FEMS Microbiology Ecology, 94, 2018, fiy140

doi: 10.1093/femsec/fiy140
Advance Access Publication Date: 19 July 2018
Research Article

RESEARCH ARTICLE

Simulation and modeling of dietary changes in the infant gut microbiome

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One sentence summary: We simulated a dietary change in the infant gut microbiome in a bioreactor, and developed a mathematical model to predict the outcome of these changes.

Editor: Cindy Nakatsu
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ABSTRACT

The early gut microbiome is essential for health, and diet has a profound influence in its composition. Oligosaccharides in breast milk or formula act as prebiotics, influencing gut microbiome structure. Here we simulated the impact of a dietary switch from fructooligosaccharides (FOS) to 2-fucosyllactose (2FL) in a continuous culture containing a consortium of species of the infant gut microbiome. During growth on FOS the consortium was dominated by *Lactobacillus acidophilus*, characterized by high amounts of lactate. Switching to 2FL led to a decrease in total biomass, and a recovery in *Bifidobacterium infantis* and *Escherichia coli* levels. While FOS was rapidly metabolized by the consortium, 2FL was utilized only after a delay. 2FL consumption was followed by a gradual switch from lactate to acetate. The activity of these bacterial species correlated well with gene expression analysis. Mathematical modeling of a multi-species consortium in continuous culture was capable to explain in great part the behavior of the system. The model was finally used to represent the outcome of the system after 48 h after each regime. This work highlights the impact of dietary changes in the gut microbiome, and provides a modeling framework to predict this influence.

Keywords: infant gut microbiome; mathematical model; continuous bioreactor; prebiotics; oligosaccharides; cross-feeding

INTRODUCTION

The intestinal microbiome goes through important changes throughout the life of an individual (Rodríguez *et al.* 2015). The establishment of the intestinal microbiota in the infant is a programmed succession of microbes (Bäckhed *et al.* 2015). Importantly, the initial patterns of colonization are thought to impact health and disease later in life (Abrahamsson *et al.* 2014; Raymond *et al.* 2015; Tamburini *et al.* 2016; Dzidic *et al.* 2017).

During this period, diet is an important factor that contributes to differences in this microbial community (Davis, Wang and Donovan 2017). Breast milk provides all the nutrients required for the infant to grow and develop properly (Smilowitz *et al.* 2013). In addition, it promotes an intestinal microbiota dominated by species of *Bifidobacterium* during the first year of life (Yatsunenko *et al.* 2012; Lewis *et al.* 2015; Matsuki *et al.* 2016). This predominance is explained in great part by the high amounts of human milk oligosaccharides (HMO) and other glycans present in breast milk that play a prebiotic role selecting for beneficial microbes (Thomson, Medina and Garrido 2017). HMO are complex molecules composed of five different monosaccharides arranged in multiple combinations, among which lacto-N-tetraose and 2-fucosyllactose (Fucα2–1Galβ1–4Glc) are dominant structures (Smilowitz *et al.* 2013). The intestinal microbiota of formula-fed infants differs

Received: 14 March 2018; **Accepted:** 17 July 2018

in overall composition, and the predominance of *Bifidobacterium* is not as pronounced (Yatsunenko *et al.* 2012; Madan *et al.* 2016). Infant formulas are normally supplemented with prebiotics such as galacto- and fructooligosaccharides (FOS), partly in order to simulate breast milk composition (Martin, Ling and Blackburn 2016). FOS are a mixture of fructose oligosaccharides in β2–1 linkage with a degree of polymerization 3–6. Prebiotic inulin-type fructans have normally been associated with the modulation of the gut microbiome (Vandeputte *et al.* 2017).

The gut microbiome could be considered as a microbial bioreactor, which continuously ferments complex dietary substrates not absorbed in the small intestine, for example polysaccharides or proteins (Korpela 2018). Short-chain fatty acids (SCFAs) are important products of microbial fermentation in the gut. Acetate, propionate and butyrate are produced in an approximate ratio of 60:20:20 in the adult (Macfarlane and Macfarlane 2003), while the feces of breast-fed infants are characterized by high concentrations of acetate and lactate (Knol *et al.* 2005; Fukuda *et al.* 2011). These acids reduce luminal pH and prevent pathogen colonization. In addition, SCFAs regulate host responses and are an important energy source for the colonic epithelium (Gao *et al.* 2009; Brahe, Astrup and Larsen 2013; Ang and Ding 2016).

In vitro bioreactor systems are useful to study the functions of the gut microbiome. Batch fermentations using fecal suspensions or defined microbes as inoculum have been used to evaluate microbial interactions and fermentability of oligosaccharides and the respective production of SCFAs (Takagi *et al.* 2016; Card *et al.* 2017; Medina *et al.* 2017b). Continuous fermentations inoculated with fecal material have been set up setting the retention time similar to flow of intestinal transit. Appropriate addition of nutrients and adjustment of environmental conditions allows culturing stable mixed communities and testing dietary interventions (Tanner *et al.* 2014; Chung *et al.* 2016). Sophisticated continuous systems such as SHIME (or also TWIN-SHIME and M-SHIME), and TIM-1 and TIM-2 systems add parallel fermenters mimicking different portions of the gastrointestinal tract (Fritz *et al.* 2013; Aguirre *et al.* 2016; García-Villalba *et al.* 2017). Recently, innovations such as HuMiX or Gut-on-a-Chip have addressed some of the limitations of *in vitro* systems, especially the interaction with human cells (Kim and Ingber 2013; Shah *et al.* 2016).

These experimental set-ups could be complemented with mathematical models in order to explain and represent the behavior of each system. These include ordinary differential equation-based models (ODE) (Kettle *et al.* 2015; Costa, Hartmann and Vinga 2016; Pinto *et al.* 2017), and genome-scale metabolic models (GSM) (Shoaie *et al.* 2013; El-Semman *et al.* 2014; Magnúsdóttir *et al.* 2016). At providing a complete representation of cellular metabolism, GSMs are useful to determine the nutritional requirements of microorganisms, in addition to predict cross-feeding (Magnúsdóttir *et al.* 2016). It has been recently shown that simple growth equations including metabolic interactions could explain and predict the composition and activity of a consortium of species of the infant gut microbiome (Pinto *et al.* 2017). These metabolic interactions could be of inhibition or cooperation (cross-feeding), and are usually mediated by macromolecule degradation products or SCFAs.

