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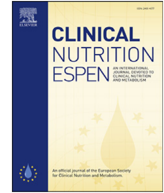

Randomized Controlled Trial

Effects of defatted rice bran-fortified bread on gut microbiome, cardiovascular risk, gut discomfort, wellbeing and gut physiology in healthy adults with low dietary fibre intake

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SUMMARY

Background & aims: Inadequate dietary fibre (DF) intake is associated with suboptimal gut function and increased risk of several human diseases. Bread is commonly consumed and is ideal to incorporate cereal bran to increase DF content. No human studies have investigated the effects of defatted rice bran (DRB) in bread, which has triple the DF of white bread, purported hypo-allergenicity and a unique nutrient profile, as a dietary intervention in healthy adults. This study aims to assess the relative abundances of a composite of key faecal microbial genera and species involved in DF fermentation and metabolism following the habitual intake of DRB-fortified bread and its influence on other biological markers of host and microbial interactions, cardiovascular risk profile, patient-reported outcomes, total DF intake, and gut physiology in healthy adults with low baseline DF intake.
Methods: Fifty-six healthy adults with low baseline DF intake (<18 g/day (females), <22 g/day (males)) completed a two-arm, placebo-controlled, double-blind, randomised, crossover study. Participants consumed three (females) or four (males) slices of DRB-fortified bread or control bread daily as part of their usual diet for four weeks, with the intervention periods separated by a two-week washout. Outcomes included faecal microbiota composite (primary outcome); relative abundances (taxa and gene); faecal moisture content and bile acid concentrations; plasma and faecal organic acid concentrations; cardiovascular risk profile; gut comfort, psychological wellbeing parameters; total DF intake; whole gut transit time, and were measured at baseline and following each intervention phase. Additionally, in a sub-study, 15 participants ingested gas-sensing capsules to assess whole and regional gut transit times, and total and regional colonic hydrogen and carbon dioxide concentrations at the same timepoints.
Results: DRB-fortified bread consumption significantly increased total DF intake from 20.7 g/day to 43.4 g/day ($p < 0.001$). No significant differences were observed in the primary outcome, microbial taxa composite within and between groups (False Discovery Rate (FDR) correction, $p > 0.10$). As compared to control, the DRB group had increased relative abundances of *Faecalibacterium prausnitzii* (unadjusted $p = 0.04$), *Bifidobacterium longum* (unadjusted $p = 0.12$), and *Bacteroides ovatus* (unadjusted $p = 0.10$); lower relative abundances in *Coprococcus* genus (unadjusted $p = 0.09$), *Roseburia faecis* (unadjusted

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$p = 0.02$) and *Prevotella copri* species (unadjusted $p = 0.05$). However, no significant differences were observed in the relative abundances of these taxa within and between groups (FDR correction $p > 0.10$) and for most of the other outcomes between groups ($p > 0.05$). Only mean serum high-density lipoprotein (HDL) concentrations significantly increased ($p = 0.006$), and mean total cholesterol (TC) to HDL concentration ratio significantly lowered ($p = 0.02$) in the DRB group compared to the control group.
Conclusion: This is the first human study to show that a high-DF DRB-fortified bread improved DF intake, HDL cholesterol profiles, and may affect the gut microbiota composition in healthy adults with low DF intake. These findings support the substitution of white bread with DRB-fortified bread as an effective method to improve DF intake, which may have subsequent benefits on gut physiology and metabolic health.
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1. Introduction

Dietary fibre (DF) has a crucial role in modulating the gut microbiota composition [1,2] and reduces the risk of developing several chronic diseases, including coronary heart disease [3], type 2 diabetes, and colorectal cancer [3,4]. The appropriate type and amount of DF consumed may modulate gut microbiota diversity, stability, and resilience [5,6]. Additionally, fermentation of DF by microbial species produces metabolites such as organic acids (including short chain fatty acids) and bile acids (BA) [3], which have roles in regulating human physiological and immunological functions [3,4]. Thus, adequate DF intake is crucial for the survival and growth of microbial species and the maintenance of general human cellular functions [7].

Although DF is found abundantly in plant-based foods, inadequate DF intake is still ubiquitous in adults globally [8–15]. Fortifying commonly consumed foods with DF may, therefore, help increase DF intake [2]. Among these foods, bread is the main source of DF worldwide [9,13,15,16] and is ideal for incorporating cereal brans to increase DF content [17,18]. Most human studies have used wheat bran or fibre, as summarised in a systematic review [19]. The reviewed studies of different cereal fibres, nonetheless, show inconsistent results, whereby the majority showed no effect or did not measure changes in microbial abundances [19]. It is noteworthy that different cereal grains have varied DF content, types, physicochemical properties, and processing methods (for instance, milling, heating, flaking, extrusion) [19]. This can further complicate their effect on the gut microbiome and their subsequent health benefits for the human host.

Rice bran is a cheap cereal by-product of rice milling [17] and has a purported hypo-allergenicity and a unique nutrient profile rich in DF, protein, antioxidants, minerals, and phytochemicals [16,20–22]. Only a limited number of human studies have been undertaken on rice bran [23–40]. Some human adult studies have reported rice bran supplementation effects on the gut microbiome. Specifically, four studies explored heat-stabilised rice bran [24–26] or rice bran [27] of different dosages, either 30 g/day [24,26] or 40 g/day [26]. Further, these studies administered their interventions either in the form of powder [25] or consumed with other foods [27]. Based on the literature, no studies have used defatted rice bran (DRB)-fortified bread as a dietary intervention in healthy adults. The process of defatting rice bran increases its insoluble DF content and, subsequently, the proportion of total DF content [17]. Thus, we hypothesised that a four-week habitual intake of DRB-fortified bread, as compared to control bread, would increase the relative abundance of a composite of selected key genera and species of the faecal gut microbiota (primary outcome), change faecal relative abundance/concentration and/or faecal or plasma biological markers of host and microbial interaction, improve blood lipid concentrations, patient-reported outcomes and gut physiology in healthy adults with low baseline DF intake.

2. Material & methods

2.1. Study design

This study is a double-blinded, randomised, placebo-controlled, crossover trial of healthy adults with low baseline DF intake. The trial design and management conformed with the CONSORT guidelines [41]. The study protocol was previously published [42]. The study was carried out in accordance with the International Conference of Harmonisation Guidelines and the Declaration of Helsinki.

2.2. Study population

Interested volunteers were assessed for eligibility for the study (**ACTRN12622000884707**). Eligible participants were of good general health; aged between 18 and 65; body mass index (BMI) between 18 and 35; low DF intake (<22 g/day for males and <18 g/day for females) as measured by a dietary fibre intake-food frequency questionnaire [43]; no known gut disorders and diseases, systemic conditions and chronic diseases; no antibiotic use within the last month; willing to consume three slices (females) and four slices (males) of bread provided during the intervention periods. Detailed eligibility criteria have been described in our published protocol [42]. All participants gave written informed consent prior to participating in the study. Their privacy rights were observed as approved by the University of Otago Human Ethics Committee for Health (H22/061), and the University of Otago Christchurch Māori Research Consultation.

2.3. Recruitment and screening

Recruitment and data collection began in June 2022 and concluded in December 2022, when the number of required participants were achieved, in Christchurch, New Zealand (NZ). Interested individuals were given a study information sheet and an online screening questionnaire to ascertain eligibility. Sixty-six individuals were screened, and 62 participants were randomised in the study. In the first allocation, 30 participants were assigned to the DRB group and 32 participants to the control group (Fig. 1).

