Nutrition Research 131 (2024) 96–110

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/NTR

Original Research

Rationale and design of a randomized placebo-controlled nutritional trial embracing a citizen science approach

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ARTICLE INFO

Article history:
Received 14 February 2024
Revised 24 July 2024
Accepted 24 July 2024

Keywords:
Behavior change
Dietary fiber
Fermented food
Gut microbiota
Tailored diet

ABSTRACT

Modulation of the gut microbiota through specific dietary interventions shows potential for maintenance and optimization of health. A dietary fiber diet and fermented foods diet appear to alter the gut microbiota, but evidence is limited. Therefore, we designed the Gut Health Enhancement by Eating Favorable Food study, a 21-week randomized controlled trial studying effects of dietary fibers and fermented foods on gut microbiota diversity and composition, while also stimulating dietary behavior changes through a citizen science (CS) approach. We hypothesized that a high-fermented food diet would increase microbial diversity, whereas a high-dietary fiber diet would stimulate the growth of specific fiber-degrading bacteria. The following elements of CS were adopted: education on the gut microbiota, tailored dietary intervention, remote data collection by participants, sharing of personal gut microbiota outcomes with participants, and vlogs by participants for dissemination of results. Here we describe the study protocol and report the flow of participants, baseline characteristics, and compliance rates. Completed in March 2024, the trial included 147 healthy adults randomized to a high-dietary fiber intervention, high-fermented food intervention, or control group. Each group received an additional study product after 2 weeks: dried chicory root, a fermented beverage, or maltodextrin (placebo). A 3-month follow-up assessed the participants' ability to sustain dietary changes. The recruitment of participants was successful, reflected by 1448 applications. The compliance with the dietary guidelines and study products was >90%. This study shows that including elements of CS in an randomized controlled trial is feasible and may help recruitment and compliance.

Abbreviations: ASVs, amplicon sequence variants; BMI, body mass index; CG, control group, CS, citizen science; DQLQ, digestion-associated quality of life questionnaire; FFF, fermented foods frequency questionnaire; GEEF, Gut Health Enhancement by Eating Favorable Food; GI, gastrointestinal; HDF, high-dietary fiber; HFF, high-fermented food; ITT, intention-to-treat; PP, per-protocol; RCT, randomized controlled trial; SCFA, short-chain fatty acids.

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https://doi.org/10.1016/j.nutres.2024.07.008

1. Introduction

The gut microbiota, which consists of trillions of microorganisms, exerts a marked influence on homeostasis and immunity of the human host [1]. A disturbed composition of the gut microbiota, known as dysbiosis, has been associated with several inflammatory and metabolic diseases, and gastrointestinal (GI) infections [2]. Diet and other lifestyle factors are among the main drivers that can alter the gut microbiota during adulthood [3]. Research on the gut microbiome has rapidly emerged over the last two decades and holds great promise for the development of personalized interventions for disease prevention and treatment [4]. There is still limited knowledge regarding the use of microbiota composition as a generic diagnostic tool, and subsequently, regarding specific dietary interventions that effectively modulate the gut microbiome to prevent or cure diseases [4,5].

Dietary fibers, which are nondigestible carbohydrates of plant origin, can be considered a key component in modulating the gut microbiota [6]. Marked differences in gut microbiota diversity and composition are observed between populations, driven by long-term differences in dietary fiber intake [7]. Observational studies with large cohorts indicate that a high intake of dietary fiber and eating diverse plant foods is associated with increased microbial diversity [8,9]. Dietary fibers are fermented in the colon by the microbiota and promote the production of short-chain fatty acids (SCFA) [10]. SCFA plays an important role in health maintenance, considering their involvement in a wide range of human metabolic pathways and their capacity to control immune homeostasis [11,12].

Fermented foods pose another dietary element that can alter microbial communities [13]. Fermented foods are defined as foods made through desired microbial growth and enzymatic conversions of food components [14]. Several potential benefits to cardiovascular, metabolic, and immune health are ascribed to fermented foods, which can be explained by the bioactive compounds and microbial metabolites produced during the fermentation process [15]. Moreover, fermented foods containing live microorganisms can introduce new, albeit transient, microbes into the gut microbiota and serve as a significant dietary source of beneficial microbial species, as many of the species found in these foods are phylogenetically related to probiotic strains [16].

Previously, Wastyk et al. [17] investigated the effects of a high-fiber diet and a high-fermented food (HFF) diet on the gut microbiota and immunity in healthy adults. During this intervention, the high-fermented-food diet steadily increased the alpha diversity of the gut microbiota. However, microbial diversity did not increase as a result of the high-fiber diet, although microbiome-encoded glycan-degrading carbohydrate-active enzymes increased. In addition, the authors of this study observed a decrease in specific inflammatory markers in the HFF group, whereas for the high-fiber diet group, three distinct immunological trajectories corresponding to baseline microbial diversity were identified [18]. Although the participants were randomized, no control group (CG) was included and the treatment groups were relatively small ($n = 18$ per arm). Larger controlled trials including a CG are needed to replicate these findings in other study populations.

Achieving sustainable changes in the dietary behavior of a large group of people remains challenging [18,19]. The average dietary fiber intake in European countries is far below the recommended intake [20]. In the Netherlands, the mean intake is 18 g/d for females and 23 g/d for males, compared to the recommended 30 to 40 g/d (14 g per 1000 kcal) [21,22]. Fermented foods are not classified (yet) as a category in the national dietary guidelines of most countries [23]. However, the inclusion of live microbes in the diet may provide an opportunity for evolutionarily important interactions with the gut microbiome and immune system, which have been reduced in our modern lifestyle [24].

Citizen science (CS) refers to a concept that can be applied to a wide range of activities, encompassing public participation and collaboration in scientific research [25]. The elements of a CS project were defined by the European Association of CS [26]. CS approaches can be classified based on the citizen scientists' (study participant) degree of involvement in the study, ranging from "contributory" (citizen scientists mainly involved in data collection) to "citizen-led" (research led by citizen scientists) [27]. From a social cognitive theory perspective, factors that promote the maintenance of behavioral change involve health knowledge, self-regulatory skills, barriers to change, self-efficacy beliefs, and outcome expectancies, which can be targeted by providing education, social support, tailoring treatments, and self-monitoring practices [28]. CS might stimulate health behavior change by involving citizens in scientific endeavors, offering learning opportunities, making (individual) study results accessible and comprehensible, and involving them in dissemination of results [29]. Traditionally, in health science, participants in clinical trials have been in a relatively passive position to ensure controlled execution and valid outcome measures, thereby counteracting self-regulation. In contrast, CS focuses on benefiting and empowering the public [30,31]. CS recognizes the knowledge, competences, and lived experiences of the participants and aims to perform research with and for them, instead of including them only as objects for scientific inquiry [32].

A qualitative systematic review suggested that contact with professionals and other participants as well as interventions that allow for tailoring and self-monitoring, increase adherence to dietary interventions [33]. Prior studies have investigated the effects of personalized dietary interventions combined with behavior change strategies, such as motivational interviewing and education [34]. However, few studies have been performed remotely [35,36]. Moreover, no trial has integrated the provision of personal gut microbiota outcomes to allow participants to monitor changes.

