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Authors	Airaksinen, K.;Yeung, N.;Lyra, A.;Lahtinen, S.J.;Huttunen, T.;Shanahan, Fergus;Ouweland, Arthur C.
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The effect of a probiotic blend on gastrointestinal symptoms in constipated patients: a double blind, randomised, placebo controlled 2-week trial

K. Airaksinen, N. Yeung, A. Lyra, S.J. Lahtinen, T. Huttunen, F. Shanahan and A.C. Ouwehand

Supplementary Materials and methods

Ethics and Good Clinical Practices (GCP)

The study was conducted per globally accepted standards of Good Clinical Practice (ICH, 1996), in agreement with the Declaration of Helsinki (WMA, 2013), and in accordance with local regulations for clinical research. The study was registered at the ISRCTN registry (ISRCTN41607808) and a notification was submitted to national authorities before commencement of the trial. The clinical study protocol including all amendments, information provided to the participants and the informed consent form were approved by the Clinical Research Ethics Committee of the Cork Teaching Hospital (ECM 4 (k) 11/08/15). The trial was conducted by Atlantia Food Clinical Trials Ltd. at two study centres in Cork and Mallow, Ireland. The intervention took place between October 2015 and March 2016. The study is reported following the CONSORT statement.

Exclusion criteria

Volunteers were excluded if they had continuous, daily use of probiotics or probiotic-containing products within one month prior to the randomization. Other exclusion criteria were: participation in a clinical trial with an IP or drug within 2 months prior to screening, likeliness to be non-compliant with the protocol or unsuitable for the study, planned major changes in lifestyle (i.e. diet, dieting, exercise level, travelling), eating disorder, history of drug or alcohol abuse, pregnancy or breastfeeding, or planning to become pregnant during the study, administrative or legal supervision, diagnosed or suspected organic gastrointestinal disease (i.e. colitis, Crohn's disease, celiac disease, recurrent diverticulitis), severely impaired general health (including cancer and cancer therapy), prior major abdominal surgery (excluding appendectomy), lactose intolerance (if the participant does not follow a lactose-restricted diet), known previous reaction (including anaphylaxis) to any substance included in the composition of the study product, consumption of or unwillingness to refrain from the use of commercial probiotics or prebiotics during the trial, laxative use within 48 hours of screening and regular use of laxatives (rescue medication was allowed), regular use of any drug or dietary supplement known to cause constipation as a common side effect (e.g. iron, opioids, sucralfate, misoprostol, 5 hydroxytryptamine [5 HT] antagonists, antacids with magnesium, calcium or aluminium, antidiarrheal medication, anticholinergic agents, calcium supplements, tricyclic antidepressants or non-steroidal anti-inflammatory drugs) within 1 month prior to study randomization, and the use of antibiotics within one month of the screening visit. These exclusion criteria for study entry were defined for safety reasons and to provide a population suitable for the study.

A non-fasted blood test was administered on Visit 1 for all participants to screen the blood safety values including C-reactive protein (CRP), and blood count. The participants with clinically significant values, based on the investigator's evaluation, were excluded from the study. A pregnancy test (urine/blood pregnancy) was administered only for female participants who were potentially premenopausal and perimenopausal, unless the participant was surgically sterile. A urine pregnancy test was carried out at visits 1, 3 and 5. When required by the radiology department performing the abdominal X-ray, a blood sample was collected at visits 2 and 4, to perform a blood pregnancy test in advance of abdominal X-ray. If the pregnancy test results were positive, the participant was excluded from the study.

Investigational product (IP)

The investigational product (IP) was a mixture of five live freeze-dried bacterial strains, *Lactobacillus acidophilus* NCFM (10^{10} cfu), *Lactobacillus paracasei* Lpc-37 (2.5×10^9 cfu), *Bifidobacterium animalis* subsp. *lactis* BI-04 (2.5×10^9 cfu), *B. animalis* subsp. *lactis* Bi-07 (2.5×10^9 cfu), and *B. animalis* subsp. *lactis* HN019 (10^{10} cfu) (Danisco USA Inc, Madison, WI, USA). Microcrystalline cellulose (MCC) was added to the active blend as an inert carrier for the probiotic capsules. Only MCC was encapsulated and used as placebo. Neither IP contained any other active ingredients than the bacterial strains. Retained stability samples were tested every 3 months for 15 months and total CFU was measured. The IP was found to be stable throughout the duration of the study.

Both groups consumed one capsule daily for 14 days. All capsules were identical in shape, texture and taste. The IPs were labelled following strict double-blind procedures. 16 capsules were available for the participants for the whole treatment period and the two extra capsules were added to allow flexibility. The five probiotic strains in the active IP have been tested in clinical trials previously and no Serious Adverse Events (SAEs) related to the IP or the trial procedures have been reported. The stability of the IP was followed for 15 months during and after the study. The stability of five-strain combination remained above the set limit (2.75×10^{10} cfu), which is considered fully acceptable for a functional probiotic product.

qPCR assays

All qPCR assays were done using 7500FAST Real-Time PCR Systems and either SYBR Fast or Taqman Fast Advanced mastermixes (Applied Biosystems, Waltham, MA, USA). Three assays were used to monitor probiotic recovery; due to the genetic homogeneity of the *B. animalis* subsp. *lactis* subspecies the only strain that was reliably detectable, separate from the others, was BI-04. *B. animalis* subsp. *lactis* strains Bi-07 and HN019 were not separately detectable. The assay for *Bifidobacterium animalis* subsp. *lactis* BI-04 was previously described in Lehtinen *et al.* (2018), and the assay for *L. paracasei* Lpc-37 was previously described in Haarman and Knol (2006). *L. acidophilus* NCFM was analysed using primers and probe designed for this study. All primers and probes were produced by Integrated DNA Technologies IDT (Coralville, IA, USA), sequences and annealing temperatures are listed in Supplementary Table S5.

