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Livaux® gold kiwifruit powder increases *Faecalibacterium prausnitzii* numbers and decreases hydrogenotrophic *Blautia* spp numbers and bloating in healthy individuals, consistent with slow fermentation. A randomised controlled trial.

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Executive Summary

The role of our gut microbiota in health are well recognised. Some key members of our microbiota such as *Faecalibacterium prausnitzii* are becoming increasingly recognised for their associations with health and/or negative correlations with disease and dysfunction.

Livaux® is a skinless, seedless, cold-processed gold kiwifruit powder, retaining whole fruit benefits, including polyphenols, vitamins, and dietary fibre content. The dietary fibre are the plant cell wall polysaccharides cellulose, hemicellulose, and pectin. These polysaccharides - particularly the high-methoxy pectin - are fermentable. A previously published clinical study showed Livaux (2,400 mg per day for 28 days) increased the relative abundance of faecal *F. prausnitzii*. In a multi-stage in vitro simulation of the gut, as a part of a synbiotic formulation, Livaux also increased the relative abundance of *F. prausnitzii*. The growth of the pure culture is already known to be supported by high-methoxy pectin.

Here we present a larger human intervention trial confirming and expanding on the *F. prausnitzii* support provided by a 600 mg daily dosage of Livaux. A randomised, double-blinded, placebo-controlled parallel study with 85 participants conducted across four North American sites were carried out where daily Livaux consumption for 28 days yielded a statistically significant increase in *F. prausnitzii*. In addition, improvements in laxation measured as >1 complete spontaneous bowel movement (CSBM) per week in individuals starting with ≤3 CSBM per week at baseline, along with improvements in constipation symptoms and quality of life indicators, such as decreased abdominal bloating and discomfort were observed. Consistent with these decreased bloating scores, a decrease in the relative abundance of hydrogenotrophs from the *Blautia* genus was observed. These data are consistent with the Livaux pectin being slowly fermented without excessive gas production.

Livaux was safe and well tolerated by participants.

This study demonstrates that once daily 600 mg Livaux consumption for 28 days conveyed significant improvements in participant *F. prausnitzii* relative abundance, gas production, bowel habits and improved stool form in healthy individuals with occasional constipation.

A patented, award-winning gold kiwifruit powder, Livaux is the only ingredient to have won the Nutraingredients award in all 3 continents, USA, Europe and Asia.

Prebiotic of the Year US, Europe 2022

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Prebiotic of the Year US, Asia 2020

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Introduction

Potential next-generation probiotics offer physiological functions that are not always conferred by currently available probiotics. *Faecalibacterium prausnitzii* exemplifies these next-generation physiological functions: it is a human faecal isolate that is known to be one of the more prevalent and abundant members of the currently cultivable human gut microbiome, where decline in numbers correlates with poor health. Formerly classified as *Fusobacterium prausnitzii* (Cato et al., 1974), *F. prausnitzii* accounts for ~5% of the total faecal microbiota in healthy individuals but can increase to ~15% in some individuals (Miquel et al., 2013; Martin et al., 2018). Reduced *F. prausnitzii* relative abundance in the faeces of individuals correlates with incidence and severity of various disorders and diseases. For example, lower abundances of *F. prausnitzii* relative to healthy controls were observed in Crohn's disease (CD) patients with endoscopic recurrence (Sokol et al., 2008); in Japanese CD patients (Fujimoto et al., 2012); in active CD and inflammatory bowel disease (IBD) patients (Sokol et al., 2009); in ulcerative colitis patients (Machiels et al., 2014; Varela et al., 2013), in irritable bowel syndrome (IBS) patients (Rajilic-Stojanovic et al., 2011); in colorectal cancer patients (Balamurugan et al., 2008; Chen et al., 2012; Wu et al, 2013); in obese subjects with diabetes (Furet et al., 2010); in prediabetic patients (Zhang et al., 2013); in obese subjects (Hippe et al., 2016); in patients with psoriasis, IBD and IBD with concomitant psoriasis (Eppinga et al., 2016), in atopic children (Candela et al., 2012); in multiple sclerosis patients (Cantarel et al., 2015); in Parkinson's disease patients (Keshavarzian et al., 2015; Unger et al., 2016; Petrov et al., 2016; Li et al., 2017); in patients with major depressive disorder (Jiang et al., 2015); in flu (H1N1) patients (Gu et al., 2020) and Covid-19 patients (Zuo et al., 2020; Yeoh et al., 2021).

These correlations suggest that increasing *F. prausnitzii* relative abundance may be beneficial to health. However, converting *F. prausnitzii* into an industrially viable probiotic for direct consumption has been conventionally regarded as problematic, as optimising their specific growth requirements and need for anaerobic conditions at scale adds complexity, time and expense. An alternative would be to provide a precision prebiotic designed to act as a specific substrate for the growth of *F. prausnitzii*.

Livaux® is a powdered health ingredient derived from New Zealand gold (*Actinidia chinensis* "Zesy002") kiwifruit from which the skin and seeds are removed, and the remaining flesh cold processed for use in food and dietary supplements (Ansell et al., 2015). Kiwifruit is an excellent source of vitamins A, C and E, potassium, polyphenols and dietary fibre, and emerging evidence suggests it may help to resolve constipation (Bayer et al., 2018). A recent randomized, double-blind, placebo-controlled clinical crossover study examining the effects of Livaux gold kiwifruit powder on stool frequency, stool form and gastrointestinal comfort in healthy and functionally constipated individuals found that supplementation with 2400 mg Livaux demonstrated a significant (two-fold) increase in *F. prausnitzii* relative abundance (Blatchford et al., 2017). Livaux as a part of a synbiotic formulation has also been shown in an artificial gut system to increase the relative abundance of *F. prausnitzii* (Duysburgh et al., 2019). These are consistent with the biology of the bacterium: most *F. prausnitzii* strains will grow well on pectin and pectin derivatives such as galacturonic acid (Lopez-Siles, et al., 2012), and pectinolytic enzymes have been encoded in the *F. prausnitzii* reference genome (Heinken, et al., 2014). It appears to prefer high methoxy pectin from apple to low methoxy pectin from citrus, signifying that *F. prausnitzii* utilises high methoxy esterified galacturonic acid as a substrate. Livaux and gold kiwifruit contain high methoxy pectin (Carnachan et al., 2012).

Livaux is derived from natural sources, is safe, bioavailable, and addresses a longstanding need for a convenient, effective precision prebiotic supporting the growth of *F. prausnitzii*. Here we report a study showing once more that Livaux increases *F. prausnitzii* relative abundance in healthy participants, whilst improving their bowel habits.

The primary clinical outcome of the current study was the efficacy of a once daily 600 mg dose of Livaux on stool frequency in participants with occasional constipation who are otherwise healthy. This was measured by participant scoring of CSBMs, as well as participant-assessed stool form, constipation symptoms and quality of life. In addition to these laxation parameters we confirmed the precision prebiotic potential of Livaux by assessing changes to participant's fecal microbiomes.

Methods and Results

Study design

This was a multi-centre, randomized, double-blind, placebo-controlled, parallel group study consisting of a single 4-week intervention period. The investigational product was 600 mg Livaux® gold kiwifruit powder, compared to a placebo (microcrystalline cellulose).

Participants had three visits: screening; baseline (start of intervention period); and 28 days later, endpoint (of intervention period).

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

Participants in the intent-to-treat population ranged from 18 to 60 years of age (Table 1). For all groups, participants were predominantly female with 68.9% in the Livaux group and 65.9% in the placebo group. The population was predominantly Western European with 51.1% in the Livaux group and 48.8% in the placebo group.

Baseline and day 28 vital signs, anthropometric measures, hematology and blood chemistry were not significantly different between groups. For within group analysis, only hemocrit (placebo group), calcium (Livaux group) and chloride (placebo group) were significantly ($p<0.05$) different (Supplementary table S1). All participants were deemed healthy by the PI.

