JPGN Journal of Pediatric Gastroenterology and Nutrition Publish Ahead of Print

DOI : 10.1097/MPG.0000000000000285

Effects of a Wheat Bran Extract containing arabinoxylan oligosaccharides on gastrointestinal parameters in healthy preadolescent children: a double-blind, randomized, placebo-controlled, crossover trial

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Short title: Wheat Bran Extract effects on gastrointestinal parameters in children

Conflicts of Interest and Source of Funding

IEJAF, OL, WSV and WFB were during the course of the study employed by Fugeia NV, which manufactures the Wheat Bran Extract product and is the sole sponsor source of funding for the studies described herein. For the remaining authors, none were declared.

The trial is registered in the clinicaltrials.gov register (NCT01001949) (http://clinicaltrials.gov/ct2/show/NCT01001949?term=AXOS&rank=4).

Word count: 4056

Number of Figures: 2

Number of Tables: 4

ABSTRACT

Objectives: We assessed whether a wheat bran extract (WBE) containing arabinoxylan oligosaccharides (AXOS) elicited a prebiotic effect and modulated gastrointestinal parameters in healthy preadolescent children upon consumption in a beverage.

Methods: This double-blind, randomized, placebo-controlled, crossover trial evaluated the effects of consuming WBE at 0 (control) or 5.0 g/day for 3 weeks in 29 healthy children (8-12 years). Faecal levels of microbiota, short-chain fatty acids, branched chain fatty acids, ammonia, moisture and faecal pH were assessed at the end of each treatment and at the end of a one-week run-in period. In addition, the subjects completed questionnaires scoring distress severity of 3 gastrointestinal symptoms. Finally, subjects recorded defecation frequency as well as stool consistency.

Results: Nominal faecal bifidobacteria levels tended to increase after 5 g/day WBE consumption ($p = 0.069$), whereas bifidobacteria expressed as percentage of total faecal microbiota was significantly higher upon 5 g/day WBE intake ($P = 0.002$). Additionally, 5 g/day WBE intake induced a significant decrease in faecal content of isobutyric acid and isovaleric acid ($P < 0.01$), markers of protein fermentation. WBE intake did not cause a change in distress severity of the 3 surveyed gastrointestinal symptoms (flatulence, abdominal pain/cramps, urge to vomit) ($P > 0.1$).

Conclusions: WBE is well tolerated at doses up 5 g/day in healthy children. In addition, intake of 5 g/day exerts beneficial effects on gut parameters, in particular increase of faecal bifidobacteria levels relative to total faecal microbiota, and reduction of colonic protein fermentation.

Key Words: Wheat Bran Extract, arabinoxylan oligosaccharides (AXOS), prebiotic, protein fermentation reduction, children

INTRODUCTION

Wheat Bran Extract (WBE), a food-grade, fiber-rich, water-soluble preparation that is produced by enzymatic extraction from wheat bran, is highly enriched in arabinoxylan-oligosaccharides (AXOS). AXOS consist of a backbone of β-1,4-linked D-xylopyranosyl residues (xylose), some of which are mono- or di-substituted at the C(*O*)2 and/or C(*O*)3 position with α-L-arabinofuranosyl residues (arabinose)[1]. Some of the xylose units in the backbone of AXOS carry glucuronic acid at the C(*O*)2 position, whereas some of the arabinose units are ester-linked at the C(*O*)5 position with ferulic acid[1]. The AXOS in WBE form a heterogeneous mixture of oligosaccharides differing in degree of polymerization and degree of substitution of the xylan backbone. Besides AXOS, WBE contains up to 15% glucans [mainly β-D-(1,3)(1,4)-linked glucan oligomers] and low levels (< 2%) of proteins, minerals and monosaccharides[2].

AXOS are non-digestible fermentable prebiotic oligosaccharides with bifidogenic activity as was demonstrated in *in vitro* studies[3], animal studies (chicken[4,5], rats[6]) and clinical trials with healthy adults[7-9]. The evidence for AXOS having prebiotic activity has been recently reviewed[1]. In addition, AXOS consumption decreases the excretion of urinary and faecal *p*-cresol, a marker of intestinal protein fermentation[7,8,10]. Colonic protein fermentation is often regarded as detrimental to host health, in particular with respect to colon toxicity, mutagenicity and carcinogenicity[11]. Proteolytic fermentation leads to the production of potentially toxic compounds such as phenolic compounds, sulfur-containing compounds, amines and ammonia [12-14]. The toxicity of these protein fermentation metabolites has mainly been established in *in vitro* studies[15-17] and animal studies[18,19].

