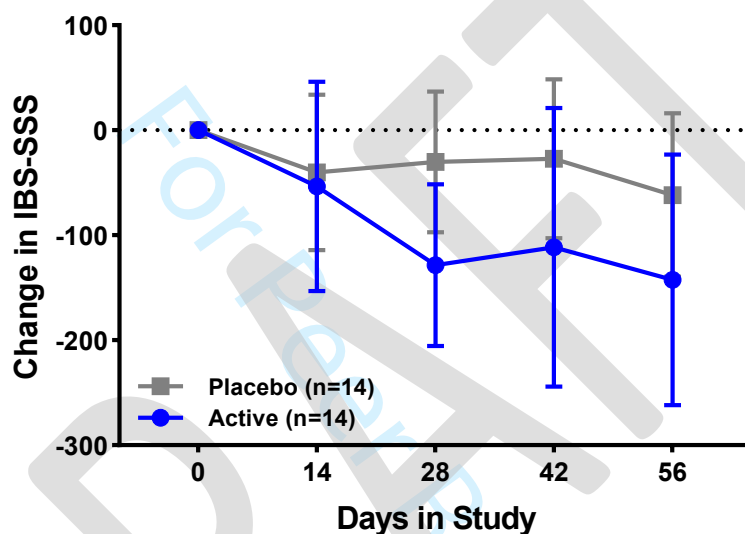


SUPPLEMENTARY INFORMATION**Clinical Trial: A double-blind, randomised, placebo-controlled study assessing the impact of probiotic supplementation on the symptoms of irritable bowel syndrome in females.**

B.H. Mullish, D.R. Michael, M. Dabcheva, T.S. Webberley, N. Coates, D. A. John, D. Wang, Y. Luo, S.F. Plummer and J.R. Marchesi



Supplementary Figure S1. Changes from baseline in IBS-symptom severity score in 18 to 40-year-old females with IBS participating in a previous intervention study with Lab4 probiotics (25 billion cfu/day)¹. The mean between group difference at 8 weeks was 80.86 points in favour of the active group.

¹Williams EA, Stimpson J, Wang D, *et al*. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Alimentary Pharmacology & Therapeutics*. 2009;29(1):97-103

Participant Number: _____ Date: dd/mm/yy _____

Day of intervention (please circle): 1 14 28 42 56

These questionnaires enable us to record and monitor the severity of your IBS symptoms. It is expected that your symptoms vary over time, so please try and answer the questions based on how you have felt over the last 14 days.

QUESTION 1:
A) Have you suffered from abdominal (tummy) pain?
YES NO
Please circle

B) If yes, please rate the severity:
Very mild 0 1 2 3 4 5 6 7 8 9 10 Very severe
Please circle

C) Number of days with pain in the past 14 days:

QUESTION 2:
A) Have you suffered from a bloated, swollen or tight tummy (that was not related to your monthly cycle):
YES NO
Please circle

B) If yes, please rate the severity:
Very mild 0 1 2 3 4 5 6 7 8 9 10 Very severe
Please circle

QUESTION 3:
How satisfied have you been with your bowel habit?
Unhappy 0 1 2 3 4 5 6 7 8 9 10 Very happy
Please circle

QUESTION 4:
Please indicate how much your Irritable Bowel Syndrome has affected your life in general.
Not at all 0 1 2 3 4 5 6 7 8 9 10 Completely
Please circle

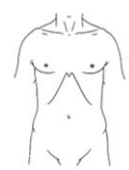
QUESTION 5:
A) What is the most number of times you have opened your bowels per day?
Number of times per day

B) What is the least number of times that you have opened your bowels?
Number of times per day

QUESTION 6:
Have your bowel movements been: *(Circle any/all that apply)*
A) normal often / occasionally / never
B) hard often / occasionally / never
C) very thin (like string) often / occasionally / never
D) in small pieces (like rabbit pellets) often / occasionally / never
E) mushy (like purée) often / occasionally / never
F) watery often / occasionally / never

QUESTION 7:
In the following questions you may circle more than one answer:
Have you: *Please circle*
A) passed mucus (or slime or jelly) during your bowel movements **YES NO**
B) passed blood during your bowel movements **YES NO**
C) had to hurry/rush to the toilet to open your bowels **YES NO**
D) Strained to open your bowels **YES NO**
E) Felt like you haven't emptied your bowel completely **YES NO**

