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Lactulose Crystals and Liquid Both Show a Dose-Dependent Prebiotic Effect in a Computer-Controlled *In Vitro* Model of the Human Proximal Colon

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Abstract

The commercially available forms of the prebiotically active disaccharide lactulose include a liquid and a crystalline form. The prebiotic effect of both forms has not been compared in a similar study setup to date. In this study we repeated the most recent experiment performed with lactulose liquid with the crystalline formulation for comparison of the prebiotic effects. Lactulose crystals were administered daily for 5 days to the *in vitro model* of the proximal colon, the TNO Intestinal Model (TIM-2). Analysis of NaOH consumption, Short-Chain Fatty Acids (SCFA), Branched-Chain Fatty Acids (BCFA) and ammonia as well as relative abundance of microbiota revealed qualitatively comparable results with slight quantitative differences. After treatment with lactulose crystals, the levels of butyrate increased even more than after administration of the previously investigated lactulose liquid.

Keywords: Lactulose; TNO Intestinal Model (TIM-2); Butyrate; *Bifidobacterium; Lactobacillus; Megasphera*

Introduction

Lactulose is an indigestible prebiotic disaccharide stimulating the growth of health-promoting bacteria like *Bifidobacterium* and *Lactobacillus* [1,2]. Besides its therapeutic use in constipation and hepatic encephalopathy, lactulose is also used as a functional food ingredient [1,3]. The commercially available forms include a liquid (syrup) and a crystalline form [1]. These two forms differ slightly in composition. While the liquid form contains small but recognizable amounts of galactose, lactose, epilactose and 3-deoxy-glyceropentulose (in total up to 37% of lactulose), these sugars are neglectable in the crystalline form (less than 3% of lactulose) [4]. Similar to lactulose, galactose, lactose, and epilactose can promote growth of *Bifidobacterium* and *Lactobacillus* [5-10]. Therefore, the results from the liquid form cannot be transferred directly to the crystalline form. To date, the two lactulose forms have not been compared in a similar study design, even though both forms show a prebiotic effect [11-16].

We hypothesized that the crystalline form of lactulose exerts less prebiotic effect than the liquid form because of the missing side sugars. This hypothesis was tested in an *in vitro* model of the proximal colon, the TNO Intestinal Model (TIM-2). In a recent study in this model, 5 g per day of liquid lactulose increased SCFA, mainly acetate and butyrate production, the abundance of *Bifidobacterium* and *Lactobacillus*, and reduced BCFA and ammonia production [12]. We repeated this study with the same design including the same batch of microbiota of human origin and administered 5 g lactulose crystals instead of lactulose liquid per day. The effects of the two lactulose forms were qualitatively and quantitatively comparable except for slight differences in the microbiota compositions. Surprisingly, lactulose crystals elicited an even stronger increase in n-butyrate compared to the liquid formulation, suggesting potential superiority with regard to gut health promotion. These results corroborate the prebiotic effect of lactulose crystals and warrant the future application of this form of lactulose in indications requiring prebiotic support.

Materials and Methods

Test Product

The substance administered in this study was the lactulose crystals form (S.C.M. Società Chimica Mugello S.r.l., Vicchio, Italy) of lactulose (CAS 4618-18-2).

Intestinal Conditions of the TIM-2 System

The TNO Intestinal Model (TIM-2) system, a dynamic *in vitro* model of the proximal colon, was inoculated with a highly metabolically active microbiota of human origin resulting from fecal donations from a group of 4 healthy volunteers (3 females, 1 male, age 38.8 ± 3.9 years; BMI (body mass index) 24.2 ± 1.5 kg/m^2) [7,16]. Such pooled inocula have been shown to properly display the overlapping functionality of individuals and to include a slightly higher biodiversity [17]. The same microbiota batch as described for the previous study with the lactulose liquid formulation was used in this study [12]. Lactulose is generally considered not to be metabolized in the small intestine. Therefore the effect on the microbiota in the distal small intestine is considered minimal and the use of a model representing the proximal colon was considered appropriate.

The microbiota was dissolved in a standardized substrate from the 'ileum' (SIEM; Standardized Ileum Efflux Medium) as described recently [12].

SIEM simulates intestinal material passing the ileocecal valve in humans. It contains the major non-digestible carbohydrates (pectin, xylan, arabinogalactan, amylopectin, starch), which are found in a normal western diet. Furthermore it contains protein (bactopepton, casein), ox-bile, Tween 80 as well as vitamins and minerals [12].