Table 1. Demographic information for participants in the ITT population (n = 86)

Variable	Livaux n(%)	Placebo n(%)
Age		
Mean ± SD (n)	40.93 ± 13.04 (45)	41.37 ± 12.21 (41)
Median (Min - Max)	43.00 (18.00 to 59.00)	43.00 (22.00 to 60.00)
Gender		
Female	31 (68.9%)	27 (65.90%)
Male	14 (31.1%)	14 (34.10%)
Ethnicity		
South American	2 (4.40%)	3 (7.30%)
Eastern European White	6 (13.30%)	8 (19.50%)
Hispanic or Latino	3 (6.70%)	2 (4.90%)
Western European White	23 (51.10%)	20 (48.80%)
South Asian	2 (4.40%)	0 (0.00%)
African American	2 (4.40%)	0 (0.00%)
Central American	0 (0.00%)	0 (0.00%)
Middle Eastern	2 (4.40%)	2 (4.90%)
African	2 (4.40%)	2 (4.90%)
East Asian	3 (6.70%)	2 (4.90%)
South East Asian	0 (0.00%)	1 (2.40%)
Native American	0 (0.00%)	1 (2.40%)
Missing	0 (0.00%)	0 (0.00%)

n, number; SD, standard deviation; Min, minimum; Max, maximum.

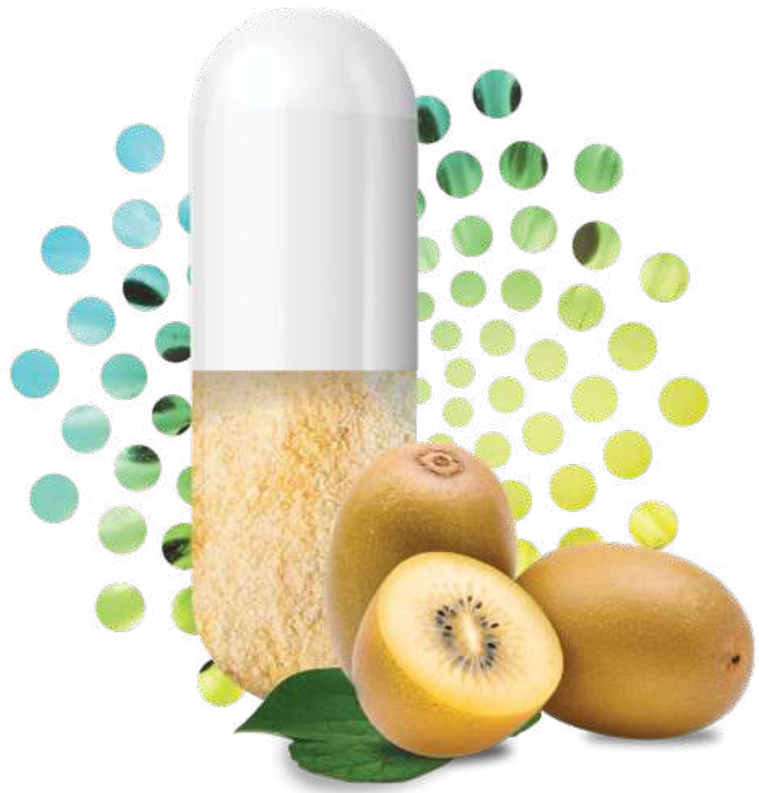
Microbiome analysis

The community composition of the fecal microbiome of participants at baseline and endpoint, analysed by Illumina Novaseq of 16SrRNA amplicons, showed no significant differences in alpha diversity (Faiths PD, Chao1, Shannon) between baseline and endpoint with either Livaux or placebo groups (data not shown).

Multivariant analyses showed no significantly distinguishable clustering by time or treatment group with Livaux and placebo (data not shown).

In terms of differences in the relative abundance of individual fecal bacterial taxa, we first examined those showing significant ($p<0.05$) fold changes from baseline to endpoint with Livaux (Figure 1), initially focusing on Faecalibacterium. Here we found the Faecalibacterium genus ($p = 0.016$ paired Wilcoxon rank test) and the F. prausnitzii species ($p = 0.047$) both showed significant increases in response to Livaux (Figure 1A).

We then examined all those other bacteria showing a significant ($p<0.05$) increase from baseline to endpoint in response to Livaux (Fig. 1B). Here we found that *Blautia* species ($p = 0.0021 – 0.038$), *Streptococcaceae* ($p = 0.012$), and *Faecalitalia* spp. ($p = 0.044$) decreased in response to Livaux, whilst *Bilophila* ($p = 0.003$), *Haemophilus* ($p = 0.0089$), *Christensenellaceae* ($p = 0.033$), *Ruminococcus* ($p = 0.018$), *Holdemania filiformis* ($p = 0.021$) and *Coprococcus eutactus* ($p = 0.0024$) increased in response to Livaux.



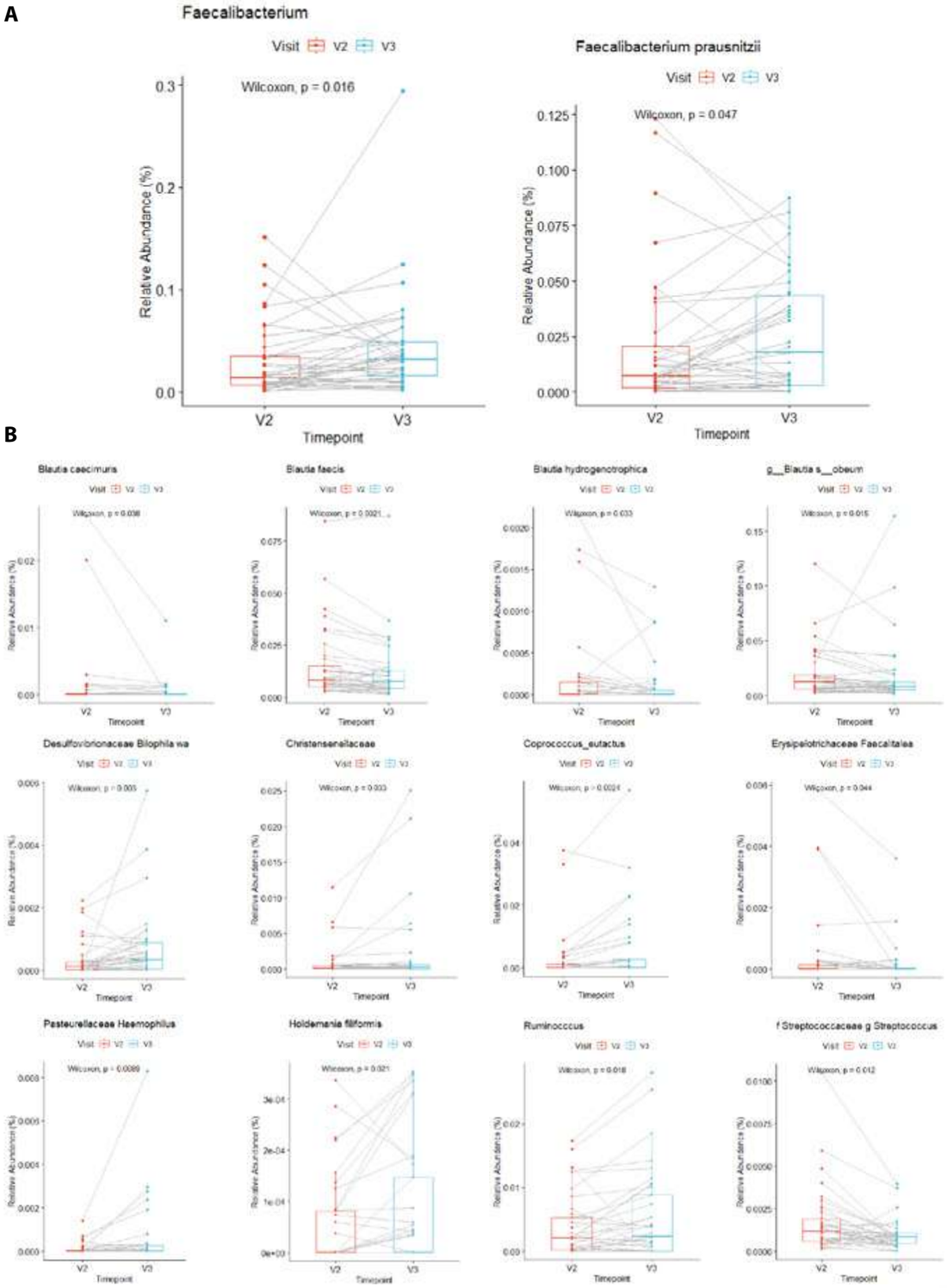


Figure 1. Livaux differentially affects key members of the faecal microbiome. Data compare baseline (V2, red) and endpoint (V3, blue). P value from paired Wilcoxon rank test shown. Only significantly different taxa represented.
1A. *Faecalibacterium* genus and *F. prausnitzii* species significantly increased in Livaux group.
1B. Different fecal bacterial taxa show fold changes in response to Livaux. *Blautia* species *(B. caecimuris, B. faecis, B. hydrogenotrophica, B. obeum*) decrease. *Bilophila wadsworthia, Christensenellaceae* and *Coprococcus eutactus* increase while *Faecalitalia* decrease, *Haemophilus, Holdemania filiformis, Ruminococcus increase* while *Streptococcus* decrease.