Including CS elements in randomized controlled trials (RCTs) may appear contradictory considering their aim to reduce bias and examine cause-and-effect relationships [37]. RCTs are focused on internal validity and reproducibility, whereas CS features feasible and remote data collection and widely accepts the assumption of measurement objectivity, even though measurements are performed by study participants [31]. In addition, active knowledge transfer and sharing of study results during study conduction may cause bias and interfere with the controlled and blind study design [37]. Although integration of CS elements requires important scien-

tific and design-related considerations, this domain may yield promising benefits for health research.

Beyond the potential of increasing participant engagement, compliance, and nurturing long-term behavior change, CS can enhance the societal relevance and acceptance of research, contributing to narrowing the gap between science and society [38]. The real-life setting and involvement of citizens in the dissemination of results make CS studies more generalizable and may ease their successful translation into practice [39]. Moreover, CS has the potential to empower communities and influence policymaking, thereby facilitating societal actions [40].

To better understand the effects of a high-dietary fiber (HDF) diet and HFF diet on the gut microbiota and immunity, while also aiming at long-term dietary behavior changes, we present here the design and methods of our RCT: the Gut Health Enhancement by Eating Favorable Food (GEEF) study. We hypothesized that the HFF diet would increase microbial alpha diversity (primary outcome). Additionally, we hypothesized that the HDF diet would not affect alpha diversity, but would stimulate specific fiber-degrading bacteria. CS elements were integrated into the study to motivate and empower citizens to adopt and maintain healthier dietary habits and to create awareness among citizens about the effects of nutrition on the gut microbiota, and subsequently its role in homeostasis and disease prevention. The GEEF trial was completed in March 2024. Data analysis has not been completed, but the flow of participants, baseline characteristics, and compliance rates are reported here. To allow the integration of CS into RCTs, we discuss several methodological considerations for the study design and execution.

2. Methods and materials

2.1. *Study design and timeline*

The GEEF study was a 21-week RCT using a remotely executed CS approach. This study was conducted between May 2023 and March 2024. The study included three treatment groups: an HDF group, an HFF group, and a CG. The study consisted of an 8-week intervention with a follow-up period of 3 months. The intervention consisted of a 2-week ramp-up period with dietary guidelines through recipe booklets, followed by a 6-week period with additional consumption of study products. Outcome measures were collected by participants at baseline, weeks 2, 8, and 21. The 3-month follow-up measurement evaluated participants' perception and awareness of food choices and assessed the ability of participants to sustain their dietary changes.

By integrating CS elements into the study design, factors of behavior change were taken into account, namely capability (e.g., providing accessible education and data collection by participants), opportunity (e.g., tailoring dietary interventions and encouraging participants to document experiences), and motivation (sharing of individual-level microbiota report and motivational videos, and allowing participants to share experiences and tips) [41]. For all groups, the intervention period started and ended with an informative group meeting, either in person or online. The initial meeting was organized separately for each group and aimed at informing participants about the research objectives, measurements, dietary guidelines (including practical tips), and providing knowledge on the gut microbiota. The main goal of the endline meeting was to enable all participants to gain knowledge about dietary fibers and fermented foods. During this endline meeting, participants shared their experiences and tips, such as how to make vegetables more palatable, how to start fermenting food products at home, and where to find specific ingredients. Throughout the study, participants were invited on a voluntary basis to record their experiences of changing their dietary habits and participating in this study using their phone cameras. For instance, they could vlog about the knowledge they had obtained, how changing their dietary habits influenced their social life, or shared tips about increasing dietary fiber or fermented food intake.

Study participants received individual gut microbiota outcomes in a report per mail, approximately 3 weeks after each fecal sampling time point. An example of the gut microbiota report (MyMicroZoo B.V., Leiden, The Netherlands, version 2022) can be found in Supplementary File 1. Videos on how to read and interpret the outcomes of the reports were published on the study websites (a unique website was created for each group). Guidance on how to compare reports was integrated into the endline meeting. Comprehensive reports offered insights into gut microbiota diversity and composition, allowing participants to monitor changes. During the intervention period, informative videos on the study measurements, gut microbiota composition, transit time, and practical tips for navigating the supermarket were shared on the study websites.

The study protocol was approved by the Medical Research Ethics Committee Brabant (P2306) and was registered at ClinicalTrials.gov (NCT05900609). All participants provided informed consent. Fig. 1 provides a schematic overview of the study design.

2.2. *Study population*

Healthy community-dwelling individuals were included in this study. Several studies have shown divergent associations of body mass index (BMI) and age with gut microbiota composition and inflammatory profiles [42,43]. For this reason, adults aged ≥18 and ≤70 years with a BMI ≥18,5 and ≤30 kg/m^2 were included. To be eligible to participate, individuals met the following additional criteria:

- Being able to read and speak Dutch;
- Willingness to keep a stable dietary pattern throughout the study, apart from the dietary advice in the study;
- Having a smartphone compatible with the LifeData application to fill out the daily questionnaires.

A number of exclusion criteria were applicable, such as having a disease or medical condition that could influence the study results, or having a relatively high average daily dietary fiber or fermented food intake. See Table 1 for all exclusion criteria.

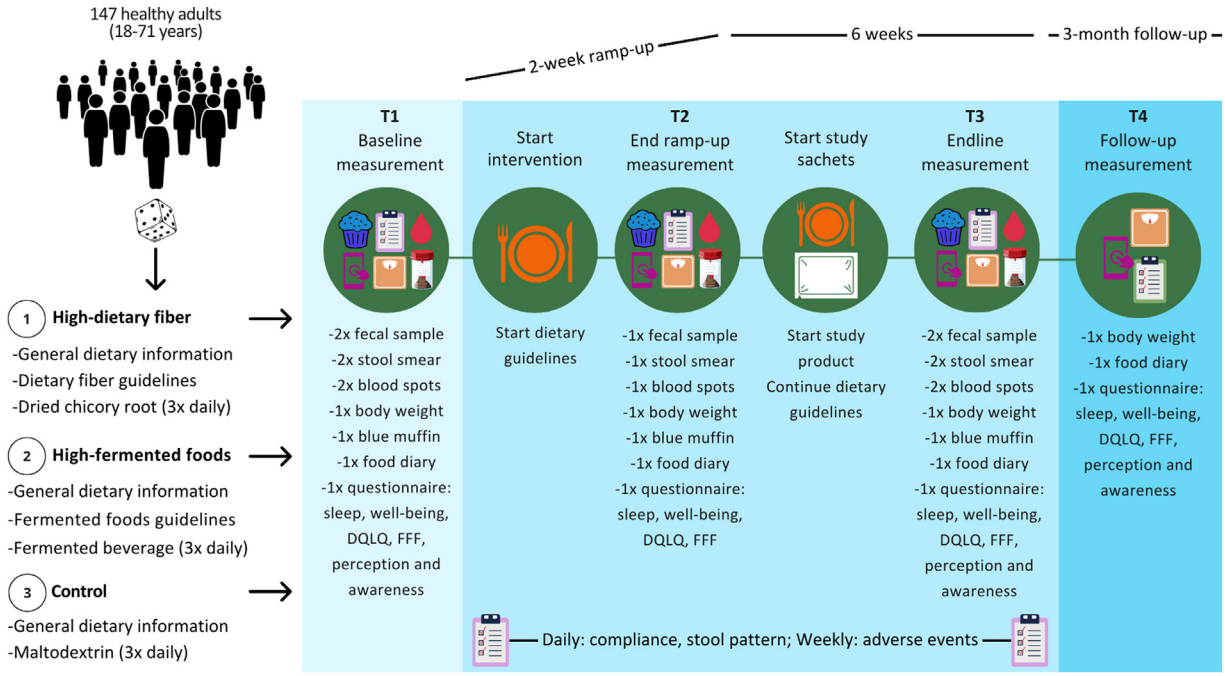

Fig. 1 – Schematic overview of the GEEF study design.

