Digestibility of branched and linear α-gluco-oligosaccharides *in vitro* and in ileal-cannulated pigs

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Abstract

Isomalto-oligosaccharides (IMOs) may promote health by modulating intestinal microbiota. We hypothesized that the proportion of α-(1→6) linkages in IMOs determines their digestibility. Ileal-cannulated pigs were fed diets containing IMO, IMO-DP3 with a greater DP and more α-(1 → 4) linkages, and digestible or resistant maltodextrins. Oligosaccharides were analysed by high-performance anion-exchange chromatography. Compared to IMO, IMO-DP3 contained more panose (18.6 vs. 10.3%) but less isomaltose (7.5 vs. 22.3%) and isomaltotriose (6.1 vs. 12.6%). The apparent ileal digestibility of dry matter were 3% greater for IMO-DP3 and digestible maltodextrin than resistant maltodextrin; the digestibility of IMO was not different from other oligosaccharides. Ileal propionate, isovalerate, and total SCFA was greater for IMO-DP3 and digestible maltodextrin than IMO. In conclusion, IMO was less digestible than IMO-DP3. Structural properties of IMOs are important determinants of their functional properties within the porcine digestive tract.

Keywords: Isomalto-oligosaccharides, prebiotic, ileal-cannulated pigs, intestinal microbiota, short chain fatty acids.

1. Introduction

Prebiotics provide substantial growth opportunities for the functional foods market (Yan, Hu, & Gänzle, 2018) and provide a tool to the food industry and consumers to increase the fibre content of western diets that are low in carbohydrate substrates for intestinal microbiota (Sonnenburg & Sonnenburg, 2014). Health benefits of prebiotics include the regulation of intestinal motility, satiety and glucose homeostasis, immune-modulation and improved epithelial barrier function, reduced colonic pH, ammonia concentration and pathogen exclusion, and improved absorption of minerals, particularly iron (Bindels, Delzenne, Cani, & Walter, 2015; Yan et al., 2018). Prebiotics were defined as dietary carbohydrates that confer health benefits through bacterial metabolism in the intestinal tract (Bindels et al., 2015). Regulatory definitions of prebiotics were not established but prebiotic carbohydrates with a degree of polymerization (DP) of 3 or higher are generally included in the widely accepted CODEX definition of dietary fibre (Howlett et al., 2010).

Beneficial health effects of dietary fibre including prebiotics are largely attributable to bacterial carbohydrate fermentation to the short chain fatty acids acetate, propionate, and butyrate, which reduce the luminal pH, serve as energy source for mucosal cells in the colonic mucosa, and elicit systemic effects through interaction with specific receptor proteins (Bindels et al., 2015; Yan et al., 2018). The consumption of non-digestible oligosaccharides, however, can also lead to adverse health effects. A high dose of non-digestible carbohydrates in the terminal ileum results in osmotic diarrhea and rapid microbial fermentation of oligosaccharides can induce bloating, flatulence, and intestinal discomfort (Oku & Nakamura, 2002; Yan et al., 2018). Prebiotic oligosaccharides are also included in the definition of FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols, which contribute to symptoms in about 30 – 50% of patients with the irritable bowel syndrome (Staudacher & Whelan, 2017).

Commercially available prebiotics include fructo-oligosaccharides (FOS), α-galacto-oligosaccharides, β-galacto-oligosaccharides (βGOS), isomalto-oligosaccharides (IMOs), and xylo-oligosaccharides. Among these, digestion, intestinal fermentation, and health benefits are best described for FOS and βGOS (Bindels et al., 2015); IMOs have the largest share of the global prebiotic market and are consumed predominantly in Asia (Goffin et al., 2011). IMOs are linear or branched oligosaccharides with a DP of two or higher and α-(1→6) and α-(1→4) linkages as predominant linkage type (Goffin et al., 2011). Commercial IMOs are produced predominantly from starch by hydrolysis to maltose followed by enzymatic conversion with transglucosidase, which forms α-(1→6) –linked oligosaccharides (Gangoiti et al., 2018; Pan & Lee, 2005). Alternative production methods include oligosaccharide synthesis with dextransucrase or α-4,6-glucanotransferase of lactic acid bacteria (Dobruchowska et al., 2012; Hu, Winter, Chen, & Gänzle, 2017). Owing to the different production methods, commercial and experimental IMOs differ substantially with respect to their composition, linkage type, and degree of polymerization (Madsen, Stanley, Swann, & Oswald, 2017; van der Zaal, Schols, Bitter, & Buwalda, 2018). Rodent models and clinical trials support prebiotic properties of commercial and experimental IMOs but also demonstrate that many commercial IMOs are partially digestible (Gu et al., 2018; Hu et al., 2017; Kohmoto et al., 1992; Oku & Nakamura, 2002). In addition, the validated enzymatic assay for quantification of dietary fibre (McCleary, 2019) does not account for most IMOs because fungal amyloglucosidase hydrolyses both α-(1→6) and α-(1→4) linked oligosaccharides (Hu et al., 2017; Tanabe, Nakamura, & Oku, 2014). We hypothesized that an increasing proportion of the proportion of α-(1→6) linkages in IMOs decreases their digestibility. The present study therefore aimed to achieve a qualitative and quantitative assessment of the digestibility of two commercial IMO preparations in an ileal-cannulated swine model. Maltodextrins and resistant maltodextrins were used as digestible and non-digestible controls. The data obtained *in vivo* were compared to an *in vitro* assay that employs intestinal brush border enzymes rather than amyloglucosidase.

2. Materials and methods

2.1. Materials

Commercial isomaltooligosaccharide preparations VitaFiber® (IMO) and VitaFiber™ (IMO-DP3) were provided by BioNeutra Inc. (Edmonton, Canada). Resistant maltodextrin Fibersol ®-2 was purchased from Matsutani America, Inc. (Clinton, Iowa, USA). Digestible maltodextrin was provided by Protein Co. (Quebec City, Canada). Mono-, di- and tri-saccharides were purchased from Sigma Aldrich.

2.2. Separation of oligosaccharides by HPAEC-PAD

Qualitative analysis of oligosaccharides was performed with high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Hu et al., 2017). In brief, samples were diluted with milliQ water and separated on a Carbopac PA20 column coupled to an ED40 chemical detector (Dionex, Oakville, Canada). Samples were eluted with water (A), 0.2M NaOH (B) and 1 M Na Acetate (NaOAc) (C) with the following gradient: 0 min, 68.3% A, 30.4% B and 1.3% C; 25 min, 54.6% A, 30.4% B and 15.0% C; 28min, 50% A and 50% C; 31 min, 10% A, 73% B and 17% C; followed by re-equilibration. The HPAEC-PAD assay was calibrated for quantification of isomaltose, isomaltotriose, maltose, and panose, and with isomaltose- and panose series oligosaccharides that were prepared with DsrM (Hu et al., 2017). Quantification of oligosaccharides was also achieved by on an Agilent 1200 series LC system (Agilent Techologies, Palo Alto, CA) equipped with a Supelcosil LC-NH_2 column (250 mm×4.6 mm, 5 µm, Sigma Aldrich) and coupled to a RI detector. Samples were eluted with acetonitrile/water at 70: 30 (v/v) at a flow rate of 0.8 mL min^{-1} and 30 °C. The method was calibrated with glucose, maltose, isomaltose, panose and isomaltotriose and linear maltodextrins. The separation of oligosaccharides was essentially based on their DP and irrespective of the linkage type. The concentration of oligosaccharides for which standards were not available was calculated by using the average response factor for all standards.

2.3. *In vitro* digestion of oligosaccharides

The digestibility was determined with brush border enzymes from the rat intestinal mucosa (Sigma) (Tsunehiro, Okamoto, Furuyama, Yatake, & Kaneko, 1999). The activity of enzymes in the rat intestinal extract matches the activity of human brush border enzymes (Oku, Tanabe, Ogawa, Sadamori, & Nakamura, 2011). Samples were dissolved in 18 MΩ water to 10 g/L, followed by addition of an equal volume of sodium maleate buffer (pH 6.0) containing 1% (w/v) rat enzymes. Digesta were agitated with glass beads at 37 °C for 4 h and the reaction was stopped by heating at approximately 100 °C for 5 min. Samples were cooled on ice, centrifuged, and the glucose concentration in the supernatant was measured with a glucose oxidase kit (Megazyme, Bray, Ireland). The digestibility of oligosaccharides was expressed relative to the digestibility of maltose as follows:

$$= \%\ \text{digestibility} = 100x\frac{([glucose]_{sample,4h} - [glucose]_{sample,0h})}{([glucose]_{maltose,4h} - [glucose]_{maltose,0h})},$$

where [glucose] represent glucose concentrations in mmol / l after 0 and 4 h of digestion.

