

Impact of 2'-fucosyllactose on gut microbiota composition in adults with chronic gastrointestinal conditions: batch culture fermentation model and pilot clinical trial findings

Article

Published Version

Creative Commons: Attribution 4.0 (CC-BY)

Open Access

Ryan, J. J. ORCID: <https://orcid.org/0000-0001-6324-9186>,
Monteagudo-Mera, A., Contractor, N. and Gibson, G. R.
ORCID: <https://orcid.org/0000-0002-0566-0476> (2021) Impact
of 2'-fucosyllactose on gut microbiota composition in adults
with chronic gastrointestinal conditions: batch culture
fermentation model and pilot clinical trial findings. *Nutrients*,
13 (3). e938. ISSN 2072-6643 doi:
<https://doi.org/10.3390/nu13030938> Available at
<https://centaur.reading.ac.uk/96897/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.3390/nu13030938>

Publisher: MDPI

including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Article

Impact of 2'-Fucosyllactose on Gut Microbiota Composition in Adults with Chronic Gastrointestinal Conditions: Batch Culture Fermentation Model and Pilot Clinical Trial Findings

Jennifer Joan Ryan ^{1,*}, Andrea Monteagudo-Mera ^{2,†}, Nikhat Contractor ³ and Glenn R. Gibson ²¹ Helfgott Research Institute, National University of Natural Medicine, Portland, OR 97201, USA² Department of Food and Nutritional Sciences, University of Reading, Reading RG6 6AD, UK; a.monteagudo@reading.ac.uk (A.M.-M.); g.r.gibson@reading.ac.uk (G.R.G.)³ Metagenics, Inc., Aliso Viejo, CA 92656, USA; nikkycontractor@metagenics.com

* Correspondence: jryan@nunm.edu; Tel.: +1-503-552-1744

† These authors contributed equally to this work.

Abstract: Intestinal dysbiosis has been described in patients with certain gastrointestinal conditions including irritable bowel syndrome (IBS) and ulcerative colitis. 2'-fucosyllactose (2'-FL), a prebiotic human milk oligosaccharide, is considered bifidogenic and butyrogenic. To assess prebiotic effects of 2'-FL, alone or in combination with probiotic strains (potential synbiotics), *in vitro* experiments were conducted on stool from healthy, IBS, and ulcerative colitis adult donors. In anaerobic batch culture fermenters, *Bifidobacterium* and *Eubacterium rectale*-*Clostridium coccooides* counts, and short-chain fatty acids (SCFAs) including butyrate increased during fermentation with 2'-FL and some of the 2'-FL/probiotic combinations. In a subsequent open-label pilot trial, the effect of a 2'-FL-containing nutritional formula was evaluated in twelve adults with IBS or ulcerative colitis. Gastrointestinal Quality of Life Index (GIQLI) total and gastrointestinal symptoms domain scores, stool counts of *Bifidobacterium* and *Faecalibacterium prausnitzii*, and stool SCFAs including butyrate, increased after six weeks of intervention. Consistent with documented effects of 2'-FL, the batch culture fermentation experiments demonstrated bifidogenic and butyrogenic effects of 2'-FL during fermentation with human stool samples. Consumption of the 2'-FL-containing nutritional formula by adults with IBS or ulcerative colitis was associated with improvements in intra- and extra-intestinal symptoms, and bifidogenic and butyrogenic effects.

Keywords: 2'-FL; dysbiosis; fucosyllactose; gut microbiota; human milk oligosaccharides; irritable bowel syndrome; inflammatory bowel disease; prebiotics; probiotics



Citation: Ryan, J.J.; Monteagudo-Mera, A.; Contractor, N.; Gibson, G.R. Impact of 2'-Fucosyllactose on Gut Microbiota Composition in Adults with Chronic Gastrointestinal Conditions: Batch Culture Fermentation Model and Pilot Clinical Trial Findings. *Nutrients* **2021**, *13*, 938. <https://doi.org/10.3390/nu13030938>

Academic Editor: Corinne Grangette

Received: 29 January 2021

Accepted: 11 March 2021

Published: 14 March 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Irritable bowel syndrome (IBS) [1], inflammatory bowel disease (IBD) [2], and celiac disease [3] are chronic gastrointestinal conditions with predominantly differing pathogenesis. Interestingly, these conditions are characterized by at least some level of chronic intestinal mucosal inflammation [4–6]. However, in contrast to patients with IBD and celiac disease, the intestinal inflammation in patients with IBS is considered low grade [4,5].

Pathophysiology shared among individuals with IBS, IBD, and celiac disease includes dysbiosis, defined as “any change to the composition of resident commensal communities relative to the community found in healthy individuals” [7]. Patients with IBS [8,9], IBD [10,11], and celiac disease [12] may have reduced stool counts of the genus *Bifidobacterium* and the butyrate-producing species *Faecalibacterium prausnitzii*.

According to recent consensus statements by the International Scientific Association for Probiotics and Prebiotics (ISAPP), a prebiotic is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” [13], probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit

on the host" [14], and a synbiotic is defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" [15]. Prebiotics and probiotics represent non-pharmacologic approaches for addressing dysbiosis associated with IBS [16–18], IBD [19,20], and celiac disease [21]. Prebiotics support the growth and activity of specific health-promoting, non-pathogenic microorganisms such as *Bifidobacterium* [13]. Probiotic mechanisms among a diversity of strains include normalization of perturbed microbiota, regulation of intestinal transit, and short-chain fatty acid (SCFA) production [14]. SCFAs are the main end products of anaerobic microbial fermentation in the mammalian colon [22,23]. Rapidly absorbed by the intestinal epithelium, the pleiotropic SCFA butyrate modulates visceral sensitivity [24] and intestinal motility [24], and regulates intestinal inflammation through multiple mechanisms including inhibition of nuclear factor-kappa B (NFkB) activation [24,25].

Dietary prebiotics can stimulate endogenous intestinal bacteria, having the effect of promoting butyrate production [22,26,27]. Due to abilities to increase the activity and growth of bifidobacteria and butyrate-producers via cross-feeding mechanisms, specific prebiotics have been described as having both bifidogenic and butyrogenic effects [26,28,29]. 2'-fucosyllactose (2'-FL) is a trisaccharide prebiotic and is the human milk oligosaccharide (HMO) most abundantly produced by the majority of nursing mothers [30]. However, whether or not a nursing mother produces 2'-FL depends on whether or not they are secretors of the fucosyltransferase 2 (FUT2) gene [31]. Serving as growth substrates for intestinal bacteria in the distal colon, HMOs act as the first prebiotics consumed by nursing infants [32–35]. In the human gastrointestinal tract, *Bifidobacterium* species are the main utilizers of HMOs, which are metabolized to the SCFA acetate and lactic acid [35,36].

Health benefits to infants are apparent for HMOs [37,38] including for supplementation of 2'-FL given to formula-fed infants [39,40]. Supplemental 2'-FL and lactose-N-neotetraose, synthesized and structurally similar to that found in human milk, has been associated with a bifidogenic effect in infants [40]. However, data on potential benefits of supplemental 2'-FL in adult populations are extremely limited. Elison et al. [41] reported that oral supplementation with 2'-FL resulted in increased stool counts of *Bifidobacterium* in healthy adults and Iribarren et al. [42] recently reported that a combination of 2'-FL and lactose-N-neotetraose resulted in increased stool counts of *Bifidobacterium*.

Given that patients with specific chronic gastrointestinal conditions exhibit dysbiosis (including reduced *Bifidobacterium*) and 2'-FL may induce bifidogenic effects, anaerobic batch culture experiments were implemented to assess prebiotic effects of 2'-FL. Alone or in combination with different probiotic strains (potential synbiotics), impact of 2'-FL on the composition and metabolic activity of stool microbiota from healthy adult volunteers and individuals with IBS or ulcerative colitis was evaluated. Several strain-identified, commercially-available probiotic species selected from the most commonly used and clinically-recommended bacterial genera of probiotics, *Lactobacillus* and *Bifidobacterium* [43], were tested. A subsequent pilot trial was conducted to examine the impact of a 2'-FL-containing nutritional formula on gastrointestinal quality of life, stool counts of commensal microbiota, and stool SCFA concentrations in adults with IBS, IBD and celiac disease.

2. Materials and Methods

2.1. In Vitro Experiments

2.1.1. 2'-FL and Probiotic Strains

Five potential synbiotic combinations were tested as described in Table 1. The 2'-FL and probiotic strains were supplied by Metagenics, Inc. (Aliso Viejo, CA, USA). Probiotic suspensions were prepared for addition to fermenter vessels by centrifuging at $14,000 \times g$ for 5 min. The supernatant was removed and cells were washed twice in anaerobic phosphate buffer (1 M, pH 7.4). Cells were re-suspended in 1 mL phosphate buffer at a concentration of 10^7 CFU/mL immediately before addition to fermenter vessels.

Table 1. Potential Synbiotic Combinations Evaluated *In Vitro*.

| | |
|------------------|---|
| Synbiotic 1 (S1) | 2'-FL + <i>Lactobacillus acidophilus</i> NCFM (ATCC 5221) + <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> Bi-07 (ATCC 5220) |
| Synbiotic 2 (S2) | 2'-FL + <i>L. acidophilus</i> NCFM + <i>B. animalis</i> subsp. <i>lactis</i> Bi-07 + <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BI-04 (ATCC 5219) + <i>Lactobacillus paracasei</i> Lpc-37 (ATCC 5275) |
| Synbiotic 3 (S3) | 2'-FL + <i>L. acidophilus</i> NCFM + <i>B. animalis</i> subsp. <i>lactis</i> Bi-07 + <i>Lactobacillus rhamnosus</i> GG (ATCC 7017) |
| Synbiotic 4 (S4) | 2'-FL + <i>L. rhamnosus</i> GG |
| Synbiotic 5 (S5) | 2'-FL + <i>Lactobacillus salivarius</i> UCC118 |