Oligosaccharides present in milk have a profound effect shaping the composition of the infant gut microbiome. Dietary changes in the infant could be observed in mixed feeding regimes, going from formula to breast milk, or a transition from breast milk to solid foods. Understanding the forces guiding the adaptations of the gut microbiome to diet are important for health. Normally, utilization of dietary substrates by the gut microbiome has been studied using either single strains or complex fecal samples, and controlled, reproducible systems with defined strains could be important to determine the impact of transition between dietary substrates in the gut microbiome. In this work we studied the behavior of a continuous bioreactor simulating the infant gut microbiome using four microorganisms, switching from a FOS-feeding regime to 2FL. We also developed a mathematical model of this dietary switch, which included multiple species and metabolic interactions, in order to explain and simulate the growth and metabolic activity of the system by performing parameter fitting.

MATERIALS AND METHODS

Microorganisms and media

Microorganisms were obtained from the UC Davis Viticulture & Enology Culture Collection (*Lactobacillus acidophilus* ATCC 4356, *Bifidobacterium longum subsp. infantis* ATCC 15 697 and *Escherichia coli* K12-MG1655), and the American Type Culture Collection (*Bacteroides vulgatus* ATCC 8482). Routine growth was performed in de Man Rogosa Sharp (MRS) broth (*L. acidophilus*) MRS supplemented with 0.05% L-cysteine-HCl (*B. infantis*), or Reinforced Clostridium Medium (Becton–Dickinson) supplemented with 1 g/L L-cysteine and 0.01 g/L hemin (Sigma-Aldrich, St. Louis, MO) for *B. vulgatus*. *Escherichia coli* was routinely grown in Standard Media Broth (Becton–Dickinson). All microorganisms except *E. coli* were grown in an anaerobic jar (Anaerocult, Merck, Germany) with anaerobic packs (Gaspak EM, Becton Dickinson, United States), at 37°C for 24–48 h. All media were prereduced in an anaerobic jar for 48 h before utilization. Previous to inoculations in the bioreactor, microorganisms were grown in mZMB, a previously reported media that allows the growth of gut microbes in the same culture medium (Medina *et al.* 2017b). Bacteria were cultured at 37°C for 24 h in mZMB supplemented with 0.05% L-cysteine-HCl, hemin (0.01 g/L, Sigma-Aldrich, St. Louis, MO) and 1% lactose as carbon source (mZMB-lac). Tryptone was autoclaved, and cysteine, lactose and hemin were 0.22 μm filter-sterilized and then added to the base media. Initial pH in the media was set at 5.5, and media was pre-reduced for 48 h before inoculations.

Bioreactor experiments

Two independent continuous fermentations were performed in a 250 mL bioreactor with automatic control (Minibio 250 connected to a MyControl controller, Applikon Biotechnology, Netherlands). The bioreactor was equipped with two six-bladed turbines operating at 100 rpm. The temperature was set at 37°C and the pH was maintained at 5.5 with automatic injection of 3 N NaOH. Dissolved oxygen was set at less than 1 ppm by automatically purging N_2 (99.99% grade) during the fermentation. The foam level was controlled adding 100 μL polydimethylsiloxane base to the initial broth (Winkler, Chile). Fresh cultures of *B. infantis*, *B. vulgatus*, *E. coli* and *L. acidophilus* grown in mZMB-lac were diluted to match the same OD (630 nm), centrifuged at 1 min 8000 x *g*, and pellets were washed in sterile and pre-reduced mZMB. After centrifugation at the same speed, all pellets were combined together in 10 mL mZMB supplemented with FOS 1% (Raftilose Synergy 1, Orafti, USA) and injected in the bioreactor. In a first phase, microorganisms were co-cultured for 12 h in a batch fermentation in a volume of 170 mL of mZMB

supplemented with 1% FOS. OD at 630 nm was continuously monitored taking 1 mL samples from the bioreactor and read in a spectrophotometer (Shimadzu UV-1280, Japan). After this period, the bioreactor was switched to continuous operation injecting mZMB-FOS 1% at a flow rate of 25 mL/h, automatically controlled using a peristaltic pump for 12 h. The media supplied to the bioreactor was maintained anaerobic in an anaerobic jar kept at 37°C in a stirred plate, connected to the bioreactor through plastic sterile tubes. A flow at the same rate than the input media was allowed to exit the bioreactor, in order to ensure a constant volume of operation. Three mL samples were taken every 2 h, which were used for OD monitoring, or to be centrifuged for 1 min at 8000 x *g* to store pellets and supernatants at −20°C until use. One mL cells were centrifuged and washed in RNA-later (Thermo Fisher Scientific), stored first at 4°C and the next day at −80°C. After 12 h in this regime, culture medium was quickly replaced by mZMB supplemented with 1% 2FL (Glycom, Denmark) as the carbon source. Flow rate was reduced to 18 mL/h to avoid extensive washing of the culture in the adaptation period. Continuous culture conditions were similar to the FOS regime, operating for additional 20 h.

Quantification of bacterial abundance by qPCR

Total DNA from each sample was purified using a phenol-chloroform modified protocol (Medina *et al.* 2017a). Extracted DNA was quantified in a NanoQuant Plate in the Tecan Infinite M200 PRO plate reader, and diluted to 10 ng/μL to be used in genus-specific qPCR reactions (Table S1, Supporting Information). qPCR reactions were carried out in an AriaMx Realtime PCR System machine (Agilent, USA), in a 10 μL volume and using the SensiFAST SYBR No-ROX kit (Bioline, UK) in MicroAmp Fast Optical plates (Applied Biosystems, USA). Cycling conditions included an initial polymerase activation for 2 min at 50°C and 2 min at 95°C, followed by 40 cycles of 3 s at 95°C, 10 s at 62°C and 20 s at 72°C. Absolute quantification was performed including a standard curve using genomic DNA from each species in 10-fold dilutions from 10 ng/μL to 0.1 pg/μL. Bacterial DNA amounts were converted into genome copy numbers as described previously (Medina *et al.* 2017a). Relative abundance was calculated considering the copy numbers from one microorganism relative to the total copy numbers in a sample, and expressed as percentage.