2.4. Animal experiments

Experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock. Pigs were handled at the Swine Research and Technology Centre of the University of Alberta in accordance according to guidelines of the Canadian Council on Animal Care (Canadian Council on Animal Care, 2009).

Eight crossbred barrows (initial body weight (BW) 31.8 ± 2.1 kg; Duroc × Large White/Landrace F_1; Genex Hybride; Hypor, Regina, SK, Canada) were surgically fitted with a T-cannula at the distal ileum, approximately 5 cm prior to the ileocecal squincter. Cannula dimension, surgical procedures and pre- and postoperative care were performed as described (Li, Sauer, & Fan, 1993; Sauer & Ozimek, 1986). After 10 d of recovery, pigs were switched to the first assigned experimental diet. Pigs were housed individually in metabolism pens (1.2 m wide, 1.5 m long, and 0.95 m high) with polyvinyl chloride walls

with windows (0.3 × 0.3 m) and slatted flooring allowing freedom of movement in a thermo-controlled room (21 ± 5°C). All pens were equipped with a stainless-steel feeder attached to the front of the pen, and a cup drinker beside the feeder to ensure free access to water.

2.5. Diets and experimental procedures

Pigs were randomly allocated to four corn starch-casein based diets formulated to include 3% of Vitafiber® (IMO), Vitafiber®-DP3 (IMO-DP3), digestible maltodextrin or non-digestible resistant maltodextrin. Diets were formulated to meet or exceed nutrient requirement for growing pigs (NRC, 2012) (Table 1).

The study was conducted as 4 (period) × 4 (diet) complete Latin square design to obtain 8 observations/diet. Each experimental period (11 days) consisted of a 7 d acclimation to the experimental diet, followed by a 2 d collection of faeces and a 2 d collection of ileal digesta. The daily feed allowance was adjusted to 3.0 times the maintenance requirement for dietary energy (DE) (3.0 × 110 kcal of DE/kg of $BW^{0.75}$; NRC, 2012) via 2 daily feedings at 08:00 and 15:00.

Faeces were collected continuously for 48 h by plastic bags attached to the skin around the anus (van Kleef, Deuring, & van Leeuwen, 1994). Digesta samples were collected between 08:00 to 16:00 h for 2 d using plastic bags containing 15 ml of 5% formic acid attached to the opened cannula barrel with rubber band. Plastic bags were replaced as soon as filled or after every 20 min. Faeces and digesta samples were pooled for each pig within the experimental period, stored at -20°C and thawed, homogenized, subsampled, and freeze-dried prior to analysis.

2.6. Chemical analysis of diets, faeces and digesta.

Diets, lyophilized faeces and digesta were ground in a centrifugal mill (Retch model ZMI; Brinkman Instruments) using a 1-mm screen. Diets, faeces, and digesta were analysed for gross energy (GE) by using an adiabatic bomb calorimeter (model 5003; Ika-Werke GmbH and Co.), moisture (method 930.15; AOAC, 2006), and crude protein by oxidation (N × 6.25 GP-428 N Determinator; Leco Corporation;

method 984.13 A-D). Diets, digesta, and faeces were analysed for titanium dioxide (TiO_2) as described (Myers, Ludden, Nayigihugu, & Hess, 2004). The apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrients in diets were calculated using the index method based on TiO_2 data (Adeola, 2000):

$$ATTD\ or\ AID\ (\%) = 100 - \left(100 \frac{[TiO_2]_{diet}}{[TiO_2]_{faeces\ or\ digesta}}\right)\left(\frac{[nutrient]_{faeces\ or\ digesta}}{[nutrient]_{diet}}\right)$$

where [TiO_2] and [nutrient] represent concentrations in[g / kg] of TiO_2 and the nutrient in question in the diet and digesta or faeces as indicated.

2.7. Qualitative analysis of oligosaccharides and quantification of glucose, α-glucans, lactate, and short chain fatty acids.

For qualitative analysis of oligosaccharides, freeze-dried ileal digesta were extracted with 18 MΩ water at 80°C for 1h and then cooled to room temperature, and centrifuged at 10000 rpm for 20 min. The pellets were re-extracted with water again and both supernatants were collected and then diluted (1:20). After filtration through a 0.22 µm filter, the oligosaccharides in the supernatant were analysed by HPAEC-PAD. Results are reported as the average signal from all chromatograms obtained from animals with the same diet.

For quantification of glucose and lactate, freeze-dried ileal digesta were extracted with water; proteins were precipitated by addition of perchloric acid to a concentration of 5%, followed by overnight incubation at 4°C and centrifugation to remove precipitates. Glucose and lactate were quantified on an Aminex HPX-87H column (Bio-Rad, Mississauga, Canada) eluted with 5mM H_2SO_4 at a temperature of 70^{o}C and a flow rate of 0.4 ml min^{-1}. Glucose was quantified with a refractive index (RI) detector and lactate was quantified with a UV (210 nm) detector. Ileal digesta were also analysed after acid hydrolysis of α-glucans with 1 M H_2SO_4 at 80 °C for 2 h, followed by addition of 2 M KOH. After centrifugation,

the supernatant was diluted and the glucose content after hydrolysis was determined by HPLC with a RI detector. Each sample was analysed with two technical repeats.

The concentration of glucose was expressed as (g glucose / kg feed dry matter) after correction for the concentration of the indigestible marker TiO_2 with the following formula:

$$glucose\ [\frac{g}{kg\ DM_{feed}}] = (glucose)[\frac{g}{kg\ DM_{digesta}}](\frac{1}{DM\ digesta})[\frac{g}{gDM}](\frac{TiO_{2digesta}}{TiO_{2feed}}),$$

where DM indicates the dry matter content; TiO_2 indicates the concentration of the indigestible marker in g / kg, and the subscripts "feed" and "digesta" indicate the respective concentrations in feed and ileal digesta.

Short chain fatty acids were quantified from frozen ileal digesta or faecal samples by gas chromatography (Htoo et al., 2007). In brief, 0.8 mL clear supernatant from digesta was mixed with 200 µL phosphoric acid (25%) and 200 µL isocaproic acid solution (22 mM) as internal standard. Faeces were combined with phosphoric acid at 4:1 and mixed with internal standard. Samples were measured by a Varian model 3400 Gas Chromatography (Varian, Walnut Creek, CA) with a Stabilwax-DA column (30 m × 0.53 mm i.d.; Restek, Bellefonte, PA). The concentrations of short chain fatty acids or lactate in digesta are reported per mL or gram digesta and the concentrations of short chain fatty acids in faeces are reported per gram feces.

2.8. Sequencing of 16S rRNA gene sequence tags

Seuquencing of 16SrRNA gene sequence tags was performed as described (Zhao et al., 2018). In brief, the V5-V6 variable region of 16S rRNA was sequenced on the Illumina Miseq platform by service of the University of Minnesota Genomics Center (Minneapolis,MN). Sequence analysis was based on the QIIME pipeline (MacQIIME1.9.1-20150604) (Caporaso et al., 2010); UCLUST (Edgar, 2013) was used for clustering of operational taxonomic units (OTU) using the Green Genes database with 97% similarity threshold. OTUs with less than 0.005% abundance were discarded. After quality control, 2,853,554

sequences with an average length of 301 bp, corresponding to 89,173 sequences per sample, were analysed. Bray Curtis distance based principle coordinates analysis was performed to quantify the compositional dissimilarity between samples.

2.9. Statistical analysis

The *in vitro* digestibility was determined relative to maltose in triplicate experiments; results are presented as means ± SEM. Data analysis was performed with Linear Regression model (PASW, Statistics 18.0, Quarry Bay, HK, China) and assessed at a 5% probability of error ($P<0.05$). The *in vivo* data was determined at two technical repeats and the results are expressed as means ± standard error of the mean. The data were analysed using the PROC MIXED of SAS (version 9.4; SAS Institute) with analysis of variance and the Tukey post hoc analysis, and assessed at a 5% probability of error ($P<0.05$). Diet and period were considered as the fixed effects with pig and period as random effects. Analysis of ileal digesta and fecal samples was carried out with two technical repeats; results are expressed as means ± standard error of the mean, or as representative chromatograms.

