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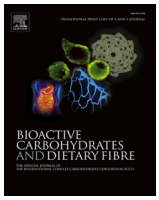

Actazin® green kiwifruit powder consumption at 600 mg per day for 28 days improves stool form and relieves occasional constipation in healthy individuals: A randomized controlled trial

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ABSTRACT

Constipation is a global issue which impacts quality of life. Kiwifruit promote laxation without the urgency induced by therapeutic laxatives. Actazin® is a skinless, seedless, cold-processed green kiwifruit powder previously shown to improve laxation at 2400 mg daily dosages. Here we investigated the laxation support provided by a 600 mg daily dosage of Actazin.

A randomized, double-blinded, placebo-controlled parallel study for 28 days across four North American sites (NCT03462199) enrolled 85 participants with non-pathological constipation who had ≤3 complete spontaneous bowel movements (CSBM) per week. Participants consuming Actazin reported improved Bristol stool form scores (BSFS) over placebo ($p < 0.05$), improving the normality of stool form. Both Actazin and placebo showed improvements of >1 CSBM per week over baseline ($p < 0.05$). Actazin was safe and well tolerated by participants and resulted in changes ($p < 0.05$) in the relative abundance of fecal bacterial taxa consistent with consumption of kiwifruit cell wall components.

This study demonstrated that once daily supplementation of 600 mg Actazin green kiwifruit powder resulted in clinically significant improvements in stool form and improved participant bowel habits in healthy individuals with occasional constipation. To the best of our knowledge this is the first recorded observation of this BSFS improvement over placebo by a kiwifruit product.

1. Introduction

Constipation is a global problem, with 10–20% of the worldwide population forced to seek medical care to improve their bowel movements and related quality of life and feeling of general well-being (Gélinas, 2013). Constipation is influenced by several risk factors including gender, age, socioeconomic status, psychological parameters, medications, physical inactivity, dietary habits, and education level (Alexandre et al., 2016; Mugie et al., 2011). Pharmacological interventions with stool softeners, osmotic laxatives, and stimulant laxatives provide therapeutic options for treating constipation, albeit with side effects (Scholar et al., 2008). There remains a need for safe and effective therapies.

A food-based approach has been considered as an effective long-term solution to constipation (Gélinas, 2013). Food ingredients such as psyllium and wheat bran are the most studied for their laxation-inducing potential. Adequate daily intake of fiber-rich fruits and vegetables with sufficient water should prevent the incidence of constipation. Kiwifruit (*Actinidia* spp.) is a source of vitamins A, C and E, potassium, polyphenols, dietary fiber and contains the kiwifruit-specific enzyme actinidin. Previous clinical studies in populations of healthy (Caballero et al., 2020; Chey et al., 2021; Rush et al., 2002; Wilkinson-Smith et al.,

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2019), constipated (Chan et al., 2007) and constipation dominant irritable bowel syndrome (IBS–C) (Chang et al., 2010) report that two whole green kiwifruit per day improves laxation by at least 1 complete spontaneous bowel movement (CSBM) per week. A recent laxative meta-analysis rates kiwifruit as having a Level I (good) quality of evidence with Grade B (moderate) efficacy (Rao & Brenner, 2021). Furthermore, green kiwifruit was approved by the European Food Safety Authority (EFSA) for the health claim "consumption of kiwifruit contributes to the maintenance of normal defecation" (EFSA NDA Panel EFSA Panel on Nutrition et al., 2021). It is thought that the unique combination of soluble and insoluble fibers, polyphenols, and the enzyme actinidin present in kiwifruit confer this and other health benefits (Ansell et al., 2015).

Fiber escapes host small intestinal digestion and enters the colon largely intact, where it may be fermented by the resident gut microbiota, resulting in increased microbial biomass and hence fecal bulking (Cummings and Spiller, 2001), leading to increased laxation (Bharucha & Lacy, 2020). The EFSA panel (EFSA NDA Panel EFSA Panel on Nutrition et al., 2021) considered the fiber composition of kiwifruit contributed to a "plausible mechanism by which green kiwifruit exerts an effect on normal defecation".

Actazin a freeze-dried fruit powder derived from green kiwifruit, is safe, bioavailable, and addresses a longstanding need for convenient, effective constipation intervention. It has been previously shown that supplementation with Actazin at both 600 mg and 2400 mg daily were well-tolerated, and at 2400 mg Actazin demonstrated a significant and clinically meaningful increase in daily bowel movements by more than 1 bowel movement per week in healthy individuals (Ansell et al., 2015). Two whole kiwifruit also increase CSBM by 1 per week (Chang et al., 2010). Thus, it appears that 2400 mg/day of Actazin is functionally equivalent (in terms of CSBM improvements) to the laxation benefits of two whole kiwifruit.

Further human efficacy studies for Actazin are necessary to substantiate its role in providing mild constipation relief. The primary aim of this study was to evaluate the efficacy of a once daily 600 mg dose of Actazin on stool form, stool frequency, constipation symptoms and quality of life in participants with occasional constipation who are otherwise healthy. We also explored the potential impact of Actazin kiwifruit fiber and other digestion-resistant constituents (polyphenols and organic acids) on gut microbial ecology by assessing changes to participant's fecal microbiome.

2. Methods and materials

2.1. Study design

This was a multi-center, randomized, double-blind, placebo-controlled study consisting of a 4-week supplementation (Fig. 1). This

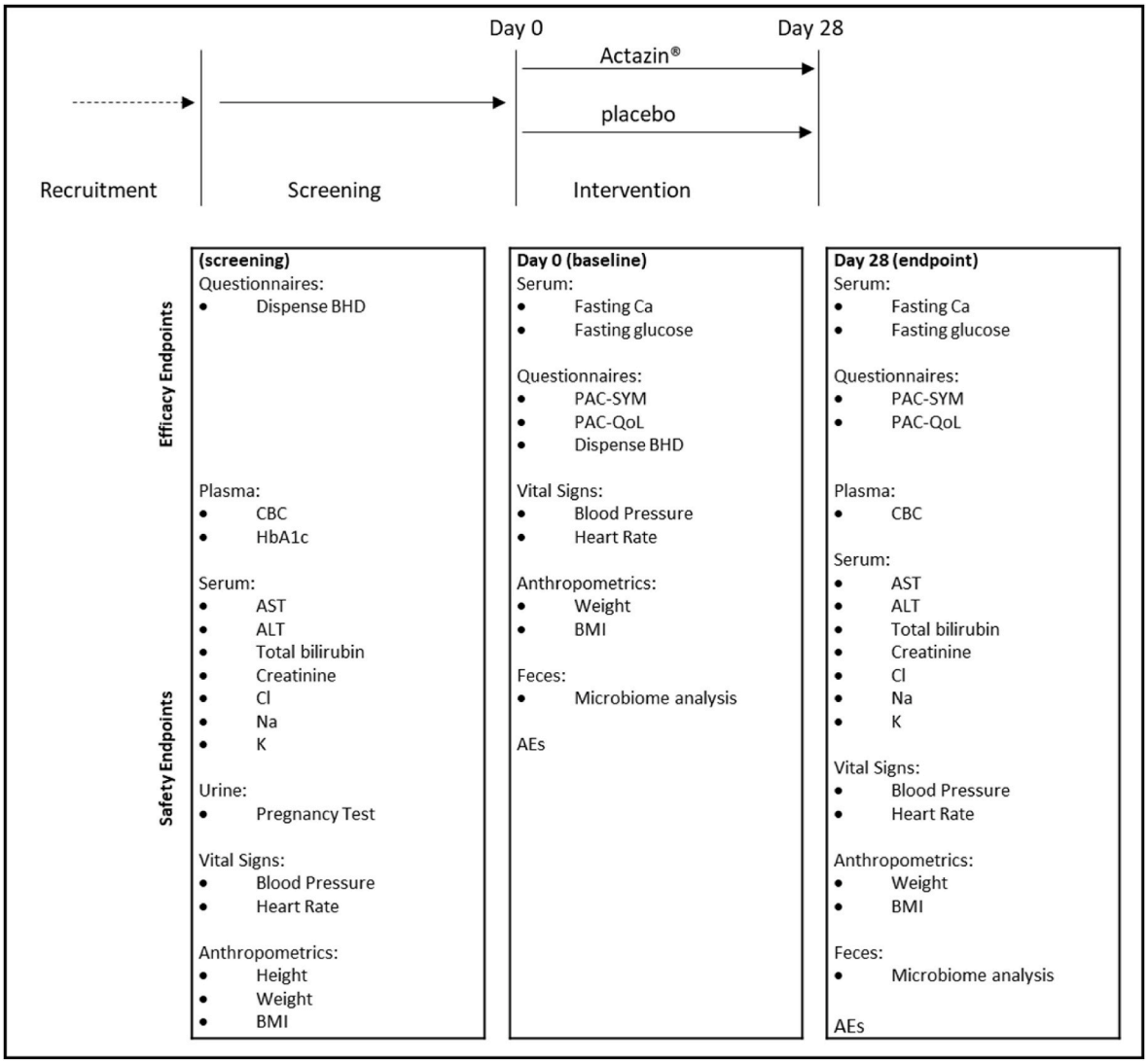

Fig. 1. Study plan.

study was conducted in accordance with the ethical principles that originate in the Declaration of Helsinki and its subsequent amendments, and in compliance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice Current Step 4 Version dated November 9, 2016. The trial was registered at Clinicaltrials.gov (NCT03462199).