Table 1 – Overview of exclusion criteria applicable in the GEEF trial.

Exclusion criteria
• Having a disease or medical condition that can influence the study results such as diabetes, cancer, diagnosed irritable bowel syndrome, renal disease, liver enzyme abnormality, malignant neoplasm, or a history of inflammatory diseases (such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease);
• Having a history of intestinal surgery that might interfere with study outcomes (this does not include appendectomy or cholecystectomy);
• Average dietary fiber intake of ≥18 g (women) or ≥22 g (men)/d, according to the fiber screen questionnaire;
• More than 3 servings of fermented foods per day as assessed with the fermented food frequency questionnaire;
• Currently following a strict diet and unwilling or unable to change; for example, a gluten-free diet or a "crash diet" using meal substitutes;
• Specific food allergies that interfere with dietary intervention (e.g., gluten or lactose);
• Use of prebiotics, probiotics, and/or symbiotics and use of fiber supplements (this should be stopped 4 wk before the start of the study);
• Use of antibiotic treatment less than 3 mo before start of the study and/or use of antibiotics during the study;
• Use of medication that can interfere with the study outcomes, as judged by the medical supervisor;
• Alcoholic use of ≥14 (women) or ≥28 (men) glasses of alcoholic beverages per week;
• Use of soft or hard drugs (should be stopped at least 4 wk before start of the study);
• Being pregnant or lactating;
• Participation in another clinical trial at the same time;
• Student or employee working at either Food, Health, and Consumer Research from Wageningen Food and Biobased Research, Systems Biology Lab from Vrije Universiteit Amsterdam, Maag Lever Darm Stichting, WholeFiber B.V., Keep Food Simple or at Ani Biome;
• Unable to follow or comply to study rules.

2.3. *Sample size*

The sample size calculation was based on the primary outcome of gut microbiota alpha diversity (microbial richness) as determined by the number of observed amplicon sequence variants (ASVs) from 16S rRNA amplicon sequencing. The numbers are based on earlier research by Wastyk et al. [17]. The numbers were kept consistent despite the assumption that the inclusion of elements of CS would induce heterogeneity in the study outcomes. This decision was based on the reasoning that the study products were part of the dietary interventions (which were not included in the study by Wastyk et al.) and would increase homogeneity, thereby balancing out the increased heterogeneity. The standard deviation in the study population was 51.1 number of observed ASVs and the clinically significant difference observed was 21.63 number of observed ASVs (between pre and postintervention) [17]. For the sample size calculation ANOVA repeated measures between factors were used in G*Power, version 3.1.9.7 [44]. Considering a difference of 21.63 number of observed ASVs, a power of 0.8, a significance level alpha of 0.05, three repeated measures and a standardized effect size of 0.42 (assumed effect size/assumed standard deviation) a sampling plan of 42 participants per group was calculated. Considering a dropout rate of 15%, we aimed to recruit 49 participants per group, comprising a total sample of 147 participants.

2.4. Recruitment

Participants were recruited through mass mailing of the volunteer database of approximately 3.700 potential volunteers at Wageningen University & Research, through social media advertising, the newsletter of the Dutch Digestive Foundation (Maag Lever Darm Stichting), and through poster and flyer distribution among Dutch universities, universities of applied sciences, local supermarkets, and general practitioner practices.

2.5. Screening, allocation and blinding

Eligibility was determined through online screening, which included attending an information meeting and completion of an eligibility questionnaire, containing questions about demographics, dietary fiber and fermented food intake, health history, lifestyle, medication and supplement use, and availability during the study period. Participation with a partner, family member, or friend was permitted if both were eligible. Participants were randomly assigned to one of the three groups. Block randomization was used to ensure a balanced distribution of the groups. Stratification was applied according to sex, age, and BMI because of the observed impact of these variables on outcome measures [45,46]. Participants from the same household were placed in the same treatment group, based on the strata of the first applicant. Participants in the HDF and HFF groups were unblinded after the initial meeting, during which they received information regarding their treatment assignments. However, they remained unaware of how their treatments differed from those of other treatment groups. The CG remained blinded until the end of the 8-week intervention period. The study personnel could not be blinded considering their role in group meetings and participant contact. Data analysis will be performed in a blinded manner.

2.6. Interventions

During the 8-week intervention period, participants in the HDF and HFF groups received dietary guidelines to increase their dietary fiber and fermented food intake, respectively. Both groups were provided a recipe booklet developed by the Dutch Digestive Foundation (Maag Lever Darm Stichting). The HDF group received a booklet dedicated to fibers, containing general information on dietary fibers, tips to increase dietary fiber intake, and recipes for breakfast, lunch, dinner, and snacks. Participants were encouraged to use 2 snacks/d and 2 recipes/d, of which five were dinner recipes per week, in accordance with their personal preferences. Adherence to these dietary guidelines would result in an estimated dietary fiber intake of 24 g/d. The HFF booklet contained general information about fermented foods, recipes to ferment food products at home, and a product list of fermented food products containing live microorganisms with corresponding serving sizes based on Wastyk et al. [17]. Fermented foods and serving sizes included: (plant-based) yogurt and quark (150 mL), buttermilk, Ayran, and milk kefir (150 mL), fermented vegetables (30 g), natto and tempeh (50 g), kombucha, (bread) kvass, water kefir and vegetable brines (150 mL) and, cottage cheese and mozzarella (50 g). The participants were encouraged to make their own fermented food products. The HFF group was advised to consume at least three additional portions of the preferred fermented foods from the list per day (either store-bought or homemade). To promote compliance, the HDF and HFF groups received a calendar to track dietary fiber and fermented food intake, respectively. All three treatment groups were advised to visit a website of the Dutch Digestive Foundation and The Netherlands Nutrition Centre (Voedingscentrum), where they found general dietary advice to positively alter the gut microbiota. Substantial and lasting dietary changes are not expected when dietary advice is shared only on a website [47].

