STATISTICAL ANALYSIS OF EFFICACY DATA

First efficacy human study (Saggioro A. Journal of Clinical Gastroenterology, 2004)

This was a placebo-controlled, randomized study. The pain at different locations in the RLQ and LLQ of the abdomen is expressed at day 0 as total scores and corresponding averages. After 14 and 28 days of supplementation the percentages of decrease of each pain score are also reported. Other symptoms typically associated with IBS (constipation, diarrhea, bloating, flatulence, nausea, cephalea and dyspepsia) are expressed at day 0 as total scores and corresponding averages. After 14 and 28 days of supplementation with probiotics or placebo the percentages of decrease of each symptom score are also included.

ANOVA analysis was used to compare the results of all parameters in the two groups. The Kruskal-Wallis test was applied only in the event of lack of homogeneity of variances (evaluated by the Bartlett test). The software used in the analysis was "Epi Info" version 6.04d. P values were calculated for each parameter at day 0, day 14 and day 28 by a comparison between each active group and placebo and also between the two active groups A and B. Where some statistically significant differences were registered at day 0 between any active group and placebo, a further statistical analysis has been conducted by comparing the differences (Δ) between day 14, or day 28, and day 0 in an active group and in placebo or in an active group compared to the other. This was aimed at highlighting whether a statistically significant improvement could still be observed after 14 or 28 days of supplementation with probiotics compared to placebo, although at day 0 differences between active groups and placebo were present.Differences were considered significant at $p \leq 0.05$.

The Group receiving the combination of *Bifidobacterium breve* BR 03 and *Lactobacillus plantarum* LP 01 was assigned the code A, while Pl was the placebo. Group B was given a different combination of two probiotic strains. 24 subjects were enrolled in Group A, 20 in the placebo.

Abdominal pain at different abdomen locations

Comparison between each active group and placebo at day 0, 14 and 28

Pain scores at different locations of the abdomen at day 0							
Pain location		Day 0			p (A vs. B)	p (A vs. Pl)	p (B vs. Pl)
		A (24)	B (26)	Pl (20)			
RLQ	total score	42	41	43	0.530	0.167	0.031
	average	1.75	1.58	2.15			
LLQ	total score	61	64	46	0.724	0.278	0.472
	average	2.54	2.46	2.30			
Epigastrium	total score	41	40	37	0.511	0.603	0.247
	average	1.71	1.54	1.85			
Back	total score	32	34	32	0.862	0.136	0.070
	average	1.33	1.31	1.60			
Other sites	total score	21	20	22	0.577	0.268	0.093
	average	0.88	0.77	1.10			
Overall	total score	197	199	180	0.306	0.159	0.034
	average	1.64	1.53	1.80			

Pain scores at different locations of the abdomen and percentages of decrease after 14 days of probiotics supplementation

Pain location		Day 14			p (A vs. B)	p (A vs. Pl)	p (B vs. Pl)
		A (21)	B (23)	Pl (18)			
RLQ	total score	31	25	40	0.102	0.005	<0.001
	% decrease	15.6	31.1	-3.4			
	average	1.48	1.09	2.22			
LLQ	total score	31	25	40	0.177	0.021	<0.001
	% decrease	41.9	55.8	3.4			
	average	1.48	1.09	2.22			
Epigastrium	total score	25	22	34	0.292	0.009	<0.001
	% decrease	30.3	37.8	-2.1			
	average	1.19	0.96	1.89			
Back	total score	13	15	18	0.890	0.141	0.160
	% decrease	53.6	50.1	37.5			
	average	0.62	0.65	1.00			
Other sites	total score	13	14	18	0.961	0.070	0.041
	% decrease	29.3	20.9	9.1			
	average	0.62	0.61	1.00			
Overall	total score	113	101	150	0.049*	<0.001	<0.001
	% decrease	34.4	42.6	7.4			
	average	1.08	0.88	1.67			

* determined by Kruskal-Wallis statistical analysis.