2.1.2. Batch Cultures

Anaerobic batch culture experiments were used to investigate the ability of stool microbiota to utilize 2'-FL and to test different potential synbiotic combinations containing 2'-FL. These were performed in three populations in triplicate using stool samples from 9 different volunteers (healthy, n = 3; IBS, n = 3; ulcerative colitis, n = 3). Donors were aged between 24 and 40 years old, who had not received antibiotics, or prebiotic or probiotics supplements for at least six months prior to sample collection. IBS donors had received a medical diagnosis and were not under any treatment at the time of the experiment. Ulcerative colitis donors were being treated with mesalazine, and all were in remission at the time of this study. Sterile gently stirred pH-controlled batch culture 10 mL fermentation vessels were aseptically filled with 9 mL of sterile (autoclaved) basal nutrient medium and sparged with O₂-free N₂ (15 mL/min) overnight to establish anaerobic conditions. The basal medium (per liter) consisted of: 2 g peptone water, 2 g yeast extract, 0.1 g NaCl, 0.04 g K₂HPO₄, 0.04 g KH₂PO₄, 0.01 g MgSO₄·7H₂O, 0.01 g CaCl₂·6H₂O, 2 g NaHCO₃, 2 mL Tween 80, 0.05 g hemin, 0.01 mL vitamin K1, 0.5 g L-cysteine-HCl, 0.5 g bile salt and 4 mL resazurin solution (0.25 g/L). 2'-FL (1% w/v) and probiotic combinations (10⁷ CFU per strain) were added to fermentation vessels just before the addition of the fecal slurry which was prepared by homogenizing fresh human feces (10%, w/v) in anaerobic phosphate-buffered saline. Vessels were kept at 37 °C using a circulating water bath and pH controlled between 6.7 and 6.9 using an automated pH controller (Fermac 260, Electrolab, Tewkesbury, UK). Each vessel was inoculated with 1 mL of fresh fecal slurry. Additionally, vessels with fructooligosaccharides (FOS)/inulin mixture (1% w/v) and without any substrate were used as positive and negative controls respectively. Batch culture fermentations were run for 24 h and samples were collected at 0, 8, and 24 h.

2.1.3. Flow Cytometry-Fluorescence In Situ Hybridization for Bacterial Enumerations

Samples (750 µL) were centrifuged at 13,000× g for 5 min at room temperature. Supernatants were collected and filtered through a 0.22 µm Millipore syringe filter for gas chromatography analysis. Pellets were fixed for further fluorescence in situ hybridization and kept at −20 °C. Briefly, after centrifugation pellets were resuspended in 375 µL of 1X PBS and 1150 µL of cold 4% (v/v) paraformaldehyde. The suspension was mixed and stored at 4 °C for 4–6 h. After incubation, samples were washed twice with 1 mL of 1X PBS. Finally, samples were centrifuged at 13,000× g for 5 min, supernatant was discarded and the pellet was resuspended in 300 µL of 1X PBS and 300 µL of ethanol. Samples were vortexed and stored at −20 °C for further analysis.

For Flow-FISH cytometry, we used 75 µL of the fixed samples. Fixed cells were washed twice with PBS and pre-treated for 10 min with lysozyme at 1 mg/mL. Cells were resuspended in 1 mL hybridization buffer. All hybridizations were performed in the dark at 35 °C overnight in the hybridization solution containing genus- and group-specific 16S rRNA-targeted oligonucleotide probes. Cells were centrifuged at 10,000× g for 3 min, resuspended in pre-warmed washing buffer and incubated at 37 °C for 20 min to remove

non-specific binding of the probe. Finally, cells were centrifuged at $10,000 \times g$ for 3 min and resuspended in PBS for flow cytometry analysis. Probes used are described in Table 2.

Table 2. 16S rRNA-Targeted Oligonucleotide Probes Used for *In Vitro* Experiments.

| Probe Name | Sequence (5' to 3') | Targeted Bacterial Group | Reference |
|------------|------------------------|--|------------------------------|
| Non Eub | ACTCCTACGGGAGGCAGC | Negative control | Wallner et al., 1993 [44] |
| Eub338 | GCTGCCTCCCGTAGGAGT | Total bacteria | Daims et al., 1999 [45] |
| Eub338II | GCAGCCACCCGTAGGTGT | Total bacteria | Daims et al., 1999 [45] |
| Eub338III | GCTGCCACCCGTAGGTGT | Total bacteria | Daims et al., 1999 [45] |
| Ato291 | GGTCGGTCTCTCAACCC | <i>Atopobium</i> cluster | Harmsen et al., 2000 [46] |
| Bac303 | CCAATGTGGGGACCTT | <i>Bacteroides/Prevotella</i> | Manz et al., 1996 [47] |
| Bif164 | CATCCGGCATTACCACC | <i>Bifidobacterium</i> spp. | Langendijk et al., 1995 [48] |
| Chis150 | TTATGCGGTATTAATCTYCCTT | <i>Clostridium histolyticum</i> (<i>Clostridium</i> cluster I and II) | Franks et al., 1998 [49] |
| Prop853 | ATTGCGTAACTCCGGCAC | <i>Clostridium</i> cluster IX | Walker et al., 2005 [50] |
| DSV687 | TACGGATTCACTCCT | <i>Desulfovibrio</i> spp. | Hold et al., 2003 [51] |
| Erec482 | GCTTCTAGTCARGTACCG | <i>Eubacterium rectale/Clostridium coccoides</i> (<i>Clostridium</i> cluster IVXa and IVXb) | Franks et al., 1998 [49] |
| Fprau655 | CGCTACCTCTGCACTAC | <i>Faecalibacterium prausnitzii</i> | Devereux et al., 1992 [52] |
| Lab158 | GGTATTAGCAYCTGTTCCA | <i>Lactobacillus/Enterococcus</i> | Harmsen et al., 1999 [53] |
| Rrec584 | TCAGACTTGCCGYACCGC | <i>Roseburia</i> spp. | Walker et al., 2005 [50] |

2.1.4. *In Vitro* SCFA Analysis

Samples from each fermentation time were centrifuged at $13,000 \times g$ for 10 min to obtain the supernatants which were kept at -20°C until analysis. Before analysis, samples were extracted and derivatised following the method described by Ferreira-Lazarte et al. [54]. A 5890 Series II Gas Chromatograph (Hewlett Packard) fitted with a Rtx-1 10 m \times 0.18 mm column with a 0.20 μm coating (Crossbond 100% dimethyl polysiloxane; Restek) was used for analysis. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were 275°C . Oven temperature was programmed from 63°C for 3 min and then heated to 190°C at a heating rate of $3^\circ\text{C}/\text{min}$ and held at 190°C for 3 min. SCFA standards analysis was also carried out to quantify concentrations of all compounds.

2.1.5. Statistical Analysis of *In Vitro* Data

Statistical analyses were performed using Graph Pad Prism 8 (Graph Pad Software Inc., San Diego, CA, USA). One-way ANOVA with Dunnet's multiple comparison test was used to determine significant differences from baseline within the same substrate. Differences were considered to be significant when $p < 0.05$.

2.2. Pilot Clinical Trial

2.2.1. Clinical Trial Design

To evaluate potential effects of a 2'-FL-containing nutritional formula, a single-arm, open-label, pilot trial was implemented. The clinical trial protocol was approved by Quorum Review Board (Seattle, WA, USA) and registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT03011593). Participants provided written informed consent prior to participation in this study.

The interventional period included two clinical visits with questionnaires administered and stool samples collected at baseline and at the study end point (after six weeks). The Gastrointestinal Quality of Life Index (GIQLI) [55], administered to all participants, was the primary outcome measure. Secondary outcome measures were the Digestive Symptom Frequency Questionnaire (DSFQ) [56] and the Inflammatory Bowel Disease Questionnaire (IBDQ) [57], administered only to participants with IBS and IBD, respectively. Exploratory outcome measures were stool CFU counts of commensal bacteria and stool SCFA levels.

2.2.2. Clinical Trial Participants and Recruitment

Up to twenty adults aged 21–75 years, with a body mass index (BMI) of 19–40 kg/m², and with previously diagnosed IBS, ulcerative colitis, Crohn's disease or celiac disease were recruited at four medical clinics in the United States (located in La Jolla, CA; Forney, TX; Avon, CT; and South Orange, NJ). Potential participants were approached by clinicians associated with this study. Target enrollment was up to twenty individuals. Exclusion criteria were as follows: taking oral or intravenous antibiotic, antiparasitic, or antifungal medications; gastrointestinal surgery within three months prior to screening; currently having a colostomy or ileostomy bag in place; cancer within the last five years; or women who were lactating, pregnant or planning pregnancy during the study period. Furthermore, participants were excluded if they had done any of the following within 28 days prior to screening: initiated or made changes to medications or supplements, initiated or made changes to an exercise regimen or food plan, participated in a significant diet or weight loss program, used recreational drugs/substances, or participated in another research study. Unless medically indicated, participants were asked to refrain from making changes to their medications or supplements, exercise routine or diet for the duration of their participation in this study.

2.2.3. Clinical Trial Intervention

The studied nutritional formula was manufactured and supplied by Metagenics, Inc. (Aliso Viejo, CA, USA) in fourteen-serving containers. The nutritional formula contained two grams per serving of 2'-FL plus micronutrients (vitamins A, B₆, B₁₂, C, D₃, and E, biotin, calcium, chromium, copper, folate, iodine, iron, magnesium, manganese, pantothenic acid, phosphorus, riboflavin, selenium, thiamin, and zinc), macronutrients (protein, carbohydrates, fat, and fiber), amino acids, and isomalto-oligosaccharide. Participants were asked to add two scoops of the nutritional formula (40 g) to chilled water or juice (8–10 ounces) and drink it as a reconstituted beverage for six weeks. Participants consumed the beverage twice per day and therefore were administered four grams 2'-FL per day.

2.2.4. Quality of Life Assessment and Stool Sample Analysis

The GIQLI, DSFQ, and IBDQ were scored as previously described [55–57]. Stool samples were shipped to Genova Diagnostics (Asheville, NC, USA) within twenty-four hours of collection; commensal gut microbiota were identified using 16S ribosomal RNA gene PCR and concentrations of SCFA were measured using gas chromatography–mass spectrometry (GC-MS) as previously described [58].

2.2.5. Statistical Analysis of Clinical Trial Data

Given that the clinical trial was a pilot trial with data collected at only two time points, statistical analyses were limited to data from participants who completed the trial. Study measures are presented as the mean and standard deviation (SD) at each time point. For questionnaire and SCFA data, changes from baseline to study completion were analyzed using paired *t*-tests to identify significant differences. Microbiota PCR data were log transformed prior to calculation of geometric mean percent change and analyzed using paired *t*-tests. Statistically-significant ($p < 0.05$) microbiota parameters were subsequently evaluated using a Wilcoxon signed rank analysis, a more conservative test, to confirm statistical significance. In the case of microbiota PCR data that were outside of laboratory detection limits, the extreme detectable value was imputed for analysis. Given the small sample size of this study, effect size as Cohen's *d* was calculated between baseline and the study end point for all continuous measures. Cohen's *d* was calculated as (week 6 mean–baseline mean)/(baseline standard deviation) and interpreted as small ($d = 0.20$), medium ($d = 0.50$), or large ($d = 0.80$) effect size. Statistical analyses were performed using R (The R Foundation for Statistical Computing, software version 3.6.0, Vienna, Austria).

