Effect of lactulose-derived oligosaccharides on intestinal microbiota during the shift between media with different energy contents

Elvira Barroso, Antonia Montilla, Nieves Corzo, Carmen Peláez, M. Carmen Martínez-Cuesta, Teresa Requena*

Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM), CEI (UAM+CSIC), Nicolás Cabrera 9, 28049 Madrid, Spain.

*Corresponding author: Tel.: +34 91 0017900; E-mail address: t.requena@csic.es

ABSTRACT

The microbiological and metabolic changes of an overweight-associated colonic microbiota after reducing *in vitro* the carbohydrate supply and its supplementation with oligosaccharides derived from lactulose (OsLu) were evaluated using a dynamic simulator of the gastrointestinal tract. The differentiation and stability of the microbial communities within each colon compartment were reached after two weeks of feeding the system with a high energy (HE) medium based on fructose and readily fermentable starches. The effect of reducing the energy content (low-energy medium, LE) and the supplementation with OsLu caused minor variations in bacterial counts, except for *Enterobacteriaceace*. The LE medium caused an effect on the microbial metabolic activity that was characterized by an absence of net butyrate production and an increase in ammonium content. This shift from fermentative to proteolytic metabolism was not observed when the LE medium was supplemented with OsLu. This oligosaccharide mixture was mainly metabolized in the proximal colonic compartment. The results obtained in this study indicate that the substitution in the diet of easily digestible carbohydrates by OsLu maintains the fermentative functionality of the intestinal microbiota, allowing the net production of butyric acid with potential beneficial effects on health, and avoiding a full transition to proteolytic metabolism profiles.

Keywords: prebiotics; OsLu; obesity; diet; gut microbiota; butyric acid

1. INTRODUCTION

Dietary habits involving high energy intake are related to the development of overweight and obesity. Monosaccharides and disaccharides such as fructose and sugar alcohols (sorbitol, lactitol and other polyols), widely used for the formulation of processed foods or beverages, can reach the large intestine when overfeeding of these sugars occurs (Payne, Chassard, & Lacroix, 2012). There is epidemiological evidence that sugar-sweetened beverages increase the risk of overweight and obesity, at all ages, and that obese individuals are reported to consume significantly more protein and sugars and lower fibre than normal-weight subjects (Lafontan, Visscher, Farpour-Lambert, & Yumuk, 2015; Requena et al., 2013). Related to it, there is an increased interest in understanding the possible effects of high energy diets in the intestinal microbiota. However, the highly personalized human microbiota shows a smaller dietary influence as the inter-individual variation decreases systematic effects (Wu et al., 2011; David et al., 2014). Salonen et al. (2014) described that studies from 14 obese males consuming fully controlled diets supplemented with resistant starch or non-starch polysaccharides and a weight-loss diet revealed that the diet explained around 10% of the total variance in microbiota composition, which was substantially less than the inter-individual variance. All these studies have noted strong individuality of the responses, the extent of which appears to depend on the initial microbiota composition (Korpela et al., 2014). The fact that the broad phylum level changes between Bacteroidetes and Firmicutes have not been found consistently (Ley, 2010; Ravussin et al., 2012) may indicate that relevant changes associated to diet-induced obesity could involve lower taxonomic levels within these phyla (Cox & Blaser, 2013).

In view of the fact that prebiotics are well-recognized to influence the gut microbiota composition, they could be consumed as part of a weight management diet. Genetically obese mice and diet-induced obese mice and rats (Alligier et al., 2014; Everard et al., 2011; Pyra, Saha, & Reimer, 2012), as well as overweight and obese adults (Parnell & Reimer, 2009) have all been reported to exhibit reduced fat mass following consumption of prebiotics. Indeed, subjects consuming diets rich in fructo-oligosacharides (FOS) and galacto-oligosacharides (GOS) show lower risk of overweight (Pérez-Cornago et al., 2015). Sarbini, Kolida, Deaville, Gibson, and Rastall (2014) described the potential of a novel dextran oligosaccharide for obesity management through *in vitro* experimentation. The degree of branching of the compound identified it as a slower-fermenting nutrient that was considered to be advantageous for obese individuals, as energy would be made available more gradually. Recently, the enzymatic synthesis of oligosaccharides derived from lactulose (OsLu) has been aimed for the production of a group of more slowly fermenting prebiotics (Cardelle-Cobas, Martínez-Villaluenga, Villamiel, Olano, & Corzo, 2008). In addition, the compounds have demonstrated to be selectively fermented by bifidobacteria and lactobacilli and to increase the concentration of short chain fatty acids (Cardelle-Cobas et al., 2012).

In this study we have used the dynamic simulator of the gastrointestinal tract described by Barroso, Cueva, Peláez, Martínez-Cuesta, and Requena (2015). The model simulates the gastric and small intestine digestion and is equipped with three-stage continuous reactors for reproducing the colon region-specific microbiota and its metabolism. The stabilization period in this study has been adapted to simulate an obese-associated microbiota by using a high energy-content medium. Changes in

68 microbiological and metabolic characteristics were assessed after lowering the energy
69 content and the supplementation with OsLu used as a potential prebiotic.

70 **2. MATERIALS AND METHODS**

71 **2.1. Dynamic simulator of the gastrointestinal tract**

72 The dynamic gastrointestinal simulator SIMGI was used in the operating mode to work
73 with the units simulating the small intestine (SI) and the ascending (AC), transverse
74 (TC) and descending colon (DC) regions (Barroso et al., 2015; Fig. S1). Therefore, the
75 three colon reactors were filled and pre-conditioned with the nutritive medium that feed
76 the system during the stabilization period. In this case, the setup was made to recreate
77 an obese-associated microbiota. For this purpose, a starting high energy (HE) medium
78 was used as described by Payne, Chassard, Banz, and Lacroix (2012), which was
79 characterized by a high content of high-glycaemic index carbohydrates (digestible
80 starch) and simple carbohydrates (fructose). The HE medium contained arabinogalactan
81 (1 g/L), pectin from apple (2 g/L), xylan (1 g/L), potato starch (6 g/L), maize starch (4
82 g/L), fructose (6 g/L), glucose (0.4 g/L), yeast extract (3 g/L), peptone (1 g/L), mucin (4
83 g/L) and L-cysteine (0.5 g/L); that is 45% more fermentable carbohydrates than the
84 standard nutritive medium (Barroso et al., 2015) to create the HE medium. The AC, TC
85 and DC units were inoculated with 20 mL of a fresh 20% (w/v) faecal sample from an
86 overweight volunteer, homogenized in anaerobic conditions with sodium phosphate
87 buffer (0.1 M, pH 7.0), containing 1 g/L sodium thioglycolate as reducing agent, as
88 described by De Boever, Deplancke, and Verstraete (2000). The development and
89 stabilization of the microbial community until steady-state conditions in the three colon
90 units was approached by feeding the small intestine with nutritive medium (75 mL, pH

2) mixed with pancreatic juice (40 mL of a solution of 12 g/L $NaHCO_3$, 6 g/L oxgall dehydrated fresh bile and 0.9 g/L porcine pancreatine) three times a day during 14 days (Van den Abbeele et al., 2010). The small intestine digestion was performed during 2 h at 37 °C and the content of the vessel was automatically transferred to the following colon compartment (AC) at a flow rate of 5 mL/min, which simultaneously activated the transit of colonic content between the AC, TC and DC compartments at the same flow rate. The overall residence time of the colon compartments was 76 h. All the vessels were maintained under anaerobic conditions by continuously flushing N_2. The stabilization of the microbial community until steady-state conditions was evaluated by sampling and measuring the production of short chain fatty acids (SCFA) and ammonium over time (see below). Stability was reached when rates of change of the parameters measured dropped below 10% for each colon compartment (Barroso et al., 2015).

After the two-week stabilization period of the colonic microbiota, the SIMGI was subjected to a 1-week experiment consisting in removing the maize starch and fructose content and reducing the potato starch content to 1.5 g/L (low energy medium; LE) and adding 10 g/L of an oligosaccharide mixture derived from lactulose (OsLu), obtained such as it will be described below. Finally, a 1-week wash-out period was included at the end of the experiment by feeding the SIMGI daily with the LE medium. During the whole study, samples were collected daily at regular time points from the three colon vessels and stored at −20 °C until further analysis.