Pain scores at different locations of the abdomen and percentages of decrease after 28 days of probiotics supplementation

Pain location		Day 28			p (A vs. B)	p (A vs. Pl)	p (B vs. Pl)
		A (21)	B (23)	Pl (16)			
RLQ	total score	19	24	35	0.496	<0.001	<0.001
	% decrease	48.3	33.8	-1.7			
	average	0.90	1.04	2.19			
LLQ	total score	19	24	35	0.582	<0.001	<0.001
	% decrease	64.4	57.6	4.9			
	average	0.90	1.04	2.19			
Epigastrium	total score	18	20	33	0.956	<0.001	<0.001
	% decrease	49.8	43.5	-11.5			
	average	0.86	0.87	2.06			
Back	total score	17	18	20	0.912	0.105	0.052
	% decrease	39.3	40.2	21.9			
	average	0.81	0.78	1.25			
Other sites	total score	17	16	20	0.519	0.059	0.013
	% decrease	7.5	9.6	-13.6			
	average	0.81	0.70	1.25			
Overall	total score	90	102	143	0.766	<0.001	<0.001
	% decrease	47.8	42.1	0.7			
	average	0.86	0.89	1.79			

Note: the percentages of decrease with a negative sign are indicative of an increase of the related parameter. The calculation of the percentages of decrease took into account any drop out occurred in the period from the previous visit.

Overall IBS symptoms

Comparison between each active group and placebo at day 0, 14 and 28

Overall symptoms scores at day 0							
Symptoms		Day 0			p (A vs. B)	p (A vs. Pl)	p (B vs. Pl)
		A (24)	B (26)	Pl (20)			
Constipation	total score	17	18	23	0.945	0.013*	0.012*
	average	0.71	0.69	1.15			
Diarrhoea	total score	54	55	58	0.581	0.002*	<0.001*
	average	2.25	2.12	2.90			
Bloating	total score	47	40	44	0.047	0.335	0.008
	average	1.96	1.54	2.20			
Flatulence	total score	51	46	45	0.126	0.575	0.050
	average	2.13	1.77	2.25			
Nausea	total score	19	18	21	0.602	0.254	0.085
	average	0.79	0.69	1.05			
Cephalea	total score	27	26	26	0.457	0.336	0.124
	average	1.13	1.00	1.30			
Dyspepsia	total score	38	34	33	0.234	0.774	0.157
	average	1.58	1.31	1.65			
Overall	total score	253	237	250	0.018	0.002	<0.001
	average	1.51	1.30	1.79			

* determined by Kruskal-Wallis statistical analysis.

Overall symptoms scores and percentages of decrease after 14 days of probiotics supplementation

Symptoms		Day 14			p (A vs. B)	p (A vs. Pl)	p (B vs. Pl)
		A (21)	B (23)	Pl (18)			
Constipation	total score	15	16	20	0.934	0.102	0.064
	% decrease	-0.8	-0.5	3.4			
	average	0.71	0.70	1.11			
Diarrhoea	total score	25	28	48	0.912	<0.001	<0.001
	% decrease	47.1	42.5	8.0			
	average	1.19	1.22	2.67			
Bloating	total score	21	23	44	0.847*	<0.001	<0.001
	% decrease	48.9	35.0	-11.1			
	average	1.00	1.00	2.44			
Flatulence	total score	19	20	39	0.901	<0.001	<0.001
	% decrease	57.4	50.9	3.7			
	average	0.90	0.87	2.17			
Nausea	total score	16	17	19	0.890	0.128	0.094
	% decrease	3.8	-6.8	-0.5			
	average	0.76	0.74	1.06			
Cephalea	total score	14	18	24	0.544	0.002	0.014
	% decrease	40.7	21.7	-2.6			
	average	0.67	0.78	1.33			
Dyspepsia	total score	21	27	30	0.423	0.008	0.041
	% decrease	36.8	10.2	-1.0			
	average	1.00	1.17	1.67			
Overall	total score	131	149	224	0.722	<0.001	<0.001
	% decrease	40.8	28.9	0.4			
	average	0.89	0.93	1.78			

* determined by Kruskal-Wallis statistical analysis.