3. Results

3.1. In Vitro Study

3.1.1. Effect of Fermentation on Bacterial Populations

Bacterial counts obtained by Flow-FISH are shown in Supplementary Figure S1 (Figure S1). The most noticeable change in the microbiota composition along the fermentations of stool samples from all groups was a significant increase in *Bifidobacterium* at 8 and 24 h of fermentation with the positive control, the 2'-FL, and several 2'-FL/probiotic combinations compared to baseline (Figure 1). Although the observed bifidogenic effect was significant overall, the increment was smaller with IBS donors due to higher inter-individual variation in the IBS donors. Closer examination, donor by donor in the IBS group, showed that this variation was due a low bifidogenic response (0.5 log) to 2'-FL and 2'-FL/probiotic combinations in one of the three IBS donors. However, the bifidogenic effect observed in the two additional IBS donors was similar to fermentations with healthy and ulcerative colitis donors. In addition, abundance of *Eubacterium rectale-Clostridium coccoides* (butyrate producers) was also significantly higher in presence of 2'-FL and 2'-FL/probiotic combinations after 8 h of fermentation (Figure 1). *Roseburia* species, another butyrate producer, also displayed a significant increment at 8 h in response of FOS/inulin mixture and 2'-FL in healthy and IBS fermentations but not in ulcerative colitis fermentations (Supplementary Figure S1). Interestingly, unlike healthy donors, stool microbiota of IBS and ulcerative colitis donors displayed a significant increase in *Atopobium* in response to 2'-FL and potential synbiotic treatments (Figure 1). Overall, no discernible differences were observed when 2'-FL/probiotic combinations were compared to 2'-FL alone (Figure 1).

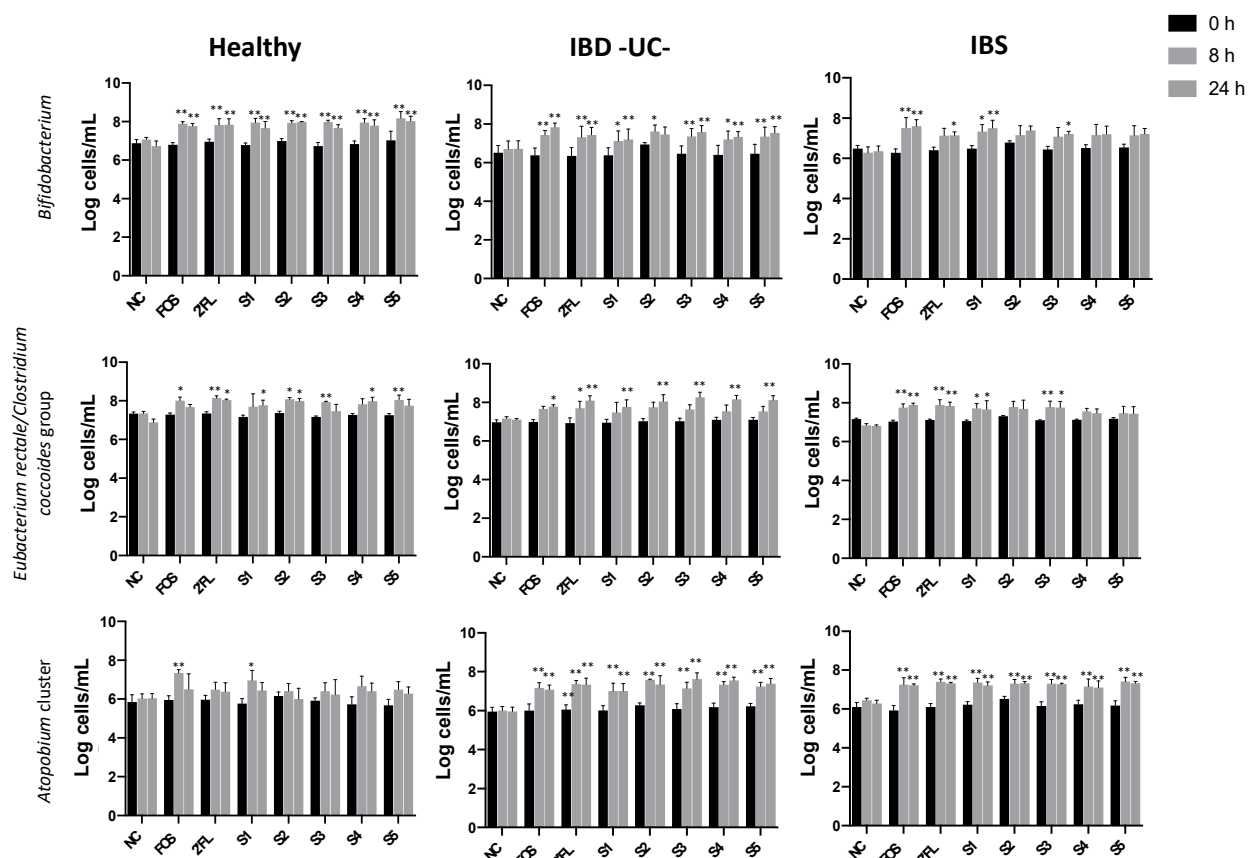


Figure 1. *Bifidobacterium*, *Clostridium coccoides*/*Eubacterium rectale* and *Atopobium* cluster populations at 0, 8, and 24 h of fermentation with no substrate/negative control (NC); positive control (FOS/inulin mixture); 2'-FL; S1: 2'-FL + *L. acidophilus* NCFM + *B. lactis* Bi-07; S2: 2'-FL + *L. acidophilus* NCFM + *B. lactis* Bi-07 + *B. lactis* BI-04 + *L. paracasei* Lpc-37; S3: 2'-FL + *L. acidophilus* NCFM + *B. lactis* Bi-07 + *L. rhamnosus* GG; S4: 2'-FL + *L. rhamnosus* GG; S5: 2'-FL + *L. salivarius* UCC118. Error bars show SEM (n = 3). Significant differences from baseline (time 0) are denoted with * ($p \leq 0.05$) or ** ($p \leq 0.01$).

3.1.2. Effect of Fermentation on Short-Chain Fatty Acid (SCFA) Production

Acetate was the main SCFA detected during the fermentation of all prebiotics and potential synbiotics tested and its production accounted for approximately 75% of total SCFAs. Significant increments of acetate and propionate (the second most prevalent SCFA) were observed at the end of fermentation for all substrates and the three donor groups (Figure 2). Moderate increments of butyrate were also observed after 24 h. Although this increment was only significant for FOS and one 2'-FL/probiotic combination in fermentations with stool microbiota of healthy donors, significant increases in butyrate with 2'-FL and 2'-FL/probiotic combinations were detected in ulcerative colitis donor fermentations and also with FOS and some 2'-FL/probiotic combinations in IBS donor fermentations (Figure 2).

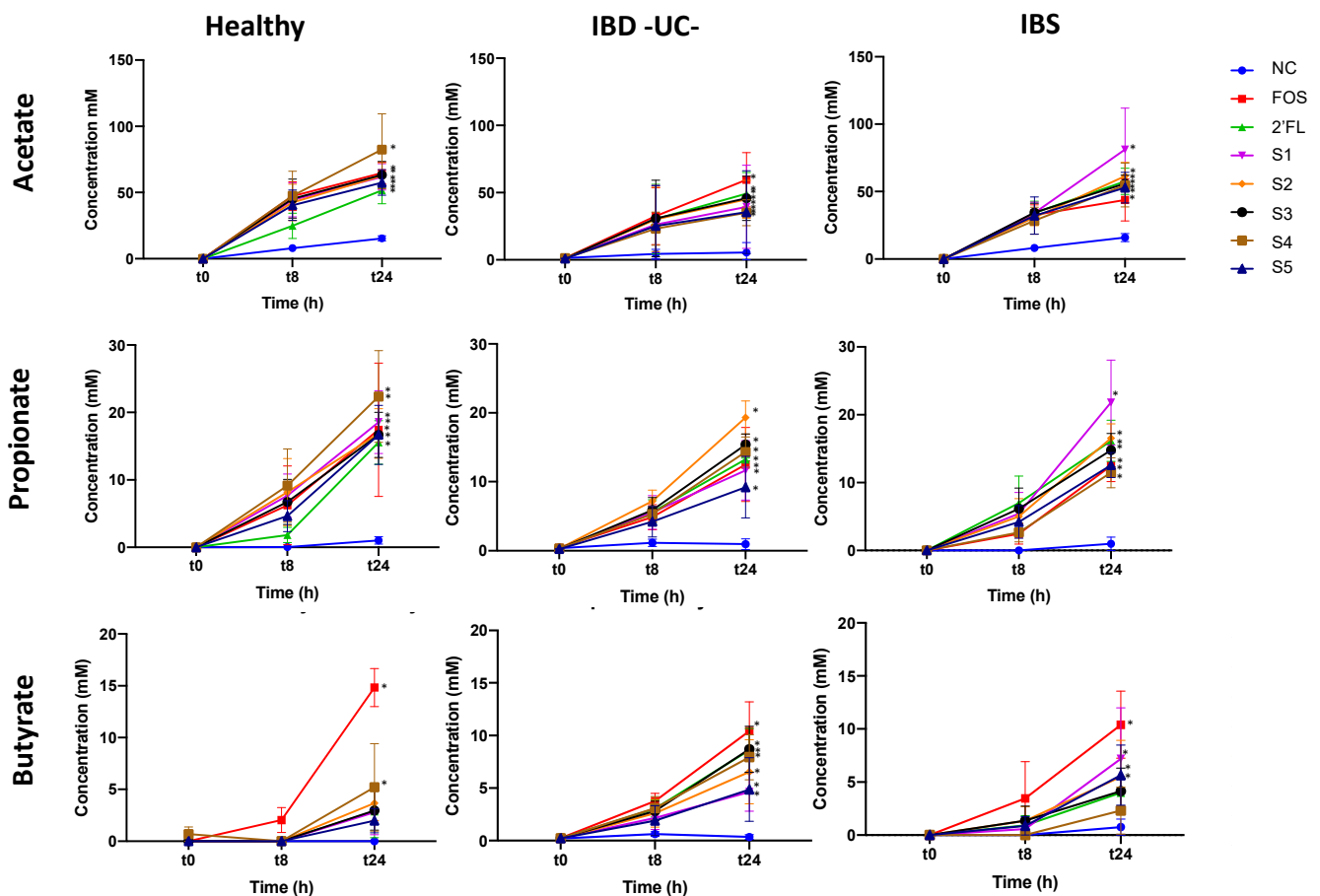


Figure 2. Acetate, propionate and butyrate concentration at 0, 8, and 24 h of fermentation with no substrate/negative control (NC); positive control (FOS/inulin mixture); 2'-FL; S1: 2'-FL + *L. acidophilus* NCFM + *B. lactis* Bi-07; S2: 2'-FL + *L. acidophilus* NCFM + *B. lactis* Bi-07 + *B. lactis* BI-04 + *L. paracasei* Lpc-37; S3: 2'-FL + *L. acidophilus* NCFM + *B. lactis* Bi07 + *L. rhamnosus* GG; S4: 2'-FL + *L. rhamnosus* GG; S5: 2'-FL + *L. salivarius* UCC118. Error bars show SEM (n = 3). Significant differences from baseline (time 0) are denoted with * ($p \leq 0.05$) or ** ($p \leq 0.01$).