At the start of the adaptation period the microbiota adapted to the model conditions and SIEM for 16 h.

Addition of Test Product

Crystalline lactulose was added daily to the system at doses of 2 g, 3 g, 4 g, and 5 g lactulose. Each dose as well as the control experiment without addition of lactulose was studied in duplicate (n = 2). The test period of the TIM-2 experiments lasted 120 h (5 consecutive days).

Sampling from TIM-2

A dialysate was continuously removed from the lumen by a semipermeable membrane and collected at the start of the test period as well as after 24, 48, 72, 96, and 120 h. At the beginning and end of the experiment (t = 0 h and t = 120 h) luminal samples were sampled as well. The samples were snap frozen in liquid nitrogen and stored at ≤ −72 ˚C until analysis. Dialysate samples were directly used; lumen samples were thawed and then centrifuged (15,300 g at 4 ˚C for 10 min in an Eppendorf Centrifuge 5417 C).

Sodium Hydroxide Usage (pH)

The pH was kept at a value of 5.8 by automatic titration with 2 M NaOH, the consumption of NaOH was monitored.

SCFA and BCFA

The lumen and dialysate fractions of TIM-2 were analyzed with gas chromatography (Shimadzu GC-2014 gas chromatograph) for SCFA (acetate, propionate and butyrate) and BCFA (iso-butyric acid and iso-valeric acid) as described previously [12].

Lactate and Ammonia

Samples for lactate and ammonia analysis were centrifuged as described above. In the supernatant, both L- and D-lactate were determined enzymatically (based on Boehringer, UV-method, Cat No.1112821035, Roche Diagnostics, West Sussex, UK). Ammonia was determined based on the Berthelot reaction [12].

16S rDNA Amplicon Sequencing

The bacterial population in the TIM-2 luminal samples was analyzed using Next Generation sequencing. Total DNA from the collected TIM-2 lumen samples at the start (t = 0 h) and at the end (t = 120 h) of the experiments was isolated as described [12].

For 16S rDNA amplicon sequencing of the V4 hypervariable region, 100 pg of purified DNA from the samples was amplified as described using 30 amplification cycles, applying F533/R806 primers [18,19]. These primers included Illumina adapters and a unique 8-nt sample index sequence key. The yield, integrity and size of the amplicons were analyzed on a Fragment Analyzer (Advanced Analytical Technologies, Inc.). The amplicon libraries were pooled in equimolar amounts and purified by using agarose gel electrophoresis and subsequent the QIAquick Gel Extraction Kit (QIAGEN). Paired-end sequencing of amplicons was conducted on the Illumina MiSeq platform (Illumina, Eindhoven, and The Netherlands).

The sequence data was processed with Mothur v.1.36.1 in line with the mothur MiSeq SOP [18]. Before merging the read pairs, low quality regions were trimmed using Btrim with a sliding window size of 5 nt and average quality score of 25 [20]. After merging,

the sequences were filtered by length (range: 243-263), while no ambiguous bases were allowed. The unique sequences were aligned to the bacterial SILVA SEED reference alignment release 102 (available at: http://www.mothur.org/wiki/Silva_reference_files); too short sequences were removed using screen.seqs with parameters "optimize=start-end, criteria=90". Chimeric sequences were identified per sample using UCHIME in de novo mode and removed. Next, sequences occurring less than 10 times in the entire dataset were removed [21]. Taxonomic names were assigned to all sequences using the Ribosomal Database Project (RDP) naïve Bayesian classifier with confidence threshold of 60% and 1000 iterations and the mothur-formatted version of the RDP training set v.9 (trainset9_032012) [22].

Statistical Analysis

Due to experimental replicate number of $n = 2$, no statistics were performed. Mean values of lactulose treated experiments were compared to mean control values.

Results

Sodium Hydroxide Usage

The addition of lactulose led to a dose dependent increase in NaOH consumption as shown in Figure 1A. The usage of NaOH was 162 ± 11 mL, 202 ± 13 mL, 223 ± 15 mL, and 223 ± 11 mL for the experiments with 2 g, 3 g, 4 g and 5 g lactulose, respectively, or 125 ± 9 mL in control runs. Both forms of lactulose led to a comparable increase in NaOH consumption at dosages of 2g and 3g (Figure 1B). While 4g and 5g lactulose liquid further increased the NaOH consumption, the same amount of lactulose crystals reached a plateau and did not increase NaOH consumption further.