Substrate consumption

Total carbohydrate quantification in supernatants was performed using the phenol-sulfuric acid method (Pinto *et al.* 2017). Briefly, 30 μL of each sample, previously diluted with distilled water to 1:20 for 2-FL and 1:125 for FOS, were loaded in a 96-well microplate (Corning incorporated, USA). 100 μL of 98% sulfuric acid and 20 μL of 5% phenol were quickly added to each well. The microplate was incubated for 5 min at 90°C into a thermostatic water cabinet (Quimis, Brasil). The microplate was cooled in ice for 5 min, and OD at 490 nm was read using a Tecan Infinite microplate reader (Tecan Trading AG, Switzerland). Carbohydrate concentration was estimated using the absorbance value obtained, extrapolated from a standard curve made with dilutions of the same carbohydrate in the same media, from 2 mg/mL to 0.016 mg/mL.

Acid production

Acetate, lactate and propionate were quantified by HPLC using an Aminex HPX-87H ion exchange carbohydrate-organic acid column (Bio-Rad, USA) at 35°C with a flow rate of 0.450 mL/min (H_2SO_4 5mM, mobile phase) on a Lachrom L-700 HPLC system (Hitachi, Japan), equipped with a Diode Array and a Refractive Index detectors as described previously (Pinto *et al.* 2017). Acid concentration in each sample (g/L) was quantified using a standard curve made with dilutions of sodium acetate and sodium propionate (SIGMA, Japan) and L−(+)−Lactic acid (SIGMA, Belgium), starting from 30 g/L to 0.07 g/L.

Gene expression analysis

Total RNA from cell cultures was extracted using a modified acid phenol-chloroform-isoamyl alcohol (25:24:1) (pH 4.5) protocol (Medina *et al.* 2017a). Extracted RNA was treated with DNAse I (OMEGA, Bio-tek, USA) and reverse transcribed to cDNA using the Affinity Script qPCR cDNA Synthesis Kit (Stratagene, USA), following manufacturer instructions. Relative quantification of gene expression of select genes was performed by qPCR using primers in Table S1 (Supporting Information). Primers were designed using Primer-BLAST. Relative gene expression was determined using a cDNA sample from each microorganism during growth in mZMB-lactose as reference control, and housekeeping genes in each microorganism were identified from the literature (Table S1, Suporting Information).

Development of the mathematical model

The model developed in this study is based on general balance equations in continuous culture, but incorporating metabolic interactions as an additional term in the Monod equation. It also allows the growth of multiple species (n = 4) (Pinto *et al.* 2017). As input for the determination of the parameters, experimental microbial abundance data from each microorganism was included, in addition to the concentration of acetate and lactate produced and the amount of substrate consumed in each condition. To simplify the modeling process was characterized by three phases, FOS and 2FL growth phases were treated separately, and we included an intermediate transition phase from one regime to the other. During the transition phase, net growth was zero therefore no calculations were attempted. We assumed that all species could consume certain FOS fractions (including small amounts of fructose in FOS for *E. coli* (Kornberg 2001)), while only *B. infantis* consumed 2FL (Medina *et al.* 2017b). *Escherichia coli* is able to assimilate acetate (Wolfe 2005), and *B. vulgatus* lactate (Schultz and Breznak 1979). The complete model had 44 parameters (Supplementary material), which were adjusted to experimental data presented in this study. The following assumptions were taken: Monod kinetics includes an inhibition term; a microorganism will prefer the utilization of an oligosaccharide if it is able to consume it; and the reported ability of a microorganism to produce or consume an intermediate product in the literature. The model was written using Matlab® R2017a. Model parameter fitting was performed by numerically solving the minimization of the sum of squared residual errors between experimental and predicted data, normalized by the respective experimental data variance. The optimization was solved using the SSmGO Toolbox, a scatter search routine programed for Matlab® (Rodriguez-Fernandez, Egea and Banga 2006; Egea, Martí and Banga 2010). The model was analyzed by parameter identifiability and sensitivity, in order to

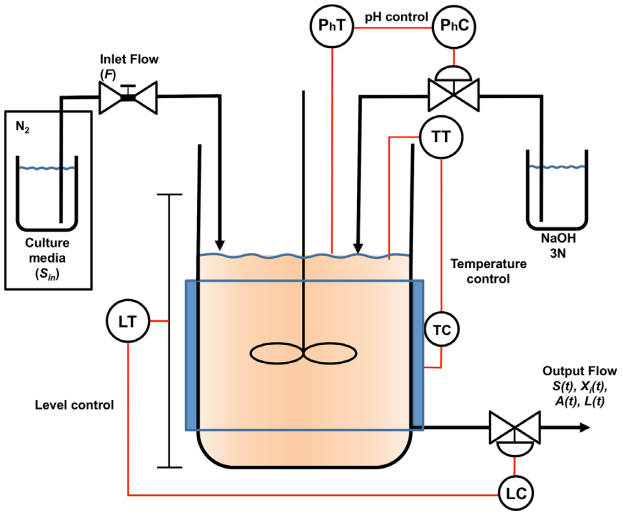

Figure 1. General representation of the continuous bioreactor used in this study. Anaerobic media containing either FOS or 2FL were fed to the system, which was stirred at 100 rpm. A constant volume was fixed by a level transmitter and controller (LT, LC). pH was set at 5.5 by adding NaOH, set by a transmitter and controller (PhT, PhC). Temperature was set at 37°C (TT, TC), and an output flow containing media and cells was recovered for analysis.

characterize the model developed and the parameters obtained. Matlab® scripts and diagnostics analyzes are presented in Supplementary Material.

RESULTS

Impact of a dietary change on microbial abundance

In this work we determined the impact of a dietary switch, from FOS to 2FL, on a continuous culture simulating the infant gut microbiome. The system contained four infant gut microbes (*L. acidophilus*, *E. coli*, *B. infantis* and *B. vulgatus*). Fig. 1 shows the set-up of the system. A first batch phase using FOS as carbon source was included to increase biomass, reaching an OD value near 7.0 after 12 h (Phase 0; Fig. 2A). This value remained stable in the last four measurements in both biological duplicates. Continuous growth in 1% FOS was allowed for 12 h at 25 mL/min (Phase I). During this phase biomass appeared stable at OD values near 7–8, suggesting exponential growth (Fig. 2A). *Lactobacillus acidophilus* increasingly dominated the culture, with a significant contribution of *E. coli* (Fig. 2B). During this phase *B. infantis* and *B. vulgatus* were minimally represented (Fig. 2B).