3.2. Pilot Clinical Trial

3.2.1. Participant Characteristics

Twenty participants enrolled in this study and twelve completed it. Demographic parameters of the twelve participants who completed this study are presented in Table 3. The mean BMI of these twelve individuals was 23.8 kg/m² (with a range from 19.4 to 29.6 kg/m²). The eight individuals who did not complete this study included two that dropped out/declined to participate before beginning the study intervention, three that dropped out due to non-serious adverse events, and three who were lost to follow up. The

participants who dropped out due to adverse events reported worsening of pre-existing gastrointestinal symptoms, gastrointestinal upset, and a non-study-related viral infection. Data from the twelve participants that completed this study were analyzed.

Table 3. Pilot Trial Participant Demographics.

| | Mean ± SD or n (%) |
|---|--------------------|
| Age (years) | 31.4 ± 10.5 |
| Sex | |
| Male | 7 (58%) |
| Female | 5 (42%) |
| Body Mass Index (kg/m ²) | 23.8 ± 3.4 |
| Race (Ethnicity) | |
| White (not Hispanic/Latino) | 8 (66.7%) |
| White/Native American (not Hispanic/Latino) | 2 (16.7%) |
| White (Hispanic/Latino) | 1 (8.3%) |
| Black (not Hispanic/Latino) | 1 (8.3%) |
| Gastrointestinal Condition | |
| Irritable bowel syndrome (IBS) | 7 (58.3%) |
| Ulcerative colitis (UC) | 4 (33.3%) |
| Celiac disease | 1 (8.3%) |

Abbreviations: kg/m², kilograms per square meter; SD, standard deviation.

3.2.2. Impact on Gastrointestinal Quality of Life

Compared to baseline, some gastrointestinal quality of life questionnaire scores improved after six weeks (Table 4). Gastrointestinal Quality of Life Index (GIQLI) (administered to all participants) total score and “gastrointestinal symptoms” domain score increased ($p < 0.05$) with a medium effect size ($d = 0.59$ and $d = 0.79$, respectively). GIQLI “Social function” domain score increased ($p < 0.01$) with a small effect size ($d = 0.42$). Inflammatory Bowel Disease Questionnaire (IBDQ) (administered only to participants with IBD) domain score increased ($p < 0.001$) compared to baseline, with a large effect size ($d = 1.65$). Digestive Symptom Frequency Questionnaire (DSFQ) (administered only to participants with IBS) score did not change.

Table 4. Pilot Trial Gastrointestinal Quality of Life Questionnaire Score Changes Over 6 Weeks.

| | Score Range | Baseline | | Week 6 | | Δ Over 6 Weeks | Mean % Δ | p ¹ | Cohen’s D |
|---------------------------------|-------------|----------|------|--------|------|----------------|----------|------------------|-----------|
| | | Mean | SD | Mean | SD | | | | |
| GIQLI Total Score | 0–144 | 94.3 | 25.5 | 109.4 | 19.2 | 15.1 | 20.8% | 0.020 | 0.59 |
| GIQLI-Gastrointestinal Symptoms | 0–76 | 53.3 | 10.3 | 61.4 | 7.7 | 8.1 | 18.1% | 0.022 | 0.79 |
| GIQLI-Physical Function | 0–28 | 15.6 | 7.4 | 17.8 | 6.1 | 2.2 | 36.5% | 0.164 | 0.30 |
| GIQLI-Social Function | 0–16 | 10.7 | 3.8 | 12.3 | 3.7 | 1.6 | 18.4% | 0.004 | 0.42 |
| GIQLI-Emotional Function | 0–20 | 12.0 | 5.8 | 14.7 | 4.5 | 2.7 | 46.5% | 0.139 | 0.47 |
| IBDQ Total Score | 32–224 | 134.0 | 52.4 | 179.0 | 36.1 | 45.0 | 43.6% | 0.078 | 0.86 |
| IBDQ-Bowel Symptoms | 10–70 | 43.0 | 19.4 | 56.3 | 11.7 | 13.3 | 44.3% | 0.165 | 0.69 |
| IBDQ-Systemic Systems | 5–35 | 16.8 | 6.2 | 27.0 | 6.3 | 10.2 | 66.9% | 0.000 | 1.65 |
| IBDQ-Social Function | 5–35 | 22.0 | 9.9 | 27.8 | 7.5 | 5.8 | 41.5% | 0.239 | 0.59 |
| IBDQ-Emotion Health | 12–84 | 51.5 | 18.7 | 68.0 | 11.7 | 16.5 | 43.3% | 0.098 | 0.88 |
| DSFQ Total Score | 0–16 | 9.0 | 3.8 | 8.4 | 5.0 | −0.6 | −10.3% | 0.522 | −0.16 |

¹ p -values calculated using paired t -tests. Abbreviations: Δ, change; DSFQ, Digestive Symptom Frequency Questionnaire; GIQLI, Gastrointestinal Quality of Life Index; IBDQ, Inflammatory Bowel Disease Questionnaire; SD, standard deviation.

3.2.3. Impact on Stool Bacteria Counts

In the clinical trial, counts of the majority of analyzed stool bacteria did not change after consuming the 2'-FL-containing nutritional formula for six weeks (Table 5). However, increases were observed in the following: *Bifidobacterium* species, *Bifidobacterium longum*, *Faecalibacterium prausnitzii*, *Anaerotruncus colihominis*, and *Pseudoflavonifractor* species ($p < 0.05$) with medium or greater effect sizes ($d \geq 0.5$).

Table 5. Pilot Trial Stool Bacteria Count Changes Over 6 Weeks.

| Phylum | Microbiota (CFU/g Stool) | Log Transformed Data | | | | | | |
|-----------------|-------------------------------------|----------------------|-----|--------|-----|------------------------------|---------|-----------|
| | | Baseline | | Week 6 | | Geometric Mean % Δ | p^1 | Cohen's D |
| | | Mean | SD | Mean | SD | | | |
| Actinobacteria | <i>Bifidobacterium</i> spp. | 20.1 | 1.6 | 21.6 | 1.7 | 356.3% | * 0.026 | 0.95 |
| | <i>Bifidobacterium longum</i> | 17.4 | 2.0 | 19.4 | 1.6 | 650.0% | * 0.003 | 0.99 |
| | <i>Collinsella aerofaciens</i> | 17.6 | 3.0 | 18.1 | 3.5 | 65.7% | 0.392 | 0.17 |
| Bacteroidetes | <i>Bacteroides-Prevotella</i> group | 20.2 | 0.9 | 21.2 | 1.3 | 169.0% | 0.101 | 1.09 |
| | <i>Bacteroides vulgatus</i> | 19.8 | 2.5 | 20.9 | 2.6 | 190.4% | 0.081 | 0.43 |
| | <i>Barnesiella</i> spp. | 16.0 | 2.0 | 15.4 | 2.9 | −46.1% | 0.468 | −0.31 |
| | <i>Odoribacter</i> spp. | 15.6 | 2.5 | 14.8 | 3.6 | −54.9% | 0.369 | −0.32 |
| | <i>Prevotella</i> spp. | 14.1 | 2.0 | 14.9 | 1.8 | 116.2% | 0.301 | 0.39 |
| Euryarchaeota | <i>Methanobrevibacter smithii</i> | 13.1 | 2.0 | 14.0 | 2.1 | 134.7% | 0.290 | 0.42 |
| Firmicutes | <i>Anaerotruncus colihominis</i> | 14.5 | 1.6 | 16.1 | 1.1 | 402.6% | * 0.018 | 1.00 |
| | <i>Butyrivibrio crossotus</i> | 9.9 | 1.5 | 10.7 | 3.1 | 111.7% | 0.422 | 0.51 |
| | <i>Clostridium</i> spp. | 21.0 | 1.6 | 21.9 | 1.9 | 156.9% | 0.125 | 0.58 |
| | <i>Coprococcus eutactus</i> | 14.1 | 2.3 | 14.1 | 2.7 | −2.4% | 0.983 | −0.01 |
| | <i>Faecalibacterium prausnitzii</i> | 20.6 | 2.2 | 22.1 | 1.8 | 353.7% | * 0.029 | 0.67 |
| | <i>Lactobacillus</i> spp. | 19.5 | 2.5 | 20.5 | 2.4 | 160.7% | 0.252 | 0.39 |
| | <i>Pseudoflavonifractor</i> spp. | 18.0 | 1.5 | 19.2 | 1.2 | 226.4% | * 0.016 | 0.80 |
| | <i>Roseburia</i> spp. | 20.7 | 1.8 | 21.9 | 1.5 | 252.8% | 0.062 | 0.68 |
| | <i>Ruminococcus</i> spp. | 17.7 | 2.0 | 18.4 | 2.1 | 97.5% | 0.273 | 0.34 |
| | <i>Veillonella</i> spp. | 16.1 | 3.0 | 16.9 | 1.7 | 118.8% | 0.331 | 0.26 |
| Fusobacteria | <i>Fusobacterium</i> spp. | 9.6 | 2.3 | 10.6 | 2.0 | 171.8% | 0.111 | 0.44 |
| Proteobacteria | <i>Desulfovibrio piger</i> | 10.3 | 2.1 | 10.5 | 2.0 | 12.2% | 0.877 | 0.06 |
| | <i>Escherichia coli</i> | 16.5 | 3.0 | 17.0 | 1.7 | 67.9% | 0.607 | 0.17 |
| | <i>Oxalobacter formigenes</i> | 14.1 | 1.7 | 13.9 | 2.5 | −16.9% | 0.833 | −0.11 |
| Verrucomicrobia | <i>Akkermansia muciniphila</i> | 12.6 | 2.0 | 12.9 | 2.0 | 33.6% | 0.702 | 0.15 |

¹ p -values calculated using paired t -tests. * p -values confirmed with a Wilcoxon signed rank test ($p < 0.05$). Abbreviations: Δ , change; CFU, colony-forming unit; g, gram; SD, standard deviation; spp., species.