2.2. Synthesis of oligosaccharides derived from lactulose (OsLu)

OsLu were synthesized following the method described by Anadón et al. (2013) by using a commercial preparation Duphalac (Abbott Biologicals B.V., Barcelona, Spain), containing 670 g/L lactulose and the β-galactosidase from *Aspergillus oryzae* (16 U/mL;

Sigma-Aldrich, St. Louis, MO, USA). Enzymatic reactions were performed at 50 °C and pH 6.5 in an orbital shaker at 300 rpm for 24 h. In order to eliminate monosaccharides, the mixture of oligosaccharides (20%, w/v) was treated with fresh *Saccharomyces cerevisiae* (1.5%, w/v) (Levital, Paniberica de Levadura S.A., Valladolid, Spain) at 30 °C for 48 h in an orbital shaker (300 rpm). Mono- and disaccharides as well as OsLu were analysed by GC with a flame ionization detector (GC-FID) as described by Montilla, Van de Lagemaat, Olano, and Del Castillo (2006).

2.3. Microbiological analyses

2.3.1. DNA extraction and purification

Microbial DNA extraction of the samples taken from the AC, TC and DC compartments was performed as described by Moles et al. (2013). Briefly, samples (1 mL) were centrifuged (10000×g, 10 min, 4 °C) and the pellet (suspended in 200 mM Tris–HCl pH 7.5, 0.5% SDS, 25 mM EDTA, 250 mM NaCl and 3 M sodium acetate) was incubated with 20 mg/mL lysozyme and 5 mg/mL lysostaphin (Sigma-Aldrich). Bacterial lysis was completed by mixing with glass beads. The DNA was extracted with phenol/chloroform/isoamyl-alcohol, precipitated by adding 0.6 volumes of isopropanol and finally resuspended in DNase, RNase free water (Sigma-Aldrich). The DNA yield was measured using a NanoDropH ND-1000 UV spectrophotometer (Nano-Drop Technologies).

2.3.2. Quantitative PCR (qPCR)

The quantitative microbiological analysis of samples was carried out by qPCR experiments that were analysed using SYBR green methodology in a ViiA7 Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). Primers, amplicon size,

annealing temperature for total bacteria, *Bacteroides*, *Bifidobacterium*, *Enterobacteriaceae*, *Lactobacillus*, *Prevotella*, the specific phylogenetic groups *Blautia coccoides-Eubacterium rectale* Cluster XIVa, *Ruminococcus* Cluster IV and *Clostridium leptum* subgroup specific cluster IV have been described previously (Barroso et al., 2013). DNA from *Escherichia coli* DH5α, *L. plantarum* IFPL935, *Bifidobacterium breve* 29M2 and *Bacteroides fragilis* DSM2151 were used for quantification of total bacteria, *Lactobacillus*, *Bifidobacterium* and *Bacteroides*, respectively. For the rest of groups analysed, samples were quantified using standards derived from targeted cloned genes using the pGEM-T cloning vector system kit (Promega, Madison, WI, USA), as described previously (Barroso et al., 2013). For the analysis of *Akkermansia* (primers AM1: CAGCACGTGAAGGTGGGGAC and AM2: CCTTGCGGTTGGCTTCAGAT), *Faecalibacterium* (Fprau 07: CCATGAATTGCCTTCAAAACTGTT and Fprau 02: GAGCCTCAGCGTCAGTTGGT) and *Roseburia* (Ros-F1: GCGGTRCGGCAAGTCTGA and Ros-R1: CCTCCGACACTCTAGTMCGAC), the samples were quantified using standards derived from clones obtained from the faecal inoculum, amplified with the mentioned primers and using the conditions described by Collado, Derrien, Isolauri, De Vos, and Salminen (2007), Sokol et al. (2008), and Ramirez-Farias et al. (2009), respectively. The PCR amplicons were cloned using the pGEM-T cloning vector system kit (Promega) as described previously (Barroso et al., 2013). The correctness of the *Akkermansia*, *Roseburia* and *Faecalibacterium* inserts was confirmed by sequence analysis.

2.3.3. PCR-DGGE

For evaluation of the microbial community evolution, DNA was amplified using the universal bacterial primers 968-F and UNI 1401-R described by Nübel et al. (1996). The primer 968-F was synthesized with a 40-bp GC clamp attached to the 3' end. Total volume for PCR reactions was 12.5 µL, composed by 0.75 µL 50 mM MgCl2; 1.25 µL Taq Buffer 10X; 0.25 µL for each primer (10 µM) and dNTPs (10 mM); 0.125 µL Taq Polimerase (5 U/µL), about 100 ng of DNA from each sample and filled up to 12.5 µL with SIGMA water. Amplification protocol was as follows: 94 ºC for 3 min 45 s, 35 cycles of 30 s at 55 ºC (annealing temperature) and 1 min at 72 ºC; and 10 min at 72 ºC. The PCR products (5 µL) were added in a 0.8% agarose gel and analysed through DGGE by a DCode system equipment (Bio-Rad Lab., Hercules, CA, USA) using a 9% polyacrylamide gel and a denaturalizing gradient from 30 to 60% of 7 M urea and 40% formamide. For electrophoresis assay, it was used TAE buffer 0.5× (20 mM Tris, 10 mM acetic acid and 0.5 mM EDTA), at 70 V and 60 ºC for 16 hours. The DGGE profiles were digitally normalized by comparison with a home-made standard using InfoQuest FP software (Bio-Rad). Clustering was performed with Pearson correlation and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

2.4. Microbial metabolism analyses

2.4.1. Short Chain Fatty-Acids (SCFA) determination

Samples from the AC, TC and DC compartments were centrifuged at 13000 ×*g* for 5 min, the supernatant was filtered and 0.2 µL were injected on a HPLC system (Jasco, Tokyo, Japan) equipped with a UV-975 detector and automatic injector. SCFA were separated using a Rezex ROA Organic Acids column (300 × 7.8 mm) (Phenomenex, Macclesfield, UK) thermostated at 50 °C following the method described by Sanz et al. (2005). The mobile phase was a linear gradient of 0.005 mM sulphuric acid in HPLC

186 grade water, and flow rate was 0.6 mL/min. The elution profile was monitored at 210
187 nm and peak identification was carried out by comparison between retention times and
188 standards. For data acquisition and processing it was used a ChromNAV Data System
189 software (Jasco). Calibration curves of acetic, propionic, butyric, formic and lactic acid
190 were built up in the range concentration of 1 to 100 mM.

191 *2.4.2. Ammonium determination*

192 Ammonium was determined directly from the supernatant fraction of samples (13000
193 ×*g*, 15 min, 4 ºC) using an ammonium ion selective electrode (NH500/2; WTW) and
194 following the manufacturer's instructions.

195 *2.5. Analysis of carbohydrates*

196 The carbohydrates not metabolized by the microbiota and therefore present in samples
197 from the AC, TC and DC vessels were determined by GC-FID. Before chromatographic
198 analysis, samples were centrifuged at 13000×g for 5 min and submitted to a clarification
199 procedure using Carrez reagents in order to remove interfering compounds (Moreno,
200 Olano, Santa-María, & Corzo, 1999). The analyses of carbohydrates were performed by
201 GC-FID (Montilla et al., 2006) as trimethyl silylated oximes (TMSO). Analysis were
202 carried out in a fused-silica capillary column SGE HT5 (5% phenyl polycarborane-
203 siloxane, 12 m × 0.32 mm × 0.10 µm thickness; SGE Analytical Science, Bellefonte,
204 USA). The oven temperature was programmed from 150 to 380 °C at a heating rate of 3
205 °C/min. Injections were made in the split mode (1:10). Data acquisition and integration
206 were done using the Agilent ChemStations Reb. 4B. 03.01 software (Wilmington, DE,
207 USA). Quantification of each sugar was performed by internal standard calibration
208 using phenyl-β-glucoside. Response factors were calculated after the analysis of
209 standard solutions of fructose, galactose, glucose, lactulose, kestose (trisaccharides) and

nystose (tetrasaccharides) over the expected concentration range in samples (1-0.01 mg/mL).