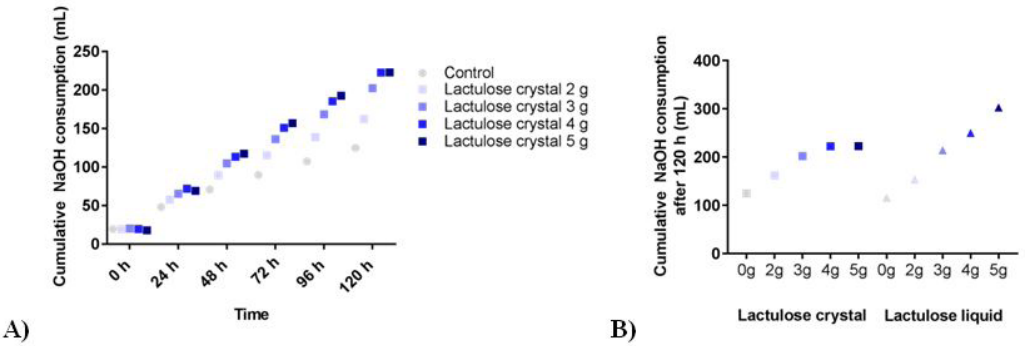
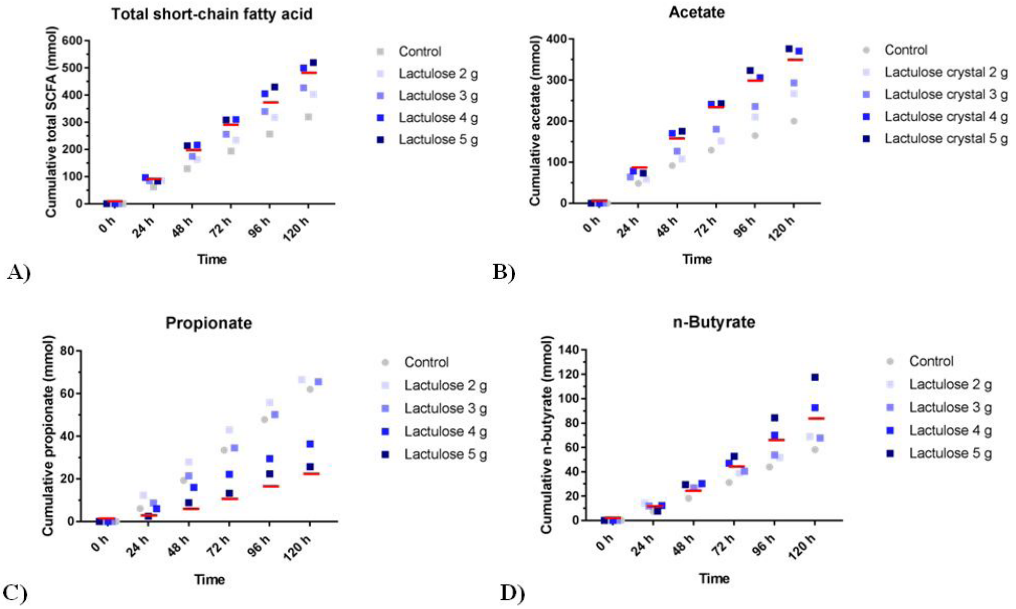


Figure 1: (**A**) Sodium hydroxide consumption during TIM-2 runs (mean of $n = 2$) with different dosages of lactulose crystals. All data points shown at the proximity of the individual time points indicated at the *X-axis* belong to these specific time points. (**B**) Cumulative sodium hydroxide consumption of lactulose crystals and lactulose liquid (taken from (12)) at t = 120 (mean from $n = 2$)