QUESTION 8:
Please mark with a cross (X) on the diagram below where you have had pain (use more than one X if necessary)

Right side  Left side

QUESTION 9:
Have you:
A) noticed your stools are more loose or frequent when you get pain
YES NO NEVER NOTICED
Please circle

B) noticed whether the pain is eased by opening your bowels
YES NO NEVER NOTICED
Please circle

QUESTION 10:
Have you been:
A) absent from work due to IBS symptoms?
Number of days

B) at work suffering from IBS symptoms
Number of days

Supplementary Figure S2: IBS-Symptom Severity Score (IBS-SSS) questionnaire

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

| Date of attempted bowel motion (dd/mm/yy) | 1) Were you able to pass a stool? (If "Yes", please answer questions 2-7) | 2) Did you feel an urgent need to pass the bowel motion? | 3) Did you strain to start the bowel motion? | 4) Were you left with a feeling of incomplete evacuation? | 5) Please rate the appearance of your bowel motion using the Bristol stool score (Circle as appropriate) | 6) Was this stool provided to the trial site? | 7) Were you taking oral antibiotics? |
|---|---|--|--|---|--|---|--------------------------------------|
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |
| / / | Y/N | Y/N | Y/N | Y/N | 1, 2, 3, 4, 5, 6, 7 | Y/N | Y/N |

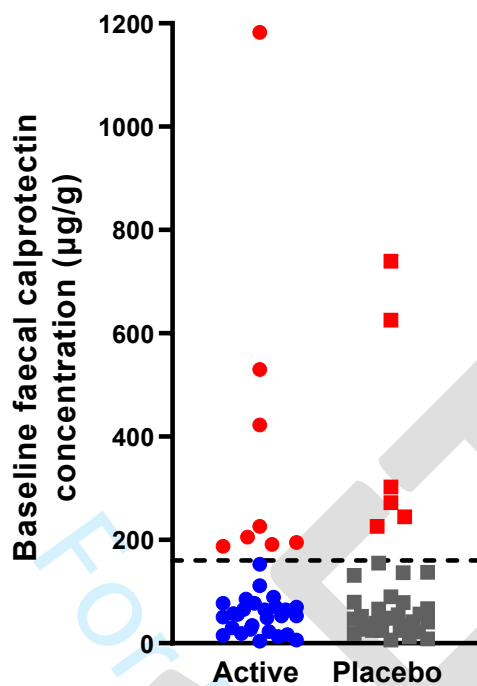
Supplementary Figure S3: Daily bowel habit diary

| Hospital Anxiety and Depression Scale (HADS) | | | |
|--|---|--|---|
| Read each item and circle the reply which comes closest to how you have been feeling. Don't take too long over your replies: your immediate reaction to each item is best. | | | |
| I feel tense or 'wound up': | | I feel as if I am slowed down: | |
| Most of the time | 3 | Nearly all of the time | 3 |
| A lot of the time | 2 | Very often | 2 |
| Time to time, occasionally | 1 | Sometimes | 1 |
| Not at all | 0 | Not at all | 0 |
| I still enjoy the things I used to enjoy: | | I get a sort of frightened feeling like 'butterflies in the stomach': | |
| Definitely as much | 0 | Not at all | 0 |
| Not quite so much | 1 | Occasionally | 1 |
| Only a little | 2 | Quite often | 2 |
| Not at all | 3 | Very often | 3 |
| I get a sort of frightened feeling like something awful is about to happen: | | I have lost interest in my appearance: | |
| Very definitely and quite badly | 3 | Definitely | 3 |
| Yes, but not too badly | 2 | I don't take as much care as I should | 2 |
| A little, but it doesn't worry me | 1 | I may not take quite as much care | 1 |
| Not at all | 0 | I take just as much care as ever | 0 |
| I can laugh and see the funny side of things: | | I feel restless as if I have to be on the move: | |
| As much as I always could | 0 | Very much indeed | 3 |
| Not quite so much now | 1 | Quite a lot | 2 |
| Definitely not so much now | 2 | Not very much | 1 |
| Not at all | 3 | Not at all | 0 |
| Worrying thoughts go through my mind: | | I look forward with enjoyment to things: | |
| A great deal of the time | 3 | A much as I ever did | 0 |
| A lot of the time | 2 | Rather less than I used to | 1 |
| From time to time but not too often | 1 | Definitely less than I used to | 2 |
| Only occasionally | 0 | Hardly at all | 3 |
| I feel cheerful: | | I get sudden feelings of panic: | |
| Not at all | 3 | Very often indeed | 3 |
| Not often | 2 | Quite often | 2 |
| Sometimes | 1 | Not very often | 1 |
| Most of the time | 0 | Not at all | 0 |
| I can sit at ease and feel relaxed: | | I can enjoy a good book or radio or TV programme: | |
| Definitely | 0 | Often | 0 |
| Usually | 1 | Sometimes | 1 |
| Not often | 2 | Not often | 2 |
| Not at all | 3 | Very seldom | 3 |