Nutrient digestibility data are presented as least-squares means and pooled SEM. Data were analysed using PROC MIXED of SAS (version 9.4; SAS Institute). Normality was confirmed by using PROC UNIVARIATE. Diet was used as fixed effect with pig and period as random effects. Multiple comparisons between least-squares means were tested by the PDIFF statement with Tukey adjustment. Significance was determined at $P < 0.05$, with $P < 0.10$ considered as a trend.

3. Results.

3.1 Composition of the α-gluco-oligosaccharides for *in vitro* and *in vivo* digestibility

Commercial IMO, IMO-DP3, digestible maltodextrin and resistant maltodextrin were used to investigate the *in vitro* and *in vivo* digestibility of the α-gluco-oligosaccharides. Resistant maltodextrin are composed of branched oligosaccharides that could not be assigned to standards used in this study (Fig. 1).

Maltodextrin were composed of linear α-(1→4) linked oligosaccharides with a DP of 2 to 9. The commercial preparations of isomalto-oligosaccharides contained isomalto-series oligosaccharides and panose-series oligosaccharides. The concentration of carbohydrates in IMO were as follows (% w/w): isomaltose, isomaltotriose, maltose and panose; DP was measured by HPLC-RID as follows (% w/w): DP2 (26), DP3 (21), DP4 (13.5), DP5 (6.6), DP6 (4.1), DP7 (2.2) and DP8 (1.3). The main composition of IMO-DP3 measured by HPAEC-PAD was as follows (% w/w): isomaltose (7.5), isomaltotriose (6.1±2.6), and panose (18.6). Compared with IMO, the maltose peak in IMO-DP3 was absent and the panose content of IMO-DP3 increased but the isomaltose and isomaltotriose content of IMO-DP3 decreased. IMO-DP3 thus contained a higher proportion of α(1→4) glycosidic bonds

3.2 *In vitro* digestibility of the α-gluco-oligosaccharides

The *in vitro* digestibility of the oligosaccharide preparations was determined with a method employing brush border enzymes (Fig. 2). Maltose served as digestible control. The method for assessment of *in vitro* digestibility indicated that digestibility of oligosaccharides decreased in the order maltose > maltodextrin and IMO-DP3 > IMO > resistant maltodextrin. The *in vitro* digestibility of IMO was lower than maltodextrin ($P<0.05$) but IMO-DP3 were as digestible as maltodextrins. The comparison of the *in vitro* digestibility of IMO-DP3 and IMO indicates that the *in vitro* digestibility of IMO with more isomaltose-series oligosaccharides is lower than IMO-DP3 with more panose-series oligosaccharides.

3.3 Nutrient digestibility *in vivo* in ileal-cannulated swine.

Digestibility of macronutrients was quantified by determination of the apparent ileal digestibility and apparent total tract digestibility of dry matter, crude protein, and gross energy (Table 2). The addition of resistant maltodextrins decreased apparent ileal digestibility of dry matter and gross energy relative to digestible maltodextrins (Table 2). Hindgut fermentation of dry matter was changed significantly by the diets; hindgut fermentation of gross energy tended (P=0.052) to be different. The addition of IMO-DP3 did not alter apparent ileal digestibility and apparent total tract digestibility relative to digestible

maltodextrins (Table 2). The apparent ileal digestibility and apparent total tract digestibility of diets containing IMO was not different from either digestible or resistant maltodextrins, confirming the intermediate digestibility observed *in vitro* (Table 2 and Fig. 1).

3.4 Composition of faecal microbiota and bacterial metabolites in faecal samples.

An altered composition of faecal microbiota and an increased concentration of faecal short chain fatty acids (SCFA) are often used as primary outcome of prebiotic intervention studies. The impact of the diet on faecal microbiota in ileal cannulated swine was assessed by high throughput sequencing of 16S rRNA sequence tags; the global microbial composition in faecal samples was assessed by principal coordinate analysis (Fig. 3). Samples from pigs fed different diets clustered together, indicating that none of the diets had a substantial impact on the overall composition of faecal microbiota (Fig. 3). A genus and family level assessment of the microbial diversity demonstrated that faecal samples from pigs fed diets with resistant maltodextrin contained less DNA from *Coprococcus* and bifidobacteria when compared to pigs fed the digestible control; *Christensellaceae* and *Blautia* increased in response to resistant maltodextrins (Table S1 of the online supplementary material). The abundance of these taxa was not significantly altered by inclusion of IMO in the diet (Table S1).

The impact of α-gluco-oligosaccharides on faecal microbiota was also assessed by quantification of SCFA (Figure 4). Acetate, propionate and butyrate were the major metabolites; branched chain fatty acids, valerate and caproate were minor components, and lactate was not detected (Fig. 4). The concentration of acetate, propionate and butyrate was not influenced by the diet; valerate and isovalerate concentrations were reduced in samples from pigs fed IMO diets relative to resistant maltodextrins (Fig. 4). Overall, the analysis of faecal samples demonstrated that the supplementation of diets with 3% digestible or indigestible α-gluco-oligosaccharides has only a minor effect on composition and activity of faecal microbiota.

3.5 Concentration of glucose and α-glucans in ileal digesta.

To determine the ileal digestibility of α-glucans, ileal samples were analysed with respect to oligosaccharide profiles, the concentration of free glucose, and the overall content of α-glucans. Oligosaccharides profiles in the ileal digesta from four diets were determined to confirm the presence of oligomeric α-glucans (Fig. 5); the analysis of digesta of animals fed digestible maltodextrins served as control. The peak patterns in ileal digesta (Fig. 5) differed from peak patterns in the oligosaccharide preparations (Fig. 1), demonstrating that oligosaccharides were modified during small intestinal digestion or small intestinal microbiota. Ileal samples from pigs fed digestible maltodextrin or IMO-DP3 showed similar patterns with only few peaks visible in ileal digesta (Fig. 5). All ileal samples from pigs fed resistant maltodextrins showed a characteristic pattern of peaks, indicating that resistant maltodextrins partially resisted hydrolysis in the small intestine. Digesta samples from pigs fed IMO showed peaks at 10.9 min and 13.6 min, corresponding to isomaltose and isomaltotriose standards, suggesting that IM2 and IM3 partially resisted hydrolysis in the small intestine. Oligosaccharide profiles thus conformed to the *in vitro* digestibility (Fig. 2).

Glucose content in ileal digesta were quantified with or without hydrolysis of α-glucans with H_2SO_4 with a protocol that hydrolyses α-glucans but not cellulose. The use of TiO_2 as indigestible marker allowed comparison of the content of α-glucans in ileal digesta to their concentration in the diet, which contained 723 g / kg digestible starch and 30 g / kg of oligosaccharides (Tab. 1). High levels of free glucose were detected only in ileal digesta of pigs fed IMO, indicating that IMO are digested or fermented in the terminal ileum. Because the site of sampling was the site of fermentation of IMO. small differences in digesta transit likely result in relatively large differences in IMO digestion and hence a high variability between samples (Fig. 6). The content of total α-glucans in the digestible control and in samples from pigs fed IMO-DP3 was less than 2 g/kg (Fig. 6), demonstrating that more than 99% of the starch and digestible oligosaccharides were hydrolysed in the small intestine. The concentration of α-glucans was

higher (P<0.05) in samples from pigs fed resistant maltodextrins than those in samples with maltodextrins; the glucan content in ileal digesta, 13 g / kg, corresponds to about 50 % of the oligosaccharide content in the diet (30 g / kg, Fig. 6 and Tab. 1). Ileal digesta from pigs fed IMO showed intermediate values and a trend (0.05<P<0.1) for a higher content of α-glucans when compared to maltodextrins.

3.6 Quantification of microbial metabolites in ileal digesta.

Bacterial fermentation of dietary glycans is initiated in the terminal ileum. Ileal microbiota are dominated by facultative aerobic microbiota that favour utilization of mono- and disaccharides over hydrolysis of polysaccharides (Regmi, Metzler-Zebeli, Gänzle, van Kempen, & Zijlstra, 2011; Zoetendal et al., 2012). To assess whether bacterial fermentation contributes to the digestion of α-glucans in the small intestine, the contents of SCFAs and lactate in ileal digesta were quantified (Fig. 7). Lactate was the most abundant metabolite, with concentrations ranging from about 60 – 80 µmol / g digesta dry weight; acetate concentrations ranged from 70 – 90 µmol / mL wet digesta. The concentration of microbial metabolites was highest when pigs were fed digestible maltodextrins or IMO-DP3. A reduced concentration of bacterial metabolites was observed in pigs that were fed IMO or resistant maltodextrins (Fig. 7). The difference was significant for lactate, propionate, isovalerate, and the sum of propionate, butyrate and valerate. These data suggest that digestible carbohydrates also increase the substrate supply for microbial fermentation in the distal ileum.