Overall symptoms scores and percentages of decrease after 28 days of probiotics supplementation							
Symptoms		Day 28			p (A vs. B)	p (A vs. Pl)	p (B vs. Pl)
		A (21)	B (23)	Pl (16)			
Constipation	total score	14	15	19	0.945	0.009	0.009*
	% decrease	5.9	5.8	-3.3			
	average	0.67	0.65	1.19			
Diarrhoea	total score	18	21	41	0.801	<0.001	<0.001
	% decrease	61.9	56.8	11.6			
	average	0.86	0.91	2.56			
Bloating	total score	15	20	41	0.470	<0.001	<0.001
	% decrease	63.5	43.5	-16.5			
	average	0.71	0.87	2.56			
Flatulence	total score	16	18	36	0.928	<0.001	<0.001
	% decrease	64.1	55.8	0.0			
	average	0.76	0.78	2.25			
Nausea	total score	12	14	18	0.823	0.009	0.007
	% decrease	27.8	12.1	-7.1			
	average	0.57	0.61	1.13			
Cephalea	total score	14	17	18	0.709	0.051	0.117
	% decrease	27.8	12.1	13.5			
	average	0.67	0.74	1.13			
Dyspepsia	total score	22	24	31	0.822	<0.001	<0.001
	% decrease	33.8	20.2	-17.4			
	average	1.05	1.04	1.94			
Overall	total score	111	129	204	0.635	<0.001	<0.001
	% decrease	49.9	38.5	-2.0			
	average	0.76	0.80	1.82			

* determined by Kruskal-Wallis statistical analysis.

Comparison of the differences (Δ) between day 14, or day 28, and day 0 in each active group and placebo

Overall symptoms scores and percentages of decrease after 14 days of probiotics supplementation

Symptoms		Day 0			Day 14			p (Δ_A vs. Δ_B)	p (Δ_A vs. Δ_{Pl})	p (Δ_B vs. Δ_{Pl})
		A (24)	B (26)	Pl (20)	A (21)	B (23)	Pl (18)			
Constipation	total score	17	18	23	15	16	20	0.491	0.787	0.785*
	% decrease				-0.8	-0.5	3.4			
	average	0.71	0.69	1.15	0.71	0.70	1.11			
Diarrhoea	total score	54	55	58	25	28	48	0.385	0.001*	0.006
	% decrease				47.1	42.5	8.0			
	average	2.25	2.12	2.90	1.19	1.22	2.67			
Bloating	total score	47	40	44	21	23	44	0.115	<0.001	0.002
	% decrease				48.9	35.0	-11.1			
	average	1.96	1.54	2.20	1.00	1.00	2.44			
Flatulence	total score	51	46	45	19	20	39	0.274	<0.001*	0.001*
	% decrease				57.4	50.9	3.7			
	average	2.13	1.77	2.25	0.90	0.87	2.17			
Nausea	total score	19	18	21	16	17	19	0.814	0.965	0.850
	% decrease				3.8	-6.8	-0.5			
	average	0.79	0.69	1.05	0.76	0.74	1.06			
Cephalea	total score	27	26	26	14	18	24	0.133	0.003	0.076
	% decrease				40.7	21.7	-2.6			
	average	1.13	1.00	1.30	0.67	0.78	1.33			
Dyspepsia	total score	38	34	33	21	27	30	0.008*	0.004	0.432
	% decrease				36.8	10.2	-1.0			
	average	1.58	1.31	1.65	1.00	1.17	1.67			
Overall	total score	253	237	250	131	149	224	0.006	<0.001	<0.001
	% decrease				40.8	28.9	0.4			
	average	1.51	1.30	1.79	0.89	0.93	1.78			

* determined by Kruskal-Wallis statistical analysis.