Switching to 2FL led to an important loss of biomass, which stabilized near 4.5 (Phase II and III; Fig. 2A). Relative abundances for each bacterium were stable for at least 4 h in the 2FL regime (Fig. 2B). However, an increase in *B. infantis* was observed from t = 18 h until the end of each run (Fig. 2B). This was accompanied by decreasing amounts of *L. acidophilus*, and an increasing representation of *E. coli* in the bioreactor (Fig. 2B). *B. vulgatus* was not detected at relevant concentrations at any phase.

Carbohydrate consumption and acid production

To determine whether the observed abundance patterns in each continuous phase correlated with substrate consumption, carbohydrate concentrations in the output flow were determined (Fig. 3A). Interestingly, FOS was not detected in the first phase in the output, indicating it was actively consumed. This also suggests FOS concentrations were limiting (Fig. 3A). These observations were confirmed in TLC plates (Fig. 3B), where we observed the absence of carbohydrates in the supernatants in this phase. This active consumption of FOS correlated with the dominance of *L. acidophilus*, and the respective large amounts of lactate produced (Fig. 3C). Later, switching to 2FL caused an initial accumulation of the substrate in the bioreactor (Phase II; Figs 3A and B). This indicates that the substrate was being consumed at a lower rate than it entered the system, or was not consumed at all. Only after 8 h in this regime 2FL concentrations started to decrease, suggesting that an adaptation period was required to utilize the oligosaccharide. No smaller degradation products were observed at any phase, such as mono or disaccharides (Fig. 3B). These observations correlated well with the increasing amounts observed of *B. infantis*, a strong reduction in lactate concentration (from 10 g/L to 2.5 g/L), and accumulation of acetate in the bioreactor (Fig. 3C).

Changes in gene expression associated to carbohydrate switch

Relative expression of select target genes was determined to further understand the behavior of the system during the dietary switch (Fig. 4). Samples for RT-qPCR were taken in the middle of the Phase I (6 h), in the transition phase (18 h), and at 24 h (Phase III). A 15-fold increase in a fructokinase gene expression was observed for *L. acidophilus* (Fig. 4A). This expression was reduced later to basal levels during 2FL growth. A gene encoding an EII from a glucose phosphotransferase system in *L. acidophilus* was also induced 4–6 fold during growth in FOS or 2FL, but not in the transition phase (Fig. 4A). None of the genes evaluated in *B. infantis* were induced during the FOS phase, while an α-fucosidase encoding gene was shown to be induced in the transition phase and mid-2FL growth (Fig. 4B) (Garrido *et al.* 2015). A galactokinase was also observed to be induced during growth in this substrate (Fig. 4B). Interestingly, *E. coli* showed the induction of a fucose permease encoding gene, suggesting it could utilize fucose left in the media (Fig. 4C). However, genes associated to lactose metabolism remained unchanged in *E. coli*. *B. vulgatus* gene expression was not clearly determined probably due to low cell levels during all phases.

Mathematical modeling of a multi-species continuous culture

We developed a kinetic black-box- ODE-based model, which represents the composition of a bacterial population, substrate consumption and SCFA production during continuous culture. The model is based on general growth equations of a continuous bioreactor culture but includes metabolic interactions and multiple species. The model included three growth phases as described above (Supplementary Material), and contained 44 parameters that were adjusted to experimental data (Fig. 5A and Fig. S1, Supporting Information). The model was characterized by parameter identifiability and sensitivity (Fig. S2 and Fig. S3, Supporting Information)

The parameters found supported the pronounced predominance of *L. acidophilus* during the FOS phase, which declined sharply during the phases II and III (Fig. 5A). The model also showed the recovery of *B. infantis* during the last 8 h of culture. However, the model did not capture the increase in *E. coli* levels in this phase (Fig. 5A). The parameters of the model also

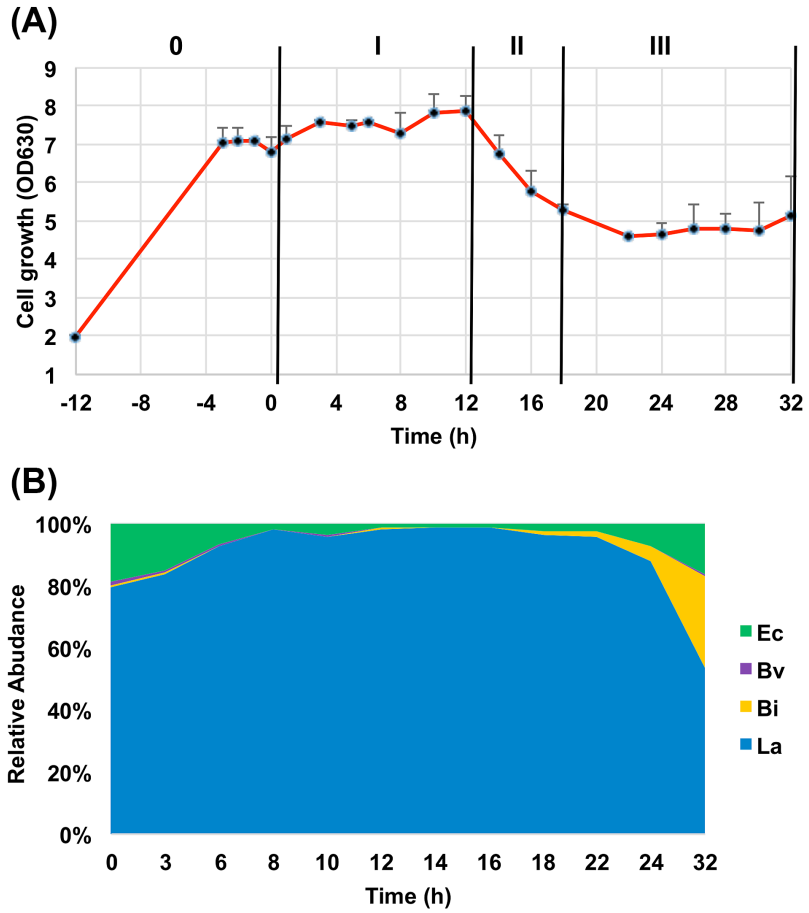

Figure 2. Changes in microbial abundance associated to the dietary switch. **A**, Total biomass in the bioreactor, measured by absorbance at 630 nm. Roman numbers indicate the growth phases of the study. **B**, Relative abundance of each member of the consortium during phases I, II and III, determined by qPCR. Ec: *E. coli*; Bv: *B. vulgatus*; Bi: *B. infantis* and La: *L. acidophilus*. Results are the average of two biological duplicates.

adjusted well to a full consumption of FOS in the first phase, the initial accumulation of 2FL later, and the gradual consumption of the oligosaccharide in the third phase (Fig. 5B). The mathematical description indicated that 2FL should stop accumulating in the bioreactor at t = 32 h, becoming the limiting substrate.