The clinical trial was conducted at four sites: KGK Science Inc. (London, On, Canada), Great Lakes Clinical Trials (Chicago, IL, USA), MB Clinical Research, LLC (Boca Raton, FL, USA), and INQUIS Clinical Research (Toronto, On, Canada). Conduct of the study was under the supervision of a qualified investigator (QI) at each site. The study was reviewed by the Natural Health Product Directorate (NHPD), Health Canada and a research ethics board. Notice of authorization was granted on February 13, 2018, by the NHPD, Ottawa, Ontario. Unconditional approval was granted on February 1, 2018, by the Institutional Review Board (IRB Services, Aurora, Ontario). Informed consent was obtained from each participant at the screening visit prior to performing any study-related activities.

This study was powered based on a previous parallel study on kiwifruit and stool frequency (Udani & Bloom, 2013) with 80% power requiring 44 subject per intervention group assuming an overall alpha of 0.05 and 20% attrition rate.

This study reports the clinical outcomes for Actazin green kiwifruit interventional product compared to cellulose placebo, from a larger study. The full study with multiple parallel arms using Actazin plus other products details recruitment and retention according to CONSORT guidelines, and covers safety and tolerability outcomes, and is reported elsewhere (Lewis et al., 2023).

2.2. Participants

Each participant fulfilled all the inclusion criteria and did not meet any of the exclusion criteria listed below:

Inclusion criteria were: males and females of 18–60 years of age; body mass index (BMI) 19–29.9 $\pm$ 1 kg/m^2; self-reported $\leq$3 CSBMs per week at screening and confirmed at baseline; fasting blood glucose $\leq$6.0 mmol/L; not consuming high fibre diets, yoghurt and fermented foods; agreed to refrain from the consumption of fresh kiwifruit 2-weeks prior to and during the study, maintained usual habitual food and beverage intake and activity level; avoided overseas travel for the duration of the study; and were healthy as determined by laboratory results, medical history, and physical exam.

Exclusion criteria included allergy or sensitivity to kiwifruit or other test product ingredients; clinically significant abnormal laboratory results at screening; use of probiotic and prebiotic dietary supplements; regular intake of nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, or other anti-inflammatory medications; use of medications for constipation, and or diarrhoea; frequent use of laxatives if greater than once per week; use of antibiotics or medications impacting gut microbes within two months, prior surgery for weight loss or weight loss of >5% within 3 months, smoking within the past 5 years and alcohol or drug abuse within 6 months of randomization; gastrointestinal alarm symptoms and major diseases of the gastrointestinal tract, pulmonary or endocrine systems, or other GI abnormalities; thyroid disease; uncontrolled hypertension; renal, hepatic, pancreatic, or biliary impairment or disease; bleeding/blood disorders; diabetes; autoimmune disease or immuno-compromised; cancer; any other condition may have adversely affected the participant's ability to complete the study or its measures or which may have posed significant risk to the participant.

Participants had three in-clinic visits: at screening, at baseline; and at 28 days post supplementation. Outcomes questionnaires were administered at baseline and day 28.

2.3. Investigational products

The investigational product (IP) Actazin was labelled per ICH-GCP guidelines and applicable local regulatory guidelines. Each capsule contained 150 mg of green kiwifruit powder from Anagenix Ltd (Auckland, New Zealand) and at a dosage of four capsules provide 600 mg/day of Actazin. Actazin green kiwifruit powder was processed using a proprietary method to maintain the key nutrients of kiwifruit such as dietary fibre, polyphenols, vitamins and minerals, and provided high levels of the enzyme actinidin with >25,000 AU/g actinidin protease activity. The soluble fiber fractions comprised predominantly xyloglucan (~40%) and kiwifruit pectin (~60%), the latter maintaining their full methylation (>50%) and branched rhamnogalacturonan I and II structures (Ian Sims, Victoria University Wellington, personal communication). Excipients included microcrystalline cellulose (Avicel PH-101, FMC Corporation, PA) (5.5% w/w), and silicon dioxide (HDK N20, Wacker Chemie AG, Germany) (2% w/w).

Placebo contained microcrystalline cellulose (Avicel PH-101, FMC Corporation, PA). Size 00 capsules were AL98014 from ACG Associated Capsules Pvt Ltd (Maharashtra, India).

Participants were instructed to take four capsules of IP or placebo daily with a glass of water and food in the morning, starting the day after randomization, for 28 days.

2.4. Randomization and blinding

Eligible participants were assigned a randomization number by a blinded investigator and allocated to each group per the order of the randomization list generated by www.randomization.com. Investigators, other site personnel, and participants were blinded to the treatment each participant received for the duration of the study.

The IP and placebo were indistinguishable by size, colour, taste, texture, or packaging. Packaging was labelled with randomization codes by site personnel not involved in any study assessments.

2.5. Bowel habits diary (BHD)

Participants were required to record number of bowel movements, and if straining to start defecation, straining to stop defecation, feelings of incomplete defecation and the use of laxatives in the BHD.

Stool form was scored according to the Bristol Stool Form Score (BSFS) (Longstreth et al., 2006; U.S. Department of Health and, 2012), a diagnostic criteria depicting the form of the feces on a 7-point scale, from hard to watery, with scores of type 3–4 considered normal and movement towards these scores ("improvements") indicative of healthier bowel function (Koh et al., 2010). The BSFS is the one most widely used diagnostic tool in both clinical and research settings (Ackley & Ladwig, 2013, p. 240; Reigler & Esposito, 2001). The BSFS has been validated as a surrogate measure for gastrointestinal transit time (Lewis & Heaton, 1997). BSFS has been suggested as a diagnostic tool for IBS-D (Longstreth et al., 2006; U.S. Department of Health and, 2012) and it is included by the Rome Foundation in the new Rome IV criteria for diagnosing FC and IBS (Schmulson & Drossman, 2017). EFSA regards Bristol stool form scoring as a validated questionnaire for measuring stool consistency in their guideline for a health claim (EFSA NDA Panel EFSA Panel on Dietetic Products and Nutrition and Allergies, 2016).

CSBM and Spontaneous Bowel Movements (SBM) were evaluated from the BHD. CSBM is an accepted and easily defined primary measure of stool frequency in clinical trials assessing bowel habits (U.S. Department of Health and, 2012). A CSBM was classified as being complete and spontaneous when a participant reported a feeling of satisfaction (complete) and no manual maneuvers, laxatives, enemas, or suppositories were used, and no assistance was needed. For SBM, it was spontaneous but there was not a feeling of satisfaction. Participants may be less comfortable following a SBM.

To assess bowel regularity, participants were provided with a series

of twelve statements at day 28 and asked to score each. Scoring for this index was based on a five-point scale for each question, from strongly disagree (0) to strongly agree (5).

2.6. Patient Assessment of constipation symptoms and Patient Assessment of Quality-of-life questionnaires

The validated Patient Assessment of Constipation Symptoms (PAC-SYM) and validated Patient Assessment of Quality-of-Life (PAC-QoL) questionnaires are patient-reported outcomes that were measured symptoms (Frank et al., 1999) and quality-of-life of people with constipation (Marquis et al., 2005), respectively. Both were a 5-point scale: the PAC-SYM questionnaire assessed constipation from a low score (0) indicating absence to a high score (4) indicating very severe (Frank et al., 1999). The PAC-QoL questionnaire assessed quality-of-life from a low score (0) indicating not at all to a high score (4) indicating extremely high (Marquis et al., 2005).