2.4. Study intervention

The main intervention was white wheat bread fortified with DRB. It is a commonly eaten cereal bran and is safe for human consumption [17,44–48]. The ingredients for the control white bread included wheat flour, water, yeast, canola oil, white vinegar, iodised salt, soy flour, emulsifier (481), cultured wheat, vitamin (folic acid), and processing aids (wheat). The DRB-fortified bread was manufactured from matched commercial white bread, where 18 % of the flour (cereal weight) was replaced with DRB. Bread slices were chosen to ensure the production of DRB-fortified bread had a texture similar to the control white bread while providing the required DF content. The nutrition information for each bread product is shown in Table 1, and was assessed in duplicate using the following methodologies: ash AOAC 942.05, protein AOAC 968.06, fat AOAC 954.02, dietary fibre, insoluble fibre and soluble fibre AOAC 991.43.

Participants were randomly assigned to a 1:1 allocation using randomised permuted blocks (block size 4) to either receive DRB or control bread in Allocation 1. Randomisation was performed by drawing a folded note from a sealed opaque box. Participants were randomised to consume either three slices (for females) or four slices (for males) of DRB-fortified bread or control bread, daily for four weeks. Following a two-week washout period, participants then crossed over to the alternate bread in Allocation 2, with those initially consuming DRB-fortified bread subsequently consuming control bread and vice versa. The study breads were provided frozen and were collected by participants from the research clinics. Participants were instructed to only replace their standard bread with study bread during the intervention phase, and maintain their usual diet and lifestyle throughout the study period. Participants completed a daily bread diary via a mobile app to assess whether they were compliant with the consumption of the intervention.

Participants were kept blinded until after the statistical analysis of the primary outcome data was completed. The bread manufacturer was responsible for labelling and adding coloured malt to the control bread to maintain blinding. Specified research team members were responsible for the randomisation, enrolment and data collection of participants, the handout of the intervention and control breads, and the management of the stock.

2.5. Study outcomes

The primary outcome of this study was the difference in the relative abundances of a composite of selected key genera and species of the gut microbiota known to be involved in plant glycan metabolism and/or known to be modulated by the DRB-fortified bread as compared to the control bread.

Key secondary outcomes were the relative abundances of individual taxa, predictive function (gene abundances) and diversity indices of the faecal gut microbiota, faecal and plasma metabolites as assessed by liquid chromatography-mass spectrometry (LCMS); cardiovascular risk profiles based on anthropometry, blood pressure, and blood lipid concentrations; gut comfort, mental health and general wellbeing (psychological wellbeing) using validated questionnaires; DF intake as assessed by three-day food diaries; whole gut transit time using blue food dye, regional and whole gut transit time and gas fermentation profiles using Atmo gas-sensing capsules.

2.6. Study measurements

2.6.1. Faecal and blood sample collections

The detailed procedures for faecal and blood sample collections, microbiome sequencing (raw sequencing data: NCBI RefSeq

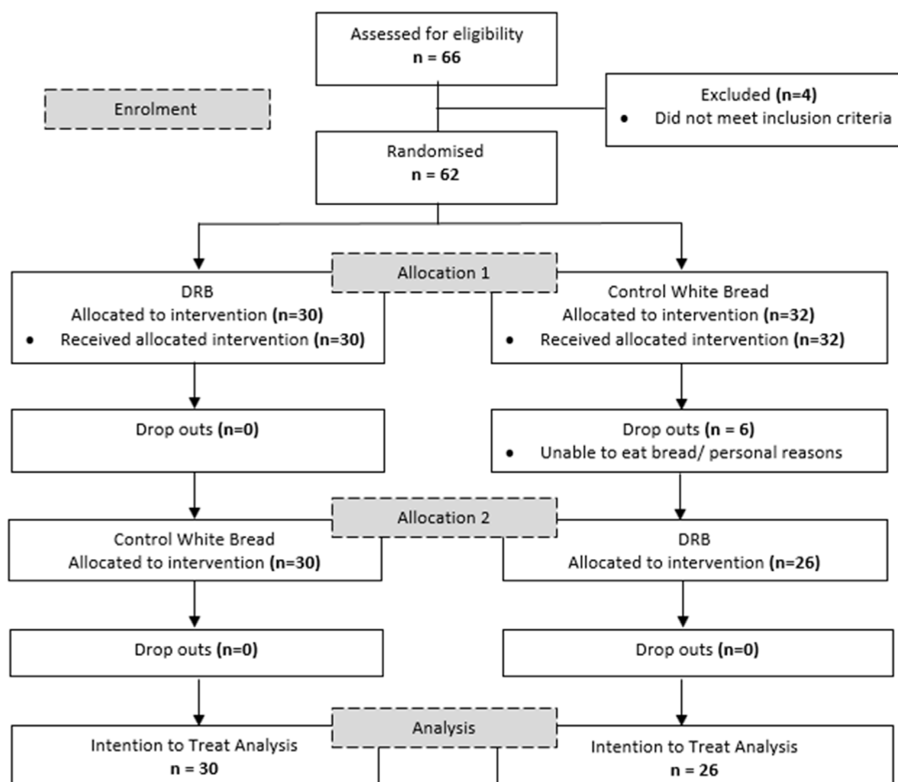

Fig. 1. Study flow diagram.

Table 1
Nutrition information for intervention bread.

	DRB-Fortified Bread			Control White Bread		
	Per 100 g	Per 3 Slices (111 g)	Per 4 Slices (148 g)	Per 100 g	Per 3 Slices (111 g)	Per 4 Slices (148 g)
Energy (kJ)	979	1086.7	1448.9	1055	1171.1	1561.4
Protein (g)	10.7	11.9	15.8	10.8	12.0	16.0
Fat, total (g)	2.9	3.2	4.3	2.3	2.6	3.4
Carbohydrate (g)	37.2	41.3	55.1	45.0	50.0	66.6
Sugars (g)	0.4	0.4	0.5	0.3	0.4	0.5
Dietary fibre (g)	7.2	8.0	10.6	2.4	2.7	3.6
Insoluble fibre (g)	6.8	7.5	10.1	2.4	2.7	3.6
Soluble fibre (g)	0.5	0.5	0.7	0.1	0.1	0.1
Starch (g)	54.8	60.8	81.1	50.4	55.9	74.6
Resistant starch (g)	0.4	0.4	0.5	0.4	0.4	0.6
Arabinoxylan (g)	2.1	2.3	3.0	0.6	0.7	0.9
Sodium (mg)	358.5	398.0	530.6	369.4	410.0	546.7

DRB: Defatted Rice bran.

database number GCF_000001405), and data processing have been previously published [42].

Briefly, participants collected a faecal sample into a provided container 24 h before each clinic visit, labelled with the time and date collected and recorded on the daily bowel movement diary app. The collected faecal samples were refrigerated at 4 °C and brought to their clinic visit.

During each clinic visit, 2 x 6 mL blood samples were drawn into lithium-heparin vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to analyse blood lipid profile (further details are described in the next section), plasma metabolome and known metabolites (BA and organic acids). One 6 mL vacutainer was kept at room temperature and delivered for analysis (Canterbury District Health Laboratory, Christchurch, NZ) within 1 h of collection. The other 6 mL vacutainer was kept on ice and centrifuged within 1 h at 4 °C. Plasma samples were distributed into 500 μL aliquots and stored at −80 °C until analysis.

2.6.2. Microbiome analysis

2.6.2.1. Faecal microbial composition and gene abundances. Taxonomic composition and gene abundance of the faecal microbiome were assessed by shotgun metagenomics as published [42]. Briefly, Auckland Genomics performed libraries for metagenomics sequencing of the extracted DNA at the University of Auckland, NZ. Microbial metagenomic libraries were then generated using the Seqwell PurePlex DNA library preparation approach. The DNA samples underwent quality control checks for purity and concentration and were shotgun sequenced using the Illumina Novaseq 6000 platform at Novogene in Singapore. Metagenomics functions were obtained through the “blastx” function of Diamond (Version 0.9.22) [49], mapping the reads against the “non-redundant” National Centre for Biotechnology Information (NCBI) database [50]. Megan (Version 6 ultimate edition) [51] was used to assign putative functions to the alignment files produced by Diamond. Differential relative abundance of taxa was performed using DESeq2 in R (Version 1.40.2) [52].