Markers of Saccharolytic Fermentation: SCFA Production



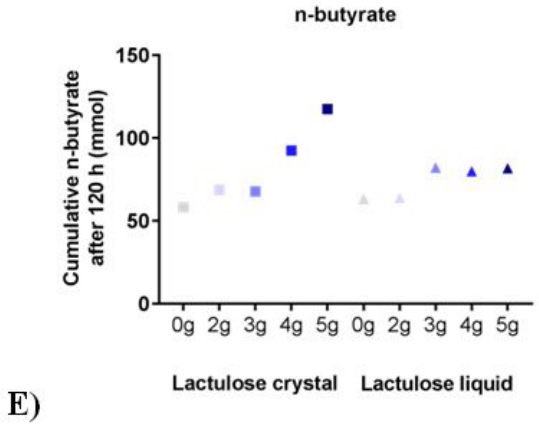


Figure 2: Production of (**A**) total short chain fatty acids (SCFA), (**B**) acetate, (**C**) propionate, and (**D**) butyrate in TIM-2 runs (mean of $n = 2$) with different dosages of lactulose crystals. Values at the start of the test period were set to zero. All data points shown at the proximity of the individual time points indicated at the *X-axis* belong to these specific time points. (E) Comparison of the cumulative *n*-butyrate production of lactulose crystals and lactulose liquid. Red horizontal bars figure 2 a-d: Amount achieved with 5 g lactulose liquid at different time points according to [12]

The different lactulose crystal doses all show a higher cumulative SCFA production as compared to the control as shown in Figure 2A. The mean amounts of total SCFA produced for the increasing daily doses of lactulose are 403 ± 5 mmol for 2 g, 427 ± 7 mmol for 3 g, 500 ± 13 mmol for 4 g, and 520 ± 7 mmol for 5 g, respectively, compared to 321 ± 12 mmol for the control. In the previous experiment the mean amount of total SCFA for 5 g lactulose liquid was less, namely 471 ± 12 mmol compared to 332 ± 34 mmol for its control [12].

The production profiles of each of the different SCFAs (Figure 2B; acetate, Figure 2C; propionate and Figure 2D; butyrate), indicate that similar to lactulose liquid, acetate is the predominantly produced SCFA [12]. The propionate production (Figure 2c) resulting from the higher doses (3, 4, and 5 g) of lactulose crystals was decreased compared to control and also compared to the amount reached with lactulose liquid 5 g treatment as recently published [12]. Lactulose crystals tended to induce increased butyrate production compared to control (Figure 2D). After 120 h, this increase was stronger when lactulose crystals were used compared to lactulose liquid (Figure 2E) [12].

Markers of Proteolytic Fermentation: BCFA and Ammonia Production

Reduced BCFA production (Figure 3A) was observed when lactulose crystals were added to the TIM-2 system. BCFA production was 9.2 ± 4.7 mmol (control), 6.9 ± 1.4 mmol (2 g lactulose crystals), 7.5 ± 1.4 mmol (3 g lactulose crystals), 3.7 ± 2.8 mmol (4 g lactulose crystals), and 4.0 ± 2.0 mmol (5 g lactulose crystals). For 5 g lactulose liquid the mean amount of total BCFA was 1.5 ± 0.2 mmol compared to 8.4 ± 4.2 mmol for the control [12].

The total amount of ammonia was measured as shown in Figure 3B. With rising doses of lactulose crystals there was a decreased mean ammonia production. Ammonia production was 108.0 ± 11.2 mmol (control), 87.7 ± 8.6 mmol (2 g lactulose), 84.8 ± 12.3 mmol (3 g lactulose), 51.1 ± 6.7 mmol (4 g lactulose), and 41.2 ± 5.2 mmol (5 g lactulose). For 5 g lactulose liquid the mean amount of ammonia produced was 30.5 ± 5.1 compared to 87.0 ± 27.9 mmol control [12].

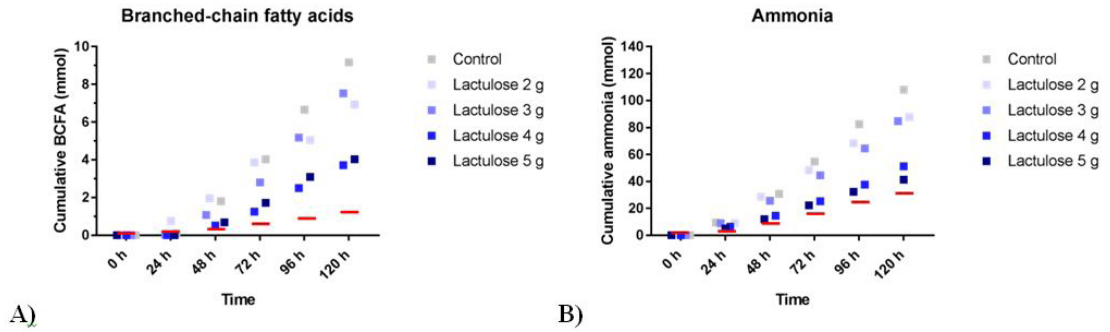


Figure 3: Cumulative branched-chain fatty acids (BCFA, Figure **3A**) or ammonia production during the 120 h test period in TIM-2 runs (mean of $n = 2$) (Figure **3B**). All data points shown at the proximity of the individual time points indicated at the *X-axis* belong to these specific time points. Red horizontal bars: Amount achieved with 5 g lactulose liquid at different time points according to (12)