2.7. Three-day food diaries

Participants recorded their food and beverage intake two weekdays and one weekend day online using DietMaster Pro (Lifestyles Technologies Inc., Grants Pass, OR, USA) prior to their baseline and day 28 clinic visits. The records were reviewed by trained staff at which time participants were reminded to maintain their normal dietary and beverage intake and physical exercise and to refrain from consuming high-fiber dietary supplements, a very high-fiber diet, fresh kiwifruit, probiotic or prebiotic supplements.

2.8. Fecal microbiome analysis

Participants provided fecal samples collected within 2 days of each clinic visit at baseline and day 28. They were instructed to freeze their samples and transport them to the clinic with supplied ice packs, ensuring the sample did not thaw during transportation. Received fecal samples were shipped frozen to the Center for Human Nutrition, University of California, Los Angeles CA, USA, for microbiome analysis.

DNA from stool was extracted using the DNeasy power soil DNA isolation kit with bead beating (Qiagen, Valencia, CA). The quality and quantity of the DNA was confirmed using a Nanodrop 1000 (Thermo Fisher Scientific, Wilmington, DE). The 16 S rRNA gene V4 variable region was amplified and barcoded using F515/R806 primers followed by 250 × 2 bp sequencing on an Illumina HiSeq 2500 (Marquis et al., 2005).

2.9. Adverse events

Participants recorded adverse events (AE) in their daily study diaries. All AEs were reviewed at the in-clinic visits and were subsequently coded with Medical Dictionary for Regulatory Activities terminology (MedRA) version 22.0. The QI assessed any AEs and decided causality, categorized as “Most Probable”, “Probable”, “Possible”, “Unlikely” or “Not Related” to the IP.

2.10. Compliance

Participants recorded IP intake in their daily study diary and were required to return all unused and open IP packages to the clinic site. Compliance was calculated by determining the number of dosage units taken divided by the number of dosage units expected to have been taken multiplied by 100. In the event of a discrepancy between the information in the participant’s diary and the amount of study product returned, study product used was based on the product returned, unless an explanation for the loss of the product was provided. Compliance was determined as >80% or <120% of IP consumed.

2.11. Statistical analysis

Analyses were conducted using R Statistical Software Package Version 3.6.1 (R Core Team, 2019). The intent-to-treat (ITT) population was analysed. This group consists of all subjects who received study product and on whom any post-randomization effectiveness information was available.

Assessment of change from baseline to Day 28 in number of BSFS, CSBM, SBM, PAC-QoL, and PAC-SYM between the intervention and placebo groups was conducted using ANOVA with post-hoc Tukey’s *t*-test.

Assessment of change in the number of BSFS, CSBM, and SBM from baseline to days 7, 14, 21, and 28 were conducted using repeated measures ANCOVA. The model included study arm, time, site, and study arm by time as fixed effects, the baseline value of the dependent variable as a covariate and subject as the random effect. Time was a categorical variable represented by day numbers. Pairwise comparisons were obtained from the model.

Sequence data was processed using the DADA2 pipeline (Callahan et al., 2016). Bacterial taxonomy was assigned using the DADA2 assign Taxonomy function (RDFP naïve Bayesian classifier) against the SILVA V132 database (https://www.arb-silva.de/documentation/release-138/) (Quast et al., 2013). The minimum bootstrap confidence threshold for the RDP classifier was set at the DADA2 default of 50.

An Amplicon Sequence Variant Table (ASV) (including taxa assignments), mapping file, and taxonomy assignments were imported into Phyloseq using R version 4.0.2 https://github.com/joey711/phyloseq) (McMurdie and Holmes, 2013). Taxonomy assignments were collapsed to the nearest common assignment from species, creating an Operational Taxonomic Unit (OTU) count table and reducing the number of amplicon assignments from 9115 to 672. Taxonomy assignments were further filtered to include only those that occurred at >20% relative abundance in at least 3 samples (from any timepoint or treatment group), further reducing the number of taxa assignments included in the analysis to 195.

Samples with lower than 10,000 total sequence counts were excluded from analysis, resulting in the inclusion of 523 samples. Samples without time-point dyads were removed from analysis to allow for pairwise significance testing.

Count tables were normalised by multiple methods (cumulative sum scaling, total sum scaling, center log ratio transformation with imputed zeros, and compositional plus Log10 transformation); ultimately cumulative sum scaling was chosen as the primary normalisation method, resulting in a relative abundance value for taxa identified in each sample.

Unpaired Wilcoxon tests were used to compare relative abundance of taxa detected in the placebo and intervention groups. Paired Wilcoxon tests were used to measure the significance of change in relative abundance of taxa within the intervention and placebo groups from baseline to day 28. Fold change was calculated on the species that were significantly different between and within groups.

3. Results

3.1. Disposition and compliance

Forty-four participants were randomized into the Actazin group and 41 randomized into the placebo group. Both groups each had one participant terminate early. Compliance was >95% in both groups and there was no difference in compliance between groups.

3.2. Demographics, anthropometric measures, vital signs, hematology and clinical chemistry

The enrolled population consisted of 85 healthy, normal weight and overweight adults, between the ages of 18 and 60 years (Table 1). Participant genders were consistent with the ratio of 2.2:1 female-to-

Table 1
Demographic and anthropometric information for participants in this study.

Category	Variable	Actazin n (%)	Placebo n (%)	
Age	Mean ± SD (n)	38.59 ± 11.80 (44)	41.37 ± 12.21 (41)	
	Median (Min - Max)	40.50 (19.00–57.00)	43.00 (22.00–60.00)	
Gender	Female	32 (72.70%)	27 (65.90%)	
	Male	12 (27.30%)	14 (34.10%)	
Ethnicity	South American	0 (0.00%)	3 (7.30%)	
	Eastern European White	11 (25.00%)	8 (19.50%)	
	Hispanic or Latino	1 (2.30%)	2 (4.90%)	
	Western European White	18 (40.90%)	20 (48.80%)	
	South Asian	3 (6.80%)	0 (0.00%)	
	African American	2 (4.50%)	0 (0.00%)	
	Central American	0 (0.00%)	0 (0.00%)	
	Middle Eastern	5 (11.40%)	2 (4.90%)	
	African	0 (0.00%)	2 (4.90%)	
	East Asian	1 (2.30%)	2 (4.90%)	
	South East Asian	3 (6.80%)	1 (2.40%)	
	Native American	0 (0.00%)	1 (2.40%)	
	Missing	0 (0.00%)	0 (0.00%)	
				Between group P-value
BMI (kg/m^2)	Baseline	25.43 ± 2.80 (44)	25.50 ± 3.38 (41)	0.919
	Day 28	25.52 ± 2.87 (43)	25.58 ± 3.55 (40)	0.932
	Change from Baseline to Day 28	0.12 ± 0.40 (43)	0.05 ± 0.42 (40)	
	P-value	0.061	0.443	
Systolic Blood Pressure (mmHg)	Baseline	117.05 ± 13.84 (44)	116.68 ± 9.87 (41)	0.890
	Day 28	115.86 ± 12.64 (43)	116.75 ± 11.33 (40)	0.737
	Change from Baseline to Day 28	−1.14 ± 9.35 (43)	0.00 ± 7.63 (40)	
	P-value	0.429	1.000	
Diastolic Blood Pressure (mmHg)	Baseline	75.09 ± 8.81 (44)	76.88 ± 7.30 (41)	0.313
	Day 28	74.86 ± 7.91 (43)	75.95 ± 9.19 (40)	0.563
	Change from Baseline to Day 28	−0.23 ± 6.81 (43)	−1.10 ± 6.52 (40)	
	P-value	0.824	0.293	

n, number; SD, standard deviation; Min, minimum; Max, maximum.
Note: one individual from each group had early termination from the study (after baseline).

male ratio of constipated individuals in the US population (Higgins & Johanson, 2004). All participants reported ≤3 CSBMs per week at screening which was confirmed during a two-week run-in period prior to baseline.

Baseline and day 28 anthropometric measures and vital signs were not significantly different between groups at baseline or day 28 (Table 1). There was no significant difference between groups or from baseline at day 28 in clinical chemistry or hematology (data not shown).

3.3. Bristol Stool Form Score (BSFS)

Actazin supplementation led to significant ($p < 0.01$) improvements in BSFS scores when compared to baseline at days 7, 14, 21, and 28, while placebo showed no significant changes at these time points. After 14 days of supplementation, the BSFS score change from Actazin consumption also showed significant ($p < 0.05$) improvements compared to the placebo (Fig. 2). Using repeated measures analysis, the Actazin BSFS were also significantly ($p = 0.037$) improved over placebo (data not shown).