Supplementary Figure S4: Hospital Anxiety and Depression Score (HADS) questionnaire

| | | | | | | | | | |
|--|---|-----------------------|---|---|---|---|---|---|--|
| Participant Number: _____ | | Date: <u>dd/mm/yy</u> | | Study visit (please circle): | | 1 | | 2 | |
| IBS-Behavioural-Responses Questionnaire (IBS-BRQ) | | | | Please consider each question and circle the number that best applies to you over the last 8 weeks: | | | | | |
| | | | | 1 = Never ⇒ 7 = Always | | | | | |
| Q1 | I eat specific foods to help me open my bowels more | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q2 | I eat specific foods to help me open my bowels less | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q3 | I strain when opening my bowels | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q4 | After opening my bowels, I check for blood | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q5 | After opening my bowels, I check my stool for abnormalities | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q6 | I spend more time on the toilet than I ideally would like | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q7 | I often go to the toilet to open my bowels and then do not pass anything | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q8 | I often go to the toilet to pass water and find I open my bowels | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q9 | I avoid exercise when I have stomach pains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q10 | I avoid certain foods when I have bowel problems | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q11 | I wear baggy clothing when my stomach feels bloated or distended | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q12 | I avoid going out in case I have problems with my IBS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q13 | I avoid making plans in case I have problems with my IBS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q14 | I carry other items (e.g. wet wipes, sanitary towels, spare underwear) in case my IBS flares up | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q15 | I take medication (e.g. before going out) just in case my IBS flares up | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q16 | I carry medication with me in case my IBS flares up | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q17 | I avoid sex in case my IBS flares up (and causes embarrassment) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q18 | When I go out I make sure I know where the nearest toilet is | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q19 | I ask for reassurance about my IBS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q20 | I avoid certain work situations (e.g. meetings) because of my IBS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q21 | I avoid certain social situations (e.g. restaurants) because of my IBS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q22 | I avoid certain foods (e.g. dairy products, spicy foods) because of my IBS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q23 | After I open my bowels I wipe more than I would like | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q24 | When I have diarrhoea I do things to ease it (e.g. take prescribed medication, take alternative medication) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q25 | I am constantly aware of my stomach | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Q26 | I avoid staying away from home overnight in case my IBS flares up | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |

Supplementary Figure S5: IBS-Behavioural Response Questionnaire (IBS-BRQ)



Supplementary Figure S6. Baseline faecal calprotectin levels of participants recruited to the active and placebo groups. The dotted line represents the IBS upper threshold of 160 µg/g. Participants highlighted in red were excluded from data analysis.

Supplementary Table S1: Changes in IBS symptom severity score over the duration of the study