Overall symptoms scores and percentages of decrease after 28 days of probiotics supplementation

Symptoms		Day 0			Day 28			p (Δ_A vs. Δ_B)	p (Δ_A vs. Δ_{Pl})	p (Δ_B vs. Δ_{Pl})
		A (24)	B (26)	Pl (20)	A (21)	B (23)	Pl (16)			
Constipation	total score	17	18	23	14	15	19	0.590	0.712	0.439
	% decrease				5.9	5.8	-3.3			
	average	0.71	0.69	1.15	0.67	0.65	1.19			
Diarrhoea	total score	54	55	58	18	21	41	0.370	0.001*	0.001
	% decrease				61.9	56.8	11.6			
	average	2.25	2.12	2.90	0.86	0.91	2.56			
Bloating	total score	47	40	44	15	20	41	0.048	<0.001	<0.001
	% decrease				63.5	43.5	-16.5			
	average	1.96	1.54	2.20	0.71	0.87	2.56			
Flatulence	total score	51	46	45	16	18	36	0.174	<0.001*	<0.001*
	% decrease				64.1	55.8	0.0			
	average	2.13	1.77	2.25	0.76	0.78	2.25			
Nausea	total score	19	18	21	12	14	18	0.591	0.066	0.121
	% decrease				27.8	12.1	-7.1			
	average	0.79	0.69	1.05	0.57	0.61	1.13			
Cephalea	total score	27	26	26	14	17	18	0.278	0.257	0.952
	% decrease				27.8	12.1	13.5			
	average	1.13	1.00	1.30	0.67	0.74	1.13			
Dyspepsia	total score	38	34	33	22	24	31	0.030	<0.001	0.021
	% decrease				33.8	20.2	-17.4			
	average	1.58	1.31	1.65	1.05	1.04	1.94			
Overall	total score	253	237	250	111	129	204	0.023	<0.001	<0.001
	% decrease				49.9	38.5	-2.0			
	average	1.51	1.30	1.79	0.76	0.80	1.82			

* determined by Kruskal-Wallis statistical analysis.

Note: the percentages of decrease with a negative sign are indicative of an increase of the related parameter. The calculation of the percentages of decrease took into account any drop out occurred in the period from the previous visit.

Abdominal pain. A statistically significant improvement was registered in both active groups compared with placebo for the majority of the locations taken into account in the study. In particular, RLQ, LLQ and epigastrium were significantly reduced also after 2 weeks of probiotics intake in Group A (p=0.005, p=0.021 and p=0.009, respectively), with no significant differences between the two active groups. Back and other sites scores were also improved in Group A compared with placebo, but not to a statistically significant extent. The overall scores recorded in the three groups confirm the effectiveness of the probiotic strains used in this study (p<0.001 in Group A after 14 and 28 days compared with placebo, p=0.766 for the comparison between group A and B after 28 days). Slightly better results were obtained in group A, treated with mixed *L. plantarum* LP 01 + *B. breve* BR 03, even if not significantly if compared with Group B (47.8% percentage decrease after 28 days compared with 42.1% recorded in Group B, p=0.766). It is interesting to note that in Group A a further

interesting reduction of overall score occurred between Day 14 and Day 28 (34.4% percentage decrease after 14 days compared with 47.8% after 28 days).

Other symptoms scores. A 4-weeks supplementation with a combination of *L. plantarum* LP 01 and *B. breve* BR 03 was able to significantly improve the scores of the majority of the other symptoms typically associated with IBS. In particular, diarrhea ($p=0.001$), bloating ($p<0.001$) and flatulence ($p<0.001$) registered the best results in Group A, with an average of 61 to 64% reduction of their scores after 28 days of probiotics intake. Dyspepsia ($p=0.004$ and $p<0.001$ after 14 and 28 days, respectively) score was also significantly improved. The overall score highlighted a strong reduction as well ($p<0.001$ compared to placebo already after 14 days). The best results were obtained in group A, receiving *L. plantarum* LP 01 + *B. breve* BR 03 (49.9% percentage decrease in the overall score after 28 days compared to 38.5% recorded in group B, $p=0.023$). Many statistically significant results were noted also after 2 weeks of probiotic strains intake, even if 2 further weeks of supplementation induced some further improvements. On the other side, constipation associated with Irritable Bowel Syndrome showed only slight improvements, but not to a statistically significant extent ($p=0.712$ in Group A). A possible explanation is that the initial incidence (Day 0) of constipation was very low if compared with other scores (average of 0.71 in Group A). Indeed, it recorded the lowest average score of 7 total parameters at Day 0. For example, diarrhea recorded a 2.25 initial average score in Group A. For this reason, constipation was the parameter most difficult to be improved during the study.