3.4. Complete spontaneous bowel movements and spontaneous bowel movements

There were significant ($p < 0.05$) improvements in frequency of CSBM and SBM in participants supplemented with Actazin and placebo from baseline to day 7, 14, 21 and 28 (Table 2). All groups reported increases of >1 CSBM per week and there were no significant between-group differences.

3.5. Patient-assessed symptoms of constipation and quality of life

There were no significant between-group differences for the change in overall PAC-SYM or PAC-QoL (Table 2) or individual scores at day 28 (Supplementary Tables S1 and S2).

For PAC-SYM, significant ($p < 0.05$) within-group improvements in abdominal, rectal and stool symptoms and overall PAC-SYM scores were reported by the Actazin group (Supplementary Table S1). Significant ($p < 0.05$) within-group changes improvements reported in placebo over time, apart from rectal symptom score (Supplementary Table S1).

For PAC-QoL, significant ($p < 0.05$) within-group improvements in physical discomfort, psychosocial discomfort, worries/concerns, and satisfaction and overall PAC-QoL scores were reported by the Actazin group as well as placebo ($p < 0.05$) (Supplementary Table S2).

3.6. Bowel regularity

There were no significant between-group differences reported for overall Bowel Regularity Index (BRI) or individual BRI scores at day 28 (Table 3). All BRI score means were higher in Actazin compared to placebo, although not significantly ($p < 0.05$) different. The overall BRI which is sum of all these questions was nearly two points higher for Actazin than placebo. This was contributed by all the individual BRI questions being slightly higher with Actazin.

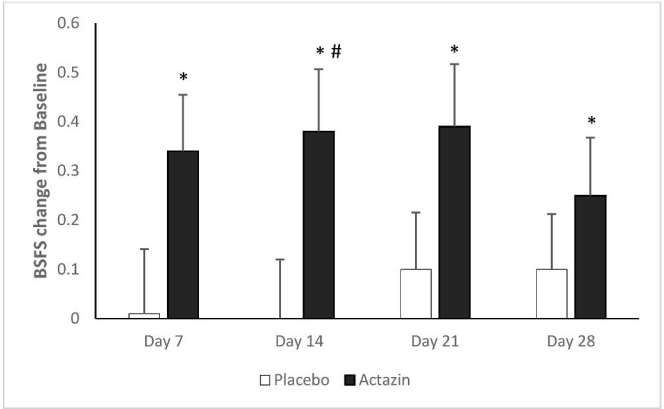

Fig. 2. Actazin shows significant ($p < 0.05$) improvements in Bristol stool form score (BSFS) compared to baseline (*), and significantly ($p < 0.05$) improved BSFS compared to placebo (#). Bar shows SD. (Actazin, black; placebo, white).

Table 2
Actazin and placebo show significant ($p < 0.05$) improvements in measured parameters over time. Weekly complete spontaneous bowel movements (CSBSM), spontaneous bowel movements (SBM) and participant assessed constipation (PAC) quality of life (QOL) and constipation symptoms (SYM) in the ITT population (n = 85).

Parameter	CSBM			SBM		
	Mean ± SD (n)	Within group P value	Between group P value	Mean ± SD (n)	Within group P value	Between group P value
Baseline						
Actazin	1.03 ± 0.87 (44)	n/a	0.372 (r)	2.08 ± 1.47 (44)		0.456 (r)
Placebo	1.32 ± 1.89 (41)	n/a		2.39 ± 2.66 (41)		
Change from Baseline to Day 7						
Actazin	+1.19 ± 1.97 (43)	<0.001 (r)	0.927	+0.87 ± 2.19 (43)	0.005 (r)	0.913 (r)
Placebo	+1.14 ± 2.81 (40)	0.001 (r)		+0.81 ± 2.73 (40)	0.010 (r)	
Change from Baseline to Day 14						
Actazin	+1.95 ± 2.40 (43)	<0.001	0.296	+1.50 ± 2.29 (43)	<0.001	0.239 (r)
Placebo	+1.36 ± 2.72 (40)	0.003		+0.81 ± 2.97 (40)	0.023 (r)	
Change from Baseline to Day 21						
Actazin	+2.12 ± 2.80 (43)	<0.001 (r)	0.820	+1.62 ± 2.59 (43)	<0.001 (r)	0.947 (r)
Placebo	+2.26 ± 3.01 (40)	<0.001		+1.66 ± 3.66 (40)	0.007	
Change from Baseline to Day 28						
Actazin	+1.44 ± 1.78 (43)	<0.001	0.671	+0.97 ± 1.64 (43)	<0.001	0.866 (r)
Placebo	+1.64 ± 2.39 (40)	<0.001 (r)		+1.04 ± 2.23 (40)	0.005	

Parameter	PAC-SYM overall score			PAC-QoL overall score		
	Mean ± SD (n)	Within group P value	Between group P value	Mean ± SD (n)	Within group P value	Between group P value
Baseline						
Actazin	1.45 ± 0.73 (44)	n/a	0.249	1.60 ± 0.82 (44)	n/a	0.427
Placebo	1.33 ± 0.52 (41)	n/a		1.47 ± 0.49 (41)	n/a	
Change from Baseline to Day 28						
Actazin	−0.77 ± 0.64 (43)	<0.001	0.524	−0.81 ± 0.85 (43)	<0.001	0.638
Placebo	−0.58 ± 0.61 (40)	<0.001		−0.63 ± 0.66 (40)	<0.001	

P-values were generated using ANOVA. P-values for change from screening/baseline generated using ANCOVA.
(r) indicates values were ranked prior to generating ANOVA/ANCOVA.
Note: one individual from each group had early termination from the study (after baseline).

3.7. Microbiome analysis

The community composition of the fecal microbiome of participants at baseline and day 28 showed no significantly distinguishable clustering by time or treatment group using principal coordinate analysis (data not shown). Based on these similarities we did not analyse enterotype. There were no significant differences in alpha and beta diversity (Faiths PD, Chao1, Shannon) between baseline and day 28 with either Actazin or placebo groups (data not shown). Nor were there any significant differences in alpha and beta diversity (Faiths PD, Chao1, Shannon) between Actazin and placebo at day 28 (data not shown). Mean Bacteroidetes: Firmicutes (synonyms: Bacteroidota:Bacillota) ratios were not significantly different between Actazin and placebo at day 28 (data not shown). There were insufficient Prevotellaceae across all participants to allow calculation of Prevotellaceae: Bacteroidaceae ratios. Collectively these data suggested there were no significant

Table 3
Bowel Regularity Index (BRI) total score at day 28 for participants in the ITT population who completed the 28-day intervention (n = 82).

Question	Actazin Mean ± SD (n) Median (Min - Max)	Placebo Mean ± SD (n) Median (Min - Max)	Between Group P-Value[a]
I feel that the product made my bowel movements more regular	3.43 ± 1.21 (42[b]) 4.00 (1.00–5.00)	3.33 ± 1.29 (40[b]) 4.00 (1.00–5.00)	0.709 (r)
I feel the product relieved my constipation	3.29 ± 1.17 (42) 4.00 (1.00–5.00)	3.15 ± 1.27 (40) 3.00 (1.00–5.00)	0.617
I feel the product eased my feelings of bloating and/or gas	3.07 ± 0.92 (42) 3.00 (1.00–5.00)	3.00 ± 1.22 (40) 3.00 (1.00–5.00)	0.765
I feel the product eased my feelings of abdominal discomfort	3.24 ± 0.93 (42) 3.00 (1.00–5.00)	3.15 ± 1.12 (40) 3.50 (1.00–5.00)	0.699
I feel the I spend less time in the toilet having taken the product	3.21 ± 1.22 (42) 3.00 (1.00–5.00)	3.15 ± 1.14 (40) 3.00 (1.00–5.00)	0.807
I feel the product increased my feelings of satisfaction with my bowel movements	3.38 ± 1.08 (42) 4.00 (1.00–5.00)	3.20 ± 1.20 (40) 3.50 (1.00–5.00)	0.475
I feel that product improved my gut health	3.43 ± 0.94 (42) 4.00 (1.00–5.00)	3.10 ± 1.01 (40) 3.00 (1.00–5.00)	0.131
I feel better having taken the product	3.45 ± 0.92 (42) 4.00 (1.00–5.00)	3.17 ± 1.11 (40) 3.00 (1.00–5.00)	0.219
I feel that the product improved my well-being	3.17 ± 0.88 (42) 3.00 (1.00–5.00)	3.08 ± 1.07 (40) 3.00 (1.00–5.00)	0.673
I tolerated the product well and had no complaints	4.14 ± 0.98 (42) 4.00 (1.00–5.00)	4.15 ± 0.80 (40) 4.00 (2.00–5.00)	0.971
I am satisfied with this product	3.67 ± 1.12 (42) 4.00 (1.00–5.00)	3.35 ± 1.12 (40) 4.00 (1.00–5.00)	0.205
I would recommend this product to others	3.60 ± 1.13 (42) 4.00 (1.00–5.00)	3.40 ± 1.13 (40) 4.00 (1.00–5.00)	0.436
Overall Bowel Regularity Index	41.07 ± 9.91 (42) 44.00 (13.00–58.00)	39.23 ± 11.54 (40) 42.50 (15.00–60.00)	0.321

n, number; SD, standard deviation; Min, minimum; Max, maximum.
[a] P-values were generated using ANOVA.
[b] One participant from each group terminated the study early. One participant from the Actazin group did not complete this diary at day 28.

community-scale differences in the fecal microbiome of participants when comparing the IPs.