Until date, the gastrointestinal effects of and tolerance effect to WBE in humans has only been investigated in adult volunteers. It is known that the composition of gut microbiota in preadolescent and adolescent children differs from that of adults[20,21], with notably a higher

abundance of Bifidobacteria in teenage children versus adults[21]. The purpose of this study therefore was to evaluate the effect of intake of 5 g/day WBE on gastrointestinal health parameters in healthy preadolescent children aged 8-12 years. The effect of WBE administration on colonic carbohydrate fermentation was investigated through measurement of faecal levels of SCFAs and the effect on colonic protein fermentation was analyzed through measurement of faecal levels of isovaleric acid and isobutyric acid. Since WBE is intended to be added to food products, including food products for children, it is also important to assess tolerance to the product and its safety profile. Tolerance to WBE was assessed through self-reported scoring by the children of distress severity of the following three gastrointestinal symptoms: flatulence, abdominal pain/cramps and urge to vomit. Safety was evaluated by assessing the occurrence of adverse events (AEs).

MATERIALS AND METHODS

Study design

Figure 1 presents a schematic overview of the randomized, placebo-controlled, double-blind, cross-over study. The study started with a one-week run-in period, followed by two 3-weeks treatment periods, during which the children had to take in placebo or 5 g/day WBE, with a 2-weeks wash out period in between the treatment periods. The WBE dose of 5 g/day was half the WBE dose that was shown in a previous trial performed on adults to raise the levels of Bifidobacteria[8], taking into consideration the lower average body weight of preadolescent children versus adults. WBE and placebo were administered as non-carbonated soft drinks of which the volunteer drank daily 70 ml after breakfast and 70 ml after dinner (140 ml per day in total). The WBE-containing soft drinks contained sucrose, colorant, flavor, citric acid and potassium sorbate. The placebo soft drink had the same composition as the WBE-containing

soft drink, except that WBE was omitted and that 0.25 g/l tricalcium phosphate was added to mimic the turbidity of the WBE-containing soft drinks. Subjects were randomly assigned to one of two randomization groups, differing in the treatment sequence by which the two types of drinks were to be consumed. The investigators who had direct contact with the subjects were blinded to the treatment since they were unaware of the randomization groups to which the subjects were assigned. Moreover, the appearance and the taste of the different soft drinks were near-identical, and the two types of soft drinks could not be discriminated from each other without careful side-to-side comparison. Side-to-side comparison of the drinks by the volunteers was not possible as only one drink type was supplied prior to each treatment period.

Study population

A total of twenty-nine healthy children (11 girls and 18 boys, all of Caucasian ethnicity) participated. Exclusion criteria were extreme dietary habits in the 6 weeks before the start of the trial, intake of antibiotics in the 3 months before the start of the trial, intake of medication or dietary supplements influencing gastrointestinal tract processes in the 2 weeks before the start of the trial, abdominal surgery in the past (with exception of appendectomy), chronic diseases/conditions, serious illness in the 3 months before the start of the trial, complete anaesthesia in the month before the start of the trial, history of chronic gastrointestinal (GI) conditions such as inflammatory bowel disease and irritable bowel syndrome, allergy to wheat products and celiac disease.

During the study, the intake of food substances containing probiotics and/or prebiotics was forbidden. The children and their parents were asked to read food labels carefully to check for absence of pro- and/or prebiotics. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of the University Hospital UZ Leuven (Belgium) under

approval number ML5282. Written informed consent was obtained from all children and their parents. The trial is registered in the clinicaltrials.gov register (NCT01001949).

Test product

Wheat Bran Extract (Brana Vita® 200) was produced from wheat bran by Fugeia NV (Leuven, Belgium), using a procedure based on that described previously[22]. WBE was analyzed for AXOS content, AXOS average degree of polymerization (avDP), arabinose/xylose ratio (A/X ratio), bound ferulic acid and glucuronic acid, glucose as part of poly/oligosaccharides, mannose as part of poly/oligosaccharides, galactose as part of poly/oligosaccharides, free monosaccharides, moisture, protein and ash by analytical procedures outlined previously[2]. Table 1 shows the composition of the WBE preparation used in the present study. It consisted of 79.0% AXOS (on dry matter basis), and the AXOS had an avDP of 5 and an A/X ratio of 0.19.