3.2.4. Impact on Stool Short-Chain Fatty Acids (SCFAs)

After six weeks, butyrate, acetate, and total stool SCFAs increased ($p < 0.05$) with large effect sizes ($d \geq 0.80$) (Table 6).

Table 6. Pilot Trial Stool Short-Chain Fatty Acids (SCFAs) Over 6 Weeks.

| SCFA (Micromol/g Stool) | Baseline | | Week 6 | | Mean % Δ | p^1 | Cohen's D |
|----------------------------|----------|-------|--------|-------|--------------------|-------|-----------|
| | Mean | SD | Mean | SD | | | |
| Total SCFA | 46.30 | 13.30 | 76.43 | 37.04 | 72.2% | 0.026 | 2.27 |
| Butyrate | 8.12 | 4.76 | 16.71 | 9.63 | 594.0% | 0.040 | 1.80 |
| Acetate | 28.24 | 7.28 | 45.65 | 23.90 | 64.3% | 0.035 | 2.39 |
| Propionate | 10.03 | 4.71 | 14.06 | 8.39 | 42.4% | 0.074 | 0.85 |

¹ p -values calculated using paired t -tests. Abbreviations: Δ , change; g, gram.

4. Discussion

In the batch culture fermentation model, 2'-FL demonstrated prebiotic effects with increases in *Bifidobacterium* and SCFAs as well as substantial increments in other beneficial groups of the microbiota as *Clostridium* cluster XIVa and *Roseburia* spp. (butyrate producers). A previous *in vitro* study has also shown the ability of *Bifidobacterium* and *Eubacterium* to utilize fucosyllactose [29].

During fermentation, 2'-FL showed a bifidogenic effect similar to the positive control FOS/inulin mixture. The impact of 2'-FL on *Bifidobacterium* after 24 h was similar in the microbiota of all donors except for one individual with IBS. In the case of stool from this volunteer, no bifidogenic effect was detected with the positive control either, although *Roseburia* spp. increased with the treatments. Interestingly, this donor showed a two-fold abundance of *Roseburia* spp. over *Bifidobacterium* at baseline. This numerical superiority possibly provided an important competitive advantage for substrate consumption. Overall, *Bifidobacterium* and *Eubacterium rectale-Clostridium coccoides* were the main groups affected by 2'-FL and 2'-FL/probiotic combinations in fermentations of samples from the three donor groups.

Interestingly, the abundance of *Atopobium* cluster was also significantly increased in the presence of prebiotics and potential synbiotic combinations in individuals with IBS and ulcerative colitis. Slight, non-significant, increments were detected in this bacterial group in healthy donors. The role of *Atopobium* cluster in the human colon is not clear. However, they are commonly isolated from healthy human stool and are typically present at 1.5–3% of the total stool population. *Atopobium* cluster comprises most *Coriobacteriaceae* species, including the *Coriobacterium* group [47].

Using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), Šuligoj et al. [59] recently reported that fermentation of 2'-FL resulted in a simultaneous increase in *Bifidobacterium* and SCFAs including butyrate in particular. In our *in vitro* experiments, fermentation of stool samples with 2'-FL also resulted in a simultaneous increase in *Bifidobacterium* and SCFAs including butyrate. As expected, the highest contribution to the SCFA increase was due to the production of acetate probably through the growth of *Bifidobacterium* spp., but significant increments of propionate and butyrate also occurred. Overall, levels of acetate, propionate and butyrate in the presence of 2'-FL were similar in the three stool donor groups after 24 h fermentation, indicating that 2'-FL could be applicable for both IBS and IBD. No major differences were observed between 2'-FL alone and the 2'-FL/probiotic combinations on the stool microbiota composition after 24 h of fermentation and some of the differences detected could be related to baseline variations. Because no additional effects were seen with the addition of the probiotic strains, we concluded that combining any of the tested probiotic strains with 2'-FL might not confer additional benefit to the host upon translation of the *in vitro* findings into human subjects. Hence we proceeded with including 2'-FL without probiotics in the nutritional formula tested in the pilot trial.

In the clinical trial, measures of gastrointestinal quality of life improved in adults with chronic gastrointestinal conditions after a six-week course of the 2'-FL-containing nutritional formula. Most notably, GIQLI total score and specifically the gastrointestinal symptom domain score improved significantly over the course of the trial. The increase in GIQLI total score (15.1 units) over six weeks exceeded what has been reported as the minimal clinically important difference (MCID) for the GIQLI (6.42 to 7.64 units) [60]. Improving gastrointestinal quality of life is meaningful given the negative impact of IBS, IBD, and celiac disease on health-related quality of life [61–63].

Over the course of the trial, stool microbiota counts increased at the genus and species levels. Consistent with our *in vitro* fermentations and other *in vitro* studies [35,64–67], the genus *Bifidobacterium* increased as expected. The increase in stool counts of *Bifidobacterium*, after consuming four grams 2'-FL per day for six weeks, is also in alignment with results demonstrating that two weeks of supplementation with ten grams per day of 2'-FL [41] or four weeks of supplementation with a ten gram mixture of 2'-FL and lacto-N-

neotetraose [42] increased stool abundance of the genus *Bifidobacterium* in healthy adults and adults with IBS, respectively.

At the species level, *Bifidobacterium longum* also increased over time. This finding was also anticipated, because *B. longum* strains produce enzymes and transporters that function in the metabolism of HMOs and several *in vitro* studies have demonstrated that specific strains of *B. longum* are able to consume 2'-FL in culture [33,64–67]. This finding was also consistent with those of Elison et al. [36] showing that the increase in sequence abundance of operational taxonomic units (OTUs) showing high sequence similarity to *B. longum* were among the species of bifidobacteria most affected by 2'-FL consumption in human subjects.

Stool counts of *Faecalibacterium prausnitzii*, *Anaerotruncus colihominis*, and *Pseudoflavonifractor* species also increased significantly over the course of the trial. The increase in *F. prausnitzii* is particularly noteworthy because multiple studies have found that stool levels of *F. prausnitzii*, which comprises about 5% of total stool microbiota in healthy individuals, are significantly lower in patients with IBS, IBD, and celiac disease [9,68–72]. Due to the association between low abundance of *F. prausnitzii* and inflammatory and metabolic diseases, *F. prausnitzii* has been described as a potential bioindicator of health [73,74]. Furthermore, *F. prausnitzii* has been described as a potential keystone species [75,76] signifying that it may be critical for maintaining the organization and diversity of the gut ecosystem through biotic interactions with other species [77]. Although changes in *F. prausnitzii* were not noted in our *in vitro* experiments, an increase in *Eubacterium rectale*/*Clostridium coccooides*, other main butyrate producer group, was detected. Interestingly, Cheng et al. [78] recently reported that when *B. longum* subsp. *infantis* and *F. prausnitzii* are co-cultured in the presence of 2'-FL, these bacteria grow faster in co-culture than in monocultures in the presence of 2'-FL. The *in vitro* results reported by Cheng et al. [78] are in alignment with our observed simultaneous increase in the species *B. longum* and the butyrate producer *F. prausnitzii* in human subjects. Furthermore, our previous work and that of other groups have also demonstrated that prebiotics can simultaneously increase stool counts of *Bifidobacterium* and *F. prausnitzii* in adult subjects [79,80].

Stool levels of butyrate, acetate, and total SCFAs also increased significantly over the course of the clinical trial. Our *in vitro* experiments as well as other pre-clinical data also support these clinical trial findings. It was reported that incubation of infant stool samples with 2'-FL resulted in significantly increased SCFA production [81]. In several *in vitro* experiments, Li et al. [82] demonstrated that incubation of crude HMOs isolated from pooled human milk samples with stool samples isolated from piglets resulted in SCFA production. Furthermore, Azagra-Boronat et al. [83] recently reported that oral 2'-FL increased cecal butyrate in healthy suckling rats.

It is possible that the observed increases in SCFAs in the clinical trial, specifically butyrate and acetate, were mechanistically related to the observed increases in commensal bacteria. Stool levels of predominant butyrate producers have been shown to correlate with stool SCFA levels [84]. Microbiota of the genera *Faecalibacterium*, *Anaerotruncus*, *Pseudoflavonifractor*, each express enzymes required for butyrate and acetate biosynthesis [85]. *A. colihominis* is also considered a major butyrate producer [86,87]. Furthermore, specific strains of *B. longum* have been shown to produce abundant acetate when cultured on 2'-FL as a growth substrate [64,65]. Potentially supporting the growth of butyrate-producing gut microorganisms as well as intestinal production of butyrate may be clinically meaningful, since it has been suggested that exogenous butyrate and butyrate-producing microorganisms, such as *F. prausnitzii*, may represent novel therapeutic approaches for IBS and IBD [13,88–92].

Strengths of the translational clinical trial design include successful collection of pilot data in individuals with various gastrointestinal conditions and concurrent evaluation of impact on quality of life, gut microbiota, and stool SCFAs following *in vitro* assessments. Limitations of the clinical trial include a high rate of withdrawal, lack of a control group, the small sample size, and completion of the trial by only one individual with celiac disease. Furthermore, although participants who completed the trial were considered normal or

overweight (but not obese), protocol eligibility criteria allowed for inclusion of a broad BMI range; future trial protocols should limit BMI to fewer categories and/or include sufficient sample size to allow for subgroup comparisons due to the influence BMI could have on results. In addition, shifts in gut microbiota composition and increases in SCFA cannot be attributed to 2'-FL solely given that the formula contained additional ingredients. Improvements in intra- and extra-intestinal symptoms also cannot be attributed only to 2'-FL given that the formula also contained macro- and micronutrients that are supportive to patients with malabsorption and malnutrition associated with chronic gastrointestinal disease including IBD [93,94].