3. RESULTS AND DISCUSSION

In addition to non-digestible polysaccharides and resistant starches, which reach the large intestine undigested due to resistance to human amylase activity (Shimaya et al., 2009), simple sugars and digestible starches, when are consumed abundantly in the diet, are capable of exceeding intestinal absorption capacity, resulting in high carbohydrate passage into the large intestine, where they are readily available for gut microbial fermentation (Payne et al., 2012). Thus, the design of a high energy mediumto simulate in the SIMGI an obesity-associated microbiota was based on a significant increase in the content of fructose and readily fermentable starches to the standard nutritious medium employed to feed the SIMGI colonic reactors (Barroso et al., 2015). This HE diet design represents the increased prevalence of high consumption of refined carbohydrates and fructose-saturated sweeteners that is correlating with the global incidence of obesity (Payne et al., 2012; Charrez, Qiao, & Hebbard. 2015).

3.1. Modulation of bacterial composition

The composition of the microbial community and the bacterial counts reached during the last three days of each fermentation period (HE, LE-OsLu and LE diets) in the AC, TC and DC compartments were evaluated by qPCR (Table 1). The end of the stabilization period with the HE medium was characterized in average by higher counts in the distal colon regions of *Bifidobacterium*, *Bacteroides*, *B. coccoides-E. rectale* group, *C. leptum*, *Ruminococcus*, *Akkermansia*, *Faecalibacterium*, *Roseburia* and *Enterobacteriacceae*. The most noticeable differences in bacterial counts observed in

the SIMGI when comparing the end of the stabilization period with the HE medium and the standard medium, which did not contain maize starch and fructose and includes half content of potato starch (Barroso et al., 2015) were the counts of *Bacteroides* and, particularly, *Enterobacteriaceae* in the three colon compartments that were higher and lower, respectively, with the HE medium. The increase of Proteobacteria is a common feature observed in colonic models (Van den Abbeele et al., 2010; Rajilić-Stojanović et al., 2010). This increase, however, did not take place during the stabilization of the SIMGI with the HE medium (Table 1). The bacterial genera *Akkermansia*, *Faecalibacterium* and *Roseburia* were not previously assayed in the SIMGI. The results indicated a predominance of the three genera in the TC and DC compartments when compared with the AC. *Akkermansia* was also found by Van den Abbeele et al. (2010) in the distal regions of the SHIME. It is important to remark the fact that mucin added to the nutritive media could be relevant for development of *Akkermansia*, as these bacteria depend on mucin as a carbon and nitrogen source (Collado et al., 2007). Furthermore, *A. muciniphila* inversely correlates with inflammation and metabolic disorders associated to obesity (Schneeberger et al., 2015). *Faecalibacterium* and *Roseburia* are butyrate-producing bacteria that have also been described to mitigate intestinal inflammation (Machiels et al., 2014). *B. coccoides-E. rectale* were, therefore, the most representative butyrate producers in the AC compartment (Table 1). The abundance of this bacterial group in the three colon compartments has also been described at the end of the stabilization period in the SHIME feed with standard nutritive medium (Barroso et al., 2014; Van den Abbeele et al., 2010).

After the 15-days stabilization period of the colonic microbiota in the SIMGI with the HE nutritive medium, a shift in diet was carried out by the suppression in the medium of simple carbohydrates and the sharp reduction of the content of readily

fermentable starches (LE medium). The carbohydrate content was replaced with the oligosaccharide mixture OsLu. Composition of the purified oligosaccharide mixture contained 76% of sugars, which corresponded to 11% of monosaccharides, 21% lactulose and 43% of OsLu, and 18% moisture, 5% salts and 1% nitrogen. The feeding of the SIMGI with OsLu showed no bifidogenic effect when compared with the HE and LE media. On the other hand, it was observed lower counts of *Enterobacteriaceae* when comparing with the feeding with the LE medium (Table 1). Overall, during the feeding of the SIMGI with the LE-OsLu medium, the highest bacterial counts were recorded for the TC compartment, including *Bifidobacterium*, *Bacteroides*, *B. coccoides-E. rectale* group, *C. leptum*, *Faecalibacterium* and *Roseburia*. *Lactobacillus* was also highly represented in the AC compartment at the end of the LE-OsLu diet. Most of the microbial changes observed between the HE and LE-OsLu mediapersisted during the feeding with the LE medium, except for the increase of *Bacteroides* and *B. coccoides-E. rectale* group (in the AC compartment), and *Enterobacteriaceace* (in all compartments) observed at the end of the experimental study with the LE diet (Table 1). However, except for *Enterobacteriaceace*, differences in bacterial counts between diets involved variations generally below 1 log units, indicating that the differences in the amount of nutrients, including the supply with 10 g/L of the OsLu mixture, were not able to cause a substantial effect in the bacterial counts such as a relevant shifting between saccharolytic and proteolytic populations. The prebiotic potential of OsLu, however, was previously demonstrated through *in vitro* batch assays using faecal slurries (Cardelle-Cobas et al., 2012) and *in vivo* assays using growing rats fed with these oligosaccharides (Algieri et al., 2014; Hernández-Hernández et al., 2012; Marin-Manzano et al., 2013). The absence of bifidogenic effect during the shift between the HE and LE-OsLu mediacould be attributed to the high fermentable carbohydrate

283 content present in the HE medium which also favoured the growth of these bacteria. As
284 mentioned above, the microbial effect persisted during feeding with the LE diet.

285 The qPCR counts were in agreement with the qualitative assessment of bacterial
286 diversity analysed by PCR-DGGE. The analysis was carried out to compare the
287 differentiation of microbial communities within each colon compartment and to
288 qualitatively detect changes to the biodiversity as a function of varied substrate
289 availability. Fig. 1 shows the microbial community profiles of the AC, TC and DC
290 compartments at the last day of each intervention period of the SIMGI with the HE
291 medium followed by the LE medium supplemented with OsLu and finally with only the
292 LE medium. The results showed that samples from the three colon compartments
293 clustered together independently of the carbohydrate content of the diets. This feature is
294 consequence of the characteristics of three-stage fermentation models that allow
295 reproducing differences from proximal, characterized by acidic pH and carbohydrate-
296 excess conditions, to distal colonic regions showing a carbohydrate-depleted and non-
297 acidic environment (Macfarlane & Macfarlane, 2007).

298 **3.2. Modulation of microbial metabolism**

299 The results of metabolic activity, determined as carbohydrate utilization and production
300 of SCFA, lactic acid and ammonium, of the microbial community during the last three
301 days of each fermentation period (HE, LE-OsLu and LE) in the AC, TC and DC
302 compartments are shown in Fig. 2 and Table 2.

303 The remaining carbohydrates in effluents from the AC compartment at the end
304 of the stabilization period with the HE medium and along the feeding period with LE-
305 OsLu and LE media are shown in Fig. 2. Previously, no degradation of oligosaccharides
306 by pancreatic enzymes in the SI compartment during the daily feeding of the dynamic
307 simulator with the OsLu mixture was observed (results not shown), supporting the

indigestible properties of OsLu. Total carbohydrate content was high in the AC compartment during the feeding with HE and LE-OsLu media and it decreased by 5-6 times with the LE medium (Fig 2). Total carbohydrates content in the TC compartment was also higher during feeding with HE and LE-OsLu, but values remained around 0.15 mg/mL (results not shown). The level of di- and oligosaccharides increased in the AC compartment during initial feeding of the simulator with the LE-OsLu mediumand they decreased thereafter indicating a net degradation of OsLu by the AC microbiota after two days of diet change adaptation. A great utilization of di- and oligosaccharides by microorganisms from LE-OsLu diet in the AC compartment was observed and only small amounts (7-16%) reached the TC. The level of fructose in AC effluents was up to 0.59 mg/mL when HE mediumwas used, indicating its consumption by the intestinal microbiota as well as the galactose and glucose derived from OsLu hydrolysis during feeding the LE-OsLu diet (Fig 2). The results of this study were in agreement with those reported by Macfarlane, Macfarlane and Gibson (1998) during inulin feeding into a three-stage continuous culture system, since the majority of carbohydrate breakdown occurred in the vessel simulating the proximal colon. Mäkelainen, Mäkivuokko, Salminen, Rautonen, and Ouwehand (2007) using a semi-continuous system with four vessels mimicking the conditions in the human large intestine from proximal to distal colon, obtained a sustained degradation of polydextrose throughout the gut model and a rapid xylitol fermentation in the proximal colonic regions.