Biochemical and microbiological analyses of faecal samples

On the evening of day 5 or during day 6 of the run-in period as well as on the evening of day 19 or during day 20 of each treatment period, one single stool was collected by each child. Faecal samples were analyzed for microbiota composition using FISH analysis. Processing of paraformaldehyde fixed samples and FISH analysis to quantify Bifidobacteria, the *Lactobacillus/Enterococcus* group, the *Clostridium histolyticum/lituseburense* group, the *Faecalibacterium prausnitzii* group and the *Roseburia/Eubacterium rectale* group was performed as described previously[8]. Concentrations of the short-chain fatty acids (SCFAs) acetate, propionate, butyrate and of the branched chain fatty acids (BCFAs) isovaleric acid and isobutyric acid were determined as described[23], using 2-methylhexanoic acid as internal standard. To determine the faecal pH, an aliquot of approximately 1 g faeces was homogenized by mixing with demineralized water (final concentration 10% (w/w)) [24]. The pH was measured immediately upon homogenization. Ammonia levels were measured on the

same faecal slurries as used for pH determination, following the procedure described previously[25].

Recording of gastrointestinal symptoms and stool parameters

Gastrointestinal symptoms were monitored daily during the run-in period and the last week of each treatment period. The volunteers were asked to grade the distress severity of abdominal pain/cramps, flatulence and urge to vomit using a 5-step scale ranging from no (0), minimal (1), mild (2), moderate (3) to severe (4) distress[7]. During the run-in period and the last week of each treatment period, the number of stools as well as stool consistency according to the Bristol Stool Form Scale[26] were recorded daily. The average stool frequency was calculated as the number of stools divided by the number of days of diary recording, the average stool consistency as the sum of Bristol Stool Form Scales divided by the number of stools, and the composite parameter of stool frequency and consistency (called Bristol composite measure) as the sum of Bristol Stool Form Scales divided by the number of days of diary recording[8,27].

Recording of Adverse Events (AEs) and treatment compliance

At the end of the run-in period and after each intake period, the children were asked to record whether they had suffered from a medical condition, had to take in medication, or had incidentally taken prebiotics or probiotics. Additionally, at each clinic visit, the children were asked these questions. This information was recorded in the appropriate section of the Case Report Form. Treatment compliance was defined as the number of times per treatment period that a serving of soft drink was not consumed. During each intake period, the children had to report daily whether they consumed the soft drinks after breakfast and after dinner.

Statistical analyses of efficacy variables

In order to test for differences at baseline, the treatment sequence groups were compared with respect to age, gender, faecal Bifidobacteria content and stool frequency. Comparison of the groups was based on a one-way analysis of variance (ANOVA) and a Chi-square test in case

of gender. The Fisher exact test was used if the chi-square test was judged inappropriate due to small cell sizes. When ANOVA analysis could not be used, the groups were compared using the non-parameteric Kruskal-Wallis test and pairwise using the Mann-Whitney U test. For each efficacy variable, the difference between both treatments was analyzed within the statistical model. A mixed model was used to capture the intra-volunteer correlation due to the repeated measurements for each volunteer. The treatment effect for the different parameters was analyzed using a linear mixed model[28]. The models were estimated using the nlme package from R's base distribution[29]. The fitted model included subject as a random effect and contained terms for treatment and treatment sequence. Additionally, the results of the run-in period were included as covariate in the linear mixed model. All tests of significance were performed at $\alpha = 0.05$ and were two-sided, unless otherwise stated. Assumptions of normality of residuals were investigated for each variable using the Shapiro-Wilk test[30]. When the data were normally distributed, linear mixed models were applied to the raw data as such, except for the microbiota data which were log transformed prior to analysis. When the distribution of the data was not approximated by a normal curve, values were ranked before analysis and the linear mixed model was performed on the rank-transformed data[31]. Ties occurring during the rank-transformation were replaced by their average rank. The data to estimate the fixed effect parameter for the run-in of the response remained unranked.

