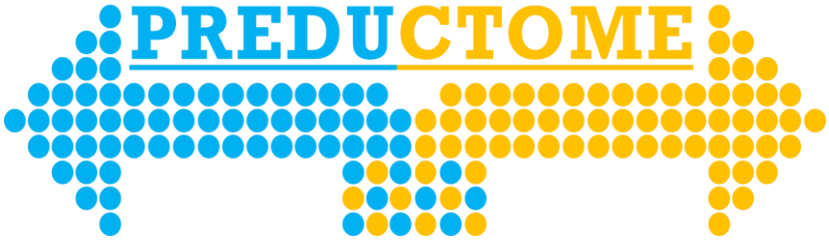
Prediction of dietary intervention efficacy in mild ulcerative colitis patients based on fecal microbiome signatures (PREDUCTOME study)



RESEARCH PROTOCOL

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PROTOCOL TITLE

Prediction of dietary intervention efficacy in mild ulcerative colitis patients based on fecal microbiome signatures

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PROTOCOL SIGNATURE SHEET

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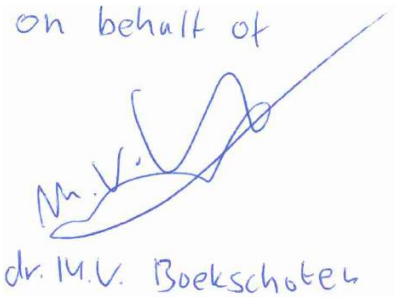
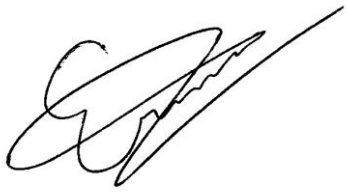
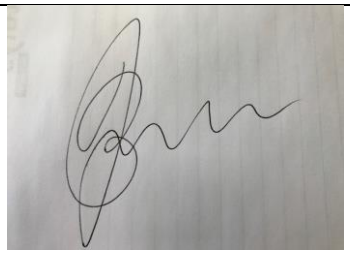
Sponsor or legal representative: Dr. Sjoukje Heimovaara, PhD Managing director Agrotechnology & Food Sciences Group Wageningen University & Research		**09-02-2022**
Coordinating Investigator/Project leader Zhuang Liu, MSc PhD candidate Laboratory of Microbiology Wageningen University & Research		**06-12-2021**
Principal Investigator Dr. Erwin G. Zoetendal, PhD Associate professor Laboratory of Microbiology Wageningen University & Research		**21-12-2021**
Medical supervisor Prof. Ben Witteman, PhD MD Division of Human Nutrition & Health Wageningen University & Research		**28-11-2021**

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
5ASA	5-Aminosalicylate
AE	Adverse Events
AG	Acacia Gum
ANOVA	Analysis of Variance
ASV	Amplicon Sequence Variant
BMI	Body Mass Index
BV	In Dutch: Besloten Vennootschap
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
COVID-19	Coronavirus disease 2019
CV	Curriculum Vitae
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
ELISA	Enzyme-linked Immunosorbent assay
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
FDR	False Discovery Rate
FFQ	Food Frequency Questionnaire
FOS	Fructo-OligoSaccharides
GBF	Germinated Barley Foodstuff
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
GG	Guar Gum
GI	Gastro-Intestinal
GMP	Good Manufacturing Practice
GP	General Practitioner
GSRS	Gastrointestinal Symptom Rating Scale
HPLC	High Performance Liquid Chromatography
IB	Investigator’s Brochure
IBD	Inflammatory Bowel Disease

ID	Identity Document
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISO	International Organization for Standardization
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
NSF	National Sanitation Foundation
PCoA	Principle Coordinate Analysis
PERMANOVA	Permutational Multivariate Analysis Of Variance
PHGG	Partially Hydrolyzed Guar Gum
P-SCCAI	Patient Simple Clinical Colitis Activity Index
qPCR	quantitative Polymerase Chain Reaction
QQ	Quantile-Quantile
RNA	Ribonucleic Acid
RS	Resistant Starch
(S)AE	(Serious) Adverse Event
SCFAs	Short Chain Fatty Acids
SD	Standard Deviation
SDL	Security Development Lifecycle
SIBDQ	Short Inflammatory Bowel Disease Questionnaire
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNF- α	Tumour Necrosis Factor alpha
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
UC	Ulcerative Colitis
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Ulcerative colitis (UC) patients respond differently to treatments/interventions (e.g. diet/fecal microbiota transplantation), but the reason for this individual specificity remains unknown. We hypothesize that the baseline fecal microbiota composition determines the efficacy of a treatment/intervention, and potential responders, i.e. patients showing symptoms improvement after treatment, can be predicted based on fecal microbiota composition.

Objective: The primary objective is to validate the prediction that prebiotics intervention boosts butyrate production and thereby induces a higher response (lower mean Patient Simple Clinical Colitis Activity Index (P-SCCAI) score) in mild UC patients with low intestinal Bacteroidetes levels (predicted responders), but not in those with high intestinal Bacteroidetes levels (predicted non-responders) at T = 8 weeks. The secondary objectives are to study the effects of prebiotics intervention on response over time (T = 0, 4, 8, 12 and 60 weeks), mucosal inflammation, gastro-intestinal (GI) complaints, stool consistency, stool frequency, fecal microbiota composition, fecal short-chain fatty acids concentrations, quality of life, medication use, and safety parameters in mild UC patients.

Study design: This study is a four-arm double-blind randomized placebo-controlled parallel trial. It consists of a screening stage in which mild UC patients will be assigned to be predicted responders or predicted non-responders based on their fecal Bacteroidetes levels. Afterwards the predicted responders and non-responders will be assigned to either the prebiotics group (arm 1 and 3) or placebo group (arm 2 and 4).

Study population: Adult subjects aged 18-65 years and body mass index 18-30 kg/m^2 with mild UC defined by P-SCCAI (3-5 points in a 19-point scale), with at least one relapse in the last two years.

Intervention: An 8-week intervention period with four parallel arms: 1) predicted responders with prebiotics treatment (acacia gum, partially hydrolyzed guar gum, and resistant starch), 2) predicted responders with placebo (maltodextrin and corn starch), 3) predicted non-responders with prebiotics treatment, 4) predicted non-responders with placebo, during which the study participants consume the respective supplement (3 grams, twice daily).

Main study parameters/endpoints: The main parameter is the response (mean P-SCCAI score) between arms at T = 8 weeks. The secondary parameters are the response over time at T = 0, 4, 8, 12, and 60 weeks, mucosal inflammation (fecal calprotectin), gastro-intestinal (GI) complaints, stool consistency, stool frequency, fecal microbiota composition, fecal short-chain fatty acids concentrations, health-related quality of life, medication use, and safety parameters.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: This study has a relatively low burden for the participants. They have to

consume the commercially available supplement twice daily for eight weeks. Besides, several questionnaires (one long FFQ questionnaire on one occasion, two short ones on five occasions, five short ones on three occasions) have to be completed, which is all possible from home. On five occasions they will collect fecal samples (which will be collected from their homes by us). There are no physical risks for the study participants, although some participants may experience minor bloating complaints at the start of using the prebiotics.