Clinical outcomes

Complete Spontaneous Bowel Movements (CSBM) and Spontaneous Bowel Movements (SBM) were evaluated using a questionnaire. CSBM is an accepted and easily defined primary measure of stool frequency in clinical trials assessing bowel habits (USDA, 2012). A CSBM classification was made if a participant reported a feeling of satisfaction (complete) and manual maneuvers, laxatives, enemas or suppositories were not used, and no assistance was needed (spontaneous). For SBM, it was spontaneous but there was not a feeling of satisfaction. Participants may be less comfortable following a SBM.

CSBMs (Table 2) showed significant ($p<0.05$) within-group improvements were reported by Livaux consumers as well as placebo at day 7, 14, 21 and 28. All groups reported increases of greater than 1 CSBM per week. However, there were no significant between-group differences in improvement of CSBM per week at day 7, 14, 21 or 28.

SBMs (Table 2) showed significant ($p<0.05$) within-group improvements in frequency were reported in Livaux and placebo from baseline to day 7, 14, 21 and 28. There were no significant overall between-group differences for the change in frequency of SBM per week at day 28.

Stool form was scored according to the Bristol Stool Form Score (BSFS) which depicts the form of the faeces on a 7-point scale, from hard to watery (Koh et al., 2010). BSFS scoring is well recognised and has been suggested as the main diagnostic criteria for IBS-D (Longstreth et al., 2006; USDA, 2012).

Significant ($p<0.05$) within-group improvements in BSFS were reported for the Livaux group at day 7, 14, 21 and 28. There were no significant between-group differences in BSFS score from baseline to day 7, 14, 21 and 28.

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 developed to measure the symptoms (Frank et al., 1999) and quality of life of people with constipation (Marquis et al., 2005), respectively. These were administered at baseline (day 0) and day 28. Both are a 5-point scale: the PAC-SYM questionnaire assessing constipation from a low score (0) indicating absent to a high score (4) indicating very severe (Frank et al., 1999), whilst the PAC-QoL questionnaire assesses quality of life from a low score (0) indicating not at all to a high score (4) indicating extremely (Marquis et al., 2005).

PAC-SYM and PAC-QoL scores (Table 2) showed there were no significant between-group differences for the change in overall PAC-SYM or PAC-QoL; or individual scores at day 28 (Supplementary tables S2 and S3, respectively). Significant ($p<0.05$) within-group improvements in abdominal symptoms, rectal and stool symptoms and overall PAC-SYM scores were reported by the Livaux group (Supplementary table S2). Significant within-group changes improvements were reported for placebo over time, with the exception of rectal symptom score (Supplementary table S2). Significant ($p<0.05$) within-group improvements in overall PAC-QoL scores, physical discomfort, psychosocial discomfort, worries/concerns and satisfaction were reported by the Livaux group as well as placebo (Supplementary table S3).

To assess bowel regularity, participants were provided with a series of twelve statements at Day 28 (±3) (Visit 3) and asked to score each. Scoring for this index is based on a five-point scale for each question, from strongly disagree (0) to strongly agree (5).

Bowel regularity index score means (Table 3) were consistently higher in Livaux compared to placebo, although not significantly ($p<0.05$) different.

Table 2. Complete spontaneous bowel movement (CSBM), spontaneous bowel movement (SBM), Bristol stool score (BSS), participant-assessed constipation symptoms (PAC-SYM) and quality of life (PAC-QoL) scores for the ITT population (n=84).

Clinical outcome	Parameter	Livaux (n=45)			Placebo (n=39)			Between group p value
		Mean		SD	Mean		SD	
CSBM	Baseline	1.10	±	0.86	1.28	±	1.91	0.58
	Day 7	2.55	±	2.07	2.41	±	2.05	0.77
	Day 14	2.68	±	2.36	2.54	±	2.18	0.78
	Day 21	3.30	±	2.32	3.44	±	2.54	0.80
	Day 28	2.89	±	2.19	2.92	±	2.55	0.95
	ΔBaseline to Day 7	1.44	±	1.80	1.13	±	2.81	0.55
	p value	<0.001	*		0.002	*		
	ΔBaseline to Day 14	1.58	±	2.12	1.26	±	2.63	0.54
	p value	<0.001	*		<0.001	*		
	ΔBaseline to Day 21	2.19	±	2.19	2.15	±	2.94	0.95
	p value	<0.001	*		<0.001	*		
	ΔBaseline to Day 28	1.78	±	2.31	1.64	±	2.39	0.79
	p value	<0.001	*		<0.001	*		
SBM	Baseline	2.07	±	1.43	2.41	±	2.69	0.47
	Day 7	3.45	±	2.55	3.21	±	2.48	0.66
	Day 14	3.77	±	2.94	3.10	±	2.33	0.26
	Day 21	4.05	±	2.70	3.95	±	2.66	0.87
	Day 28	3.89	±	2.49	3.44	±	2.48	0.42
	ΔBaseline to Day 7	1.26	±	1.98	0.86	±	2.78	0.29
	p value	<0.001	*		0.014	*		
	ΔBaseline to Day 14	1.56	±	2.49	0.91	±	3.14	0.15
	p value	<0.001	*		0.034	*		
	ΔBaseline to Day 21	1.88	±	2.47	1.73	±	3.64	0.65
	p value	<0.001	*		0.005	*		
	ΔBaseline to Day 28	1.70	±	2.35	1.19	±	2.38	0.13
	p value	<0.001	*		0.014	*		
BSFS	Baseline	2.27	±	1.31	2.50	±	1.31	0.42
	Day 7	2.27	±	1.23	2.78	±	1.27	0.07
	Day 14	2.25	±	1.05	2.59	±	1.24	0.20
	Day 21	2.35	±	1.29	2.73	±	1.31	0.19
	Day 28	2.37	±	1.11	2.86	±	1.37	0.08
	ΔBaseline to Day 7	0.00	±	0.83	0.28	±	0.83	0.14
	p value	0.009	*		0.419			
	ΔBaseline to Day 14	-0.02	±	0.76	0.08	±	0.88	0.59
	p value	0.368			0.983			
	ΔBaseline to Day 21	0.08	±	0.72	0.23	±	0.86	0.42
	p value	0.003	*		0.414			
	ΔBaseline to Day 28	0.10	±	0.71	0.36	±	0.97	0.18
	p value	0.007	*		0.433			
PAC-SYM	Baseline	1.441	±	0.547	1.333	±	0.507	0.249
Overall	Day 28	0.752	±	0.534	0.761	±	0.479	0.524
score	p value (paired)	<0.001	*		<0.001	*		
PAC-QoL	Baseline	1.548	±	0.615	1.557	±	0.509	0.947
Overall	Day 28	0.870	±	0.575	0.928	±	0.604	0.659
score	P value (paired)	<0.001	*		<0.001	*		

n, number; SD, standard deviation, Δ, change. * P values were generated using ANOVA. P values Δbaseline generated using paired Wilcoxon signed rank test.

Table 3. Bowel Regularity Index (BRI) total score at day 28.