2.6.2.2. Faecal and blood metabolites. Faecal and plasma organic acids (including short chain fatty acids) were extracted and measured as previously described [53–55] using MS-probe and stable isotope techniques combined with targeted LCMS [53–55]. Faecal BA were extracted following those outlined previously [56] and measured using high-resolution LCMS [57].

2.6.2.3. Faecal and blood metabolome. Metabolomics was used to assess the relative intensity of faecal and blood metabolites in the polar, semi-polar, and non-polar fractions of faecal and plasma samples, as previously described [58–60].

Faecal samples were freeze-dried under vacuum and extracted with a bi-phasic extraction for polar and semi-polar analytes from the aqueous layer and lipids from the non-aqueous layer [61]. The metabolite profiling analyses were carried out using high-resolution LCMS on a Shimadzu 9030 quadrupole time-of-flight mass spectrometry equipped with electrospray [58]. An aliquot of the polar extract was analysed using hydrophilic interaction liquid chromatography [58], and semi-polar metabolites were resolved using reverse-phase chromatography [59]. The organic phase was analysed using the reverse-phase lipidomic methodology [60].

Plasma polar metabolites were extracted using a single-phase extraction [59] with pre-chilled acetonitrile:water (9:1 v/v). Plasma semi-polar metabolite was extracted using ice-cold chloroform:methanol (1:1 v/v) [62]. Lipid extraction was performed using butanol:methanol (1:1 v/v) and then spiked with an internal standard SPLASH mix (Avanti Lipids, Merck KGaA, Darmstadt, Germany) [60]. Extractions of plasma polar, semi-polar and non-polar extracts were analysed using the same LCMS methods as for faecal samples above [58–60].

2.6.3. Cardiovascular risk profile

2.6.3.1. Anthropometry. Height, weight, BMI, and waist circumference were measured using calibrated equipment at each visit according to the previously established procedures [63]. All measurements were conducted twice, with a third reading when the two readings varied by more than 0.5 units.

2.6.3.2. Blood pressure. Blood pressure was also measured at each morning clinic visit according to the Australian Expert Consensus recommendations [64]. Measurements were taken on their non-dominant arm.

2.6.3.3. Blood lipid profile. The full blood lipid profile was measured at each morning clinic visit according to the recommendations of the Canterbury District Health Laboratory (Christchurch, NZ). The lipid profile consists of five components: total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), and TC/HDL ratio.

2.6.4. Patient-reported outcomes

2.6.4.1. Daily bowel movement and daily bread diaries. Throughout the study period, participants were asked to complete a daily bowel movement and bread diary via an app. The bowel movement diary provided a comprehensive record of the bowel habits of the participants; the daily bread diary assessed study

compliance, whereby the proportion of participants meeting above 50 % was deemed compliant.

2.6.4.2. Gut comfort. The Gastrointestinal Symptom Rating Scale (GSRS) was used to assess gut comfort at baseline and during the intake of DRB-fortified bread and control bread. The GSRS has a one-week recall that assesses gut symptom severity using a seven-grade Likert scale, ranging from 1 ("no discomfort at all") to 7 ("very severe discomfort") [65–67].

2.6.4.3. Psychological wellbeing. Participants were also instructed to complete five psychological wellbeing (mental health and general wellbeing) questionnaires, i.e., Patient-Reported Outcome Measurement Information System: Anxiety, Depression [68,69]; World Health Organisation - Five Question Well-Being Index [70]; Warwick-Edinburgh Mental Wellbeing Scales [71]; Multidimensional Fatigue Inventory Short Form [72]; Subjective Vitality Scale [73], at each of the baseline and post intervention phases. Detailed information on each questionnaire has been described in the previously published protocol [42].

2.6.5. Dietary intake

Participants completed non-consecutive three-day food diaries, one weekend and two weekdays, to ascertain their usual food intake before and after each intervention. The food diaries were then entered into a dietary analysis software, FoodWorks Online Professional (Version 1.0, Xyris Software, Brisbane, Australia, 2021) [74]. After the primary outcome was analysed and the study was unblinded to the intervention, the macro- and micro-nutrients from the study bread were manually entered into the dataset based on the reported bread consumption.

2.6.6. Gut physiology

2.6.6.1. Blue food dye. Blue food dye was used to assess whole gut transit time upon passing of bowel motion via visual confirmation [75,76]. At each baseline and post intervention visit, participants ingested one teaspoon of blue food colouring in water. The intake time and date were recorded, and the participant recorded visual confirmation on the daily bowel movement diary app.

2.6.6.2. Atmo gas-sensing capsule sub-study. Fifteen participants received a separate participant information sheet and completed a specific consent form for colonic gas profiling and assessment of whole and regional gut transit time by the Atmo gas-sensing capsule [77,78]. The capsule was ingested at each baseline and post-intervention visit before being fitted with a sling bag containing an Atmo transponder. Upon excretion of the capsule or the return of the transponder, the unidentified data from the Atmo app were then reviewed and analysed by trained Atmo team members.

2.7. Statistical analysis

Sample size calculations were based on the GutFeelingKB cohort [79]. The percentage relative abundance of a composite microbiome index (incorporating 15 operational taxonomic units) was estimated to be 28.3 % with a standard deviation of 14 %. The sample size needed was 60 participants in this crossover study to account for a 15 % dropout rate. The composite microbiome index and statistical plan were described in detail in our previously published protocol [42]. However, briefly, the mean differences from the baseline of most outcomes were compared between groups and referred to as between group comparisons using 2-tailed (parametric) t-tests and (non-parametric) tests for symmetrically and asymmetrically distributed data. Within-group analyses (post intervention compared to baseline) for most outcomes were also performed. A two-tailed $p < 0.05$ was determined as statistical significance. A $p \leq 0.10$ was considered a trend for most outcomes except for the microbiome data.

To determine the primary outcome, the microbiome composite, the selected genera, and species were summed to give a composite relative abundance for each participant. For other faecal microbiome measures, univariate and multivariate statistical analyses were used to assess within and between groups (within groups and post intervention values between groups only for metabolome). To identify the most discriminant features, metabolites with a variable importance in the projection (VIP) score>1 were selected for filtered partial least squares projection-discriminant analysis (PLS-DA) modelling. These models were validated using the predictive ability of the model (Q2) and cross-validation ANOVA (CV-ANOVA) methods. Pathway analysis and metabolite compounds identification using KEGG database were described previously [80]. The Benjamini-Hochberg method was applied to control the false discovery rate (FDR) inherent in multiple-hypothesis testing. $P < 0.05$, Q2 values > 0.1 (for metabolome analyses) and FDR of $p < 0.10$ were deemed significant, with a probable biological significant difference (trend) assumed at unadjusted $p \leq 0.10$. These VIP-filtered and optimized models generated were not significant ($p > 0.05$) or robust, with all four models having Q2 values < 0.1.

3. Results

A total of 62 participants were eligible and were randomised in the study. In the first allocation, six participants dropped out of the control group for personal reasons or not being able to consume the number of slices required for the study. No dropouts were observed in the second allocation. A total of 56 participants (33 females and 23 males) were included in the analysis (30 in the DRB group and 26 in the control group). As depicted in Table 2, the mean age for the entire cohort was 40.4 (SD: 13.4) years with a mean BMI of 26.4 (SD: 3.9) kg/m^2, and a mean daily DF intake of 20.7 ± 7.2 g/day.

3.1. Microbiome and biological markers of host and microbial interactions

3.1.1. Faecal microbiome compositional composite

A total of 102 samples were analysed (n = 51 for the DRB group and n = 51 for the control group). No significant difference was observed in the primary outcome, the microbial composite, between groups (adjusted $p = 0.91$; Supplementary Table 1). Based on Cohen's guidelines, the effect sizes using standardised mean differences drawn between DRB and control groups were considered 'very small' (Cohen's d = 0.05, 95 % CI: −0.23, 0.32) [81,82]. This indicates a minimal intervention effect of DRB-fortified bread on the faecal microbial compositional composite.