Microbiota Composition

Amplicon sequencing of the TIM samples resulted in a total of 9,092,516 reads with an average of 287,547 reads per sample in a range of 207,253 up to 370,474 reads per sample. The total read length varied from 252 to 254 bases. Alignments were done against the Silva database (see method) and for classification of the reads, RDP was used. The β-diversity index represented by the constrained analysis plot of principal coordinates is shown in Figure 4. It shows effects of the lactulose dose and type of lactulose on the microbiota composition after 120 h. While after 120 h the lower lactulose doses (2 g and 3 g) are most similar in microbial diversity to the 0 g lactulose (control), higher doses (4 g and 5g) are most distinct from the 0 g lactulose.

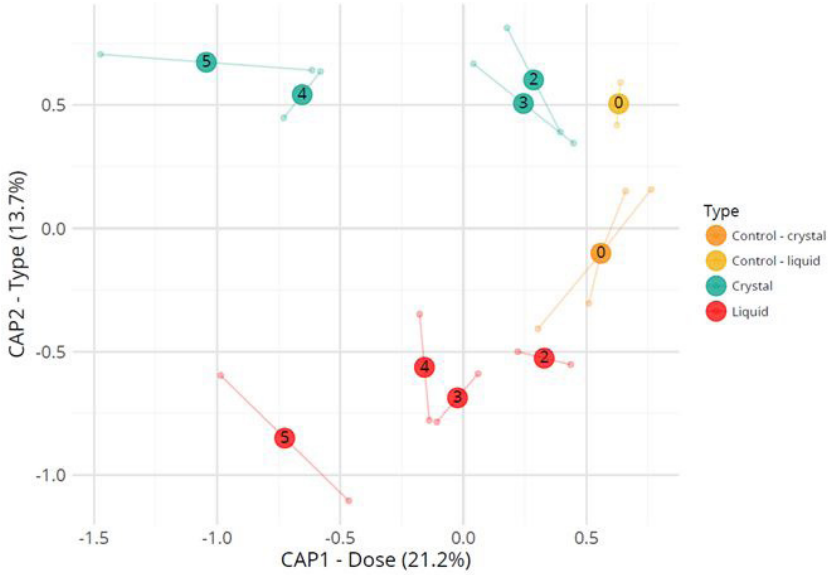


Figure 4: Constrained analysis plot of principal coordinates provides a visual representation of the microbiota pattern of proximities (i.e., similarities or distances) among the set of TIM exposures to the different types of lactulose (crystal- and liquid lactulose) as well as amounts of lactulose (0, 2, 3, 4 and 5 g lactulose) after 120 h (mean of *n*=2 TIM-2 runs)

The genus level abundancies for the control and test conditions resulting from analysis with mass V4 16S rDNA amplicon sequencing after 120 h in TIM-2 are shown in Table 1. Lactulose crystals increased the abundance of *Bifidobacterium and Lactobacillus*. The increase was most prominent at a dose of 4 g lactulose crystals (> 4-fold increase in *Bifidobacterium* and > 100-fold increase in *Lactobacillus*). In the previous study lactulose liquid led to > 6-fold elevated relative abundance of Bifidobacterium and > 2-fold elevated relative abundance of Lactobacillus [12]. In contrast to lactulose liquid, no increase in *Anaerostipes* was observed after treatment with lactulose crystals [12]. However, another butyrate-producing bacterium, *Megasphera*, showed elevated relative abundance (> 20-fold after treatment with 4 g lactulose crystals and > 60-fold after treatment with 5 g lactulose crystals).