After following the dietary guidelines for 2 weeks, all participants began using an additional study sachet three times daily. In the HDF group, sachets contained 3.3 g dried chicory root (WholeFiber Holding BV, The Netherlands). Consumption of 3 sachets/d resulted in an additional dietary fiber intake of 7.9 g, supporting the HDF participants in reaching the recommended 30 to 40 g of fiber per day (also indicated as 14 g per 1000 kcal) and enhancing gut microbiota modulation [21,48]. Dried chicory root was chosen because it is minimally processed and its unprocessed fiber structures allow gradual release of fermentable carbohydrates in the distal intestinal location, where SCFA are produced and absorbed [49]. This will lead to a presumed enhanced effect on gut microbiota modulation and inflammatory markers in comparison to single or purified dietary components, such as inulin-type fructans [49]. A strong modulatory effect of 5-week dried chicory root consumption (2 weeks 15 g/d and 3 weeks 30 g/d) on β-diversity has been observed, as well as increased fecal butyrate concentrations [48]. Moreover, substantial increases in the relative abundance of *Anaerostipes* and *Bifidobacterium* spp. were observed in a dose-dependent manner [48]. The participants of the HFF group were provided with study sachets containing 19 mL of fermented beverage (Ani Biome, Zagreb, Croatia). Consumption of 3 sachets/d contributes to the daily consumption of six portions of fermented foods [17]. Study sachets of the CG contained 3.3 g of maltodextrin (Glucidex IT19, Roquette Freres SA, France) as a placebo supplement [50]. Nutritional values of the study products are listed in Table 2. The investigational products were added after the 2-week ramp-up, to prevent GI discomfort resulting from the increase of the amount of dietary fiber and fermented foods in a short period of time.

2.7. Study outcomes

Study assessments were performed daily and at the following four-time points: baseline (at the start of the study), week 2 (end ramp-up phase), week 8 (end intervention phase), and week 21 (follow-up). The daily assessments included a brief questionnaire. At baseline, week 2, and week 8, participants collected fecal and blood samples, consumed two blue muffins, made a stool smear picture, logged their dietary intake for three consecutive days, and completed questionnaires. At baseline and week 8, two fecal samples and two blood samples were collected on different occasions to account for intraindividual variations. The weekly questionnaire assessed adverse events and changes in medication and supplement use. Participants received home sampling kits for fecal sampling and blood collection and were requested to re-

Table 2 – Average nutritional content of the total daily intake of study product as part of the HDF, HFF, or CG intervention within the GEEF trial.

	HDF group	HFF group	CG
Study product	Dried chicory root (WholeFiber Holding BV, The Netherlands)	Fermented beverage "Agebiotics Boost" (Ani Biome, Zagreb, Croatia)	Maltodextrin (Glucidex IT19, Roquette Freres SA, France)
Commercially available at	wholefiber.nl	anibiome.ai	roquette.com
Amount per day	9.9 g	57.0 mL	9.9 g
Energy (kcal)	18.6	8.2	37.6
Fat (g)	<0.1	0.0	0.0
Protein (g)	0.5	0.0	0.0
Carbohydrates (g)	0.5	0.2	9.4
/of which sugars (g)	0.3	0.1	0.7
Fibers (g)	7.9	0.0	0.0
/of which inulin (g)	6.5		
/of which pectin (g)	0.9		
/of which cellulose and hemicellulose (g)	0.5		

Abbreviations: CG, control group; g, grams; HDF, high-dietary fiber; HFF, high-fermented food; kcal, kilocalories; mL, milliliters.

turn them by post. Blue muffins, material to make a stool smear, and paper and video instructions on how to perform all measurements were also provided. The schedule of the study timeline and all the assessments are presented in Table 3.

2.7.1. *Primary outcome*

Based on the earlier observation that an HFF diet increases alpha diversity, the primary outcome measure in the GEEF trial will be the change in gut microbiota alpha diversity (number of ASVs) after the 8-week dietary intervention, determined from fecal samples assessed using V3-V4 16S rRNA gene amplicon sequencing [17]. Other alpha diversity indices will include Chao1, Shannon, abundance-based coverage estimator index, and Fisher's exact test. The 16S rRNA gene amplicon sequencing was conducted by MyMicroZoo B.V. (Leiden, The Netherlands). In accordance with previous studies, microbiota data analysis will be carried out using QIIME2 version 2023.2.0 [51]. Data will be denoised and unique ASVs will be identified using DADA2, version 1.26 [52].

2.7.2. *Secondary outcomes*

2.7.2.1. Beta-diversity and gut microbiota composition To determine beta diversity, Bray-Curtis diversity will be calculated. Phylogenetic positions will be assigned to ASV's using the Green Genes classifier for QIIME2 [53,54].

2.7.2.2. Inflammatory markers To obtain insights into the participants' immune status, dried blood spot samples were collected and used to assess 92 protein biomarkers associated with inflammatory and immune responses. Participants were instructed to send samples per post to the Vrije Universiteit Amsterdam after drying them horizontally for 24 hours. The samples were stored at –80°C until processing. The samples were randomly distributed across and within the plates, with all samples from the same participant placed on the same plate. Per plate, samples were thawed and 1.2 mm punches were taken from the blood spot and placed in the corresponding well. The filled plates were stored at –80°C until protein biomarker assessments were performed. All samples underwent the same number of freeze-thaw cycles and were kept outside the freezer for an equivalent duration to collect the punches. Plates were transported on dry ice to the University Medical Center Utrecht for protein biomarker assessment using the Target 96 Inflammation Panels, Olink Uppsala, Sweden [55]. Olink uses a multiplex proximity extension assay technology, a pair of oligonucleotides linked to antibodies for each target protein that undergoes a real-time polymerase chain reaction, amplifying a sequence that is then quantified [55]. Protein biomarkers include proinflammatory cytokines (e.g., tumor necrosis factor), anti-inflammatory cytokines (interleukin 10), chemokines (e.g., C-C motif chemokine 13), growth factors (e.g., vascular endothelial growth factor A) and cell adhesion molecules (e.g., signaling lymphocytic activation molecule). However, protein biomarkers have not yet been analyzed. Protein expression will be reported as normalized protein expression values, a relative quantification unit logarithmically related to protein concentration.

2.7.2.3. Gut transit time Gut transit time was measured using the blue dye method, which enabled participants to obtain awareness of their stool patterns [56]. This method has been validated in approximately 600 people and correlates well with stool consistency, stool frequency, and gut microbiome diversity and composition [56]. Blue cakes were prepared following the method described by ZOE [57]. One day during the first three time points, participants consumed two blue muffins in the morning after fasting for approximately 12 hours. The blue muffin breakfast weighed 136 g, consisting of 61.7 g carbohydrates, 1.4 g of fiber, 17.1 g of fat, and 4.4 g of protein, providing a total of 422 kilocalories. Participants were instructed to avoid consuming any food or drink, except water, while eating the muffins and during the 3 hours after finishing the consumption. Participants recorded the time of starting the blue muffin consumption (start time) and the time of first observing blue stool (end time) within the LifeData application "RealLife Exp" [58]. The transit time

Table 3 – SPIRIT diagram of study timeline and assessments in the GEEF trial.