Statistical methods

The total sample size of 152 randomized participants (76 per group) was based on the following parameters for the primary endpoint, weekly average VAS of bloating: Two-sided alpha: 0.05, 80% statistical power, mean reduction in VAS of 15 mm in the active group and 5 mm in the placebo group, VAS standard deviation of 20 mm, and 15% participant attrition. Similarly, for the secondary endpoint, CTT, the sample size calculation was based on the following parameters: Two-sided alpha: 0.05, 80% statistical power, estimated mean transit time decrease by 20 hours in the active group and 5 hours in the placebo group, mean transit time standard deviation of 30 h, two-group comparison of means (t-test), and 15% participant attrition: 15%. These conditions yielded to 129 evaluable participants. This sample size was also adequate to detect a general effect size of 0.5 for scale outcome and an effect size in ordinal scaled secondary outcomes with probiotic blend of 12.5% (SD=25%) and an absolute difference of 25% for categorical endpoints.

The primary analyses were performed for three different study populations: All safety analyses were based on the safety analysis population which included all participants that received at least one dose of IP. The Intention-to-treat (ITT) population was considered the primary efficacy analysis population and included all randomized participants who had taken at least one dose of IP, with a valid baseline measurement (Day 0). In case of missing data, Last Observation Carried Forward (LOCF) method be applied to conduct the statistical analysis for the ITT population. The Per-Protocol (PP) population included all randomized participants with a valid Baseline measurement (Day 0), a valid measurement at the end of study for CTT (Day 14) and at least 5/7 of bloating scores answered during the baseline and treatment periods, and who consumed $\geq 12/14$ of IP capsules and 100% of ROMs. Screening failures and withdrawn participants and those who had major protocol deviations were excluded from the PP analysis. Hence, the PP population represents a more accurate measure of therapeutic efficacy, with no evident bias. In addition to the analysis of the overall study population, a cohort analysis was performed on the participants enrolled before and after Christmas. Cohort analyses were performed for the PP population for primary and secondary outcomes.

The primary efficacy analysis for abdominal bloating followed the multivariate approach (ANCOVA) controlling for Baseline on the average VAS score (including all VAS values, also 0) for the change from Baseline (Day -7 to Day 1) to Treatment period (Day 7 to Day 13). The change in average VAS scores from the Baseline to the Treatment period, controlling for baseline, was compared between the active and placebo groups.

The secondary efficacy analyses for digestive symptoms in addition to bloating followed the same multivariate approach. The CTT on Day 14 and change from Day 0 to Day 14 were analysed using the multivariate approach controlling for Day 0. Constipation-related PAC-SYM and PAC-QoL scores were analysed consistent with the CTT. The number of defecations per week and stool consistency percentages of each defecation event during the Run-in, Baseline, Treatment, and On-treatment periods were summarized for each arm. These were analysed consistent with the digestive symptoms scored with daily VAS. In addition, the BSS scores were categorized as constipation (1,2); optimal (3,4,5); or diarrhoea (6,7), and were compared with a chi-square test for homogeneity. McNemar's test was used to analyse how many participants moved between the categories, indicating direction of the movement. Overall product

satisfaction was assessed at Day 14 with a 5-point ordinal Likert Scale (1 to 5) and summarized for each arm. These were evaluated using chi-square tests of homogeneity.

A post-hoc analysis was conducted to determine the differences in subgroups regarding VAS items (bloating, flatulence, abdominal pain, and burbling), CTT and bowel movement frequency. CTT and faecal microbial data (qPCR) were investigated further to define five subgroups for the post-hoc analysis: 1) ITT, 2) PP, 3) PP + CTT extremes excluded (PP + CTT), 4) PP + qPCR non-compliers excluded (PP + qPCR), 5) PP + CTT extremes excluded + qPCR non-compliers excluded (PP + CTT + qPCR). A few participants were found to have abnormal CTT values (less than 24 hours or more than 100 hours during the treatment period), thus these participants were excluded from the subgroup 3. The qPCR results were used to ensure that the IP were properly consumed, thus additional subgroups 4 and 5 were formed.

Post-hoc analyses included longitudinal repeated measures analysis to consider the non-linear nature of the VAS items between adjacent days. The area under the curve (AUC) was calculated for each visit individually in cumulative manner. AUC values were compared between the treatment groups at certain timepoints by using non-parametric Wilcoxon Two-Sided test. P-values are based on normal approximation. Repeated measures Analysis-of-Variance (RMANOVA) model was fitted to the data by using Compound symmetry covariance structure to consider multiple measures from the same participant over time. The degrees-of-freedom were adjusted using Kenward-Rogers method. RMANOVA model was fitted for the actual VAS scores and the post-hoc derived AUCs. The estimates for between-treatment differences were calculated as active–placebo. All models were adjusted for age, gender, BMI, bowel movement frequency and study site. Also, the time to significant relief (decrease) in VAS scores was calculated. An equal or greater than 20% relative decrease or equal or greater than 10 points absolute decrease in score was considered as a significant relief and the day count after the baseline (Day 0) was recorded. Generalized linear model was fitted to this data to see if one of the treatment groups would reach the relief faster than the other. In addition, CTT was compared between the treatment groups on Day 0 and Day 14 using Wilcoxon Two-Sided test. Adjusted model similar to repeated measures analyses was fitted for change from Baseline of CTT in each subgroup. Bowel movement frequency was calculated over Days -7 to -1 and Days 8 to 13 to form frequency count at Baseline and Treatment periods, respectively. Treatment group differences were analysed with Wilcoxon Two-Sided test at Baseline, Treatment, and unadjusted change from Baseline. Adjusted model similar to repeated measures analyses was fitted for change from baseline of bowel movement frequency in each subgroup.