	Livaux (n=45)			Placebo (n=39)			Between group p value
	Mean	±	SD	Mean	±	SD	
I feel that the product made my bowel movements more regular	2.40	±	1.22	2.31	±	1.28	0.73
I feel the product relieved my constipation	2.26	±	1.31	2.13	±	1.26	0.65
I feel the product eased my feelings of bloating and/or gas	2.17	±	1.09	2.05	±	1.18	0.65
I feel the product eased my feelings of abdominal discomfort	2.19	±	1.05	2.13	±	1.11	0.80
I feel the I spend less time in the toilet having taken the product	2.21	±	1.06	2.13	±	1.14	0.73
I feel the product increased my feelings of satisfaction with my bowel movements	2.48	±	1.14	2.18	±	1.20	0.26
I feel that product improved my gut health	2.29	±	1.03	2.08	±	1.00	0.36
I feel better having taken the product	2.38	±	1.07	2.15	±	1.10	0.35
I feel that the product improved my well-being	2.24	±	0.92	2.05	±	1.06	0.41
I tolerated the product well and had no complaints	3.36	±	0.95	3.15	±	0.80	0.31
I am satisfied with this product	2.55	±	1.18	2.33	±	1.12	0.41
I would recommend this product to others	2.50	±	1.16	2.38	±	1.12	0.66

Integrating microbiome data with symptom scores

Given the decrease in hydrogenotrophic *Blautia* species and increase in *Bilophila*, also hydrogenotrophic but requiring a much lower hydrogen threshold concentration, we hypothesized this may be a consequence of changes in fermentative hydrogen gas production. We examined participant-assessed gas and bloating and associated comfort scores from individual questions in the PAC-QoL and PAC-SYM questionnaires (Table 4). Improvements in these parameters was seen for both treatment groups, although the Livaux group trended (i.e. not significant (p>0.05)) towards greater improvements to bloating questions than pain questions.

Table 4. Improvements in participant-assessed bloating and associated symptom scoring (n=85). Standard error shown.

Question	Livaux		Placebo	
(PAC-QoL, Q1) Have you felt bloated, to the point of bursting?	-0.8	± 0.1807	-0.75	± 0.1916
(PAC-SYM, Q1) Discomfort in your abdomen?	-0.5778	± 0.1354	-0.875	± 0.1436
(PAC-SYM, Q2) Pain in your abdomen?	-0.3556	± 0.1275	-0.55	± 0.1352
(PAC-SYM, Q3) Bloating in your abdomen?	-0.6667	± 0.144	-0.5385	± 0.1546

We checked whether changes in *Blautia* and *Bilophila* relative abundances correlated with the improvements in bloating and comfort scores (Table 5). There was an overall positive correlation of changes (decreases) in *Blautia* relative abundance with improvements in bloating scores. This was accompanied with negative correlation between changes (increases) in *Bilophila wadsworthia* relative abundance.

Table 5. Correlations between changes in relative abundance of hydrogenotrophic bacteria and participant-assessed bloating and associated symptom scoring. (n=85).

Taxa	PAC-SYM			PAC-QoL	
	Q1 Abdominal Discomfort	Q2 Abdominal Pain	Q3 Abdominal Bloating	Q1 Bloated to bursting	p.value V2 vs V3
Blautia genus	0.143	0.246	0.343	0.053	0.055
Blautia caecimuris	-0.044	0.162	0.209	0.042	0.038
Blautia faecis	0.073	-0.058	0.181	0.046	0.002
Blautia hydrogenotrophica	-0.047	-0.382	-0.028	0.040	0.033
Blautia obeum	0.018	-0.049	-0.164	-0.056	0.015
Bilophila wadsworthia	-0.115	-0.008	-0.201	-0.102	0.003

Gas production in the gut is a consequence of rapid fermentation. Given that fermentation has occurred, as a kiwifruit pectin-utilising bacterium *F. prausnitzii* and other saccharolytic bacteria (*Christensenellaceae* and *Ruminococcus* spp.) did significantly ($p<0.05$) increase in the Livaux group, the decreases in gas scores and decrease in hydrogenotrophic bacterial taxa reliant on that gas suggest that fermentation must have occurred at a slower rate.

Safety and tolerance

A total of 39 adverse events (AE) were reported in this study by 21 participants (Table 6).

Five pre-emergent AE were reported by 3 participants in this study. These were reported by two participants in the Livaux group and 1 participant in the placebo group. They were categorized by the Quality Investigator (QI) as Not Related to the IP.

Thirty-nine post-emergent AE were reported by 18 participants in this study. Of these, 21 AE were reported by nine participants in Livaux and 18 AE were reported by nine participants in placebo.

Of the 21 AE reported by participants in the Livaux group, eight were classified as Unlikely, six as Not Related, and seven as Possibly related to the IP. These latter seven comprised abdominal cramps, bloating, chest congestion, headache, hunger, and nausea. Of the 18 AE reported by participants in the placebo group, three were classified as Unlikely, six as Not Related, and nine as Possibly related to the IP. These latter nine were: abdominal cramps, epigastric pain, bloating, burping, nausea, dizziness, gas and hunger. None of the AE were categorised as Probably related to the IP.

All AE were resolved by the end of the study, with the exception of one AE. For this one AE of fatigue (Livaux), multiple attempts to follow up with this participant were made, but they did not want further contact.

All reported AE were only mild. There were no moderate AE, severe AE or deaths to report in this study.

Table 6. Number of participants experiencing at least one pre-emergent or post-emergent Adverse Events (AE).

Variable	Livaux n(%)	Placebo n(%)
No pre-emergent AE	43 (95.60%)	40 (97.60%)
At least one pre-emergent AE	2 (4.40%)	1 (2.40%)
No post-emergent AE	36 (80.00%)	32 (78.00%)
At least one post-emergent AE	9 (20.00%)	9 (22.00%)

Discussion

Livaux® is a powdered health ingredient derived from New Zealand gold (*Actinidia chinensis* "Zesy002") kiwifruit from which the skin and seeds are removed, and the remaining flesh cold processed for use in food and dietary supplements (Ansell et al., 2015). A recent randomized, double-blind, placebo-controlled clinical crossover study examining the effects of Livaux gold kiwifruit powder (2400 mg) and Actazin® green kiwifruit powder (600 mg and 2400 mg daily) on stool frequency, stool form and gastrointestinal comfort in healthy and functionally constipated individuals found that both investigational products were well-tolerated, and that supplementation with 2400 mg Livaux demonstrated a significant (two-fold) increase in *F. prausnitzii* relative abundance. The results reported here for 600 mg Livaux are consistent with that previous study.

Livaux improved CSBM over baseline by >1 per week, improved BSFS scores over baseline, and improved participant-assessed symptoms and quality of life (PAC-SYM and PAC-QoL, respectively) scores over baseline. This is as expected. A body of evidence already exists that kiwifruit contribute to the maintenance of normal laxation. Previous clinical studies in populations of healthy (Rush et al., 2002, Wilkinson-Smith et al., 2018; Caballero et al., 2020; Chey et al., 2021), constipated (Chan et al., 2007) and constipation-dominant irritable bowel syndrome (IBS-C) (Chang et al, 2015) participants show that whole (green) kiwifruit can improve laxation by at least 1 CSBM per week. Furthermore, (green) kiwifruit have now been approved by the European Food Safety Authority (EFSA) for the health claim "consumption of kiwifruit contributes to the maintenance of normal defecation" (EFSA, 2021), where two large kiwifruit (e.g. around 200 g flesh) should be consumed. It is likely the unique combination of soluble and insoluble fibre, polyphenols, and the enzyme actinidin that are present in kiwifruit, confer this and other health benefits (Ansell et al., 2015).

The allowed EFSA (2021) claim suggests kiwifruit fibre as the most plausible mechanism by which kiwifruit may contribute to normal defecation. Livaux contains a similar type of kiwifruit fibre, and as dosage may not be a factor in the role of fibre for laxation (Thompson et al., 2017), the clinically meaningful (≥1 CSBM/day) improvements reported here suggest that it is also plausible that Livaux kiwifruit fibre is the mechanism by which these improvements may be explained.

This study also observed a high placebo result for most outcome measures. In retrospect, the use of non-digestible, poorly fermentable fibre which survives intact to and throughout the length of the colon and may contribute to faecal bulking and increased transit time (Nsor et al., 2017) was unfortunate. Nevertheless, Livaux compared well despite a lower absolute amount of fibre 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 laxation outcomes.