Time point	Screening	T1[a]		T2[b]	T3[c]	Daily	Weekly	T4[d]
Wk		–1	0	2	8			21
ENROLLMENT								
Screening questionnaire including fiber screen and FFF	x							
Baseline characteristics	x							
INTERVENTION								
High-dietary fiber			x	x	x			
High-fermented food			x	x	x			
Control			x	x	x			
ASSESSMENTS								
Body weight	x	x		x	x			x
Fecal sample		x (2)		x	x (2)			
Stool smear image		x (2)		x	x (2)			
Dried blood spots		x (2)		x	x (2)			
Blue muffins		x		x	x			
Food diary		x		x	x			x
Sleep quality		x		x	x			x
DQLQ		x		x	x			x
Wellbeing		x		x	x			x
Perception and awareness		x		x	x			x
FFF		x		x	x			
Stool frequency						x		
Stool consistency						x		
GI complaints						x		
Compliance						x		
Adverse events							x	
Supplement use	x						x	
Medication use	x						x	
Evaluation dietary advice					x			
Evaluation								x

Abbreviations: DQLQ, digestion-associated quality of life questionnaire; FFF, fermented foods frequency questionnaire; GI, gastrointestinal.
[a] Baseline measurement.
[b] End ramp-up measurement.
[c] End intervention measurement.
[d] Follow-up measurement.

will be derived by subtracting the start time from the end time.

2.7.2.4. *Gut microbiota outcomes using fecal smear images* Fecal smear images were incorporated into the study and will be analyzed using Microbelink by HORAIZON (www.horaizon.nl), Delft, the Netherlands. Participants were provided with an A4 template and received instructions to make a fecal smear on this template using a scalpel and cotton bud, and then take a picture of the fecal smear. The Microbelink application employs advanced computer vision techniques to extract information from the picture, predicting whether the fecal sample has high or low alpha diversity and high or low relative abundance of several microbial taxa at the genus level [59]. This process involves segmenting the fecal smear from the background, followed by rescaling and normalizing the images to prepare them for analysis. The algorithm then uses a pretrained vision transformer model to classify the images based on microbial composition [60]. Microbelink offers a fast, noninvasive, and affordable method to track gut microbiota composition in large cohorts over time, which is particularly interesting for CS research. By leveraging deep learning, this approach significantly reduces the time and costs associated with traditional microbial analysis methods. Additionally, the outcomes of the fecal smear images will be coupled with the results of 16S rRNA gene amplicon sequencing using a multiomics approach, providing a comprehensive understanding of the gut microbiota. This multiomics integration enables cross-validation of the predictions made by Microbelink, ensuring robustness and accuracy in identifying microbial taxa and assessing the alpha diversity.

2.7.2.5. *Dietary intake* Dietary intake was assessed via 3-day food diaries using the Traqq app [61]. Participants were instructed to log their dietary intake for 2 weekdays and 1 day on the weekend. The following variables will be derived from the dietary intake assessment: energy (average, kilocalories/d), macronutrient distribution (average, g/d, and energy percentages), dietary fiber intake (average, g/d), and fermented food intake (average, servings/d).

2.7.2.6. *Questionnaires* The following validated questionnaires were completed at baseline, weeks 2, 8, and 21: the digestion-associated quality of life questionnaire, to measure

digestion-associated quality of life [62], the Athens insomnia scale, to measure sleep quality [63], and the World Health Organization five well-being index, to measure well-being [64]. Additionally, several validated psychological questionnaires measured the perception and awareness of food choices, including subjective health, intention to stay healthy [65], dietary intrinsic motivation [66], dietary self-efficacy [67], self-regulation [68] and subjective knowledge [69]. The average scores for digestion-associated quality of life, sleep quality, well-being, and perception and awareness of food choices, will be calculated using applicable scoring systems. In addition, questions regarding the evaluation of study participation and appreciation and acceptance of dietary guidelines and study products were incorporated into the week 8 questionnaire.

Daily questionnaires using the application RealLife Exp were added to monitor stool frequency, stool consistency, GI complaints, and adherence to the dietary guidelines and study products. Stool consistency was measured using the Bristol stool chart and the mode per day will be calculated [70]. GI complaints including bloating, abdominal pain, and flatulence were measured on a 10-point Likert scale and will be reported per day.

2.7.2.7. *Compliance* Treatment compliance was assessed for each group for the completion of daily questionnaires, adherence to dietary guidelines (self-reported), and intake of the study sachets (self-reported) over the 8-week intervention. Compliance with completion of the daily questionnaires was calculated as the sum of the completed questionnaires divided by the total number of questionnaires. Compliance with the intake of study sachets and dietary guidelines was calculated only for participants with at least 75% completed daily questionnaires. This arbitrary threshold of 75% for adequate compliance with completing daily questionnaires was chosen based on the idea that it would distinguish participants who consistently did not complete questionnaires from those who occasionally (once or twice per week) forgot to complete a questionnaire.

For the intake of study sachets, compliance was calculated as the sum of reported consumed sachets divided by the number of completed questionnaires. For the HDF group, compliance with the dietary guidelines was calculated as the average adherence to the following criteria: using two recipes daily, using five dinner recipes weekly, and consuming two snacks daily. Each day, a participant who reported having used at least two recipes was scored as 1, and having used only one recipe was scored as 0.5. The total adherence to using two recipes daily was calculated by dividing the sum of scores by the number of completed daily questionnaires. Similarly, adherence to weekly dinner recipe use (≥5 recipes scored as 1 and 3-4 recipes as 0.5) and daily snack consumption (≥2 snacks scored as 1 and 1 snack as 0.5) were calculated. For the HFF group, the daily consumption of at least three servings of fermented foods was scored as 1, and the consumption of two servings as 0.5. Compliance with dietary guidelines for the HFF group was calculated by dividing the sum of the scores by the number of completed daily questionnaires.

2.8. *Statistical analysis plan*

2.8.1. *Analysis populations*

The intention-to-treat (ITT) population will be defined as all participants who were randomized and received an intervention. The per-protocol (PP) population will be defined as all participants who:

- Completed the 8-week intervention;
- Completed at least 75% of daily questionnaires;
- Sufficiently complied with the dietary guidelines and intake of study sachets:
  - HDF: ≥80% intake of study sachets and ≥80% adherence to dietary guidelines;
  - HFF: ≥80% intake of study sachets and ≥80% adherence to dietary guidelines;
  - CG: ≥80% intake of study sachets.

Arbitrary thresholds of ≥80% compliance with dietary guidelines and study sachets were chosen to exclude nonadherent participants while maintaining a sufficiently large sample size to detect statistically significant effects [71]. In addition to the abovementioned exclusions, specific data points will be excluded for participants with major protocol deviations, such as those using antibiotic treatment, as determined on a per-subject basis by the study team and medical investigator, immediately before database lock.

2.8.2. *Statistical analyses*

Statistical analyses will be performed using R studio. Treatment groups will be tested 2-sided and a *P*-value of $P < 0.05$will be considered statistically significant. All data will be tested for normality using the Shapiro–Wilk test. Both ITT and PP population analyses will be performed for the primary and secondary outcomes.

The significance of differences in the primary outcome, alpha microbial diversity metrics, between groups will be determined using nonparametric statistics for the analysis of longitudinal data in factorial experiments, assuming data will not be normally distributed [72]. In case of normal distributed data, a mixed model analysis for repeated measures will be used with as fixed effects “treatment” (fiber/fermented/control), “time point” (WK0, WK2, WK8), and “treatment x time point.” The subject will be added as a random effect. Terms of sex, age, and BMI will be included in the model as covariates in the analysis over time.