Discussion

Swine as model for carbohydrate digestion. Swine are more suitable than other non-primate models for assessment of carbohydrate digestion in humans; they are of comparable size, consume a comparable diet, and the anatomy and physiology of the swine intestinal tract is similar to humans (Heinritz, Mosenthin, & Weiss, 2013). Nevertheless, several factors confound the comparison of carbohydrate

digestion in swine and humans (Champ, Martin, Noah, & Gratas, 1999). When compared to humans, swine have a higher capacity to digest dietary fibre through bacterial fermentation in the caecum, which is absent in humans. The microbial consortia of humans and swine intestinal microbiota that carry out colonic carbohydrate fermentation differ (Wang et al., 2019; Ze, Duncan, Louis, & Flint, 2012). In addition, non-secretory epithelia of the swine stomach are densely colonized by lactobacilli, which remain present in high cell counts and metabolically active throughout gastro-intestinal transit (Bach Knudsen, Hedemann, & Lærke, 2012; Leser et al., 2002; Walter, 2008). *L. reuteri* and other swine intestinal lactobacilli preferentially metabolize maltose and IMOs with DP 2 and 3 (Hu, Ketabi, Buchko, & Gänzle, 2013) but the abundance of active bacterial cells and hence the capacity to metabolize di- and trisaccharides in the human small intestine is much lower (Walter & Ley, 2011). In human nutrition, metabolic activity of lactobacilli is achieved in food fermentations but not in the intestinal tract (Gänzle, 2019; Højberg, Canibe, Knudsen, & Jensen, 2003).

The use of a highly purified diet in this study facilitated the qualitative and quantitative analysis of α-glucans in ileal digesta but likely also influenced starch digestion when compared to a human diet. Corn starch is highly digestible when used in a purified diet with a low content of fibre but even moderate inclusion of fibre in the diet decreases starch digestibility and increases ileal dry matter flow into the large intestine (Metzler-Zebeli et al., 2010; Regmi et al., 2011; Tian et al., 2017). Accordingly, rodent studies observed functional benefits of prebiotics when added to an undefined chow but not when added to a chemically defined and highly digestible diet (Koleva, Ketabi, Valcheva, Gänzle, & Dieleman, 2014). In summary, the pig model employed in the present study likely over-estimates the overall digestibility of α-glucans when compared to humans but results can be used for qualitative comparison of different α-glucans.

Digestibility of α-glucans in swine. Pancreatic amylases degrade α-(1 → 4)-linked linear glucans to maltose (Bach Knudsen et al., 2012; Zijlstra, Jha, Woodward, Fouhse, & van Kempen, 2012). Linear α-

(1 → 4)- and α-(1 → 6)-linked gluco-oligosaccharides are hydrolysed by brush border sucrase / isomaltase and maltase / glucoamylase activities (Hooton, Lentle, Monro, Wickham, & Simpson, 2015; Oku et al., 2011). Oligosaccharide hydrolysis by brush border enzymes is limited by diffusion and therefore dependent on the molecular weight as well as the linkage type of α-glucans (Gangoiti et al., 2018; Hooton et al., 2015; Kaulpiboon, Rudeekulthamrong, Watanasatitarpa, Ito, & Pongsawasdi, 2015), however, the digestibility of individual components of commercial IMOs are poorly documented. Microbial fermentation substantially contributes to oligosaccharide digestion in the small intestine of humans (Zoetendal et al., 2012), swine (Tian et al., 2017), and milk-fed calves (Gilbert et al., 2015). The present study assessed the digestibility of α-gluco-oligosaccharides through a comprehensive analysis of ileal digesta with respect to the apparent ileal digestibility of dry matter and gross energy, detection of oligosaccharides, and the quantification of bacterial metabolites (Tab. 2, Fig. 5 and Fig. 6). In keeping with prior studies (Regmi et al., 2011; Tian et al., 2017), corn starch and maltodextrins were highly digestible as demonstrated by the high apparent ileal digestibility of dry matter and gross energy, the low content of glucose and gluco-oligosaccharides in ileal digesta, and the high concentration of bacterial metabolites. About 15 g / kg DM feed of resistant maltodextrins, corresponding to about 50% of the amount added to feed, escaped small intestinal digestion and fermentation, this consistently reduced the apparent ileal digestibility of dry matter, and increased the concentration of α-glucans in ileal digesta (Fig. 5 and 6). Resistant maltodextrins also reduced the ileal concentration of bacterial metabolites. This result is counter-intuitive but matches prior observations with different levels of resistant starch (Regmi et al., 2011) or pectin (Tian et al., 2017) in swine diets, and likely reflects the metabolic focus of swine ileal microbiota on the starch hydrolysis products maltose and glucose (Gänzle & Follador, 2012; Hu et al., 2013). Data for IMO consistently document intermediate digestibility. Owing to the small differences between digestible and indigestible controls and the large variability between pigs, the differences were not significant for the apparent ileal digestibility of gross energy and dry matter and observed only as a

trend ($0.1 > P > 0.05$) for the α-glucan content of ileal digesta. The oligosaccharide patterns and the concentration of microbial metabolites in ileal digesta consistently or significantly, respectively, differed from the digestible control. IMO-DP3 were as digestible as the digestible control, which likely reflects the higher content of α-(1→4) linked oligosaccharides including panose-series oligosaccharides in IMO-DP3 versus IMO and the slower hydrolysis of α-(1→6) linked oligosaccharides by brush border enzymes (Gangoiti et al., 2018). In keeping with the rapid digestion or fermentation of α-gluco-oligosaccharides in the distal ileum or the proximal large intestine, indigestible maltodextrins or IMO had little impact on composition of faecal microbiota.

Digestibility of α-glucans in humans and rodent models. Data on IMO digestibility obtained in the current study in a swine model confirm and extend past studies in human and rodent models. Prebiotic properties of IMOs are not as well documented as those of FOS or β-GOS (Goffin et al., 2011) but are supported by a substantial number of animal and human studies. In rats, the same commercial IMO preparation increased the number of faecal lactobacilli and the total number of bacterial in faecal samples (Ketabi, Dieleman, & Gänzle, 2011). In keeping with the partial digestibility of IMO, the dose of IMO that was required to achieve beneficial physiological effects was higher when compared to FOS (Koleva et al., 2014; Lin et al., 2012) and depended on the molecular weight and linkage type (Iwaya et al., 2012). Human studies also support the finding that IMOs are partially digested by small intestinal enzymes and partially fermented by intestinal microbiota. Intake of 10 g/day of IMOs with DP2 or 5 g/day of IMOs of DP3 increased the number of bifidobacteria in faeces, suggesting that different components of IMOs have different digestibility in the small intestine (Kaneko et al., 1994). The digestibility of a commercial IMOs preparation was also assessed by using ^{13}C labels substrates, followed by quantification of $^{13}CO_2$ in breath, blood glucose, and blood insulin (Kohmoto et al., 1992). IMOs were partially digestible and increased breath $^{13}CO_2$, serum glucose and serum insulin but were also partially fermented in the large intestine (Kohmoto et al., 1992). Only few clinical trials document functional benefits of IMOs in clinical

trials. Supplementation of 10 g / day IMOs relieved constipation in elderly patients (Yen, Tseng, Kuo, Lee, & Chen, 2011).

Implications for food applications. The comparison of the digestibility of different IMOs provides consistent *in vivo* data on the digestible and indigestible portions in these products and complements currently available and partially conflicting data from humans and rodent models. In particular, current and proposed methods for quantification of dietary fibre in foods employ amyloglucosidase, which rapidly hydrolyses α-(1→6)-linked glucans and thus over-estimates the digestible portion of IMOs (McCleary, 2019; Tanabe et al., 2014). The digestibility of IMOs as predicted by an assay employing commercially available rat brush border enzymes, which have similar hydrolytic activities as human brush border enzymes (Oku et al., 2011), was in good agreement with *in vivo* data, and may be useful for analysis of the estimation of the content of non-digestible oligosaccharides in foods. A similar agreement of *in vitro* and *in vivo* data was also reported for fructo-oligosaccharides and other dietary oligosaccharides (Ferreira-Lazarte, Olano, Villamiel, & Moreno, 2017; Ohtsuka et al., 1990)Improved data on the digestibility of IMOs will also inform their use and dosage for future clinical studies to establish physiological benefits in humans.