5. Conclusions

Consistent with documented effects of 2'-FL, our *in vitro* work demonstrated bifidogenic and butyrogenic effects of 2'-FL during fermentation with human stool samples. With no additional effects seen with the addition of probiotic strains, only 2'-FL was included in the nutritional formula when the *in vitro* results were translated to human subjects. Consumption of the 2'-FL-containing nutritional formula by adults with IBS or ulcerative colitis was associated with improvements in intra- and extra-intestinal symptoms, and bifidogenic and butyrogenic effects. Based on the promising yet preliminary clinical trial results, future research should further explore relationships between potential impacts of the nutritional formula on quality of life and gut microbiota composition and metabolism, including SCFA production. To better isolate the impact of 2'-FL, the effect of the 2'-FL-containing formula could be compared to 2'-FL only or the formula without the inclusion of 2'-FL. Patient populations tested should include individuals with gastrointestinal conditions associated with intestinal dysbiosis, including, but not limited to IBS, IBD, and celiac disease. In addition, impact on the prevention of these conditions could be explored. Furthermore, additional batch culture fermentation experiments could explore effects of 2'-FL during fermentation with human stool samples from additional clinical populations, including those with other gastrointestinal conditions. However, the present results are promising, particularly in the context of the limited research on HMOs in adult populations with chronic gastrointestinal conditions.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2072-6643/13/3/938/s1>, Figure S1: Bacterial populations at 0 h, 8 h, and 24 h of fermentation with no substrate/negative control (NC), positive control (FOS/inulin mixture) and the prebiotic 2'FL.

Author Contributions: Conceptualization, J.J.R., A.M.-M., N.C. and G.R.G.; methodology, J.J.R., A.M.-M. and G.R.G.; formal analysis, J.J.R., A.M.-M. and G.R.G.; investigation, J.J.R., A.M.-M. and G.R.G.; resources, N.C. and G.R.G.; writing—original draft preparation, J.J.R., A.M.-M. and G.R.G.; writing—review and editing, J.J.R., A.M.-M., N.C. and G.R.G.; visualization, J.J.R. and A.M.-M.; supervision, J.J.R., N.C. and G.R.G.; project administration, N.C. and G.R.G.; funding acquisition, N.C. and G.R.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research and the APC was funded by Metagenics, Inc.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki. Ethical approval for the pilot clinical trial was obtained from Quorum Review Board (Seattle, WA, USA; protocol code 30872, 09/08/15).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Data is contained within the article and supplementary material.

Acknowledgments: The authors thank Genova Diagnostics (Asheville, NC, USA) for providing their clinical laboratory services in kind. We thank the study participants and Robert Bonakdar, Kevin Holder, Cory Rice, and Jennifer Stagg for implementing aspects of the clinical study within their medical practices. We also thank Douglas A. Hanes for assistance with some of the clinical trial data analysis and Kimberlee Koch for contributions to this work.

Conflicts of Interest: J.J.R., A.M.-M. and G.R.G. report grants from Metagenics during the conduct of the work. N.C. is an employee of Metagenics.

References

1. Saha, L. Irritable bowel syndrome: Pathogenesis, diagnosis, treatment, and evidence-based medicine. *World J. Gastroenterol.* **2014**, *20*, 6759–6773. [[CrossRef](#)]
2. Zhang, Y.-Z.; Li, Y.-Y. Inflammatory bowel disease: Pathogenesis. *World J. Gastroenterol.* **2014**, *7*, 91–99. [[CrossRef](#)]
3. Gujral, N.; Freeman, H.J.; Thomson, A.B.R. Celiac disease: Prevalence, diagnosis, pathogenesis and treatment. *World J. Gastroenterol.* **2012**, *18*, 6036–6059. [[CrossRef](#)]
4. Barbara, G.; De Giorgio, R.; Stanghellini, V.; Cremon, C.; Corinaldesi, R. A role for inflammation in irritable bowel syndrome? *Gut* **2002**, *51* (Suppl. 1), i41–i44. [[CrossRef](#)]
5. Ng, Q.X.; Soh, A.Y.S.; Loke, W.; Lim, D.Y.; Yeo, W.-S. The role of inflammation in irritable bowel syndrome (IBS). *J. Inflamm. Res.* **2018**, *11*, 345–349. [[CrossRef](#)] [[PubMed](#)]
6. Pascual, V.; Dieli-Crimi, R.; López-Palacios, N.; Bodas, A.; Medrano, L.M.; Núñez, C. Inflammatory bowel disease and celiac disease: Overlaps and differences. *World J. Gastroenterol.* **2014**, *20*, 4846–4856. [[CrossRef](#)] [[PubMed](#)]
7. Petersen, C.; Round, J.L. Defining dysbiosis and its influence on host immunity and disease. *Cell. Microbiol.* **2014**, *16*, 1024–1033. [[CrossRef](#)] [[PubMed](#)]
8. Pimentel, M.; Lembo, A. Microbiome and Its Role in Irritable Bowel Syndrome. *Dig. Dis. Sci.* **2020**, *65*, 829–839. [[CrossRef](#)]
9. Liu, H.-N.; Wu, H.; Chen, Y.-Z.; Chen, Y.-J.; Shen, X.-Z.; Liu, T.-T. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: A systematic review and meta-analysis. *Dig. Liver Dis.* **2017**, *49*, 331–337. [[CrossRef](#)]
10. Kostic, A.D.; Xavier, R.J.; Gevers, D. The Microbiome in Inflammatory Bowel Disease: Current Status and the Future Ahead. *Gastroenterology* **2014**, *146*, 1489–1499. [[CrossRef](#)] [[PubMed](#)]
11. Lopez-Siles, M.; Duncan, S.H.; Garcia-Gil, L.J.; Martinez-Medina, M. *Faecalibacterium prausnitzii*: From microbiology to diagnostics and prognostics. *ISME J.* **2017**, *11*, 841–852. [[CrossRef](#)]
12. Valitutti, F.; Cucchiara, S.; Fasano, A. Celiac Disease and the Microbiome. *Nutrients* **2019**, *11*, 2403. [[CrossRef](#)] [[PubMed](#)]
13. Gibson, G.R.; Hutkins, R.; Sanders, M.E.; Prescott, S.L.; Reimer, R.A.; Salminen, S.J.; Scott, K.; Stanton, C.; Swanson, K.S.; Cani, P.D.; et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 491–502. [[CrossRef](#)]
14. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 506–514. [[CrossRef](#)] [[PubMed](#)]
15. Swanson, K.S.; Gibson, G.R.; Hutkins, R.; Reimer, R.A.; Reid, G.; Verbeke, K.; Scott, K.P.; Holscher, H.D.; Azad, M.B.; Delzenne, N.M.; et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2020**, *17*, 687–701. [[CrossRef](#)] [[PubMed](#)]
16. Staudacher, H.M.; Whelan, K. Altered gastrointestinal microbiota in irritable bowel syndrome and its modification by diet: Probiotics, prebiotics and the low FODMAP diet. *Proc. Nutr. Soc.* **2016**, *75*, 306–318. [[CrossRef](#)]
17. Didari, T.; Mozaffari, S.; Nikfar, S.; Abdollahi, M. Effectiveness of probiotics in irritable bowel syndrome: Updated systematic review with meta-analysis. *World J. Gastroenterol.* **2015**, *21*, 3072–3084. [[CrossRef](#)]
18. Moayyedi, P.; Ford, A.C.; Talley, N.J.; Cremonini, F.; Fox-Orenstein, A.E.; Brandt, L.J.; Quigley, E.M.M. The efficacy of probiotics in the treatment of irritable bowel syndrome: A systematic review. *Gut* **2008**, *59*, 325–332. [[CrossRef](#)]
19. Eom, T.; Kim, Y.S.; Choi, C.H.; Sadowsky, M.J.; Unno, T. Current understanding of microbiota and dietary-therapies for treating inflammatory bowel disease. *J. Microbiol.* **2018**, *56*, 189–198. [[CrossRef](#)]
20. Ganji-Arjenaki, M.; Rafieian-Kopaei, M. Probiotics are a good choice in remission of inflammatory bowel diseases: A meta analysis and systematic review. *J. Cell. Physiol.* **2018**, *233*, 2091–2103. [[CrossRef](#)]
21. Marasco, G.; Cirotta, G.G.; Rossini, B.; Lungaro, L.; Di Biase, A.R.; Colecchia, A.; Volta, U.; de Giorgio, R.; Festi, D.; Caio, G. Probiotics, Prebiotics and Other Dietary Supplements for Gut Microbiota Modulation in Celiac Disease Patients. *Nutrients* **2020**, *12*, 2674. [[CrossRef](#)]
22. Pryde, S.E.; Duncan, S.H.; Hold, G.L.; Stewart, C.S.; Flint, H.J. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett.* **2002**, *217*, 133–139. [[CrossRef](#)] [[PubMed](#)]
23. Hamer, H.; Jonkers, D.; Venema, K.; Vanhoutvin, S.; Troost, F.J.; Brummer, R. Review article: The role of butyrate on colonic function. *Aliment. Pharmacol. Ther.* **2007**, *27*, 104–119. [[CrossRef](#)] [[PubMed](#)]
24. Canani, R.B.; Costanzo MDi Leone, L.; Pedata, M.; Meli, R.; Calignano, A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World J. Gastroenterol.* **2011**, *17*, 1519–1528. [[CrossRef](#)]
25. Żółkiewicz, J.; Marzec, A.; Ruszczyński, M.; Feleszko, W. Postbiotics—A step beyond pre- and probiotics. *Nutrients* **2020**, *12*, 2189. [[CrossRef](#)]
26. Rivièrè, A.; Selak, M.; Lantin, D.; Leroy, F.; De Vuyst, L. Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human Gut. *Front. Microbiol.* **2016**, *7*, 979. [[CrossRef](#)] [[PubMed](#)]
27. Scott, K.P.; Antoine, J.-M.; Midtvedt, T.; Van Hemert, S. Manipulating the gut microbiota to maintain health and treat disease. *Microb. Ecol. Health Dis.* **2015**, *26*, 25877. [[CrossRef](#)]
28. De Vuyst, L.; Leroy, F. Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifidobacterial competitiveness, butyrate production, and gas production. *Int. J. Food Microbiol.* **2011**, *149*, 73–80. [[CrossRef](#)] [[PubMed](#)]