1. INTRODUCTION AND RATIONALE

The incidence and prevalence of ulcerative colitis (UC) are increasing worldwide [1]. It is a disease that causes inflammation extending from rectum to colon, and it is one of the two major types of inflammatory bowel disease (IBD) associated with abdominal pain, bloody diarrhea, and urgency to defecate [2]. Compared with the general population, patients with longstanding UC, regardless of clinical activity, have an increased risk for colorectal cancer [2, 3]. Unfortunately, there is no cure for UC and hence, treatments focus on dampening the severity of symptoms. Besides medical or surgical treatments, about half of patients with UC have mild symptoms in which a dietary recommendation, including increasing dietary fiber intake, could help reduce inflammation [4-6]. Although several successes for inducing remission in UC have been reported with treatments including dietary modification and fecal microbiota transplantation, there is no golden grail treatment for all subjects. Some individuals respond well to a certain treatment strategy while others do not. The reason for these differences between individual responses remains speculative. Therefore, it is important to find biomarkers that enable clinicians to identify patients more likely to respond to specific dietary treatments beforehand, and push them back to remission as fast as possible. As a consequence, most therapeutic approaches of UC start with classifying patients according to the severity of the disease [7].

Diet could extensively affect the environment of human intestine, which harbors trillions of microbes, commonly referred to as the “intestinal microbiota”, and “microbiome” (the collection of microbes and all the genes and functionalities they encode [8]). Numerous studies have suggested that the intestinal microbiota is closely associated with overall human health [9, 10]. More importantly, the human gut microbiome is a rich pool of potential biomarkers that have been previously shown to be associated with disease states including IBD [11, 12]. Microbiome-based stratification strategies of patients groups have been widely employed to guide dietary intervention studies [13]. Recently, a low-fat, high-fiber diet has been found to reduce inflammatory markers and alter the microbiome in patients with mild UC or UC in remission [14]. Another similar study demonstrated that a fiber-based prebiotic resulted in less abdominal pain and cramping in UC patients [15]. This indicates that increasing dietary fiber intake could be beneficial for maintaining periods of remission or improving clinical outcomes in patients with UC. Furthermore, short chain fatty acids (SCFAs) such as butyrate are produced by intestinal bacteria when they break down dietary fibers and these are considered beneficial for gut health. In a pilot study, it was demonstrated that UC patients could take a fiber-rich diet to increase the fecal butyrate level [5]. However, response to a treatment differs drastically between subjects, with some patients entering remission (responder), while others fall or remain into relapse (non-responders). The reasons for this inconsistent response between subjects remain speculative but may be the result of differences in the personal gut microbiota composition [16].

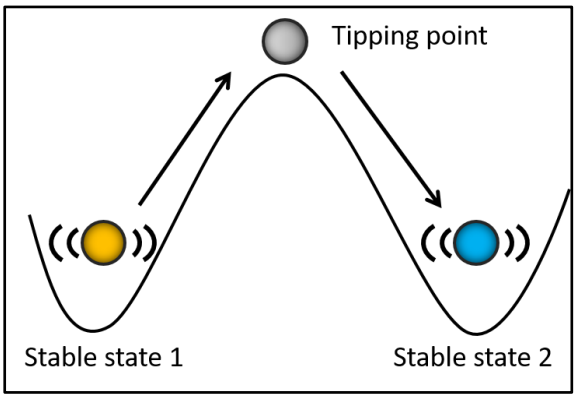


Figure 1 Existent tipping point in ecosystem. (adapted from reference 17 and 18.)

Looking from an ecological perspective, ecosystems are generally consisting of alternative stable states that are resilient to (minor) perturbations. Changing from one stable state into another stable state needs a strong perturbation in which a tipping point needs to be crossed (**Figure 1**) [17, 18]. For example, both deserts and rainforests are stable states, and only a drastic perturbation, like deforestation, allows a rainforest to cross a tipping point to become a desert, and similarly, it will need a lot of effort to have it return again from a desert into a rainforest in which a tipping point needs to be crossed as well. We believe that similar ecological principles exist for intestinal microbial ecosystems. Recently, we have published a review in which groups of microbiomes containing certain characteristics can be considered as alternative ecosystems states that are generally resilient to perturbations [19]. These stable states can both be homeostatic microbiomes in healthy subjects as well as microbiomes associated with disease, such as UC, and switching from one to the other needs strong perturbations (such as diseases/infections causing diarrhea) or treatments (such as antibiotics use or prebiotic consumption) that result in crossing the tipping point.

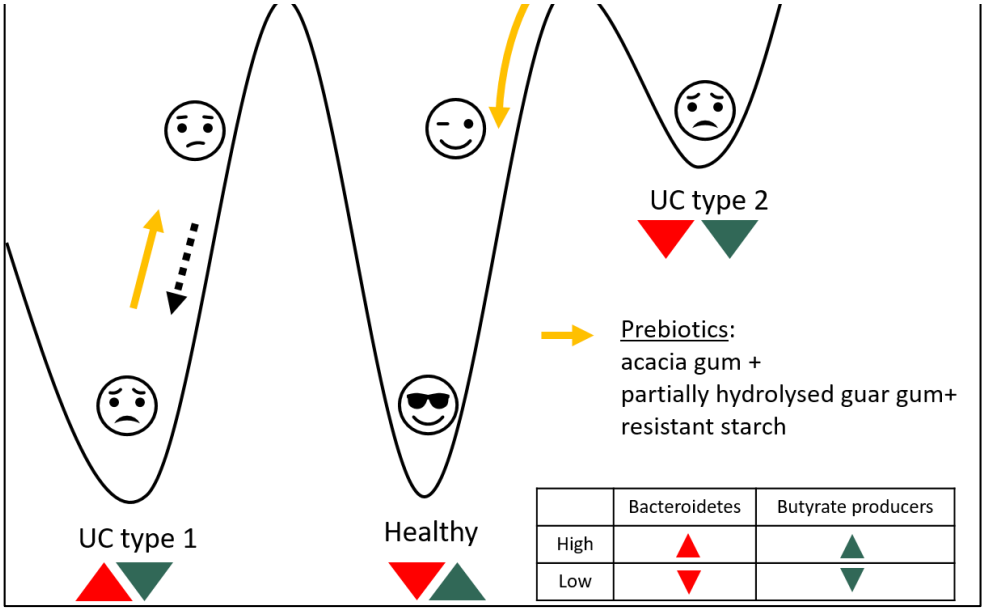
Generally, UC patients have a gut microbiota composition that is different from that observed in healthy subjects. In UC patients, alterations of the intestinal microbiome have been observed in many clinical studies, characterized by a general reduction in fecal microbiota diversity, along with phylum-level increases in Bacteroidetes and decreases in Firmicutes, and lower levels of butyrate [20-22]. Nevertheless, there is also a clear subject-specific impact on the gut microbiota composition. For healthy subjects, different alternative healthy states of the gut-microbiome have been identified, and we speculate that different alternative states also exist for UC patients. Indeed a recent study on the rat model for UC has indicated the existence of health states and disease active states [23]. Similarly, alternative intestinal ecosystem states have also been observed in pediatric UC and these could affect remission in UC [24].

The Bacteroidetes phylum is one of the most abundant phyla in the human gut microbiome and dramatic differences at this phylum level have been observed [20, 25]. Remarkably, induction of sustained remission via fecal microbiota transplantation was associated with a baseline microbiota that was more similar to that of healthy individuals which have a relatively low Bacteroidetes level (average 5% and 8%, respectively), while non-responders have a relatively high baseline Bacteroidetes level (average 19%) [20]. Moreover, the induction of sustained remission was associated with an improved butyrate production capacity. Hence, we hypothesize that the gut microbiome of UC patients with low intestinal Bacteroidetes levels are characterized as an alternative state that has a tipping point that is

easier to be crossed by an intervention compared to those with higher Bacteroidetes levels. We speculate that crossing this tipping point can be reached by stimulating intestinal butyrate production capacity.