Evaluations of the effects of treatment on the efficacy variables were completed on an efficacy evaluable (EE) population, defined as all randomized subjects who received placebo and at least one serving of WBE containing soft drink and who provided at least one post-randomization outcome data point during each of the two treatment phases. The per-protocol (PP) population was defined as the subset of EE subjects who completed the study, did not take excluded medications (e.g. antibiotics) or products and had no major protocol violations.

In the present study the PP population coincided with the EE population, and statistical analyses were not repeated for the PP population.

Treatment effects as well as treatment by treatment sequence interaction effects were tested with linear mixed models using conditional F- and t-tests[28] (significance at $\alpha = 0.1$). The single-step Tukey post-hoc multiple comparison procedure was used for the pairwise comparisons of the treatments, using R's multcomp package[32]. In case no significant interactions were found, treatment differences were evaluated based on the main effect model. In case of significant interactions, treatment differences were evaluated within each treatment sequence group (results not shown). Next to that, the overall differences were also analyzed by aggregating over the interaction effects, which was performed by setting up a linear combination of the treatment differences for each treatment sequence group, giving equal weights to both treatment sequence groups. For the distress severity of the 3 gastrointestinal symptoms, an analysis was performed using binary data. Although the symptoms were scored daily during one week on an ordinal scale and were subsequently aggregated (averaged) over the 7 days, lack of variation in the symptom scores obliged us to use binary response models. In this case, all volunteers who indicated for an aggregated gastrointestinal symptom score "no distress" were regarded as "0". All persons who indicated an aggregated distress severity score differing from "no distress" were regarded as "1". The level of the *Clostridium histolyticum/lituseburense* group was in a large proportion of the volunteers below the detection limit ($\log_{10}$ 5.65 per g wet faeces), leading to a binary distribution of this dataset ("1" = content of *Clostridium* group $\geq \log_{10}$ 5.65 per g wet faeces; "0" = content of *Clostridium* group $< \log_{10}$ 5.65 per g wet faeces). For the analysis of binary data, a generalized linear mixed-effects was used as described previously[8].

Statistical analysis of safety parameters and treatment compliance

The safety population was defined as all randomized subjects who received at least one serving of WBE. Safety was analyzed using the emergent AEs in the safety population. An AE was attributed to the treatment period during which the AE started. An AE that started during a wash out period was attributed to the treatment preceding the specific wash out period. McNemar's test was used to compare differences in AE frequencies among the two treatments ($\alpha = 0.025$, Sidak correction for 2 comparisons)[33]. Statistical analysis of the number of non-consumed SSD servings was performed using a Poisson mixed effect model with similar fixed effects as for the efficacy variables but excluding the runin period as there were no consumptions at runin. For the analysis of Poisson data, a generalized linear mixed-effects model[34] with log link function was used with equal random and fixed effects as in the linear mixed model. For the Poisson data, a similar approach as for the continuous data was used starting from a basic model which contained the main effects: the value at baseline, treatment and treatment sequence. The test for a significant interaction between treatment and treatment sequence was done based on a likelihood ratio test[34]. P-values were obtained similarly as described for the models of the the efficacy variables. Evaluation of a significant treatment effect was done by comparing the basic model to a model containing only treatment sequence and run-in. Adding and evaluating the interaction effects was done stepwise by adding treatment sequence based on the most significant likelihood ratio test. In case of not enough data in each interaction cell (<4), the final model to evaluate the treatment was the basic model and interactions were disregarded. For models with interaction effects and inflated standard errors of the treatment differences, the model containing only main effects was referred to.

RESULTS

Study population

The disposition of all study participants is presented in Figure 2. A total of 30 children were screened and 29 were randomized to the 2 different randomization groups. Since all children received WBE, the 29 children were included in the safety population. Of these, one child was excluded from the Efficacy Evaluable (EE) population since no data points were obtained from this volunteer during the placebo treatment. Hence, the EE population consisted of 28 children. Since none of them had to take in antibiotics and all children were compliant, therefore, the Per Protocol (PP) was the same as the EE population.

Baseline characteristics for the EE/PP population are presented for both randomization groups in Table 2. No significant differences could be observed at baseline between both randomization groups with respect to gender, age, stool frequency and faecal bifidobacteria level.

Treatment compliance

The number of times per treatment period that the volunteers of the PP population did not take in a serving of soft drink was on average very low (2.0% during the placebo intake period and 1.8% during the WBE-intake period) indicating a good compliance to the study based on self-reporting by the volunteer. No statistically significant differences ($P > 0.1$) were observed between the treatments. None of the volunteers reported that they had incidentally taken in prebiotics or probiotics during the study. Hence, overall compliance to the study was considered to be good.