3.1.2. Differences in microbial relative abundances

A total of 108 samples were analysed for microbial relative abundances in the DRB group and 111 samples in the control group. Relative abundances within groups are shown in bar charts and dot plots at different taxonomic levels (Supplementary Figs. 1–8). Differences in relative abundances for phylum, family, genus, and species levels within and between groups are displayed in Supplementary Tables 2–5.

Following FDR correction, no significant differences were observed in all taxa at each level within groups and between groups (adjusted FDR of $p > 0.10$). However, when probabilities were unadjusted, the DRB group, as shown in Fig. 2 and Supplementary Table 5, had increased relative abundances in species *Faecalibacterium prausnitzii* (family Ruminococcaceae, phylum Firmicutes) (mean change from baseline: 0.007; unadjusted $p = 0.04$),

Table 2
Baseline demographics.

Characteristics		DRB then Control Bread	Control then DRB Bread	All
		n = 30	n = 26	n = 56
Sex	Female	20 (66.7)	13 (50.0)	33 (58.9)
	Male	10 (33.3)	13 (50.0)	23 (41.1)
Age		41.5 ± 12.9	38.5 ± 11.8	40.4 ± 13.4
Body Mass index (kg/m^2)[a]		25.7 ± 3.7	27.1 ± 3.8	26.4 ± 3.9
Ethnicity	NZ European	24 (80.0)	17 (65.4)	41 (73.21)
	Māori	1 (3.3)	0 (0.0)	1 (1.79)
	Chinese	2 (6.7)	3 (11.5)	5 (8.93)
	Indian	1 (3.3)	0 (0.0)	1 (1.79)
	Other[b]	2 (6.7)	7 (26.9)	9 (16.07)
Highest level of qualification	University graduate (≥3 years)	11 (36.7)	7 (26.9)	18 (32.14)
	Postgraduate qualifications	7 (23.3)	8 (30.8)	15 (26.79)
	2 years at university	9 (30.0)	6 (23.1)	15 (26.79)
	NCEA level 3/University bursary	3 (10.0)	3 (11.5)	6 (10.71)
	Completed high school	0 (0.0)	2 (7.7)	2 (3.57)
Dietary Intake	Energy (kCal/day)	1899.4 ± 518.6	2101.7 ± 626.5	1993.3 ± 575.0
	Protein (g/day)	75.0 ± 27.3	95.8 ± 33.2	84.7 ± 31.7
	Total fat (g/day)	86.5 ± 29.8	99.3 ± 38.7	92.5 ± 34.5
	Carbohydrate (g/day)	183.6 ± 50.2	185.8 ± 49.6	184.6 ± 49.5
	Sugars (g/day)	81.5 ± 36.6	74.7 ± 30.7	78.3 ± 33.8
	Dietary fibre (g/day)	20.2 ± 5.9	21.3 ± 8.4	20.7 ± 7.2
	Vitamin C (mg/day)	63.5 ± 68.4	41.0 ± 28.5	53.0 ± 54.4
	Sodium (mg/day)	2695.9 ± 794.0	2775.5 ± 965.9	2732.9 ± 870.7
	Iodine (μg/day)	122.1 ± 199.8	77.8 ± 39.7	101.5 ± 149.2

Values are presented as counts (percentage) or mean ± SD.
DRB: defatted rice bran; NCEA: national certificate of educational achievement.
[a] Body Mass Index (BMI) is calculated as weight in kilograms (kg) divided by the square of height in metres (m) (kg/m^2).
[b] Others include British, Irish, Filipino, Indonesian, Latin American, South African, Latvian, Hispanic, and Egyptian.

Bifidobacterium longum (family Bifidobacteriaceae, phylum Actinobacteria) (mean change from baseline: 0.01; unadjusted p = 0.12) and *Bacteroides ovatus* (family Bacteroidaceae, phylum Bacteroidetes) (mean change from baseline: 0.0007; unadjusted p = 0.10) than the control group, which aligns with previous rice bran studies and is relevant for DF degradation and metabolism. However, the DRB group had lower relative abundance in the genus *Coprococcus* (unadjusted p = 0.09), *Roseburia faecis* (family Lachnospiraceae, phylum Firmicutes) (unadjusted p = 0.02) and *Prevotella copri* (family Prevotellaceae, phylum Bacteroidetes) (unadjusted p = 0.05) than the control group (Supplementary Table 4).

3.1.3. Alpha diversity

Table 3 shows the alpha diversity (Chao1 and Shannon) indices within and between groups. Chao1 index assesses species richness or the total number of species in a sample [83,84]. Shannon Index has a higher sensitivity for richness but can measure both richness and evenness [85,86]. A total of 107 samples (n = 52 for the DRB group, n = 55 for the control group) for baseline and post intervention were analysed. As measured by the Chao1 index, there was a significant difference in alpha diversity between groups, with a decrease in the DRB group as compared to the control group (difference between DRB and control: −8.07 ± 26.79; adjusted FDR of p = 0.02). However, no significant difference was observed for the Shannon Index between groups, with a decrease in the DRB group as compared to the control group (difference between DRB and control: −0.12 ± 0.62; adjusted FDR of p = 0.14) (Table 3).

Boxplots within groups and between groups are shown for the Chao1 Index (Fig. 3) and Shannon Index (Fig. 4). These figures showed no significant change in alpha diversity with both indices in the DRB group (p = 0.14 for Chao1 and p = 0.35 for Shannon

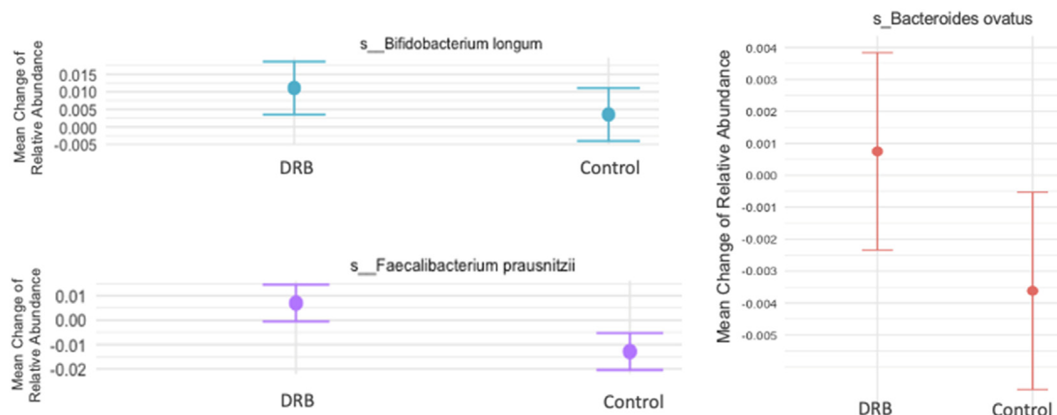

Fig. 2. Dot plots of relative abundances of microbial taxa at the species level within groups with error bars represent the standard deviation of the mean.

Table 3
Alpha diversity indices of the faecal microbiota within and between groups.

Alpha Diversity Index	Timepoints	DRB Bread			Control Bread			Mean Differences (DRB-Control)	Adjusted p value (FDR corrected) between groups
		Mean	SD	Adjusted p value (FDR corrected) within group	Mean	SD	Adjusted p value (FDR corrected) within group		
Chao 1 index	Baseline	111.87	13.15		108.72	12.84			
	Post intervention	108.31	14.18		113.24	12.81			
	Mean change	−3.56	17.90	0.14	4.51	19.93	0.06	−8.07 ± 26.79	0.02
Shannon index	Baseline	3.05	0.31		3.00	0.30			
	Post intervention	3.01	0.33		3.07	0.28			
	Mean change	−0.05	0.44	0.35	0.07	0.45	0.20	−0.12 ± 0.62	0.14

DRB: Defatted Rice Bran; SD: Standard Deviation.
Baseline and Post Intervention: n = 52 (DRB bread), n = 55 (control bread).
Mean differences are presented as mean ± standard deviation.

indices), while the control group showed a trend in increasing Chao1 (p = 0.06) but no change in Shannon index (p = 0.20) (Table 3).