Table S1. Demographic and baseline characteristics of the intention-to-treat (ITT) population (n=156 enrolled in the study).

		Active (n=78)	Placebo (n=78)	Total (n=156)
Age (years)	Mean (range)	42.15 (18–66)	40.95 (19–67)	41.55 (18–67)
Sex	Female, n (%)	71 (91)	68 (87.2)	139 (89.1)
	Male, n (%)	7 (9)	10 (12.8)	17 (10.9)
Ethnicity	Caucasian, n (%)	78 (100)	73 (93.6)	151 (96.8)
	Asian, n (%)	0 (0)	1 (1.3)	1 (0.6)
	Afro-Caribbean, n (%)	0 (0)	4 (2.6)	4 (2.6)
Smoking status	Ex-smoker, n (%)	10 (12.8)	10 (12.8)	20 (12.8)
	Current smoker, n (%)	27 (34.6)	16 (20.5)	43 (27.6)
	Never a smoker, n (%)	41 (52.5)	52 (66.7)	93 (59.6)

Activity level (IPAQ)	Category 1: Inactive, n (%)	20 (25.6)	14 (18.4) ¹	34 (22.1) ²
	Category 2: Minimally active, n (%)	58 (74.4)	62 (81.6) ¹	120 (77.9) ²
	Category 3: Highly active (HEPA), n (%)	25 (32.1)	27 (35.5) ¹	52 (33.8) ²
Physical characteristics (mean [SD])	Body temperature, °C	36.17 (0.41)	36.23 (0.38)	36.20 (0.39)
	Systolic blood pressure, mmHg	124 (14.46)	125.6 (17.11)	124.8 (15.81)
	Diastolic blood pressure, mmHg	78.26 (10.71)	77.17 (10.68)	77.71 (10.67)
	Pulse rate, bpm	72.33 (11.2)	71 (10.92)	71.67 (11.05)
	BMI, kg/m ²	26.00 (3.89)	27.74 (5.26)	26.87 (4.69)

BMI = body mass index; HEPA = health enhancing physical activity; IPAQ = International Physical Activity Questionnaire; SD = standard deviation.

¹ n=76 in the placebo group for baseline IPAQ score.

² Total n=154 for the IPAQ score.

Table S2. Summary of adverse events (AEs).

Population	Active (n=78) n (%)	95% CI	Placebo (n=78) n (%)	95% CI	Total (n=156) n (%)	95% CI
At least one AE	30 (38.5)	27.7%, 50.2%	36 (46.2)	34.8%, 57.8%	66 (42.3)	34.4%, 50.5%
Intensity of AE						
Any severe AE	0	0%, 4.6%	0	0%, 4.6%	0	0%, 2.3%
Any moderate AE	6 (7.7)	2.9%, 16%	2 (2.6)	0.3%, 9%	8 (5.1)	2.2%, 9.9%
Any mild AE	26 (33.3)	23.1%, 44.9%	35 (44.9)	33.6%, 56.6%	61 (39.1)	31.4%, 47.2%
Any event leading to death	0		0		0	
Any event leading to treatment discontinuation*	2 (2.6)		0		2 (1.3)	
Any SAE	0	0%, 4.6%	0	0%, 4.6%	0	0%, 2.3%
Relationship to study IP						
Any definitely related AE	0		0		0	
Any probably related AE	0		0		0	
Any possibly related AE	12 (15.4)		13 (16.7)		25 (16.0)	
Any unlikely related AE	13 (16.7)		14 (17.9)		27 (17.3)	
Any unrelated AE	14 (17.9)		15 (19.2)		29 (18.6)	

* Treatment was discontinued because of use of antibiotics to treat the AE.

Table S3. Mean values of primary and secondary outcomes in intention-to treat (ITT) and per-protocol (PP) populations. Primary and secondary parameters measured at baseline (Day -7 to Day 1) and treatment (Day 7 to Day 13) periods or at Visit 3 (Day 0) and Visit 5 (Day 14).

