the effect of IMO on the abundance of bifidobacteria appears to differ between rodent models and human studies.

Although studies in humans demonstrated increased SCFA concentrations after consumption of up to 10 g per day of IMO (Chen *et al.* 2001; Yen *et al.* 2010), this study found decreased acetate and total SCFA in rats fed IMO. Ninety-five per cent of SCFA produced by intestinal bacteria are rapidly absorbed by the colon, only unabsorbed SCFA are detected in the faeces (Topping and Clifton 2001; Wong *et al.* 2006). IMO likely stimulates lactate and SCFA production in the upper intestine of rats, resulting in SCFA absorption in the intestine and decreased concentrations in faeces.

In conclusion, IMO exhibited a remarkable selectivity and particularly increased the abundance and biodiversity of lactobacilli. Structural differences in nondigestible carbohydrates substantially influence their effect on the



**Figure 4** Short-chain fatty acids concentration in the faecal samples of rats fed commercial inulin (black symbols), isomalto-oligosaccharides (grey symbols) or control diet (white symbols). Panel a: acetate (▲), propionate (●), butyrate (■), Panel b: isobutyrate (▲), isovalerate (●), valerate (■), caproate (◆), \*: significantly different,  $P < 0.05$  ( $n = 6$ ).

intestinal microbiota (Kleessen *et al.* 2001; Nilsson and Nyman 2005; Metzler-Zebeli *et al.* 2010). Studies in humans indicated that the dose required to achieve bifidogenic effects with IMO with a DP of 3–4 is lower when compared with IMO with a DP of 2–3 (Kaneko *et al.* 1994). The effect of polymers with mixed  $\alpha$ -(1  $\rightarrow$  4) and  $\alpha$ -(1  $\rightarrow$  6) linkages analogous to IMO, reuteran (Kralj *et al.* 1999), on intestinal microbiota has not been studied. Resistant starch with  $\alpha$ -(1  $\rightarrow$  4) linkages has specific bifidogenic properties both in rats and humans (Bouhnik *et al.* 2004). It may thus be possible to alter the specificity of IMO by modifying their degree of polymerization.

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