3.1.4. Beta diversity

Baseline and post intervention Bray–Curtis Dissimilarity Index beta diversity values were determined using the Principal Coordinate Analysis and the Non-metric multidimensional scaling (NMDS) analysis. These data were widespread, and no clustering was observed at baseline and post intervention within groups (Supplementary Figs. 9–11). There were also similar total percentages of variance explained by both principal components (47 %) at baseline (Supplementary Fig. 9) and post intervention (Supplementary Fig. 10). Using Permutational multivariate analysis of variance (PERMANOVA) analysis, no significant differences were observed at baseline (p = 0.94) and post intervention (p = 0.43) (data not shown). This non-significance was also shown using Analysis of dissimilarity (ADONIS) analysis, comparing groups (p = 0.72) and the interaction within groups and between groups (p = 0.55) (data not shown).

3.1.5. Microbial gene relative abundance

A total of 13,916 genes were identified. There were no significant differences within groups, as indicated in the Principal Component (PC) analysis plots in Supplementary Figs. 12 and 13 (PC1 score: p = 0.78 for the DRB group, p = 0.20 for the control group; PC2 score: p = 0.51 for the DRB group, p = 0.20 for the control group).

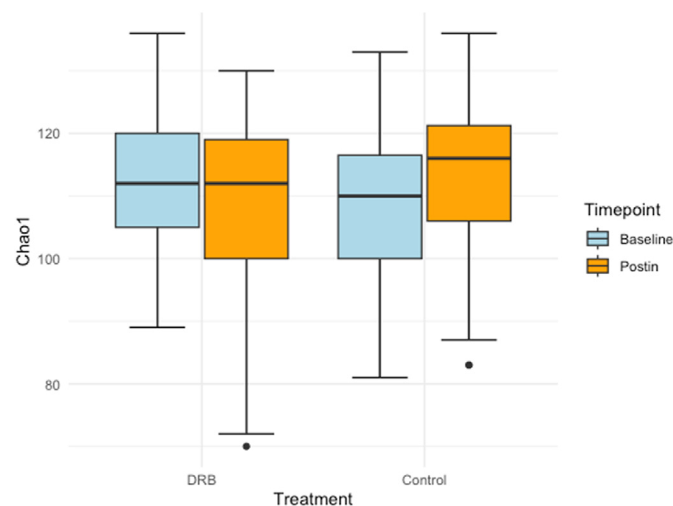

Fig. 3. Boxplot of the Alpha Diversity of the Faecal Microbiota as measured using the Chao1 Index Within and Between Groups.
DRB: Defatted Rice Bran; Postin: Post Intervention.

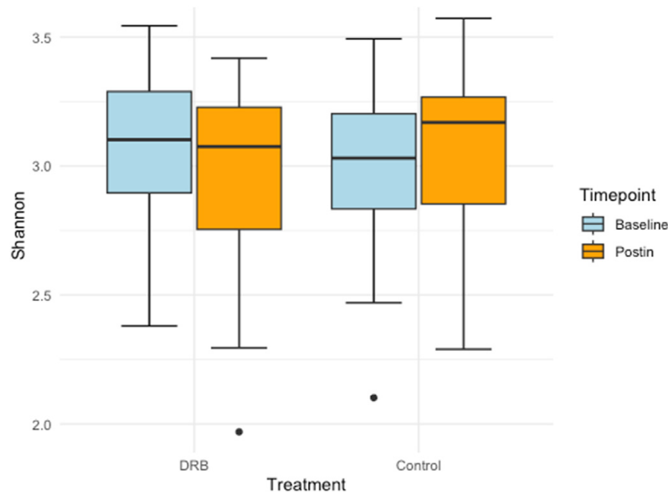

Fig. 4. Boxplot of the Alpha Diversity of the Faecal Microbiota as measured using the Shannon Index Within and Between Groups.
DRB: Defatted Rice Bran; Postin: Post Intervention.

When probability was unadjusted, at post intervention, genes with higher expression levels in the DRB group were associated with peptidases and inhibitors (log fold change (FC) = 1.30; unadjusted p = 0.005). The other genes with higher expression levels in the control group were related to glycan metabolism (logFC = 1.46; unadjusted p = 0.001), enzymes (logFC = 1.40; unadjusted p = 0.011), transporters (logFC = 1.28; unadjusted p = 0.003), biosynthesis of siderophore group non-ribosomal peptides (logFC = 1.26; unadjusted p = 0.006), purine metabolism (logFC = 1.24; unadjusted p = 0.024), and a two-component regulatory system (logFC = 1.21; unadjusted p = 0.028). Following FDR correction, no significant differences were observed in the differentially expressed microbial genes between groups post intervention (FDR of p = 1.00) (Supplementary Table 6).

3.1.6. Faecal moisture content and bile acid concentrations

A total of 112 samples [(DRB group (n = 56) and control group (n = 56)] were analysed for faecal moisture content and BA concentration at baseline and post intervention. The proportion of faecal moisture content and distribution of secondary BA were similar between groups. Total primary BA concentration (sum of cholic acid and chenodeoxycholic acid) also showed no significant difference between groups (p = 0.83) (Supplementary Table 7).

3.1.7. Faecal and plasma organic acid concentrations

A total of 108 faecal organic acid samples (n = 53 for the DRB group, n = 55 for the control group) and 100 plasma organic acid

samples ($n = 49$ for the DRB group, $n = 51$ for the control group) were analysed at baseline and post intervention. No significant differences were observed for faecal and plasma organic acids concentration between groups ($p > 0.05$) (Supplementary Tables 8 and 9).

3.1.8. Faecal and plasma metabolome and lipidome

Metabolomic and lipidomic analyses were performed on faecal ($n = 55$) and plasma samples ($n = 50$). A total of 283 metabolites and 108 metabolites annotated in faecal and plasma samples, respectively. The classes of identified faecal metabolites used for statistical analysis were: alpha-amino acids, amino purines, purine nucleosides, phenolic glycosides, bile acids, glycosyl compounds, xanthines, flavins, bilirubins, peptides, dipeptides, carnitines, aminobenzoic acids, alkaloids, salicylamides, gingerols, aporphines, stilbenes, hydroxycinnamic acids, isoflavones and catechols. The lipidome datasets comprised of a total of 294 faecal lipid species from 17 different faecal lipid classes, and 295 plasma lipid species from 18 plasma lipid classes for all participants (Supplementary Table 10), which were used for statistical analysis. The PLS-DA of metabolite and lipid relative intensities of the DRB group (vs the control group) were, as expected, similar at baseline and washout (CV-ANOVA $p > 0.05$, Q2 value $= 0.07$ for plasma and -0.21 for faecal metabolomes and lipidomes) (Supplementary Figs. 14 and 15).

When comparing within groups, the PLS-DA score plots showed no significant differences in the faecal metabolome (Supplementary Fig. 16), plasma metabolome and plasma lipidome (CV-ANOVA, $p > 0.05$, Q2 value close to zero) (Supplementary Fig. 17).

When comparing between groups at post intervention, the PLS-DA score plots showed differences in faecal metabolome (but not in faecal lipidome) between DRB and control bread, and this model was further examined using a data-driven feature selection technique. The filtered PLS-DA modelling produced 50 metabolites as the most discriminant features between groups at post intervention (PLS-DA CV-ANOVA $p = 2.530e\text{-}07$, $Q2 = 0.29$, metabolites in Supplementary Table 11). However, after FDR correction no significant differences between groups at post intervention were observed (FDR>0.05). Heatmap visualisation highlighted a considerable variation in metabolite relative intensities among participants (Supplementary Fig. 18). Although these 50 features were driving metabolite variation between samples using a multivariate approach, their back-transformed concentrations were not statistically significant using FDR corrected pairwise comparisons. Similarly, the PLS-DA score plots showed no significant difference in the plasma metabolomes and lipidomes between DRB and control group samples post intervention (CV-ANOVA $p > 0.05$) (Supplementary Fig. 19).