Adverse Events

Adverse Events (AEs) were categorized in 6 categories according to the National Cancer Institute Common Terminology Criteria for Adverse Events (v3.0) prior to the unblinding of the study. During the run-in period, placebo intake period and WBE intake period, 4, 7 and 5

AEs occurred, respectively. Statistical analysis of the AEs in the safety population revealed no difference between the placebo and WBE treatment in frequency of any of the different AE categories ($P > 0.1$).

Analysis of efficacy variables

Conditional F-tests showed overall WBE-related significant treatment effects for 5 parameters (Table 3): faecal levels of bifidobacteria, percentage of bifidobacteria in faeces, faecal levels of isobutyric acid, faecal levels of isovaleric acid and faecal levels of total BCFAs ($P < 0.1$). The main results of the subsequent pairwise comparisons of these parameters will be discussed below.

Levels of faecal microbiota

In the PP population, WBE intake selectively increased bifidobacteria levels in the faeces (Table 3). Intake of 5 g/d WBE tended to increase the levels of bifidobacteria in the faeces relative to placebo intake by 0.19 log units ($P = 0.069$). The percentage of Bifidobacteria relative to the total bacterial content in faeces upon 5 g/d WBE intake increased by 1.7-fold relative to placebo intake ($P = 0.002$). The faecal levels of the other bacterial groups analyzed, the *Lactobacillus/Enterococcus* group, the *Clostridium histolyticum/lituseburense* group, the *Faecalibacterium prausnitzii* group and the *Roseburia/Eubacterium rectale* group, remained unchanged after WBE intake.

Biochemical parameters in faeces

Intake of 5 g/d WBE decreased the level of total faecal BCFAs and the levels of isobutyric acid and isovaleric acid by about 28% relative to placebo intake ($P < 0.05$) (Table 3).
WBE intake did not affect the percentage moisture in faeces, nor did it influence faecal ammonia levels, faecal SCFAs levels and faecal pH ($P > 0.1$).

Bowel habits: defecation frequency and stool consistency

WBE intake did not influence the number of bowel movements per day, nor did it modulate stool consistency as measured using the Bristol Stool Form Scale ($P > 0.1$).

Analysis of tolerance variables

Tolerability was assessed through self-reported scoring by the children of the distress severity of flatulence, urge to vomit, and abdominal pain/cramps, using a 5-step scale ranging from no (0), minimal (1), mild (2), moderate (3) to severe (4) distress. In Table 4, an overview of the scoring of the distress severity of the three surveyed gastrointestinal symptoms can be found. Statistical analysis of the distress severity of the three GI symptoms in the PP population demonstrated no difference between placebo and WBE treatment ($P > 0.1$, binary mixed model).

DISCUSSION

This study investigated, for the first time, the effect of WBE consumption in healthy preadolescent children (aged 8-12 years). The effects of WBE consumption at a dose of 5 g/day on following gastrointestinal parameters were analyzed: faecal levels of microbiota, SCFAs, BCFAs, ammonia, faecal pH and faecal moisture. In addition, using self-reported scoring of the distress severity of three gastrointestinal symptoms, tolerance to WBE was assessed in children.

WBE consumption by healthy children during 3 weeks at a daily dosage of 5 g led to an increase in faecal bifidobacteria levels, expressed as percentage of total microbiota, relative to placebo intake. These data extend earlier studies evaluating the effect of WBE and WBE-like material on faecal microbiota in healthy adult volunteers[7-9, 35], despite the fact that the relative level of faecal bifidobacteria in preadolescent children is higher than in adults[24]. As was also observed in adult volunteers, intake of WBE by children only modulated faecal

levels of Bifidobacteria. The levels of the other bacterial groups analyzed, i.e. the *Lactobacillus/Enterococcus* group, the *Clostridium histolyticum/lituseburense* group, the *Faecalibacterium prausnitzii* group and the *Roseburia/Eubacterium rectale* group, were not modulated upon WBE intake. This points to a selective increase of faecal bifidobacteria levels relative to total faecal microbiota, upon WBE intake by healthy children.