29. Schwab, C.; Ruscheweyh, H.; Bunesova, V.; Pham, V.T.; Schwab, C. Trophic interactions of infant bifidobacteria and *Eubacterium hallii* during L-fucose and fucosyllactose degradation bacterial strains and culture conditions. *Front. Microbiol.* **2017**, *8*, 1–14. [[CrossRef](#)]
30. Castanys-Muñoz, E.; Martin, M.J.; Prieto, P.A. 2'-fucosyllactose: An abundant, genetically determined soluble glycan present in human milk. *Nutr. Rev.* **2013**, *71*, 773–789. [[CrossRef](#)]
31. Salminen, S.; Stahl, B.; Vinderola, G.; Szajewska, H. Infant Formula Supplemented with Biotics: Current Knowledge and Future Perspectives. *Nutrients* **2020**, *12*, 1952. [[CrossRef](#)] [[PubMed](#)]
32. Bode, L. Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology* **2012**, *22*, 1147–1162. [[CrossRef](#)]
33. Garrido, D.; Barile, D.; Mills, D.A. A Molecular Basis for Bifidobacterial Enrichment in the Infant Gastrointestinal Tract. *Adv. Nutr.* **2012**, *3*, 415S–421S. [[CrossRef](#)]
34. Coppa, G.V.; Bruni, S.; Morelli, L.; Soldi, S.; Gabrielli, O. The first prebiotics in humans: Human milk oligosaccharides. *J. Clin. Gastroenterol.* **2004**, *38*, 80–83. [[CrossRef](#)]
35. Musilova, S.; Rada, V.; Vlkova, E.; Bunesova, V. Beneficial effects of human milk oligosaccharides on gut microbiota. *Benef. Microbes* **2014**, *5*, 273–283. [[CrossRef](#)] [[PubMed](#)]
36. Triantis, V.; Bode, L.; Van Neerven, R.J.J. Immunological Effects of Human Milk Oligosaccharides. *Front. Pediatr.* **2018**, *6*, 190. [[CrossRef](#)] [[PubMed](#)]
37. Nolan, L.S.; Rimer, J.M.; Good, M. The Role of Human Milk Oligosaccharides and Probiotics on the Neonatal Microbiome and Risk of Necrotizing Enterocolitis: A Narrative Review. *Nutrients* **2020**, *12*, 3052. [[CrossRef](#)]
38. Zuurveld, M.; Van Witzenburg, N.P.; Garssen, J.; Folkerts, G.; Stahl, B.; Land, B.V.; Willemsen, L.E.M. Immunomodulation by Human Milk Oligosaccharides: The Potential Role in Prevention of Allergic Diseases. *Front. Immunol.* **2020**, *11*. [[CrossRef](#)]
39. Reverri, E.J.; Devitt, A.A.; Kajzer, J.A.; Baggs, G.E.; Borschel, M.W. Review of the Clinical Experiences of Feeding Infants Formula Containing the Human Milk Oligosaccharide 2'-Fucosyllactose. *Nutrients* **2018**, *10*, 1346. [[CrossRef](#)]
40. Vandenplas, Y.; Berger, B.; Carnielli, V.P.; Ksiazyk, J.; Lagström, H.; Luna, M.S.; Migacheva, N.; Mosselmans, J.-M.; Picaud, J.-C.; Possner, M.; et al. Human Milk Oligosaccharides: 2'-Fucosyllactose (2'-FL) and Lacto-N-Neotetraose (LNnT) in Infant Formula. *Nutrients* **2018**, *10*, 1161. [[CrossRef](#)]
41. Elison, E.; Vigsnaes, L.K.; Krogsgaard, L.R.; Rasmussen, J.; Sørensen, N.; McConnell, B.; Hennes, T.; Sommer, M.O.A.; Bytzer, P. Oral supplementation of healthy adults with 2'-O-fucosyllactose and lacto-N-neotetraose is well tolerated and shifts the intestinal microbiota. *Br. J. Nutr.* **2016**, *116*, 1356–1368. [[CrossRef](#)]
42. Iribarren, C.; Törnblom, H.; Aziz, I.; Magnusson, M.K.; Sundin, J.; Vigsnaes, L.K.; Amundsen, I.D.; McConnell, B.; Seitzberg, D.; Öhman, L.; et al. Human milk oligosaccharide supplementation in irritable bowel syndrome patients: A parallel, randomized, double-blind, placebo-controlled study. *Neurogastroenterol. Motil.* **2020**, *32*, e13920. [[CrossRef](#)]
43. Islam, S.U. Clinical Uses of Probiotics. *Medicine* **2016**, *95*, e2658. [[CrossRef](#)] [[PubMed](#)]
44. Wallner, G.; Amann, R.; Beisker, W. Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry* **1993**, *14*, 136–143. [[CrossRef](#)]
45. Daims, H.; Brühl, A.; Amann, R.; Schleifer, K.-H.; Wagner, M. The Domain-specific Probe EUB338 is Insufficient for the Detection of all Bacteria: Development and Evaluation of a more Comprehensive Probe Set. *Syst. Appl. Microbiol.* **1999**, *22*, 434–444. [[CrossRef](#)]
46. Harmsen, H.J.; Wildeboer-Veloo, A.C.; Grijpstra, J.; Knol, J.; Degener, J.E.; Welling, G.W. Development of 16S rRNA-based probes for the Coriobacterium group and the Atopobium cluster and their application for enumeration of Coriobacteriaceae in human feces from volunteers of different age groups. *Appl. Environ. Microbiol.* **2000**, *66*, 4523–4527. [[CrossRef](#)]
47. Manz, W.; Amann, R.; Ludwig, W.; Vancanneyt, M.; Schleifer, K.H. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology* **1996**, *142*, 1097–1106. [[CrossRef](#)] [[PubMed](#)]
48. Langendijk, P.S.; Schut, F.; Jansen, G.J.; Raangs, G.C.; Kamphuis, G.R.; Wilkinson, M.H.; Welling, G.W. Quantitative fluorescence In Situ hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. *Appl. Environ. Microbiol.* **1995**, *61*, 3069–3075. [[CrossRef](#)]
49. Franks, A.H.; Harmsen, H.J.M.; Raangs, G.C.; Jansen, G.J.; Schut, F.; Welling, G.W. Variations of bacterial populations in human feces measured by fluorescent In Situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* **1998**, *64*, 3336–3345. [[CrossRef](#)]
50. Walker, A.W.; Duncan, S.H.; Leitch, E.C.M.; Child, M.W.; Flint, H.J. pH and Peptide Supply Can Radically Alter Bacterial Populations and Short-Chain Fatty Acid Ratios within Microbial Communities from the Human Colon. *Appl. Environ. Microbiol.* **2005**, *71*, 3692–3700. [[CrossRef](#)] [[PubMed](#)]
51. Hold, G.L.; Schwierz, A.; Aminov, R.I.; Blaut, M.; Flint, H.J. Oligonucleotide Probes That Detect Quantitatively Significant Groups of Butyrate-Producing Bacteria in Human Feces. *Appl. Environ. Microbiol.* **2003**, *69*, 4320–4324. [[CrossRef](#)]
52. Devereux, R.; Kane, M.D.; Winfrey, J.; Stahl, D.A. Genus- and Group-Specific Hybridization Probes for Determinative and Environmental Studies of Sulfate-Reducing Bacteria. *Syst. Appl. Microbiol.* **1992**, *15*, 601–609. [[CrossRef](#)]
53. Harmsen, H.J.M.; Elfferich, P.; Schut, F.; Welling, G.W. A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in faecal samples by fluorescent In Situ hybridization. *Microb. Ecol. Health Dis.* **1999**, *11*, 3–12.