Lactate was the major acid produced during the first phase at high and stable concentrations. However, the model indicated a more gradual decrease in lactate production during FOS utilization (Fig. 5C). This could be explained by the prioritization that the optimization process gives to biomass production, compared to other parameters such as acid production. In the model lactate levels remained low during the 2FL phase but they increased due to *B. infantis* growth (Fig. 5C) (Garrido *et al.* 2013). Acetate concentration was well adjusted by the model in both phases (Fig. 5D).

Mathematical representation of the dietary change for extended time

The mathematical model was used later to simulate the outcome of the four-species continuous culture in an extended FOS regime of 48 h, switched to 2FL for 48 h, and returning to a FOS regime for additional 48 h. Each transition included a 4 h lag period (Fig. 6A). This analysis indicates that extending the FOS regime to 48 h should consolidate the growth of *L. acidophilus*, resulting in full FOS consumption, and displacing all other microbes. Lactate levels decrease probably due to a lower biomass/acetate yield value found for this bacterium by the model.

The switch to 2FL at t = 48 h was predicted to cause a large reduction in *L. acidophilus* that at t = 100 h should be at its minimum. This was accompanied by the corresponding predominance of *B. infantis*. No major presence of *B. vulgatus* or *E. coli* was estimated by the simulation. Switching to 2FL showed an initial accumulation of the substrate, as well as increasing concentrations of lactate and acetate that reached a maximum at t = 80 h. This occurred at the same time when 2FL became the limiting substrate, indicating that the 2FL input rate matched the substrate consumption rate in the bioreactor.

The final switch, from 2FL to FOS, caused a second wave of substrate accumulation, necessary for the system to match consumption rates to the substrate input rate. This accumulation of FOS appeared smaller compared to 2FL, probably due to faster growth rates in the FOS regime dictated by the parameters. Transition from 2FL to FOS in the model allowed a full recovery of *L. acidophilus*, the wash out of *B. infantis* cells, and decreasing amounts of lactate and acetate.

DISCUSSION

There is great interest in identifying, understanding and predicting factors influencing the composition of the gut microbiome

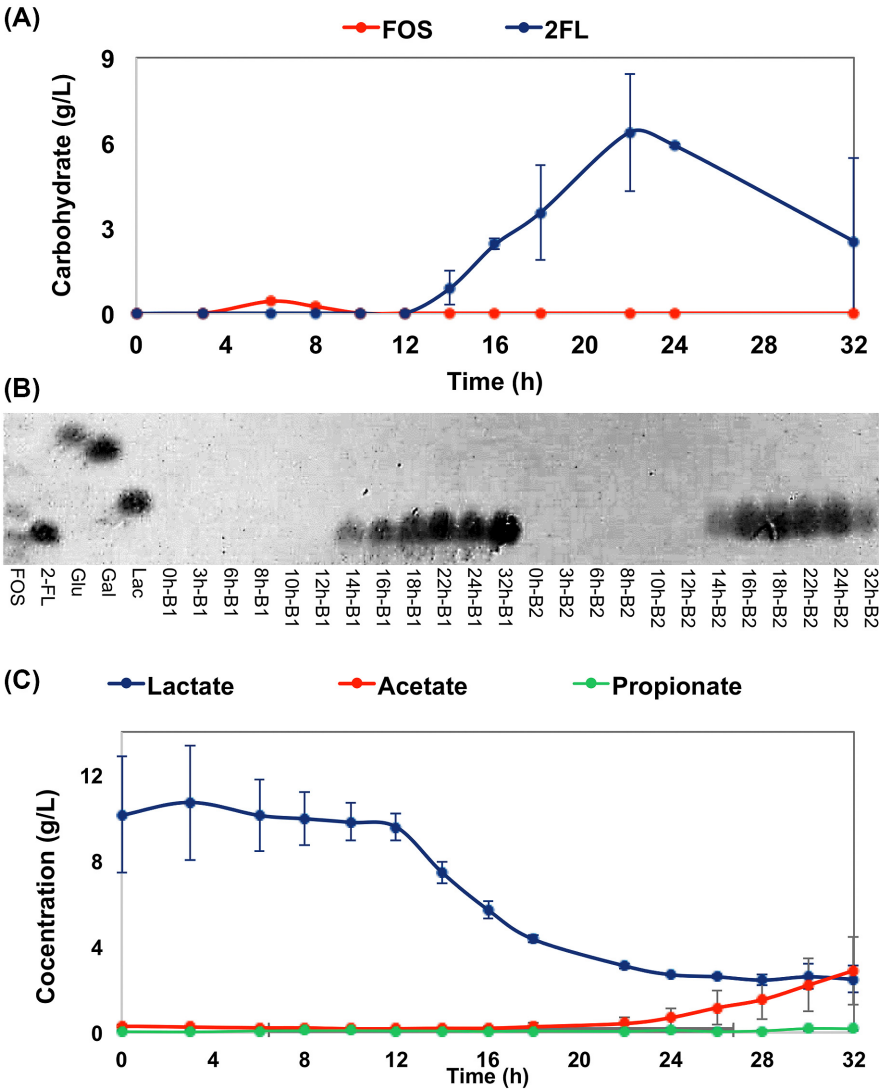

Figure 3. Carbohydrate consumption and acid production during continuous culture. **A**, Carbohydrate left in the media during the FOS phase or 2FL phase. **B**, TLC analysis of bioreactor supernatants. Glu: Glucose standard; Gal: galactose standard; Lac: lactose standard. Bioreactor samples spotted are indicated by time (h) sample was taken, and B1 and B2 indicate each bioreactor replicate. **C**, Acid production quantified in bioreactor supernatants by HPLC.