Microbial richness and specific microbial abundances *versus* stool consistency (Falony et al., 2016; Vandeputte et al., 2016) were examined using the BSFS data but there were no significant correlations between these factors.

Next, we compared changes in the relative abundance of individual taxa within the treatment groups. We considered that taxa changing from baseline to day 28 in the Actazin group were only important if they also changed when comparing Actazin at day 28 with placebo at day 28, thereby excluding changes common to both treatments, as these changes may not solely be in response to Actazin. Those taxa that showed significant ($p < 0.05$) fold changes from baseline to day 28 with Actazin which also showed significant ($p < 0.05$) fold changes in comparison to the placebo at day 28 are presented in Fig. 3.

Actazin group participants had a 1.4-fold increase from baseline to day 28, and a 1.3-fold increase in the Actazin group over the placebo at day 28 for the genus *Faecalibacterium* (CM04.06: not *F. prausnitzii*) of the Ruminococcaceae family within the Firmicutes phylum, order *Clostridiales*. Other *Clostridiales* decreased in the Actazin group from baseline to day 28 and decreased at day 28 compared with placebo at day 28 by 1.1-fold and 1.3-fold respectively for Peptococcaceae family, 2.0-fold for both comparisons for the *Intestinibacter bartlettii* of the Peptostreptococcaceae family, and 1.5-fold and 1.3-fold respectively for *Blautia massiliensis* of the Lachnospiraceae family. The only other Firmicutes decreasing from baseline to day 28 in the Actazin group and in the Actazin group at day 28 compared to placebo at day 28 were *Holdemania* of the Erysipelotrichaceae family by 3.7-fold and 1.7-fold, respectively. Of the Bacteroidetes phylum, only *Alistipes indistinctus* of the Rikenellaceae family showed significant fold changes for both comparisons, decreasing from baseline to day 28 in the Actazin group by 2.8-fold and increasing in Actazin at day 28 compared to placebo at day 28 by 1.8-fold, consistent with a greater decline from baseline to day 28 in the placebo group than the Actazin group. Of the Actinobacteria, *Adlercreutzia* of the Eggerthellaceae family showed significant fold changes, decreasing in Actazin from baseline to day 28 and comparing Actazin at day 28 with placebo at day 28 by 1.5-fold and 1.4-fold respectively. The fecal bacterial taxa that were significantly different by pairwise Wilcoxon *t*-test between baseline and day 28 in the Actazin intervention group are summarised in Supplementary Table S3; and between Actazin intervention group at day 28 and placebo group at day 28 are summarised in Supplementary Table S4.

3.8. Compliance, safety and tolerance

There were no moderate AEs, severe AEs, or deaths to report in this study. Forty-eight post-emergent AEs categorized as 'unlikely or 'not related' were reported by 22 participants in this study. Of these, 30 AEs were reported by 13 participants in the Actazin group, and 18 AEs were reported by 9 participants in the placebo group. Of the 30 AEs reported by participants in the Actazin group, none were classified as 'probably' related to the Actazin and were: abdominal distension, hypertension, and weight gain. Of the 18 AEs reported by participants in the placebo group, nine were categorized as 'possibly' related. These nine were from two participants: one reported abdominal cramps, epigastric pain, bloating, burping, nausea, and dizziness. The other reported gas and hunger.

All AEs were resolved by the end of the study, except for one. An AE of weight gain in the Actazin group was classified as unrelated.

4. Discussion

Actazin consumption for 28 days in a population with ≤3 CSBM/week showed significantly improved BSFS score, a measure of stool consistency. Participants consuming Actazin reported significant improvements in BSFS score from baseline throughout the 28-day study and significant improvements over placebo at 14 days after supplementation, as well as over 28 days using repeated measures analysis. To the best of our knowledge this is the first study to report on BSFS improvement over placebo by a kiwifruit product. Previous studies examining Actazin supplementation, or whole kiwifruit consumption in populations of healthy and functionally constipated, did not observe any significant changes in BSFS score (Ansell et al., 2015; Chan et al., 2007; Chang et al., 2010). Normalising stool form (scored by BSFS) is associated with improved quality-of-life (Ohkubo et al., 2021).

Along with improvements to BSFS scores, significant improvements in CSBM frequency of greater than one were reported by the Actazin group after 28 days of supplementation ($p < 0.05$). The FDA has advised that in a symptomatic population an increase in one BM per week is considered a clinically meaningful magnitude (U.S. Department of Health and, 2012). Consistent with this observation, in a previous randomized, double-blind, placebo-controlled, cross-over study with functionally constipated (based on Rome III criteria) participants, those responders in the subgroup analysis supplemented with once daily 600 mg of Actazin for 28 days reported a greater mean increase in the number of daily BMs than those receiving 2400 mg Actazin per day (Ansell et al., 2015). In that study, and for both healthy and functionally constipated populations, the 600 daily dose of Actazin resulted in improvements of at least one BM per week from the washout period, the same as seen in the placebo group (Ansell et al., 2015).

While it may be hypothesized that higher doses should be more beneficial than lower doses, a recent meta-analysis examining different

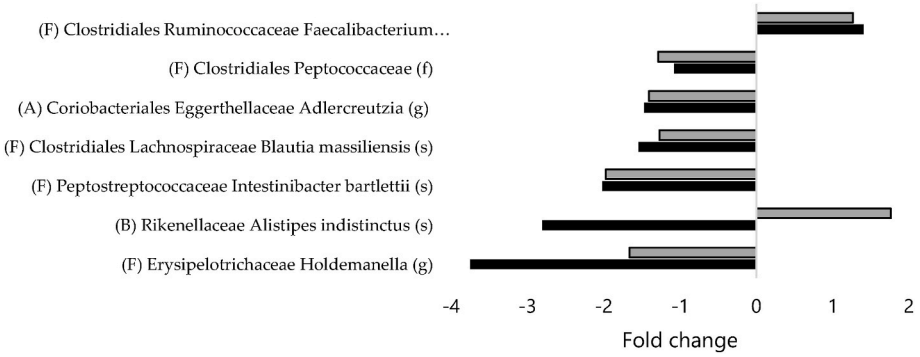

Fig. 3. Fecal bacterial taxa from Actazin treatment group at day 28 which both showed significant ($p < 0.05$) fold changes (x-axis) compared to the Actazin group at baseline (black bar) and also showed significant ($p < 0.05$) fold changes compared to the placebo group at day 28 (grey bar). *Faecalibacterium* showed significant fold increases from baseline to day 28 and also in comparison to placebo at day 28. *Alistipes* showed significant ($p < 0.05$) fold decrease from baseline to day 28 and a significant ($p < 0.05$) fold increase vs. placebo at day 28. All other bacteria showed significantly ($p < 0.05$) decreased fold change from baseline to day 28 and vs. placebo at day 28.

types of soluble fiber supplementation on anthropometric and metabolic outcomes reported that fiber supplementation was not dose dependant (Thompson et al., 2017) suggesting health benefits in even slight increases in fiber intake. This is true, particularly for those with a low dietary fiber intake which includes over 95% of North American adults (Quagliani & Felt-Gunderson, 2017). Participants included in the current study were not consuming high fiber diets (defined as >30 g/day), therefore improvements in BSFS and CSBM frequency at the 600 mg doses of Actazin suggests the IP was relevant as a supplement for the general population.