Beneficial effects of bifidobacteria on host health have been demonstrated through placebo-controlled clinical trials involving direct oral supplementation with viable bifidobacteria. For instance, oral intake of bifidobacteria by healthy infants was shown to lower the risk of experiencing respiratory infections[36]. In addition, studies performed on patients suffering mild to moderate irritable bowel syndrome showed that supplementation with bifidobacteria improves symptoms of abdominal pain/discomfort, distension/bloating, and bowel movement difficulty[37,38]. The mechanisms responsible for such effects have not been fully elucidated, yet may involve modification of the gut microbiota, competitive adherence to the intestinal mucosa and epithelium, strengthening of the gut epithelial barrier, and/or modulation of the immune system through interaction with pattern recognition receptors on gut epithelial cells[39].

Intake of 5 g/d WBE resulted in a marked reduction of the faecal levels of BCFAs isobutyric acid and isovaleric acid by 28% as compared to the faecal BCFAs levels after placebo intake. BCFAs are not produced by human enzymes and are therefore unique bacterial metabolites. Isobutyric acid and isovaleric acid are produced from the fermentation of valine and leucine, respectively[40]. As a consequence, excretion of BCFAs is often considered as a marker for the degree of protein fermentation in the colon[41]. The reduction in protein fermentation observed in this study confirms previous results in adults, which showed a reduction in urinary and faecal p-cresol[7,8,10] or a beneficial modulation of the colonic ammonia metabolism (both protein fermentation metabolites)[42] after intake of WBE or WBE-like material. Colonic

fermentation of proteins results in the formation of ammonia, nitrosamines, thiols and phenolic compounds, which are generally believed to be potentially harmful. Hence, reduction of colonic protein fermentation is believed to be beneficial to human health[43].

Consumption of 5 g/day WBE did not result in increased faecal levels of the carbohydrate fermentation products acetate, propionate and butyrate. François and co-workers showed increased faecal levels of these SCFAs after intake of 10 g/day WBE in healthy adults, but not after intake of 3 g/day WBE[8]. The fact that an increase in faecal SCFA levels was not observed could be due to the intake of a WBE dose that was too low to modulate faecal SCFA levels. However, it should be kept in mind that faecal SCFA levels are the result of both colonic SCFAs production and mucosal absorption of these SCFAs[44]. As such, the absence of increased faecal SCFA levels does not exclude an increased colonic fermentation following WBE fermentation.

Intake of 5 g/d WBE by healthy children did not affect stool frequency, stool consistency or the composite Bristol measure. In addition, 5 g/ day WBE consumption did not have an effect on any of the three surveyed gastrointestinal symptoms: flatulence, abdominal pain/cramps, urge to vomit. In healthy adult volunteers, a mild increase of flatulence was observed at 10 g/d WBE intake[11]. Mild to moderate flatulence is observed in studies with other prebiotic compounds such as inulin and fructooligosaccharides, which is caused by the production of gases upon fermentation of the prebiotic compound[45-49].

The low incidence of GI complaints together with the absence of a difference between the placebo and WBE treatment in occurrence frequency of the AE categories provides evidence for the excellent tolerability and safety of WBE in children. This is important since addition of WBE to food products intended for children may be a way to increase the fiber intake by these children. Indeed, intake data from the USA indicate that dietary fiber consumption is

inadequate in most children, especially from low-income and minority backgrounds[50]. In this respect, it is also important to note that WBE is water-soluble and does not have a pronounced taste which makes it easy to formulate in food products without affecting their texture or taste. Other fiber sources, such as insoluble fibers, can disturb the taste or texture of a food product and thereby make it less attractive for consumption, in particular by children.

In conclusion, intake of 5 g/d WBE exerts beneficial effects on gut parameters, in particular reduction of colonic protein fermentation and increase of relative faecal bifidobacteria levels. Moreover, WBE is well tolerated and does not cause adverse effects at up to 5 g/d in healthy children.

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FIGURE & Table Legends

FIGURE 1. Schematic representation of the study design. The study started with a one-week run-in (RI) period, followed by two 3-week treatment periods in which Wheat Bran Extract (WBE) was taken in by the subjects at a dose of 0 g/d (placebo) or 5 g/d (with the order differing between the two randomisation groups). The treatment periods were separated by a wash out (WO) period. Faecal samples were taken at different, indicated time points. During the run-in period and the last week of both treatment periods, the subjects completed daily a questionnaire assessing the distress severity of flatulence, urge to vomit and abdominal pain/cramps. Additionally, subjects recorded in the Bowel Habits Diary the number of bowel movements and stool consistency during the one-week run-in period and during the last week of each 3-week treatment period. RI = run-in; WO = wash out; GI = gastrointestinal.