There is a growing recognition that not an individual fiber but a mixture of them are more likely to promote the growth of SCFAs producing bacteria, because individual or consortia of bacteria play different, sometimes complementary, roles in the fiber degradation process. Therefore, by using a fiber mixture, the physiological effects of fiber (e.g., SCFAs production and bowel movement modulation) would be stimulated [26]. To identify a potential treatment targeting the butyrate-producing capacity that could be used in an intervention study, we applied an *in vitro* screening of different dietary fiber mixtures (so-called “butyrate boosters”) on the gut microbiome of UC patients to test our hypothesis. From this pilot study, we identified a promising fiber prototype product (mixture of acacia gum, partially hydrolyzed guar gum, and resistant starch) that boosted butyrogenic activity in microbiota with low Bacteroidetes levels, while it remained unaffected in those with high Bacteroidetes levels (unpublished results). To further test our hypothesis, we will conduct a dietary intervention study with a mixture of butyrate boosting fibers (hereinafter referred to as prebiotics) of which we predict they will induce a higher response in mild UC patients with a low intestinal Bacteroidetes level, while mild UC patients with a high intestinal Bacteroidetes level will be less affected (**Figure 2**).

Figure 2 PREDUCTOME study : hypotheses



2. OBJECTIVES

Primary Objective:

The primary objective is to validate the prediction that prebiotics intervention boosts butyrate production and thereby induces a higher response (lower mean Patient Simple Clinical Colitis Activity Index (P-SCCAI) score) in mild UC patients with low intestinal Bacteroidetes levels (predicted responders), but not in those with high intestinal Bacteroidetes levels (predicted non-responders) at T = 8 weeks

Secondary Objective(s):

1. To study the effects of the prebiotic intervention on response over time at T = 0, 4, 8, 12, and 60 weeks in mild UC patients
2. To study the effects of the prebiotic intervention on mucosal inflammation (fecal calprotectin levels) in mild UC patients
3. To study the effects of the prebiotic intervention on gastro-intestinal (GI) complaints (e.g., indigestion, abdominal pain) in mild UC patients
4. To study the effects of the prebiotic intervention on stool consistency and frequency in mild UC patients
5. To study the effects of the prebiotic intervention on the gut microbiota composition and SCFAs profile in mild UC patients
6. To study the effects of the prebiotic intervention on health-related quality of life in mild UC patients
7. To study the effects of the prebiotic intervention on medication use in mild UC patients
8. To study the effects of the prebiotic intervention on safety parameters in mild UC patients

3. STUDY DESIGN

This study is a double-blind randomized placebo-controlled parallel trial. A summary of the study set-up and workflow is shown in **Figure 3**.

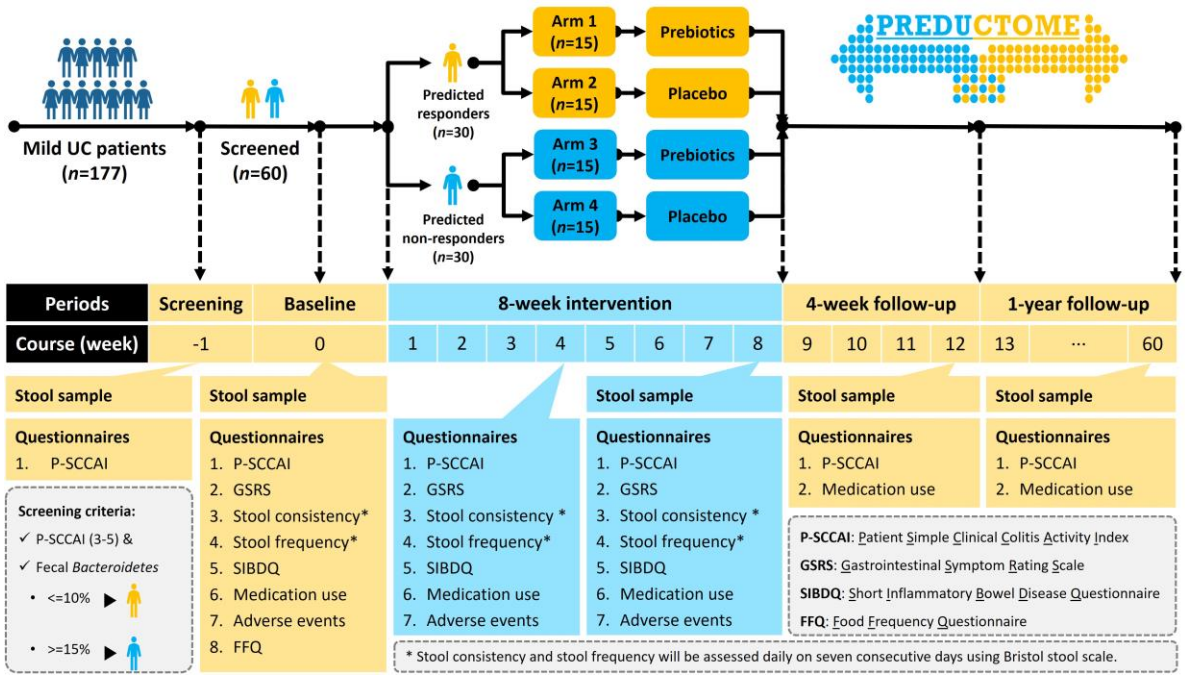


Figure 3 PREDUCTOME study: set-up and workflow

The study consists of the screening, intervention, and the follow-up periods.

During the **screening** period, approximately one hundred and seventy-seven UC patients will be recruited. P-SCCAI score data will be collected to select mild UC patients which is defined as 3-5 points on a 19-point scale. A stool sample will also be provided by patients to determine the Bacteroidetes level by quantitative polymerase chain reaction (qPCR) quantification of the number of total bacteria and Bacteroidetes. Based on the baseline microbiota from a subject in a fecal microbiota transplantation trial [20], in which healthy donors, sustained responding UC patients, and non-responding UC patients had an average relative abundance of 8%, 5%, and 19% Bacteroidetes, respectively, predicted responders are defined as UC patients having with a relative abundance of Bacteroidetes <=10% in their feces, while predicted non-responders are defined as UC patients with a relative abundance of Bacteroidetes >=15% in their feces. A total of sixty screened mild UC patients will continue with this study that consists of thirty predicted responders and thirty predicted non-responders.

To assess the habitual dietary intake of the last month, a validated food frequency questionnaire (FFQ, see F1) will be taken at the baseline. The data will also be used for potential correlation analysis with other parameters as diet is considered an important modulator of the intestinal microbiota. For the intervention, at **baseline**, selected participants

need to fill out several questionnaires including P-SCCAI (once), gastrointestinal symptom rating scale (GSRS, once), stool consistency (Bristol stool scale, daily for 7 consecutive days), stool frequency (daily for 7 consecutive days), short inflammatory bowel disease questionnaire (SIBDQ, once). Information of medication use and adverse events will be extracted from patient record and diary. In addition, they have to collect a stool sample for determining calprotectin level, microbiota composition, and SCFAs profile.

Sustained remission in UC patients in the published fecal microbiota transplantation trial was associated to an increased fraction of butyrate producers [20]. Our *in vitro* incubation studies with fecal inocula from UC patients showed that a prebiotic mixture of acacia gum, partially hydrolyzed guar gum, and resistant starch, showed the highest butyrate boosting capacity in subjects with low fecal Bacteroidetes levels. Hence, demonstrating the perfect candidate for our study.
Based on published studies on interventions on inducing clinical remission, an 8-week intervention should be sufficient for the prebiotics interventions to result in significant effects [4, 16, 27, 28]. An 8-week **intervention** period with four parallel arms:
1) predicted responders with prebiotics treatment (acacia gum, partially hydrolyzed guar gum, and resistant starch)
2) predicted responders with placebo (maltodextrin and corn starch)
3) predicted non-responders with prebiotics treatment (acacia gum, partially hydrolyzed guar gum, and resistant starch)
4) predicted non-responders with placebo (maltodextrin and corn starch)

During the intervention period, the subjects will consume the respective supplement (3 grams/sachet, 2 sachets per day, one in the morning and one in the evening). To avoid GI complaints due to a sudden intake of the prebiotics/placebo supplement, there will be a gradual increase in dosage: only 1 sachet will be consumed per day in the first week of the intervention period, followed by 2 sachets per day for the remainder of the intervention. At week 4 and week 8, they will also complete short questionnaires including P-SCCAI (once a week), GSRS (once a week), stool consistency (a 7-day record at each week), stool frequency (a 7-day record at each week), SIBDQ (once a week). Information of medication use and adverse events will be extracted from patient record and diary (once a week). At the end of the intervention period (T= 8 weeks), the participants will provide a second stool sample for fecal calprotectin measurements, microbiota composition, and SCFAs profile analysis. We will determine response with the P-SCCAI score at T= 8 weeks.