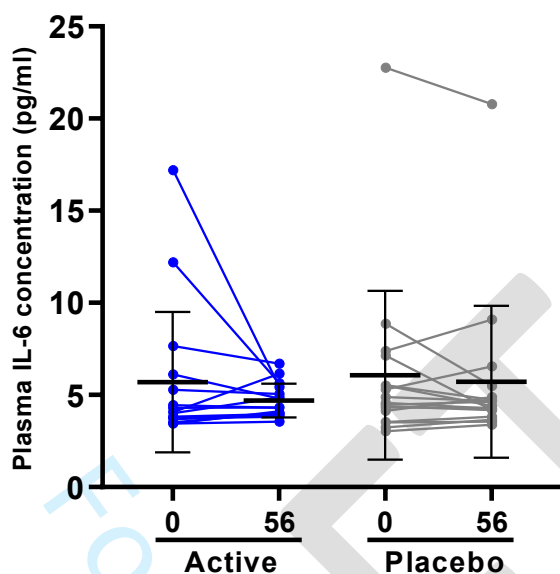
| | Active (n=27) | Placebo (n=29) |
|---|--|---------------------------------------|
| IBS symptom severity score | | |
| Day 14 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value) | -6.31(-22.37,9.74; <i>p</i> =0.4386) | 4.30(-11.18,19.78; <i>p</i> =0.5840) |
| Difference between groups in LSM(95% CI; <i>p</i> value) | -10.62(-33.12,11.89; <i>p</i> =0.3530) | |
| Day 28 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value) | -37.95(-54.01,-21.90; <i>p</i> <0.0001) | -1.86(-17.34,13.63; <i>p</i> =0.8132) |
| Difference between groups in LSM(95% CI; <i>p</i> value) | -36.10(-58.60,-13.59; <i>p</i> =0.0018) | |
| Day 42 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value) | -59.70(-75.76,-43.64; <i>p</i> <0.0001) | 2.23(-13.25,17.72; <i>p</i> =0.7762) |
| Difference between groups in LSM(95% CI; <i>p</i> value) | -61.93(-84.44,-39.43; <i>p</i> <0.0001) | |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value) | -78.43(-94.49,-62.37; <i>p</i> <0.0001) | 6.52(-8.96,22.00; <i>p</i> =0.4070) |
| Difference between groups in LSM(95% CI; <i>p</i> value) | -84.95(-107.45,-62.44; <i>p</i> <0.0001) | |
| Abdominal pain severity | | |
| Day 14 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -3.66(-7.86,0.54; <i>p</i> =0.0869) | -0.38(-4.43,3.67; <i>p</i> =0.8526) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -3.28(-9.15,2.58; <i>p</i> =0.2706) | |
| Day 28 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -12.18(-16.38,-7.98; <i>p</i> <0.0001) | -0.38(-4.43,3.67; <i>p</i> =0.8526) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -11.80(-17.66,-5.94; <i>p</i> =0.0001) | |
| Day 42 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -16.26(-20.46,-12.06; <i>p</i> <0.0001) | -0.04(-4.09,4.02; <i>p</i> =0.9857) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -16.22(-22.08,-10.36; <i>p</i> <0.0001) | |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -18.85(-23.05,-14.65; <i>p</i> <0.0001) | 0.31(-3.74,4.36; <i>p</i> =0.8809) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -19.16(-25.02,-13.29; <i>p</i> <0.0001) | |
| Days with Abdominal pain (%) | | |
| Day 14 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -0.57(-4.01,2.87; <i>p</i> =0.7432) | -0.70(-4.02,2.62; <i>p</i> =0.6774) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | 0.13(-4.65,4.91; <i>p</i> =0.9577) | |
| Day 28 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -8.51(-11.94,-5.07; <i>p</i> <0.0001) | -1.68(-5.00,1.63; <i>p</i> =0.3171) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -6.82(-11.60,-2.05; <i>p</i> =0.0054) | |
| Day 42 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -10.62(-14.06,-7.19; <i>p</i> <0.0001) | -2.42(-5.74,0.89; <i>p</i> =0.1508) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -8.20(-12.98,-3.42; <i>p</i> =0.0009) | |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -13.80(-17.24,-10.36; <i>p</i> <0.0001) | -0.21(-3.52,3.11; <i>p</i> =0.9020) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -13.59(-18.37,-8.81; <i>p</i> <0.0001) | |

Abbreviations: LSM, least square mean; CI, confidence interval

Supplementary Table S1 continued.