Parameter	ITT			PP		
	Active	Placebo	Total	Active	Placebo	Total
Bloating VAS						
Baseline	39.683 (17.558)	39.961 (18.742)	39.823 (18.104)	40.641 (17.742)	38.165 (19.911)	39.427 (18.783)
Treatment	29.829 (21.671)	30.905 (18.889)	30.367 (20.269)	30.675 (21.033)	28.144 (16.724)	29.434 (18.992)
Change from Baseline to Treatment	-9.199 (16.364)	-9.311 (15.511)	-9.255 (15.888)	-9.966 (15.312)	-10.021 (15.092)	-9.993 (15.13)
Treatment difference ^a	-0.039 (95% CI: 4.796, -4.875)			-0.834 (95% CI: 4.641, -6.309)		
Flatulence VAS						
Baseline	32.976 (18.59)	34.133 (20.959)	33.558 (19.761)	34.158 (19.019)	32.546 (21.327)	33.368 (20.099)
Treatment	28.57 (22.005)	30.286 (20.641)	29.428 (21.282)	30.645 (20.953)	27.266 (18.144)	28.988 (19.603)
Change from Baseline to Treatment	-3.747 (12.294)	-4.248 (15.116)	-3.999 (13.744)	-3.514 (12.454)	-5.28 (15.686)	-4.38 (14.088)
Treatment difference ^a	-0.304 (95% CI: 3.948, -4.557)			-2.205 (95% CI: 2.878, -7.289)		
Abdominal pain VAS						
Baseline	24.801 (18.144)	27.504 (19.5)	26.161 (18.826)	25.589 (19.568)	27.793 (21.002)	26.669 (20.213)
Treatment	19.677 (19.932)	22.862 (21.023)	21.269 (20.481)	20.579 (20.734)	20.631 (19.821)	20.604 (20.191)
Change from Baseline to Treatment	-4.627 (11.279)	-5.015 (15)	-4.822 (13.24)	-5.01 (10.539)	-7.162 (14.204)	-6.065 (12.455)
Treatment difference ^a	0.045 (95% CI: 4.169, -4.078)			-1.736 (95% CI: 2.901, -6.374)		
Burbling VAS						
Baseline	23.334 (18.717)	22.187 (19.993)	22.757 (19.314)	25.184 (19.503)	22.07 (19.961)	23.658 (19.693)
Treatment	18.698 (20.65)	19.215 (19.569)	18.957 (20.053)	20.478 (21.382)	18.408 (19.566)	19.463 (20.437)
Change from Baseline to Treatment	-4.294 (12.855)	-3.203 (15.674)	-3.745 (14.307)	-4.706 (13.196)	-3.662 (15.427)	-4.194 (14.271)
Treatment difference ^a	0.826 (95% CI: 5.162, -3.51)			0.348 (95% CI: 5.686, -4.99)		
Colonic Transit Time						
Visit 3 (Day 0)	66.846 (33.275)	65.031 (33.543)	65.938 (33.314)	67.477 (35.972)	67.632 (33.002)	67.553 (34.378)
Visit 5 (Day 14)	60.985 (34.179)	61.938 (35.612)	61.462 (34.793)	57.646 (36.287)	63.84 (34.527)	60.682 (35.397)
Change from Day 0 to Day 14	-5.862 (28.26)	-3.092 (29.76)	-4.477 (28.959)	-9.831 (28.469)	-3.792 (31.566)	-6.871 (30.031)

Parameter	ITT			PP		
	Active	Placebo	Total	Active	Placebo	Total
Treatment difference ^b	2.167 (95% CI: 10.616, -6.281)			6.093 (95% CI: 16.806, -4.62)		
Defecation frequency						
Baseline	2.346 (0.835)	2.5 (0.734)	2.423 (0.787)	2.212 (0.75)	2.5 (0.544)	2.353 (0.67)
Treatment	3.077 (1.642)	2.949 (1.528)	3.013 (1.582)	3.231 (1.676)	2.92 (1.322)	3.078 (1.514)
Change from Baseline to Treatment	0.731 (1.688)	0.449 (1.374)	0.59 (1.54)	1.019 (1.639)	0.42 (1.326)	0.725 (1.517)
Treatment difference ^a	-0.223 (95% CI: 0.254, -0.701)			-0.474 (95% CI: 0.112, -1.06)		
Stool consistency						
Baseline						
Constipation (1,2)	68	68		68	68	
Optimal (3,4,5)	8	9		8	9	
Diarrhea (6,7)	1	0		1	0	
Treatment						
Constipation (1,2)	47	43		47	43	
Optimal (3,4,5)	27	32		27	32	
Diarrhea (6,7)	0	3		0	3	
PAC-SYM median (min, max)						
Visit 3 (Day 0)	2.000 (0.636, 3.918)	1.917 (0.250, 3.668)	1.999 (0.250, 3.918)	1.875 (0.636, 3.918)	1.917 (0.250, 3.668)	1.917 (0.250, 3.918)
Visit 5 (Day 14)	1.250 (0.166, 3.501)	1.083 (0.083, 3.334)	1.182 (0.083, 3.501)	1.181 (0.166, 3.501)	1.083 (0.083, 2.582)	1.166 (0.083, 3.501)
Change from Day 0 to Day 14	-0.596 (-2.832, 0.750)	-0.584 (-2.917, 1.082)	-0.585 (-2.917, 1.082)	-0.584 (-2.832, 0.667)	-0.667 (-1.834, 1.082)	-0.585 (-2.832, 1.082)
PAC-QoL median (min, max)						
Visit 3 (Day 0)	2.125 (0.714, 3.607)	2.000 (0.393, 3.714)	2.000 (0.393, 3.714)	2.143 (0.714, 3.607)	1.875 (0.393, 3.536)	1.929 (0.393, 3.607)
Visit 5 (Day 14)	1.268 (0.143, 3.643)	1.250 (0.000, 3.357)	1.250 (0.000, 3.643)	1.304 (0.143, 3.643)	1.143 (0.000, 2.893)	1.250 (0.000, 3.643)
Change from Day 0 to Day 14	-0.518 (-2.750, 0.516)	-0.643 (-3.607, 0.679)	-0.536 (-3.607, 0.679)	-0.536 (-2.269, 0.516)	-0.661 (-2.179, 0.643)	-0.571 (-2.269, 0.643)
Product satisfaction (Visit 5, Day 14)						
Very dissatisfied	1 (0.6%)	0 (0%)	1 (0.6%)	0 (0%)	0 (0%)	0 (0%)
Dissatisfied	1 (0.6%)	4 (2.6%)	5 (3.2%)	1 (0.8%)	4 (3.1%)	5 (3.8%)
Neutral	31 (19.9%)	25 (16%)	56 (35.9%)	28 (21.5%)	22 (16.9%)	50 (38.5%)
Satisfied	28 (17.9%)	32 (20.5%)	60 (38.5%)	23 (17.7%)	26 (20%)	49 (37.7%)
Very satisfied	15 (9.6%)	17 (10.9%)	32 (20.5%)	14 (10.8%)	12 (9.2%)	26 (20%)

^a Treatment difference for Change Scores from Baseline to Treatment (Controlling for Baseline): Placebo – Active.