The increase in CSBM by one per day from once daily 600 mg Actazin is functionally equivalent to (i.e., consumption yields the same results as) those increases with higher (2400 mg) doses of Actazin (Ansell et al., 2015), and those increases from consuming two whole kiwifruit (Caballero et al., 2020; Chey et al., 2021) for which an EFSA claim for maintenance of normal defecation has been allowed (EFSA NDA Panel EFSA Panel on Nutrition et al., 2021). The allowed EFSA claim states that kiwifruit fiber is a plausible mechanism by which kiwifruit may contribute to normal defecation (EFSA NDA Panel EFSA Panel on Nutrition et al., 2021). Actazin contains the same type of kiwifruit fiber as whole kiwifruit, and as dosage may not be a factor (Thompson et al., 2017), the clinically meaningful improvements reported here suggest that it is also plausible that Actazin kiwifruit fibre is the mechanism by which these improvements may be explained.

Kiwifruit fiber's capacity of swelling, defined as the volume occupied by fiber in water after passively settling (Robertson et al., 2000), is one and a half times higher than psyllium and greater than six times higher than apple fiber (Sims & Monro, 2013). Kiwifruit fiber has high water retention capacity (Mishra & Monro, 2012; Sims & Monro, 2013), defined as volume of water bound to insoluble fiber and not separated by centrifugation (Robertson et al., 2000). Kiwifruit fibre is fermentable (Parkar et al., 2012; Rosendale et al., 2012) and will contribute to colonic microbial biomass (Rosendale et al., 2017). Other constituents may survive intact to the colon (polyphenols, organic acids, other dietary fibers from the diet) and may contribute to water holding and act as microbial fermentation substrates. These should contribute to fecal bulking (Bayer et al., 2018). The role of kiwifruit polyphenols and the kiwifruit protease actinidin in conferring laxative properties to Actazin have been suggested previously (Ansell et al., 2015). The Actazin used in this study possessed actinidin activity more than the minimum 25,000 AU/g product specification, indicating that processing from the fruit retained kiwifruit bioactives with minimal structural changes. The potential role of other components in contributing to osmotic laxative function such as kiwifruit organic acids (Bayer et al., 2018) are plausible, although yet to be determined. Collectively, these factors may have contributed to daily Actazin consumption improving stool form scores and regularity of BMs in a functionally constipated cohort.

All participants reported significant improvements in abdominal, rectal and stool symptoms, and overall PAC-SYM scores from baseline. Additionally, participants supplemented with Actazin reported significant improvements in PAC-QoL from baseline. While there is some debate as to whether constipation is a consequence or cause of poor quality of life, current evidence suggests that treating constipation symptoms increases health-related quality of life (Dennison et al., 2005). A meta-analysis of adults with chronic constipation found that loss of productivity and activity impairment was significantly greater than healthy controls due to their constipation (Sun et al., 2011). Improvements in BM frequency and stool form in constipated individuals may not only treat physical symptomology but also contribute to the larger picture of the person's overall health and wellbeing.

Several studies have looked at the association between stool consistency and microbial richness and specific microbial abundances (Falony et al., 2016; Vandeputte et al., 2016). These associations are likely due to the bacteria most appropriate for the conditions of the stool such as water content and availability of fermentable material being most abundant (Shah et al., 2020). Despite Actazin having a significant improvement in BSFS over placebo and over baseline, none of the associations with microbial parameters were found in this group or in the placebo group. However, significant modification to participant fecal microbiomes from the Actazin group were consistent with that seen following the consumption of cell wall polysaccharide-containing material. These modifications were fold-changes in relative abundance of distinct bacteria rather than overall changes to community structure. *Faecalibacterium* genera showing a significant but small fold improvement after Actazin consumption may include genera able to grow on high methoxy pectins (Lopez-Siles et al., 2012). Actazin and green kiwifruit contains high methoxy pectins (Carnachan et al., 2012). The health-promoting properties of a species of this genus, *F. prausnitzii*, are widely recognised (Martín et al., 2023). Note that *F. prausnitzii* itself was not amongst those significantly increased upon Actazin consumption, unlike observations with a similar gold kiwifruit powder (Blatchford et al., 2017), indicating that individual species substrate utilization (or pectin structures in individual kiwifruit cultivars) vary. Bacteria showing significant decreases from baseline in response to Actazin were Peptococcaceae and Peptostreptococcaceae, the latter being relevant as observed in cats (where Peptostreptococcaceae is one of the numerically predominant species in the feces of younger animals) a decrease occurs upon moving from a meat diet to a kibble diet containing plant cell wall polysaccharides (Bermingham et al., 2018). *Peptostreptococcaceae* have been observed in higher abundance in colorectal cancer patients (Ahn et al., 2013), suggesting a decrease in response to Actazin is a good thing. *Alistipes indistinctus* also decreased in Actazin consumer feces from baseline to day 28, consistent with this bacterium's only weak saccharolytic ability (Nagai et al., 2010). *Alistipes* have been associated with increased risk of cancer and metal health issues, while being protective against gut inflammation and cardiovascular risk (Parker et al., 2020). Similarly, *Adlercreutzia* decreased in Actazin consumer feces, also consistent with its only weak ability to access plant cell wall-derived sugars such as rhamnose (pectin) (Willems et al., 1997). These fold change comparisons held true for the comparison of Actazin with placebo at day 28, with the interesting exception of the same *Alistipes*, which was 1.8-fold increased at day 28 for the Actazin group vs. placebo, suggesting the increased abundance of this bacterium following Actazin consumption over placebo is related to this bacterium's ability to use rhamnose and weak ability to use arabinose (Nagai et al., 2010), pectin constituent sugars which Actazin contains but the placebo does not. Collectively these data show that while overall microbiome community structures do not drastically change in response to 600 mg daily consumption of Actazin, some resident gut taxa do respond to the intervention at the family-to-species levels. These changes are consistent with bacterial taxa responding to Actazin fermentable kiwifruit constituents, which has been previously observed with kiwifruit cell wall polysaccharides (Parkar et al., 2012; Rosendale et al., 2012).

This study demonstrated that 28 days of Actazin supplementation was safe and well tolerated with few AEs "possibly" related to the IP. This is consistent with the long history of safe human consumption of kiwifruit and kiwifruit products by non-allergic consumers.

There were limitations of this study that warrant consideration. There was a high placebo effect observed. The placebo effect is common to gastrointestinal studies, especially those of less than 12-week duration, with up to 50% of the placebo effect due to the spontaneous waning and waxing of symptoms (Enck & Klosterhalfen, 2020). This was exacerbated by the choice of placebo material: microcrystalline cellulose (MCC). This was chosen on the basis that it is inert, indigestible, and poorly fermentable. In retrospect, the use of non-digestible, poorly fermentable agent which survives intact in the colon and may contribute to fecal bulking and increased transit time (Nsor-Atindana et al., 2017) and may have been less optimal than the use of a completely digestible material such as maltodextrin, which would not be expected to reach the colon in significant amounts. Alternatively, as a recent meta-analysis shows even maltodextrin placebos may yield effects (Almutairi et al., 2022), it may be worth considering the ethics of future gastrointestinal

intervention studies using just a control group, receiving no placebo at all. Nevertheless, in the present study Actazin outperformed the placebo for BSFS at Day 14 despite a lower absolute amount of fiber per dose, showing the greater efficacy of the collective kiwifruit cell wall fibers (cellulose, hemicellulose, and pectin), enzymes, vitamins, minerals, organic acids and polyphenols towards improving bowel habits and stool form.

Practical application: this study illustrates the benefits to occasionally constipated healthy individuals of consuming gently processed kiwifruit powder containing plant cell wall polysaccharides (pectin, hemicellulose and cellulose), organic acids and polyphenols for maintenance of gut health, normalising laxation and stool form, and microbiome modulation. It occurs to us that perhaps daily consumption of other freeze-dried fruit powders may confer the benefits associated with their whole fruit consumption.

5. Conclusions

In a healthy population with $\leq$3 CSBM per week, 600 mg of Actazin per day for 28 days significantly improved stool consistency (BSFS) compared to placebo as soon as 14 days. To the best of our knowledge this is the first report of this BSFS improvement over placebo by a kiwifruit product. Actazin consumption resulted in changes in specific microbial taxa in the gut, such as genus *Faecalibacterium*. This was accompanied by significant improvements in the frequency of CBSM and SBM. The benefits of improvements in bowel habits are evidenced by reported constipation symptom and quality of life improvements. Supplementation with Actazin was safe and well tolerated during the 28-day study period. Novelty: this study illustrates that low doses (600 mg) consumed daily have the same benefits to laxation as consuming two whole fruit (approx. 184 g), and showed improvements to stool form with kiwifruit powder for the first time. The results of the study support a role for Actazin in improving stool form and bowel habits in healthy populations at risk of occasional constipation.