Regarding formation of microbial metabolites, except for lactic and formic acids, the SCFA and ammonium concentrations gradually increased from the AC to the DC compartment because of the accumulation of products in the system, consistent with operation of three-stage culture reactors without absorption steps (Cinquin, Le Blay, Fliss, & Lacroix, 2006; Possemiers, Verthe, Uyttendaele, & Verstraete, 2004). Lactic

and formic acids were only produced in the AC compartment and the latest only with the HE and LE-OsLu diets. Lactic acid can be further metabolized within the colon and turned into butyric and propionic acids through cross-feeding by gut bacteria (Duncan, Louis, & Flint, 2004; Reichardt et al., 2014). Likewise, formic acid is a component in the mutualistic interaction between fermentative bacteria and syntrophic metabolizers and has an important role in anaerobic metabolism via interspecies cross-feeding interactions (Louis, Hold, & Flint, 2014). *Bifidobacterium* has been described to produce formic acid from several carbohydrate sources, whereas some *Lactobacillus* species undergo a metabolic shift towards acetate and formate production, at the expense of lactate production, when growing on non-digestible oligosaccharides (Tabasco, Fontecha, Fernández de Palencia, Peláez, & Requena, 2014). The metabolic shift has been associated with more ATP production, resulting in a more efficient use of the available energy source (Van der Meulen, Makras, Verbrugghe, Adriany, & De Vuyst, 2004).

The effect of reducing the carbohydrate loading on microbial metabolic activity was characterized by an overall 2-fold decrease in the average content of total SCFA, mainly associated with acetic acid changes, of the three colon compartments with the LE diet compared to the HE intake period. Within the SCFA analyzed, propionic acid production was the least affected by nutrient load, whereas butyric acid production was practically stopped with the LE diet (Table 2). These results point toward the microbial utilization of the butyric acid via methanogenesis or sulphate reduction (Worm et al., 2014). The absence of net butyric acid production could be restored by supplementing the LE medium with OsLu. Additionally, the shift from high to low energy medium caused a 1.5-fold increase in the ammonium content of the TC compartment and a remarkable 5.5-fold increase in the proximal colon compartment (AC). This shift from

fermentative to proteolytic metabolism in the AC was not observed when the LE medium was supplemented with OsLu (Table 2; Fig. S1). The SFCA and ammonium results could be compared with *in vivo* data from obese subjects, where a significant decrease of SCFA, particularly acetic and butyric acids, and an increase of proteolytic products were observed when the individuals consumed diets high in protein and reduced in total carbohydrates (Russell et al., 2011). There is evidence from both humans and animal models that dietary supplementation with non-digestible carbohydrates can decrease protein fermentation in the large intestine, which concurs with a decrease in the genotoxicity of faecal water (Windey, De Preter, & Verbeke, 2012). Likewise, Algieri et al. (2014) observed in colitic rats a significant reduction in the production of SCFA as a consequence of the colonic inflammatory process that was restored by feeding the rats with lactulose and OsLu.

4. Conclusions

In conclusion, the results obtained in this study indicate that except for *Enterobacteriaceae*, characterized by becoming great competitors in carbohydrate scarcity, stability of the microbial populations was the dominant pattern. Community structure clusters were predominately a function of the specific-region colonic conditions, suggesting that community structures are relatively robust with little substantial change during shifts in nutrient supply. Furthermore, metagenomic studies are consistently showing that inter-individual differences in gut microbiota in terms of microbial composition can be over 90% (Dorrestein, Mazmanian, & Knight R, 2014), but that there is an assembly of functional communities that share similarities in their metabolic pathways (Shafquat, Joice, Simmons, & Huttenhower, 2014). It implies that distinct microbial species may be responsible for specific functions and adapt themselves to environment and diet affecting human homeostasis and health status. The

383 results obtained in this study indicate that substitution of easily digestible carbohydrates
384 by OsLu allows the development of a fermentative functionality, maintaining the net
385 production of butyric acid with potential beneficial effects on health, and avoiding a full
386 transition to proteolytic metabolism profiles.

387

388 **Acknowledgments**

389 The authors acknowledge funding from the Spanish Ministry for Science and
390 Innovation (AGL2012-35814 and AGL2014-53445-R), INIA (RM2011-00003-00-00)
391 and CSIC (COOPB-20099). The authors thank Iván Álvarez-Rodríguez for his valuable
392 assistance with HPLC and quantitative-PCR results and the technical contributions of
393 Ignacio Sánchez-García and Lidia Torre-Albarsanz.

394 **REFERENCES**

395 Algieri, F., Rodríguez-Nogales, A., Garrido-Mesa, N., Vezza, T., Garrido-Mesa, J.,
396 Utrilla, M.P., Montilla, A., Cardelle-Cobas, A., Olano, A., Corzo, N., Guerra-
397 Hernández, E., Zarzuelo, A., Rodríguez-Cabezas, M.E., & Gálvez, J. (2014).
398 Intestinal anti-inflammatory effects of oligosaccharides derived from lactulose in the
399 trinitrobenzenesulfonic acid model of rat colitis. *Journal of Agricultural and Food*
400 *Chemistry*, *62*, 4285–4297.
401 Alligier, M., Dewulf, E.M., Salazar, N., Mairal, A., Neyrinck, A.M., Cani, P.D., Langin,
402 D., & Delzenne, N.M. (2014). Positive interaction between prebiotics and
403 thiazolidinedione treatment on adiposity in diet-induced obese mice. *Obesity*, *22*,
404 1653–1661.
405 Anadón, A., Martínez, M.A., Ares, I., Castellano, V., Martínez-Larrañaga, M.R., Corzo,
406 N., Olano, A., Montilla, A., Recio, I., Martínez-Maqueda, D., Miralles, B., Fornari,
407 T., García-Risco, M.R., González, M., & Reglero, G. (2013). Acute and repeated
408 dose (28 days) oral safety studies of ALIBIRD in rats. *Journal of Food Protection*,
409 *76*, 1226–1239.

Barroso, E., Cueva, C., Peláez, C., Martínez-Cuesta, M.C., Requena, T. (2015). Development of human colonic microbiota in the computer-controlled dynamic simulator of the gastrointestinal tract SIMGI. *LWT Food Science and Technology*, *61*, 283–289.

Barroso, E., Van de Wiele, T., Jiménez-Girón, A., Muñoz-González, I., Martín-Alvarez, P.J., Moreno-Arribas, M.V., Bartolomé, B., Peláez, C., Martínez-Cuesta, M.C., & Requena, T. (2014). *Lactobacillus plantarum* IFPL935 impacts colonic metabolism in a simulator of the human gut microbiota during feeding with red wine polyphenols. *Applied Microbiology and Biotechnology*, *98*, 6805–6815.

Barroso, E., Sánchez-Patán, F., Martín-Alvárez, P.J., Bartolomé, B., Moreno-Arribas, M.V., Peláez, C., Requena, T., Van de Wiele T, & Martínez-Cuesta MC (2013). *Lactobacillus plantarum* IFPL935 favors the initial metabolism of red wine polyphenols when added to a colonic microbiota. *Journal of Agricultural and Food Chemistry*, *61*, 10163–10172.

Cardelle-Cobas, A., Martínez-Villaluenga, C., Villamiel, M., Olano, A., & Corzo, N. (2008). Synthesis of oligosaccharides derived from lactulose and Pectinex Ultra SP-L. *Journal of Agricultural and Food Chemistry*, *56*, 3328−3333.

Cardelle-Cobas, A., Olano, A., Corzo, N., Villamiel, M., Collins, M., Kolida, S., & Rastall, R.A. (2012). *In vitro* fermentation of lactulose-derived oligosaccharides by mixed fecal microbiota. *Journal of Agricultural and Food Chemistry*, *60*, 2024−2032.

Charrez, B., Qiao, L., & Hebbard, L. (2015). The role of fructose in metabolism and cancer. *Hormone molecular biology and clinical investigation*, *22*, 79–89.

Cinquin, C., Le Blay, G., Fliss, I., & Lacroix, C. (2006). Comparative effects of exopolysaccharides from lactic acid bacteria and fructooligosaccharides on infant gut microbiota tested in an *in vitro* colonic model with immobilized cells. *FEMS Microbiology Ecology*, *57*, 226–238.

