

Introduction

Isomalto-oligosaccharides are oligosaccharides with $\alpha(1\rightarrow6)$ and $\alpha(1\rightarrow4)$ glucosidic linkages. Isomaltose, isomalto-triose, panose and isomalto-tetraose are major compounds of commercial IMO (Kohomoto, 1991). Isomalto-oligosaccharides are partially digested in the small intestine and undigested portion is fermented by gastrointestinal microbiota. Isomalto-oligosaccharides are used as low-calorie sweeteners in food products. There are only a few studies on the effect of IMO on intestinal microflora. Human studies indicated that cell counts of bifidobacteria were increased when IMO were added to diet (Kaneko, 1994, Kohomoto, 1988, Kohomoto, 1991). Bifidogenic oligosaccharides such as inulin have laxative effects when taken in high dosages. Isomalto-oligosaccharides, however, are tolerated in high dosages without laxative effects. In this communication, the effect of IMO on change of microbiota and short chain fatty acids (SCFA) was investigated in the intestine of rats. Three groups of 6 littermates of F344 Fisher rats were fed IMO, inulin, or a control diet for 6 weeks. Inulin or IMO were added to commercial laboratory rodent diet (5004 lab diet) at a level of 8 g/kg body weight. Samples were taken at the start (5 weeks of age), 3 and 6 weeks after feeding inulin or IMO and compared to control diet. Intestinal microbiota were characterised by PCR- denaturing gradient gel electrophoresis (PCR-DGGE). Short chain fatty acids were measured by gas chromatography (GC). Specific bacterial taxa were quantified by quantitative PCR (qPCR).



Table 1- Effect of diet, time and interaction of diet and time on DNA copy numbers of bacteria

Bacterial group	Weeks			Pooled SEM	Statistical significance of effect: P		
	5 wk	8wk	11wk		Diet	Time	Diet x Time
Lactobacilli							
Control							*
IMO	7.82	7.74	7.46	0.17			
	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					ns	ns	ns
Control	8.18	8.45	8.13	0.14			
IMO	8.48	8.26	8.29	0.14			
Cluster IV					ns	ns	ns
Control	8.10	8.30	8.11	0.14			
IMO	8.08	8.33	8.30	0.14			
Bacteroides					ns	ns	ns
Control	9.84	9.99	9.61	0.17			
IMO	9.82	9.61	9.58	0.17			
Cluster I					ns	ns	ns
Control	8.69	8.81	8.58	0.16			
IMO	8.34	8.59	8.35	0.16			
Enterobacteriaceae					ns	ns	*
Control	8.02	8.03	7.34	0.16			
IMO	7.94	7.98	7.54	0.16			
Cluster XI					ns	ns	*
Control	7.43	7.18	6.93	0.15			
IMO	7.60	6.99	7.04	0.15			
But CoA- CoA transferase					ns	ns	ns
Control	6.64	6.89	6.59	0.19			
IMO	6.77	6.53	6.54	0.19			
Total bacteria					*	ns	ns
Control	10.10	10.15	9.85	0.14			
IMO	10.44	10.28	10.28	0.14			

* Significant (P< 0.05)
ns: not significant

Quantitative PCR was used to target major bacterial groups present in the intestine of rats. Total number of bacteria was increased by IMO diet compared to control diet. Lactobacilli were increased and bifidobacteria were decreased in rats fed IMO compared to control diet (Table 1). Lower number of bifidobacteria in rats fed IMO was not only related to diet, but was also influenced by time. Number of bifidobacteria was low unlike that in human colonic content. In rats, fermentation starts in fore-stomach. PCR- DGGE of fecal samples of rats fed IMO, inulin or control diets was run with 16s rRNA universal primers. Cluster analysis showed rats fed inulin almost separated from rats fed IMO or control diets at 8 weeks of age whereas there is no clear diet-based separation at 11 weeks of age. DGGE with lactobacilli-specific primers was performed to determine the effect of IMO on biodiversity. Biodiversity of lactobacilli was increased in addition to quantity at 8 and 11 weeks of age (Figure 1). Except a few, band b (*L. reuteri*) was absent in control samples. Band c (*L. animalis*) was mainly present in rats fed IMO.

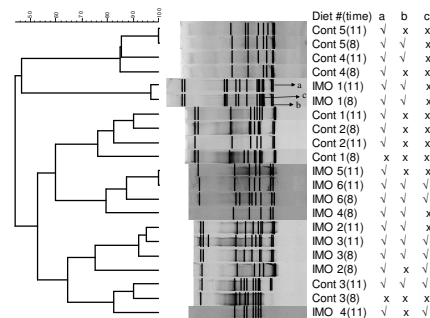


Figure 1- PCR- DGGE of fecal samples at 8 and 11 weeks of age fed IMO or control diets with lactobacilli- specific primers (Dice coefficient, Tol: 1%, Opt: 0.5%). Diet: IMO or control, #: rat number, a: *Lactobacillus reuteri* b: *Lactobacillus reuteri* and c: *Lactobacillus animalis* (excised from the gel and sequenced). Numbers in brackets are ages of rats in weeks. (v): band present, (x): band absent.

Results

Acetate, butyrate and propionate were dominant short chain fatty acids (SCFA) detected in fecal samples of rats fed IMO, inulin or control diets. Iso-butyrate, valerate, iso-valerate and coproate were minor SCFA. Among all SCFA, acetate decreased in the fecal samples of rats fed IMO (Figure 2)

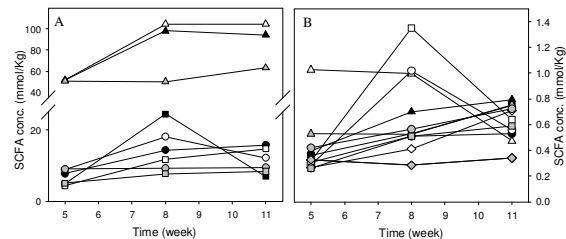


Figure 2- SCFA concentrations in fecal samples of rats fed IMO (grey), inulin (black) or control (white) diets at 5, 8 and 11 weeks of age measured with GC used iso-caproic acid as internal standard (n=6). Panel A: acetate (▲), propionate (●), butyrate (■). Panel B: iso-butyrate (▲), iso-valerate (●), valerate (■), coproate (◆).

Conclusions

- ❑ Lactobacilli and total number of bacteria were increased in rats fed IMO diet, compared to those fed a control diet.
- ❑ PCR- DGGE with lactobacilli-specific primers on fecal samples showed increased diversity at 8 and 11 weeks of age in rats fed IMO. *Lactobacillus animalis* was mainly present in rats fed IMO after 8 and 11 weeks, whereas it was absent in the majority of rats fed control diet.
- ❑ Acetate, butyrate and propionate were dominant SCFA detected in the fecal samples of rats fed IMO, inulin or control diets. Iso-butyrate, valerate, iso-valerate and coproate were present in smaller concentrations. Acetate was decreased in fecal samples of rats fed IMO compared to control diet.
- ❑ IMO selectively stimulated lactobacilli among other bacteria and increased their diversity. Therefore, IMO could be recognized as prebiotics in rats; however, the gastrointestinal system of rodents is different from that of human. Future experiments need to be performed in humans to determine the effect of IMO on intestinal microbiota.