^b Treatment difference for Change Scores from Day 0 to Day 14 (Controlling for Day 0): Placebo – Active.

CI = confidence interval; PAC-SYM = Participant assessment of constipation-related symptoms; PAC-QoL = Participant assessment of constipation-related quality of life; VAS = visual analogue scale. Values are means (standard deviation, SD).

Table S4. Faecal recovery qPCR results, shown as qualitatively positive by assay and, the criteria of being positive for 2 out of 3 or 3 out of 3 of the assays concurrently.

	Total (n)	NCFM ^a (n)	BI04 ^b (n)	Lpc-37 ^c (n)	2/3 (n)	3/3 (n)
Placebo	63	7	2	22	4	0
Active	60	21	47	47	43	21

^a *Lactobacillus acidophilus* NCFM.

^b *Bifidobacterium animalis* subsp. *lactis* BI04.

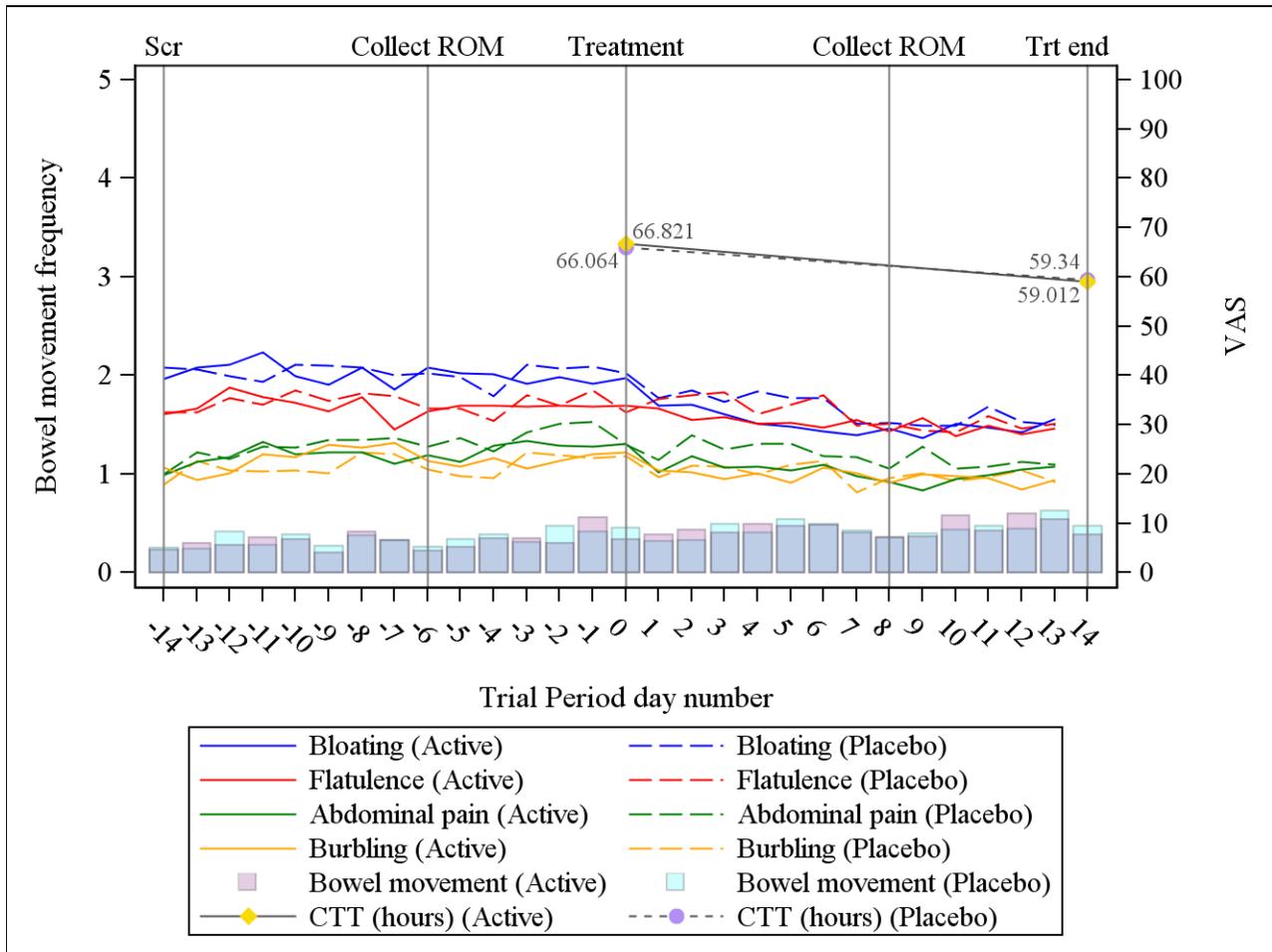
^c *Lactobacillus paracasei* Lpc-37.

Table S5. Primer sequences and annealing temperatures.