The intervention will be followed by a 4-week and 1-year **follow-up** period. At the end of each follow-up (T= 12, and 60 weeks), the participants will collect a stool sample for the same measurements as those at the end of the intervention period (fecal calprotectin level, microbiota composition, and SCFAs profile), and complete the questionnaires including P-SCCAI (once a week). Information of medication use will be extracted from patient record and diary (once a week).

As the intervention will take place at home, the study can be conducted in a merely coronavirus (COVID-19)-proof setting.

4. STUDY POPULATION

4.1 Population (base)

This study will be performed in adult UC patients with mild severity of the disease. UC severity will be determined by P-SCCAI score (see F1) [29], with a 3-5 score on a 19-point scale selected as mild UC inflammatory activity [30, 31]. Call for the participation of this study will be first spread among patients base (more than 1200 IBD patients) of Ziekenhuis Gelderse Vallei in Ede. In case of under-recruitment, UC patients of Rijnstate in Arnhem will be included as well.

In order to be eligible to participate in this study, a subject must meet all of the following inclusion criteria and none of the exclusion criteria.

4.2 Inclusion criteria

1. Male and female subjects aged 18 to 65 years*
2. Body Mass Index (BMI) between 18 and 30 kg/m^2 (self-reported)
3. Ulcerative Colitis confirmed via previous endoscopy and histology
4. Mild active UC as defined by P-SCCAI score of 3 to 5 (range 0 to 19)
5. Frequent relapse (at least one exacerbation in the last two years)
6. No known allergy to any components of the study product (self-reported)
7. Signed informed consent
8. Stable UC medication defined as no switch to other medication or no dose change
9. Mobile phone on which apps (used for questionnaires) can be downloaded (iOS version 9 and newer, Android version 4.4 and newer. Phones manufactured after 2013 are usually suitable)
10. Stable dietary pattern during the study

* We only include adults (age 18-65) as previous studies have shown that adolescent and elderly microbiota as well as UC presentation in adolescents can be different from adults[32, 33].

4.3 Exclusion criteria

1. Any other underlying disease of the GI-tract or previous bowel surgery, except cholecystectomy and appendectomy
2. Pregnancy or intending to become pregnant during the study
3. Use of medication that can interfere with the study outcomes, as judged by the medical supervisor
4. The need for antibiotic use during the intervention period
5. Systemic antibiotics and proton pump inhibitors (except for omeprazole and pantoprazole with dosage <20 mg), prebiotic supplements, probiotic supplements four weeks prior to study start
6. Currently participating in another intervention study
7. Acquaintances of anyone in the research team

4.4 Sample size calculation

The primary objective of the trial is to validate the prediction that a higher response (lower mean P-SCCAI score) is induced at T= 8 weeks by the prebiotics intervention in mild UC patients with low Bacteroidetes levels (predicted responders), but not in those with high Bacteroidetes levels (predicted non-responders) in their gut. Mild UC is defined as a P-SCCAI score of 3 to 5. According to an extensive study regarding UC disease activity conducted in the Netherlands, mild UC patients have a P-SCCAI score of 3.5 ± 0.5 (Mean ± standard deviation (SD)), and patients in remission score at 1.0 ± 0.33 (Mean ± SD)[34, 35].

In a series of germinated barley foodstuff (GBF) intervention studies, 10 mild to moderately active UC patients received 30 grams of GBF (equals to 10 grams fiber) daily for 4 weeks [4], and researchers found a significant decrease in clinical activity index scores. In a later study, another group of 11 mildly to moderately active UC patients consuming 20-30 grams of GBF (equals to 6.66-10 g fiber) daily for 4 weeks showed similar results, with decreases in clinical activity index scores [28].

Based on two-sided statistical testing for unpaired continuous data, an α=0.05 (chance on type-I error) and β=0.10 (chance on type-II error), and assuming a 20% difference of mean P-SCCAI score between predicted responders with prebiotics and predicted non-responders with prebiotics, it was calculated that at least 11 subjects per group are needed for the primary outcome P-SCCAI (μ1=3.5; SD1=0.5; μ2=2.8). According to previous trials on prebiotics on IBD patients, a 20% of drop-out rate is commonly seen. Given that the intervention duration is 8 weeks long, followed by a 1 year follow-up, higher dropout rate (30%) is expected. To compensate for a 30% drop-out rate, 15 subjects per group are needed.

To reach inclusion of 30 predicted responders and 30 predicted non-responders, we made an estimate how many subjects we need to screen based on the earlier obtained microbial composition of stool samples from UC patients in the Ede area. Around 50% of these subjects were categorized as low Bacteroidetes subjects (predicted responders) and while 17% of them as high Bacteroidetes subjects (predicted non-responders). To include these responders we will need to screen 177 mild UC patients.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

In this study, all 60 participants will undertake an 8-week intervention with either a prebiotics supplement (arm 1 and 3) or a placebo supplement (arm 2 and 4), which will be consumed once per day (1 sachet in the morning) during the first week, and twice per day (1 sachet in the morning and 1 sachet in the evening) for the remainder of the intervention. During the entire trial, research subjects will maintain their normal routines concerning their diet, medication use, and exercise pattern.

Prebiotics supplement: Prebiotics preparation is composed of three individual fibers (40% acacia gum, 20% partially hydrolyzed guar gum, and 40% resistant starch) from Winclove Probiotics B.V. (Amsterdam, the Netherlands).

Placebo (control): Placebo preparation is composed of food-grade corn starch and maltodextrin from Winclove Probiotics B.V. (Amsterdam, the Netherlands).

5.2 Use of co-intervention (if applicable)

Not applicable.

5.3 Escape medication

Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Prebiotics: acacia gum (AG), partially hydrolyzed guar gum (PHGG), and resistant starch (RS).

- Acacia gum (AG) also known as gum arabic, is obtained from the stems and branches of *Acacia senegal* and *Acacia seyal* trees. AG is a nonviscous, soluble, highly branched fiber, and its main components are arabinose and galactose [36]. It is a safe, natural, and plant-based food additive used for many centuries. For further details see product brochure (D6).
- Partially hydrolyzed guar gum (PHGG) is a soluble fiber produced by controlled partial enzymatic hydrolysis of GG which is derived from guar seeds, and it consists of galactomannan, which is characterized by high viscosity. In the modern food industry, GG is used as a food additive in various food products for thickener, emulsion stabilizer and as fiber source [37, 38]. For further details see product brochure (D6).
- Resistant starch (RS) refers to the fraction of starch which is not hydrolyzed in the small intestine, but is fermented by bacteria in the colon. It exists in many common foods, including legumes, cereals, grains and vegetables (especially potatoes) [39]. For further details see product brochure (D6).
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The prebiotics preparation is composed of three non-digestible carbohydrates (40% acacia gum, 20% partially hydrolyzed guar gum, and 40% resistant starch). In total 3 grams of prebiotic preparation contains 1.2 grams acacia gum, 0.6 gram partially hydrolyzed guar gum, and 1.2 grams of resistant starch.