Rapid ileal or colonic fermentation of non-digestible oligosaccharides results in osmotic diarrhea and intestinal bloating and a threshold dose of about 0.3 g / kg and day is exceeded (Oku & Nakamura, 2002; Yan, Hu, & Gänzle, 2018). Adverse effects of non-digestible oligosaccharides, particularly fructans, have gained increasing relevance for food product development after their contribution to the irritable bowel syndrome was established (Staudacher & Whelan, 2017). Owing to the partial digestibility of IMOs, their threshold dose for adverse symptoms is higher when compared to FOS or βGOS (Oku & Nakamura, 2002; Yan et al., 2018).

In conclusion, this study documented that an IMO preparation with a high proportion of α-(1→6) linkages was only partially digested by brush border enzymes and fermented by small ileal and colonic microbiota; an increase of the proportion of α-(1→4) linkages in IMO-DP3 increased digestibility to a level that was indistinguishable from digestible maltodextrins. The *in vitro* assay employing brush border enzymes reflected the relative digestibility of maltodextrins, resistant maltodextrins, and IMO or IMPO-DP3. The linkage type and degree of polymerization is readily manipulated as a multitude of carbohydrate-active enzymes have been employed for production of IMOs (Casa-Villegas, Marín-Navarro, & Polaina, 2018; Dobruchowska et al., 2012; Gangoiti et al., 2018; Hu et al., 2017). Only few of these enzymes, particularly GH13 family glycosyl hydrolases and dextransucrases, are currently employed for industrial production of IMOs (Madsen et al., 2017). The availability of multiple enzymes for synthesis of IMOs in combination with a validated *in vitro* assay to estimate their digestibility will nevertheless allow fine-tuning of sweetness, digestibility, and the potential for adverse symptoms through enzymatic manufacturing processes that are tailored for specific applications or populations.

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612 **Figure legends**

613 **Figure 1.** Composition of commercial and experimental IMO preparations. Peaks identified by HPAEC-
614 PAD are indicated as follows: M2, maltose; M3; maltotetraose, M4, M5 etc, maltodextrins with DP4,
615 DP5 etc; IM2, isomaltose; IM3, isomaltotriose, 6-glucosyl panose; α-D-glc*p*-(1→6)-panose; 6'6'-
616 diglucosyl-pannose, α-D-glc*p*-(1→6)-α-D-glc*p*-(1→6)-panose. Chromatograms were offset by 500 nC.

617 **Figure 2.** *In vitro* digestibility of α-glucans as measured by rat intestinal enzymes. Digestibility is
618 expressed as % digestibility relative to maltose. Bars without a common superscript differ ($P<0.05$).

619 **Figure 3.** Bray Curtis based principle coordinate analysis (PCoA) of the fecal microbiota of ileal
620 cannulated pigs. Each dot represents an individual sample fed with digestible maltodextrin (n = 8, Δ),
621 IMO (n = 8, ■), IMO-DP3 (n= 8, ▲) and resistant maltodextrin (n = 8,□).

622 **Figure 4. .** Short chain fatty acid concentrations in the faeces of swine. Within each analyte, bars without
623 a common superscript differ ($P<0.05$).

624 **Figure 5.** Separation of oligosaccharides from ileal digesta of swine fed digestible or non-digestible
625 α-glucans by HPAEC-PAD. Chromatograms represent the average of eight samples each, and were offset
626 by 50 nC.

627 **Figure 6.** Content of total α-glucans and of free glucose in ileal digesta. The glucose concentration was
628 determined directly; the content of total α-glucans was determined after hydrolysis of glucans with 2N
629 H_2SO_4. The glucose concentration was expressed relative to feed dry matter. Within each analyte, bars
630 without a common superscript differ ($P<0.05$).

631 **Figure 7.** Lactate and short chain fatty acid concentrations in ileal digesta of swine. SUM refers to the
632 sum of the concentration of propionate, butyrate, and valerate. Within each analyte, bars without a
633 common superscript differ ($P<0.05$).

Table 1. Ingredient composition and analysed nutrient content of experimental diets

Item, %	Diet			
	IMO	IMO-DP3	Maltodextrins	Resistant maltodextrins
Corn starch[1]	72.30	72.30	72.30	72.30
Casein[2]	16.00	16.00	16.00	16.00
Test product	3.00	3.00	3.00	3.00
Cellulose[3]	3.00	3.00	3.00	3.00
Canola oil	1.00	1.00	1.00	1.00
Mono/di-calcium phosphate	1.20	1.20	1.20	1.20
Limestone	0.90	0.90	0.90	0.90
TiO_2	0.50	0.50	0.50	0.50
Mineral and vitamin premix[4]	0.50	0.50	0.50	0.50
K_2CO_3	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
MgO	0.10	0.10	0.10	0.10
Analysed nutrient content of experimental diets, %				
Dry matter	91.7	91.7	92.1	91.9
Crude protein	16.5	17.9	17.4	16.7
Gross energy (Mcal/kg)	4.26	4.29	4.31	4.24

[1]Melojel (National Starch and Chemical Co., Bridgewater, NJ, USA).

[2]Calcium Caseinate 380 (Clover Fonterra, Roodepoort, South Aftrica).

[3]Solka-floc, International Fiber Corp., North Tonawanda, NY, USA.

[4]Provided the following per kilogram of diet: 7500 IU of vitamin A, 750 IU of vitamin D, 50 IU of vitamin E, 37.5 mg of niacin, 15 mg of pantothenic acid, 2.5 mg of folacin, 5 mg riboflavin, 1.5 mg pyridoxine, 2.5 mg of thiamine, 2000 mg of choline, 4 mg of vitamin K, 0.25 mg of biotin and 0.02 mg of vitamin B_{12}.

Table 2. Apparent ileal digestibility (AID), apparent total tract digestibility (ATTD, and hindgut fermentation (ATTD-AID) of energy and nutrients in growing pigs fed two different isomalto-oligosaccharides, digestible and resistant maltodextrin[a]. Within a row, means of diets without a common superscript differ ($P < 0.05$).

	Diet					
Item	IMO	IMO-DP3	digestible malto-dextrin	resistant malto-dextrin	Pooled SEM	P
			Gross energy, %			
AID	89.1^{xy}	90.9^{x}	91.1^{x}	87.6^{y}	1.1	0.019
ATTD	94.4	94.5	94.3	94.0	0.25	0.336
ATTD - AID	5.36	3.57	3.22	6.43	1.2	0.052
			Dry matter, %			
AID	88.0^{xy}	89.8^{x}	89.9^{x}	86.5^{y}	1.1	0.022
ATTD	93.0	92.9	93.0	92.7	0.23	0.705
ATTD - AID	5.01	3.15	2.90	6.21	1.2	**0.044**
			Crude protein, %			
AID	86.2	88.1	88.0	87.6	1.3	0.478
ATTD	95.7 ^{xy}	96.5^{x}	96.3^{xy}	95.5^{y}	0.28	**0.012**
ATTD - AID	9.54	8.35	8.38	7.55	1.4	0.576

[a]Least-squares means based on 7-8 pigs per diet.

Figure 1.