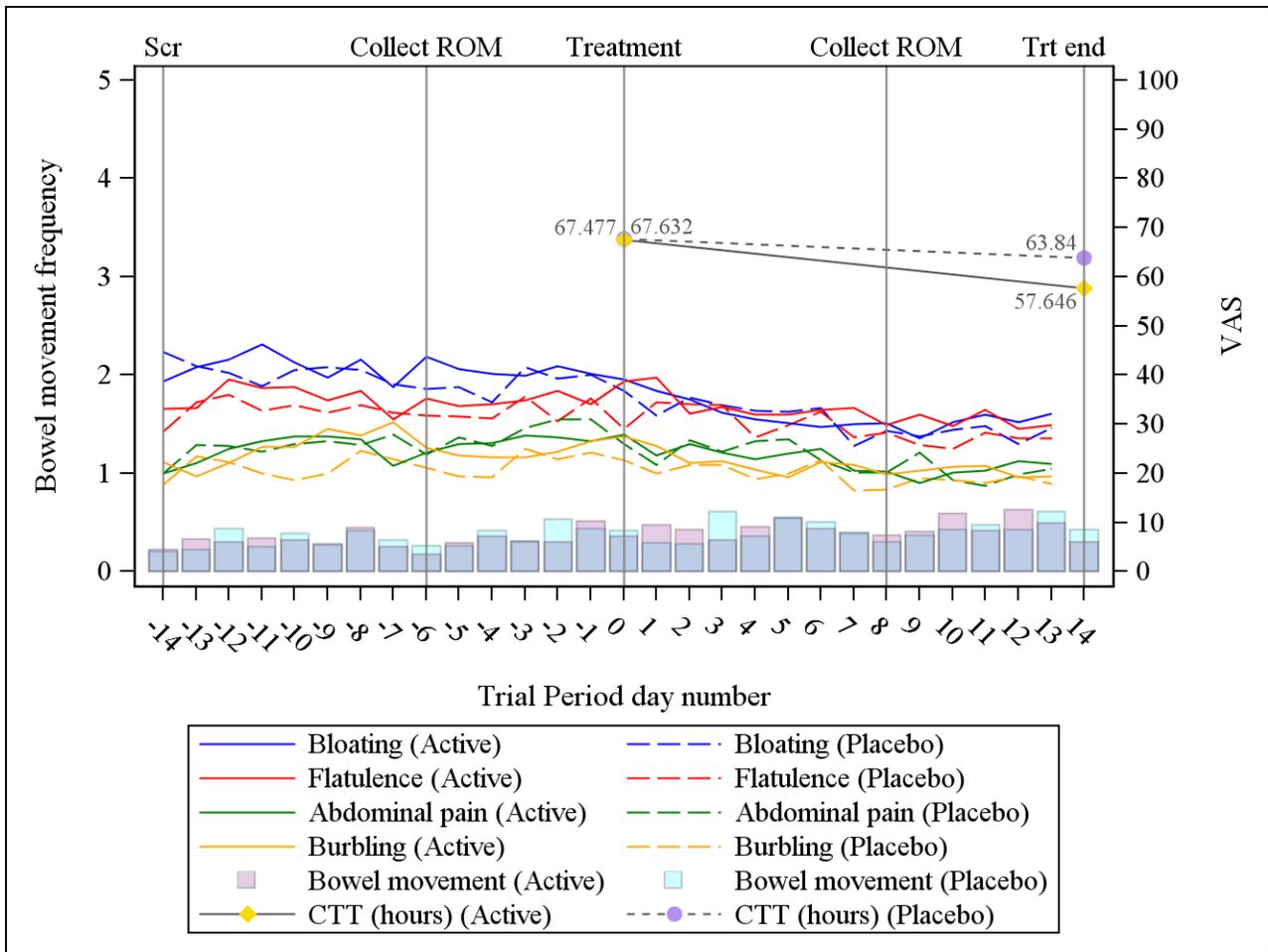
Target species	Name	Sequence 5' to 3'	Annealing temperature (°C)	Reference
<i>Lactobacillus acidophilus</i> NCFM	Laci_NCFMMJ_RTfwd	CCACGACCAGATGTAACCAA	62	this study
	Laci_NCFM_Rtrev	TTAGAAGATGCCAACGTCGAG		
	Laci_NCFM_probe	HEX-TAAGCCGAA/ZEN/CAATGCTGAAACGAT-IABkFQ		
<i>Lactobacillus paracasei</i> Lpc-37	F_paca_IS	ACATCAGTGTATTGCTTGTGTCAGTGAATAC	60	Haarman and Knol, 2006
	R_paca_IS	CCTGCGGGTACTGAGATGTTTC		
	P_paca_IS	TGCCGCCGGCCAG		
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> B1-04	B104_for	CTTCCCAGAAGGCCGGGT	60	Lehtinen <i>et al.</i> , 2018
	B104_rev	CGAGGCCACGGTGCTCATATAGA		

Figure S1. eDiary average response curves in different subgroups: (A) ITT, (B) PP, (C) PP + CTT, (D) PP + qPCR, and (E) PP + CTT + qPCR.