Collado, M.C., Derrien, M., Isolauri, E., De Vos, W.M., & Salminen, S. (2007). Intestinal integrity and *Akkermansia muciniphila*, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Applied and Environmental Microbiology*, *73*, 7767–7770.

Cox, L.M., & Blaser, M.J. (2013). Pathways in microbe-induced obesity. *Cell Metabolism*, *17*, 883–894.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., Biddinger, S.B., Dutton, R.J., & Turnbaugh, P.J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*, 559–563.

De Boever, P., Deplancke, B., & Verstraete, W. (2000). Fermentation by gut microbiota cultured in a simulator of the human intestinal microbial ecosystem is improved by supplementing a soygerm powder. *Journal of Nutrition*, *130*, 2599–2606.

Dorrestein, P.C., Mazmanian, S.K., Knight, R. (2014). Finding the missing links among metabolites, microbes, and the host. *Immunity*, *40*, 824–832.

Duncan, .SH., Louis, P., & Flint, H.J. (2004). Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Applied and Environmental Microbiology*, *70*, 5810–5817.

Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G.G., Neyrinck, A.M., Possemiers, S., Van Holle, A., François, P., De Vos, W.M., Delzenne, N.M., Schrenzel, J., & Cani, P.D. (2011). Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes*, *60*, 2775–2786.

Hernández-Hernández, O., Marín-Manzano, M.C., Rubio, L.A., Moreno, F.J., Sanz, M.L., & Clemente, A. (2012). Monomer and linkage type of galacto-oligosaccharides affect their resistance to ileal digestion and prebiotic properties in rats. *Journal of Nutrition*, *142*, 1232–1239.

Korpela, K., Flint, H.J., Johnstone, A.M., Lappi, J., Poutanen, K., Dewulf, E., Delzenne, N., De Vos, W.M., & Salonen, A. (2014). Gut microbiota signatures predict host and microbiota responses to dietary interventions in obese individuals. *PLoS One*, *9*, e90702.

Lafontan, M., Visscher, T.L.S., Farpour-Lambert, N., & Yumuk, V. (2015). Opportunities for intervention strategies for weight management: global actions on fluid intake patterns. *Obesity Facts*, *8*, 54–76.

Ley, R.E. (2010). Obesity and the human microbiome. *Current Opinion in Gastroenterology*, *26*, 5–11.

Louis, P., Hold, G.L., & Flint, H.J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews in Microbiology*, *12*, 661–672.

475 Macfarlane, G.T., & Macfarlane, S. (2007). Models for intestinal fermentation:
476 association between food components, delivery systems, bioavailability and
477 functional interactions in the gut. *Current Opinion in Biotechnology*, *18*, 156–162.
478 Macfarlane, G.T., Macfarlane, S., & Gibson, G.R. (1998). Validation of a three-stage
479 compound continuous culture system for investigating the effect of retention time on
480 the ecology and metabolism of bacteria in the human colon. *Microbial Ecology*, *35*,
481 180–187.
482 Machiels, K., Joossens, M., Sabino, J., De Preter, V., Arijs, I., Eeckhaut, V., Ballet, V.,
483 Claes, K., Van Immerseel, F., Verbeke, K., Ferrante, M., Verhaegen, J., Rutgeerts,
484 P., & Vermeire, S. (2014). A decrease of the butyrate-producing species *Roseburia*
485 *hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with
486 ulcerative colitis. *Gut*, *63*, 1275–1283.
487 Mäkeläinen, H.S., Mäkivuokko, H.A., Salminen, S.J., Rautonen, N.E., & Ouwehand,
488 A.C. (2007). The effects of polydextrose and xylitol on microbial community and
489 activity in a 4-stage colon simulator. *Journal of Food Science*, *72*, M153–M159.
490 Marín-Manzano, M.C., Abecia, L., Hernández-Hernández, O., Sanz, M.L., Montilla, A.,
491 Olano, A., Rubio, L.A., Moreno, F.J., & Clemente, A. (2013). Galacto-
492 oligosaccharides derived from lactulose exert a selective stimulation on the growth of
493 *Bifidobacterium animalis* in the large intestine of growing rats. *Journal of*
494 *Agricultural and Food Chemistry*, *61*, 7560–7567.
495 Moles, L., Gómez, M., Heilig, H., Bustos, G., Fuentes, S., De Vos, W., Fernández, L.,
496 Rodríguez, J.M., & Jiménez, E. (2013). Bacterial diversity in meconium of preterm
497 neonates and evolution of their fecal microbiota during the first month of life. *PLoS*
498 *One*, *8*, e66986.
499 Montilla, A., Van de Lagemaat, J., Olano, A., & Del Castillo, M. D. (2006).
500 Determination of oligosaccharides by conventional high-resolution gas
501 chromatography. *Chromatographia*, *63*, 453–458.
502 Moreno, F.J., Olano, A., Santa-María, C., & Corzo, N. (1999). Determination of
503 maltodextrins in enteral formulations by three different chromatographic methods.
504 *Chromatographia*, *50*, 705–710.
505 Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R.I., Ludwig, W.,
506 & Backhaus, H. (1996). Sequence heterogeneities of genes encoding 16S rRNAs in
507 *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis.
508 *Journal of Bacteriology*, *178*, 5636–5643.

Parnell, J.A., & Reimer, R.A. (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *American Journal of Clinical Nutrition, 89*, 1751–1759.

Payne, A.N., Chassard, C., Banz, Y., & Lacroix, C. (2012). The composition and metabolic activity of child gut microbiota demonstrate differential adaptation to varied nutrient loads in an *in vitro* model of colonic fermentation. FEMS Microbiol Ecol 80:608-23.

Payne, A.N., Chassard, C., & Lacroix, C. (2012). Gut microbial adaptation to dietary consumption of fructose, artificial sweeteners and sugar alcohols: implications for host-microbe interactions contributing to obesity. *Obesity Reviews, 13*, 799–809.

Pérez-Cornago, A., Martínez-González, M.A., Ruiz-Canela, M., Jaurrieta, I., Carlos, S., Sayon-Orea, C., & Bes-Rastrollo, M. (2015). Prebiotic consumption and the incidence of overweight in a Mediterranean cohort: the Seguimiento Universidad de Navarra Project. *American Journal of Clinical Nutrition, 102*, 1554–1562.

Possemiers, S., Verthe, K., Uyttendaele, S., & Verstraete, W. (2004). PCR-DGGE-based quantification of stability of the microbial community in a simulator of the human intestinal microbial ecosystem. *FEMS Microbiology Ecology, 49*, 495–507.

Pyra, K.A., Saha, D.C., & Reimer, R.A. (2012). Prebiotic fiber increases hepatic acetyl CoA carboxylase phosphorylation and suppresses glucose-dependent insulinotropic polypeptide secretion more effectively when used with metformin in obese rats. *Journal of Nutrition, 142*, 213–220.

Rajilić-Stojanović, M., Maathuis, A., Heilig, H.G., Venema, K., De Vos, W.M., & Smidt, H. (2010). Evaluating the microbial diversity of an *in vitro* model of the human large intestine by phylogenetic microarray analysis. *Microbiology, 156*, 3270–3281.

Ramirez-Farias, C., Slezak, K., Fuller, Z., Duncan, A., Holtrop, G., & Louis, P. (2009). Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *British Journal of Nutrition, 101*, 541–550.

Ravussin, Y., Koren, O., Spor, A., LeDuc, C., Gutman, R., Stombaugh, J., Knight, R., Ley, R.E., & Leibel, R.L. (2012). Responses of gut microbiota to diet composition and weight loss in lean and obese mice. *Obesity, 20*, 738–747.

Reichardt, N., Duncan, S.H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K.P., Flint, H.J., & Louis, P. (2014). Phylogenetic distribution of three pathways for

propionate production within the human gut microbiota. *The ISME Journal*, *8*, 1323–1335.

Requena, T., Cotter, P., Shahar, D.R., Kleiveland, C.R., Martínez-Cuesta, M.C., Peláez, C., & Lea, T. (2013). Interactions between gut microbiota, food and the obese host. *Trends in Food Science and Technology*, *34*, 44–53.