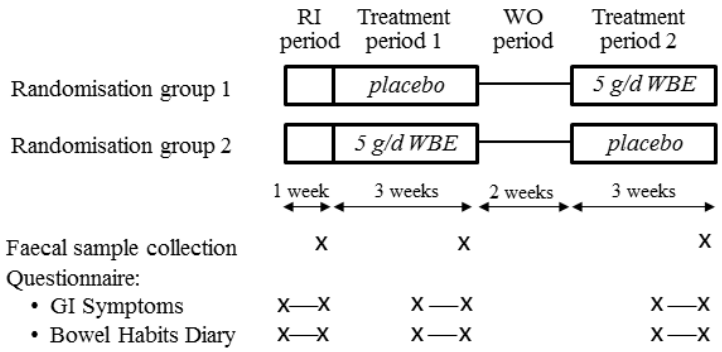
FIGURE 2. Volunteer disposition in the study.

TABLE 1. Characterization of the WBE preparation

TABLE 2. Baseline data of the EE/PP population of children participating to the study

TABLE 3. Efficacy variables during the intervention study following intake of placebo and 5 g/d WBE. P is the p-value for the pairwise comparison between 5 g/d WBE and placebo

TABLE 4. Overview of the scoring of the three surveyed gastrointestinal symptoms



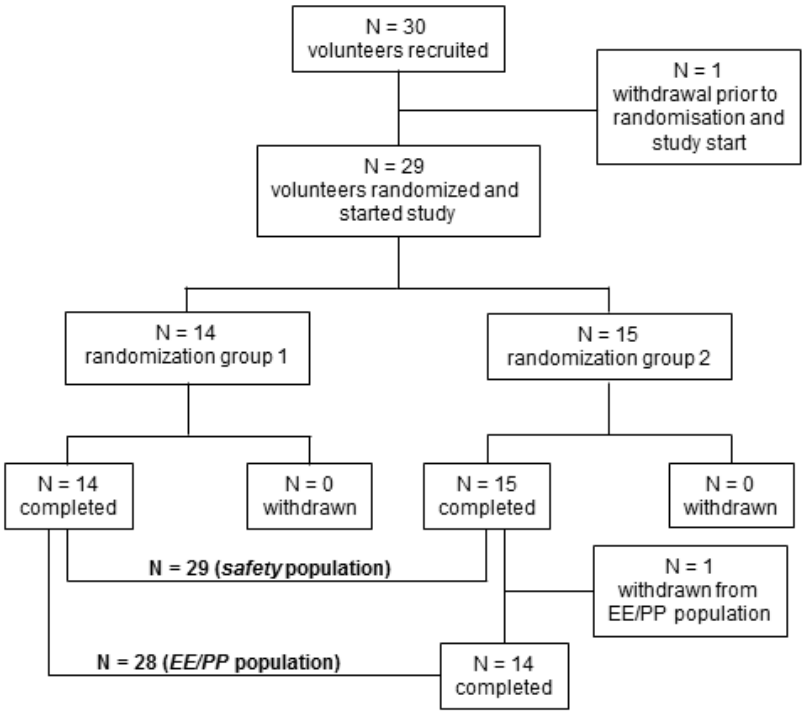


TABLE 1. Characterization of the WBE preparation

Constituent	Concentration (% of dry matter)
Arabinoxylan-oligosaccharides (AXOS)	79.0
of which xylo-oligosaccharides (XOS_{DP3-9})	39.5
of which xylobiose (XOS_{DP2})	22.2
Glucuronic acid bound to AXOS	1.0
Ferulic acid bound to AXOS	1.5
Glucose (as part of poly/oligosaccharides)	12.2
Galactose (as part of poly/oligosaccharides)	1.5
Total free monosaccharides	0.5
Protein (N x 6.25)	0.6
Total lipids	<0.5
Ash	0.2
Dry matter (%)	96.4

DP = degree of polymerization

TABLE 2. Baseline data of the EE/PP population of children participating to the study