The significance of the differences in beta diversity and microbial composition will be determined in a manner similar to that of alpha diversity. If the data are not normally distributed, nonparametric statistics for the analysis of longitudinal data in factorial experiments will be applied. Normally distributed data will be analyzed using a mixed model of repeated measures, including microbiota composition, (1) time point, and (2) treatment, as described previously. Inflammation markers will be analyzed using the same approach as that used for the microbiota data. Inflammation markers occurring in 75% of the samples will be included in the data analysis [17]. When applicable, a moderated F-test for each marker will be conducted to detect differences between the control and treatment curves, and the false discovery rate will be calculated.

Other secondary outcome parameters include transit time, fiber intake, fermented food intake, sleep quality, quality of life, well-being, and the different scales for perception/awareness and will be analyzed by mixed model analysis for repeated measures, using "treatment" (fiber/fermented/placebo), "time point" (WK0, WK2, WK8, WK20) and "treatment x time point" as fixed effects and the subject as random effect. Time will be included as a factor, comparing week 2, week 8, and the 3 months measurement with week 0 (reference). Additionally, a model will be used with week 8 as a reference, comparing the follow-up meetings at 3 months, and week 2 will be compared to week 8. Daily questions regarding GI complaints, stool frequency, and stool consistency result in repeated measurements. These outcome measures will be analyzed by mixed model analysis, using "treatment" (fiber/fermented/placebo), time (continuous), and "treatment x time point" as fixed effects and the subject as random effect. Before the data analysis, the data will be visually checked for linearity. If the data are linear, time will be included as continuous data, based on the slope over time. If the data do not seem to be linear, time (days) will be recoded into periods:

- Baseline: day 0 to 2 (T1)
- Ramp-up: day 3 to 14 (T2)
- Intervention: days 15 to 56 (T3)

Associations between microbial abundance and secondary outcome parameters and other covariates, such as BMI, sex, age, dietary intake, compliance, and treatment group, will be investigated using multivariate analysis of compositional data. Random Forest (with leave-one-out cross-validation) will be used to investigate the predictive potential of each of the data types, composition, and secondary outcomes for treatment groups, and regression (zero-inflated random effects model with beta distribution) will be used to discover associations between clustered, correlated microbial taxa, secondary outcome measures, and other covariates. When applicable, false discovery rates will be estimated using the Benjamini–Hochberg method.

3. Results

3.1. Participant flow

A total of 1448 individuals showed interest in the study. Of these, 552 individuals attended an information meeting and signed informed consent, and 509 individuals were screened for eligibility; of these, 334 did not meet the inclusion criteria and 9 withdrew. Upon attaining the required number of participants ($n = 147$), the final applicants ($n = 19$) were unable to participate. All participants were randomized, with the exception of one participant in the CG (started antibiotic use) who received the allocated intervention (ITT population: $n = 146$). Two participants discontinued the intervention because of GI complaints (HDF) and antibiotic use (HFF). The PP population will consist of 133 participants. For the microbiota and inflammatory data analyses, two additional participants will be excluded from the PP population because of antibiotic use at week 8. Fig. 2 shows the flow diagram of the participants.

3.2. Baseline characteristics

The baseline characteristics of participants in each treatment group are presented in Table 4. Approximately 75% of the participants were female. Mean age of all participants was 44.5 years (range 19-69 years) with an average BMI of 24.1 kg/m^2 (range 18.8-30 kg/m^2). Sex, age, and BMI were equally distributed among the treatment groups. The average daily dietary fiber and fermented food intake, as assessed by the screening questionnaire, was similar across all groups.

3.3. Participant compliance and adverse events

During the 2-week ramp-up and 6-week intervention period, compliance with the dietary guidelines was high (HDF, 91.8%; HFF, 93.8%). Moreover, compliance with the consumption of the study products throughout the intervention period was high in all treatment groups (HDF, 94.5%; HFF, 92.7%; CG, 96.6%). Twelve adverse events classified as possibly or probably related to the study product were reported, all involving mild GI complaints (HDF, $n = 1$; HFF, $n = 7$; CG, $n = 4$). One adverse event related to blue muffin consumption (a mild GI complaint) was reported.

4. Discussion

Manipulating the composition of the gut microbiota holds promise for preventing or delaying the onset of several chronic diseases. However, evidence from RCTs on the effects of specific dietary advice on gut microbiota composition and whether changes impact other health outcomes, such as inflammatory markers in the blood, remains limited. Therefore, we designed the GEEF study, which was an RCT that investigated the effects of an HDF diet and an HFF diet, compared to a control treatment, on gut microbiota composition and several secondary outcomes. Given that poor dietary intake and long-term dietary behavior change remain public health challenges, several elements of CS were integrated into the study design. We aimed to stimulate sustained improvements in dietary behavior through active involvement of the participants in our study. The elements of CS included (1) interactive education sessions where participants learned about the gut microbiota and could exchange experiences and tips with each other, (2) a tailored dietary intervention through recipe booklets, (3) remote data collection by participants themselves, (4) providing insight into individuals' stool pattern and gut microbiota composition throughout the study and (5) involvement of participants in the dissemination of results through vlogs. The study protocol, as well as the initial results on the flow of participants and adherence rates, suggest that the application of a CS approach in RCTs is feasible and might benefit participant recruitment and study compliance [73,74].

A gut microbiota dysbiosis, broadly defined as any change in the composition of commensal microbial communities relative to those found in healthy individuals, has been associated with intestinal disorders, metabolic syndrome, cardiovascular disease, and obesity [75]. Dysbiosis can be characterized by a loss of microbial diversity, an increase in potentially harmful microbes, and/or a reduced abundance of mi-