Russell, W.R., Gratz, S.W., Duncan, S.H., Holtrop, G., Ince, J., Scobbie, L., Duncan, G., Johnstone, A.M., Lobley, G.E., Wallace, R.J., Duthie, G.G., & Flint, H.J. (2011). High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *American Journal of Clinical Nutrition*, *93*, 1062–1072.

Salonen, A., Lahti, L., Salojärvi, J., Holtrop, G., Korpela, K., Duncan, S.H., Date, P., Farquharson, F., Johnstone, A.M., Lobley, G.E., Louis, P., Flint, H.J., & De Vos, W.M. (2014). Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *The ISME Journal*, *8*, 2218–2230.

Sarbini, S.R., Kolida, S., Deaville, E.R., Gibson, G.R., & Rastall, R.A. (2014). Potential of novel dextran oligosaccharides as prebiotics for obesity management through in vitro experimentation. *British Journal of Nutrition*, *112*, 1303–1314.

Sanz, M.L., Polemis, N., Morales, V., Corzo, N., Drakoularakou, A., Gibson, G.R., & Rastall, R.A. (2005). *In vitro* investigation into the potential prebiotic activity of honey oligosaccharides. *Journal of Agricultural and Food Chemistry*, *53*, 2914–2921.

Schneeberger, M., Everard, A., Gómez-Valadés, A.G., Matamoros, S., Ramírez, S., Delzenne, N.M., Gomis, R., Claret, M., & Cani, P.D. (2015). *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Scientific Reports*, *5*, 16643.

Shafquat, A., Joice, R., Simmons, S.L., & Huttenhower, C. (2014). Functional and phylogenetic assembly of microbial communities in the human microbiome. *Trends in Microbiology*, *22*, 261–266.

Shimaya, S., Shimoyama, T., Fukuda, S., Matsuzaka, M., Takahashi, I., Umeda, T., Chinda, D., Saito, D., Sakamoto, J., Nagura, T., Danjo, K., & Nakaji, S. (2009). The recovery rate at the human terminal ileum of an orally administered non-digestive

oligosaccharide (raffinose). *International Journal of Food Science and Nutrition*, *60*, 344–351.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L.G., Gratadoux, J.J., Blugeon, S., Bridonneau, C., Furet, J.P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottière, H.M., Doré, J., Marteau, P., Seksik, P., & Langella, P. (2008). *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 16731–16736.

Tabasco, R., Fontecha, J., Fernández de Palencia, P., Peláez, C., & Requena, T. (2014). Competition mechanisms of probiotic bacteria: fermentative metabolism and colonization. *LWT Food Science and Technology*, *55*, 680–684.

Van den Abbeele, P., Grootaert, C., Marzorati, M., Possemiers, S., Verstraete, W., Gérard, P., Rabot, S., Bruneau, A., El Aidy, S., Derrien, M., Zoetendal, E., Kleerebezem, M., Smidt, H., & Van de Wiele, T. (2010). Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for *Bacteroidetes* and *Clostridium* cluster IX. *Applied and Environmental Microbiology*, *76*, 5237–5246.

Van der Meulen, R., Makras, L., Verbrugghe, K., Adriany, T., & De Vuyst, L. (2006). *In vitro* kinetic analysis of oligofructose consumption by *Bacteroides* and *Bifidobacterium* spp. indicates different degradation mechanisms. *Applied and Environmental Microbiology*, *72*, 1006–1012.

Windey, K., De Preter, V., & Verbeke, K. (2012). Relevance of protein fermentation to gut health. *Molecular Nutrition and Food Research*, *56*, 184–196.

Worm, P., Koehorst, J.J., Visser, M., Sedano-Núñez, V.T., Schaap, P.J., Plugge, C.M., Sousa, D.Z., & Stams, A.J. (2014). A genomic view on syntrophic versus non-syntrophic lifestyle in anaerobic fatty acid degrading communities. *Biochimica et Biophysica Acta*, *1837*, 2004–2016.

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F.D., & Lewis, D.J. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, *334*, 105–108.

609

610 **Legend to figures**

611

612 Fig. 1. Clustering tree of total bacteria DGGE profiles of samples from the ascending
613 (CA; green), transverse (CT; orange) and descending (CD; fuchsia) colon compartments
614 at the last 1-2 days of feeding the dynamic simulator of the gastrointestinal tract
615 (SIMGI) with the HE (red; days 1-14), LE (violet; days 15-21) and LE with OsLu (blue;
616 days 22-28)) media.

617

618 Figure 2.- Evolution of carbohydrate content (mg/mL) in the ascending colon (AC)
619 compartment during feeding of the dynamic simulator of the gastrointestinal tract
620 (SIMGI) with the high energy (HE; days 1-14) medium and the low energy (LE)
621 medium with (days 15-21) and without (days 22-28) oligosaccharides derived from
622 lactulose (OsLu). Oligosaccharides: carbohydrates with degree of polymerization ≥ 3.

623

Table 1. Mean ± SD of quantitative PCR counts (log copy number/ml) for the different microbial groups analysed in the ascending (AC), transverse (TC) and descending colon (DC) of the SIMGI at the end of the stabilization period with the high energy (HE; days 1-14) medium and at the end of the feeding with the low energy (LE) medium with (days 15-21) and without (days 22-28) oligosaccharides derived from lactulose (OsLu).

Bacterial group	Compartment	Medium		
		HE	LE OsLu	LE
Total bacteria	AC	8.83 ± 0.29	8.92 ± 0.42	9.11 ± 0.23
	TC	9.14 ± 0.23	9.19 ± 0.66	9.45 ± 0.17
	DC	9.42 ± 0.18	8.87 ± 0.12	9.26 ± 0.10
Lactobacillus	AC	7.36 ± 0.32	7.74 ± 0.38	7.74 ± 0.46
	TC	7.41 ± 0.08	7.45 ± 0.18	7.49 ± 0.23
	DC	7.20 ± 0.13	6.83 ± 0.11	6.94 ± 0.33
Bifidobacterium	AC	6.45 ± 1.04	5.22 ± 0.04	5.81 ± 1.58
	TC	7.30 ± 0.52	7.62 ± 0.48	7.87 ± 0.25
	DC	7.45 ± 0.16	7.46 ± 0.25	7.92 ± 0.14
Bacteroides	AC	8.66 ± 0.38	9.00 ± 0.33	9.52 ± 0.16
	TC	9.29 ± 0.13	9.51 ± 0.08	9.51 ± 0.10
	DC	9.32 ± 0.22	8.98 ± 0.27	9.24 ± 0.12
Blautia coccoides-	AC	7.38 ± 1.00	7.19 ± 0.39	8.05 ± 0.41
Eubacterium rectale	TC	8.50 ± 0.14	8.46 ± 0.11	8.56 ± 0.07
	DC	8.69 ± 0.18	7.97 ± 0.42	8.16 ± 0.18
Clostridium leptum	AC	2.70 ± 0.77	2.11 ± 0.28	2.28 ± 0.10
	TC	7.24 ± 0.16	6.92 ± 0.10	6.96 ± 0.09
	DC	7.00 ± 0.20	6.48 ± 0.24	6.69 ± 0.03
Ruminococcus	AC	1.73 ± 1.23	3.43 ± 1.78	1.94 ± 0.94
	TC	4.67 ± 0.08	4.85 ± 0.06	5.53 ± 0.36
	DC	5.51 ± 0.01	5.47 ± 0.20	5.89 ± 0.13
Prevotella	AC	4.16 ± 0.61	4.54 ± 1.20	5.18 ± 0.05
	TC	4.62 ± 0.46	4.96 ± 0.62	5.56 ± 0.28
	DC	4.72 ± 0.21	4.46 ± 0.09	4.56 ± 0.01
Akkermansia	AC	2.20 ± 0.79	2.80 ± 0.16	3.18 ± 0.26
	TC	3.57 ± 0.05	3.52 ± 0.55	3.39 ± 0.41
	DC	3.46 ± 0.04	3.89 ± 0.57	3.91 ± 0.13
Roseburia	AC	2.68 ± 0.17	2.04 ± 0.14	2.63 ± 0.42
	TC	8.22 ± 0.05	7.89 ± 0.36	7.84 ± 0.21
	DC	7.32 ± 0.03	7.23 ± 0.77	7.58 ± 0.28
Faecalibacterium	AC	n.d.	n.d	n.d.
	TC	6.23 ± 0.01	5.48 ± 0.76	6.06 ± 0.33
	DC	6.14 ± 0.87	5.11 ± 0.28	6.05 ± 0.12
Enterobacteriaceae	AC	2.84 ± 0.17	5.56 ± 0.28	7.43 ± 0.13
	TC	6.18 ± 0.03	7.56 ± 0.08	7.75 ± 0.16
	DC	5.95 ± 0.19	6.72 ± 0.23	7.88 ± 0.16