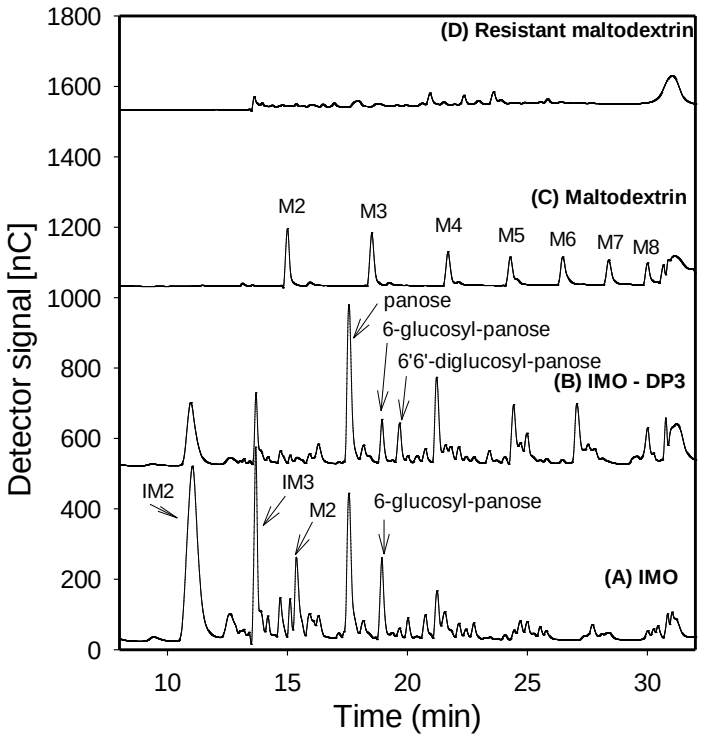

(D) Resistant maltodextrin
(C) Maltodextrin
M2
M3
M4
M5
M6
M7
M8
panose
6-glucosyl-panose
6'6'-diglucosyl-panose
(B) IMO - DP3
IM2
IM3
M2
6-glucosyl-panose
(A) IMO
Detector signal [nC]
Time (min)
0
200
400
600
800
1000
1200
1400
1600
1800
10
15
20
25
30

Figure 2.

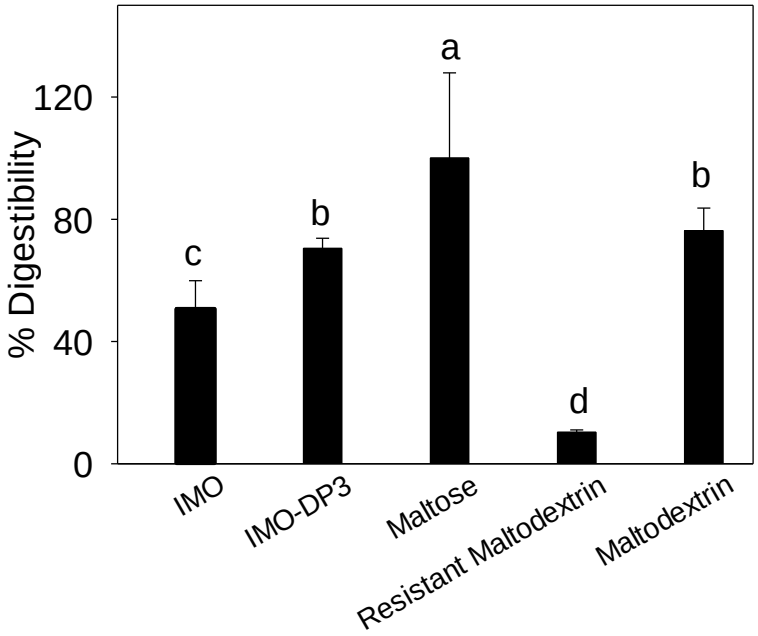

% Digestibility
120
80
40
0
a
b
c
d
b
IMO
IMO-DP3
Maltose
Resistant Maltodextrin
Maltodextrin

Figure 3.

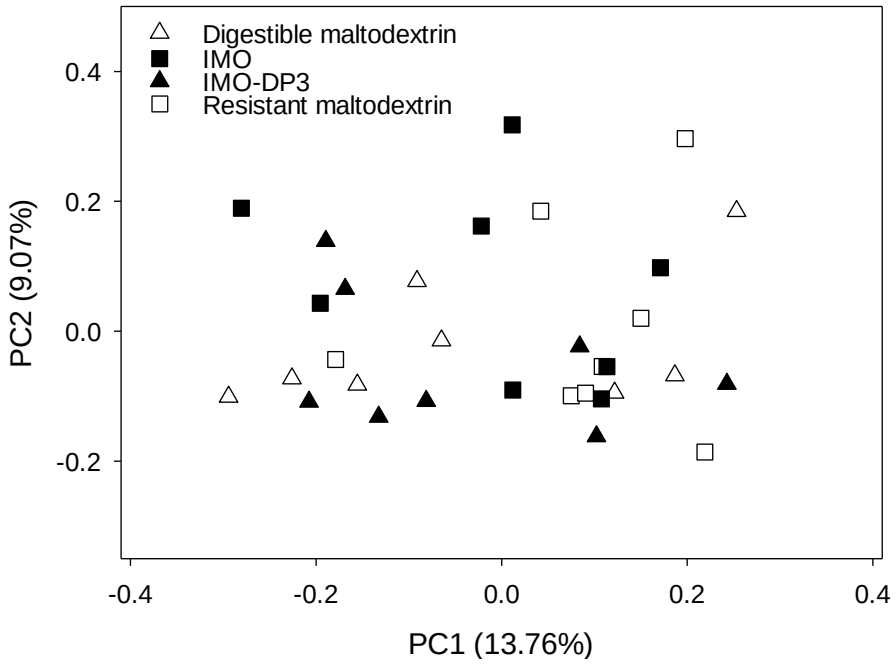

Digestible maltodextrin
IMO
IMO-DP3
Resistant maltodextrin
0.4
0.2
0.0
-0.2
PC2 (9.07%)
-0.4
-0.2
0.0
0.2
0.4
PC1 (13.76%)

Figure 4

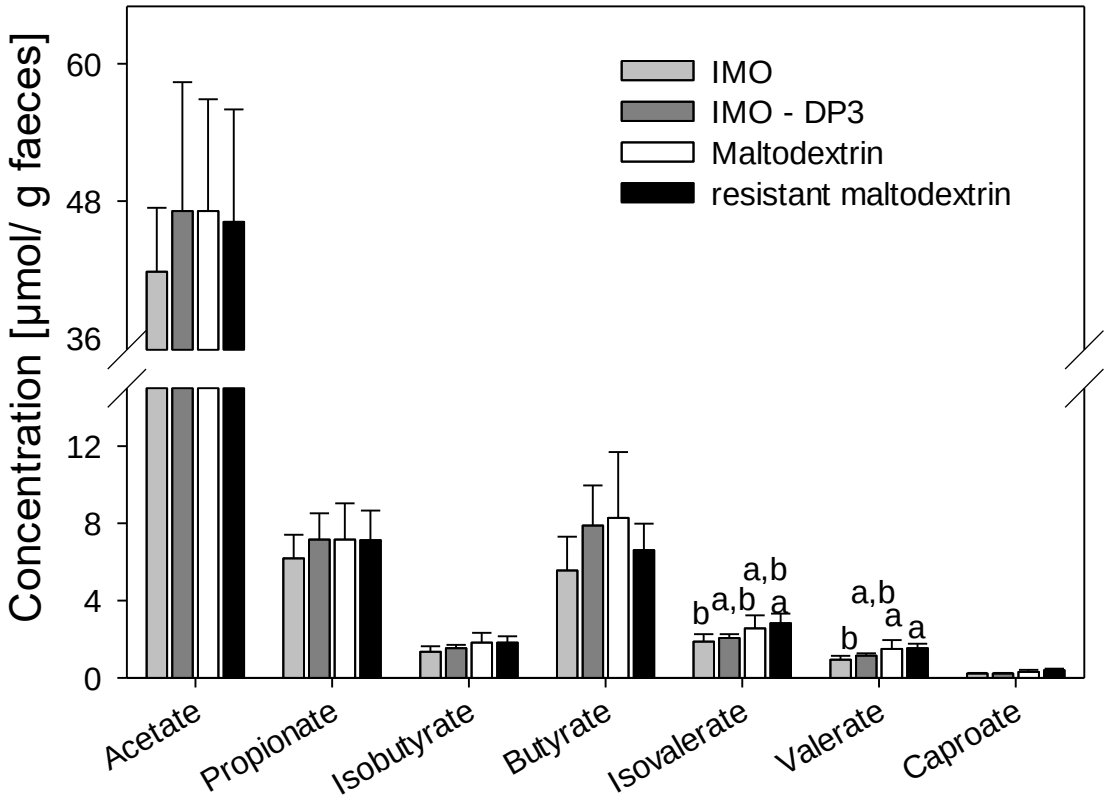

Concentration [µmol/ g faeces]
60
48
36
12
8
4
0
IMO
IMO - DP3
Maltodextrin
resistant maltodextrin
b
a,b
a
a,b
b
a,b
a
a
Acetate
Propionate
Isobutyrate
Butyrate
Isovalerate
Valerate
Caproate

Figure 5.