| | Active (n=27) | Placebo (n=29) |
|---|---|-------------------------------------|
| Dissatisfaction with bowel habit | | |
| Day 14 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -0.48(-5.03,4.06; <i>p</i> =0.8341) | 0.10(-4.28,4.49; <i>p</i> =0.9624) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -0.59(-6.91,5.73; <i>p</i> =0.8544) | |
| Day 28 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -7.15(-11.70,-2.60; <i>p</i> =0.0022) | -2.65(-7.04,1.73; <i>p</i> =0.2340) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -4.50(-10.81,1.82; <i>p</i> =0.1619) | |
| Day 42 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -14.56(-19.10,-10.01; <i>p</i> <0.0001) | 2.17(-2.21,6.56; <i>p</i> =0.3292) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -16.73(-23.05,-10.41; <i>p</i> <0.0001) | |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -23.08(-27.62,-18.53; <i>p</i> <0.0001) | 1.48(-2.90,5.87; <i>p</i> =0.5050) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -24.56(-30.88,-18.24; <i>p</i> <0.0001) | |
| Bloating severity | | |
| Day 14 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -1.05(-7.53,5.43; <i>p</i> =0.7491) | 5.81(-0.44,12.05; <i>p</i> =0.0683) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -6.86(-15.97,2.26; <i>p</i> =0.1393) | |
| Day 28 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -5.87(-12.35,0.61; <i>p</i> =0.0757) | 4.77(-1.48,11.02; <i>p</i> =0.1334) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -10.64(-19.75,-1.52; <i>p</i> =0.0225) | |
| Day 42 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -8.46(-14.94,-1.98; <i>p</i> =0.0108) | 2.01(-4.23,8.26; <i>p</i> =0.5255) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -10.47(-19.59,-1.36; <i>p</i> =0.0246) | |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -11.05(-17.53,-4.57; <i>p</i> =0.0009) | 3.74(-2.51,9.99; <i>p</i> =0.2392) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -14.79(-23.90,-5.67; <i>p</i> =0.0016) | |
| IBS impact on everyday life | | |
| Day 14 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -2.01(-6.32,2.31; <i>p</i> =0.3569) | 0.83(-3.32,4.99; <i>p</i> =0.6930) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -2.84(-8.88,3.20; <i>p</i> =0.3551) | |
| Day 28 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -5.71(-10.02,-1.40; <i>p</i> =0.0098) | -0.55(-4.70,3.61; <i>p</i> =0.7953) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -5.16(-11.20,0.88; <i>p</i> =0.0935) | |
| Day 42 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -11.26(-15.57,-6.95; <i>p</i> <0.0001) | 1.87(-2.29,6.02; <i>p</i> =0.3764) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -13.13(-19.17,-7.09; <i>p</i> <0.0001) | |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | -13.12(-17.43,-8.81; <i>p</i> <0.0001) | 2.56(-1.60,6.71; <i>p</i> =0.2263) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -15.67(-21.71,-9.63; <i>p</i> <0.0001) | |

Abbreviations: LSM, least square mean; CI, confidence interval



Supplementary Figure S7. Changes in plasma IL-6 over the duration of the intervention period.

The data is presented as individual values of 33 participants (Active, n=16; Placebo, n=17) at day 0 and day 56 overlaid with the group mean \pm standard deviation (SD). Values of p were calculated using the Wilcoxon matched pairs signed ranked test comparing day 0 vs day 56 in the same group or the Kruskal-Wallis test with Dunn's *post hoc* analysis comparing variations between groups.

Supplementary Table S2: Changes in general well-being over the duration of the study

| | Active (n=27) | Placebo (n=26) |
|---|-------------------------------------|------------------------------------|
| General wellbeing | | |
| Day 0 | | |
| Mean(SD): | 8.04(1.48) | 8.00(1.60) |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | 0.42(-0.12,0.95; <i>p</i> =0.1220) | 0.60(0.06,1.15; <i>p</i> =0.0298) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -0.19(-0.95,0.57; <i>p</i> =0.6233) | |
| State of health | | |
| Day 0 | | |
| Mean(SD): | 7.85(1.59) | 8.42(1.65) |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | 0.60(0.11,1.09; <i>p</i> =0.0171) | 0.61(0.11,1.11; <i>p</i> =0.0173) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -0.01(-0.71,0.69; <i>p</i> =0.9759) | |
| State of mood | | |
| Day 0 | | |
| Mean(SD): | 7.63(1.69) | 8.35(1.65) |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | 0.38(-0.15,0.92; <i>p</i> =0.1572) | 0.29(-0.25,0.84; <i>p</i> =0.2839) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | 0.09(-0.69,0.86; <i>p</i> =0.8193) | |
| State of energy | | |
| Day 0 | | |
| Mean(SD): | 7.19(1.73) | 7.58(1.88) |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | 1.07(0.46,1.69; <i>p</i> =0.0010) | 0.73(0.11,1.36; <i>p</i> =0.0231) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | 0.34(-0.54,1.22; <i>p</i> =0.4444) | |
| Sleep quality | | |
| Day 0 | | |
| Mean(SD): | 6.93(1.77) | 7.12(2.25) |
| Day 56 | | |
| Change from day 0, LSM(95% CI; <i>p</i> value): | 0.98(0.41,1.56; <i>p</i> =0.1220) | 1.60(1.01,2.18; <i>p</i> <0.0001) |
| Difference between groups in LSM(95% CI; <i>p</i> value): | -0.62(-1.44,0.21; <i>p</i> =0.1382) | |