Pathway analysis of the 50 faecal metabolites based on the KEGG database, shown in Supplementary Table 12, found none of the pathways achieved a significant p value, and based on a normalised data, the high variance between individual samples was the dominant pattern (Supplementary Fig. 18).

3.2. Cardiovascular risk profile

3.2.1. Anthropometry

Participants maintained their weight, BMI and waist circumference during the study. No significant difference was noted for these parameters between groups ($p > 0.05$) (Supplementary Table 13). These results indicate that neither DRB-fortified bread nor control bread was associated with an increased risk of weight gain during each of the four-week intervention period.

3.2.2. Blood pressure

The blood pressure for participants were within the normal range ($\leq$120 mm (mm) of Mercury (Hg) for systolic and $\leq$80 mm of Hg diastolic). There were no significant differences in the systolic and diastolic blood pressure between groups (Supplementary Table 14).

3.2.3. Blood lipid profile

As observed in Table 4, there was a significant increase in HDL cholesterol concentration between groups, mean HDL cholesterol was 0.11 mmol/L (SD: 0.24, $p = 0.006$) higher for the DRB group compared to the control group. With the increase in HDL cholesterol, the TC/HDL ratio was significantly lower for the DRB group compared to the control group (Mean difference: -0.15, SD: 0.43, $p = 0.02$). No difference was observed in the remaining parameters of the lipid profile between groups ($p > 0.05$).

3.3. Patient-reported outcomes

3.3.1. Daily bowel movement and daily bread diaries

No significant difference was observed between groups for total BM, CSBM, straining, manual manoeuvre or BSF score between groups ($p > 0.05$) (Supplementary Table 15).

Table 5 shows study adherence, based on the number of slices and days of bread consumed for both females and males, and the difference between groups for both females and males. Compliance was reached if the proportion of participants met above 50 % for slices consumed and days of intervention. As shown in Table 5, females and males were more than 80 % compliant with the DRB-fortified bread and control bread. Females were more compliant than males with both study interventions ($p < 0.001$) and had a higher intake of control bread than DRB-fortified bread ($p = 0.02$). However, males consumed similar amounts of both interventions ($p = 0.31$). There were no significant differences in the proportion of days that females ($p = 0.44$) and males ($p = 0.45$) consumed the study interventions. Further, there were no sequence effects on gut symptoms, including diarrhoea, indigestion, constipation, abdominal pain and reflux, as well as psychological wellbeing domains (all $p > 0.05$) (Supplementary Tables 16 and 17).

3.3.2. Gut comfort and psychological wellbeing

As compared to the control bread, the intake of DRB-fortified bread led to more positive mean change scores, i.e., fewer gut symptoms. However, the DRB group was not significantly different from the control group for all gut symptom domains (all $p > 0.05$). For the DRB group, there were fewer constipation ($p = 0.17$) and reflux ($p = 0.31$) symptoms reported as compared to the control group, despite not reaching statistical significance (Supplementary Table 18 and Supplementary Fig. 20). Similarly, no significant differences were observed for all psychological wellbeing domains within and between groups (all $p > 0.05$) (Supplementary Table 19 and Supplementary Fig. 21).

3.4. Dietary intake

Table 6 shows the comparison of nutrient intake within groups [$n = 56$ (DRB bread) and $n = 54$ (control bread)] and between groups ($n = 54$). All nutrient intake increased following the consumption of DRB-fortified bread and control bread. Specifically, there were significant increases in energy, protein, carbohydrate, DF and sodium consumption ($p < 0.01$). Notably, DF intake was increased by 22.7 g/day ($p < 0.001$) and 2.7 g/day following the consumption of the DRB-fortified bread and control bread, respectively ($p < 0.01$). Compared to the control bread, there was also a significant increase in DF intake with the DRB-fortified bread

Table 4
Blood lipid profile within and between groups.

Lipid variable	Timepoint	DRB Bread	Control Bread	Mean Difference[b]	P value
TC (mmol/L)	Baseline	5.12 ± 0.95	5.22 ± 1.00		
	Post intervention	5.14 ± 0.93	5.16 ± 0.95		
	Mean change[a]	0.02 ± 0.50	−0.06 ± 0.52	0.11 ± 0.78	0.34
TG (mmol/L)	Baseline	1.07 ± 0.50	1.10 ± 0.66		
	Post intervention	1.00 ± 0.38	1.06 ± 0.56		
	Mean change[a]	−0.08 ± 0.41	−0.04 ± 0.38	−0.01 ± 0.43	0.91
HDL (mmol/L)	Baseline	1.56 ± 0.33	1.60 ± 0.37		
	Post intervention	1.61 ± 0.35	1.55 ± 0.34		
	Mean change[a]	0.05 ± 0.16	−0.05 ± 0.18	0.11 ± 0.24	0.006
LDL (mmol/L)	Baseline	3.09 ± 0.81	3.13 ± 0.81		
	Post intervention	3.09 ± 0.70	3.13 ± 0.78		
	Mean change[a]	0.004 ± 0.40	0.002 ± 0.43	0.02 ± 0.64	0.87
TC/HDL	Baseline	3.40 ± 0.85	3.37 ± 0.79		
	Post intervention	2.30 ± 0.80	3.44 ± 0.79		
	Mean change[a]	−0.10 ± 0.16	0.07 ± 0.27	−0.15 ± 0.43	0.02

Values are presented as mean ± SD. Significance is based on two-sided t-tests p < 0.05.
DRB: Defatted Rice Bran; TC: Total cholesterol; TG: Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TC/HDL: ratio of total cholesterol and high-density lipoprotein.
[a] Mean Change: Post intervention minus Baseline.
[b] Mean Difference: DRB minus control group (n = 56).

Table 5
Study adherence based on slices and days of bread consumed for both females and males.

		Females	Males	P value	Females	Males	P value	Females		Males	
		DRB bread			Control bread			Mean Difference[a]	P value	Mean Difference[a]	P value
Slices (%)	Required (F:3; M:4)	98.72 ± 10.77	81.09 ± 9.79	<0.001	102.65 ± 7.13	83.92 ± 12.51	<0.001	−3.93 ±9.12	0.02	−2.84 ± 12.96	0.31
Days (%)	Required (based on calculated duration)	92.57 ± 13.51	90.28 ± 14.52	0.55	90.10 ± 14.56	92.39 ± 10.29	0.52	2.47 ±18.05	0.44	−2.11 ± 13.15	0.45

Values are presented as mean percentage ± SD. Required slices: females (3 slices); males (4 slices).
The duration of required days that the intervention should be eaten is calculated based on the start of the intervention (start of record of bread diary) and one day prior to the post intervention visit.
Significance is based on two-sided p < 0.05.
DRB: Defatted Rice Bran.
[a] Mean difference is based on the difference of DRB minus control group.

(p < 0.001) (Table 6). However, consumption of all other nutrients was similar between groups (p > 0.05).

3.5. Gut physiology

3.5.1. Blue food dye

No significant difference (Mean difference: −1.74 h, SD: 30.62, p = 0.72) was observed for the whole gut transit time (WGT) between groups (Supplementary Table 20).

3.5.2. Atmo gas-sensing capsule

In a sub-study, sixteen participants were invited to ingest the ATMO gas-sensing capsule. A total of 62 capsules were ingested, with 15 participants completing all 4 ingestions. However, one participant could only ingest one capsule at baseline and could not ingest the capsule post intervention. They were subsequently withdrawn from the sub-study.