Kiwifruit fibre's capacity of swelling, defined as the volume occupied by (green kiwifruit) fibre 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 that for apple fibre (Sims and Monro, 2013). Kiwifruit fibre also has high water retention capacity (Mishra and Monro, 2012; Sims and Monro, 2013), defined as volume of water bound to insoluble fibre and not separated by centrifugation (Robertson et al., 2000). Other constituents may survive intact to the colon (polyphenols, organic acids, other dietary fibre from the diet) and may contribute to water holding and act as microbial fermentation substrates for increased colonic microbial biomass. Collectively these should contribute to faecal bulking (Bayer et al., 2018). It is likely that gold kiwifruit constituents behave similarly to their green kiwifruit relatives. Fibre and other constituents escaping host small intestinal digestion and entering the colon largely intact where it may be fermented by the resident gut microbiota resulting in increased microbial biomass and hence fecal bulking (Cummings, 2001) should also lead to increased laxation (Bharucha and Lacy, 2020).

However, fermentation may also lead to increased gastrointestinal gas. Fermentable materials increase gas production as methane, hydrogen and carbon dioxide are waste products of anaerobic microbial metabolism. Most gases escape by diffusing into blood and hence expelled as breath (Fritscher-Ravens et al., 2014). Some gut microbes use these gases (e.g. hydrogen by hydrogenotrophs) as redox electron acceptors for fermentation of substrates, to generate soluble acids (e.g. acetate by homoacetogenic bacteria (acetogens)), or methane by methanogens. Gas production exceeding this disposal capacity is expelled as flatus (Fritscher-Ravens et al., 2014).

However, the period between gas production and gas expulsion may be a problem for some people, especially if much is generated and trapped in the proximal colon, a long way from the exit. A recent multinational study of more than 73,000 respondents found that functional gastrointestinal disorders (FGIDs) affect more than 40% of people (Sperber et al., 2021), where the prevalence of functional abdominal bloating was 3.5%. Therapeutic approaches to functional bloating (Malagelada et al., 2017) currently include non-fermentable non-bulky laxatives (effective); dietary restriction (restricting lactulose, FODMAPs, etc.: effective); antibiotics (poor results); probiotics (minor effects); prebiotics (aggravate bloating 1-2 weeks, may help after that); attenuation of visceral perception (distraction, antispasmodics, antidepressants, and anxiolytics: varies depending on individual responsiveness). Mechanisms by which food exacerbate bloating include food maldigestions/malabsorption, food allergy, and visceral hypersensitivity (Fritscher-Ravens et al., 2014). Fermentable prebiotics or other oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) of plant and dairy origin reaching the colon can aggravate bloating through their rapid fermentability (Barrett et al., 2010).

Thus, the rate and extent of fermentation of material within the colon are important. Faster fermentation leads to increased gas production (Hernot et al., 2009). Such gases then support other bacteria, such as hydrogenotrophs. Hydrogenotrophs are typically at lower densities than fermentative bacteria but consume a substantial amount of hydrogen (Strocchi and Levitt, 1992). The main hydrogenotrophs of the gut microbiome which utilise hydrogen include acetogens, methanogens, and sulfur-reducing bacteria (SRB) (Nakamura et al., 2010). For example, acetogens such as *Blautia* species (e.g. *B. hydrogenotrophica*) (Liu et al., 2021) consume hydrogen and carbon dioxide using the Wood–Ljungdahl pathway to create acetate. Acetogenesis, methanogenesis or sulfur reduction are the most important types of microbial hydrogen utilisation, others are quantitatively less important (Nakamura et al., 2010). Hydrogenotrophic populations are governed by the hydrogen threshold theory, wherein the (numerically) dominant hydrogenotrophs maintain their local hydrogen concentration at the lowest level capable of sustaining them (Cord-Ruwisch et al., 1988), to exclude other hydrogenotrophs with higher concentration requirements. It follows that should hydrogen gas levels drop below the concentration required to sustain them, the previously dominant hydrogenotroph levels will also decline until a hydrogenotrophic population with lower threshold requirement dominates (Kim, 2012). In order of hydrogen threshold requirements from highest to lowest are acetogens, methanogens, then SRB.

The hydrogenotrophic *Blautia* species relative abundance of our participants were determined. Interestingly, consumption of Livaux resulted in a decline in relative numbers of these acetogenic hydrogenotrophs. There was a commensurate rise in *Bilophila wadsworthia*: SRB which have a lower hydrogen threshold than *Blautia*. This suggested that microbial hydrogen production in the colons of participants consuming Livaux was low. This does not appear to be due to an absence of fermentation: Livaux polysaccharides - particularly the pectin - are fermentable (Parkar et al., 2012; Rosendale et al., 2012). Livaux has been previously shown to support the growth of *F. prausnitzii* (Blatchford et al.,

2017), a gut bacterium known to ferment pectin (Lopez-Siles et al., 2012). *F. prausnitzii* relative abundance also increased in the participants consuming Livaux reported here, as did saccharolytic *Christensenellaceae* and *Ruminococcus* spp., indicating that fermentation had still occurred. Pectin fermentation has been previously indicated to result in gas production, albeit to a lesser extent than the shorter prebiotic oligosaccharides GOS (galactooligosaccharides), FOS (fructooligosaccharides) or inulin (a long chain fructopolysaccharide) (Hernot et al., 2009). Given that fermentation of Livaux has clearly occurred, the lower relative abundance of higher hydrogen threshold hydrogenotrophs suggests that microbial fermentative hydrogen output was lower, and hence the rate of fermentation must have been lower (Hernot et al., 2009). This slower fermentation may be attributed to the increased complexity of pectin relative to FOS, GOS and inulin. Pectin rhamnogalacturonan II side chains are the most complex glycans in nature (Ndeh et al., 2017). These must be removed by a consortium of enzymes by one or more cooperating bacteria before, for example, *F. prausnitzii* can utilise the pectin galacturonate core.

We considered clinical scores for abdominal bloating from the same participants. Consistent with the hydrogenotrophic bacteria findings, Livaux-consuming participants' self-assessed symptom scores for gas, bloating and abdominal comfort decreased (i.e. improved). These scores were drawn from questions which are part of the validated Patient Assessment of Quality of Life (PAC-QoL) and Patient Assessment of Constipation Symptoms (PAC-SYM) questionnaires. There were mild correlations of these scores with the changes in hydrogenotrophic bacterial relative abundances.

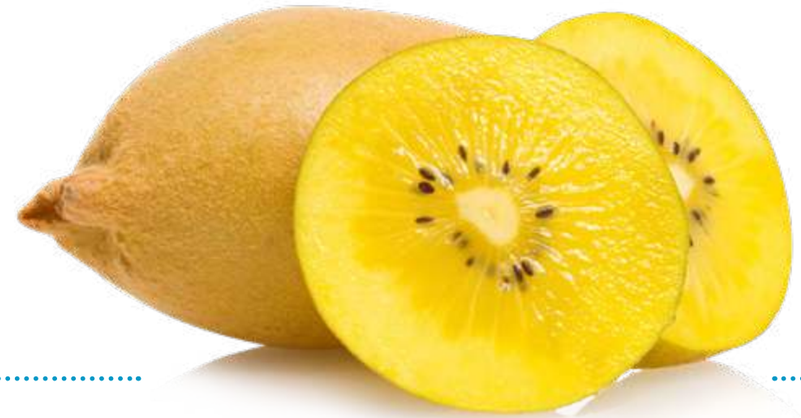
Collectively, these data suggest that Livaux is a fermentable prebiotic with the interesting attribute for a fermentable substrate of reducing gas and bloating, and consistent with the hydrogen threshold theory, reducing the numbers of gas-utilizing bacterial species within the gut.

Support for this substantial-but-slow fermentation hypothesis can be drawn from a recent in vitro experiment using the simulator of human intestinal microbial ecosystem (SHIME®) model. This is a multi-stage simulator of the entire human intestine from stomach to rectum, importantly featuring compartments equating to the three main physiologically distinct regions of the colon: proximal, transverse and distal (Duysburgh et al., 2019). Livaux, as a part of a synbiotic combination with *Bacillus* spp. probiotics and other prebiotics (GOS and PreticX®, a xylo-oligosaccharide from corn cob), was shown to increase *F. prausnitzii* relative abundance in progressively more distal compartments. This increase was accompanied with increases in other saccharolytic bacteria and increased end-point fermentation byproducts propionate and butyrate in the transverse and distal compartments. Had the substrate been rapidly fermented (such as FOS, GOS or inulin), then greater increases in saccharolytic bacteria and intermediate byproducts such as lactate would have been expected in the proximal compartment than were reported. Collectively, the more distal fermentation and completeness of the fermentation suggested that fermentation proceeded all the way through the length of the colon, like that observed from the faecal samples of Livaux-consuming participants in this study.