Abbreviations: SD, Standard deviation; LSM, least square mean; CI, confidence interval

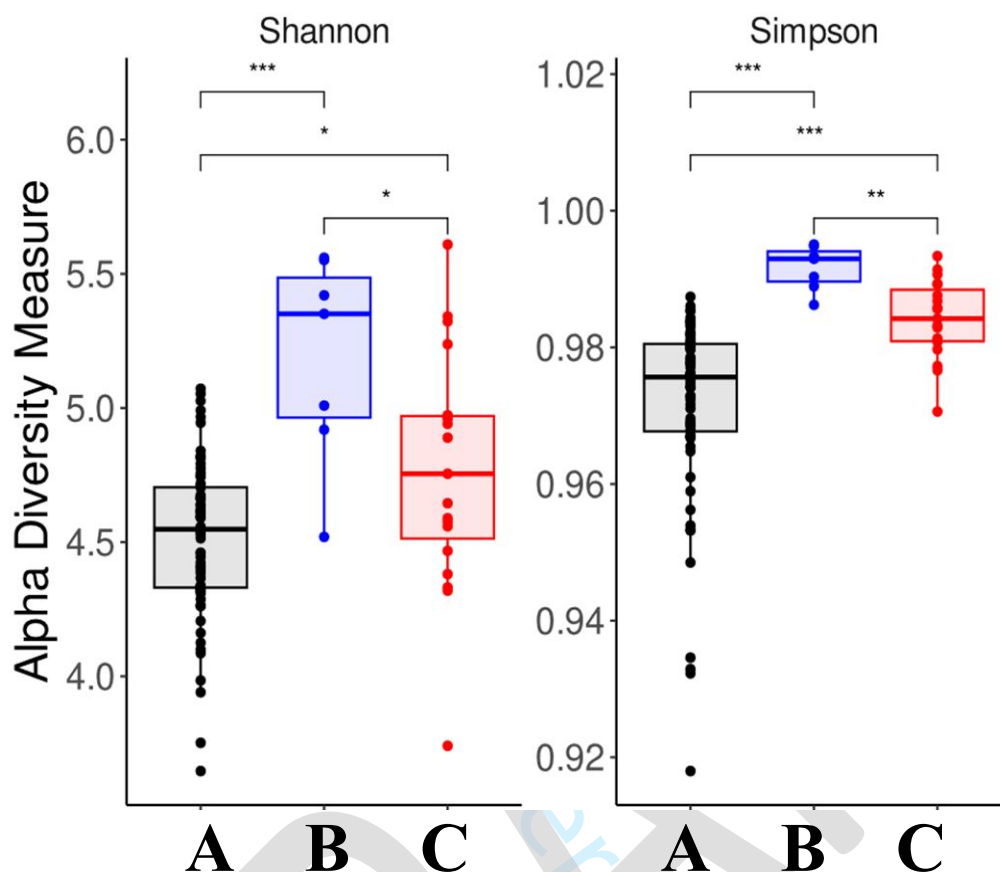
Supplementary Table S3: Changes in outcomes of the modified Stroop word colour test over the duration of the study

| | Active (n=26) | Placebo (n=26) |
|--|--|--------------------------------------|
| Total number of answers(per minute) | | |
| Day 0 | | |
| Mean(SD): | 32.64(8.17) | 33.85(10.26) |
| Day 56 | | |
| Change from day 0, mean(95% CI; <i>p</i> value): | 2.12(-2.20,6.44, <i>p</i> =0.4574) | 0.12(-4.12,4.35, <i>p</i> =0.9975) |
| Difference between groups in mean(95% CI; <i>p</i> value): | 2.01(-2.20,6.22, <i>p</i> =0.4855) | |
| Latency to correct answer(ms) | | |
| Day 0 | | |
| Mean(SD): | 2007.26(991.35) | 1908.59(619.03) |
| Day 56 | | |
| Change from day 0, mean(95% CI; <i>p</i> value): | -114.0(-511.6,283.6, <i>p</i> =0.7612) | 84.12(-305.8,4.74, <i>p</i> =0.8563) |
| Difference between groups in mean(95% CI; <i>p</i> value): | -198.2(-585.8,189.5, <i>p</i> =0.4352) | |
| Accuracy(% correct answers) | | |
| Day 0 | | |
| Mean(SD): | 95.92(5.59) | 94.34(9.19) |
| Day 56 | | |
| Change from day 0, mean(95% CI; <i>p</i> value): | 2.34(-1.28,5.97, <i>p</i> =2646) | 3.62(0.06,7.18, <i>p</i> =0.0453) |
| Difference between groups in mean(95% CI; <i>p</i> value): | -1.28(-4.81,2.26, <i>p</i> =0.6568) | |