The prebiotics, consisting of a mix of non-digestible fermentable fibers described above has been selected on the basis of (1) Total butyrate production, (2) Gradual fermentation throughout the entire colon (instead of fast fermentation in the proximal colon), (3) A good prebiotic tolerability index (low gas production), and (4) Commercial considerations.

Placebo: corn starch and maltodextrin.
Corn starch and maltodextrin are digestible carbohydrates, that are completely digested and absorbed in the proximal small intestine, and thus do not reach the colon, which makes them suitable placebo dietary compounds and the reason why they are widely used in dietary intervention studies.
The placebo consists of two digestible carbohydrates (20% maltodextrin and 80% corn starch). In total 3 grams of placebo preparation contains 0.6 gram of maltodextrin and 2.4 grams of corn starch). A Technical Data Sheet has been provided for both prebiotic as well as placebo product. For further details see product brochure (D6).

All ingredients are non-pathogenic, non-toxigenic and are classified as Generally Recognized As Safe (GRAS) as food ingredients. The prebiotics and placebo supplements are produced by Winclove Probiotics B.V., Amsterdam, the Netherlands. Winclove is a National Sanitation Foundation (NSF) International Certified Good Manufacturing Practice (GMP) Facility for manufacturing dietary supplements. Winclove's food safety management system is International Organization for Standardization (ISO) 22000:2005 certified for the development and production of pre- and probiotics.

6.2 Summary of findings from non-clinical studies

Prebiotics: acacia gum (AG), partially hydrolyzed guar gum (PHGG) and resistant starch (RS).
In vitro and animal models have been employed to study AG, PHGG or RS, with regard to their potential beneficial effect on health.

- Using an *in vitro* colon model inoculated with healthy human fecal material, AG showed the potential prebiotic effects, as it promoted the SCFAs production, especially butyrate [40].
- In a mice colitis model, rectal administration of AG (30 mg/kg) showed significant effects in reducing the inflammatory markers, and the stronger therapeutic effect were observed when combining with anti-inflammatory drug 5-Aminosalicylate (5ASA) [41].
- In a mice colitis model, PHGG and RS boosted SCFAs and butyrate production in the colon. In addition, they reduced intestinal inflammation [42] and induced proliferation of butyrate producing bacteria [43].

As described earlier in sections 1 and 3, we have tested the mix of AG, PHGG and RS using *in vitro* fecal batch fermentation models. In this study, four prebiotics candidates were incubated with fresh stool samples from UC patients in remission or from UC patients with mild active disease over a 48 hour period. The prebiotic (acacia gum, partially hydrolyzed guar gum, and resistant starch), that is selected for this PREDUCTOME study showed the most promising effects, namely (unpublished results);

- Increased production of butyrate and other SCFAs
- Promotion of the growth of butyrate producing bacteria
- Higher prebiotics tolerance defined as stimulated SCFAs production while minimal gas production

Collectively, these pre-clinical data show that the selected prebiotics could potentially have a beneficial effect on the course of UC by modulating butyrate production.

6.3 Summary of findings from clinical studies

Prebiotics: acacia gum (AG), partially hydrolyzed guar gum (PHGG), and resistant starch (RS).

Many clinical studies with the supplementation of AG, PHGG, or RS have been performed to investigate prebiotics effects (e.g., modulating the gut microbiome and boosting butyrate production) and GI tolerance.

- AG is slowly fermented compared with other fermentable fibers [44], therefore it is well-tolerated, i.e., less GI symptoms after consumption. Cherbut et al found AG did not induce adverse GI effects even when consumed up to a daily dose of 30 grams in healthy subjects [45]. A study by Marzorati et al also observed that blending AG with fructo-oligosaccharides (FOS, a well-researched prebiotic) can help to extend the time of fermentation of FOS in the colon [46].
- Partially hydrolyzed guar gum (PHGG) showed no side effects when consumed in 20-40 grams per day [47]. Besides of favouring butyrate-producing bacteria [48], PHGG also benefits bowel function. Increased stool frequency in constipated individuals and improved stool consistency and abdominal pain have previously been reported [49, 50].
- In general, in healthy adults, doses of RS up to 30 grams per day are well tolerated [51]. A dose-response study indicated that a daily dose of 20-50 grams of RS supplementation for one week resulted in beneficial changes in fecal microbiota and fecal metabolites in healthy individuals [52].

6.4 Summary of known and potential risks and benefits

No serious adverse events are expected from the administration of the prebiotics/placebo. If adverse effects occur, they are likely to relate to minor GI symptoms, such as bloating or flatulence or a minor change in defecation, especially when one uses prebiotics for the first time [53]. In our case, UC patients are already used to GI complaints, hence they are expected to report less bloating and flatulence. These effects are expected to disappear within a few days.

Furthermore, to minimize GI complaints due to a sudden intake of the prebiotics/placebo supplement, there will be a gradual increase in dosage: only 1 sachet will be consumed per day in the first week of the intervention period, followed by 2 sachets per day for the remainder of the intervention.

Nevertheless, in case of suspected serious adverse events, the coordinating investigator will immediately notify the Ethics Committee, all study personnel and the manufacturer of the product about the nature of the event. A decision on continuation or discontinuation of the trial will be made by the coordinating investigator in agreement with the Ethics Committee. All the adverse events also will be noted in the Case Report Forms.

6.5 Description and justification of route of administration and dosage

The boxes of prebiotics/placebo will be given to subjects by the coordinating investigator. Boxes include prebiotics/placebo sachets and Winclove's user instruction. Subjects

should consume prebiotics/placebo twice a day in the morning before breakfast and in the evening before dinner during the 8-week intervention period. Prebiotics/placebo will be administered orally. The content of the sachet needs to be dissolved in a glass of water or juice and should be stirred before consumption. The sachets need to be stored at room temperature in their original package.

6.6 Dosages, dosage modifications and method of administration

3 grams of prebiotics per time, twice a day for 8 weeks.
3 grams of placebo per time, twice a day for 8 weeks.
Prebiotics and placebo and powders are similar in colour, solubility, and taste.

The dose of non-digestible fermentable fibers has been selected to maximize the potential benefits but to limit potential GI side effects and is based on previous studies that have been conducted.

6.7 Preparation and labelling of Investigational Product

Preparation and labelling will be conducted at Winclove Probiotics B.V. All components are legally admitted as food additives or food components. Winclove is a NSF International Certified GMP Facility for manufacturing dietary supplements. Winclove works with the food safety management system ISO 22000:2005 and is certified for the development and production of pre- and probiotics.

After block randomization, the sachets will be labelled with: study name, instruction for use, expiration date and storing conditions. For further details see the product label in product brochure (D6). The products will be dispensed by the coordinating investigator.

6.8 Investigational Product accountability

The boxes of prebiotics/placebo will be given to subjects by the coordinating investigator (and are supplied to the subjects for the entire study period). Boxes include sachets and Winclove's user instruction. The shipment with boxes and sachets will be delivered to Laboratory of Microbiology, Wageningen University & Research, Wageningen by courier. The food supplements will be safely stored and locked by key at the Laboratory of Microbiology and only accessible by the research team (a list of the randomization codes is provided, which can only be opened in case of emergency). The product will be kept at room temperature. The coordinating investigator will distribute the boxes to participants in person. For compliance reasons, the patients will be asked to save all the empty sachets, and return everything (used and unused sachets) to the coordinating investigator after the end of the intervention. After counting the empty sachets, to get numbers on protocol compliance, all study material can be destroyed. As this is a food supplement, there is no need for special waste treatment.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Response (mean P-SCCAI score) between arms at T = 8 weeks:
At T = 8 weeks (the end the intervention), the P-SCCAI score will be collected via the P-SCCAI questionnaire. As main endpoint, the mean P-SCAAI score for each arm will be determined and compared to each other to determine the difference in response at T = 8 weeks.