Table 7 shows that anatomical landmarks such as the gastroduodenal junction (GDJ), ileocaecal junction (ICJ), and capsule exit were successfully identified. The GDJ was not identifiable in 18 % of the ingestions, the ICJ in 11 %, and the capsule exit in 5 %. Of the three ingestions where the capsule exit was not identified via the app, two participants confirmed capsule exit by visual inspection, and one participant underwent an X-ray to confirm capsule exit.

No significant difference was observed in regional transits, including gastric emptying (GET), ICJ, small bowel transit time (SBTT), orocaecal colonic transit (OCTT), colonic transit time (CTT), and WGT (p > 0.05) transit times between groups (Supplementary Table 21). Although non-significant, GET, OCTT, CTT, and WGT were longer and SBTT shorter in the DRB group compared to the control group.

When compared based on the method of measurement (blue food dye and ATMO gas-sensing capsule), the WGT showed no significant difference, suggesting a good agreement between both measurement tools (p > 0.05). Figure 5 shows the correlation between both methods. The Pearson's correlation coefficients for WGTT between both methods was r = 0.5 (p < 0.001, n = 45).

3.5.2.1. Colonic fermentation. Non-significant but distinct total and regional colonic hydrogen (H_2) and carbon dioxide (CO_2) concentration patterns were observed between groups. The total H_2 concentration was higher for the control group compared to the DRB group (p > 0.05). The mean and median CO_2 concentrations were numerically (non-significantly) lower for the DRB group; area under the curve and total area under the curve normalised to transit time were higher for the DRB group compared to the control group (Supplementary Tables 22 and 23).

To further explore the colonic H_2 and CO_2 production pattern, the colon was divided into four quartiles: quartiles 1 and 2 represent the proximal colon and quartiles 3 and 4 illustrate the distal colon. No significant difference in H_2 and CO_2 production patterns was seen between groups, although there was a numerical lower H_2 concentration in quartile 3 (non-significant, p = 0.13) (Supplementary Tables 24 and 25). The H_2 and CO_2 production continued in the distal colon in both the DRB and control group (Supplementary Fig. 22).

Table 6
Comparison of nutrient intake within and between groups.

Nutrient	Timepoint	DRB Bread (n = 56)	Control Bread (n = 54)	Mean Difference[c] (DRB-Control)	P value
Energy (kCal/day)	Baseline	1993.29 ± 574.99	2055.91 ± 583.72		
	Post intervention	2648.57 ± 645.96	2707.38 ± 648.17		
	Mean change[a]	655.28 ± 522.73[d]	651.47 ± 509.71[d]	3.83 ± 750.69	0.80
Protein (g/day)	Baseline	84.66 ± 31.67	84.04 ± 27.35		
	Post intervention	116.19 ± 33.57	112.62 ± 31.13		
	Mean change[a]	31.53 ± 23.50[d]	28.58 ± 26.82[d]	2.55 ± 33.39	0.58
Total fat (g/day)	Baseline	92.45 ± 34.50	90.20 ± 28.54		
	Post intervention	100.03 ± 35.89	98.30 ± 32.94		
	Mean change[a]	7.59 ± 35.75	8.10 ± 32.07	0.45 ± 49.73	0.95
Carbohydrate (g/day)	Baseline	184.64 ± 49.47	202.95 ± 83.01		
	Post intervention	284.85 ± 70.59	312.59 ± 75.49		
	Mean change[a]	100.21 ± 62.04[d]	109.64 ± 76.29[d]	−5.84 ± 95.05	0.65
Dietary fibre (g/day)	Baseline	20.69 ± 7.15	22.86 ± 9.43		
	Post intervention	43.40 ± 11.16	25.59 ± 7.11		
	Mean change[a]	22.71 ± 10.23[d]	2.73 ± 7.10[b]	20.06 ± 13.78	<0.001
Sugar (g/day)	Baseline	78.31 ± 33.84	80.77 ± 47.73		
	Post intervention	86.48 ± 40.89	87.15 ± 38.14		
	Mean change[a]	8.17 ± 33.39	6.38 ± 39.64	2.30 ± 53.93	0.76
Sodium (mg/day)	Baseline	2732.86 ± 870.66	2757.22 ± 888.93		
	Post intervention	3551.47 ± 983.48	3601.81 ± 1107.01		
	Mean change[a]	818.61 ± 951.24[d]	844.59 ± 983.89[d]	−25.98 ± 1445.00	0.99

Values are presented as mean ± SD based on n = 56 (DRB bread) and n = 54 (control bread).
Significance was based on two-sided $p < 0.05$.
DRB: Defatted Rice Bran; g: gram: kCal: kilocalorie; mg: milligram.
[a] Mean changes are calculated as post intervention minus baseline.
[b] Significance of p = 0.01.
[c] Values for the mean difference between groups are based on n = 54.
[d] Significance of $p < 0.001$.

Table 7
Anatomical landmark identification as measured by Atmo gas-sensing capsule.

	Number of ingestions (n)	GDJ[a] n (%)	ICJ[a] n (%)	Capsule exit n (%)
ATMO capsule	61[a]	50 [82]	54 [89]	58 [95]

GDJ: gastroduodenal junction; ICJ: ileocaecal junction.
[a] 1 participant was excluded due to inability to complete capsule ingestion.

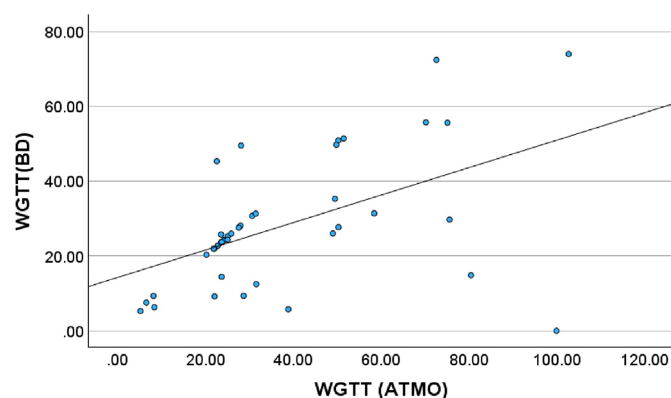

Fig. 5. Correlation of whole gut transit (WGT) between blue food dye and ATMO gas sensing capsule. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.6. Adverse events

This study was considered low-risk. All participants were provided with information about managing possible side effects. Only one participant underwent an X-ray solely to confirm the excretion of the Atmo-sensing capsule. No adverse events relating to the study were reported by participants.

4. Discussion

This is the first human study to assess the effect of DRB-fortified bread on the gut microbiome, gut physiology, patient-reported outcomes and cardiovascular risk profile in healthy adults with low baseline DF intake. Whilst no differences were observed in the primary and most secondary outcomes, consumption of DRB-fortified bread was associated with a significant increase in DF intake, serum HDL concentration and a significant reduction in the ratio of TC to HDL concentrations compared to control bread.

4.1. Biological markers of host and microbial interactions

DRB-fortified bread contains bran which is a chemically complex substance [7]. The cell walls of bran are high in lignin [87,88]. Lignin is low in fermentability and is non-degradable in the colon [89]. Therefore, DRB may be more resistant to degradation by gut microbes. The DRB-fortified bread also contains many intact DF structures and other bioactive components, including phenolic acids [90,91], which may support the growth of microbes with different degrading capabilities [92,93]. These embedded components in DRB-fortified bread have a multifaceted nature [94], which could affect the primary outcome, the microbial composite.