54. Ferreira-Lazarte, A.; Kachrimanidou, V.; Villamiel, M.; Rastall, R.A.; Moreno, F.J. In Vitro fermentation properties of pectins and enzymatic-modified pectins obtained from different renewable bioresources. *Carbohydr. Polym.* **2018**, *199*, 482–491. [[CrossRef](#)] [[PubMed](#)]
55. Eypasch, E.; Williams, J.I.; Wood-Dauphinee, S.; Ure, B.M.; Schmulling, C.; Neugebauer, E.; Troidl, H. Gastrointestinal Quality of Life Index: Development, validation and application of a new instrument. *Br. J. Nutr.* **1995**, *82*, 216–222. [[CrossRef](#)] [[PubMed](#)]
56. Azpiroz, F.; Guyonnet, D.; Donazzolo, Y.; Gendre, D.; Tanguy, J.; Guarner, F. Digestive Symptoms in Healthy People and Subjects With Irritable Bowel Syndrome. *J. Clin. Gastroenterol.* **2015**, *49*, e64–e70. [[CrossRef](#)]
57. Guyatt, G.; Mitchell, A.; Irvine, E.J.; Singer, J.; Williams, N.; Goodacre, R.; Tompkins, C. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology* **1989**, *96*, 804–810. [[CrossRef](#)]
58. Chen, L.; Reynolds, C.; David, R.; Brewer, A.P. Development of an Index Score for Intestinal Inflammation-Associated Dysbiosis Using Real-World Stool Test Results. *Dig. Dis. Sci.* **2020**, *65*, 1111–1124. [[CrossRef](#)]
59. Šuligoj, T.; Vigsnaes, L.K.; Abbeele, P.V.D.; Apostolou, A.; Karalis, K.; Savva, G.M.; McConnell, B.; Juge, N. Effects of Human Milk Oligosaccharides on the Adult Gut Microbiota and Barrier Function. *Nutrients* **2020**, *12*, 2808. [[CrossRef](#)]
60. Jayadevappa, R.; Cook, R.; Chhatre, S. Minimal important difference to infer changes in health-related quality of life—A systematic review. *J. Clin. Epidemiology* **2017**, *89*, 188–198. [[CrossRef](#)]
61. Coffin, B.; Dapoigny, M.; Cloarec, D.; Comet, D.; Dyard, F. Relationship between severity of symptoms and quality of life in 858 patients with irritable bowel syndrome. *Gastroenterol. Clin. Biol.* **2004**, *28*, 11–15. [[CrossRef](#)]
62. Borgeonkar, M.; Irvine, E. Quality of life measurement in gastrointestinal and liver disorders. *Gut* **2000**, *47*, 444–454. [[CrossRef](#)]
63. Casellas, L.R.F.; Rodrigo, L.; Vivancos, S.R.P.; Riestra, S.; Pantiga, C.; Baudet, J.S.; Junquera, F.; Diví, V.P.; Abadia, C.; Papo, M.; et al. Factors that impact health-related quality of life in adults with celiac disease: A multicenter study. *World J. Gastroenterol.* **2008**, *14*, 46–52. [[CrossRef](#)]
64. Yu, Z.-T.; Chen, C.; Kling, D.E.; Liu, B.; McCoy, J.M.; Merighi, M.; Heidtman, M.; Newburg, D.S. The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. *Glycobiology* **2013**, *23*, 169–177. [[CrossRef](#)]
65. Yu, Z.-T.; Chen, C.; Newburg, D.S. Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology* **2013**, *23*, 1281–1292. [[CrossRef](#)] [[PubMed](#)]
66. Ruiz-Moyano, S.; Totten, S.M.; Garrido, D.A.; Smilowitz, J.T.; German, J.B.; Lebrilla, C.B.; Mills, D.A. Variation in Consumption of Human Milk Oligosaccharides by Infant Gut-Associated Strains of *Bifidobacterium breve*. *Appl. Environ. Microbiol.* **2013**, *79*, 6040–6049. [[CrossRef](#)]
67. Bunesova, V.; Lacroix, C.; Schwab, C. Fucosyllactose and L-fucose utilization of infant *Bifidobacterium longum* and *Bifidobacterium kashiwanohense*. *BMC Microbiol.* **2016**, *16*, 1–12. [[CrossRef](#)] [[PubMed](#)]
68. Martín, R.; Bermúdez-Humarán, L.G.; Langella, P. Searching for the Bacterial Effector: The Example of the Multi-Skilled Commensal *Bacterium Faecalibacterium prausnitzii*. *Front. Microbiol.* **2018**, *9*, 346. [[CrossRef](#)]
69. Rajilić-Stojanović, M.; Biagi, E.; Heilig, H.G.; Kajander, K.; Kekkonen, R.A.; Tims, S.; de Vos, W.M. Global and Deep Molecular Analysis of Microbiota Signatures in Fecal Samples From Patients with Irritable Bowel Syndrome. *Gastroenterology* **2011**, *141*, 1792–1801. [[CrossRef](#)] [[PubMed](#)]
70. Cao, Y.; Shen, J.; Ran, Z.H. Association between *Faecalibacterium prausnitzii* Reduction and Inflammatory Bowel Disease: A Meta-Analysis and Systematic Review of the Literature. *Gastroenterol. Res. Pr.* **2014**, *2014*, 1–7. [[CrossRef](#)]
71. Sokol, H.; Seksik, P.; Furet, J.P.; Firmesse, O.; Nion-Larmurier, I.; Beaugerie, L.; Cosnes, J.; Corthier, G.; Marteau, P.; Doré, J. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm. Bowel Dis.* **2009**, *15*, 1183–1189. [[CrossRef](#)] [[PubMed](#)]
72. De Palma, G.; Nadal, I.; Medina, M.; Donat, E.; Ribes-koninckx, C.; Calabuig, M.; Sanz, Y. Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children. *BMC Microbiol.* **2010**, *10*, 1–7. [[CrossRef](#)]
73. Verhoog, S.; Taneri, P.E.; Díaz, Z.M.R.; Marques-Vidal, P.; Troup, J.P.; Bally, L.; Franco, O.H.; Glisic, M.; Muka, T. Dietary Factors and Modulation of Bacteria Strains of *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*: A Systematic Review. *Nutrients* **2019**, *11*, 1565. [[CrossRef](#)] [[PubMed](#)]
74. Ferreira-Halder, C.V.; Faria, A.V.D.S.; Andrade, S.S. Action and function of *Faecalibacterium prausnitzii* in health and disease. *Best Pr. Res. Clin. Gastroenterol.* **2017**, *31*, 643–648. [[CrossRef](#)]
75. Miquel, S.; Martín, R.; Lashermes, A.; Gillet, M.; Meleine, M.; Gelot, A.; Eschalier, A.; Ardid, D.; Bermúdez-Humarán, L.G.; Sokol, H.; et al. Anti-nociceptive effect of *Faecalibacterium prausnitzii* in non-inflammatory IBS-like models. *Sci. Rep.* **2016**, *6*, 19399. [[CrossRef](#)]
76. Fang, S.; Wu, D.; Yang, Z.; Jiao, N.; Lin, Y.; Li, X.; Xiao, J.; Yuan, M.; Zhu, R.; Zhu, L. Keystone species in the pathogenic process of NAFLD. *FASEB* **2019**, *33*, 496.40.
77. Trosvik, P.; De Muinck, E.J. Ecology of bacteria in the human gastrointestinal tract—Identification of keystone and foundation taxa. *Microbiome* **2015**, *3*, 1–12. [[CrossRef](#)]
78. Cheng, L.; Kiewiet, M.B.G.; Logtenberg, M.J.; Groeneveld, A.; Nauta, A.; Schols, H.A.; Walvoort, M.T.C.; Harmsen, H.J.M.; De Vos, P. Effects of Different Human Milk Oligosaccharides on Growth of Bifidobacteria in Monoculture and Co-culture with *Faecalibacterium prausnitzii*. *Front. Microbiol.* **2020**, *11*, 569700. [[CrossRef](#)]

79. Dewulf, E.M.; Cani, P.; Claus, S.P.; Fuentes, S.; Puylaert, P.G.B.; Neyrinck, A.M.; Bindels, L.B.; De Vos, W.M.; Gibson, G.R.; Thissen, J.-P.; et al. Insight into the prebiotic concept: Lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* **2012**, *62*, 1112–1121. [[CrossRef](#)] [[PubMed](#)]
80. Ramirez-farias, C.; Slezak, K.; Fuller, Z.; Duncan, A.; Holtrop, G.; Louis, P. Effect of inulin on the human gut microbiota: Stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br. J. Nutr.* **2009**, *101*, 541–550. [[CrossRef](#)]
81. Van den Abbeele, P.; Duysburgh, C.; Vazquez, E.; Chow, J.; Buck, R.; Marzorati, M. 2'-Fucosyllactose alters the composition and activity of gut microbiota from formula-fed infants receiving complementary feeding in a validated intestinal model. *J. Funct. Foods* **2019**, *61*. [[CrossRef](#)]
82. Li, M.; Bauer, L.L.; Chen, X.; Wang, M.; Kuhlenschmidt, T.B.; Kuhlenschmidt, M.S.; Fahey, G.C.; Donovan, S.M. Microbial Composition and In Vitro Fermentation Patterns of Human Milk Oligosaccharides and Prebiotics Differ between Formula-Fed and Sow-Reared Piglets. *J. Nutr.* **2012**, *142*, 681–689. [[CrossRef](#)]
83. Azagra-Boronat, I.; Massot-Cladera, M.; Mayneris-Perxachs, J.; Knipping, K.; Land, B.V.; Tims, S.; Stahl, B.; Garssen, J.; Franch, À.; Castell, M.; et al. Immunomodulatory and Prebiotic Effects of 2'-Fucosyllactose in Suckling Rats. *Front. Immunol.* **2019**, *10*, 1773. [[CrossRef](#)]
84. Kumari, R.; Ahuja, V.; Paul, J. Fluctuations in butyrate-producing bacteria in ulcerative colitis patients of North India. *World J. Gastroenterol. WJG* **2013**, *19*, 3404–3414. [[CrossRef](#)] [[PubMed](#)]
85. Polansky, O.; Sekelova, Z.; Faldynova, M.; Sebkova, A.; Sisak, F.; Rychlik, I. Important Metabolic Pathways and Biological Processes Expressed by Chicken Cecal Microbiota. *Appl. Environ. Microbiol.* **2016**, *82*, 1569–1576. [[CrossRef](#)] [[PubMed](#)]
86. Lawson, P.A.; Song, Y.; Liu, C.; Molitoris, D.R.; Vaisanen, M.-L.; Collins, M.D.; Finegold, S.M. *Anaerotruncus colihominis* gen. nov., sp. nov., from human faeces. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 413–417. [[CrossRef](#)] [[PubMed](#)]
87. Louis, P.; Flint, H.J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* **2009**, *294*, 1–8. [[CrossRef](#)]
88. Vergara, D.M.; Gonz, E. Short chain fatty acids (butyric acid) and intestinal diseases. *Nutr. Hosp.* **2017**, *34*, 58–61.
89. Borycka-Kiciak, K.; Banasiewicz, T.; Rydzewska, G. Butyric acid—A well-known molecule revisited. *Gastroenterol. Rev.* **2017**, *2*, 83–89. [[CrossRef](#)]
90. Załęski, A.; Banaszkiwicz, A.; Walkowiak, J. Butyric acid in irritable bowel syndrome. *Gastroenterol. Rev.* **2013**, *8*, 350–353. [[CrossRef](#)]
91. Soldavini, J.; Kaunitz, J.D. Pathobiology and potential therapeutic value of intestinal short-chain fatty acids in gut inflammation and obesity. *Dig. Dis. Sci.* **2013**, *58*, 2756–2766. [[CrossRef](#)] [[PubMed](#)]
92. Martín, R.; Miquel, S.; Benevides, L.; Bridonneau, C.; Robert, V.; Hudault, S.; Chain, F.; Berteau, O.; Azevedo, V.; Chatel, J.M.; et al. Functional Characterization of Novel *Faecalibacterium prausnitzii* Strains Isolated from Healthy Volunteers: A Step Forward in the Use of *F. prausnitzii* as a Next-Generation Probiotic. *Front. Microbiol.* **2017**, *8*, 1226. [[CrossRef](#)] [[PubMed](#)]
93. Forbes, A.; Escher, J.; Hébuterne, X.; Kłęk, S.; Krznaric, Z.; Schneider, S.; Shamir, R.; Stardelova, K.; Wierdsma, N.; Wiskin, A.E.; et al. ESPEN guideline: Clinical nutrition in inflammatory bowel disease. *Clin. Nutr.* **2017**, *36*, 321–347. [[CrossRef](#)] [[PubMed](#)]
94. Mullin, G.E. Micronutrients and Inflammatory Bowel Disease. *Nutr. Clin. Pract.* **2012**, *27*, 136–137. [[CrossRef](#)]