nd: not detected

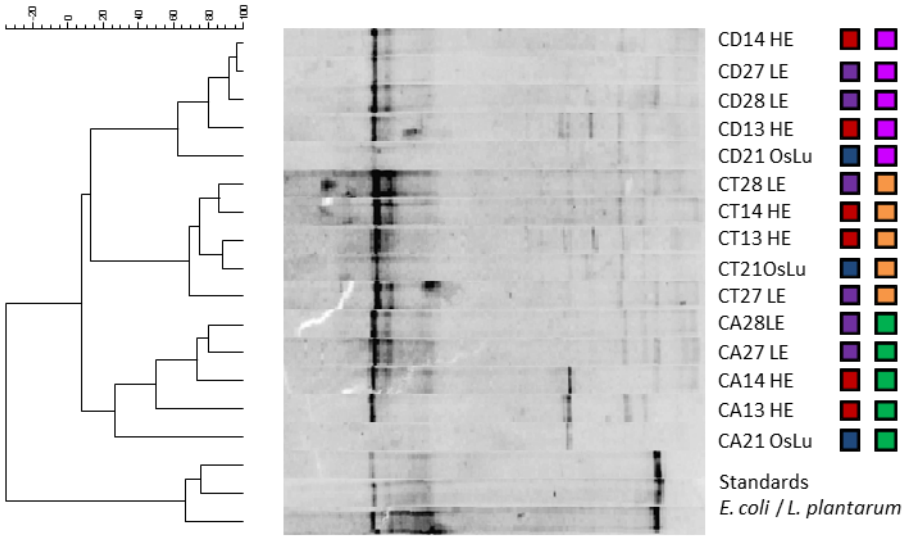
631 Table 2. Changes in concentration (mM; mean ± SD) of SCFA and ammonium in the
632 ascending (AC), transverse (TC) and descending colon (DC) of the SIMGI at the end of
633 the stabilization period with the high energy (HE) medium (days 1-14) and at the end of
634 the feeding with the low energy (LE) medium with (days 15-21) and without (days 22-
635 28) oligosaccharides derived from lactulose (OsLu).

Compound		HE	LE OsLu	LE
Total SCFA	AC	88.24 ± 2.53	59.17 ± 12.36	36.54 ± 0.74
	TC	110.04± 14.50	94.30± 16.12	50.89± 1.36
	DC	120.35 ± 2.50	86.88 ± 0.21	54.80 ± 0.21
Acetic acid	AC	45.46 ± 3.79	33.91 ± 2.08	22.81 ± 0.86
	TC	57.63 ± 6.27	46.43 ± 7.55	30.84 ± 0.33
	DC	62.84 ± 1.61	44.03 ± 0.44	30.70 ± 0.47
Propionic acid	AC	16.36 ± 0.30	17.27 ± 4.84	11.06 ± 0.14
	TC	28.05 ± 4.64	25.60 ± 4.94	16.69 ± 1.16
	DC	32.17 ± 1.32	26.48 ± 0.55	20.55 ± 0.34
Butyric acid	AC	21.39 ± 0.43	13.58 ± 4.29	0.05 ± 0.08
	TC	27.53 ± 4.24	22.28 ± 3.63	3.36 ± 0.13
	DC	25.34 ± 0.43	16.38 ± 0.77	3.34 ± 0.62
Lactic acid	AC	3.85 ± 0.00	2.53 ± 0.00	2.62 ± 0.05
	TC	nd	nd	nd
	DC	nd	nd	nd
Formic acid	AC	3.12 ± 1.32	2.90 ± 0.00	nd
	TC	nd	nd	nd
	DC	nd	nd	nd
Ammonium	AC	4.05± 1.19	7.07± 3.09	22.39± 4.97
	TC	34.80± 15.79	50.61± 6.44	50.44± 6.99
	DC	55.68± 19.23	67.50± 5.15	63.10± 2.47

636 nd: not detected

637

638



639

640

641

642 Figure 1

643

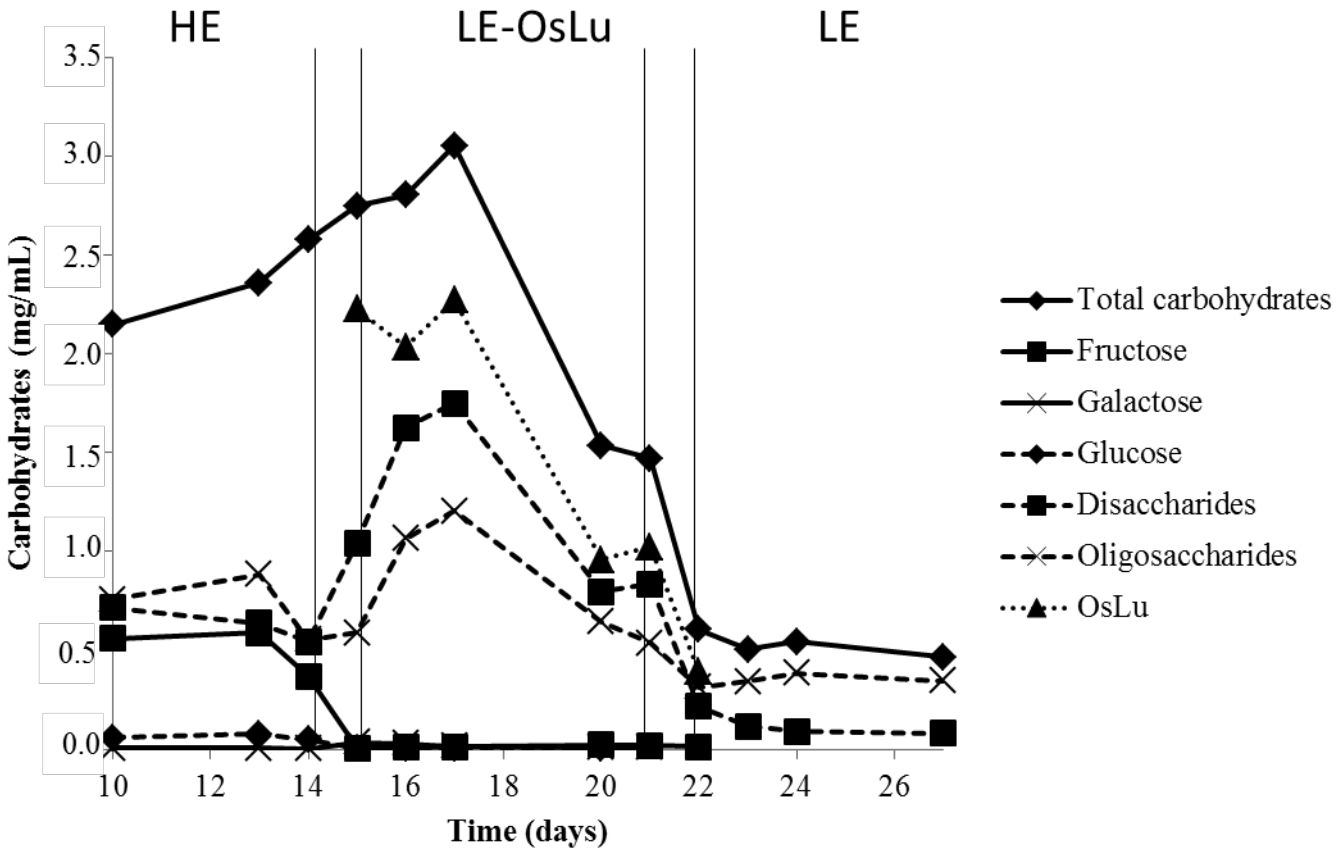

HE
LE-OsLu
LE
Carbohydrates (mg/mL)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10
12
14
16
18
20
22
24
26
Time (days)
Total carbohydrates
Fructose
Galactose
Glucose
Disaccharides
Oligosaccharides
OsLu

Figure 2.