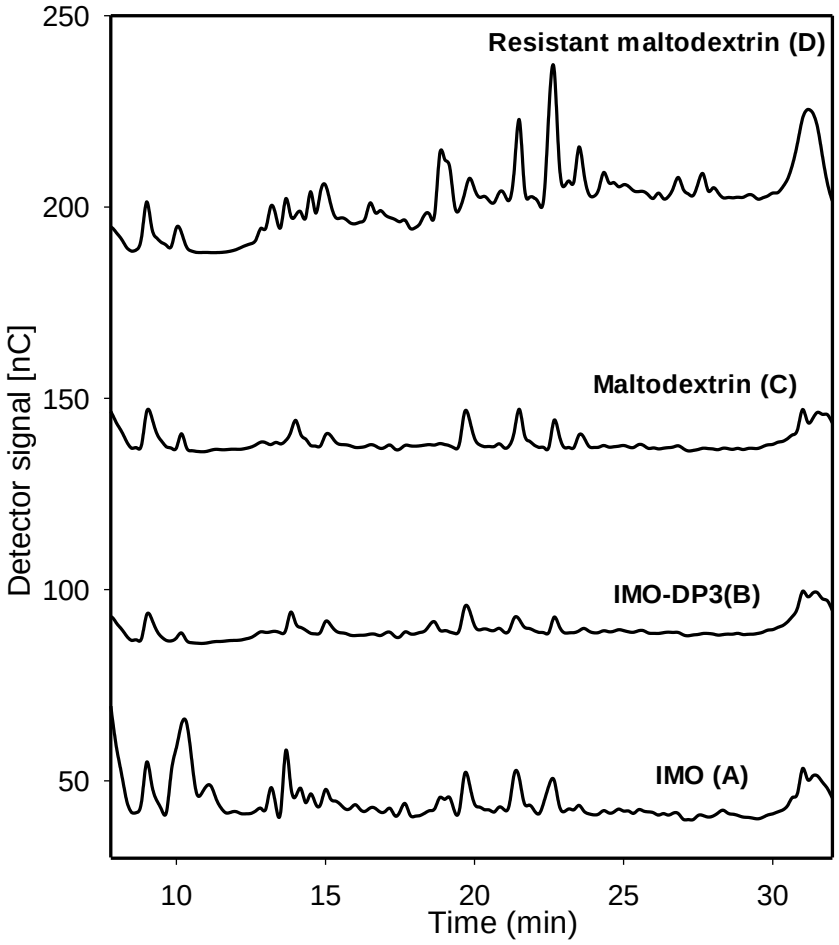

Resistant maltodextrin (D)
Maltodextrin (C)
IMO-DP3(B)
IMO (A)
Detector signal [nC]
250
200
150
100
50
10
15
20
25
30
Time (min)

Figure 6.

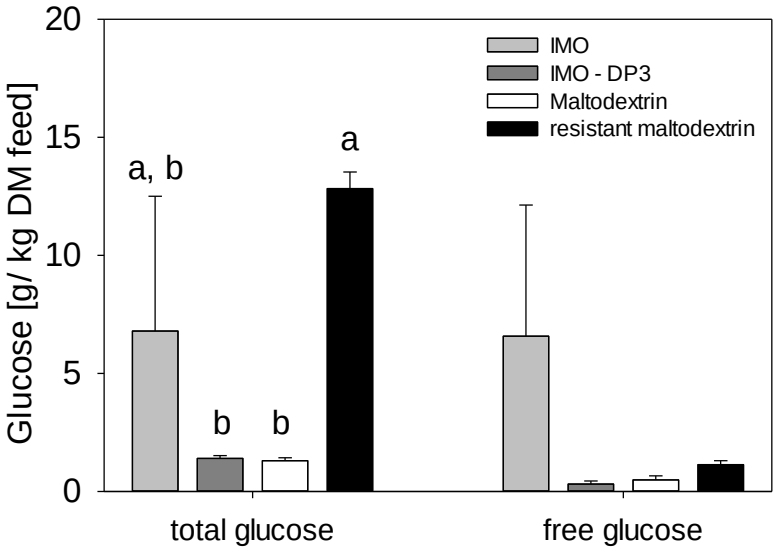
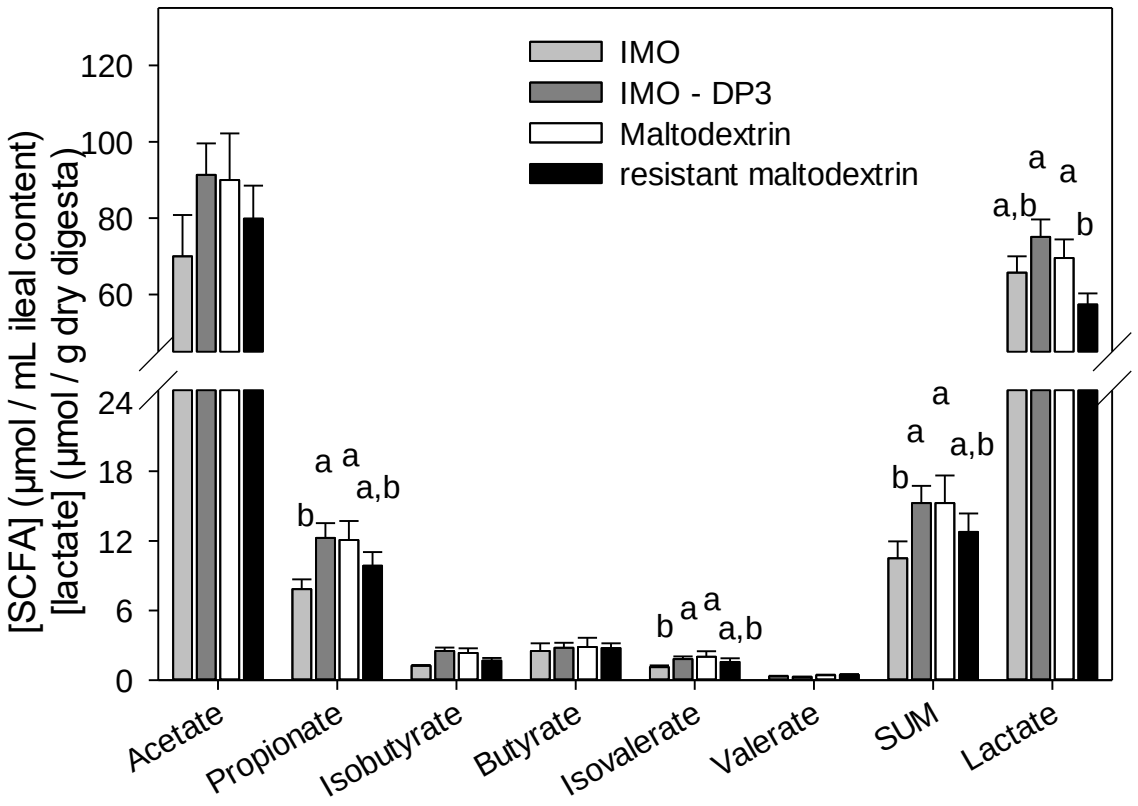

20
15
10
5
0
Glucose [g/ kg DM feed]
IMO
IMO - DP3
Maltodextrin
resistant maltodextrin
a, b
b
b
a
total glucose
free glucose

Figure 7.


IMO
IMO - DP3
Maltodextrin
resistant maltodextrin
[SCFA] (µmol / mL ileal content)
[lactate] (µmol / g dry digesta)
120
100
80
60
24
18
12
6
0
a,b
a
a
b
a,b
a
a
b
a
a
b
a,b
a
a
b
Acetate
Propionate
Isobutyrate
Butyrate
Isovalerate
Valerate
SUM
Lactate

Digestibility of branched and linear α-gluco-oligosaccharides *in vitro* and in ileal-cannulated pigs

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Online supplementary material:

Table S1. Composition of fecal microbiota.

Table S1. Relative abundance (%) of bacterial genera in fecal microbiota of pigs fed with different diets, determined by sequencing of 16S rRNA tags. Data were analyzed by QIIME pipeline and are represented as mean ± SD. Only Data in the same row that do not share a common superscript are significantly different ($P < 0.05$).