Similar to other cereal grains, rice bran contains arabinoxylans [95], a hemicellulose polymer [96]. Arabinoxylans may be prebiotics, which selectively stimulate beneficial gut microbes,

including the *Bifidobacterium* genus [19,97,98], as observed for the DRB group post intervention. This concurs with other studies, with either 30 g/day to 40 g/day of heat-stabilised or powder form of rice bran supplementation following two or four weeks post intervention compared to baseline [24–26], and 24 weeks compared to control [27]. *B. longum,* a known DF degrader species in adults, can metabolise hemicellulose, including arabinoxylans [99]. Additionally, DRB-fortified bread intake led to a non-significant increase in the relative abundances of the *B. ovatus* from the *Bacteroides* genus as compared to control bread. *B. ovatus* utilises DF for growth and has specific polysaccharide utilisation loci or degradation genes that can degrade structurally complex xylans [100], and a specific enzymatic pathway [101] for metabolising hemicellulose [100,102]. These suggest the potential role of arabinoxylan in DRB in modulating the potentially beneficial microbial species.

The relative abundance of *Faecalibacterium prausnitzii* increased, although non-significantly, following DRB-fortified bread intake. *F. prausnitzii* is a known butyrate producer; butyrate is an important energy source for colonocytes [93]. However, two recent systematic reviews reported that most studies showed no effect of DF intake on the relative abundances of *F. prausnitzii* [92,93]. Further research is required to elucidate the role of DF related to DRB in modulating the relative abundance of *F. prausnitzii* and its implications for gut health.

No between group differences were observed in other biological markers of host and microbial interactions. Gut microbiota derived metabolites may undergo rapid metabolism or uptake by colon tissues, leading to their depletion or transformation before they reach the systemic bloodstream or faeces [103]. Hence, plasma or faecal concentrations of metabolites might not accurately represent their production by the gut microbiota [103]. Additionally, participants were in a free living setting throughout the study duration. This contributes to inter-individual variation in metabolite profiles and metabolism [104–107], increasing the challenge to find a significant difference between interventions. Further studies, whether habitual or acute, are warranted to provide a better understanding of their production and absorption following DRB-fortified bread and its impact on host health.

4.2. Cardiovascular risk profile

This is the first dietary intervention study to demonstrate significant increases in serum HDL cholesterol levels and decreases in serum TC to HDL ratio following four weeks of DRB-fortified bread intake in healthy adults. Only two whole-grain rice studies have shown increased HDL cholesterol levels in high-risk populations for metabolic diseases [108,109]. A recent study demonstrated a non-significant reduction in serum lipid profile with 30 g/day DRB supplementation in adults with hypercholesterolemia [110]. This may be explained by the insoluble DF in DRB, which increased during the defatting process, and may have slowed macronutrient absorption in the small bowel [111,112]. However, their underlying mechanism was not investigated, thus warranting further evaluation.

4.3. Patient-reported outcomes and gut physiology

An abrupt change or addition of DF can cause gut symptoms or change in bowel habits, especially in individuals with low DF intake [113,114]. However, DRB-fortified bread, which had triple the DF content of control bread, did not affect patient-reported outcomes in this cohort. Further, there was no difference in the gut transit time as measured by blue dye or Atmo gas-sensing capsule. Although not statistically significant, the DRB group had a longer GET and shorter SBTT, aligning with previous studies [115–118]. These findings can be explained by the higher insoluble DF content and structures in DRB-fortified bread. These may help in bulking the luminal content, increasing the passage from the small bowel, thus reducing macronutrient absorption rate, and promoting laxation [111,112]. Notably, this study's participants had no diagnosed gastrointestinal or psychological conditions. As this was the first study using DRB-fortified bread as a dietary intervention, it was decided to recruit healthy adults to inform public health policy. Nonetheless, the findings show that DRB-fortified bread is acceptable and should be considered a food option to improve DF intake in healthy individuals.

4.4. Dietary intake

Bread is the main contributor to DF intake among the NZ population [15]. Hence, this study implemented a practical approach to increasing DF intake. As observed in this study, participants used their preferred spread to consume the study interventions as part of their usual diet. Consequently, DF intake increased by 22.7 g/day following DRB-fortified bread (an increase of 20.1 g/day compared to control bread) and potentially increasing participants' overall diet quality. Nonetheless, more research is warranted to assess overall diet quality using DRB-fortified bread.

4.5. Limitations and strengths

Some limitations were identified. Participants with low DF intake were screened using a validated FFQ [43]. Three-day food diaries were further completed throughout the study. However, these dietary assessment methods are prone to self-reported bias [119]. Additionally, there are no biological markers to assess DF intake; future considerations should include subgroup analysis or performing statistical adjustments based on baseline gut microbiota composition status which may reduce the varied effects of baseline status [120,121] and identify potential responders to the dietary intervention.

Variations in collection times and storage methods by participants may have introduced potential confounding factors, thus affecting the gut microbiome results. Differences in storage conditions could lead to microbial DNA degradation, overgrowth, and viability loss in faecal samples [122]. Nonetheless, the DNA yield for this study passed quality control parameters.

It is noteworthy that taxonomic and other biological changes can be challenging to interpret, as they can be affected and rapidly change by factors including high inter-individual variability [104–107]. Therefore, observing temporal changes in the gut microbiome can guide researchers in investigating underlying mechanisms and potential interventions.

Study strengths include a blinded crossover study design, thus allowing participants to be their own control [123]. Additionally, bowel habits can vary across the menstrual cycle [124], which could mask the true effect of the intervention. Therefore, the four-week intervention timeframe may balance out the effect of where female participants (60 % in this study) are in their cycle during subjective assessments. Furthermore, sequence effects were included in the analysis, and no crossover effects were observed.

5. Conclusion

Collectively, these findings support the substitution of white bread with DRB-fortified bread as an effective method to improve DF intake, HDL cholesterol profiles and potentially have beneficial effects on the gut microbiome in healthy adults with low DF intake. These gut microbiome findings should be used to design more adequately powered studies to substantiate the effect of the DRB-

fortified bread on the gut microbiome and related gut physiological as well as metabolic health changes.

Author contribution

CW: Formal Analysis, Supervision, Writing – review & editing. CG: Formal Analysis, Investigation, Supervision, Writing – review & editing. CF: Formal Analysis, Supervision, Writing – review & editing. DC: Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. HN: Data curation, Formal Analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. JMu: Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. JC: Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. JM: Data curation, Formal Analysis, Investigation, Project administration, Writing – review & editing. KF: Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. MF: Formal Analysis, Supervision, Writing – review & editing. NR: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. RG: Conceptualisation, Investigation, Methodology, Supervision, Writing – review & editing. SB: Investigation, Supervision, Validation, Writing – review & editing. TT: Investigation, Methodology, Writing – review & editing. WM: Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare no conflicts of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All authors work at independent research facilities or businesses and are not employees of the industry partner. The industry partner has had input into the study design; however, they were not involved in the data collection nor will be involved in the analysis or interpretation of results, manuscript writing or in the decision of results publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2025.03.045.

Abbreviations

%	Percent
ADONIS	Analysis of dissimilarity
ANOVA	Analysis of variance
ANZCTR	Australia New Zealand Clinical Trial Registration
BMI	Body mass index
BREAD	Bread Related Effects on microbiAl Distribution
Bp	Basepair
CO_2	Carbon dioxide
CTT	Colonic transit time
CV-ANOVA P	Cross-validated predictive residuals
DF	Dietary fibre
DNA	Deoxyribonucleic acid
DRB	Defatted rice bran
FDR	False discovery rate
GET	Gastric emptying
GDJ	Gastroduodenal junction
H_2	Hydrogen
HDL	High-density lipoprotein
Hg	Mercury
ICJ	Ileocaecal junction
LCMS	Liquid chromatography mass spectrometry
LDL	Low-density lipoprotein
MS	Mass Spectrometry
NCBI	National Centre for Biotechnology Information
NMDS	Non-metric multidimensional scaling
NZ	New Zealand
OCTT	Orocaecal colonic transit
PC	Principle Component
PERMANOVA	Permutational multivariate analysis of variance
PLS-DA	Partial least squares projection-discriminant analysis
Q2	Predictive ability of the model
RCT	Randomised controlled trial
SBTT	Small bowel transit time
SPSS	Statistical package for social sciences
WGT	Whole gut transit

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