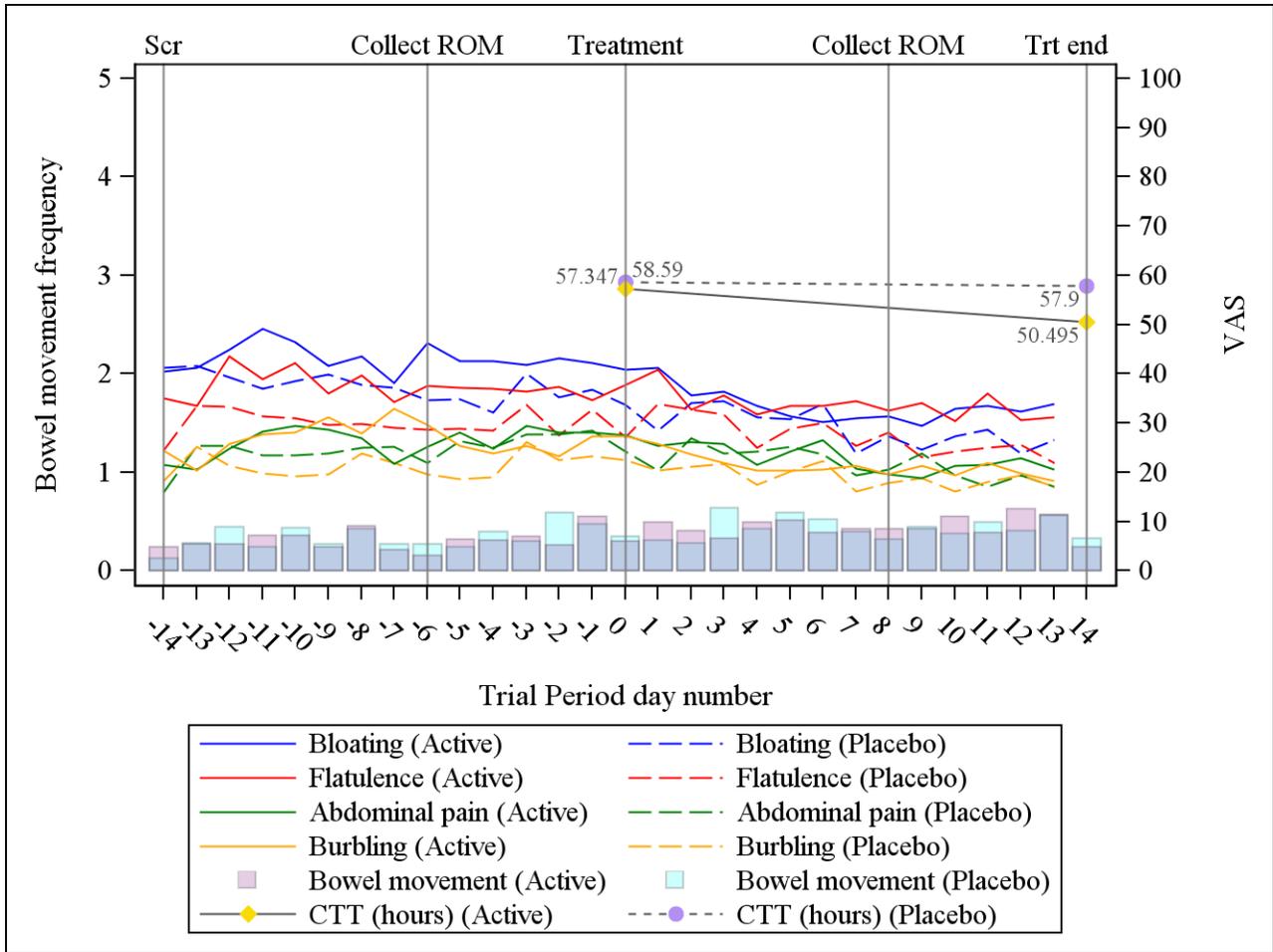
Electronic diary data is expressed using averaged line plots. The components of the response curve plots are bowel movement frequency as an underlaid bars, each VAS item as coloured line, and the average CTT values at baseline and post-intervention plotted for both active and placebo groups. The subgroups are intention-to-treat (ITT), per-protocol (PP), PP with extreme colonic transit time values excluded (PP + CTT), PP with qPCR non-compliers excluded (PP + qPCR), and PP with extreme CTT values and qPCR non-compliers excluded (PP + CTT + qPCR).



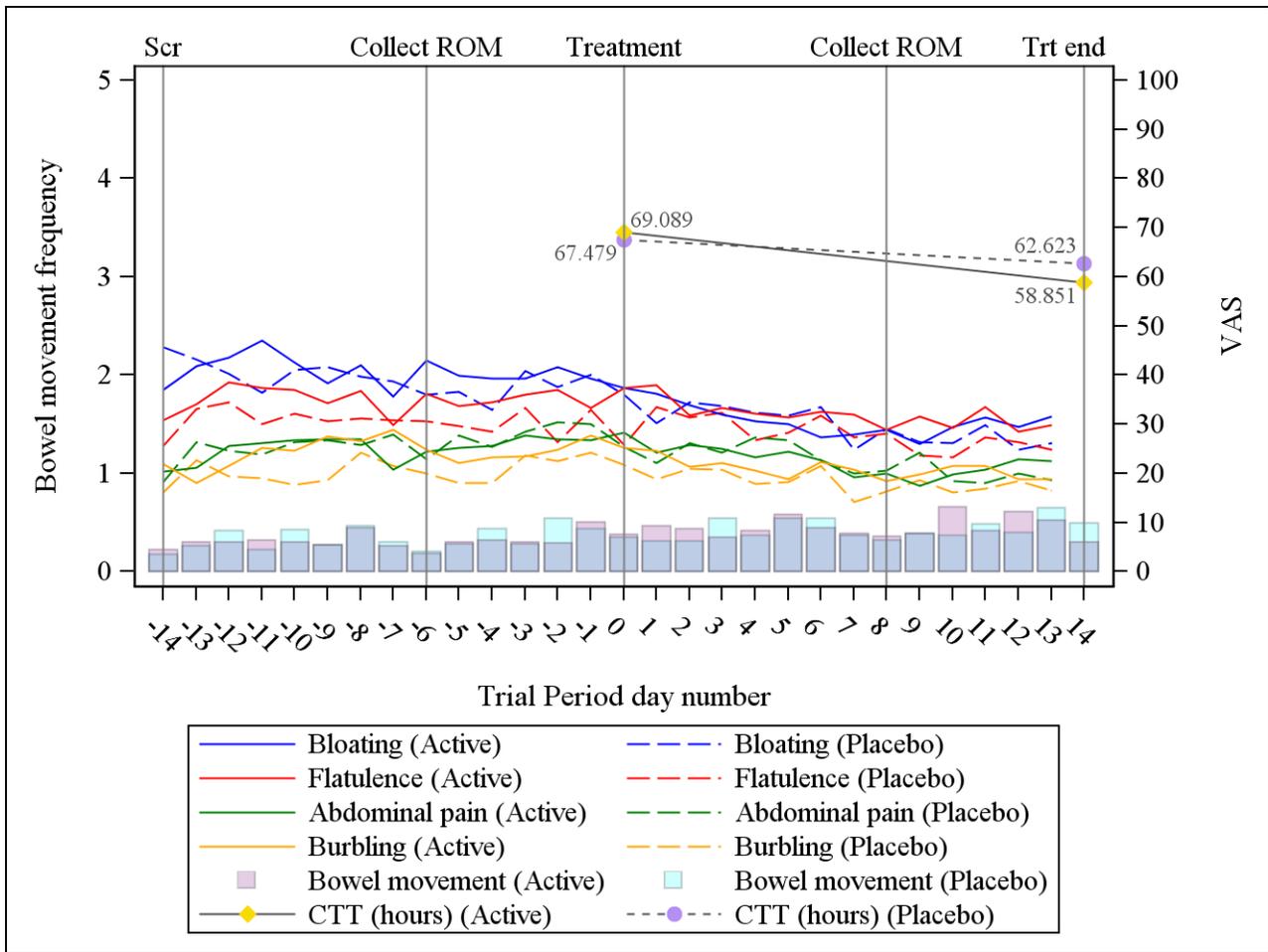
S1A. eDiary average response curves (ITT)