(Shoaie *et al.* 2013; Kettle *et al.* 2015). The infant gut microbiome is characterized as a dynamical system, with a relative simple composition and shaped by diet (Yatsunenko *et al.* 2012; Bäckhed *et al.* 2015). *In vitro* systems emulating the gut microbiome have been helpful to study the impact of this microbial community on host health. In this study, we set up a simple system in a continuous bioreactor to test the impact of a dietary change, from FOS to 2FL. We used a growth media that resembles some of the properties of the proximal colon: a partially degraded protein source derived from casein, a fixed pH of 5.5, and oligosaccharides as the sole carbon source (Medina *et al.* 2017b). Therefore, the only difference in media composition between the phases of the study was the oligosaccharide provided to the system. A bioreactor offers a controlled environment in terms of temperature and anaerobic atmosphere, low speed stirring and continuous input and output of nutrients. Results were in general very similar in two independent experiments, suggesting a high reproducibility of the system. Flow rates in the bioreactor were estimated and set to match intestinal flow rate (Weaver and Steiner 1984; Macfarlane, Macfarlane and Gibson 1998). This resulted in substrate limitation, especially during the FOS regime, which was completely consumed during continuous operation and theoretically allowed exponential growth of the dominant microbe in this phase.

A continuous system presents several advantages compared to a batch system, for example preventing product accumulation and reaching a steady state. Continuous systems are harder to control and set-up, and they are prone to microbial contamination. One important disadvantage of this set-up is the lack of mechanisms for SCFA absorption. 95% of these acids could be absorbed by the colonic epithelium (Ruppin *et al.* 1980). We consider that the system studied here could be more representative of the intestinal lumen. This set-up could be improved by adding a mucin layer that permits bacterial adhesion for certain sessile microorganisms.

The results obtained in terms of overall abundance were expected and could resemble the influence of these oligosaccharides *in vivo* (Costalos *et al.* 2008; Salminen *et al.* 2016). We have previously determined that microbial interactions in the

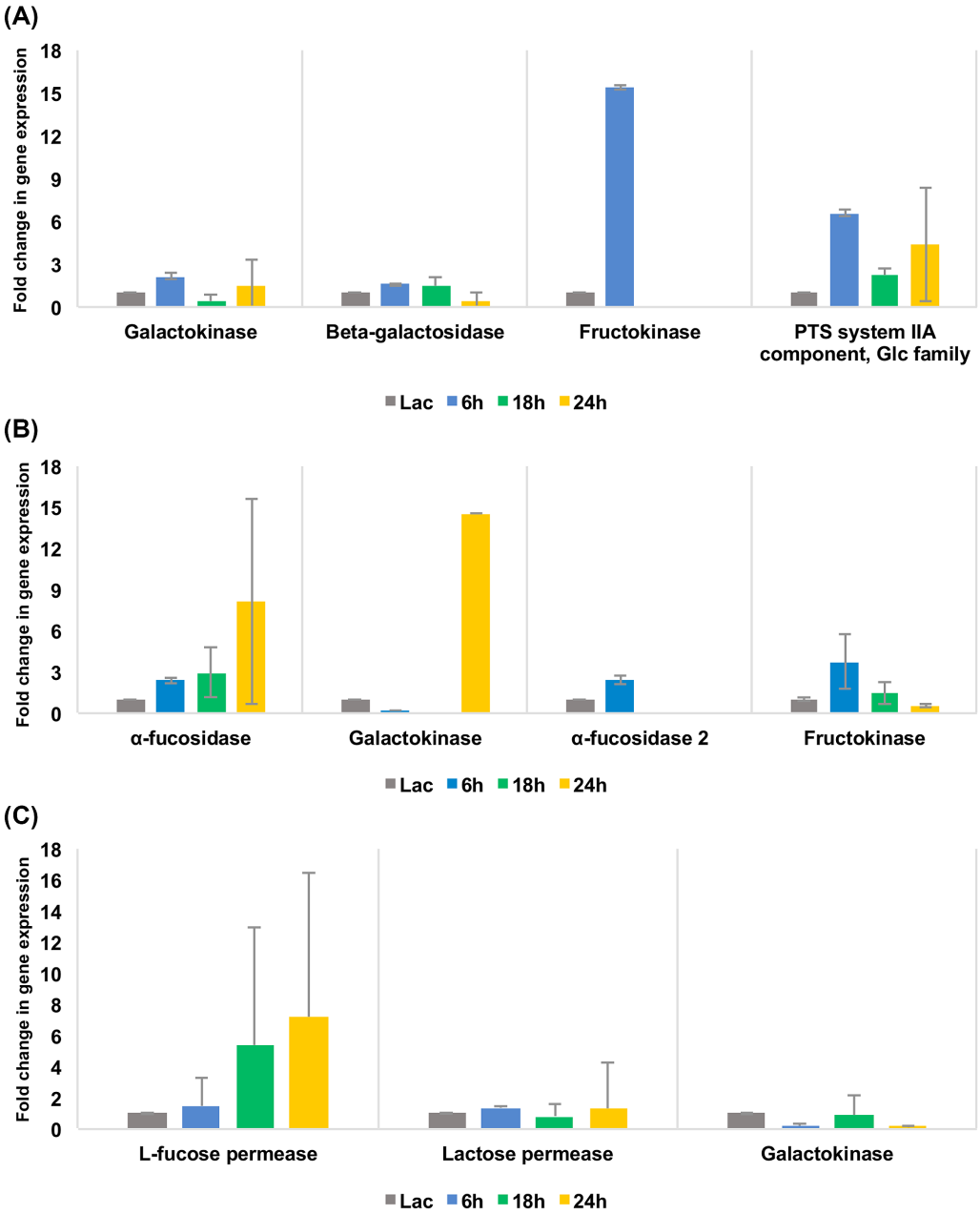

Figure 4. Gene expression qPCR analysis of select genes in the microorganisms in the consortium. Time points represent the three phases of continuous growth. Locus tags and additional gene information is shown in Table S1 (Supporting Information). **A**, *L. acidophilus*; **B**,*B. infantis*. **C**, *E. coli*. Relative gene expression was normalized to growth on lactose (lac).

consortium of these microorganisms are dependent on the substrate, and mostly of competition during growth on FOS (Medina *et al.* 2017b). All microorganisms in the consortium could use certain fractions of FOS (Kaplan and Hutkins 2000), however *L. acidophilus* was the most active microorganism in the FOS phase, which was confirmed by gene expression analysis. The predominance of *L. acidophilus* on FOS could be explained by its markedly higher growth rate on the substrate (Pinto *et al.* 2017). Growth rates on each substrate, determined in the model presented in this study, could be used to predict microbial abundance in more complex systems. Finally, *B. infantis* was very active in the 2FL phase, which resulted in increased expression of genes associated to the metabolism of the oligosaccharide, a reduction in 2FL in spent media, and increasing concentrations of acetate. The utilization of HMO by this bacterium has been well studied (Garrido *et al.* 2015).