Finally, the ability of Livaux to act as a precision prebiotic by being used as a substrate for the growth of *F. prausnitzii* has been confirmed in a second study, the first being reported in 2017 (Blatchford et al., 2017). This time a 600 mg daily dosage was used, in contrast to the previous 2,400 mg daily dosage over a similar 28 day period. *F. prausnitzii* is one of the most abundant bacteria in the human gut ecosystem. Low numbers have been associated with numerous disorders and diseases. Due to its oxygen sensitivity and fastidious nature, *F. prausnitzii* is challenging to introduce into the gut via direct probiotic supplementation. The growing desire to specifically target *F. prausnitzii* has led to emerging developments in faecal transplants via stool delivery or oral administration – treatments that are not necessarily appealing or accessible options for everyday consumers. Livaux is a natural and novel prebiotic supplement derived entirely from New Zealand gold kiwifruit, which is well tolerated and safe. This research shows that it helps *F. prausnitzii* communities in the gut to flourish.

Conclusion

In conclusion, a randomised, double-blinded, placebo-controlled parallel study with 85 participants conducted across four North American sites was carried out where daily 600 mg Livaux consumption for 28 ± 3 days yielded a statistically significant increase in *F. prausnitzii* and other saccharolytic bacteria. In addition, improvements in laxation along with improvements in constipation symptoms and quality of life indicators such as decreased abdominal bloating and discomfort, were measured. Consistent with these decreased bloating scores, a significant decrease in the relative abundance of hydrogenotrophs from the *Blautia* genus was observed. These data are consistent with the Livaux pectin being slowly fermented throughout the length of the colon without excessive gas production, while being safe and well-tolerated.



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Appendix One

Methods and Materials

Study design

This was a multi-centre, randomized, double-blind, placebo-controlled, parallel group study consisting of a single 4-week intervention period. The investigational product was 600 mg Livaux® gold kiwifruit powder, compared to a placebo (microcrystalline cellulose). Participants had three visits: screening; baseline (start of intervention period); and 28 days later, endpoint (of intervention period).

The clinical trial was conducted at 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) from 22 February 2018 to 01 February 2020 under the supervision of a qualified investigator (QI) at each site. This study was reviewed by the Natural Health Product Directorate trr(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. This 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, including the archiving of essential documents. The trial was registered at Clinicaltrials.gov (NCT03462199). Informed consent was obtained from each participant at the screening visit prior to performing any study-related activities.

Participants

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

Inclusion criteria consisted of males and females of 18 to 60 years of age, inclusive at baseline; female participants were not of child bearing potential; body mass index (BMI) between 19 and 29.9 ± 1 kg/m2 at screening, inclusive; self-reported ≤ 3 CSBMs per week at screening and confirmed in a bowel habits diary during the run-in period for enrolment at baseline; people who were not regular consumers of high fibre diets, yoghurt, fermented foods, refrained from the consumption of high-fiber dietary supplements including Metamucil, Benefibre, and Phloe; refrained from the consumption of fresh kiwifruit 2-weeks prior to and during the study; maintained their habitual food and beverage intakes; maintained current physical activity patterns; avoided overseas travel for the duration of the study due to the impact this may have on diet and gastrointestinal health; fasting blood glucose ≤ 6.0 mmol/L at screening; were healthy as determined by laboratory results, medical history, and physical exam as assessed by the QI; were willing to complete questionnaires, records, and diaries associated with the study, collect stool samples, and to complete all clinic visits; and had given voluntary, written, informed consent to participate in the study.

Exclusion criteria included participation in a clinical research trial within 30 days prior to randomization; blood donation during the study or within 30 days of completing the study; vegan, raw food, or very high-fiber diet, including regular consumption of foods labeled as supplemented with fiber; weight loss of > 5% within the past 3 months; frequent use of laxatives defined as greater than once per week; use of medications such as antibiotics that have major impact on gut microbes 2 months prior to baseline and as assessed case by case by the QI; 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 as assessed by QI; allergy or sensitivity to kiwifruit or other test product ingredients; prior surgery for weight loss (lap band or gastric bypass); gastrointestinal alarm symptoms including blood in stools, frequent diarrhea, and unremitting abdominal pain, and major diseases of the gastrointestinal tract (such as IBS, Crohn's, etc.), pulmonary or endocrine systems, or other GI abnormalities; gastroparesis or lactose intolerance; current, or history of, thyroid disease; uncontrolled hypertension (SBP ≥160 mmHg) assessed by QI; renal, hepatic, pancreatic, or biliary impairment or disease as disclosed or detected (if applicable) by chemistry and hematology taken at screening; current, or history of, bleeding/blood disorders; Type I and Type II diabetes; autoimmune disease or immuno-compromised (i.e. HIV positive, use of anti-rejection medication, rheumatoid arthritis, Hepatitis B/C positive); cancer, except skin cancers completely excised with no chemotherapy or radiation with a follow up that is negative. Volunteers with cancer in full remission for more than five years after diagnosis were considered as per the QI's opinion; clinically significant abnormal laboratory results at screening; alcohol or drug abuse within the last 6 months; participants with a history of cigarette smoking within the past 5 years; individuals who were cognitively impaired and/or who were unable to give informed consent; and any other condition which in the qualified investigator's opinion 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.

Investigational products

Investigational products (IP) were labelled according to the requirements of ICH-GCP guidelines and applicable local regulatory guidelines. The active IP was Livaux®. Livaux capsules each contained 600 mg of the gold kiwifruit powder from Anagenix Ltd, Auckland, New Zealand. Livaux gold kiwifruit powder contains dietary fibre, actinidin protease activity and kiwifruit vitamins, minerals, organic acids and polyphenols.

The soluble fiber fractions from Livaux comprise 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, pers. comm) due to proprietary processing methods.

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).

Size 00 capsules (AL98014, ACG Associated Capsules Pvt Ltd, Maharashtra, India) comprised hydropropylmethylcellulose, purified water, carrageenan, potassium acetate, and titanium dioxide.

Placebo contained microcrystalline cellulose ((Avicel PH-101, FMC Corporation, PA) 600 mg), hydropropylmethylcellulose, purified water, carrageenan, potassium acetate, and titanium dioxide.

Participants were instructed to take their dosage of placebo or IP daily with a glass of water and food in the morning, starting the day after randomization (day 1), for 28 days.

Randomization and blinding

This study design was double-blinded. Participants were assigned a participant number at their screening visit, and if they met all the inclusion criteria and did not meet any of the exclusion criteria at their baseline visit, a randomization number was assigned to the participant by an investigator blinded to the treatment groups, per the order of the randomization list generated by www.randomization.com. During the 28 day supplementation period, one group received the active IP Livaux® and one group received placebo (cellulose). Investigators, other site personnel, and participants were blinded to the treatment each participant received.

Bowel Habits Diary (BHD)

Bowel Habits were captured in a diary based on the number of bowel movements, straining to start defecation, straining to stop defecation, feelings of incomplete defecation and the use of laxatives. Timing of bowel movements were also recorded.

Complete Spontaneous Bowel Movements (CSBM) and Spontaneous Bowel Movements (SBM) were evaluated from this diary. CSBM is an accepted and easily defined primary measure of stool frequency in clinical trials assessing bowel habits (USDA, 2012). A CSBM classification was made if a participant reported a feeling of satisfaction (complete) and manual maneuvers, laxatives, enemas or suppositories were not used, and no assistance was needed (spontaneous). 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 (±3) (Visit 3) and asked to score each. Scoring for this index is based on a five-point scale for each question, from strongly disagree (0) to strongly agree (5).

Stool form was scored according to the Bristol Stool Form Score (BSFS) which depicts the form of the faeces on a 7-point scale, from hard to watery (Koh et al., 2010). BSFS scoring is well recognised and has been suggested as the main diagnostic criteria for IBS-D (Longstreth et al., 2006; USDA, 2012).