Randomisation group	Subjects (No)	Gender		Age (years)		Stool frequency		Bifidobacteria (log_{10} cells/g dry faeces)	
		Female (No)	Male (No)	Mean	SD	Mean	SD	Mean	SD
1	14	4	10	9.86	1.46	1.06	0.55	9.05	0.55
2	14	6	8	9.79	1.19	1.00	0.43	8.72	0.34
All	28	10	18	9.82	1.31	1.03	0.09	8.89	0.48
Test statistic		0.70[1]		0.81[2]		0.96[2]		0.068[3]	

[1] Fisher's Exact Test for Count Data test

[2] Mann-Whitney U-test

[3] One-Way ANOVA

TABLE 3. Efficacy variables during the intervention study follwing intake of placebo and 5 g/d WBE. *P* is the p-value for the pairwise comparison between 5 g/d WBE and placebo

	Placebo treatment period		5 g/d treatment period		
	Mean	SD	Mean	SD	*P*
Levels of faecal microbiota					
Total bacteria (log_{10} cells/g dry faeces)	10.79	0.41	10.74	0.34	0.403
Bifidobacteria (log_{10} cells/g dry faeces)	9.17	0.47	9.36	0.41	**0.069**
Percentage Bifidobacteria	3.25	2.66	5.67	3.89	**0.002**
Lactobacilli (log_{10} cells/g dry faeces)	5.73	2.20	5.98	2.04	0.962
F. prausnitzii (log_{10} cells/g dry faeces)	9.32	0.73	9.35	0.49	0.843
C. histolyticum/lituseburense (log_{10} cells/g dry faeces)	3.52	1.77	3.14	1.19	0.390
Roseburia/Eubacterium rectale (log_{10} cells/g dry faeces)	8.97	1.79	8.99	1.67	0.979
Biochemical parameters of faeces					
Acetic acid (µmol/g dry faeces)	357.44	210.85	348.61	110.68	0.845
Propionic acid (µmol/g dry faeces)	79.61	47.54	78.65	40.41	0.915
Butyric acid (µmol/g dry faeces)	84.18	55.15	67.83	33.25	0.143
Total SCFAs[1] (µmol/g dry faeces)	521.23	292.82	495.09	160.68	0.791
Isobutyric acid (µmol/g dry faeces)	11.37	6.10	8.10	3.94	**0.005**
Isovaleric acid (µmol/g dry faeces)	14.36	7.76	10.55	5.62	**0.008**
Total BCFAs[2] (µmol/g dry faeces)	25.74	13.81	18.65	9.52	**0.006**
Stool pH	7.28	0.58	7.05	0.82	0.110
Stool moisture (%)	72.03	6.41	70.04	5.89	0.230
Ammonia (mg/g dry faeces)	2.21	0.81	2.25	1.06	0.874
Bowel habits					
Stool frequency (# bowel movements/day)	0.99	0.65	0.99	0.53	1.000
Stool consistency (average stool consistency/bowel movement)	3.36	0.96	3.01	0.82	0.308
Bristol composite measure (average stool consistency/day)	2.50	1.27	2.41	1.07	0.775

[1] Total SCFAs levels are defined as the sum of the levels of acetic acid, propionic acid and butyric acid;
[2] Total BCFAs levels are defined as the sum of the levels of isobutyric acid and isovaleric acid.

TABLE 4. Overview of the scoring of the three surveyed gastrointestinal symptoms

		Placebo treatment period		5 g/d WBE treatment period	
GI symptom	Average score[1]	Number[2]	Percentage[3]	Number	Percentage
Abdominal pain	0	20	71	17	61
	> 0 -0.5	6	21	7	25
	> 0.5 - 1	2	7	3	11
	> 1 - 1.5	1	4	1	4
Urge to vomit	0	27	96	27	96
	> 0 -0.5	1	4	1	4
Flatulence	0	13	46	11	39
	> 0 -0.5	8	29	7	25
	> 0.5 - 1	5	18	7	25
	> 1 - 1.5	1	4	2	7
	> 1.5 - 2	1	4	1	4

[1] The average score is the average for the 7 days period during which symptoms were surveyed;
[2] The number of volunteers with a specified average gastrointestinal (GI) symptom severity score is shown for each treatment;
[3] The percentage of volunteers with a specified average GI symptom severity score is shown (denominator for the calculation of the percentage is the number of volunteers in the specified treatment period).