S1B. eDiary average response curves (PP)



S1C. eDiary average response curves (PP + CTT Extremes excluded)



S1D. eDiary average response curve (PP + qPCR non-compliance excluded)

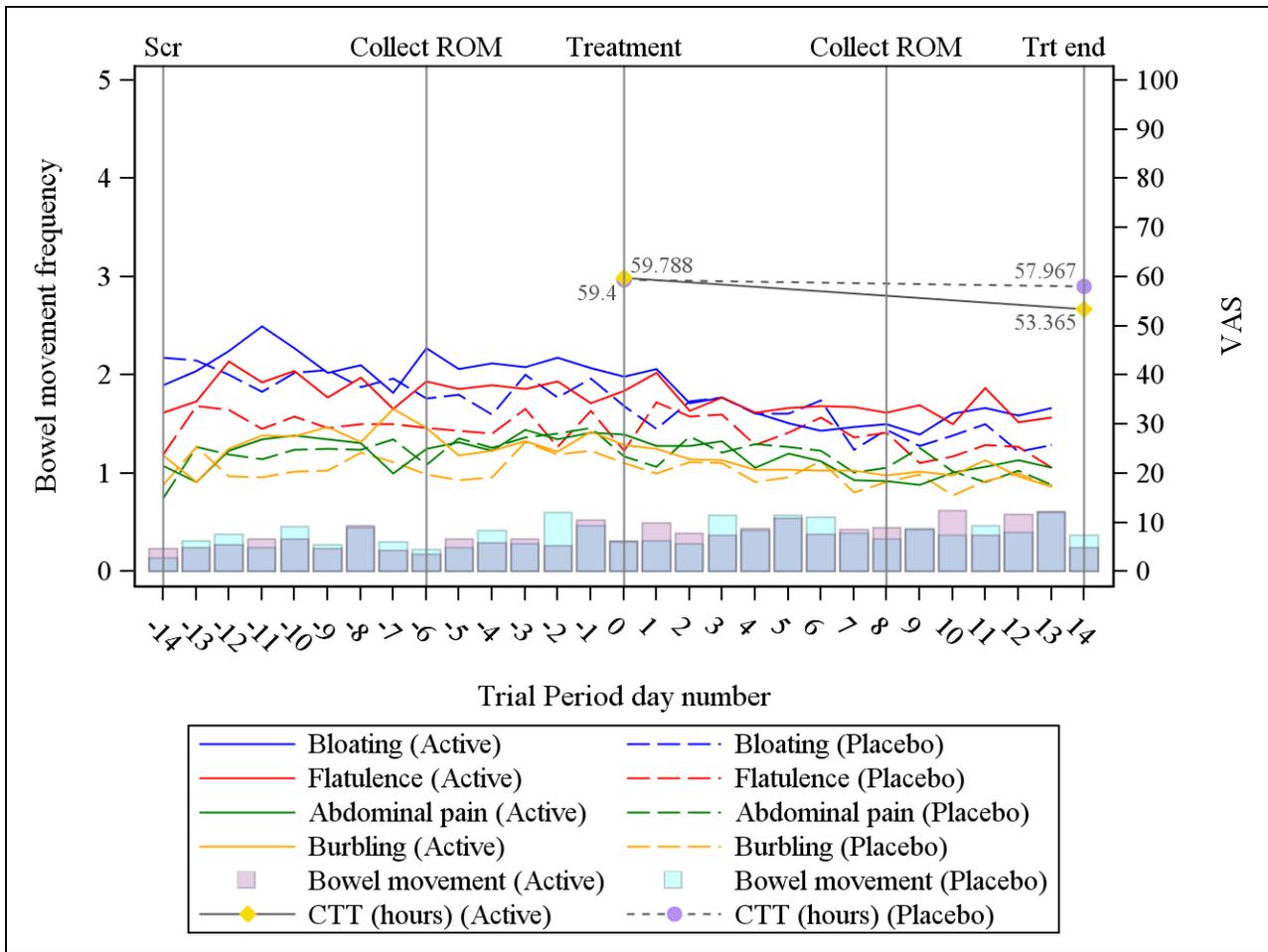


Figure S1E. eDiary average response curves (PP + CTT Extremes & qPCR non-compliant excluded)