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 developed to measure the symptoms (Frank et al., 1999) and quality of life of people with constipation (Marquis et al., 2005), respectively. They were administered at baseline (day 0) and day 28. Both are a 5-point scale: the PAC-SYM questionnaire assessing constipation from a low score (0) indicating absent to a high score (4) indicating very severe (Frank et al., 1999), whilst the PAC-QoL questionnaire assesses quality of life from a low score (0) indicating not at all to a high score (4) indicating extremely (Marquis et al., 2005).

2.7 Food Diaries

Participants used an online food recording application DietMaster Pro (Lifestyles Technologies Inc., Grants Pass, OR, USA) to record their three-day food records prior to Visit 2 and 3. All participants were provided with instructions on how to use the DietMaster Pro food diary. Participants used this tool to track their 3-day food and beverage intake, which began the day prior to their screening visit. The food records were reviewed by trained staff at each visit and a copy of the 3-day food diary was dispensed to participants at their intervention start (Visit 2). Participants were reminded to maintain their normal dietary and beverage intake and physical exercise. Participants were also reminded to refrain from consuming high-fiber dietary supplements, a very high-fiber diet, fresh kiwifruit, probiotic or prebiotic supplements.

Faecal microbiome analysis

Faecal samples were collected from participants at baseline (day 0) and the end of the intervention period (day 28). Participants collected their own samples as close as possible to, but before, their visit (within 48 h). 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. At the conclusion of all participant visits, all 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 16S rRNA gene V4 variable region was amplified and barcoded using F515/R806 primers followed by 250x2 bp sequencing on an Illumina HiSeq 2500 (Jacobs et al., 2017).

Adverse Events

During the study, participants recorded adverse events (AE) in their diaries 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.

Compliance

Clinic staff instructed participants to save all unused and open IP packages and return them to the clinic site for a determination of compliance. Compliance was determined as >80% of IP consumed.

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 differences 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 assignTaxonomy 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) (McMurdy 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, centre 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.

Appendix Two

Supplementary Tables

Table S1. Change in vital signs, anthropometric measurements, clinical chemistry and hematology parameters from baseline to day 28 in the ITT population (n=84).

Outcome		Livaux (n=39)			Placebo (n=45)			Between group p value
		Mean	±	SD	Mean	±	SD	
Average weight (kg)	Baseline	72.43	±	11.33	71.21	±	13.22	0.66
	Day 28	72.60	±	11.44	71.38	±	13.65	0.66
	p value (paired)	0.25			0.36			
BMI (kg/m2)	Baseline	25.30	±	3.03	25.53	±	3.42	0.75
	Day 28	25.36	±	3.09	25.58	±	3.55	0.77
	p value (paired)	0.26			0.42			
Heart Rate (bpm)	Baseline	71.64	±	12.00	70.59	±	10.07	0.67
	Day 29	71.00	±	13.23	71.95	±	9.78	0.72
	p value (paired)	0.49			0.20			
Systolic Blood Pressure (mmHg)	Baseline	117.20	±	10.70	116.85	±	9.96	0.88
	Day 28	116.45	±	13.51	117.03	±	11.19	0.84
	p value (paired)	0.70			0.68			
Diastolic Blood Pressure (mmHg)	Baseline	75.41	±	8.21	77.28	±	7.16	0.28
	Day 28	73.70	±	8.96	76.33	±	8.86	0.19
	p value (paired)	0.18			0.55			
Hemoglobin (g/L)	Screening	136.50	±	14.53	139.250	±	11.950	0.32
	Day 28	135.79	±	13.77	136.923	±	14.373	0.69
	p value (paired)	0.51			0.064			
Hematocrit (L/L)	Screening	0.40	±	0.04	0.415	±	0.031	0.13
	Day 28	0.40	±	0.04	0.407	±	0.035	0.50
	p value (paired)	0.39			0.015			
White Blood Cell Count (xE9/L)	Screening	5.68	±	1.60	6.080	±	1.579	0.38
	Day 28	5.70	±	1.61	5.910	±	1.841	0.60
	p value (paired)	0.70			0.402			
Red Blood Cell Count (xE12/L)	Screening	4.64	±	0.49	4.717	±	0.359	0.38
	Day 28	4.64	±	0.48	4.678	±	0.373	0.61
	p value (paired)	0.64			0.102			
Mean corpuscular Volume (MCV) (fl)	Screening	87.36	±	4.87	87.950	±	4.480	0.59
	Day 28	87.02	±	5.39	87.256	±	5.079	0.88
	p value (paired)	0.36			0.225			
Mean corpuscular Hemoglobin (MCH) (pg)	Screening	29.50	±	2.19	29.503	±	2.042	0.96
	Day 28	29.35	±	2.18	29.274	±	2.249	0.85
		0.44			0.830			
Mean corpuscular Hemoglobin Concentration (MCHC) (g/L)	Screening	337.68	±	11.17	335.150	±	10.845	0.25
	Day 28	337.35	±	9.21	335.667	±	10.766	0.45
	p value (paired)	0.96			0.220			
Red Cell Distribution Width (RDW) (%)	Screening	12.86	±	1.19	13.005	±	0.982	0.52
	Day 28	12.88	±	1.44	12.921	±	0.951	0.81
	p value (paired)	0.24			0.227			
Platelet Count (xE9/L)	Screening	248.34	±	77.99	256.000	±	58.891	0.69
	Day 28	255.49	±	52.17	254.436	±	56.336	0.99
	p value (paired)	0.94			0.964			
Absolute Neutrophil Count (NEUTS) (xE9/L	Screening	3.28	±	1.24	3.555	±	1.174	0.39
	Day 28	3.37	±	1.39	3.415	±	1.557	0.89
	p value (paired)	0.85			0.460			
Absolute Lymphocyte Count (LYMP) (xE9/L)	Screening	1.78	±	0.46	1.865	±	0.559	0.64
	Day 28	1.69	±	0.50	1.851	±	0.541	0.17
	p value (paired)	0.74			0.764			
Absolute Monocyte Count (MONO) (xE9/L)	Screening	0.45	±	0.13	0.503	±	0.163	0.22
	Day 28	0.47	±	0.12	0.467	±	0.128	0.91
	p value (paired)	0.59			0.298			
Absolute Eosinophil Count (EOS) (xE9/L)	Screening	0.15	±	0.07	0.133	±	0.081	0.29
	Day 28	0.14	±	0.09	0.149	±	0.096	0.73
	p value (paired)	0.24			0.082			
Absolute Basophil Count (BASO) (xE9/L)	Screening	0.02	±	0.04	0.030	±	0.046	0.37
	Day 28	0.02	±	0.04	0.028	±	0.044	0.41
	p value (paired)	0.78			0.346			
Fasting glucose (mmol/L)	Screening	4.88	±	0.45	4.966	±	0.494	0.43
	Day 28	4.94	±	0.40	4.955	±	0.457	0.91
	p value (paired)	0.55			0.561			
Creatinine (µmol/L)	Screening	72.85	±	14.67	69.791	±	10.554	0.31
	Day 28	72.01	±	15.41	70.351	±	11.458	0.60
	p value (paired)	0.28			0.871			
Estimated Glomerular Filtration Rate (eGFR) (ml/min/1.73 m2)	Screening	97.48	±	16.22	101.024	±	14.608	0.27
	Day 28	98.07	±	16.81	100.523	±	14.684	0.47
	p value (paired)	0.50			0.596			
Sodium (mmol/L)	Screening	141.32	±	2.58	140.854	±	1.799	0.43
	Day 28	141.02	±	2.43	140.875	±	1.944	0.84
	p value (paired)	0.16			0.981			
Potassium (mmol/L)	Screening	4.60	±	0.44	4.570	±	0.355	0.29
	Day 28	4.52	±	0.51	4.615	±	0.395	0.35
	p value (paired)	0.36			0.488			
Chloride (mmol/L)	Screening	102.43	±	2.90	101.878	±	2.002	0.30
	Day 28	102.44	±	2.44	102.675	±	2.183	0.63
	p value (paired)	0.81			0.019			
Total Bilirubin (µmol/L)	Screening	8.13	±	4.71	8.512	±	3.286	0.83
	Day 28	8.67	±	7.48	7.816	±	3.181	0.53
	p value (paired)	0.77			0.205			
Aspartate Transaminase (AST) (U/L)	Screening	19.07	±	4.23	18.675	±	3.634	0.28
	Day 28	19.19	±	4.62	19.100	±	6.263	0.97
	p value (paired)	0.99			0.753			
Alanine Transaminase (ALT) (U/L)	Screening	18.11	±	8.22	18.756	±	10.534	0.64
	Day 28	17.65	±	8.19	17.625	±	7.769	1.00
	p value (paired)	0.93			0.488			
Calcium (mmol/L)	Baseline	2.40	±	0.09	2.357	±	0.098	0.08
	(mmol/L)	2.37	±	0.09	2.350	±	0.089	0.40
	p value (paired)	0.05			0.795			

n, number; SD, standard deviation
* P-values were generated using ANOVA. P-values for change from screening/baseline generated using paired Wilcoxon signed rank test.