Total biomass produced in the bioreactor during the FOS phase was higher compared to 2FL phase. It could be expected that dietary switches induce great changes in the total number of microorganisms in the intestine. One important consequence observed after the dietary switch was the adaptation time required by the system, which lasted 6 h. During this period we observed a marked washout of acids and microorganisms from the system, and the concomitant accumulation of substrate. While it took 6 h to *E. coli* to induce a fucose permease, this time was higher for *B. infantis* to show full induction of its fucosidase and galactokinase (Fig. 4B). Dietary alterations are characterized by an adaptation period. Short dietary changes in healthy

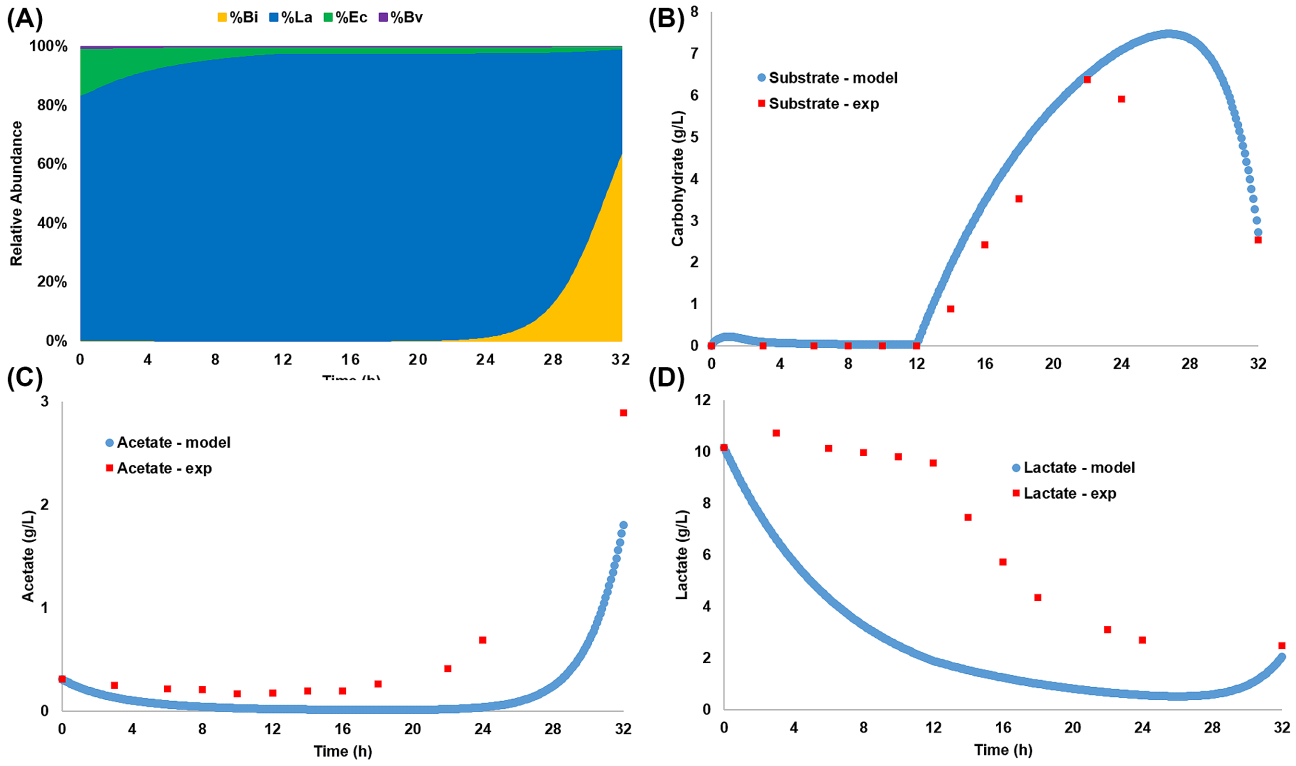

Figure 5. Parameter fitting to experimental data in the model. Mathematical modeling of relative microbial abundance (A) and substrate consumption (B) in continuous culture during the FOS and 2FL regime. Parameter estimation was also run for lactate concentration (C) and acetate (D). Experimental data is shown in red dots. Solid lines indicate the three phases modeled.