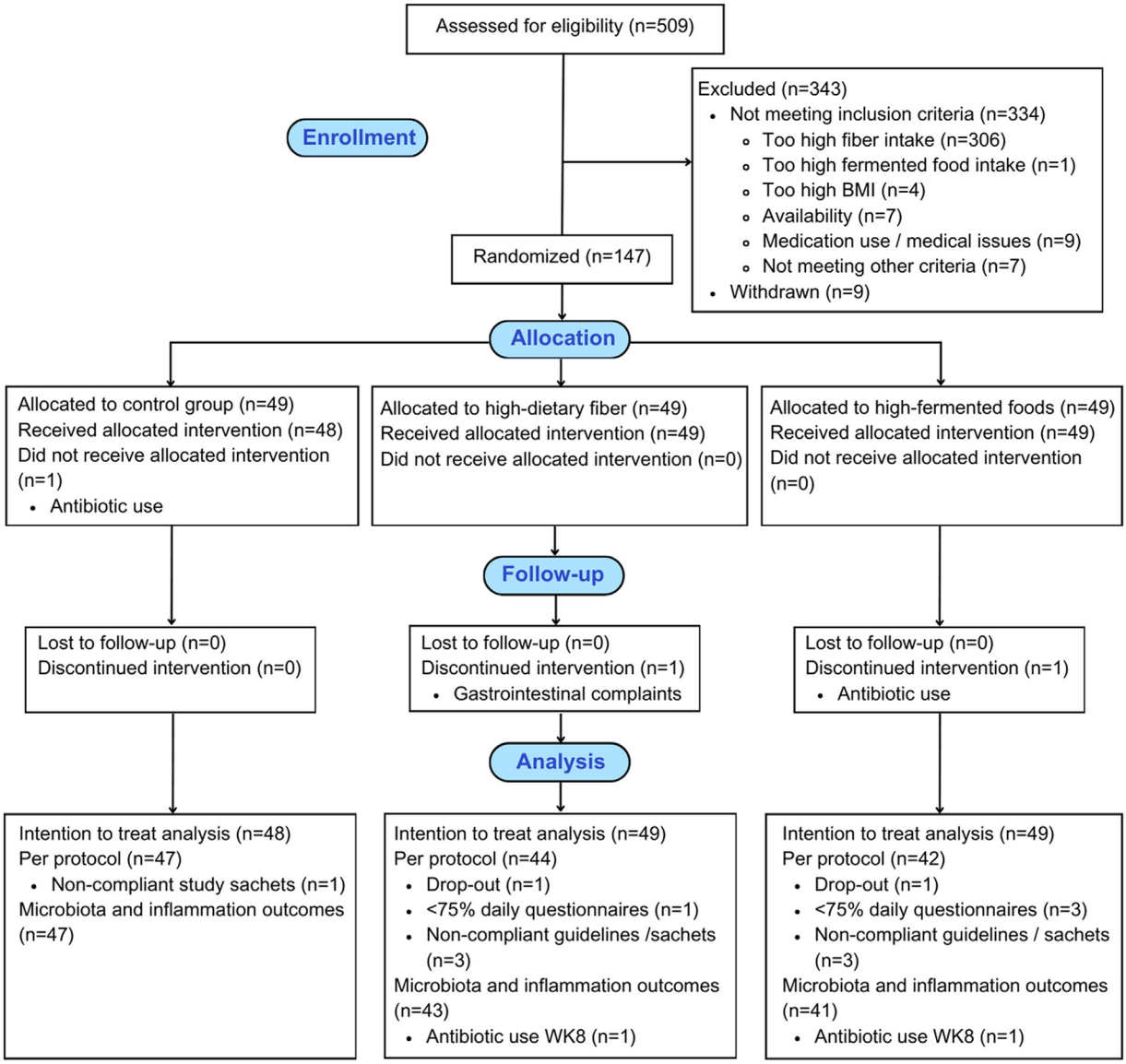

Fig. 2 – CONSORT flow diagram of study participants in the GEEF trial.

Table 4 – Baseline characteristics of study participants in the GEEF trial, including healthy adults from 19 to 69 years old (ITT population, *n* = 146).

	HDF group (*n* = 49)	HFF group (*n* = 49)	CG (*n* = 48)
Sex			
Female, *n* (%)	36 (73.5)	35 (71.4)	36 (75.0)
Male, *n* (%)	13 (26.5)	14 (28.6)	12 (25.0)
Age (y), average (±SD)	43 (14.0)	46 (13.1)	45 (14.6)
BMI (kg/m^2), average (±SD)	23.8 (2.9)	24.3 (2.6)	24.0 (2.3)
Dietary fiber intake[a] (g/d), average (±SD)	14.2 (2.9)	14.8 (3.7)	15.9 (2.7)
Fermented food intake[b] (portions/d), average (±SD)	0.6 (0.7)	0.7 (0.7)	0.7 (0.6)

Abbreviations: BMI, body mass index; CG, control group; HDF, high-dietary fiber; HFF, high-fermented food; ITT, intention to treat; SD, standard deviation.

[a] Based on fiber screen questionnaire.

[b] Based on self-developed fermented foods frequency questionnaire.

crobes associated with health benefits [76]. The composition of the microbial community influences a balanced immune response and perturbations can lead to low-grade inflammation [77]. Increasing microbial diversity or changing the microbiota composition (i.e., increasing the relative abundance of microbes associated with beneficial health effects and/or decreasing the relative abundance of potentially harmful microbes) could improve health status and prevent or delay several chronic diseases mediated by reduced low-grade inflammation. A previous clinical trial by Wastyk et al. [17] found that a 10-week fermented food diet increased alpha diversity, whereas a high-fiber diet increased microbiome-encoded glycan-degrading carbohydrate-active enzymes. Building on these earlier findings, the GEEF study included a CG, a large sample of study participants, and elements of CS. The intervention comprised a tailored approach involving recipe booklets, from which participants were free to choose, and a study product aimed at modulation of the gut microbiota. With this combined approach, we aimed to study the effect of dietary fiber and fermented foods on the gut microbiota, as well as to achieve long-term behavioral changes toward a healthier diet that supports the gut microbiota. This approach presented some challenges, which will be discussed in the following paragraph.

The main aim of CS research is to involve participants in scientific endeavors. This is something that we tried to prioritize in our RCT without compromising the integrity, validity, and reliability of the study regarding scientific outcomes. Several methodological and practical considerations have emerged during the design of the research methodology. While some of these considerations arose from our objective to engage participants in research, others mirror the challenges encountered in tailored dietary intervention studies or remote RCTs.

The tailored approach introduced heterogeneity in the treatment groups. Therefore, we decided to include study sachets with dietary fiber or fermented food products, as these offered a solution to attain a more homogenous treatment. Another challenge posed by the tailored approach is obtaining accurate information on dietary intake. The daily questionnaires broadly assessed adherence to dietary recommendations; however, collecting detailed information on product or recipe use would impose a significant strain on the participants. Therefore, we decided to rely on the 3-day food diaries collected at weeks 2 and 8, even though dietary intake could have fluctuated significantly due to the personal approach.

Another obstacle related to the tailored intervention and CS approach was the ambition to inform and involve participants at an early stage without unblinding the CG or inducing an enhanced placebo effect in the dietary intervention groups. To overcome this, initial meetings were convened per group, and general information about the role of the gut microbiome in host physiology was shared. The health effects of dietary fiber and fermented foods were mentioned in the respective groups, but the health claims were nuanced in nature. The inclusion of a study product provided the opportunity to include placebo sachets, mimicking some of the potential placebo effects in the dietary intervention groups. Participants who recorded vlogs during the study period were requested to refrain from sharing them with relatives or on social media platforms until the completion of the 8-week intervention period. To provide all participants with knowledge on how the gut microbiota might be modulated through diet, the end meeting was organized for all groups combined, and the study materials of all groups were made available for all participants.

The final challenge related to the CS approach involves the choice of outcome measurements. Data were self-reported or derived from the biological samples collected by the participants themselves. To make instructions understandable for everyone and to minimize measurement errors, instructional videos were published on the study websites in addition to paper instructions. Methodologies that measure low-grade inflammation based on the induction of an inflammatory change in response to a biological challenge, such as oral glucose load or administration of bacterial lipopolysaccharides, are preferred over blood cellular markers [78]. Although these measurements have been used in earlier nutritional trials, they cannot be performed at home or without an expert. High-throughput biological sample characterization of targeted immune biomarkers, measured using dried blood spots, provided the opportunity to include a minimally invasive sampling technique that could be performed by the participants themselves. Regarding gut microbiota outcomes, we opted for 16S amplicon rRNA sequencing over shotgun whole-genome sequencing despite the multiple advantages of the latter [79]. Because we wanted to provide participants with individual-level gut microbiota outcomes,16S amplicon rRNA sequencing was selected from the company that delivered the most comprehensive customer reports (MyMicroZoo B.V., Leiden, The Netherlands). Genus abundances fluctuate substantially on a daily basis around a stable state, making single measurements unreliable for reflecting a person's temporal average [80]. To minimize intraindividual variation, two different fecal samples were collected at the start and end time points.