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CRediT authorship contribution statement

Emma Graham: Conceptualization, Writing – review & editing. **Starin McKeen:** Formal analysis, Writing – review & editing. **Erin D. Lewis:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Malkanthi Evans:** Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing. **Zhaoping Li:** Formal analysis, Investigation, Writing – review & editing. **Susanne M. Henning:** Formal analysis, Methodology, Writing – review & editing. **Neville Jopson:** Formal analysis, Software, Writing – review & editing. **Jennifer Gu:** Conceptualization, Project administration, Writing – review & editing. **Doug Rosendale:** Formal analysis, Writing – original draft.

Declaration of competing interest

I hereby disclose that Doug Rosendale, Emma Graham, Starin McKeen and Jennifer Gu have conflicts. Doug Rosendale, Emma Graham and Starin McKeen are employees of Anagenix Ltd., manufacturer of the ingredients that are the subject of the investigation, and co-sponsor of this study. Jennifer Gu is an employee of AIDP Ltd., distributor of the ingredients that are the subject of the investigation, and co-sponsor of this study. Doug Rosendale is a scientific consultant to AIDP Ltd.

Erin Lewis, Malkanthi Evans, Susanne Henning, Zhaoping Li and Neville Jopson do not have any personal circumstances or interests that may be perceived as inappropriately influencing the representation or interpretation of reported results and thus have no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcdf.2024.100436.

References

Ackley, B. J., & Ladwig, G. B. (2013). *Nursing diagnosis handbook, an evidence-based guide to planning care, 10: Nursing diagnosis handbook*. Elsevier Health Sciences. ISBN 9780323085496.

Ahn, J., Sinha, R., Pei, Z., et al. (2013). Human gut microbiome and risk for colorectal cancer. *Journal of the National Cancer Institute, 105*(24), 1907–1911. https://doi.org/10.1093/jnci/djt300

Alexandre, V., Bertin, C., Boubaya, M., Airinei, G., Bouchoucha, M., & Benamouzig, R. (2016). Randomized clinical trial: Efficacy of a food supplement, TRANSITECH, on healthy individuals with mild intermittent constipation. *European Journal of Gastroenterology and Hepatology, 28*(9), 1087–1093. https://doi.org/10.1097/meg.0000000000000672

Almutairi, R., Basson, A. R., Wearsh, P., Cominelli, F., & Rodriguez-Palacios, A. (2022). Validity of food additive maltodextrin as placebo and effects on human gut physiology: Systematic review of placebo-controlled clinical trials [published online ahead of print, 2022 mar 1]. *European Journal of Nutrition*. https://doi.org/10.1007/s00394-022-02802-5

Ansell, J., Butts, C. A., Paturi, G., Eady, S. L., Wallace, A. J., Hedderley, D., & Gearry, R. B. (2015). Kiwifruit-derived supplements increase stool frequency in healthy adults: A randomized, double-blind, placebo-controlled study. *Nutrition Research, 35*(5), 401–408.

Bayer, S. B., Gearry, R. B., & Drummond, L. N. (2018). Putative mechanisms of kiwifruit on maintenance of normal gastrointestinal function. *Critical Reviews in Food Science and Nutrition, 58*(14), 2432–2452. https://doi.org/10.1080/10408398.2017.1327841

Bermingham, E. N., Young, W., Butowski, C. F., Moon, C. D., Maclean, P. H., Rosendale, D., Cave, N. J., & Thomas, D. G. (2018). The fecal microbiota in the domestic cat (Felis catus) is influenced by interactions between age and diet; A five year longitudinal study. *Frontiers in Microbiology, 9*, 1231. https://doi.org/10.3389/fmicb.2018.01231

Bharucha, A. E., & Lacy, B. E. (2020). Mechanisms, evaluation, and management of chronic constipation. *Gastroenterology, 158*, 1232–1249.e3. https://doi.org/10.1053/j.gastro.2019.12.034

Blatchford, P., Stoklosinski, H., Eady, S., Wallace, A., Butts, C., Gearry, R., Gibson, G., & Ansell, J. (2017). Consumption of kiwifruit capsules increases Faecalibacterium prausnitzii abundance in functionally constipated individuals: A randomised controlled human trial. *Journal of Nutrition Sciences, 6*. https://doi.org/10.1017/jns.2017.52

Caballero, N., Benslaiman, B., Ansell, J., & Serra, J. (2020). The effect of green kiwifruit on gas transit and tolerance in healthy humans. *Neuro-Gastroenterology and Motility*. , Article e13874. https://doi.org/10.1111/nmo.13874

Callahan, B., McMurdie, P., Rosen, M., et al. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*, 581–583. https://doi.org/10.1038/nmeth.3869

Carnachan, S., Bell, T., Mishra, S., Monro, J., & Sims, I. (2012). Effects of simulated digestion in vitro on cell wall polysaccharides from kiwifruit (Actinidia spp.). *Food Chemistry, 133*, 132–139. https://doi.org/10.1016/j.foodchem.2011.12.084

Chan, A. O. O., Leung, G., Tong, T., & Wong, N. Y. H. (2007). Increasing dietary fiber intake in terms of kiwifruit improves constipation in Chinese patients. *World Journal of Gastroenterology, 13*, 4771–4775.

Chang, C.-C., Lin, Y.-T., Lu, Y.-T., Liu, Y.-S., & Liu, J.-F. (2010). Kiwifruit improves bowel function in patients with irritable bowel syndrome with constipation. *Asia Pacific Journal of Clinical Nutrition, 19*(4), 451–457.

Chey, S. W., Chey, W. D., Jackson, K., & Eswaran, S. (2021). Exploratory comparative effectiveness trial of green kiwifruit, psyllium, or prunes in US patients with chronic constipation. *American Journal of Gastroenterology*. https://doi.org/10.14309/ajg.0000000000001149

Cummings, J. (2001). The effect of dietary fiber on fecal weight and composition. In G. Spiller (Ed.), *CRC handbook of dietary fiber in human nutrition* (pp. 183–252). Boca Raton, Florida, USA: CRC Press.

Dennison, C., Prasad, M., Lloyd, A., Bhattacharyya, S., Dhawan, R., & Coyne, K. (2005). The health-related quality of life and economic burden of constipation. *PharmacoEconomics*.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2016). Guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms. *EFSA Journal, 14*(1), 4369. https://doi.org/10.2903/j.efsa.2016.4369ISSN:1831-4732, 2016.

Enck, P., & Klosterhalfen, S. (2020). Placebo responses and placebo effects in functional gastrointestinal disorders. *Frontiers in Psychiatry, 11*, 797. https://doi.org/10.3389/fpsyt.2020.00797

Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., Kurilshikov, A., Bonder, M. J., Valles-Colomer, M., Vandeputte, D., & Tito, R. Y. (2016). Population-level analysis of gut microbiome variation. *Science, 352*(6285), 560–564. https://doi.org/10.1126/science.aad3503

Frank, L., Kleinman, L., Farup, C., Taylor, L., & Miner, P. J. (1999). Psychometric validation of a constipation symptom assessment questionnaire. *Scandinavian Journal of Gastroenterology, 34*(9), 870–877. https://doi.org/10.1080/003655299750025327

Gélinas, P. (2013). Preventing constipation: A review of the laxative potential of food ingredients. *International Journal of Food Science and Technology, 48*(3), 445–467.

Higgins, P. D., & Johanson, J. F. (2004). Epidemiology of constipation in North America: A systematic review. *American Journal of Gastroenterology, 99*(4), 750–759. https://doi.org/10.1111/j.1572-0241.2004.04114.x

Koh, H., Lee, M. J., Kim, M. J., Shin, J. I., & Chung, K. S. (2010). Simple diagnostic approach to childhood fecal retention using the Leech score and Bristol stool form scale in medical practice. *Journal of Gastroenterology and Hepatology, 25*, 334–338. https://doi.org/10.1111/j.1440-1746.2009.06015.x

Lewis, E. D., Crowley, D. C., Trivedi, R., Wolever, T., Buggia, M. A., Graham, E., McKeen, S., Gu, j., Rosendale, D., & Evans, M. (2023). Green and gold kiwifruit powders consumed for 28 days by healthy individuals with occasional constipation are safe and well tolerated: A randomized controlled trial. *Bioactive Carbohydrates and Dietary Fibre* (in press).

Lewis, S. J., & Heaton, K. W. (1997). Stool form scale as a useful guide to intestinal transit time. *Scandinavian Journal of Gastroenterology, 32*, 920–924.