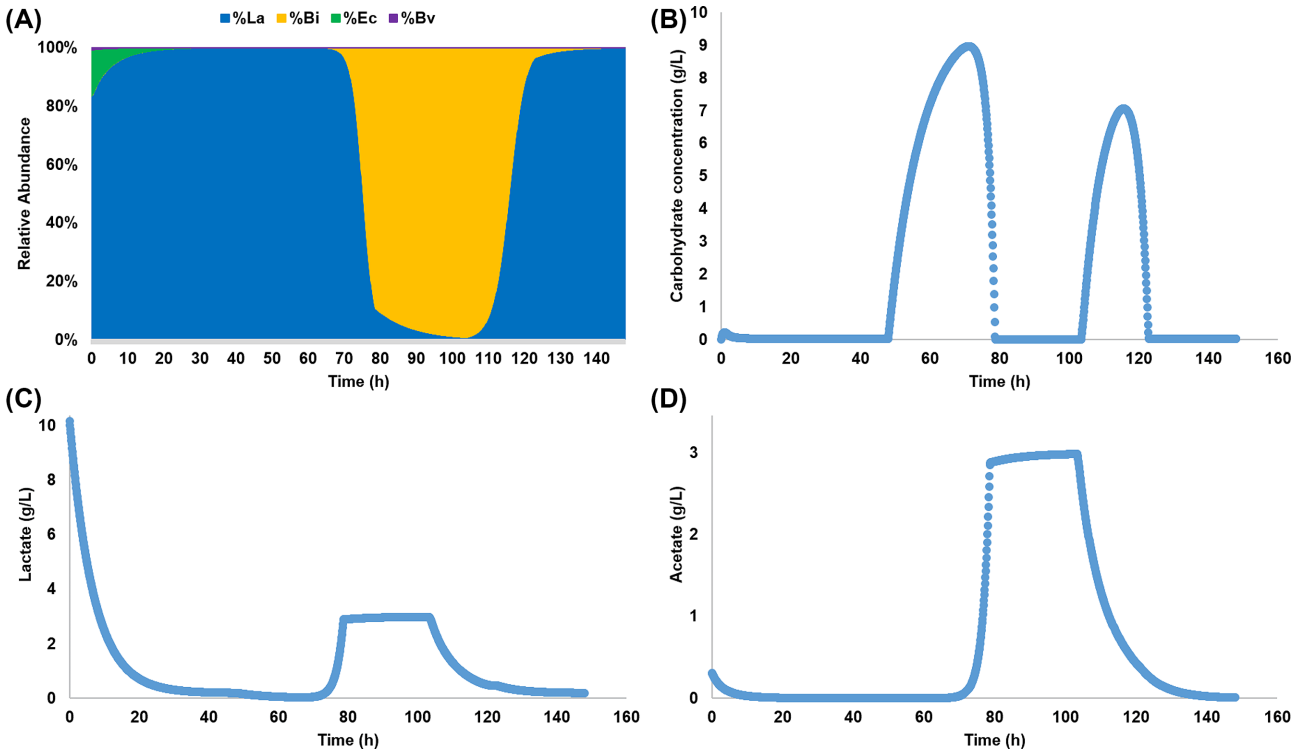

Figure 6. Model simulation of extending feeding time and crossed dietary switches. Using the parameters estimated in the model, these were extended for 48 h, and the transition 2FL to FOS was added. **A**, estimated relative abundance for the microorganisms in the consortium. **B**, estimated total carbohydrate concentration in the bioreactor. **C**, acetate concentration estimated in the three growth phases. **D**, lactate concentration estimated in the three growth phases. Solid lines indicate the transition phases simulated.

subjects such as consumption of animal or plant-based diets induce rapid changes in the gut microbiome of young adults. Interestingly, these changes are reversible after two days (David et al. 2014).

Escherichia coli was not very active during the FOS phase, but its relative abundance increased importantly during the 2FL phase (Fig. 2B and Fig. S1, Supporting Information). This growth could be explained for example by cross-feeding of fucose, acetate or protein consumption. We did not observe free monosaccharides at any time in the output flow during the 2FL phase, however this could indicate that if fucose is released, it is immediately consumed inside the bioreactor. *Escherichia coli* has the machinery to grow on the monosaccharide (Pacheco et al. 2012), and in this study we observed the induction of a fucose permease. Another plausible explanation for *E. coli* growth in this phase is by acetate utilization. The bacterium is able to assimilate acetate in a phenomenon known as 'acetate switch' (Treves, Manning and Adams 1998; Wolfe 2005). Cross-feeding has also been observed in the infant gut using lactate as exchangeable metabolite (Pham et al. 2016).

We previously developed and validated a mathematical model showing that metabolic interactions of inhibition and cross-feeding could explain the relative abundance of species and acid profiles of a simple microbiome consortium (Pinto et al. 2017). Experimental data in this study was used to develop an ODE-based mathematical model, which contains general continuous bioreactor growth equations, modified to include multiple species and allowing metabolic interactions. Adjusting the parameters of the equations used to experimental data, allowed a good interpretation of the observed results. This indicates that the parameters found and the equations included explain the behavior of the system in the three phases, in terms of relative microbial abundance, substrate consumption and acid production. The development of the model prioritizes the reduction of the error between experimental data and modeled parameters, especially those related to biomass production and substrate consumption. This is why the parameters that generate the smaller error in the objective function are growth rates and biomass/substrate yields. This could explain the poor adjustment of lactate production during the FOS phase.

Parameter identifiability allowed to find terms in the model that are directly or inversely correlated locally in a given time interval (Fig. S2, Supporting Information). It is calculated by the correlation matrix between each parameter in the model calculated previously on the sensitivity matrix. If two parameters are highly correlated, these parameters affect the measured variable in exactly the same way. Given the experimental data generated, not all the parameters were identifiable that indicates that model presented needs extra experimental data. As shown in Fig. S3 (Supporting Information), the parameter sensitivity analysis indicates that the effects of the parameters initially set are important in every ODE, due to the fact that *L. acidophilus* and *B. infantis* appear to be dominant microorganisms in the consortium during FOS or 2FL consumption respectively. Specifically, the fifth parameter of the model (Bi's μmax in 2FL) has the highest influence on carbon consumption and acid production on all microbes. Additionally, we observed that *L. acidophilus* displays a large influence on the growth especially of E. coli during the FOS phase.

In addition, the model developed was used to expand the time of each phase for 48 h, or four residency times. The simulation was also performed to determine the outcome in the system when switching from 2FL to FOS. The results of the simulation are expected in terms of microbial abundance, and clearly show the accumulation of substrate before it could be used by the consortium. They also show the importance of the time point when the substrate becomes a limiting substrate, which results in later changes in the concentration of other products such as acetate and lactate. A good parameter fit was observed between the experimental data and the modeling results, resulting in a good representation of the interactions in the consortium. This suggests, we generate a model structurally capable of determining the composition and functions of a consortium of species of the gut microbiome. However, it is necessary to consider that the parameters obtained must be validated with new experimental data to evaluate the predictive power of the model.

CONCLUSIONS

Here we determined the impact of a dietary change in a continuous bioreactor simulating the gut microbiome. The switch reduced the total biomass in the system and changed microbial abundance in the consortium, adjusting to a new steady state. Changes were correlated with substrate consumption and acid production. The results obtained indicated also that a transition period is required for the dietary switch, which could cause loss of certain species and transiently alter SCFA profiles. A mathematical model was also developed to explain the variations in abundance and performance of the system, as well as to simulate long-term outcomes. A combination of *in vitro* and in silico analyses could be scaled to study the impact of dietary and non-dietary alterations on larger consortia of gut microorganisms.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

ACKNOWLEDGEMENTS

This work was funded by Fondecyt de Iniciacion 11130518, Fondecyt de Postdoctorado 3160525 and FONDEF IDEA ID16i10045. We thank Diego Sandoval for his technical support.

Conflicts of interest. None declared.

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