P-SCCAI is derived from the original SCCAI questionnaire by translating into patients' comprehensible language, and has been validated to give substantial agreement with the original SCCAI [54]. P-SCCAI refers to disease symptoms during the previous week. It contains 13 items clustered into six domains: bowel frequency (during the day), bowel frequency (during the night), urgency of defecation, blood in stool, general well-being, and a number of defined extracolonic features of UC (i.e. arthritis, erythema nodosum, pyoderma angrenosum, uveitis).

8.1.2 Secondary study parameters/endpoints

Response over time
In addition to week 8, subjects need to fill out the P-SCCAI questionnaire at T = 0, 4, 12, and 60 weeks to determine the response over time. Comparisons will be made between groups as well as within subjects over time. With P-SCCAI score <= 2 being regarded as clinical remission, and a decrease in the P-SCCAI score by more than two points from baseline being regarded as clinical response [30, 31, 55](see F1). We will also determine whether subjects have reached clinical remission and/or showed a clinical response over time.

Mucosal inflammation
At T= 0, 8, 12, and 60 weeks, a fecal sample will be provided by subjects for calprotectin measurement.

GI complaints: (e.g. abdominal pain, bloating, flatulence)
At T= 0, 4, and 8 weeks, the GI complaints will be assessed by the GSRS questionnaire. The GSRS contains 15 items clustered into five domains (reflux, abdominal pain, indigestion, diarrhea and constipation) addressing different gastrointestinal symptoms [56]. It has a seven-point graded scale where 1 represents the absence of troublesome symptoms and 7 represents very

troublesome symptoms (see F1). The reliability and validity of the GSRS are well-documented, and norm values for a general population are available [57].

Stool consistency and frequency
At T = 0, 4, and 8 weeks, subjects will be asked to keep diaries to record stool frequency (bowel movement per day) and consistency of each stool using Bristol stool form scale (see F1) on a daily basis for 7 days.

Gut microbiota and SCFAs: (e.g. microbiota composition, SCFAs profile)
At T = 0, 8, 12, and 60 weeks, a fecal sample will be provided by subjects for 16S rRNA gene-based microbiota profiling and SCFAs measurement. Subsequent analyses of a variety of microbial ecological characteristics, including microbiota composition, α-diversity (diversity within the sample), β-diversity (diversity between the samples), population dynamics over time, and group differences will also be conducted.

Health-related quality of life
At T = 0, 4, and 8 weeks, health-related quality of life will be assessed by the SIBDQ questionnaire (see F1). SIBDQ is a 10 item shortened version of the original IBDQ which was 32 items. It has a seven-point graded scale where 1 represents very troublesome symptoms and 7 represents the absence of troublesome symptoms Measures quality of life as measured in four domains (bowel symptoms, emotional health, systemic systems and social function) [58]. Its reliability and responsiveness to change has been validated in UC [59].

Medication use
At T= 0, 4, 8, 12 and 60 weeks, information of medication use will be extracted from patient record and diary. It consists of the current medication use (e.g., aminosalicylates, corticosteroids, immunosuppressive agents, antimicrobial agents, and inhibitors of tumour necrosis factor-alpha (TNF- α)). Medication changes at week 4, 8, 12, and 60 provide information on disease development, with a downgrade means improvement and an upgrade means worsening of UC.

Safety parameters
At T= 0, 4, and 8 weeks, safety parameters (i.e., incidence of adverse events) will be monitored by patient record and diary. In this diary, all relapse-relevant information, including the need for systemic steroids, hospitalization, and surgery will be collected.

8.1.3 Other study parameters

Habitual food intake

At T= 0 (baseline), habitual dietary intake of the last month will be assessed by a validated food frequency questionnaire (FFQ, see F1) via FFQ-tool, a web-based interface tool. It provides data that will be used for potential correlation analysis with other parameters.

Participants demographics and characteristics

At T= 0 (baseline), general information (e.g., sex, age, BMI, disease duration, age at diagnosis, smoking habit, UC location, type of treatment) will be collected.

8.2 Randomisation, blinding and treatment allocation

During the screening period, participants will provide a fecal sample for microbiome signature measurement. This information will be used to assign subjects to four intervention arms by block randomization. All subjects screened for the trial will be assigned a unique participant ID number. Suitable participants will subsequently be randomised to prebiotics or placebo group using an online randomisation system accessible via password-protected access.

All sachets will be uniformly packaged to ensure double-blinding. Research teams and trial participants will be blinded to the treatment group for the duration of the trial. After collection of all laboratory & questionnaire data and performing all analysis of the baseline/intervention period the study (up to the 4-week follow-up; T=12 weeks) will be unblinded for the investigators. At the end of the study (T=60 weeks), the participants will be informed of the kind of intervention they received. (only when approved in the informed consent).

8.3 Study procedures

A detailed description of procedures is shown in **Table 1**.

Table 1 Procedures

Taking supplements				√	√	√	√	√	√	√	√							
Questionnaire	FFQ		√															
	P-SCCAI	√	√				√				√				√			√
	GSRS		√				√				√							
	Stool consistency*		√				√				√							
	Stool frequency*		√				√				√							
	SIBDQ		√				√				√							
	Medication use		√				√				√				√			√
	Adverse events		√				√				√							
Stool sample	Bacteroidetes level	√																
	Microbiota composition		√								√				√			√
	SCFAs profile		√								√				√			√
	Calprotectin level		√								√				√			√
	Trial course (week)	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**…**	**60**
		Screening	**Baseline**	**8-week intervention**								**4-week follow-up**				**1-year follow-up**		

FFQ = Food Frequency Questionnaire, **P-SCCAI** = Patient Simple Clinical Colitis Activity Index, **GSRS** = Gastrointestinal Symptom Rating Scale, **SIBDQ** = Short Inflammatory Bowel Disease Questionnaire

* Stool consistency and stool frequency will be assessed daily on seven consecutive days using Bristol stool form scale.

Informed consent procedure

One or more information meetings will be organized prior to the study. In this meeting, the investigators will explain the background, objectives and study procedures. During this meeting, there will be time for the potential research subjects to ask questions. After the information event, subjects have two weeks to decide whether they are interested in participation or not. Research subjects who are willing to participate will receive the informed consent and the screening package which includes the P-SCCAI questionnaire and one stool collection kit. Then within one week, subjects will receive packages with corresponding products and instructions.

If COVID-19 restrictions are still in place, we would also provide the option of having online information meetings via Microsoft Teams ("Teams is designed and developed in compliance with the Microsoft Trustworthy Computing Security Development Lifecycle (SDL), https://docs.microsoft.com/en-us/microsoftteams/teams-security-guide). No personal data will be collected or send via this route. If preferred, research subjects will always have the option to attend a physical meeting.

Screening

Figure 4 Stool collector

Subjects who fulfil all inclusion criteria and none of the exclusion criteria will be invited to a screening.
One stool sample will also be provided via the specific stool collectors (**Figure 4**). For female participants, pee gutters will also be provided to accurately separate stool from urine. After collection, stool material will be immediately frozen at the home freezers of the subjects, transported on dry ice (via courier) to the laboratory and subsequently frozen at -80 °C until further analysis. DNA will be isolated from part of the stool samples and subsequently be used for qPCR. Primers targeting total bacteria and Bacteroidetes will be used to quantify the total gene copies and calculate the relative abundance of Bacteroidetes in the stool. Subjects that have a relative abundance of Bacteroidetes <=10% will be assigned to the predicted responders group, while those who have a relative abundance of Bacteroidetes >= 15% will be assigned to the predicted non-responders group.