SUPPLEMENTARY MATERIAL

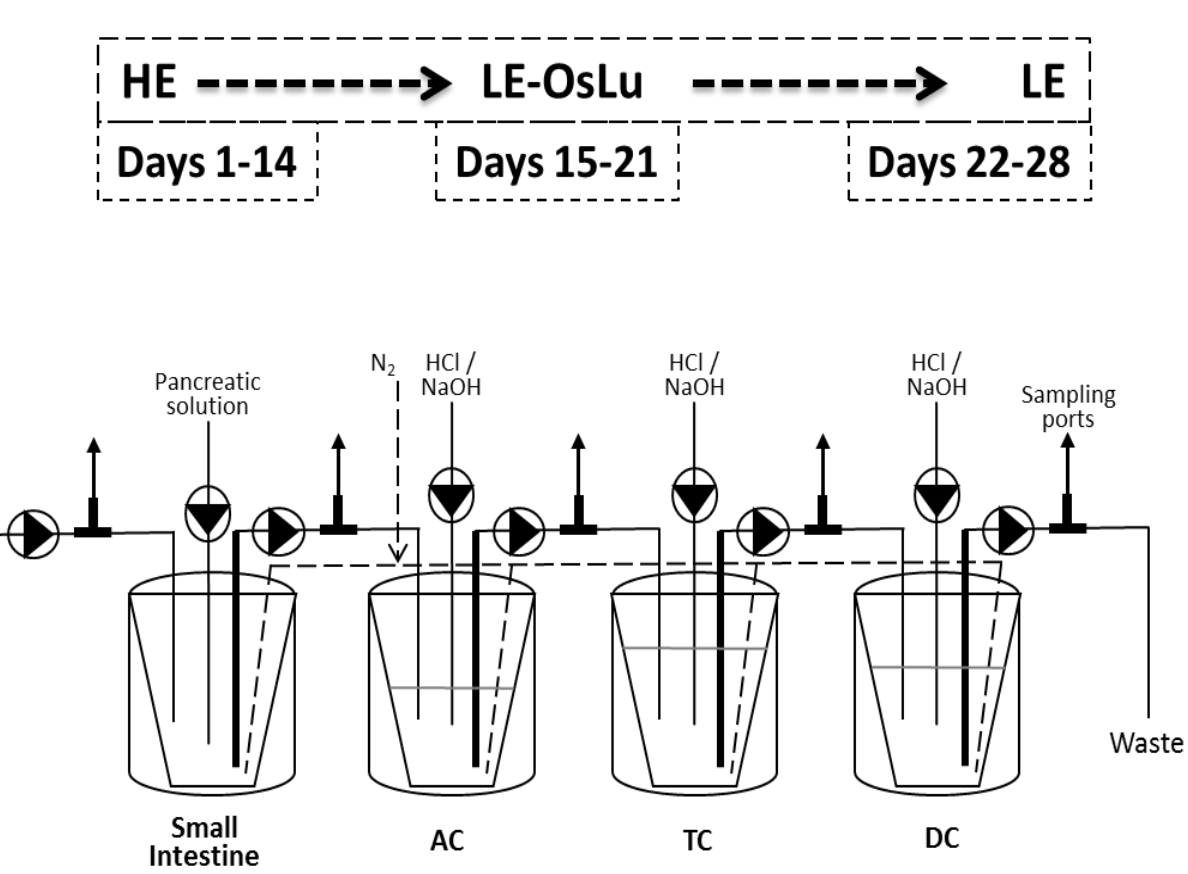


Fig. S1. Schematic diagram of the dynamic simulator of the intestinal microbiota, including the small intestine and the ascending (AC), transverse (TC) and descending colon (DC) compartments. The setup included the feeding with a starting high energy (HE) medium for 14 days, a 1-week experiment with a low energy (LE) medium with the oligosaccharide mixture derived from lactulose (OsLu) and a 1-week wash-out period with the LE medium.

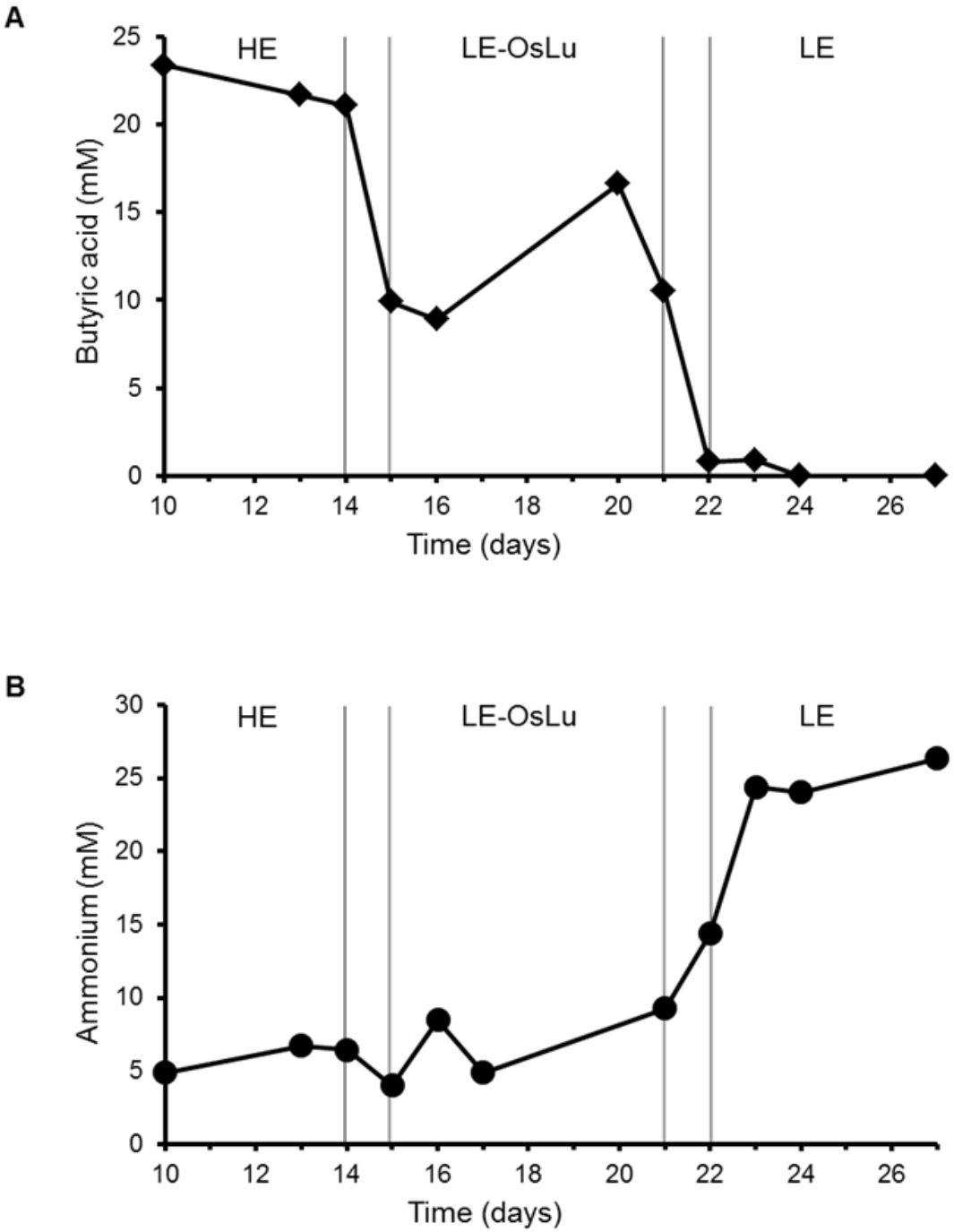


Fig. S2. Changes in concentration (mM) of butyric acid (A) and ammonium (B) in the ascending colon of the SIMGI at the end of the stabilization period with the high energy (HE; days 1-14) medium and during the feeding with the low energy (LE) medium with (days 15-21) and without (days 22-28) oligosaccharides derived from lactulose (OsLu).