Genus	Digestible maltodextrin	Resistant maltodextrin	IMO	IMODP3	*P*-value
Archaea					
Methanobrevibacter	1.78±1.23	1.92±1.39	1.68±1.95	2.13±1.13	0.75
vadinCA11	0.20±0.27	0.23±0.16	0.59±0.67	0.24±0.47	0.21
Actinobacteria					
Actinomyces	0.49±0.25	0.37±0.25	0.69±0.86	0.42±0.36	0.60
Corynebacterium	0.05±0.08	0.04±0.06	0.02±0.02	0.08±0.10	0.42
[F:Micrococcaceae]	0.05±0.06	0.03±0.03	0.04±0.03	0.09±0.11	0.46
Bifidobacterium	0.07±0.12 A	0.00±0.01 B	0.00±0.01 AB	0.01±0.02 AB	***0.04***
[F:Coriobacteriaceae]	0.96±0.65	0.93±0.58	0.78±0.56	1.26±0.76	0.46
Collinsella	0.26±0.32	0.89±0.79	0.29±0.26	0.92±1.19	0.05
Bacteroidetes					
[O:Bacteroidales]	1.42±1.33	1.83±2.56	1.14±1.01	1.05±0.76	0.58
Bacteroides	4.73±1.99	4.50±3.32	5.32±5.06	5.34±3.45	0.90
Parabacteroides	4.82±3.42	8.89±4.90	5.12±3.73	6.53±4.04	0.12
Prevotella	0.04±0.04	2.42±0.48	0.09±0.11	0.10±0.13	0.37
[F:Rikenellaceae]	0.02±0.02	0.07±0.08	0.04±0.08	0.04±0.05	0.06
[F:S24-7]	0.54±0.97	0.83±0.72	0.56±0.68	0.67±0.67	0.45
ButyricPnas	0.44±0.29	0.63±0.50	0.50±0.58	0.46±0.37	0.56
[F:Paraprevotellaceae]	0.08±0.06 AB	0.08±0.07 AB	0.14±0.11 A	0.05±0.05 B	***0.05***
Chlamydiae					
[F:Chlamydiaceae]	0.02±0.03	0.02±0.02	0.01±0.02	0.05±0.12	0.47
Deferribacteres					
Mucispirillum	0.05±0.10	0.02±0.03	0.01±0.03	0.03±0.05	0.57
Firmicutes					
Enterococcus	0.93±0.94	0.59±0.78	0.56±0.55	0.45±0.28	0.62
Lactobacillus	0.01±0.03	0.04±0.07	0.04±0.09	0.18±0.50	0.69
Streptococcus	0.68±0.85	0.35±0.58	0.68±0.72	0.87±0.88	0.44
Turicibacter	2.97±2.34	4.20±4.30	3.25±6.68	1.21±0.95	0.32
[C:Clostridia]	0.02±0.02	0.01±0.01	0.02±0.02	0.03±0.04	0.69
[O:Clostridiales]	16.68±2.50	17.28±14.58	18.74±8.41	17.03±7.16	0.24
[F:Christensenellaceae]	5.62±2.92 B	8.23±3.38 A	5.00±3.11 B	4.71±3.47 B	***0.00***
Christensenella	0.02±0.02 B	0.09±0.07 A	0.02±0.02B	0.03±0.03 B	***0.00***
[F:Clostridiaceae]	4.87±2.92	9.09±5.81	3.29±1.40	4.35±2.20	0.12
Clostridium	1.11±0.82	1.36±1.50	1.60±2.18	0.82±0.51	0.91
[F:Dehalobacteriaceae]	0.23±0.15	0.32±0.27	0.24±0.09	0.29±0.17	0.71
Dehalobacterium	0.03±0.02	0.02±0.01	0.02±0.01	0.02±0.01	0.43
Pseudoramibacter_Eubacterium	0.06±0.12	0.01±0.01	0.03±0.05	0.03±0.04	0.43

[F:Lachnospiraceae]	4.67±2.45	11.83±8.61	3.47±1.22	4.94±1.94	0.91
Blautia	0.16±0.14 B	0.91±0.69 A	0.31±0.23 B	0.39±0.34 B	***0.02***
Coprococcus	2.30±3.32 AB	0.11±0.23 C	1.08±1.51 BC	4.70±5.15 A	***0.00***
Dorea	2.21±1.71	1.24±0.64	2.09±1.27	1.59±0.98	0.19
Epulopiscium	0.14±0.20	0.26±0.68	0.12±0.21	0.09±0.15	0.98
Ruminococcus	0.32±0.73	0.18±0.42	0.05±0.11	0.47±0.84	0.32
Peptococcus	0.03±0.04	0.03±0.05	0.05±0.06	0.05±0.07	0.91
rc4-4	0.05±0.05	0.04±0.09	0.09±0.12	0.01±0.02	0.16
[F:Peptostreptococcaceae]	0.81±0.89	0.48±0.42	1.05±1.19	0.78±0.77	0.36
Peptostreptococcus	0.11±0.09	0.12±0.15	0.25±0.49	0.17±0.26	0.86
[F:Ruminococcaceae]	14.43±5.49	15.72±14.42	14.91±6.75	13.83±3.90	0.82
Oscillospira	2.68±1.18	2.09±1.21	1.70±1.05	1.93±0.97	0.24
Ruminococcus	6.32±5.33	7.50±3.74	6.81±3.54	5.81±2.21	0.78
Acidaminococcus	0.02±0.05 AB	0.06±0.13 A	0.02±0.05 AB	0.00±0.00 B	***0.02***
Phascolarctobacterium	0.46±0.32	0.65±0.65	0.42±0.19	0.34±0.25	0.43
[F:Mogibacteriaceae]	0.80±0.27	4.68±3.85	0.66±0.29	0.97±0.39	0.11
Anaerovorax	0.13±0.16	0.14±0.21	0.05±0.09	0.11±0.12	0.54
Mogibacterium	0.25±0.40	0.03±0.08	0.36±0.75	0.60±1.11	0.42
ParvPnas	0.01±0.02	0.02±0.04	0.10±0.27	0.00±0.01	0.67
[F:Erysipelotrichaceae]	1.09±0.68	5.60±2.05	1.05±1.02	0.81±0.80	0.53
Bulleidia	0.35±0.61	0.40±0.27	0.20±0.20	0.26±0.17	0.38
Catenibacterium	0.01±0.02	1.43±3.60	0.04±0.07	0.05±0.08	0.10
L7A_E11	0.05±0.06 A	0.01±0.02 AB	0.02±0.02 AB	0.00±0.01 B	***0.03***
Eubacterium	0.29±0.17	0.42±0.37	0.33±0.40	0.49±0.53	0.76
p-75-a5	0.36±0.32 A	0.12±0.26 B	0.27±0.39 AB	0.44±0.52 AB	***0.04***
Fusobacteria					
Fusobacterium	0.45±0.34	0.41±0.46	1.67±3.18	0.49±0.53	0.11
Planctomycetes					
[F:Pirellulaceae]	0.85±0.73	0.87±0.64	1.35±1.10	0.74±0.45	0.58
Proteobacteria					
Sutterella	0.33±0.29	0.25±0.18	0.48±0.34	0.27±0.17	0.37
Bilophila	0.02±0.02	0.02±0.02	0.02±0.02	0.06±0.16	0.77
Desulfovibrio	1.23±0.52	1.62±0.95	1.16±0.66	1.25±0.47	0.36
[O:gMD14H09]	0.03±0.05	0.04±0.07	0.01±0.01	0.02±0.04	0.18
[F:Enterobacteriaceae]	2.84±2.34	3.41±1.93	4.66±2.29	3.73±2.04	0.18
Erwinia	0.04±0.03	0.05±0.05	0.08±0.08	0.05±0.05	0.20
Spirochaetes					
Treponema	0.70±1.46	0.11±0.10	0.29±0.59	0.33±0.46	0.17
Synergistetes					
TG5	0.09±0.06	0.04±0.04	0.05±0.05	0.05±0.05	0.24
[F:Synergistaceae]	0.78±0.65	0.37±0.43	1.00±1.56	1.27±2.36	0.51
TM7					
f_f16]	0.10±0.19	0.04±0.04	0.07±0.09	0.40±0.73	0.41

Tenericutes					
[O:RF39]	1.57±1.98	1.06±0.69	0.87±0.93	1.12±1.79	0.80
Verrucomicrobia					
Akkermansia	1.72±4.87	0.00±0.00	0.00±0.00	0.03±0.08	0.39
Unassigned	1.95±1.35	1.56±0.58	2.26±0.95	1.88±1.36	0.40

Unassigned genera are presented with upper level of family (F) or order (O) in square brackets. "Unassigned" means a good hit to a poorly defined taxonomy sequence.