Abbreviations; SD, Standard deviation; CI, confidence interval

Supplementary Table S4: Relative abundance estimates of the differentially abundant bacteria taxa within the probiotic or placebo groups

| Study Group | ASV | Genus | Relative Abundance | | P value |
|--|--------|------------------------------|--------------------|-------------|----------|
| | | | Probiotic | Placebo | |
| Different between groups at endpoint (but not at baseline): | | | | | |
| Placebo | ASV207 | Bacteroides | 0.020774564 | 0.326174145 | 2.24E-13 |
| Placebo | ASV260 | Blautia | 0.054300735 | 0.086622797 | 4.11E-15 |
| Probiotic | ASV478 | Roseburia | 0.091249086 | 0.022347656 | 8.82E-15 |
| Placebo | ASV504 | Prevotella_9 | 0.009852596 | 0.028124955 | 0.0421 |
| Probiotic | ASV221 | Agathobacter | 0.058569823 | 0.105182849 | 9.97E-17 |
| Probiotic | ASV225 | Holdemanella | 0.031579312 | 0.106195098 | 2.93E-14 |
| Probiotic | ASV227 | Blautia | 0.029289306 | 0.066828914 | 4.31E-19 |
| Probiotic | ASV258 | Blautia | 0.10761913 | 0.034481941 | 7.53E-15 |
| Placebo | ASV536 | Roseburia | 0.026654628 | 0.017667497 | 1.85E-14 |
| Placebo | ASV211 | Rumiococcus | 0.00987689 | 0.060027811 | 1.73E-20 |
| Placebo | ASV425 | Anaerostipes | 0.001835654 | 0.020658827 | 1.05E-12 |
| Different between groups at baseline: | | | | | |
| Placebo | ASV303 | Dorea | 0.007471204 | 0.110316564 | 1.19E-12 |
| Placebo | ASV308 | Undefined | 0.005665221 | 0.107211365 | 3.05E-14 |
| Placebo | ASV356 | Clostridium sensu stricto 1 | 0.000000000 | 0.049122948 | 4.14E-14 |
| Placebo | ASV379 | Bacteroides | 0.003278554 | 0.021258427 | 3.05E-14 |
| Probiotic | ASV459 | Roseburia | 0.045824732 | 0.002186901 | 3.05E-14 |
| Placebo | ASV539 | Clostridium sensu stricto 1 | 0.000000000 | 0.021483320 | 2.38E-12 |
| Probiotic | ASV541 | Anaerostipes | 0.038655216 | 0.003439796 | 9.07E-13 |
| Probiotic | ASV581 | [Ruminococcus] torques group | 0.028850609 | 0.000000000 | 1.88E-12 |
| Placebo | ASV628 | Terrisporobacter | 0.000000000 | 0.023694766 | 3.05E-14 |



Supplementary Figure S8. Comparison of the alpha diversity (Shannon and Simpson indices) of the baseline faecal samples from (A) the current study with (B) age, gender and geographically matched non-IBS subjects from Michael *et al* 2020¹ and (C) age and gender matched non-IBS subjects from Mullish *et al* 2023². Participant numbers were 70, 15 and 19 respectively and values of p were calculated using Wilcoxon rank sum test where * p <0.05, ** p <0.01 and *** p <0.001.

1. Michael DR, Jack AA, Masetti G, et al. A randomised controlled study shows supplementation of overweight and obese adults with lactobacilli and bifidobacteria reduces bodyweight and improves well-being. *Sci Rep.* 2020;10(1):4183.
2. Mullish BH, Michael DR, Webberley TS, et al. The gastrointestinal status of healthy adults: a post hoc assessment of the impact of three distinct probiotics. *Benef Microbes.* 2023:1-14.