Our study design has several limitations. In exchange for greater external validity, which aligns with CS research, the study's internal validity may be diminished. Although we enrolled a CG, a double-blind study design was not feasible. Consequently, the treatment groups may inadvertently have preconceived dietary interventions as effective. Informing these groups about the health benefits of their dietary intervention may have augmented this placebo effect on subjective outcome measures. On the other hand, the high compliance rates may enhance internal validity. In addition, the data analysis will be performed in a blinded manner, even though the study personnel could not be blinded. The provision of information on the gut microbiome and gut microbiota reports constitutes another source of confounding as it might have stimulated dietary behaviors other than those intended. To mitigate this, specific dietary and other lifestyle-related recommendations were excluded from the report, and participants were asked on a weekly basis if they had made any changes to their lifestyle, and their dietary intake was monitored.

Despite the abovementioned challenges and limitations, the inclusion of CS elements in our study protocol may offer several benefits. First, participant recruitment might have been expedited. Participant recruitment started on May 4, 2023, and was planned to last until the beginning of September 2023. A vast number of individuals expressed interest in par-

ticipation, leading to the cessation of recruitment on August 9, 2023, due to the attainment of the required number of eligible participants. On average, we recruited and randomized around 49 participants per month, which is high compared to a review that concluded that rates for RCTs are likely to be 4 to 10 participants per center per month [73]. However, it should be noted that recruiting healthy individuals from across the Netherlands might be easier than recruiting specific populations or individuals from a certain geographical location. The potential of CS for recruitment has been demonstrated in an earlier trial that reported a large number of applications [81]. The willingness of participants to contribute to research might be enhanced when they get the opportunity to gain knowledge not easily obtained elsewhere, when they receive feedback on individual study outcomes, such as via the comprehensive gut microbiota reports, and when they are provided with motivational videos [82].

Second, compliance with the dietary guidelines and study products was high (>90%) [83]. This can probably not be attributed to the tailored dietary guidelines, as a systematic review found that personalized nutrition does not improve compliance compared to conventional dietary advice [34]. Receiving education and a personal gut microbiota report, along with group meetings and daily availability of the researchers by mail and phone, may have created an avenue to keep in close contact with the study participants and might have positively impacted compliance [84].

Third, we speculate that the CS approach might have encouraged participants to take control of their own health, potentially leading to sustained behavioral changes. This will be reflected by an increased intake of dietary fiber and fermented foods during the follow-up measurement compared to baseline. It has been demonstrated that provision of biological information conveying health status may promote motivation to change behavior [85]. Nevertheless, the impact of CS on compliance and long-term behavioral changes has not yet been evaluated in an RCT that included a treatment arm with CS and a control arm without CS, although we did find a study protocol for such a trial [86]. However, we did identify a trial in the environmental domain that demonstrated benefits in the CS arm on self-efficacy and well-being parameters compared to a control arm [87]. Such improvements might support the sustainability of behavioral changes [88].

Finally, the adoption of CS elements is anticipated to strengthen the generalizability of the study findings, given their influence on the determinants affecting external validity. These determinants include the inclusion of the general public, the pragmatic and tailored dietary recommendations, the real-life setting of the study, and the inclusion of a follow-up measurement concerned with behavior change [89]. To make this more concrete, we included a diverse group (aged 18-70 years) of healthy Dutch individuals, likely representative of the healthy Dutch population. The pragmatic and tailored dietary recommendations can be adopted more easily by the general public because they allow for personal implementation. The real-life setting of the study makes the findings more reflective of how individuals in the real world would adopt dietary recommendations, and the follow-up measurement provides insight into the sustainability of these recommendations, which can be considered during implementation. The opinions of the participants regarding the dissemination of the results will further augment the implementation of the study findings.

Various approaches will be applied to disseminate the findings of this study. To reach a wide audience and make the results permanently available to researchers, healthcare professionals, and others interested, the results will be published in an open-access peer-reviewed journal. The metadata will be deposited in a publicly available repository. Additionally, the findings of the study will be communicated by consortium partners through newsletters and social media. The societal and personal relevance of the findings and their potential for implementation in practice, together with preferred channels and tools for communication to the general public, will be discussed with the participants during a group meeting in which the study results will be shared. These findings will be presented to the Dutch Digestive Foundation for integration into campaigns and materials for patients to reach the public.

Most importantly, in close collaboration with the Dutch Digestive Foundation, a campaign will be created about the GEEF study and its findings. This campaign includes a platform on which Dutch citizens can join a dietary challenge (i.e., consuming more dietary fibers or fermented foods for 8 weeks). Participation in this challenge allows the use of study materials (recipe booklets, motivational videos, and vlogs by the study participants) through which participants will gain knowledge to make informed dietary adjustments. Tips and experiences of the study participants, which were captured in vlogs throughout the study, will be used to create informative videos that will help motivate the general public to take part in the challenge of increasing dietary fiber and fermented food intake in the long term.

To leverage the potential of CS in RCTs, a better understanding of its impact on long-term behavioral changes and societal implementation is required. If participants in the GEEF study were able to maintain dietary changes need to be demonstrated in its findings. Further research is needed to evaluate the effectiveness of RCTs using a CS approach for participant recruitment, retention, and compliance. It would also be interesting to examine whether the adoption of CS in RCTs results in significant differences in effect sizes compared to RCTs without CS.

In conclusion, the incorporation of CS elements in our RCT provided an opportunity to engage study participants in the study, while concurrently advancing scientific knowledge on the effects of an HDF diet and HFFs diet on the gut microbiota. Our study design and results on the flow of participants and compliance rates demonstrate that including elements of CS in an RCT is feasible and may aid the recruitment of study participants and enhance compliance.

CRediT authorship contribution statement

Marieke van de Put: Writing – original draft, Visualization, Project administration, Investigation. **Maartje van den Belt:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation. **Nicole de Wit:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Remco Kort: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Acknowledgment

The authors acknowledge the valuable collaboration with Bouwens, M., Copini, AJ. and Rook, M. from the Dutch Digestive Foundation; Pintar, N., Hlapčić, I. and Balen, B. from Ani Biome; van Straaten, J. P. and Voragen, F. from Keep Food Simple; Kaper, F., Rijnaarts, I. and Borkent, J. from WholeFiber Holding BV; van Eeden, F. and Seegers, J. from MyMicroZoo and Levin, E. from HORAIZON. The authors gratefully acknowledge the anonymous reviewers for their valuable suggestions. The graphical abstract was created with BioRender.com. The project Gut Health Enhancement by Eating Favorable Food (the GEEF study) was funded by the Dutch Digestive Foundation (Maag Lever Darm Stichting), Amersfoort, The Netherlands WOO 22-03 grant; Ani Biome; Keep Food Simple and WholeFiber Holding BV. The study sponsors had no role in the planning, design, data collection, analysis, or interpretation, except for the Dutch Digestive Foundation, which developed the recipe booklets with the dietary guidelines for the RCT. The authors declare that they have no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nutres.2024.07.008.

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