Genus	Lactulose 0 g	Lactulose 2 g	Lactulose 3 g	Lactulose 4 g	Lactulose 5 g
Prevotella	155,025	138,346	149,013	36,489	21,998
Bifidobacterium	28,345	24,781	17,424	135,876	116,792
Blautia	13,703	26,237	34,513	20,943	10,562
Ruminococcus	28,669	31,462	39,882	50	218
Faecalibacterium	18,663	17,834	12,275	15,666	11,755
Megasphera	769	3,614	3,123	17,857	46,716
Lactococcus	3,233	6,700	6,958	14,619	8,934
Unclassified_ Lachnospiraceae	11,086	4,102	1,859	1,615	492
Unclassified_ Ruminococcaceae	4,962	3,739	1,334	2,092	3,537
Lactobacillus	82	1,623	1,504	12,047	912

Table 1: The table indicates the average number n of the different bacterial genera with a relative abundance of >1% in the 5 different lactulose conditions 0 g, 2 g, 3 g, 4 g, and 5 g, respectively, after 120 h of exposure in TIM-2 (mean of *n*=2 TIM-2 runs)

Discussion

The main finding of this study is that the prebiotic effect of lactulose crystals is qualitatively and quantitatively comparable to the

prebiotic effect of lactulose liquid with few quantitative differences. Both formulations increase *Bifidobacterium* and *Lactobacillus*. The relative increase in *Bifidobacterium* is comparable for the two forms of lactulose, while lactulose crystals lead to a more pronounced growth of *Lactobacillus* compared to lactulose liquid.

Both forms of lactulose increased the consumption of NaOH in this model, reflecting acidification of the colonic content. Such acidification is mainly due to the production of SCFA. *In vivo*, the colonic pH would be shifted to neutral by the uptake of SCFA by the epithelial cells and buffered by the excretion of bicarbonate [23]. The buffering of the pH by epithelial bicarbonate excretion is modeled in our study by the NaOH consumption. Daily administration of lactulose liquid to the TIM-2 model lead to a dose-dependent increase in NaOH consumption, which was also seen with lactulose crystals. After 5 days administration of lactulose crystals, however, the NaOH consumption of the 4g and the 5g runs were similar, while the NaOH consumption still increased when 5g lactulose liquid were compared to 4g of this formulation. This difference between the formulations was also reflected in the total amount of SCFA: Here the 4g and 5g runs of lactulose crystal were similar, while the levels further increased when 5g lactulose liquid were compared to 4g lactulose liquid. Even though the intraluminal pH is one of the modulators of bacterial growth, the impact of this slight difference in intraluminal acidification between the two formulations of lactulose is questionable due to the presence of bicarbonate buffering *in vivo* [23].

Both forms of lactulose increased the levels of SCFA and decreased the levels of BCFA and ammonia. The crystalline formulation led to a greater elevation of butyrate levels compared to lactulose liquid and to a slightly lower production of propionate. These two SCFA differ in their fate and effect. Butyrate is used preferentially as an energy source by the gut mucosa, while propionate contributes to gluconeogenesis in the liver [24]. Both have an anti-inflammatory effect with regard to cytokines, but butyrate in addition also inhibits production of reactive oxygen species [25]. Further postulated effects of butyrate are prevention of cancer, obesity and diabetes type 2 as reviewed in [26]. Propionate has been shown to lower liver lipogenesis and cholesterol levels, increase plasma leptin levels and satiety and to exert an anti-proliferative effect on cancer cells *in vitro* [27-32]. To date, nothing is known about the ideal intestinal ratio of propionate and butyrate and thus the consequences of the changes in SCFA after lactulose administration for gut health will have to be tested in vivo before final conclusions can be drawn.

The reason for the differences in butyrate levels between lactulose crystals and lactulose liquid may be due to differences in microbiota composition. Lactulose liquid leads to a strong increase in *Anaerostipes* growth, which was among others considered responsible for the increase in butyrate levels [12]. Nevertheless, although the relative abundance of *Anaerostipes* was not increased after administration of lactulose crystals, still the cumulative amount of butyrate in the lumen exceeded the levels induced with lactulose liquid. This effect may be related to butyrate production by other bacteria like *Megasphaera*, which increased in relative abundance after treatment with lactulose crystals, but not with lactulose liquid [33,34]. The increased relative abundance of *Anaerostipes* after treatment with lactulose liquid, which is missing after administration of lactulose crystals, may have been due to the additive effects of galactose and lactose, which are present in higher amounts in the lactulose liquid formulation. Both galactose and lactose have been shown to be even more growth promoting to the *Anaerostipes* strain *Anaerostipes caccae* than lactulose itself [35,36].