Longstreth, G. F., Thompson, W. G., Chey, W. D., Houghton, L. A., Mearin, F., & Spiller, R. C. (2006). Functional bowel disorders. *Gastroenterology, 130*(5), 1480–1491. https://doi.org/10.1053/j.gastro.2005.11.061. Erratum in: Gastroenterology. 2006 Aug;131(2):688. PMID: 16678561.

Lopez-Siles, M., Khan, T. M., Duncan, S. H., Harmsen, H. J., Garcia-Gil, L. J., & Flint, H. J. (2012). Cultured representatives of two major phylogroups of human colonic Faecalibacterium prausnitzii can utilize pectin, uronic acids, and host-derived substrates for growth. *Applied and Environmental Microbiology, 78*(2), 420–428. https://doi.org/10.1128/AEM.06858-11

Marquis, P., De, La L. C., Dubois, D., McDermott, A., & Chassany, O. (2005). Development and validation of the patient assessment of constipation quality of life questionnaire. *Scandinavian Journal of Gastroenterology, 40*(5), 540–551. https://doi.org/10.1080/00365520510012208

Martín, R., Rios-Covian, D., Huillet, E., Auger, S., Khazal, S., Bermúdez-Humarán, L. G., Sokol, H., Chatel, J. M., & Langella, P. (2023). Faecalibacterium: A bacterial genus with promising human health applications. *FEMS Microbiology Reviews*, fuad039.

McMurdie and Holmes. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One, 8*(4), Article e61217.

Mishra, S., & Monro, J. A. (2012). Kiwifruit remnants from digestion in vitro have functional attributes of potential importance to health. *Food Chemistry, 135*, 2188–2194.

Mugie, S. M., Benninga, M. A., & Di Lorenzo, C. (2011). Epidemiology of constipation in children and adults: A systematic review. *Best Practice & Research Clinical Gastroenterology, 25*(1), 3–18. https://doi.org/10.1016/j.bpg.2010.12.010

Nagai, F., Morotomi, M., Watanabe, Y., Sakon, H., & Tanaka, R. (2010). Alistipes indistinctus sp. nov. and Odoribacter laneus sp. nov., common members of the human intestinal microbiota isolated from faeces. *International Journal of Systematic and Evolutionary Microbiology, 60*(Pt 6), 1296–1302. https://doi.org/10.1099/ijs.0.014571-0

Nsor-Atindana, J., Chen, M., Goff, H. D., Zhong, F., Sharif, H. R., & Li, Y. (2017). Functionality and nutritional aspects of microcrystalline cellulose in food. *Carbohydrate Polymers, 172*, 159–174. https://doi.org/10.1016/j.carbpol.2017.04.021

Ohkubo, H., Yoshihara, T., Misawa, N., et al. (2021). Relationship between stool form and quality of life in patients with chronic constipation: An internet questionnaire survey. *Digestion, 102*(2), 147–154. https://doi.org/10.1159/000502815

Parkar, S. G., Rosendale, D., Paturi, G., et al. (2012). In vitro utilization of gold and green kiwifruit oligosaccharides by human gut microbial populations. *Plant Foods for Human Nutrition, 67*, 200–207. https://doi.org/10.1007/s11130-012-0293-1

Parker, B. J., Wearsch, P. A., Veloo, C. M., & Rodriguez-Palacios, A. (2020). The genus Alistipes: Gut bacteria with emerging implications to inflammation, cancer and mental health. *Frontiers in Immunology, 11*, 906.

Quagliani, D., & Felt-Gunderson, P. (2017). Closing America's fiber intake gap: Communication strategies from a food and fiber summit. *American Journal of Lifestyle Medicine, 11*(1), 80–85. https://doi.org/10.1177/1559827615588079

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research, 41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219, 1 January 2013.

Rao, S., & Brenner, D. M. (2021). Efficacy and safety of over-the-counter therapies for chronic constipation: An updated systematic review. *American Journal of Gastroenterology, 116*(6), 1156–1181. https://doi.org/10.14309/ajg.0000000000001222

Reigler, G., & Esposito, I. (2001). Bristol scale stool form. In *A still valid help in medical practice and clinical research – techniques in coloproctology*. Springer.

Robertson, J. A., de Monredon, F. D., Dysseler, P., Guillon, F., Amado, R., & Thibault, J.-F. (2000). Hydration properties of dietary fibre and resistant starch: A European collaborative study. *LWT–Food Science and Technology, 33*, 72–79.

Rosendale, D. I., Blatchford, P. A., Sims, I. M., Parkar, S. G., Carnachan, S. M., Hedderley, D., & Ansell, J. (2012). Characterizing kiwifruit carbohydrate utilisation in vitro and its consequences for human faecal microbiota. *Journal of Proteome Research*. https://doi.org/10.1021/pr300646m

Rosendale, D. I., Vetharaniam, I., Kelly, W. J., Upsdell, M., Cookson, A. L., & Roy, N. (2017). A case study: Using microbial abundance data to mathematically calculate organic acid production by human faecal microbiota within an in vitro batch fermentation. *Bioactive Carbohydrates and Dietary Fibre, 9*, 28–38. https://doi.org/10.1016/j.bcdf.2016.12.004

Rush, E. C., Patel, M., Plank, L. D., & Ferguson, L. R. (2002). Kiwifruit promotes laxation in the elderly. *Asia Pacific Journal of Clinical Nutrition, 11*, 164–168.

Schmulson, M. J., & Drossman, D. A. (2017). What is new in Rome IV. *J Neurogastroenterol Motil, 23*(2), 151–163. PMCID: PMC5383110.

Scholar, E. (2008). Laxatives. In S. J. Enna, & D. B. Bylund (Eds.), *xPharm: The comprehensive pharmacology reference*. Elsevier Inc.. ISBN: 978-0-08-055232-3.

Shah, R. M., McKenzie, E. J., Rosin, M. T., Jadhav, S. R., Gondalia, S. V., Rosendale, D., & Beale, D. J. (2020). An integrated multi-disciplinary perspective for addressing challenges of the human gut microbiome. *Metabolites, 10*, 94. https://doi.org/10.3390/metabo10030094

Sims, I. M., & Monro, J. A. (2013). Fiber: Composition, structures, and functional properties. *Advances in Food & Nutrition Research, 68*, 81–99.

Sun, S. X., Dibonaventura, M., Purayidathil, F. W., Wagner, J. S., Dabbous, O., & Mody, R. (2011). Impact of chronic constipation on health-related quality of life, work productivity, and healthcare resource use: An analysis of the national health and wellness survey. *Digestive Diseases and Sciences, 56*(9), 2688–2695. https://doi.org/10.1007/s10620-011-1639-5

Thompson, S. V., Hannon, B. A., An, R., & Holscher, H. D. (2017). Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: A systematic review and meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition, 106*(6), 1514–1528. https://doi.org/10.3945/ajcn.117.163246

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Knutsen, H. K., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Thies, F., Tsabouri, S., Vinceti, M., Bresson, J.-L., & Siani, A. (2021). Opinion of the scientific panel on green kiwifruit (lat. Actinidia deliciosa var. Hayward) and maintenance of normal defecation: Evaluation of a health claim pursuant to article 13(5) of regulation (EC) No 1924/2006. *EFSA Journal, 19*(6), 6641. https://doi.org/10.2903/j.efsa.2021.6641, 2021.

Udani, J. K., & Bloom, D. W. (2013). Effects of kivia powder on gut health in patients with occasional constipation: A randomized, double-blind, placebo-controlled study. *Nutrition Journal, 12*, 78. https://doi.org/10.1186/1475-2891-12-78

U.S. Department of health and human Services FDA, & centre for drug evaluation and research (CDER) guidance for Industry: Irritable bowel syndrome-clinical evaluation of drugs for treatment.(2012).

Vandeputte, D., Falony, G., Vieira-Silva, S., et al. (2016). Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut, 65*, 57–62. https://doi.org/10.1136/gutjnl-2015-309618

Wilkinson-Smith, V., Dellschaft, N., Ansell, J., Hoad, C., Marciani, L., Gowland, P., & Spiller, R. (2019). Mechanisms underlying effects of kiwifruit on intestinal function shown by MRI in healthy volunteers. *Alimentary Pharmacology & Therapeutics*. https://doi.org/10.1111/apt.15127

Willems, A., Moore, W. E., Weiss, N., & Collins, M. D. (1997). Phenotypic and phylogenetic characterization of some eubacterium-like isolates containing a novel type B wall murein from human feces: Description of Holdemania filiformis gen. Nov., sp. nov. *International Journal of Systematic Bacteriology, 47*, 1201–1204.