Supplementary Table S2. Change in Patient Assessment of Constipation Symptoms Questionnaire (PAC-SYM) score at baseline and at day 28 for participants in the ITT population (n = 84).

		Livaux n(=45)			Placebo (n=39)			Between group p value
		Mean	±	SD	Mean	±	SD	
Abdominal symptoms	Baseline	1.244	±	0.694	1.250	±	0.599	0.969
	Day 28	0.711	±	0.570	0.660	±	0.570	0.688
	p value (paired)	<0.001	*		<0.001	*		
Rectal Symptoms	Baseline	0.822	±	0.752	0.641	±	0.664	0.254
	Day 28	0.385	±	0.592	0.376	±	0.529	0.942
	p value (paired)	0.001	*		0.046	*		
Stool Symptoms	Baseline	1.969	±	0.762	1.813	±	0.625	0.318
	Day 28	1.004	±	0.762	1.072	±	0.605	0.662
	p value (paired)	<0.001	*		<0.001	*		
Overall	Baseline	1.441	±	0.547	1.333	±	0.507	0.249
	Day 28	0.752	±	0.534	0.761	±	0.479	0.524
	p value (paired)	<0.001	*		<0.001	*		

n, number; SD, standard deviation

* P-values were generated using ANOVA. P-values for change from screening/baseline generated using paired Wilcoxon signed rank test.

Supplementary Table S3. Change in Patient Assessment of Constipation Quality of Life Questionnaire (PAC-QoL) score at baseline and at day 28 for participants in the ITT population (n=84).

		Livaux n(=45)			Placebo (n=39)			Between group p value
		Mean	±	SD	Mean	±	SD	
Physical Discomfort	Baseline	1.678	±	0.692	1.699	±	0.754	0.896
	Day 28	0.783	±	0.647	0.872	±	0.638	0.536
	P value (paired)	<0.001	*		<0.001	*		
Psychosocial Discomfort	Baseline	0.775	±	0.610	0.763	±	0.565	0.929
	Day 28	0.422	±	0.432	0.449	±	0.557	0.809
	P value (paired)	<0.001	*		0.002	*		
Worries & Concerns	Baseline	1.378	±	0.790	1.376	±	0.664	0.991
	Day 28	0.764	±	0.629	0.793	±	0.614	0.834
	P value (paired)	<0.001	*		<0.001	*		
Satisfaction	Baseline	3.119	±	0.704	3.156	±	0.604	0.798
	Day 28	1.889	±	1.172	2.036	±	1.328	0.596
	P value (paired)	<0.001	*		<0.001	*		
Overall	Baseline	1.548	±	0.615	1.557	±	0.509	0.947
	Day 28	0.870	±	0.575	0.928	±	0.604	0.659
		<0.001	*		<0.001	*		

n, number; SD, standard deviation

* P-values were generated using ANOVA. P-values for change from screening/baseline generated using paired Wilcoxon signed rank test.

Supplementary Table S4. Faecal bacterial taxa that were significantly different by pairwise Wilcoxon t-test between baseline and endpoint in the Livaux intervention group. P values shown. Asterisk in the rightmost column denotes significantly responding taxa in both comparison of Livaux at baseline vs endpoint (this table) and Livaux vs placebo at endpoints (Supplementary table S5), and these taxa are presented in Fig. 2.

Phylum	Class	Order	Family	Genus	Species	p value	
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Streptococcus*		0.012	
	Clostridia	Clostridiales	Christensenellaceae			0.033	*
			Lachnospiraceae	*Blautia*	*faecis*	0.002	
					obeum	0.015	
					hydrogenotrophica	0.033	
					caecimuris	0.038	*
				Coprococcus 2	*eutactus*	0.002	
			Ruminococcaceae	*Faecalibacterium*		0.016	*
					prausnitzii	0.047	
				Ruminococcus 1		0.018	
	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	*Faecalitalea*		0.044	
				Holdemanella	*filiformis*	0.021	
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	*Bilophila*	*wadsworthia*	0.003	
	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	*Haemophilus*		0.009	

Supplementary Table S5. Faecal bacterial taxa that were significantly different by pairwise Wilcoxon t-test between the Livaux intervention group and Placebo at Endpoint. Asterisk in rightmost column denotes significantly responding taxa in both comparison of Livaux vs placebo at endpoints (this table) and Livaux at baseline vs endpoint (Supplementary table S4), and are those presented in Fig. 2.

Phylum	Class	Order	Family	Genus	Species	p value
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	*Actinomyces*	*odontolyticus*	0.007
	Coriobacteriia	Coriobacteriales	Eggerthellaceae			0.003
		Coriobacteriales	Eggerthellaceae	*Gordonibacter*		0.023
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*		0.000
					caccae	0.014
					eggerthii	0.000
					massiliensis	0.007
					ovatus	0.003
					plebeius	0.006
			Barnesiellaceae	*Barnesiella*		0.001
			Marinifilaceae	*Butyricimonas*		0.033
			Muribaculaceae			0.016
			Prevotellaceae	*Paraprevotella*		0.000
				Prevotella 9		0.000
					copri	0.001
			Rikenellaceae	*Alistipes*		0.000
					putredinis	0.000
					shahii	0.045
			Tannerellaceae	*Parabacteroides*		0.002
Cyanobacteria	Melainabacteria	Gastranaerophilales				0.000
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	*Methanobrevibacter*		0.001
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*		0.000
			Lactobacillaceae	*Lactobacillus*		0.000
			Streptococcaceae	*Lactococcus*		0.000
	Clostridia	Clostridiales				0.000
			Christensenellaceae			0.000 *
				Catabacter		0.009
			Clostridiaceae_1	*Clostridium_sensu_stricto_1*		0.042
			Family_XIII			0.026
				Family_XIII_AD3011_group		0.000
			Lachnospiraceae	*Blautia*	*caecimuris*	0.000 *
					hydrogenotrophica	0.010
					luti	0.000
					stercoris	0.000
				Coprococcus_1	*catus*	0.035
				Dorea		0.012
					formicigenerans	0.046
				Eisenbergiella	*massiliensis*	0.000
				GCA		0.000
				Lachnoclostridium		0.001
				Lachnospira	*pectinoschiza*	0.001
				Lachnospiraceae FCS020 group		0.000
				Lachnospiraceae NK4A136 group		0.000
				Lachnospiraceae UCG		0.008
				Marvinbryantia		0.004
				Sellimonas	*intestinalis*	0.036
				Shuttleworthia		0.026
			Ruminococcaceae			0.001
				Angelakisella		0.010
				Candidatus_Soleaferrea		0.002
				DTU089		0.022
				Faecalibacterium		0.000 *
					CM04	0.000
				Hydrogenoanaerobacterium		0.002
				Intestinimonas	*massiliensis*	0.027
				Ruminiclostridium_6		0.040
				Ruminiclostridium_9		0.000
				Ruminococcaceae_UCG		0.000
					010	0.032
				Ruminococcus 1	*bicirculans*	0.035
					callidus	0.000
				Ruminococcus_2		0.000
				UBA1819		0.001
	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	*Catenibacterium*	*mitsuokai*	0.021
				Dubosiella	*newyorkensis*	0.000
				Erysipelatoclostridium		0.001
					ramosum	0.020
	Negativicutes	Selenomonadales	Veillonellaceae	*Dialister*		0.014
				Megamonas	*funiformis*	0.002
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Parasutterella*	*excrementihominis*	0.031
		Enterobacteriales	Enterobacteriaceae	*Escherichia*		0.000
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas*		0.047
Tenericutes	Mollicutes	Mollicutes RF39				0.007
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	*Akkermansia*		0.018
					muciniphila	0.005

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