The reduction of BCFA levels, reflecting less proteolytic fermentation, was more pronounced after lactulose liquid treatment together with increased saccharolytic fermentation. Due to the reduced amount of additional sugars, administration of lactulose crystals provides less saccharides than lactulose liquid. Thus, the microbiota has to rely more on proteolytic fermentation with lactulose crystals and this could indicate why the amount of BCFA is less reduced. Interestingly, ammonia levels were comparable in both lactulose forms, excluding a harmful influence of the lessened decrease in proteolytic fermentation.

A limitation of this study is the low number of runs which precludes statistical analysis. The low number of runs was considered appropriate to gather first data for the lactulose crystal formulation as according to the former experience with the TIM-2 system the variation between runs of the TIM-2 system is rather small. Albeit the reproducible narrow variability these results require confirmation in future studies with a higher number of runs to determine statistical significance of the findings. A second limitation is the total amount of carbohydrates administered with the lactulose liquid formulation due to the higher number of side sugars. The effect of this rise in total carbohydrates on the microbiota composition cannot be excluded.

In conclusion, lactulose crystals exert a pronounced prebiotic effect *in vitro* comparable to the lactulose liquid formulation. The levels of butyrate after 120 h lactulose crystal treatment exceed those evoked by lactulose liquid, suggesting an even more positive effect on gut health.

Acknowledgment

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Author Contributions

"M.K.B., A.K.-K., A.K., S.S.-G., J.F.S., A.J.H.M., and M.E.K. conceived and designed the experiments; K.L., A.J.H.M. and J.M.B.M.V. performed the experiments; S.B., K.L. and J.M.B.M.V. analyzed the data; M.K.B. wrote the paper."

Conflicts of Interest

M.K.B., A.K.-K., A.K., S.S.-G., and J.F.S. are employees of Fresenius Kabi, the sponsor of this study.

References

1. Panesar PS, Kumari S (2011) Lactulose: production, purification and potential applications. Biotechnol Adv 29: 940-8.

2. Ackerman DL, Craft KM, Townsend SD (2017) Infant food applications of complex carbohydrates: Structure, synthesis, and function. Carbohydr Res 437: 16-27.

3. Aït-Aissa A, Aïder M (2014) Lactulose: production and use in functional food, medical and pharmaceutical applications. Practical and critical review. Int J Food Sci Tech 9: 1245-53.

4. European Pharmacopeia 9.0. Lactulose liquid, 07/2018:0924. p. 5712-5.

5. European Pharmacopeia 7.0. Lactulose, 01/2009:1230. p. 2234-5.

6. Anvari M, Khayati G, Rostami S (2014) Optimisation of medium composition for probiotic biomass production using response surface methodology. J Dairy Res 81: 59-4.

7. Banerjee D, Chowdhury R, Bhattacharya P (2017) Optimization of extraction process of inulin from Indian millets (jowar, bajra and ragi)-characterization and cost analysis. J Food Sci Technol 54: 4302-14.

8. Watanabe J, Nishimukai M, Taguchi H, Senoura T, Hamada S, et al. (2008) Prebiotic properties of epilactose. J Dairy Sci 91: 4518-26.

9. Amaretti A, Bernardi T, Tamburini E, Zanoni S, Lomma M, et al. (2007) Kinetics and metabolism of Bifidobacterium adolescentis MB 239 growing on glucose, galactose, lactose, and galactooligosaccharides. Appl Environ Microbiol 73: 3637-44.

10. Ortakci F, Broadbent JR, Oberg CJ, McMahon DJ (2015) Growth and gas production of a novel obligatory heterofermentative Cheddar cheese nonstarter lactobacilli species on ribose and galactose. J Dairy Sci 98: 3645-54.

11. Aguirre M, Jonkers DM, Troost FJ, Roeselers G, Venema K (2014) In vitro characterization of the impact of different substrates on metabolite production, energy extraction and composition of gut microbiota from lean and obese subjects. PloS one 9: e113864.

12. Bothe MK, Maathuis AJH, Bellmann S, van der Vossen J, Berressem D, et al. (2017) Dose-Dependent Prebiotic Effect of Lactulose in a Computer-Controlled In Vitro Model of the Human Large Intestine. Nutrients 9.

13.Bouhnik Y, Attar A, Joly FA, Riottot M, Dyard F, et al. (2004) Lactulose ingestion increases faecal bifidobacterial counts: a randomised double-blind study in healthy humans.Eur J Clin Nutr 58: 462-6.