Baseline

At baseline, selected participants need to fill out several questionnaires P-SCCAI, FFQ [60], GSRS [61], Bristol stool form scale [62], stool frequency, SIBDQ [58], information of medication use and adverse events will be extracted from patient record and diary. All questionnaires can be found in F1 Questionnaires. The GSRS questionnaire provides baseline GI complaints and tolerance information. P-SCCAI provides disease activity information. FFQ provides information regarding the usual foods consumed and the frequency of consumption. Bristol stool form scale provides stool consistency information. SIBDQ provides information on health-related quality of life.

One stool sample will also be provided. The sample will undergo the same collection and storage procedures as those in the screening period. DNA will be isolated from part of the stool samples and subsequently be used for 16S rRNA gene-based microbiota profiling using NG-Tax [63]. The stool sample will be also used for measurements of fecal calprotectin using enzyme-linked immunosorbent assay (ELISA) methods [64], and SCFAs concentrations using high-performance liquid chromatography (HPLC) [65].

Dietary intervention period (8 weeks)

During the intervention period, the subjects will consume the respective supplement (3 grams/sachet) twice per day. To minimize potential GI complaints that might occur due to intake of the prebiotics/placebo supplement, there will be a gradual increase in dosage that only 1 sachet daily will be consumed in the first week of the intervention period, followed by 2 sachets daily for the remainder of the intervention. The patients will be asked to save all the empty sachets.

At T= 4, and 8 weeks, subjects will also need to complete short questionnaires on response (P-SCCAI, once a week), GI complaints and tolerance (GSRS, once a week), stool frequency and consistency (Bristol stool form scale, a 7-day record at each week), SIBDQ (once a week). Information of medication use and adverse events will be extracted from patient record and diary (once a week).

At the end of the intervention period (T= 8 weeks), the participants will provide a second stool sample for fecal calprotectin measurements, 16S rRNA gene-based microbiota profiling, and SCFAs profile analysis. We will determine response with P-SCCAI score.

At the end of the intervention period (T= 8 weeks), the participants will be asked to return everything (used and unused sachets) to the coordinating investigator to determine compliance to the study.

Follow-up period (4 weeks and 52 weeks)
During the follow-up period, participants will not take supplements anymore.

At T=12, and 60 weeks, P-SCCAI (once a week) and medication use (once a week) will be completed.

At T=12, and 60 weeks, one time stool sample will be collected at each time point. These samples will be used for fecal calprotectin measurements, 16S rRNA gene-based microbiota profiling, and SCFAs profile analysis.

We will determine response with P-SCCAI score at T= 12, and 60 weeks.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without providing a reason and without any consequences. The investigator can decide (upon consultation with the medical supervisor) to withdraw a subject from the study for urgent medical reasons, such as serious GI complaints, negative reactions upon supplement intake, etc., or when study participants have to start medication that interferes with outcomes during the study period.

8.4.1 Specific criteria for withdrawal

A subject will be withdrawn from the study in the case of serious gastro-intestinal complaints or an exacerbation of UC reported by the subjects (such as continuous diarrhea or constipation, bloating, abdominal pain).

8.5 Replacement of individual subjects after withdrawal

Not applicable.

8.6 Follow-up of subjects withdrawn from treatment

After possible withdrawal, no follow-up of research subjects will take place, except in case of withdrawal for other medical reasons.

8.7 Premature termination of the study

A premature termination of the study is not expected to happen as there are only limited safety risks. Adverse events are not expected, since maltodextrin and dietary prebiotics have been proved to be safe. In addition, subjects will be checked on the remission status, so any complaints will be reported in time. In case of unexpected adverse events that harm the subjects, the study will be stopped immediately. In case of premature termination, we still intend to analyse and publish the results of the part of the study that has been completed.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise the subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product/trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life-threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by

a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. In case of AEs or SAEs, the study may be prematurely terminated if the health or safety of the subject is at risk.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till the end of study within the Netherlands, as defined in the protocol.

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

Not applicable.

10. STATISTICAL ANALYSIS

Statistical analyses will be done using the statistical program embedded within R. The threshold for significance will be set at $p<0.05$, with a FDR correction for 16S rRNA gene sequence data. In general, the continuous data will be presented as mean ± standard deviation or mean and interquartile range when skewed, and categorical data as counts and percentages. Normality will be checked by visual inspection and QQ plots, followed by the Shapiro Wilk test for testing if data show a normal distribution.

10.1 Primary study parameter(s)

Response (mean P-SCCAI score) between arms at T = 8 weeks

To assess the response, data of mean P-SCCAI score within each arm at T = 8 weeks (after the intervention) will be obtained.

Firstly, the mean P-SCCAI score between the predicted responders with prebiotics and predicted non-responders with prebiotics will be compared by using a Student T-test if normally distributed, otherwise a Mann-Whitney U test when skewed.

Secondly, the mean P-SCCAI score between prebiotics and placebo groups within predicted responders or predicted non-responders will be compared by using a Student T-test if normally distributed, otherwise a Mann-Whitney U test when skewed.

10.2 Secondary study parameter(s)

Response over time

To assess the response over time, data of mean P-SCCAI score within each arm at T = 0, 4, 8, 12 and 60 weeks will be obtained. Comparisons will be made between groups (as done for T = 8 for the primary study parameter) as well as within subjects over time. With P-SCCAI score <= 2 being regarded as clinical remission, and a decrease in the P-SCCAI score by more than two points from baseline being regarded as clinical response, we will determine whether subjects have reached clinical remission and/or showed a clinical response over time.

To assess the response within each subject (clinical remission and/or clinical response) over time, data of sum P-SCCAI score within each subject at T = 0, 4, 8, 12 and 60 weeks will be obtained. For comparative analyses between baseline and intervention, the parametric paired sample T-test or the non-parametrical Kruskal-Wallis or Mann-Whitney

U test will be used, depending on the distribution of the samples. Differences between treatments before and after supplement intake will also be analysed by linear mixed models for repeated measures, using “treatment” [prebiotics/placebo], “responding” [responders/non-responders], “time point” [0, 4, 8, 12 and 60 weeks] and “treatment x responding x time point” as fixed effects and subject as random effect. Clinically relevant variables such as BMI, age, sex, and medication use will be taken into account.

Mucosal inflammation

Fecal calprotectin data will be analysed in the same manner as described above (Response over time).

GI complaints:

Data from GSRS will be computed into scores, and the score of each item will be pooled together as the sum score, and the score of each item within each domain will be pooled as well. All data will be analysed in the same manner as described above (Response over time). Differences over time within and between groups will be tested with mixed model analysis. Variables such as timepoint, group, age, sex will be taken into account.

Stool consistency and frequency

Average bowel movement per day and dynamic change on stool consistency will be calculated and collected. All data will be analysed in the same manner as described above (Response over time)..

Gut microbiota and SCFAs

Fecal SCFAs data will be analysed in the same manner as described above (Response over time

16S rRNA gene sequencing provides sequence read counts data. It will be normalized to microbial relative abundance, and microbiota diversity indices (Shannon, Simpson, and inverse Simpson) are calculated at amplicon sequence variant (ASV) level, as implemented in the Picante [66] and Phyloseq [67] packages in R, respectively. Wilcoxon test will be applied to determine whether diversity as well as the relative abundance of specific bacterial taxa, were significantly different between groups since the data was non-parametric. For microbiota analysis, *p*-values will be corrected for multiple comparisons using the Benjamini–Hochberg procedure. To correct for a false discovery rate (FDR), a FDR adjusted *p*-values will be computed, a so-called *q*-value [68]. A corrected *q*-value of less than 0.2 was considered significant. Paired tests will be used to

compare the effects of prebiotics. Pairwise weighted Unifrac [69] and unweighted UniFrac [70] distance-based principle coordinate analysis (PCoA) will be used to visualize microbial community variation at the ASV level. Permutational multivariate analysis of variance (PERMANOVA) will be used to test for significant differences between groups, as implemented in the Vegan package [71].