14. Bouhnik Y, Neut C, Raskine L, Michel C, Riottot M, et al. (2004) Prospective, randomized, parallel-group trial to evaluate the effects of lactulose and polyethylene glycol-4000 on colonic flora in chronic idiopathic constipation. Aliment Pharmacol Ther 19: 889-99.

15. Tayebi-Khosroshahi H, Habibzadeh A, Niknafs B, Ghotaslou R, Yeganeh Sefidan F, et al. (2016) The effect of lactulose supplementation on fecal microflora of patients with chronic kidney disease; a randomized clinical trial. J Renal Inj Prev 5: 162-7.

16. Venema K, van Nuenen M, van den Heuvel E, Pool W, van der Vossen J (2003) The Effect of Lactulose on the Composition of the Intestinal Microbiota and Short-chain Fatty Acid Production in Human Volunteers and a Computer-controlled Model of the Proximal Large Intestine. Microbiol Eco Health Dis 15: 94-105.

17. M A, J R-G, ME K, K V(2014) To pool or not to pool? Impact of the use of individual and pooled fecal samples for in vitro fermentation studies. J Microbiol Methods 107: 1-7.

18. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79: 5112-20.

19. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, et al (2011) Global patterns of 16S rrna diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America 108: 4516-22.

20. Kong Y (2011) Btrim: a fast, lightweight adapter and quality trimming program for next-generation sequencing technologies. Genomics 98: 152-3.

21. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194-200.

22. Qiong Wang, George M. Garrity, James M. Tiedje, James R. Cole (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73: 5261-7.

23.Cremer J, Arnoldini M, Hwa T (2017) Effect of water flow and chemical environment on microbiota growth and composition in the human colon. Proc Natl Acad Sci U S A 114: 6438-43.

24. Morrison DJ, Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes 7: 189-200.

25. Bolognini D, Tobin AB, Milligan G, Moss CE (2016) The Pharmacology and Function of Receptors for Short-Chain Fatty Acids. Mol Pharmacol 89: 388-98.

26. McNabney SM, Henagan TM (2017) Short Chain Fatty Acids in the Colon and Peripheral Tissues: A Focus on Butyrate, Colon Cancer, Obesity and Insulin Resistance. Nutrients 9: doi: 10.3390/nu9121348.

27. Delzenne NM, Williams CM (2002) Prebiotics and lipid metabolism. Curr Opin Lipidol 13: 61-7.

28. Adam A, Levrat-Verny MA, Lopez HW, Leuillet M, Demigne C, et al. (2001) Whole wheat and triticale flours with differing viscosities stimulate cecal fermentations and lower plasma and hepatic lipids in rats. J Nutr 131: 1770-6.

29. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, et al. (2004) Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc Natl Acad Sci U S A 101: 1045-50.

30. Ruijschop R, Boelrijk AEM, MCT G. (2008) Satiety effects of a dairy beverage fermented with propionic acid bacteria. Int Dairy J 18: 945-50.

31. Jan G, Belzacq AS, Haouzi D, Rouault A, Metivier D, et al. (2002) Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. Cell Death Differ 9: 179-88.

32. Scheppach W, Bartram HP, Richter F (1995) Role of short-chain fatty acids in the prevention of colorectal cancer. Eur J Cancer 31: 1077-80.

33. Counotte GH, Prins RA, Janssen RH, Debie MJ (1981) Role of Megasphaera elsdenii in the Fermentation of dl-[2-C]lactate in the Rumen of Dairy Cattle. Appl Environ Microbiol 42: 649-55.

34. Hashizume K, Tsukahara T, Yamada K, Koyama H, Ushida K (2003) Megasphaera elsdenii JCM1772T normalizes hyperlactate production in the large intestine of fructooligosaccharide-fed rats by stimulating butyrate production. J Nutr 133: 3187-90.

35. Chia LW, Aalvink S, Wopereis H, Knol J, deVos WM, et al. (2014) Cross feeding of Akkermansia muciniphila and Anaerostipes caccae. ENGIHR Conference: The Gut Microbiota Throughout Life; September Max Rubner-Insitut, Karlsruhe, Germany.

36. Elshaghabee FM, Bockelmann W, Meske D, de Vrese M, Walte HG, et al. (2016) Ethanol Production by Selected Intestinal Microorganisms and Lactic Acid Bacteria Growing under Different Nutritional Conditions. Front Microbiol 7: 47.