Health-related quality of life

Data from SIBDQ will be computed into scores, and the score of each item will be pooled together as the sum score, and the score of each item within each domain will be pooled as well. All data will be analysed in the same manner as described above (Response over time).

Medication use

Type, Dose, frequency and duration of each medication use will be collected. All data will be analysed in the same manner as described above (Response over time).

Safety parameters

Incidence rate of each adverse event is recorded and calculated. All data will be analysed in the same manner as described above (Response over time).

10.3 Other study parameters

Habitual food intake

Data from FFQ will be analysed in the same manner as described above (Response over time). Also, it will be used for Pearson r correlation coefficient calculation, or a Spearman rank when not normally distributed, with other parameters.

Participants demographics and characteristics

Data will be analysed in the same manner as described above (Response over time). Correlational analysis will be used to explore possible associations between symptoms and baseline characteristics.

10.4 Interim analysis (if applicable)

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (64th WMA Assembly, October 2013), and in accordance with the Medical Research Involving Human Subjects Act (WMO 1998), and other guidelines, regulations and acts.

11.2 Recruitment and consent

There are more than 1200 IBD patients in the patients base of Ziekenhuis Gelderse Vallei (ZGV). With around half UC patients have mild activity, this guarantees that enough potential patients will be reached out. Previous surveys performed in ZGV on dietary beliefs and behaviors showed that around 60% of IBD patients believe that diet could make a difference on the disease course. This increases the chance to involve enough patients who actually support the dietary strategy.

Recruitment will be done by the investigators. During the outpatient clinic visit, the UC patients of ZGV will be informed by nurses about this study. For questions, potential subjects can contact the investigators through e-mail or phone for further explanation of study procedures. Only when the patients agree to participate the study, their contact information will be shared with investigators, who will send the invitation for information meeting. Interested subjects will be invited for (digital) information events, where additional information will be given, and subjects have the chance to ask questions. After the information event, subjects have two weeks to decide whether they are interested in participating or not. If they wish to enter the study, they are asked to sign the informed consent form at home, and then send it to the researcher. The participants will be notified in the consent form to give permission to share the information of unexpected new findings regarding the (future) health of them with their General Practitioner (GP). The subject will receive a copy of the signed informed consent directly after signing. After this, subjects will receive the fecal material collection kit and information to provide the first fecal sample for pre-screening. Then within one week, subjects will receive packages with corresponding products and instructions.

Data will be stored at secured hard drives of Wageningen University, and will be locked with a password. Only members of the research team will have access to these data and only after they sign a confidentiality agreement. Paper data such as informed consent will be stored in a cabinet with a lock. Subjects will be given a study code, and only members of the research team will have access to the files decrypting the study code.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

There is negligible risk for the participants. Dietary prebiotics has been proved to help with remission in UC patients. Participants may potentially benefit from the dietary intervention with improved disease activity.

Intervention products

The prebiotics used in this study has been *in vitro* tested to be effective in boosting butyrate production in healthy individuals and it also showed the same ability in mild UC patients *in vitro* beforehand. Maltodextrin and corn starch as a placebo has been widely used in many clinical trials, no adverse effects has been reported.

Time investment research participants

Research participants will invest approximately 9 hours during the trial. A schematic overview of the time investments is listed in **Table 2**.

Table 2: Overview of time investment of the research subjects

Items	Time (minutes)	Number	Time in total
Information meeting	60	1	60
Taking supplement	2	105	210
Long questionnaire	45	1	45
Short questionnaires	3-5	40	160
Stool collection	15	5	75
Total investment			550 min ≈ **9 hours**

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

Based on burden calculations, after completion of the full study, each participant will receive a financial compensation of €105,-. Participants not selected for the study after the screening will receive €17. Research participants are free to withdraw from further participation for any reason and at any time during the trial and will receive a proportional repayment for the effort they made. However, if a participant needs to withdraw because of adverse events, or because of medical advice, he or she would receive the full financial compensation.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Before the start of the study, subjects will be assigned to a study code that will not change during the study. This number is linked with the name, address, date of birth, and telephone number of the subject in a password-protected file that is stored separately from the study data. Only members of the research team involved in the study logistics (e.g. collecting samples) can access this file. For all other purposes, the study code will be used for subject identification. The informed consent will be stored separately from all other information. All researchers have signed a confidential statement. The handling of personal data will be done according to the General Data Protection Regulation (GDPR), which is enforced on the 25th of May 2018. According to standard data management procedures, all research data are stored for a period of 10 years after collection. Thereafter, data and documents will be deleted and destroyed (e.g. by a shedder). Biological samples will be stored for a maximum of 10 years after study end, defined as the last data collection point, and then disposed of according to biomedical waste procedures. In case we want to use the collected material for additional research, which includes research in relation to the health of the patients or other research activities that are not covered by this study, we will provide our plans to the medical ethics committee Oost Nederland for evaluation.

12.2 Monitoring and Quality Assurance

Not applicable.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. Non-substantial changes (such as typing errors, administrative changes like changes in names, telephone numbers and other contact details of involved persons mentioned in the submitted study documentation) will not be notified to the reviewing METC and the competent authority, but will be recorded and filed by the sponsor.

Substantial amendments will be notified to the METC that gave a favourable opinion. The documentation that will be included in the submission should cover the following information:

- Covering letter, including the reasons for the amendment in one or two sentences, a brief description of the changes that are included in the amendment and the name of the documents that are modified;
- An extract of the modified documents, where applicable, showing both the previous and new wording, where applicable;
- The new version of the modified documents, where applicable, identified with updated number of version and date.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the collection of the last data.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.
In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

Subjects will be informed about the results of the study. Results will be presented in agreement with the CCMO publication statement. Results will be published in scientific peer-reviewed papers and possible also in professional journals by the investigators. The authorship of the article shall be determined in appropriate consultations based on a considerable contribution to the set-up and execution of the study and active participation in publication. Outcomes of the study will be presented at conferences and meetings; data will be reported anonymously.

None of the collaborating parties will withhold the public disclosure of study results. Collaborating parties may delay publication for up to three months after analysing the research results if it is applying for a patent or for other important reasons. All collaborating parties are entitled to examine any form of public disclosure prior to submission.

The study protocol will be registered at a public database (www.clinicaltrials.gov).

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable. All ingredients are non-pathogenic, non-toxigenic and are classified as Generally Recognized As Safe (GRAS) as food ingredients.

13.2 Synthesis

The following actions will be taken to reduce the small risks of this study:

- The following aspects point towards a safe use of the dietary prebiotic supplements:
    - They are commonly consumed throughout the world.
    - All compounds are commercially available and tested safe for human consumption.
    - Prebiotic supplements were selected on the basis of expected low GI tolerance issues.
    - Prebiotic supplements will be given in a relatively low daily dose.
    - To avoid gastrointestinal complaints, due to a sudden intake of fiber supplements during the first week, the study participants will consume only one sachet per day, followed by two sachets per day for the remainder of the study.
- The following aspects include safety monitoring throughout the study:
    - We will monitor GI complaints via questionnaires throughout the study.
    - A medical doctor has been appointed for this study to check and discuss reported AEs with the subjects.
- The following aspects point towards a low-risk patient group:
    - Only UC patients with mild disease activity are included.
    - Subjects with other digestive tract disorders that are expected to interfere with this study are excluded from participation.
- The following aspects point towards a non-burdensome sampling procedure
    - The stool sample collection aligns with their routine clinical check and will therefore not cause an extra burden.
    - We will take the applicable COVID-19 regulation into account when it comes to potential contact with the subjects (e.g., information meeting).

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